



UNIVERSITY OF AGRONOMIC SCIENCES
AND VETERINARY MEDICINE OF BUCHAREST
FACULTY OF AGRICULTURE



SCIENTIFIC PAPERS

SERIES A. AGRONOMY

VOLUME LXIX, No. 1



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SOIL SCIENCES

SOIL FERTILITY DYNAMICS ON A SOWN GRASSLAND UNDER THE COMBINED INFLUENCE OF MINERAL FERTILIZATION AND WATER EROSION

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Abstract

This study investigates the temporal dynamics of key agrochemical properties of soil in a sown grassland under the influence of mineral fertilization and water erosion on a slope of 10-15%, at the Preajba Experimental Center, Gorj County. Research was conducted on a stagnic luvisol using three experimental treatments: unfertilized control (N0P0K0), fertilization with N60P60K60, and fertilization with N100P90K60, each replicated three times in a randomized block design. Soil samples were collected from the upper and lower positions of the plots at three distinct time points: pre-fertilization (April), post-fertilization (May), and at the end of the growing season (October). The results demonstrated highly significant increases in hay yield in the fertilized treatments, while soil agrochemical properties exhibited moderate changes, characteristic of soils with high buffering capacity and pronounced chemical inertia. Mineral fertilization induced slight acidification and increases in available phosphorus and potassium, whereas organic matter and total nitrogen evolved gradually. These findings provide a scientific basis for the development of sustainable management strategies for sown grasslands on sloped terrains.

Key words: dynamics, mineral fertilization, sown grassland, water erosion, agrochemical indicators, soil fertility.

INTRODUCTION

Modern agriculture operates under increasing pressures generated by population growth, climate variability, and the depletion of natural resources, while simultaneously being required to reduce its environmental impact (Leoveanu-Soare et al., 2020; Mărin N. et al., 2023, Nițu et al., 2023).

In this context, agricultural production fundamentally relies on soil, water, and atmospheric resources, rendering agroecosystems particularly vulnerable to extreme meteorological phenomena such as drought and unevenly distributed precipitation (Bonea 2020; Cioboată et al., 2025, Nițu et al., 2025). In parallel, to meet crop nutritional requirements, various fertilization strategies and technologies are applied, whose efficiency and limitations vary depending on species, pedological conditions, and agroclimatic factors; consequently, fertilizer use remains a major concern (Cioboată et al., 2025; Prioteasa et al., 2025). Soil degradation processes, particularly erosion and organic matter

depletion, reduce soil stability and its capacity to sustain agricultural productivity (Ailincăi et al., 2008; Popescu et al., 2024). Balanced nutrient management therefore represents a core pillar of sustainable agriculture, enhancing system resilience to climatic and economic pressures. In modern agriculture, soil fertility is the result of the interaction between natural fertility and fertility induced by agrochemical management, including mineral and organic fertilization, soil reaction amendment, and the application of technologies aimed at conserving soil organic matter.

Optimizing fertility is an essential condition for achieving high agricultural productions that are economically efficient and compatible with the principles of sustainable agriculture (Popescu & Balan, 2024).

The application of fertilizers on eroded soils contributes to increased plant density, enhanced aboveground biomass and root system development, thereby improving water infiltration into the soil and increasing the protective capacity of vegetation against erosion processes (Iancu et al., 2009).

Grasslands represent highly complex ecosystems of considerable ecological and economic importance, determined by their global extent and the multiple functions they perform (Rotar, 1997). They support livestock production by providing forage resources with high nutritional value and deliver a wide range of ecosystem services, including soil fertility conservation, carbon sequestration, regulation of the hydrological cycle, climate stabilization, and enhancement of landscape and recreational value (Vargová et al., 2020).

In Gorj County, agriculture and livestock production represent essential components of the local economy, with grasslands constituting a central element of the agricultural system (Balan et al., 2024; Balan & Nițu, 2025). Approximately half of the county's agricultural area (50.07%), corresponding to 122,070 ha out of a total of 243,768 ha, is occupied by pastures and hayfields (Balan & Popescu, 2024), highlighting the strategic importance of these ecosystems. The management of grasslands decisively influences their ecological status, being sensitive to both abandonment and overexploitation (Briemle & Opperman, 2003; Bogdan, 2012).

Mineral fertilization and management intensity induce significant changes in soil fertility, floristic composition, and productivity, with management intensification often being associated with a reduction in plant species diversity.

The floristic composition and productivity of grasslands are the result of complex interactions between edaphic, climatic and technological factors, and their response to mineral fertilization differs depending on the type of grassland and soil conditions (Rotar, 2003; Rotar et al., 2016; Cirebea, 2017). In this context, detailed knowledge of the soil and the processes that influence productive capacity becomes essential for the implementation of sustainable management strategies (Balan et al., 2024; Păcurar, 2005). A balanced approach to fertilization and grassland utilization is required to optimize forage productivity while simultaneously maintaining biodiversity and the functional integrity of grassland ecosystems (Naie et al., 2017).

Soil erosion is one of the main degradation processes, particularly affecting the top layer of

soil (0-30 cm), which is rich in organic matter and biological activity. The removal of this layer leads to significant losses of humus and essential nutrients, such as nitrogen, phosphorus and potassium, with negative effects on physicochemical properties, microbiological activity and nutrient supply capacity (Ailincăi et al., 2008; Mușat et al., 2023; Balan et al., 2024; Popescu et al., 2024).

Overall, these transformations lead to a decline in soil fertility and a reduction in the productive potential of grasslands, underscoring the need to assess the impact of erosion on nutrient dynamics as a critical factor in ensuring sustainable land use and the functional integrity of grassland ecosystems (Balan et al., 2024).

The aim of this study was to evaluate the impact of erosion processes on nutrient dynamics and soil fertility in sown grassland ecosystems of Gorj County in relation to the applied management practices. Specifically, the study aimed to: characterize changes in soil physicochemical properties induced by erosion processes and mineral fertilization; analyzing variations in humus content and the main nutrient elements (nitrogen, phosphorus, and potassium) in sown grassland soils; and assess the effects of these changes on the productivity and functionality of sown grassland ecosystems.

MATERIALS AND METHODS

The research was conducted at the Preajba Experimental Grassland Center (Gorj County, Romania), belonging to the University of Craiova. The site is located in the Subcarpathian region, on Bălănești Hill, at an altitude of approximately 300 m, on the highest terrace of the Jiu River (fifth terrace), with south-eastern exposure and a slope of 10-15%. The area is characterized by a humid hilly climate, with mean annual precipitation of 736.9 mm, of which 434.1 mm occurs during the growing season. Mean air temperatures range from -1.1°C in January to 21.9°C in July.

The research was carried out on a Stagnic Luvisol located on land with a slope of 10-15%, developed on fluvial terrace deposits. The groundwater table is situated at depths ranging between 5 and 10 m, and the natural vegetation is represented by an acidophilous grassland. The soil profile at the experimental site is of the Aț-

Ao–El(w)–ElBt–Bt₁(w)–Bt₂(w) type. From a chemical perspective, the soil exhibits a strongly acidic reaction, with pH values ranging from 5.1 to 5.4 throughout the entire profile.

With respect to chemical fertility, the soil is characterized by a very low content of available phosphorus, approximately 6 ppm in the surface horizon, decreasing to about 1 ppm in the underlying horizons. The supply of available potassium is good in the surface A_{tel} and Ao horizons (174 ppm and 144 ppm, respectively), moderate in the AEI (76 ppm) and EI (46 ppm) horizons, and low in the Bt₁, BC, and C horizons, where concentrations are below 40 ppm.

The experiment consisted in the placement of an experiment with grassland sown with 3 variants and 3 repetitions, according to the method of isolated blocks, in order to highlight the dynamics of soil fertility under the influence of erosion and mineral fertilization.

The experimental plots were rectangular, measuring 25 m in length (oriented along the maximum slope and runoff direction) and 2.5 m in width, with a total area of 62.5 m². In April, the soil was prepared using a rotary tiller, manually leveled, and manually sown with a grass-legume mixture composed of 60% grasses and 40% legumes, as follows: *Dactylis glomerata* (20%, 210 g), *Lolium perenne* (20%, 228 g), *Phleum pratense* (20%, 228 g), *Trifolium pratense* (15%, 120 g), and *Lotus corniculatus* (25%, 282 g).

Immediately after sowing in April, the experimental plots were fertilized as follows: the control variant V₁ was left as an unfertilized control, the V₂ variant was fertilized with N₆₀P₆₀K₆₀, and the V₃ variant was fertilized with N₁₀₀P₉₀K₆₀.

The experimental variants were mowed after each flowering phase, in order to determine the yields obtained as a result of the application of mineral fertilization. At the same time, to assess changes in soil agrochemical properties under the influence of mineral fertilization and water erosion processes, soil samples were collected using an agrochemical probe, with two composite samples taken for each treatment.

Thus, sample A was collected from the upstream area of the plot, respectively from its upper half, from 10 randomized points, and sample B was taken from the downstream area of the plot,

corresponding to the lower half, also from 10 randomized points. In order to highlight the dynamics of the agrochemical properties of the soil, the collection of samples was carried out in three stages: in April, before the application of fertilizers, two weeks after fertilization and at the end of the vegetation period (October 1).

Laboratory analyses of the soil samples were performed in accordance with ICPA methodology. The following analytical methods were applied for the determination of soil chemical properties:

- pH (potentiometric method in aqueous suspension at soil/water ratio of 1/2.5 - SR 7184 /13-2001);
- Sum of basic exchange basic ions (SB) by hydrochloric acid extraction 0.1 N according to the Kappen method, Chiriță modified;
- hydrolytic acidity, extraction with sodium acetate at pH 8.2;
- base saturation degree, V% (Kappen Schofield method).
- organic matter (humus): volumetric determination (Walkley-Black humidification method, STAS 7184/21-82);
- the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases (IN = humus x V/100);
- mobile phosphorus content (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
- mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry);

RESULTS AND DISCUSSIONS

Mineral fertilization had a pronounced and statistically significant effect on hay production in the sown meadow.

The unfertilized control variant (N₀P₀K₀) registered a production of 2.82 t·ha⁻¹, while the application of the N₆₀P₆₀K₆₀ dose increased the production to 5.97 t·ha⁻¹, corresponding to an increase of 110% compared to the control.

The highest production was obtained in the N₁₀₀P₉₀K₆₀ variant (6.65 t·ha⁻¹), which represents an increase of 133.7% compared to the non-fertilized variant. The differences in production between the fertilized and control variants were very statistically significant, as illustrated in Table 1 and Figure 1.

Table 1. Influence of mineral fertilization on hay production

Variant	Dose	Production			Significance
		t·ha ⁻¹	%	±d/mt	
V ₁	N ₀ P ₀ K ₀ (Ct)	2.82	100	—	—
V ₂	N ₆₀ P ₆₀ K ₆₀	5.97	210	3.15	***
V ₃	N ₁₀₀ P ₉₀ K ₆₀	6.65	233.7	3.83	***

DL 5% = 0.259 t·ha⁻¹
DL 1% = 0.426 t·ha⁻¹
DL 0.1% = 0.800 t·ha⁻¹

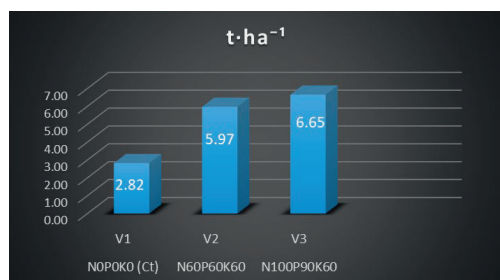


Figure 1. Influence of mineral fertilization on hay production

The values determined for the main soil agrochemical indicators - namely aqueous suspension pH, sum of exchangeable bases (SB), hydrolytic acidity (Ah), exchangeable aluminum (Al), humus content (H), total nitrogen (N), available phosphorus (PAL), and available potassium (KAL) - as well as the calculated fertility indicators, including total cation exchange capacity (T), base saturation degree (V), and nitrogen index (IN), are presented in Tables 2-4. The seasonal dynamics of these parameters reflect the complex interaction between climatic conditions and management practices, requiring an integrative approach to the interpretation of annual variations.

The agrochemical indicator values recorded during the study period indicate a moderate seasonal dynamics of soil reaction and nutrient content, which remained within relatively narrow ranges.

Table 2. Values of the main agrochemical soil indicators in the experimental plots in April

Experimental plots	pH	SB	Ah	T	Al	V %	H %	IN	Nt %	P _{AL} ppm	K _{AL} ppm
		me/100 g sol									
Sown grassland Control (Ct) A	4.90	5.45	5.27	10.72	0.20	50.83	2.82	1.43	0.124	9.20	48.72
Sown grassland Control (Ct) B	5.00	5.51	5.40	10.91	0.40	50.50	2.82	1.42	0.124	9.40	48.55
Sown grassland N60P60K60 A	4.95	5.47	5.21	10.68	0.38	51.22	2.77	1.42	0.122	10.23	49.20
Sown grassland N60P60K60 B	4.97	5.44	5.28	10.72	0.64	49.25	2.87	1.41	0.125	9.11	49.35
Sown grassland N100P90K60 A	4.85	5.28	5.23	10.51	0.44	49.76	2.79	1.39	0.123	8.70	49.70
Sown grassland N100P90K60 B	4.95	5.39	5.35	10.74	0.84	49.81	2.80	1.39	0.124	8.13	48.84

Table 3. Values of the main agrochemical soil indicators in the experimental plots in May

Experimental plots	pH	SB	Ah	T	Al	V %	H %	IN	Nt %	P _{AL} ppm	K _{AL} ppm
		me/100 g sol									
Sown grassland Control (Ct) A	5.07	5.07	5.16	10.23	0.46	49.56	2.84	1.41	0.121	9.15	50.21
Sown grassland Control (Ct) B	5.10	4.67	5.10	9.77	0.72	47.80	2.76	1.32	0.122	9.55	50.03
Sown grassland N60P60K60 A	5.01	4.88	5.08	9.96	0.40	49.00	2.74	1.34	0.123	11.02	50.70
Sown grassland N60P60K60 B	5.04	5.11	5.25	10.36	0.48	49.32	2.87	1.42	0.124	9.20	50.86
Sown grassland N100P90K60 A	5.03	4.78	5.12	9.90	0.40	48.28	2.79	1.35	0.124	9.00	51.22
Sown grassland N100P90K60 B	5.07	5.09	5.21	10.30	0.42	49.42	2.85	1.41	0.125	8.54	50.33

Table 4. Values of the main soil agrochemical indicators in the experimental plots in October

Experimental plots	pH	SB	Ah	T	Al	V %	H %	IN	Nt %	P _{AL} ppm	K _{AL} ppm
		me/100 g sol									
Sown grassland Control (Ct) A	4.95	5.23	5.25	10.48	0.70	49.90	2.81	1.38	0.122	9.21	48.65
Sown grassland Control (Ct) B	5.00	5.15	5.20	10.35	1.12	49.76	2.80	1.37	0.124	9.42	48.40
Sown grassland N60P60K60 A	4.87	5.10	5.12	10.22	0.36	49.90	2.79	1.37	0.123	10.29	49.84
Sown grassland N60P60K60 B	5.03	5.05	5.18	10.23	0.82	49.36	2.78	1.37	0.125	9.18	50.00
Sown grassland N100P90K60 A	4.85	5.21	5.21	10.42	0.40	50.00	2.82	1.40	0.124	8.85	50.35
Sown grassland N100P90K60 B	4.89	5.26	5.28	10.54	0.58	49.90	2.83	1.42	0.125	8.35	49.70

Seasonal fluctuations in humus, nutrients and soil reaction are the result of the complex interaction between climatic factors (amount and intensity of precipitation), erosion processes, peak consumption phenophases of plants, microbiological activity dependent on temperature, aeration and pH, as well as the doses and nature of the fertilizers applied. Some of these factors can act synergistically, favoring nutrient losses, while others have a compensatory effect, contributing to their accumulation in the soil. Overall, the data presented in Tables 2-4 indicate the maintenance of a relative soil homeostasis as an ecological system, despite the concomitant influence of fertilization and erosion processes, which are analyzed in more detail by comparing the initial and final values of the agrochemical indicators presented in Tables 5-9.

Soil reaction (pH) variation

The analysis of soil reaction variation (Table 5) reveals only minor pH changes across all experimental treatments, indicating a good buffering capacity of the soil. In the unfertilized control, soil reaction showed a very slight increase of approximately 0.5%, with minimal differences between upslope and downslope positions, suggesting high pH stability in the absence of mineral fertilization.

Under the N₆₀P₆₀K₆₀ fertilization rate, soil pH exhibited a slight average decrease (approximately 0.2%), with differences between upslope and downslope positions attributable to the physiologically acidifying effect of fertilizers and to local runoff conditions. At the higher fertilization rate (N₁₀₀P₉₀K₆₀), soil reaction decreased by approximately 0.6% on average, with a more pronounced reduction in downslope positions, confirming the cumulative acidifying effect of mineral fertilization.

Table 5. Variation in soil reaction between initial and final soil pH values

Experimental plots	Soil reaction (pH units)		Soil reaction variation (%)	Range of soil reaction variation
	Initial values (April)	Final values (October)		
N ₀ P ₀ K ₀ Mt A	4.90	4.95	1.02	4.85 – 5.10
N ₀ P ₀ K ₀ Mt B	5.00	5.00	0.00	
N ₆₀ P ₆₀ K ₆₀ A	4.95	4.87	-1.62	
N ₆₀ P ₆₀ K ₆₀ B	4.97	5.03	1.21	
N ₁₀₀ P ₉₀ K ₆₀ A	4.85	4.85	0.00	
N ₁₀₀ P ₉₀ K ₆₀ B	4.95	4.89	-1.21	

Variation in humus content

The data presented in Table 6 indicate differentiated variations in humus content

depending on the fertilization level. In the unfertilized control, humus content decreased slightly (approximately 0.5%) as a result of natural organic matter mineralization processes. Under the N₆₀P₆₀K₆₀ fertilization rate, humus content showed an average decrease of approximately 1.2%, with more pronounced differences between upslope and downslope positions, indicating increased spatial variability in organic matter decomposition processes. In contrast, at the higher fertilization rate (N₁₀₀P₉₀K₆₀), humus content increased uniformly by approximately 1.1%, as a result of enhanced plant biomass production and a greater return of organic residues to the soil.

Table 6. Variation in soil humus content between initial and final values

Experimental plots	Humus content %		Humus content variation (%)	Range of variation of humus content
	Initial values (April)	Final values (October)		
N ₀ P ₀ K ₀ Mt A	2.82	2.81	-0.35	2.78-2.83
N ₀ P ₀ K ₀ Mt B	2.82	2.80	-0.71	
N ₆₀ P ₆₀ K ₆₀ A	2.77	2.79	+0.72	
N ₆₀ P ₆₀ K ₆₀ B	2.87	2.78	-3.14	
N ₁₀₀ P ₉₀ K ₆₀ A	2.79	2.82	+1.08	
N ₁₀₀ P ₉₀ K ₆₀ B	2.80	2.83	+1.07	

Variation in total nitrogen content

Variations in total nitrogen content follow a pattern similar to that of humus, which can be explained by the close relationship between these two components, given that about 92-98% of total soil nitrogen is found in organic matter. Thus, according to the data in Table 7, in the control version, the total nitrogen content decreased on average by about 0.8%, as a result of nitrogen consumption by plants.

Table 7. Variation in total soil nitrogen content between initial and final values

Experimental plots	Total nitrogen content %		Total nitrogen content variation (%)	Range of variation in total nitrogen content (%)
	Initial values (April)	Final values (October)		
N ₀ P ₀ K ₀ Mt A	0.124	0.122	-1.61	0.122-0.125
N ₀ P ₀ K ₀ Mt B	0.124	0.124	0.00	
N ₆₀ P ₆₀ K ₆₀ A	0.122	0.123	0.82	
N ₆₀ P ₆₀ K ₆₀ B	0.125	0.125	0.00	
N ₁₀₀ P ₉₀ K ₆₀ A	0.123	0.124	0.81	
N ₁₀₀ P ₉₀ K ₆₀ B	0.124	0.125	0.81	

The application of the N₆₀P₆₀K₆₀ fertilization rate resulted in a slight increase in total nitrogen content (approximately 0.4%), with only minor differences between upslope and downslope

positions. Under the higher fertilization rate ($N_{100}P_{90}K_{60}$), total nitrogen content increased uniformly, confirming the effectiveness of nitrogen inputs through mineral fertilization.

Variation in available phosphorus content

Available phosphorus content exhibited more pronounced variations compared with the other analyzed nutrients (Table 8). In the unfertilized control treatment, available phosphorus increased only marginally (approximately 0.2%). Application of the $N_{60}P_{60}K_{60}$ fertilization rate resulted in a moderate increase in available phosphorus content (approximately 0.68%). The most substantial increase was observed under the $N_{100}P_{90}K_{60}$ treatment, where available phosphorus content increased by approximately 2.22%. Differences between positions A and B indicate the influence of plot position on phosphorus distribution. The high variability of available phosphorus can be explained by its uneven distribution within the soil profile, with maximum concentrations located in the erosion-prone surface layer, as well as by precipitation processes leading to the formation of sparingly soluble phosphate compounds in the presence of elevated levels of exchangeable aluminum.

Table 8. Variation in available soil phosphorus content between initial and final values

Experimental plots	Available phosphorus content (ppm)		Variation in available phosphorus (%)	Range of variation of mobile phosphorus, ppm
	Initial values (April)	Final values (October)		
$N_0P_0K_0$ Mt A	9.20	9.21	0.11	8.13 – 10.29
$N_0P_0K_0$ Mt B	9.40	9.42	0.21	
$N_{60}P_{60}K_{60}$ A	10.23	10.29	0.59	
$N_{60}P_{60}K_{60}$ B	9.11	9.18	0.77	
$N_{100}P_{90}K_{60}$ A	8.70	8.85	1.72	
$N_{100}P_{90}K_{60}$ B	8.13	8.35	2.71	

Variation in available potassium content

The data presented in Table 9 indicate a distinct evolution of available potassium content depending on fertilization treatment. In the unfertilized control, available potassium content decreased slightly (approximately 0.23%) as a result of plant uptake. Application of the $N_{60}P_{60}K_{60}$ fertilization rate resulted in a clear and relatively uniform increase in available potassium content (approximately 1.31%). At the higher fertilization rate ($N_{100}P_{90}K_{60}$), the increase in available potassium was more pronounced (approximately 1.54%), with higher values

recorded in downslope positions, suggesting the influence of transport and redistribution processes within the plot. These increases reflect the compensation of potassium losses through mineral fertilizer inputs.

Table 9. Variation in available soil potassium content between initial and final values

Experimental plots	Available potassium content (ppm)		Variation in available potassium (%)	Range of variation in available potassium content
	Initial values (April)	Final values (October)		
$N_0P_0K_0$ Mt A	48.72	48.65	-0.14	48.40-50.35
$N_0P_0K_0$ Mt B	48.55	48.40	-0.31	
$N_{60}P_{60}K_{60}$ A	49.20	49.84	1.30	
$N_{60}P_{60}K_{60}$ B	49.35	50.00	1.32	
$N_{100}P_{90}K_{60}$ A	49.70	50.35	1.31	
$N_{100}P_{90}K_{60}$ B	48.84	49.70	1.76	

The results obtained demonstrate that mineral fertilization influences both grassland productivity and the dynamics of the main soil agrochemical indicators, in agreement with numerous studies showing that nutrient availability represents a major limiting factor for grassland ecosystem productivity (Whitehead, 2000; Hopkins & Del Prado, 2007). The yield increases observed in fertilized treatments confirm the role of nitrogen, in combination with phosphorus and potassium, in stimulating plant biomass growth (Radu et al., 2021). The moderate decreases in soil pH observed in the fertilized treatments are consistent with the physiologically acidifying effect of mineral fertilizers, particularly nitrogen-based fertilizers, through nitrification processes and alterations in the ionic balance of the soil adsorption complex (Guo et al., 2010). The relatively low amplitude of the changes indicates that, in the short term, the soil has sufficient buffering capacity to limit the sharp variations in the reaction.

The differences observed between upslope and downslope positions may reflect spatial soil heterogeneity and the influence of microrelief on water redistribution processes (Brady & Weil, 2016). The evolution of humus content reflects the complex interaction between fertilization, biomass production, and mineralization processes. The slight decreases in humus observed in unfertilized or weakly fertilized treatments can be associated with intensified organic matter decomposition and reduced inputs of plant residues. In contrast, appropriate fertilization, by stimulating vegetation growth

and root system development, can increase organic carbon inputs to the soil, thereby promoting the maintenance or even enhancement of humus reserves (Six et al., 2002; Lal, 2004; Acatrinei et al., 2024).

The literature indicates that the effect of fertilization on soil organic matter depends on the balance between residue inputs and the intensity of mineralization, being influenced by pedoclimatic conditions and the duration of treatment application (Chen et al., 2018). In this context, the results obtained suggest that the fertilization-induced increase in biomass production may contribute to humus stabilization.

Total nitrogen dynamics closely follow humus variations, which is consistent with the fact that most soil nitrogen is stored in organic fractions. The slight decreases observed in the control variant can be attributed to nitrogen consumption by plants and slow replenishment through mineralization processes, while the low increases in the fertilized variants are compatible with exogenous nitrogen intake and intensification of its internal cycling (Stevenson, 1994; Brady & Weil, 2016).

The relatively small magnitude of the variations suggests that, in the short term, changes in total nitrogen content are limited and influenced by spatial variability, which requires a cautious interpretation of the results, supported by statistical analysis.

The more pronounced variability of available phosphorus is characteristic of acidic soils, where the availability of this element is strongly controlled by sorption and precipitation processes involving aluminum and iron oxides and hydroxides (Hinsinger, 2001; McLaughlin et al., 2011). Under these conditions, even following fertilization, a substantial proportion of the applied phosphorus may be rapidly immobilized in sparingly soluble forms.

The spatial differences observed between positions A and B can be explained by heterogeneity in exchangeable aluminum content and by the uneven distribution of phosphorus along the soil profile.

The evolution of available potassium primarily reflects the balance between removal through harvest and inputs via fertilization, under conditions of relatively good retention on the soil cation exchange complex.

The increases observed in fertilized variants are consistent with the literature indicating that potassium fertilization can compensate for nutrient losses and maintain adequate soil potassium levels (Sparks, 2003; Havlin et al., 2014).

Overall, the results support the hypothesis that rationally applied mineral fertilization can simultaneously contribute to increasing grassland productivity and maintaining soil fertility. At the same time, the early signals of acidification observed at higher doses underscore the need for long-term monitoring of soil reaction and indicators that control organic matter stability and nutrient availability, as recommended by synthesis studies on sustainable grassland management (Tilman et al., 2002; Lal, 2015).

CONCLUSIONS

The study demonstrates that mineral fertilization represents a key determinant of productivity enhancement in sown grasslands, while simultaneously influencing soil agrochemical functioning. The positive system response to nutrient inputs confirms the importance of fertilization management in grassland ecosystems characterized by limited nutrient availability.

The relatively small changes observed in the main agrochemical indicators indicate short-term functional stability of the soil, despite seasonal variability and the influence of fertilization. This stability suggests the presence of internal regulatory mechanisms that allow the maintenance of soil fertility under climatic and anthropogenic pressures.

Overall, the results highlight the need for an integrated management approach to sown grasslands, in which mineral fertilization is applied rationally and coupled with continuous soil monitoring, as an essential prerequisite for ensuring the sustainability of these ecosystems.

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STUDIES ON THE ACCUMULATION OF HEAVY METALS IN *Trifolium pratense* L. PLANTS ON AN ARTIFICIALLY POLLUTED SOIL IN THE GREENHOUSE

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Abstract

This study aims to investigate the accumulation of heavy metals (Cd, Pb, Zn and Cu) from soil in red clover (Trifolium pratense L.) plants in experiments with single-element induced pollution, carried out in the greenhouse. The soil used comes from an unpolluted permanent meadow in the Copsa Mica area and was analyzed from a physico-chemical point of view. The total contents of heavy metals fall within the normal limits according to Order 756/1997 on the assessment of environmental pollution. To perform the experiments with induced pollution, the soil material was treated with variable amounts of metal (Cd, Cu, Pb and Zn) from their soluble salts, and for each element 6 experimental variants were established depending on the metal content in the soil. The results show that for the total cadmium and lead contents in the soil and the metal content in the plant, extremely significant linear correlation indices were obtained and for zinc and copper very significant. The mobile metal contents in the soil and the metal content in Trifolium pratense plants have a very significant linear correlation index.

Key words: heavy metals, pollution, soil, *Trifolium pratense* L.

INTRODUCTION

Soil is a complex system composed of mineral and organic fractions, water and air, inhabited by diverse biota that vary depending on environmental and climatic conditions (Giunchino et al., 2026).

The rapid growth of industrialization in the last century has led to the intense and often uncontrolled exploitation of natural resources, having a significant impact on the environment. Industrial activities have led to the emergence of numerous pollutants, including soil contamination with heavy metals (Briffa et al., 2020, cited by Cakaj, 2023).

Heavy metal pollution is a major problem due to its persistence, high toxicity and bioaccumulative potential present in the environment (Abhinav et al., 2024). They accumulate very easily in soils and transform into more toxic methyl compounds, and their elimination is a long-term process, which can take tens or even hundreds of years (Abd Elnabi et al., 2023, cited by Cakaj, 2024; Li et al., 2018, cited by Zhao et al., 2022; Denchylia-Sakal et al., 2019).

Soils contaminated with heavy metals have a negative impact on living organisms and the food chain (Tomaskin et al., 2013; Zhao et al., 2022).

Forage crops grown on heavy metal contaminated soils tend to accumulate excessive amounts of heavy metals, posing a severe risk to human and animal health (Rattan et al., 2005; Kulhari et al., 2013, cited by Bhatti et al., 2016). Heavy metals, both essential and non-essential, have similar harmful effects on plants. Plants have developed detoxification mechanisms to reduce the negative effects of heavy metal exposure (Collin et al., 2022)

Although heavy metals such as zinc and copper are essential for the growth and development of plants and animals, in concentrations above maximum permissible limits, they disrupt the normal functioning of organisms (Ali et al., 2012; Jolly et al., 2013; Abuzaid et al., 2021).

Cadmium and lead are non-essential heavy metals, known to be highly toxic and carcinogenic to animals and humans (Rajaganpathy et al., 2011). Since the heavy metal content in soil is strongly affected by soil characteristics, and the bioaccumulation of

heavy metals in forage crops is a major pathway for heavy metals to enter livestock systems (Bhatti et al., 2016).

Cadmium is easily absorbed and accumulated by plants and affects physiological and biochemical processes such as seed germination, growth, photosynthesis, and antioxidant systems. It also blocks the absorption and transport of vital elements, leading to critical nutritional deficiencies (Haider et al., 2021; Wu et al., 2022).

The non-biodegradable nature of lead, like cadmium, is the main reason for its prolonged persistence in the environment (Flora et al., 2012; Alisha and Gupta, 2018).

The most polluted areas in Romania are Baia Mare, Zlatna and Copsa Mica where the highest levels of contamination are with cadmium, lead, copper and zinc, which mainly come from anthropogenic activities (Damian et al., 2008; Mihali et al., 2013).

When these heavy metals are present in the soil in low concentrations, plants continue to grow evenly despite the accumulation of these metals. The ability of plants to accumulate heavy metals in their organs can therefore be used to monitor soil pollution with heavy metals (Malizia et al., 2012).

Trifolium pratense L. is a perennial herbaceous species of the legume family (fabaceae) widely cultivated in temperate regions. It has a high protein content, large and tender leaves, and is a soil nitrogen fixer. It is widely used as pasture or for hay production, and is also used as a green manure and for soil and water conservation, contributing to the improvement of soil fertility (Meng et al., 2022; Mo et al., 2022; Mu et al., 2025; Valujeva et al., 2025).

Studies have shown that *Trifolium pratense* L. has a strong photosynthetic capacity, superior antioxidant activity and low root mobility towards heavy metals, indicating that it may have good potential to resist heavy metal toxicity (Meng et al., 2022; Mu et al., 2025).

Cakaj et al. (2024) note that *Trifolium pratense* L. has a high bioaccumulation potential for zinc, cadmium and nickel, both in the roots and leaves. Thus, due to its ability to accumulate heavy metals in the soil, it has been proposed as a potential bioindicator plant of heavy metals. Thus, it is used as an indicator plant for

biological monitoring of metals in polluted soils (Shahbaz et al., 2018).

The results presented by Kujawska et al. (2018) indicate that red clover plants accumulated high levels of copper, zinc, and nickel from soil-like substrates, but can be used as animal feed.

In studies conducted by Ali et al. (2012), it is shown that *Trifolium alexandrinum* has the ability to accumulate heavy metals (Zn, Pb, Cu and Cd) from artificially contaminated soils.

The values of the bioaccumulation coefficient in the roots were significantly higher, with the highest values for zinc. The translocation of heavy metals from the roots to the stems was limited, however, *Trifolium alexandrinum* plants are recommended in the phytoremediation of contaminated soils.

Bidar et al. (2009) indicate that the bioaccumulation of heavy metals from soil in *Trifolium repens* L. plants is mainly in the roots (Cd>Zn>Pb) compared to the aerial parts of the plants.

The results of the experiments with induced pollution (Cd and Zn) conducted by Stravinskiene & Racaitė (2014) showed that heavy metals have an inhibitory effect on the development of white clover plants (*Trifolium repens* L.). The inhibitory effect of cadmium was statistically significantly greater than that of zinc on the growth and development of white clover roots and shoots. In the studies conducted by Sotiriou et al. (2023) mention that *Trifolium pratense* L. has a moderate capacity to accumulate Cd and Zn compared to a weak capacity to accumulate Pb. Similar results are also shown by Lanier et al. (2016), emphasizing that cadmium accumulates more in roots than in leaves, and the presence of lead in the soil slightly reduces the bioaccumulation of cadmium in both the underground and aerial parts of the plant.

Memic et al. (2023) show that white clover behaves differently towards metals depending on the type of metal, its location in the soil and the season.

Trifolium pratense L. was selected for the experiment because it was one of the most common plant species in the legume family in the Copsa Mica area and known in the literature for its capacity to accumulate heavy metals. The aim of this study is to evaluate the effects of increasing concentrations of heavy metals (Cd,

Pb, Zn and Cu) in the soil on *Trifolium pratense* L. plants and to evaluate its capacity to accumulate these elements in plant tissues.

MATERIALS AND METHODS

This paper presents a study conducted in 2025, regarding the accumulation of heavy metals (Cd, Pb, Zn and Cu) in *Trifolium pratense* L. plants, in experiments with induced pollution. The experiments were carried out in the greenhouse of INCDPAPM-ICPA Bucharest, in pots with 10 kg of soil.

For the experiments, soil material was collected from an unpolluted permanent meadow in the Copsa Mica area. The soil material was harvested from the upper 0-20 cm layer, homogenized and dried at air temperature.

The experiments were carried out on artificially contaminated soil. Thus, the soil material was treated with amounts of metals (Cd, Pb, Zn and Cu) derived from their soluble salts (acetate). For each element, 5 experimental variants with treated soil and the control were established, as follows:

- Cd: control; 3 mg·kg⁻¹; 6 mg·kg⁻¹; 9 mg·kg⁻¹; 12 mg·kg⁻¹; 15 mg·kg⁻¹;
- Pb: control; 50 mg·kg⁻¹; 100 mg·kg⁻¹; 150 mg·kg⁻¹; 200 mg·kg⁻¹; 250 mg·kg⁻¹;
- Zn: control; 300 mg·kg⁻¹; 600 mg·kg⁻¹; 900 mg·kg⁻¹; 1200 mg·kg⁻¹; 1500 mg·kg⁻¹;
- Cu: control; 100 mg·kg⁻¹; 200 mg·kg⁻¹; 300 mg·kg⁻¹; 400 mg·kg⁻¹; 500 mg·kg⁻¹;

When establishing these concentrations, previous studies were taken into account, as well as the limit values from Order 756/1997. The soil was carefully prepared to ensure a uniform distribution of the applied treatments.

Trifolium pratense L. (red clover) of the David Liv variety from SCDA Livada was selected as the test plant. The plants were harvested 18 weeks after sowing.

Before preparing the experiments, the soil material used in the experiments was analyzed from a physicochemical point of view. The collected soil samples were dried at room temperature, then ground and sieved through a sieve with 2 mm and 0.2 mm mesh. The samples were carried out in four repetitions.

The soil texture was determined by the sieving and sedimentation method.

Soil pH was determined using the potentiometric method (soil: water ratio 1:2.5 g/v), organic matter content was determined using the Walkley-Black method, modified after Gogoasa. Total nitrogen was determined using the Kjeldahl method. Soil available phosphorus and potassium were determined using the Egner-Riehm-Domingo method.

For the determination of heavy metals content was used atomic absorption spectrometry, after the extraction by the aqua regia–microwave digestion method (Ethos Easy Microwave Dygestion System from Milestone).

Microwave digestion was performed using 10 mL of aqua regia (7.5 mL HCl and 2.5 mL HNO₃) at 140°C for 30 min, method developed according to SR ISO 11466:1999.

DTPA extractable heavy metals were extracted from soil (10 g) with 20 ml of extraction solution (0.05 M DTPA (diethylenetriaminopentacetic acid), 0.01 M CaCl₂, and 0.1 M TEA (tetraethylammonium), according to SR ISO 14870:2002. ICE3000 (1M) atomic absorption spectrophotometry was used.

Trifolium pratense L plant samples were harvested, oven-dried at 60°C, chopped, ground and treated with nitric acid in a microwave digestion system (Ethos Easy Microwave Dygestion System from Milestone).

Microwave digestion was performed using 10 mL mixture of HNO₃ + H₂O₂ (9 mL HNO₃ and 1 mL H₂O₂) at 210°C for 20 min, method developed in-house.

Microsoft Excel was used for statistical data processing.

RESULTS AND DISCUSSIONS

Table 1 presents the main characteristics of the soil material used in the induced pollution experiments. The results of laboratory determinations indicated a fine textured, medium clayey (LL) soil. As granulometric fractions (in mm, % of the mineral part of the soil), they contain 12.0% coarse sand (2.0-0.2 mm), 44.0% fine sand (0.2-0.02 mm), 19.3% silt (0.02-0.002 mm) and 24.2 clay (<0.002 mm).

The analysed soil has a weak alkaline reaction (pH = 7.94), low humus content (2.71%), medium nitrogen (0.183 %) and potassium (186

mg·kg⁻¹) input, and a high phosphorus content (53 mg·kg⁻¹).

Table 1. Physico-chemical characteristics of the soil material (n = 4)

Characteristics	Mean value
Coarse sand (%) 2.0-0.2 mm	12.0
Fine sand (%) 2.0-0.02 mm	44.0
Silt (%) 0.02-0.002 mm	19.3
Clay (%) < 0.002 mm	24.7
Texture	LL
pH _{water}	7.94
Humus (%)	2.71
Total N (%)	0.183
Available P (mg·kg ⁻¹)	53
Available K (mg·kg ⁻¹)	186
Cd (mg·kg ⁻¹)	0.771
Pb (mg·kg ⁻¹)	41.1
Zn (mg·kg ⁻¹)	124
Cu (mg·kg ⁻¹)	27.8
Cr (mg·kg ⁻¹)	43.9
Mn (mg·kg ⁻¹)	579
Ni (mg·kg ⁻¹)	37.2

In terms of heavy metal content, it is observed that the values obtained are within the normal limits according to Order 756/1997 on the assessment of environmental pollution. The heavy metal contents indicated the following average values: 0.771 mg·kg⁻¹ Cd, 41.1 mg·kg⁻¹ Pb, 124 mg·kg⁻¹ Zn, 27.8 mg·kg⁻¹ Cu, 43.9 mg·kg⁻¹ Cr, 579 mg·kg⁻¹ Mn and 37.2 mg·kg⁻¹ Ni.

The data obtained in the experiments with single-element induced pollution were used to parameterize and validate stochastic models, which allowed the estimation of the bioaccumulation of heavy metals (Cd, Pb, Zn and Cu) in *Trifolium pratense* L. plants depending on the total content of the metal in the soil and its mobile fraction (DTPA extractable form).

Figure 1 presents regression equations estimating the stochastic dependence between total soil cadmium content, mobile soil cadmium content and cadmium content in the aerial part of *Trifolium pratense* L. plants. The value of the linear correlation coefficient (Cd_{total} r=0.9891^{***}) is highly significant, showing a very strong correlation between total soil cadmium content and cadmium content accumulated in *Trifolium pratense* L. plants (Figure 1a). Figure 1b presents the logarithmic diagram between mobile soil cadmium content and cadmium content in the aerial part of *Trifolium pratense* L. plants, indicating a distinctly significant linear correlation coefficient (Cd_{DTPA} r=0.9618^{**}). Increasing the bioavailability of cadmium in the soil favors a more intense absorption and a higher accumulation of the metal in the tissues of *Trifolium pratense* L. plants.

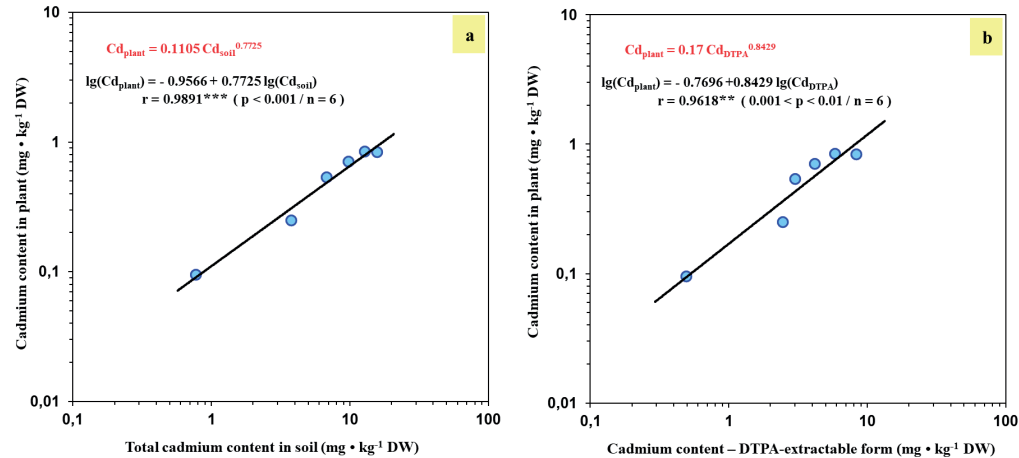


Figure 1. Regression equations estimating the stochastic dependence between total soil cadmium content (a), soil mobile cadmium content - DTPA extractable form (b) and cadmium content in the aerial part of *Trifolium pratense* L. plants harvested from an experiment with induced pollution

The regression equations estimating the stochastic dependence between the total lead content in the soil (Figure 2a), the mobile lead

content in the soil (Figure 2b) and the lead content in the aerial part of *Trifolium pratense* L. plants are shown in Figure 2. Extremely

significant linear correlation indices (Pb_{total} $r=0.9846^{***}$) and respectively distinctly significant (Pb_{mobile} $r=0.9735^{**}$) were obtained. The transfer of zinc content from soil to the aerial part of *Trifolium pratense* L. plants is represented by regression equations that estimate the stochastic dependence between the

zinc content in soil and that accumulated in plants (Figure 3). The results show a distinctly significant linear correlation between the total zinc content in soil (Figure 3a), the mobile zinc content in soil (Figure 3b) and that in the aerial part of plants, with high values in both cases (Zn_{total} $r=0.9535^{**}$ and Zn_{mobile} $r=0.9673^{**}$).

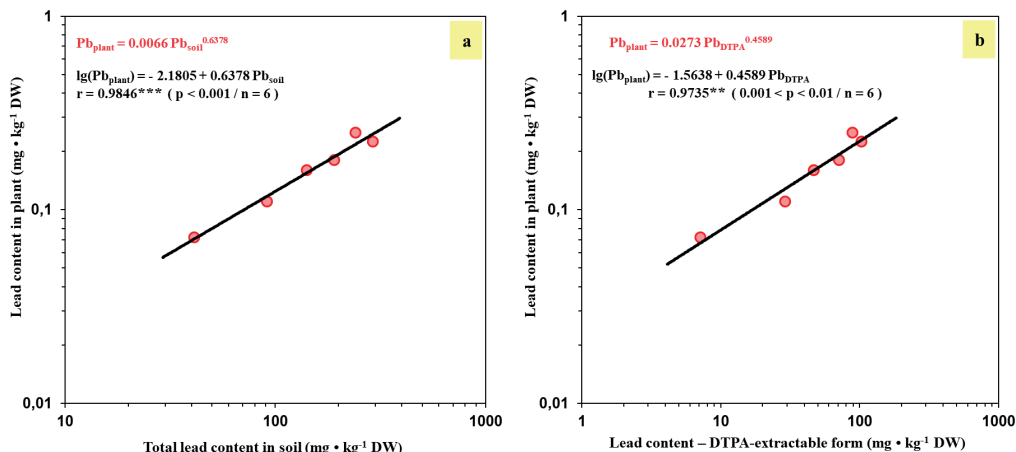


Figure 2. Regression equations estimating the stochastic dependence between total soil lead content (a), soil mobile lead content - DTPA extractable form (b) and lead content in the aerial part of *Trifolium pratense* L. plants harvested from an experiment with induced pollution

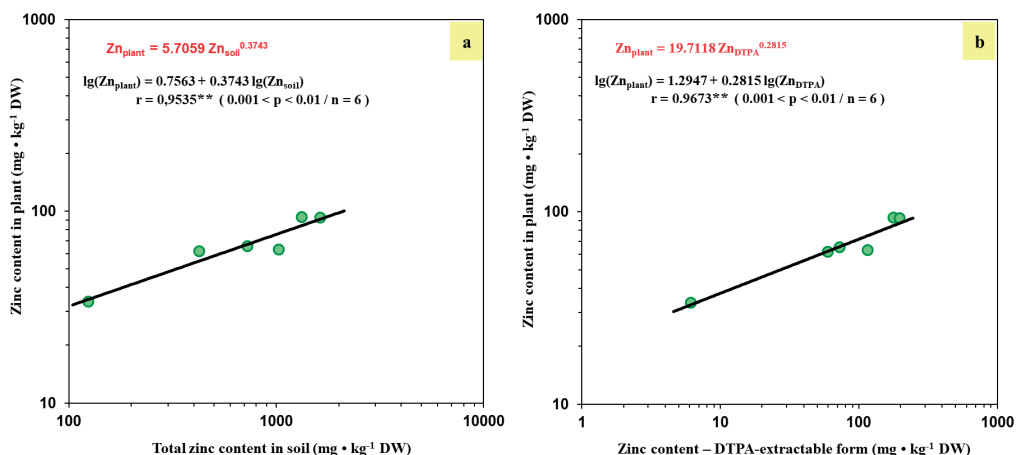


Figure 3. Regression equations estimating the stochastic dependence between total soil zinc content (a), soil mobile zinc content - DTPA extractable form (b) and zinc content in the aerial part of *Trifolium pratense* L. plants harvested from an experiment with induced pollution

Studies by Denchylia-Sakal et al. (2019) on soil artificially polluted with zinc and copper show that as the concentration of the metal in the soil increases, the leaf area decreases. *Trifolium pratense* L. plants can accumulate significant amounts of zinc, with higher values in the roots and leaves compared to the shoots.

Figure 4 shows regression equations that estimate the stochastic dependence between the total copper content in the soil (Figure 4a), the mobile copper content in the soil (Figure 4b) and the copper content in the aerial part of *Trifolium pratense* L. plants. The linear correlation index

is distinctly significant in both cases: C_{total} $r=0.965^{**}$ and C_{DTPA} $r=0.9518^{**}$. Cakaj et al. (2023) showed that *Trifolium pratense* L. accumulated the highest concentration of copper in the roots – $20.38 \text{ mg} \cdot \text{kg}^{-1}$, while in the leaves $9.20 \text{ mg} \cdot \text{kg}^{-1}$. Metal concentrations in the Zlatna and Copsa Mica areas exceed the maximum permissible limits according to Order 756/1997. In Copsa

Mica the maximum permissible limits for lead are exceeded by 5.61-27.68 times, zinc: 2.16-25 times and cadmium: 5.99-133.3 times. For Zn and Cd, the total concentrations exceeded both the maximum permissible limit and the intervention threshold in the clay-rich horizons, at variable depths, in both areas (Damian et al., 2008).

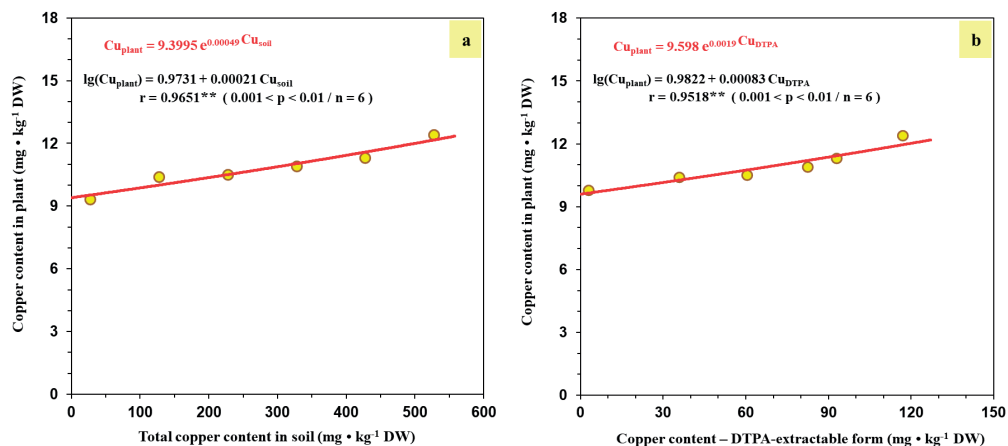


Figure 4. Regression equations estimating the stochastic dependence between total soil copper content (a), soil mobile copper content – DTPA extractable form (b) and copper content in the aerial part of *Trifolium pratense* L. plants harvested from an experiment with induced pollution

CONCLUSIONS

This study shows that *Trifolium pratense* L. has the capacity to accumulate heavy metals (Cd, Pb, Zn and Cu) from artificially contaminated soils. The soil material used for the experiments in the greenhouse is a soil with a fine texture, weak alkaline reaction and medium nutrient supply. The contents of heavy metals fall within the normal limits according to the order 756/1997 on the assessment of environmental pollution.

The results of the experiments carried out in the greenhouse show a strong correlation between the total and mobile content of heavy metals in the soil and that accumulated in the aerial part of *Trifolium pratense* L. plants. Also, a direct relationship was observed between the increase in the solubility of metals in the soil material and the applied doses. It is noted that for the total cadmium and lead contents in the soil and the metal content in the plant, very significant linear correlation indices were obtained, and for zinc and copper distinctly significant. The mobile

metal contents in the soil and the metal content in *Trifolium pratense* L. plants have distinctly significant linear correlation indices.

ACKNOWLEDGEMENTS

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IMPACT OF INNOVATIVE SOIL CULTIVATION DISCS ON AGRONOMICALLY VALUABLE SOIL

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Abstract

The final stage of the mechanical action of soil tillage machinery is the attainment of a target aggregate composition, defined by the size distribution of the resulting soil fractions. This study investigates the performance of innovative disk type working bodies and their effectiveness in achieving a specific degree of soil fragmentation. Agronomically valuable structure is characterized by aggregates ranging from 1 to 25 mm, which ensure optimal aeration, water regime, and root development. The experimental setup includes three operating speeds of the machine-tractor unit and two different soil terrains. The tests were conducted using a tillage mill equipped with non-standard disk working bodies with a horizontal axis of rotation, designed for enhanced kinematic and energy efficiency. For each experimental configuration, the degree of fragmentation was determined by collecting five evenly spaced soil samples along the length of the test plot. The results indicate that, under specific combinations of soil type, moisture content, and operating speed, the innovative disk working bodies achieve optimal aggregate composition, confirming their applicability for high efficiency soil tillage processes.

Key words: soil tillage machines, agronomically valuable soil, percentage of soil fragmentation.

INTRODUCTION

Tillage machines play a fundamental role in agricultural production systems, as they form the physical environment necessary for the optimal development of cultivated plants (Mitkov, 2023). Their main function is to modify the soil structure in such a way as to ensure a favorable water-air regime, uniform germination and stable initial development of crops. In addition, tillage is a key factor in maintaining soil fertility, which in turn determines the long-term productivity of agroecosystems. Soil is a complex, dynamic and multi-component natural system formed as a result of long-term pedogenetic processes occurring on geological time scales. It occupies the surface horizon of the lithosphere, usually 20-50 cm thick, in specific cases reaching 1-1.5 m. Its physical, chemical and biological properties are highly sensitive to external influences and are constantly changing depending on the structure, aggregate state and the ratio between solid, liquid and gas phases. Fertility - an integrative indicator of soil quality - is determined by its ability to provide plants with accessible nutrients, water and oxygen, as well as its biological activity.

The technological properties of the soil, including its workability, density, plasticity and resistance to deformation, depend to a significant extent on the water regime and aeration (Hristova et al., 2022). Within traditional intensive agricultural systems, the number of tillage operations can reach 10-12 per year, which leads to significant energy costs and a load on production resources. In addition to economic disadvantages, frequent mechanical impacts cause degradation processes – soil profile compaction, destruction of the aggregate structure, reduction of porosity and increased risk of water and wind erosion, especially in the absence of vegetation cover (Mandradzhiev, 1982). These negative trends require the development of strategies for soil cultivation optimization, aimed at minimizing mechanical interventions and increasing the sustainability of agroecosystems. Combining shallow cultivation with periodic deep interventions, the introduction of high-performance machinery and the application of reduced or zero-tillage technologies are key directions in modern agro-engineering practice (Bakhtin, 1969). These approaches are the basis of the concept of regenerative agriculture, which aims to restore soil ecosystem functions, limit degradation processes and

increase the long-term sustainability of agricultural production.

Regenerative agriculture is an integrated system of principles and management practices aimed at restoring, maintaining and long-term improving agro-ecosystems. Its philosophy is based on the understanding that sustainable agriculture cannot be achieved without purposefully strengthening the ecological functions of the soil, protecting natural resources and creating conditions for improving human health and the economic viability of farms. In this context, regenerative practices emphasize the restoration of soil health as a key factor for increasing the productivity and sustainability of agricultural systems.

A key element in this approach is the cessation of degradation processes and the limitation of erosion, which are leading threats to soil fertility. Restoring the soil to its natural functional state requires minimizing mechanical interventions, maintaining a biologically active environment and stimulating natural biogeochemical cycles. Therefore, regenerative agriculture conceptually and technologically builds on the principles of Conservation Agriculture - a system developed for more than six decades within the framework of industrial agriculture.

Conservation agriculture is based on three fundamental pillars: minimal mechanical impact on the soil (including zero tillage), maintaining a constant soil cover and a high level of plant diversity. These principles aim to improve biodiversity and activate natural biological processes in both the soil profile and above-ground plant biomass. As a result, the efficiency of water and nutrient use increases, leading to more sustainable and stable production.

Modern research shows that the implementation of innovative active tillage tools can accelerate the transition to regenerative practices, allowing reduced tillage, precise incorporation of preparations and minimal disturbance of the soil structure (Dallev, 2014). These technological solutions are especially important in the context of climate change and increased pressure on soil resources.

For the conduct of scientific research, as well as for the effective. Improving soil structure through reduced-intensity and regenerative tillage practices has become a central objective

in modern sustainable agriculture (Manhart and Delibaltova, 2022). Active-drive working bodies, which combine rotational and translational soil movement, offer potential advantages in achieving optimal aggregate formation while minimizing soil disturbance (Dallev, 2012). The present study investigates a novel configuration of disk-type working bodies mounted on a horizontal shaft, designed to enhance soil fragmentation and promote the formation of stable aggregates within the agronomically preferred size range.

MATERIALS AND METHODS

The research builds upon the need for efficient surface tillage tools capable of operating effectively in heavy clay soils, which are often characterized by high cohesion and resistance to mechanical breakdown. By integrating innovative working body geometry with controlled kinematic parameters, the study aims to evaluate the capacity of the prototypes to produce a soil structure suitable for regenerative agricultural systems (Dimov, 1979). The experimental work includes the design and fabrication of the working bodies, their assembly and testing under field conditions, and a detailed assessment of soil aggregate distribution following cultivation. The purpose of the present study is to examine newly developed active-drive working bodies intended for shallow soil cultivation. The investigated concept integrates the motion characteristics of a tillage machine with a horizontal rotational axis and the lateral displacement of soil achieved through a disk-type working element (Dallev, 2015). This combination is expected to produce a soil structure that meets agronomic requirements. To fulfil the research objective, the following activities were carried out:

- Development and fabrication of prototype working bodies.
- Installation of the working bodies on a horizontal shaft at a predefined operational angle.
- Characterization and analysis of the soil conditions at the test site.
- Execution of field trials under controlled parameters.
- Processing, evaluation, and interpretation of the collected data.

Table1. Interpretation of the collected data

N	Wa %	V m/s	1-25 mm (%)
1	11.5	0.69	47.6
2	12.8	0.69	49.8
3	14.8	0.69	50.7
4	15.8	0.69	55.2
5	17.8	0.69	54.4
6	18.9	0.69	56.3
7	22.1	0.69	59.7
8	23.5	0.69	58.9
9	25.7	0.69	64.1
1	12.8	1.2	59.7
2	14.8	1.2	62.4
3	15.8	1.2	62.6
4	17.8	1.2	67.9
5	18.9	1.2	66.3
6	22.1	1.2	71.4
7	23.5	1.2	71.2
8	25.7	1.2	70.6
9	11.5	2.08	74
1	12.8	2.08	23.5
2	14.8	2.08	25.1
3	15.8	2.08	28.6
4	17.8	2.08	26.7
5	18.9	2.08	30.4
6	22.1	2.08	30.3
7	23.5	2.08	35
8	25.7	2.08	36.1
9	11.5	2.08	41.6

Research data summarized in Table 1

The innovative working body is made of 65G steel. The experimental work was carried out in the city of Plovdiv at the base of the Agricultural University-Plovdiv on land previously subjected to plowing (Figure 1). The soil in the area is classified as a heavy clay type (TGP), containing approximately 50% physical clay. The machine used in the trials was aggregated with a TK-80 tractor and equipped with an active tillage implement with a working width of 0.76 m. The PTO operated at 540 min^{-1} , while the forward speeds tested were 0.69, 1.2, and 2.08 m/s. The working depth was maintained at 12 cm, and soil moisture during the experiments varied between 11% and 26%. Laboratory analyses performed using the Kaczynski pipette method confirmed the soil's classification as heavy clay with 50% physical clay content. For each experimental run, soil fragmentation into aggregates was assessed. Five samples were collected at equal intervals along the experimental plot. Sampling was performed by inserting a bottomless metal box with dimensions $400 \times 230 \times 230 \text{ mm}$ into the soil, placing a base plate underneath, and

removing the box together with the enclosed soil.



Figure 1. Presents the location of the experiments performed

The samples were air-dried indoors and subsequently separated into fractions using sieves with openings of 1 mm and 25 mm. The acceptable deviation in aggregate distribution was limited to $\pm 5\%$. According to agronomic standards, optimal soil structure after cultivation is achieved when:

- 70% of the aggregates fall within the 1-25 mm size range;
- aggregates smaller than 1 mm do not exceed 30%;
- and the soil surface remains even and free of excessive compaction.

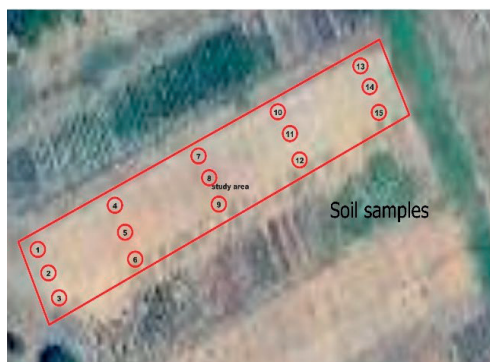


Figure 2. General view of the innovative working bodies

The study area is located in Agricultural university-Plovdiv, Bulgaria. The study territory is overspread nearest 140 m^2 . The location maps are generated by GIS platform named QGIS (Figure 2). For more good and presentative effects is used open sources data from Google Satellite. On the map, it is presented the

experimental field and points of soil samples. The field is with size 7 m x 20 m. The area allows to be prepared 15 soil samples. The results of them are presented on the study below.

Used GIS product is QGIS program. Selected data is from open source information-Google Satellite.

The present study presents the concept, design characteristics and agro-ecological effects of a newly developed multifunctional machine designed to implement regenerative agricultural practices through a single mechanized pass. The machine combines localized loosening, precise crushing and obtaining agronomically valuable soil in a single pass (Figure 3). The results show a significant improvement in soil structure, increased water holding capacity, increased biological activity and reduced energy costs. The technology demonstrates the potential for sustainable management of soil resources in the face of climate change and increased production requirements.



Figure 3. General view of the innovative working bodies

The aim of this paper is to analyze the influence of soil humidity (W_a) and processing speed (V) on the 1-25 mm fraction, using a multiple linear regression model and a sensitivity analysis of the process when using a new soil tillage machine. The study is based on experimental data showing the changes in the percentage of the 1-25 mm fraction as a function of soil moisture (W_a) and machine speed (V). The data were collected from tests conducted with a new model of tillage machine and cover different values of these variables.

RESULTS AND DISCUSSIONS

Although all these requirements are of great importance, the leading factor remains the size of the soil aggregates, which determines both the physical properties of the soil and the effectiveness of subsequent treatments. The profile of the already treated area is directly dependent on the type and geometry of the working bodies that have passed through it. Different machines leave a characteristic structural imprint, determined by their design features.

The materials used in the production of agricultural machinery are decisive for the durability and quality of treatment. The literature indicates recommended materials for wear-resistant coatings of parts, assemblies and elements of soil-tillage machines, as well as their areas of application (Penyashki et al., 2003). The presented graph illustrates a three-dimensional surface dependence between the moisture content of the material (W_a , %), the process speed (Velocity, m/s) and the relative share of the 1-25 mm fraction (%) (Figure 4). The surface is built as an interpolation network (wireframe), allowing visualization of the combined influence of the two independent parameters on the output characteristic of the process.

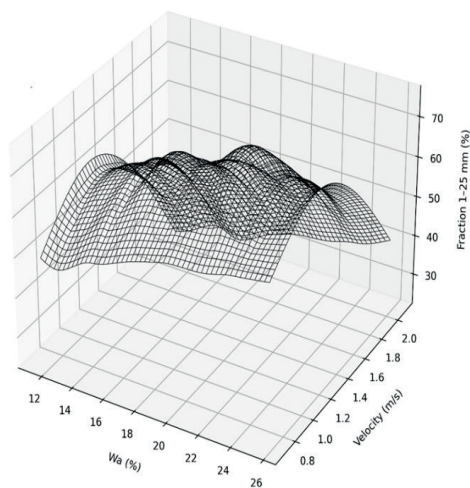


Figure 4. Three-dimensional surface dependence between the moisture content of the material (W_a , %), the process speed (Velocity, m/s) and the relative share of the 1-25 mm fraction (%)

The graph below shows the comparison between the actual values of the 1-25 mm fraction and the predicted values calculated by multiple regression (Figure 5). The graph shows that by analyzing the sensitivity of the results to changes in W_a and V , we have established the following conclusions:

- W_a has a positive influence on the 1-25 mm fraction. This means that increasing humidity leads to an increase in this fraction size, which is desirable in agronomic practices.
- V has a negative influence. High processing speeds lead to the disintegration of larger particles and a decrease in the stability of the 1-25 mm fraction.

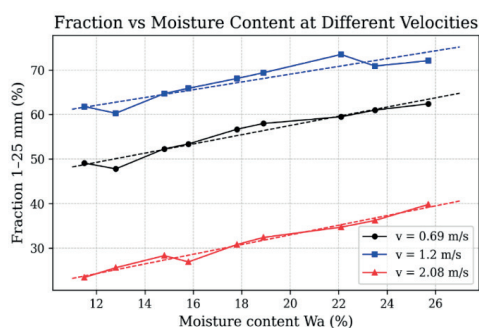


Figure 5. Comparison between the actual values of the 1-25 mm fraction and the predicted values calculated by multiple regression

The surface analysis shows a clearly pronounced nonlinear dependence of the 1-25 mm fraction content on the studied factors. In the range of speeds 0.8-1.2 m/s, the highest values of the fraction (up to approximately 70-75%) are observed, which indicates favorable conditions for the process at lower kinetic intensity. With an increase in the speed above 1.6-1.8 m/s, a steady trend towards a decrease in the relative share of the target fraction (up to about 40-50%), which can be associated with increased dispersion, secondary crushing or poor separation efficiency, is observed.

The influence of moisture content (W_a) also exhibits a nonlinear character. In the interval of approximately 18-22%, a local maximum of the response function is formed, which suggests the presence of an optimal humidity at which the mechanical and rheological properties of the material contribute to the formation or preservation of a fraction of 1-25 mm. At lower

or higher values of W_a , a certain decrease in the output indicator is observed.

The combined effect of the two factors indicates the presence of an optimization area characterized by moderate to increased moisture content and lower process speed. The shape of the surface suggests the existence of an interaction effect between the variables, which should be confirmed by regression analysis or experimental design methodology (e.g. Response Surface Methodology).

The results obtained show that the speed is a dominant factor in terms of the influence on the relative share of the fraction, while the moisture content plays a regulating role, determining the optimal operating range. The practical significance of the analysis is expressed in the possibility of determining rational technological parameters leading to maximizing the yield of the target fraction 1-25 mm with minimal energy and process losses.

The data from the conducted study show a clear relationship between the air flow velocity and the separation efficiency of the 1-25 mm fraction. At low and medium speeds (0.69 and 1.2 m/s), increasing humidity leads to a gradual increase in the fraction of the separated fraction. The highest and most stable efficiency is observed at 1.2 m/s. At the highest speed (2.08 m/s), the results become more heterogeneous, which suggests the occurrence of turbulent effects or a change in the behavior of particles at high kinetic energy. This indicates that the optimal speed for the process is about 1.2 m/s, at which the best balance between air flow and material humidity is achieved.

CONCLUSIONS

The newly developed multifunctional machine represents a significant step towards sustainable and resource-efficient agriculture. It allows the implementation of regenerative practices through a single mechanized pass, combining agronomic, environmental and economic advantages. The technology has the potential for widespread implementation in modern agricultural systems, especially in regions with degraded soils and climate risks. The results show that the new model of tillage machine has significant potential for improving the 1-25 mm fraction at optimized moisture values and tillage

speed. To achieve the best results, it is advisable to use an optimal speed of about 1.2 m/s and to control the soil moisture to improve the structure.

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FUNCTIONAL ALTERATION AND DOMINANCE-CODOMINANCE PATTERNS IN DECOMPOSITION FUNCTIONAL MICROBIOMES FROM LONG-TERM FERTILIZED AND AMENDED EXPERIMENTS

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Abstract

The application of fertilizers and amendments in agroecosystems is a necessary management strategy to sustain crop productivity, but in the long term it causes changes in soil microbiomes. The aim of the research was to analyze in detail the changes on decomposition functional microbiome induced by both types of inputs in a long-term experiment. The functional microbial associations were analyzed using the DEMSA tool, for each of the experimental variants being extracted the segments of the ecological niche: intensification, expansion, narrowing and contraction. The functional alteration varied greatly ranging between 0.4% and 26.7%, with most of the communities showing a dominance of carbohydrates guilds (intensification and narrowing niche) and carboxylic acids (expansion and contraction niche). Within the communities, the dominant groups were associated mostly with amendments for the activities that exceeded the control. In contrast, the reduction of activities within the microbiomes was correlated with the applied fertilizers. Each of the analyzed microbiome segments presented a specific pattern of dominance-codominance of functional guilds and groups that were associated with the synergic impact of long-term applied amendments and fertilizers.

Key words: functional ecological niches, long-term experiment, microbial assemblage changes, straw decomposition, substrate microbial reaction.

INTRODUCTION

Soil microorganisms are important in maintaining ecosystem health and respond to long-term fertilization by changing the structure of their communities. The microorganisms create complex communities which support soil and plant health by regulating the nutrient cycle and protecting the ecosystem from biotic and abiotic stresses (Bhattacharjee et al., 2022).

The high diversity of microorganisms in soil and limited understanding of how they function often lead to their omission in carbon cycle models. However, microbial decomposers are responsible for the breakdown of most organic matter in the biosphere and play an important role in the carbon and nutrient cycles (Soares & Rousk, 2019).

Microbial coexistence means that multiple species can live together, even if they compete for the same resources, through mechanisms that reduce direct competition or balance the advantages of each species. Understanding these interactions, based on modern coexistence

theory, shows how niche differences allow microbial communities to maintain to maintain stability and functionality. Applying this concept in agriculture can help build and manage microbial communities to improve soil health and agroecosystem productivity (Zhang et al., 2021; Chang et al., 2023).

According to Vidican et al., 2017 on the assemblage of community, significant attention is given to dominant and co-dominant groups and provides an insight on the internal dynamics of ecosystems. Soil microbiome is structured around these dominant groups, which influence both the composition and functioning of the microbial community. The dominance of certain groups is closely linked to their ability to adapt to environmental conditions, allowing rapid responses to changes in resources or management activities. In contrast, co-dominant groups are more unstable, susceptible to pronounced variations due to environmental factors (Stoian et al., 2022).

Soil microbial communities possess functional redundancy, even though species have different

metabolic niches. All microorganisms perform basic functions, but only a few participate in rare processes. The conservation of phylogenetic niches explains this redundancy, as essential genes have been preserved over billions of years and transmitted between microorganisms. Soil provides many different microhabitats, and species from the same evolutionary branch may have overlapping niches, making them functionally redundant in certain processes.

In this context, the Detailed Exploration of Microbial Sociological Assemblages (DEMSA) method was created to improve the limitations of standard CLPP analyses by using Biolog EcoPlate substrates as chemically defined groups, facilitating the detailed study of microbial niche evolution in soil (Stoian et al., 2022). The soil microbiome is a dynamic micro-ecosystem that regulates biological processes vital to global ecosystems. Preserving it as "next-generation biological" could support future applications, but the challenge lies in finding storage methods that maintain its functionality. Current research is analyzing the integrity of the preserved microbiome by assessing its structural diversity and functional potential.

The aim of this research was to analyze in-depth the impact of long-term fertilizer and liming application on the functional microbiome assemblage associated with straw decomposition. The hypotheses proposed and tested were formulated as research questions: i) treatment combination show a visible pattern on the functional microbiome activity; ii) each treatment induce a specific increase or decrease in metabolic activities recorded in microbiome; iii) functional group analysis reveal specific patterns of ecological niche as defined by the activity level; iv) the use of DEMSA model is suitable for the extraction of relevant indicators of long-term treatments.

MATERIALS AND METHODS

The study was conducted in an experimental field with long-term fertilization (over 60 years), located at ARDS Livada, Satu Mare county, and aimed to investigate the decomposition process of wheat straw using the litterbag method. The experimental protocol was adapted from Gheorghită et al. (2024), with minor

modifications to the DEMSA model applied to the dataset.

Based on the analysis of activity trends in functional microbial profiles involved in organic matter decomposition across 10 variants (V2-V11) in experimental treatments after 60 years of long-term soil fertility management, this study aims to investigate the interactions between functional microbial communities and their diversity in long-term fertile soils and to determine if decomposition rates are associated with overall microbial diversity or with specific key species linked to the applied inputs. The DEMSA model by Stoian et al. 2022, evaluates the response of microbial communities involved in organic matter decomposition to long-term fertilization by comparing carbon substrate utilization against a control interval. It classifies functional shifts into four ecological niche segments-intensification, expansion, narrowing, and contraction-thereby revealing changes in microbial functional diversity and decomposition stability in soil.

The experiment included ten experimental variants (V2-V11), corresponding to different levels of liming (amendment): medium amendment (2.5 t ha^{-1}) for variants V2-V6 and intensive amendment (5 t ha^{-1}) for variants V7-V11. Variants V2 and V7 included only the application of the amendment (from moderate to intensive), without mineral fertilization. Variants V3 and V8 were fertilized with nitrogen ($\text{N} - 100 \text{ kg ha}^{-1}$), followed by V4 and V9 fertilized with phosphorus ($\text{P} - 70 \text{ kg ha}^{-1}$), V5 and V10 with nitrogen and phosphorus ($\text{NP} - 100+70 \text{ kg ha}^{-1}$), and V6 and V11 with nitrogen, phosphorus, and potassium ($\text{NPK} - 100+70+60 \text{ kg ha}^{-1}$). A major difference in the use of the DEMSA model is the comparison of all experimental variants with the control V1, represented by unfertilized soil (native soil). The Biolog EcoPlate protocol involved the use of plant material harvested after a 30-day decomposition period, followed by sample processing and serial dilutions up to 10^{-4} . The resulting suspensions were then inoculated into microplates, $120 \mu\text{L}$ per well. The microplates were incubated at room temperature in the dark, and readings were taken at 24, 48, 72, and 96 hours using a spectrophotometer (Gheorghită et al., 2024).

Within the methodology used, the substrates were classified as functional groups, each being assigned to a functional guild based on chemical indicators (Stoian et al., 2022). The impact of organic matter decomposition on the microbiome in soils with long-term fertilization was assessed using the functional groups: carbohydrates (CH), polymers (P), carboxylic acids (CX), amino acids (AA), and amines/amides (AM).

The entire database was explored using RStudio (R Core Team, 2024). Basic statistics were obtained using the 'psych' package (Revelle, 2024), while differences between variants (V2-V11) were evaluated using ANOVA and the LSD test from the 'agricolae' package (de Mendiburu, 2023). The stability of functional microbiomes was evaluated using diversity indices and NMDS ordination, calculated with the vegan package (Oksanen et al., 2022), and correlations vectors and communities were explored at niche level.

RESULTS AND DISCUSSIONS

The functional traits of microorganisms are measurable characteristics that show the performance of an organism under specific environmental conditions. These traits can be studied in microbial ecology to improve biogeochemical models and predict how ecosystems respond to global changes.

Patterns of functional proliferation in microbial communities under long-term application of treatments

In soils, the intensification process in microbial community implies an increase in activity that is maintained in the average-to-maximum range of the native soil. These values can be used as an indicator of natural activity associated with the decomposition process, which overscores the native potential but do not affect the natural balance of community. Expansion can be defined as a significant or considerable increase in the activity of decomposing microorganisms, that perform an accelerated process due to the significant increase in the abundance and variety of several functional groups.

Carbohydrates (CH) show a relatively homogeneous distribution of the intensity of microbial metabolism involved in the decomposition of organic matter. Microbial

activity associated with the use of carbohydrates, as well as other functional groups (P, CX, AA), remains relatively stable between different inputs applied over the long term. Of all the guilds analysed, carbohydrates show the most pronounced increase in activity in microbial communities, suggesting that they are preferred and more easily accessible substrates for soil microorganisms.

In the amine guild (AM1 and AM2), variants V5 and V6 - treated with organic amendment and 2.5 t ha⁻¹ CaCO₃ - recorded zero values, which indicates the absence of stimulation of the metabolic activity of microorganisms involved in the decomposition of organic matter (Table 1). This suggests that, under long-term fertilization conditions, microbial communities belonging to this functional guild respond by reducing their metabolic processes. Consequently, the additional application of mineral fertilizers (NP or NPK) does not result in higher values. Also, in the intensively amended variant (V8), no increase in activity is observed, even though nitrogen is present. This may indicate either a lower functional activity of the microorganisms in this guild or the fact that amine-specific substrates are more difficult to degrade, requiring metabolic conditions or a longer adaptation of the microbial community.

Table 1. Microbial functional guilds and diversity in Intensification and Expansion range of activities

	Guild performance in Intensification niche				
	Px	CH	CX	AA	AM
V2	19.8±3.9ab	41.4±3.4ab	17.5±1.5ab	18.4±2.9bcd	2.7±1.6bc
V3	14.4±3.4ab	38.5±3.5ab	26.5±7.3a	18.1±3.4bcd	2.2±2.2bc
V4	20.0±1.8ab	38.4±8.7ab	25.6±4.7a	14.7±4.1d	1.1±1.1c
V5	13.2±2.9b	39.3±7.7ab	17.9±2.3ab	29.4±5.6ab	
V6	15.1±1.3ab	42.2±2.5ab	16.1±1.0ab	26.4±3.0abc	
V7	13.3±5.3b	26.3±2.7b	24.9±5.4a	30.8±2.4a	4.4±2.2bc
V8	29.7±13.6a	42.8±7.8ab	8.5±2.0b	18.7±5.6bcd	
V9	17.1±2.8ab	52.3±5.0a	11.1±3.2b	17.8±3.6cd	1.5±1.5bc
V10	10.5±3.4b	31.8±7.9b	25.5±4.2a	21.0±2.9abcd	11.0±0.9a
V11	18.8±4.9ab	35.0±10.1ab	17.9±6.5ab	21.3±4.1abcd	6.7±4.5ab
	Guild performance in Expansion niche				
	Px	CH	CX	AA	AM
V2	21.1±5.7ab	43.8±9.1a	15.2±1.7ab	14.7±2.9b	4.9±3.2b
V3	7.6±2.1b	61.7±5.1a	16.7±6.0ab	13.4±5.1b	0.4±0.4b
V4	20.8±6.3ab	49.9±14.3a	18.1±6.4ab	10.4±7.6b	0.6±0.6b
V5	8.8±1.4b	63.1±2.5a	6.2±2.7b	21.7±5.3ab	
V6	10.9±1.5b	46.9±10.0a	9.0±4.5ab	33.0±11.0a	
V7	6.4±4.3b	42.9±12.5a	25.1±9.0a	22.2±5.9ab	3.1±2.1b
V8	30.3±13.3a	51.9±11.3a	12.5±1.7ab	5.1±2.0b	
V9	11.2±3.9b	67.6±11.6a	12.2±6.2ab	8.5±3.1b	0.3±0.3b
V10	9.1±2.4b	39.5±14.0a	23.8±6.7ab	12.3±3.3b	15.1±4.7a
V11	15.4±3.7ab	48.7±11.8a	15.2±9.7ab	14.9±7.7b	5.6±3.6b

Note: V2-V6 - liming L1 (2.5 t ha⁻¹ calcium carbonate), V7-V11 - liming L2 (5 t ha⁻¹ calcium carbonate), V2-V7 - liming without mineral fertilizer, V3-V8 - liming with N100, V4-V9 - liming with P70, V5-V10 - liming with N100P70, V6-V11 - liming with N100P70K60. Legend: Px - polymers; CH - carbohydrates; CX - carboxylic acids; AA - amino acids; AM - amines/amides. Values ± s.e. followed by different letters present significant differences according to LSD (p<0.05).

The microbial diversity of the soil responsible for decomposing organic matter can expand, leading to a richer microbiome in terms of groups involved in decomposition.

The V5 and V10 variant treated with NP, but different liming intensities show that an increase in the level of amendment does not lead to a significant increase in the microbiome diversity. The carbohydrate guild generates the highest values, as these substances are more easily solubilised and used as an energy source by decomposing microorganisms in the soil. Due to their simpler chemical structure and rapid degradation, microbial diversity expands within a short period of time. As a result, there is a significant increase in both the number of microorganisms and their diversity, a phenomenon that indicates a marked stimulation of biological processes in the soil.

The general trend of expansion indicates a shift towards L1, and in the case of polymers the amendment applied at the average level results in overall slightly higher values. A similar pattern can be observed in carboxylic acids, where there is a moderate expansion in values, also in L1. It appears that applying only 2.5 t ha⁻¹ of amendment is sufficient to stimulate higher microbial diversity.

Narrowing niche of the soil microbiome refers to the decrease or reduction in the microbiome in the average-minim range compared to the native community. Overall, the dominant microbial groups involved in decomposition processes become less abundant. In terms of contraction niche the microbiome suffers a compaction process and its diversity is reduced. The affected populations decrease considerably under the minimum native potential and the groups involved in the decomposition decrease or even disappear due to the application of different inputs.

Growth strategies in Intensification and Expansion niches

The microbial diversity involved in the decomposition process extends to the soil microbiome under long-term fertilization conditions. In the L1 variants, characterized by a moderate level of amendment, the CH guild shows an increase in microbial activity, as this level of amendment supports a highly active microbiome. Under these conditions, intensive amendment (L2) does not generally lead to

higher diversity values, except in variants V7 and V8.

A similar behaviour is observed for the P, CX, and AA guilds, where microbial diversity remains higher under L1 conditions. The carbon sources associated with these guilds seem to be preferred by microbial communities involved in decomposition when there is a buffering effect generated by soil pH correction. A particular case is represented by the AM guild (amines), which are more difficult to metabolize by the decomposing microbiome, which explains the lack of diversity expansion under conditions of intensive amendment (L2). In most situations, intensive amendment applied alone generates higher microbial indicator values compared to variants combined with chemical fertilizers (Liu et al., 2009). Thus, soil pH adjustment becomes a main factor for the structure and functioning of the microbiome (Hartmann & Six, 2023).

The intensification in the activity of microbial communities indicates a relative homogeneity of the soil microbiome, with no major functional differences between the guilds analysed under conditions offered by L1 and L2 (Ortega et al., 2024). Microorganisms generally respond similarly to the decomposition process, suggesting a relatively uniform microbial structure between guilds. The exception is the AM guild, which, in certain situations, does not show increases in microbial activity intensity.

Functional diversity supports stable metabolic activity at an optimal level of amendment (L1), correlated with expansion and intensity indicators. Amendment (L2), the increase in intensity is not accompanied by an expansion of the diversity of the agricultural soil microbiome, which indicates a selection effect on the dominant microbial communities. This is assured by a shift from a generalist to a specialized community, depending both on the inputs applied over a long period of time and on the availability of carbon sources in Biolog EcoPlate (Gheorghiu et al., 2026).

A significant positive influence is exerted by the application of calcium carbonate, which results in high values in the treated variants, with no major differences in microbial metabolism at the intensive dose of 5 t ha⁻¹. An average dose of 2.5 t ha⁻¹ CaCO₃ is sufficient to maintain the balance of the agricultural soil microbiome.

The expansion of microorganisms responsible for decomposition becomes more efficient in the presence of a moderate amount of fertilizer, which facilitates and stimulates a visible proliferation of microorganisms. This manifests itself both in the increase in the diversity of communities across a wide range and in the intensification of their metabolic functions, their activity becoming more pronounced (Maron et al., 2018).

Patterns of functional decline in microbial communities under long-term application of treatments

In conditions of long-term fertilization (Table 2), the narrowing manifests as a reduction in functional microbiome involved in the decomposition of organic matter, as a result of the rich supply of organic and inorganic nutrients in the soil. This phenomenon generates a limited or restricted number of groups adapted to nutrient-rich conditions, to the detriment of a diverse microbial community. The dominance of decomposing microorganism species is reduced, and mineralization processes become more limited in terms of functional diversity (Gessner et al., 2010).

Table 2. Microbial functional guilds and diversity in Narrowing and Contraction range of activities

	Guild performance in Narrowing niche				
	Px	CH	CX	AA	AM
V2	6.3±4.6b	18.1±6.6ab	41.4±4.9ab	25.8±6.6ab	8.1±0.8abc
V3	12.9±4.5ab	17.4±6.1ab	37.6±5.0ab	20.5±2.6abc	11.3±2.9a
V4	8.1±2.9b	17.0±4.2ab	36.6±2.5ab	27.9±4.6a	10.1±1.3a
V5	10.8±2.1ab	23.8±3.9ab	38.5±1.0ab	16.8±2.5abc	9.9±0.3ab
V6	9.3±0.6ab	21.2±2.2ab	45.3±2.5a	13.0±4.6c	10.9±0.7a
V7	13.4±2.2ab	33.2±3.6a	31.4±4.7b	16.4±1.9abc	5.4±0.1bc
V8	9.1±2.0ab	20.3±6.7ab	43.8±5.7ab	16.5±1.9abc	10.0±1.3a
V9	7.8±1.8b	11.1±3.1b	48.8±1.9a	22.3±2.7abc	9.7±1.5ab
V10	17.5±3.9a	26.1±9.1ab	40.7±7.2ab	15.6±2.1bc	
V11	11.5±2.0ab	18.9±7.8ab	42.6±3.4ab	21.7±6.2abc	5.1±2.9c
	Guild performance in Contraction niche				
	Px	CH	CX	AA	AM
V2	6.2±6.1b	10.1±3.6b	42.7±10.2ab	26.7±8.5a	14.0±2.8ab
V3	17.1±7.0b	11.2±8.2b	39.7±6.3ab	15.3±4.9ab	16.5±2.1a
V4	9.6±3.2b	16.4±6.5ab	41.3±6.2ab	22.8±4.8ab	9.7±3.2abc
V5	14.2±7.1b	13.7±3.3ab	44.6±4.0ab	13.9±2.2ab	13.4±1.6ab
V6	4.6±1.6b	21.9±5.8ab	42.7±5.6ab	17.1±7.8ab	13.4±3.1ab
V7	11.7±3.2b	31.9±4.3a	32.6±4.4ab	18.9±3.2ab	4.7±1.5bc
V8	10.8±4.2b	21.6±8.0ab	37.4±7.2ab	15.9±5.8ab	14.0±2.4ab
V9	12.8±2.0b	6.6±3.6b	50.5±5.0a	18.6±0.4ab	11.1±2.2ab
V10	34.0±9.7a	25.5±9.2ab	30.6±7.0b	9.8±3.7b	
V11	14.9±5.8b	22.5±12.0ab	38.3±8.4ab	9.3±5.2b	14.7±8.5ab

Note: V2-V6 - liming L1 (2.5 t ha⁻¹ calcium carbonate), V7-V11 - liming L2 (5 t ha⁻¹ calcium carbonate), V2-V7 - liming without mineral fertilizer, V3-V8 - liming with N100, V4-V9 - liming with P70, V5-V10 - liming with N100P70, V6-V11 - liming with N100P70K60. Legend: Px - polymers; CH - carbohydrates; CX - carboxylic acids; AA - amino acids; AM - amines/amides. Values ± s.e. followed by different letters present significant differences according to LSD (p<0.05).

The most pronounced reduction in microbial metabolism is observed in the case of carboxylic acids, with values ranging from 40.7% in variant V10 (treated with NP and intensively amended) to 48.8% in variant V9 (intensively treated and fertilized with P). These results indicate that the types of fertilizers applied lead to a decrease in microbial metabolic activity (Ge et al., 2010). Under these conditions, carboxylic acids are carbon sources that are more difficult to solubilize in the presence of added inputs.

A relatively uniform distribution of the communities involved in the decomposition of organic matter is particularly noticeable in the polymer guild, where narrowing in microbial metabolism are lower compared to the CX guild. The assessment of contraction phenomenon can be used to describe a sharp decrease in microbial activity, manifested by a reduction in metabolism below the minimum observed in native community (Table 2). This phenomenon can occur during the decomposition process, when the microorganisms involved are strongly influenced by environmental factors, which can lead to the inhibition of microbiome development and the cessation of its proliferation (Islam et al., 2020).

When contraction occurs, a relatively heterogeneous loss in microbiome can be observed in all treatments. Thus, microbial communities maintain their structure in a similar range of losses. Overall, they do not suffer an abrupt change into their metabolic activity, proliferation rate, or biological functions, even in the presence of different variants or specific types of substrates (Poyraz et al., 2021). This stability leads to a uniform loss of microbial groups, with no visible differences in the entire vital process of decomposition (Khatoun et al., 2017).

Carboxylic acids show the highest values in the contraction phenomenon analysed. As a result of prolonged exposure to the different types of inputs, the diversity of microbial communities tends to decrease significantly, and the metabolic activity of microorganisms is affected (Macias-Benitez et al., 2020). Over time, part of biological functions is inhibited or even completely inactivated, as the conditions created by the constant presence of treatments limit the development and adaptation of microbial populations (Gonzalez & Aranda, 2023).

Reduction strategies in Narrowing and contraction niches

The functional diversity involved in the decomposition process, in the context of the contraction phenomenon, is strongly influenced by the application of N, P, and NP fertilizer complexes (Luo et al., 2018). These treatments cause significant reductions in microbial diversity, especially in guilds associated with the use of carboxylic acids, which implicitly leads to a decrease in the microbial metabolism of various C sources.

Microbial diversity losses double in volume during the contraction phenomenon at the total CX guild level, reaching its maximum value in variant V9, characterized by the application of an intensive amendment treatment and phosphorus in the form of mineral fertilizer. In this variant, the soil microbiome is significantly reduced, followed by variant V5 fertilized with NP and variant V6 fertilized with NPK complex. In the case of carboxylic acids, the functional diversity of microorganisms involved in decomposition processes plays a significant role in the structure of this guild. The ability to use the 9 carboxylic acids may be limited by fertilization conditions and the type of amendment applied, as well as soil pH and nutrient availability (Zhang et al., 2023). Under these conditions, decomposing microorganisms will metabolize these compounds less efficiently, leading to a reduction in the metabolic activity of microorganisms (Phale et al., 2007).

On the other hand, the CX guild, both in the narrowing and contraction niches, causes significant decreases in both functional diversity and the structure of the communities of microorganisms responsible for decomposition, under conditions of fertilization with N, P, NP, and NPK.

At the same time, the NP and NPK fertilizer complex causes functional decreases in the Px, CH, and CX guilds. An exception is the AA guild, where the contraction phenomenon is reduced by almost half compared to Px. In the case of the AM guild, the absence of the contraction phenomenon in variant V10 is notable, a trend that is also maintained in the narrowing phenomenon.

In the CH and AA guilds, there is a relatively uniform decreasing distribution of microbial

diversity, with N and P treatments causing similar effects of microbial diversity reduction (Wang et al., 2023).

These two phenomena are closely related in terms of reducing both microbial diversity and the structure of communities, as the reduction in the availability of certain metabolic resources and the modification of soil fertilization conditions favour the selection of dominant microbial groups, while other groups become less active or are even eliminated, thus leading to a more selective microbiome (Islam et al., 2020).

Niche-functional alteration produced by long-term application of treatments

Within the positive phenomena (Table 3), the highest index of expansion and intensification is recorded, indicating a significant increase in the diversity of microbial communities, as well as an amplification of their metabolic activity when applying only moderate fertilization (L1), without the addition of mineral fertilizers. In this context, soil pH is a key factor in the decomposition process of straw (Yang et al., 2023).

Table 3. Microbial functional activity and changes in the range of the functional niches activities

	Sum Int	Index of Int	Sum Exp	Index of Exp
V2	0.6±0.1ab	1.6±0.1ab	5.8±2.1a	13.8±4.0a
V3	0.5±0.1ab	1.5±0.1ab	4.9±1.8ab	12.2±3.4ab
V4	0.6±0.1ab	1.5±0.3ab	4.2±1.1ab	11.0±2.7ab
V5	0.5±0.0ab	1.5±0.0ab	2.9±0.3ab	8.4±1.0ab
V6	0.6±0.1ab	1.8±0.1ab	3.9±0.9ab	10.1±2.0ab
V7	0.5±0.0ab	1.4±0.1ab	1.8±0.3b	5.1±0.9b
V8	0.4±0.1b	1.2±0.2b	3.1±1.5ab	8.5±3.8ab
V9	0.6±0.1ab	1.6±0.2ab	3.3±1.1ab	8.7±2.7ab
V10	0.7±0.0a	1.9±0.1a	3.9±0.8ab	10.0±1.9ab
V11	0.6±0.1ab	1.6±0.3ab	3.4±0.9ab	8.9±1.9ab
	Sum Narr	Index of Narr	Sum Contract	Index of Contract
V2	1.2±0.1ab	3.2±0.4ab	2.8±0.4b	7.3±1.3b
V3	1.4±0.2ab	4.2±1.1ab	4.2±1.3ab	12.5±4.9ab
V4	1.5±0.2ab	4.1±0.9ab	3.3±0.7b	9.2±2.4b
V5	1.6±0.0a	4.8±0.2ab	4.5±0.2ab	13.±2±0.8ab
V6	1.4±0.2ab	3.8±0.7ab	3.2±0.7b	9.0±2.5b
V7	1.6±0.1ab	4.7±0.3ab	3.7±0.4ab	10.9±1.6ab
V8	1.7±0.2a	5.5±1.2a	5.7±1.1a	18.0±4.8a
V9	1.5±0.1ab	4.3±0.7ab	3.6±0.8ab	10.4±3.1ab
V10	1.0±0.0b	2.7±0.2b	2.3±0.3b	6.2±0.9b
V11	1.4±0.2ab	3.9±0.9ab	2.7±0.7b	7.7±2.2b

Note: values ± s.e. followed by different letters present significant differences according to LSD ($p < 0.05$). Legend: Int - Intensification niche, Exp - Expansion niche, Narr - Narrowing niche, Contract - Contraction niche; Sum - the total recorded sum of activities in each functional niche, Index of - The index of general changes in community assemblage pattern recorded in each functional niche.

In contrast, variants V3, V4, and V6 show relatively similar values, but slightly lower than V2. The application of mineral fertilizers (N, P, and NPK) contributes to maintaining an active, more uniform, and balanced microbiome in soils treated in the long term. Although microbial

diversity reacts quickly to environmental changes, a functional balance and long-term adaptability are maintained within microbial communities. At the same time, the NP fertilizer complex in variant V10 (L2+NP) produces values like those observed previously, so this combination of inputs has a synergistic downgrade effect comparable to that observed in variant V6.

Significant decreases are registered in the V8 variant, fertilized with N, associated with an intensively applied amendment (L2). Under these conditions, the total microbial diversity is reduced, and within the microbial communities, there is a decrease in the microbiome involved in decomposition processes.

In the case of variants V5 (L1+NP) and V3 (L1+N), there is also a slight reduction in the microbiome associated with the phenomenon of narrowing and contraction, but the effect is less pronounced compared to V8.

At the same time, variants V7 and V9 (P as mineral fertilizer), characterized by the application of an intensive amendment (L2), show a relatively homogeneous dispersion of the contraction phenomenon. This uniform distribution suggests that the application of L2, both individually and in combination with P, contributes to the similar manifestation of this pattern in the analysed variants.

The intensification segment in microbial communities is highlighted by a star-like distribution in the NMDS projection (Figure 1). The carbohydrate vector shows an increase, based on the amplified microbial activity in the presence of nitrogen and organic amendments, regardless of their intensity. The intersection of this vector with phosphorus and the NP combination suggests an intensification in carbohydrate guild when additional mineral inputs are available.

Nitrogen treated communities are distributed pointwise, with a stellar structure characterized by unequal arms, ranging from medium to intensive organic amendments, with obvious differences depending on the microbial guild. Thus, microbial communities intensify their carbohydrates and polymers metabolism, while amine-associated activity is absent. In contrast, fertilization with NP and NPK has a significant effect on soil microorganisms, visibly stimulating the rate of amine guild metabolism.

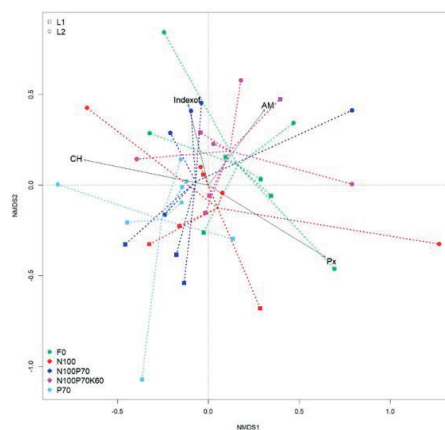


Figure 1. Functional microbiome dispersion in Intensification niche

Microbial communities associated with the guild of amines and polymers show a pronounced overlap and intensified metabolic activity in the absence of mineral fertilizers, but under conditions of organic amendments at both medium and intensive levels. In soils fertilized with NPK, microbial communities generate multiple intersection points across the entire NMDS projection, crossed by both polymers and amines vectors. This dynamic indicates an acceleration in the metabolism of these two guilds, associated with subtle but relevant functional changes in the structure of microbial communities.

The vector associated with the carbohydrate guild (CH) is the only one that significantly exhibits the phenomenon of expansion (Figure 2). This is an important image provided by the NMDS analysis, highlighting the dominant guild that expands along the microbial diversity gradient, consisting of a microbiome capable of metabolizing carbohydrates and amplifying its functional activity.

The absence of mineral fertilizers, in the context of the sole application of amendments L1 and L2, has a significant effect on soil microbiome diversity, as microorganisms are strongly and positively influenced by the CH vector. This process leads to a marked increase in microbiome diversity. The CH vector overlaps three distinct branches, corresponding to a gradient of amendment intensity, ranging from medium to intensive.

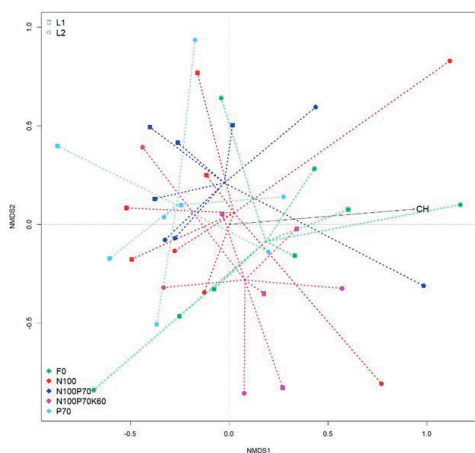


Figure 2. Functional microbiome dispersion in Expansion niche

Within this unique vector, it is also observed that the application of N and NP treatments leads to the formation of an extremely active microbiome, and the expansion of microorganisms involved in the decomposition of organic matter has a synergistic effect on the functionality of the soil ecosystem.

In the context of narrowing, microorganisms from the carbohydrate and amine guild vectors overlap directly with L1 and NP complexes (Figure 3). The carbohydrate-associated vector (CH) also interacts with the structure of microbial communities shaped by N, P, and NPK treatments. Given that the centroid of the NP complex also overlaps with that of NPK, suggest that the microorganisms responsible for the degradation of organic matter have a uniform and comparable effect in both types of fertilization systems.

In contrast, the application of amendments at an intense level significantly interferes with the amine vector, suggesting that high amendment input favours the formation of a microbial community like that developed under the influence of NP and NPK mineral input complexes. This indicates a convergence of microbial structure in response to intensified amendments.

The cumulative activity of microorganisms is modified by the long-term application of mineral fertilizers, which is reflected in a gradual decrease in metabolic activity associated with adaptation processes to a constant nutrient supply.

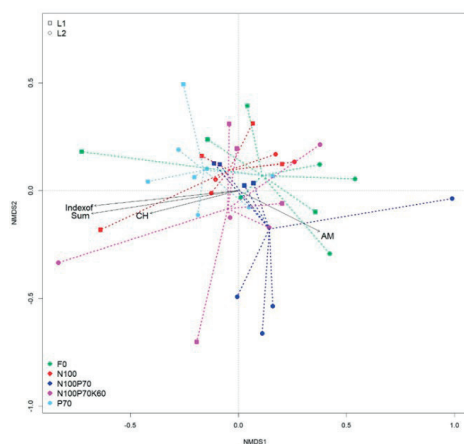


Figure 3. Functional microbiome dispersion in Narrowing niche

The NMDS of the contraction phenomenon show a more dispersed microbiome at a similar pattern with the narrowing (Figure 4). The CH vector from overlaps with the microbiome of unfertilized soils, but amended both moderately and intensively, through four points that intersect this vector. Under these conditions, the diversity of dominant microorganisms involved in the decomposition of organic matter is significantly reduced. An additional synergistic effect is exerted by N and NP fertilization, which leads to a decrease in diversity and, implicitly, in the activity of microorganisms in agricultural soils subjected to long-term fertilization.

Also, the base of the CH vector is positioned at a very short distance from the NPK complex, which indicates a close association between these factors. The proximity suggests that the presence of complete NPK fertilization further contributes to the inhibition of microbial community activity, through nutritional imbalances and changes in the physical-chemical conditions of the soil.

NMDS analysis reveals a star-like distribution of points, indicating the existence of relatively high structural microbial diversity. However, the reduced dispersion and partial overlap of the groups suggest a decrease in the abundance of microorganisms, as well a direction through a functional homogenization in communities, especially in soils affected by intense and long-term fertilization.

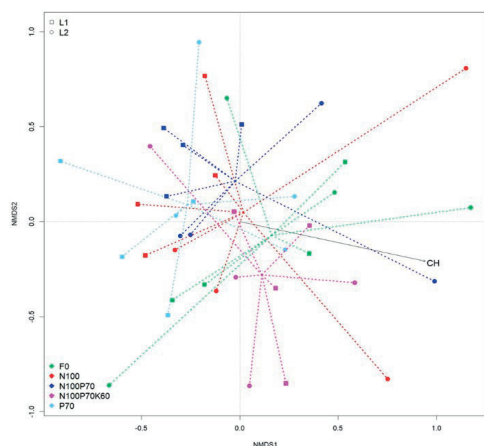


Figure 4. Functional microbiome dispersion in Contraction niche

Long-term fertilization, within the proliferation/decline phenomena, allows data to be divided into 4 quartiles (Q1-Q4) based on the general functional alteration, allowing the

evaluation of the microbial response to applied treatments (Figure 5). This phenomenon scores the functionality of the microbiome from low to high values.

Q1 and Q2 represent the quartiles with higher values, both in terms of positive and negative values recorded in microbial metabolism. On the other hand, Q3 and Q4 are represented by the quartiles with the lowest values, both positive and negative.

In the variants subjected to moderate amendment treatments, it can be observed that, in the upper quartile (Q1), variants V2 and V3(+N) reach a maximum of metabolic activity and diversity. Variant V2, which is subjected only to liming inputs of 2.5 t ha⁻¹, records a maximum of 26% in Q1.

Q4 records, in 5 variants, represent functionally reduced values, with positive values between 0.43 and 5.81, both recorded in L2, while the maximum value was recorded in V8 and the minimum value in V9.

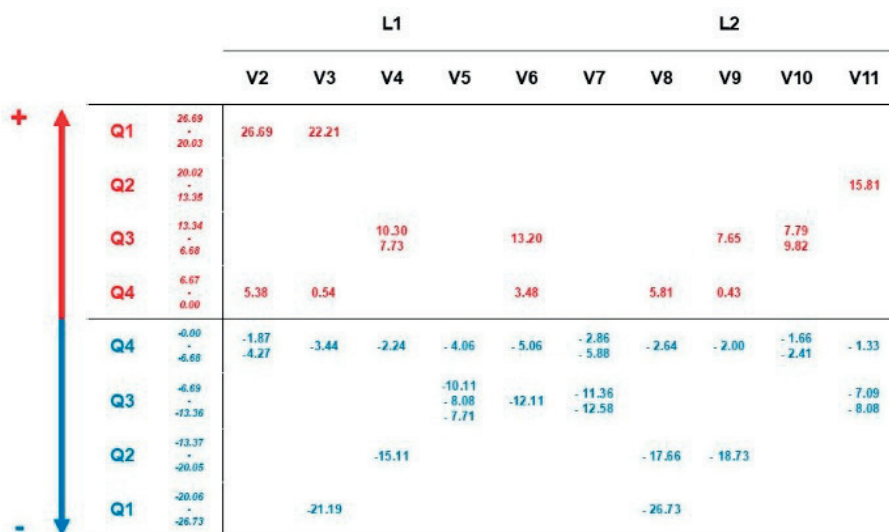


Figure 5. Microbial response as total functional alteration compared to native community.

Legend: Table representation by quartiles: Q1 - high values; Q2 - medium-high values;
Q3 - medium-low values; Q4 - low values

Q3 predominates in variants V4, V6, V9, and V10, with semi-reduced values. Two approximately equal values are observed between V4, treated with medium-level amendment and fertilized with P, and V10, amended at an intensive level and treated with NP, which does not generate significant

differences between treatments in terms of the decomposer microbiome.

Regarding the lower negative quartiles, there is a general downward trend in microbial activity associated with specific treatments. Thus, in V5, fertilized with NP, activity decreases especially in Q3 and Q4. In contrast, in V6, amended at a

medium level and fertilized with NPK, there is a maximum decrease in activity in Q3. For V7, amended at an intensive level, there are significant decreases in activity in Q3, with values remaining low in Q4.

In the negative Q4 there is homogeneity between values, ranging from -1.33 in V11, treated intensively with CaCO_3 + NPK, to -5.88 in V7, treated only with intensive amendment. Metabolic activity shows a slight decrease in all variants analyzed. An interesting aspect is that this trend is present in all variants, the functional microbiome is influenced by long-term inputs. This distribution of the functional microbiome responds differently depending on the type and intensity of the treatments applied, and the effects are maintained in the long term, demonstrating that proper management of fertilization and amendments is vital for maintaining the balance and functionality of the soil microbiome.

Variant V8, intensively treated with CaCO_3 and fertilized with N, showed the highest decrease in Q1, reaching 26%, in the metabolic activity of the functional microbiome responsible for decomposition. A second case is represented by V3, amended at a medium level and fertilized with N, which showed a slight decrease of 5% from the initial value (-21%). The application of these inputs leads to some of the lowest values of metabolic activity, generating significant changes in the structure of the microbiome, from generalist species to more specialized species (Ren et al., 2023). As a general image on how soil microbiome change its functionality due to long-term treatment application, the intensification and expansion represent the processes by which microorganisms grow in number and activity, and their diversity expands, stimulated by favorable conditions, such as the application of a moderate amount of fertilizer, resulting in more intense metabolic activity and more varied communities (Dincă et al., 2022). On the other hand, contraction and narrowing represent the opposite process whereby the diversity of microbial communities shrinks and becomes more selective: certain dominant groups continue to function, while others decline in activity or disappear, reducing the diversity and functionality of the agricultural soil microbiome (Coban et al., 2022).

Metabolic plasticity provides a new way to differentiate generalist microorganisms from specialist ones (Chen et al., 2021). In microbial biology, generalists and specialists are often defined by their frequency in different habitats: microbes that show up in lots of environments have a broad metabolic niche and are considered generalists, while those with limited distribution have a narrower metabolic niche and are considered specialists (Liao et al., 2016). Generalist microorganisms have a wide distribution and a large metabolic niche, and can adapt their metabolism to various environmental conditions, while specialists, with a restricted distribution and a smaller metabolic niche, have fewer biological mechanisms for adaptation (Malard & Guisan, 2023; Vidican et al., 2025). Using the DEMSA model, it was beneficial to refine the biological framework related to organic matter decomposition for the specific objectives of our study. Since the model describes general functional microbial responses, we introduced more specific links between microbial indices and the stages of the decomposition process of wheat straw. Thus, microbial indices were correlated with the complex dynamics of organic matter decomposition to improve the interpretation and visualization of the dataset and to better highlight functional microbial diversity and activity in long term fertilized soil.

CONCLUSIONS

Fertilization and liming influence soil microbiome functioning, generating variations in metabolic activity reflected in quartile distributions. Moderate liming stimulates the diversity and density of microbial activity, particularly in Q1 and Q2, while intensive applications reduce activity in Q3 and Q4 and cause negative values, showing a decrease in the functionality of microorganisms involved in the decomposition of organic matter.

Moderate amendment (L1) stimulates the expansion and intensification of soil microbial communities, leading to higher functional diversity and more active decomposition processes. Soil pH correction supports a stable and balanced microbiome, while intensive amendment (L2) tends to select specialized microorganisms.

Application of mineral fertilizers (N, P, NP, NPK) leads to narrowing and contraction of microbial diversity. Changes in nutrient availability, along with modifications in soil chemistry, encourage the selection of dominant microbial groups, causing a decline in overall functional diversity.

The DEMSA model shows how long-term fertilization changes the microbiome, shifting from generalist microbes, which can adapt to various conditions, to specialist microbes, which are better suited to a specific environment. This transition indicates that inputs not only affect which species live in the soil, but also how they adapt their metabolism to the available resources.

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IMPROVEMENT OF SOIL PHYSICAL PROPERTIES THROUGH THE INTEGRATION OF GREEN MANURE CROPS UNDER THE PEDO-CLIMATIC CONDITIONS OF BRĂILA

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Abstract

During the agricultural years 2022-2023 and 2023-2024, a study was carried out at SCDA Brăila, under the specific pedo-climatic conditions of the area, to evaluate the effect of green manures on selected soil physical properties, namely soil penetration resistance and bulk density. The experiment included autumn pea, mustard, rye, rapeseed, and a mustard + rapeseed mixture as green manure crops, compared with a control without green manure. Measurements were performed along the soil profile, assessing depth-dependent changes in penetration resistance and bulk density as indicators of soil structure and compaction. The results obtained over the two years show that green manure use led to an overall improvement in the analyzed soil physical parameters. Soil penetration resistance decreased in the upper soil layers, while bulk density improved compared with the control, indicating more favorable conditions for root growth and for water and air movement. Although the magnitude of the effect varied among species, all green manure treatments contributed to reducing soil compaction and improving soil physical quality, supporting the role of green manures in conservative soil management.

Key words: green manures, soil penetration resistance, bulk density.

INTRODUCTION

The intensification of agricultural systems, characterized by repeated soil tillage and the predominant use of mineral fertilization, has frequently led to soil structure degradation, a reduction in organic matter content, and decreased aggregate stability. Under the pedo-climatic conditions of the Brăila area, where Chernozems soils predominate and the rainfall regime is often limiting, maintaining soil physical properties is a key prerequisite for yield stability and efficient water use.

Green manures represent a viable agronomic alternative for restoring soil fertility and improving soil structure. The incorporation of fresh plant biomass contributes to increased organic matter content, enhanced particle aggregation, and improved water retention capacity. Green manure crops can supply approximately 80-100 kg N/ha within 40-50 days of growth, while leguminous species provide an additional contribution through biological nitrogen fixation. In addition, the input of organic residues promotes an increase in soil organic carbon and improves soil structure through the formation of stable

aggregates. The input of plant biomass promotes the formation and stabilization of structural aggregates, reduces bulk density, and increases soil porosity, with direct effects on air and water movement within the soil profile. The root systems of crops used as green manures also contribute to the natural loosening of the soil and to improved water infiltration and retention, supporting the development of subsequent crops and the functional stability of soil in sustainable agricultural systems. (Sumiahadi et al., 2020; Gulaiya et al., 2024).

These crops contribute to improving the physical, chemical, and biological properties of soil by increasing organic matter content and nutrient availability. They reduce soil compaction, improve soil structure and aggregation, and stimulate microbial activity. Green manure crops also increase soil water retention capacity, reduce bulk density, and limit nutrient losses through leaching. Species such as rye (*Secale cereale* L.), triticale (*Triticosecale rimpaui*), and barley (*Hordeum vulgare* L.) are commonly used to protect soil against erosion, while legumes such as grass pea (*Lathyrus sativus* L.) and clover (*Trifolium* spp.) contribute to

improved nutrient cycling and the maintenance of soil fertility (Topa et al., 2025).

Analyses reported in the scientific literature show that the use of cover crops can reduce soil bulk density by about 4% on average, depending on the species, soil type, and management system. Species such as rye (*Secale cereale*), hairy vetch (*Vicia villosa*), sunn hemp (*Crotalaria juncea*), and pearl millet (*Pennisetum glaucum*) have demonstrated the ability to reduce soil compaction and increase total porosity, thereby improving the physical conditions for root development as well as water and air movement in the soil (Haruna et al., 2020).

It has been demonstrated that annual legumes such as red clover (*Trifolium pratense*) and common vetch (*Vicia sativa*) are frequently associated with increased structural stability and reduced soil bulk density. Species such as yellow lupine (*Lupinus luteus*) contribute to improving soil texture and reducing compaction and are particularly recommended for lighter or degraded soils. In addition, crops such as mustard (*Brassica juncea*) and forage rape, used as green manures, are associated with increased total porosity and improved soil structure (Sumiahadi et al., 2020); they can be easily integrated into crop rotations in Romania due to their low technological requirements and good climatic adaptability.

The use of green manures contributes not only to the improvement of the physical characteristics of the soil but also to the enhancement of its chemical properties by increasing organic carbon content and nutrient availability. Species such as clover, barley, rye, and triticale used as green manure have been shown to increase soil organic carbon, phosphorus, and potassium levels compared with the control treatment (Soleymani & Shahrajabian, 2011).

In an experiment conducted on a clay soil cultivated under an organic system, soil bulk density was higher in the control treatment (approximately 1.30 g cm^{-3}), while the application of green manure reduced this value to about 1.22 g cm^{-3} , with the effect being more pronounced in the surface soil layer (Műjdecı et al., 2020).

In experiments conducted on eroded soils in north-western Romania, organic fertilization

with green manure crops led to a reduction in soil bulk density by approximately 2.1-4.7% compared with the control treatment. Lower values were recorded in plant mixtures (for example vetch + oat + ryegrass or lupine + oat + rape) compared with single crops. At the same time, the use of these crops reduced soil penetration resistance from 26.8 kgf/cm^2 in the control treatment to values ranging from about 19.9 to 21.5 kgf/cm^2 in the green manure treatments, indicating improved loosening of the arable layer and more favorable conditions for plant root development (Pantiş, 2016). In a similar experiment conducted under the same conditions on an eroded soil, the use of green manure crops led to a decrease in soil bulk density from 1.53 g/cm^3 in the control treatment to values between 1.38 and 1.43 g/cm^3 , depending on the species or mixture used. At the same time, penetration resistance values decreased from 25.6 kgf/cm^2 in the control treatment to approximately $19\text{-}21 \text{ kgf/cm}^2$ in the treatments with green manure crops (Domuţa et al., 2010).

In a study conducted on a cambic chernozems, the incorporation of vetch (*Vicia sativa*) biomass as green manure led to an improvement in the structure of the arable layer and an increase in organic matter content. Experimental data showed that in the surface layer (0-10 cm), soil bulk density decreased from 1.24 g/cm^3 in the control treatment to 1.16 g/cm^3 after the incorporation of two green biomass harvests, while penetration resistance decreased from 13 to 9 kgf/cm^2 , indicating better soil loosening and increased porosity (Leah, 2017).

Soils in south-eastern Romania frequently face pedoclimatic constraints that limit soil fertility and agricultural productivity. The more arid climate of this region favors water deficit and a lower organic matter content in the soil, which negatively affects soil structure and water-holding capacity. These characteristics can limit root system development, restrict the movement of water and air in the soil, and contribute to the degradation of soil physical properties (Păltineanu et al., 2025)

To address these constraints and evaluate the role of green manure crops in improving soil physical properties, an experiment was conducted at ARDS Brăila, a representative area for south-eastern Romania. The experiment

included autumn pea, mustard, rye, rapeseed, and a mustard + rapeseed mixture as green manure crops, compared with a control treatment without green manure. Measurements were performed along the soil profile, analyzing depth-dependent variations in penetration resistance and bulk density as indicators of soil structure and compaction.

MATERIALS AND METHODS

The study was conducted during the 2022-2023 and 2023-2024 agricultural years at SCDA Brăila, under the local pedoclimatic conditions. The experiment was set up at the Chiscani Experimental Center on a Calcaric Alluvial Chernozem (SRTS, 2012).

For the implementation of the experiment, green manure crops were sown under the following treatments:

- a1 - unfertilized with green manure (control);
- a2 - autumn pea green manure;
- a3 - mustard green manure;
- a4 - rye green manure;
- a5 - mustard + rapeseed green manure;
- a6 - rapeseed green manure.

The green manure crops were sown in the last decade of August during both experimental years. The chopping and incorporation of the green manure were carried out in the first decade of April in all three years of the experiment, at the budding-flowering stage, respectively at the stem elongation-booting stage in rye, using disk tillage. The mustard green manure treatment entered the winter at the budding–flowering stage and, depending on annual conditions, was partially or completely affected by frost.

Measurements of soil bulk density and penetration resistance were performed 30 days after the incorporation of the green manure crops.

Bulk density was determined in undisturbed soil samples taken by steel cylinders (Blake and Hartge, 1986).

In the experimental research presented in this study, a digital electronic cone penetrometer, FieldScout SC 900 (Figure 1), was used to measure the resistance force to cone penetration and the penetration depth in the soil. Penetration resistance was determined at each of the three points at 2.5 cm intervals, down to a depth of 45 cm, using the FieldScout SC 900 penetrometer.



Figure 1. FieldScout SC 900 penetrometer

RESULTS AND DISCUSSIONS

Green manure influence on bulk density

In 2023, soil bulk density recorded lower values in all treatments following the incorporation of green manures compared with the control (1.37 g/cm³), indicating an improvement in soil physical condition. The measured values ranged between 1.27 and 1.32 g/cm³, corresponding to 92.8-96.2% of the control.

The greatest reduction in bulk density was observed in the mustard treatment (1.27 g/cm³; -0.10), followed by the mustard + rapeseed and rye treatments (1.29 g/cm³; -0.08). Rapeseed and autumn pea showed more moderate decreases of -0.06 and -0.05 g/cm³, respectively (Table 1).

Table 1. Green manure influence on bulk density, 2023

Green manure	Bulk density		Difference	Signif..
	(g/cm ³)	%		
Autumn pea	1.32	96.2	-0.05	o
Mustard	1.27	92.8	-0.10	ooo
Rapeseed	1.31	95.9	-0.06	o
Mustard + rapeseed	1.29	94.4	-0.08	oo
Rye	1.29	94.4	-0.08	oo
Control	1.37	100	-	Control
LSD 5% = 0.04; LSD 1% = 0.06; LSD 0.1% = 0.09				

The results highlight the beneficial effect of green manures, particularly mustard grown as a sole crop as well as in mixture with rapeseed, on reducing soil compaction and improving soil physical properties.

In 2024, soil bulk density was lower in all green manure treatments compared with the control (1.34 g/cm³), confirming their beneficial effect on soil physical condition. The recorded values ranged from 1.26 to 1.33 g/cm³, corresponding to 94.0-99.5% of the control.

The greatest reductions in bulk density were observed in the rye treatment (1.26 g/cm³; -0.08) and in the mustard + rapeseed mixture (1.27 g/cm³; -0.07), followed by mustard (1.29 g/cm³; -0.05). Rapeseed and autumn pea showed smaller decreases of -0.03 and -0.01 g/cm³, respectively (Table 2).

Statistical analysis indicates that the rye and mustard + rapeseed treatments showed distinctly significant differences compared with the control (LSD 5% = 0.05), whereas mustard had a weaker effect and the other treatments did not exceed the significance threshold.

Table 2. Green manure influence on bulk density, 2024

Green manure	Bulk density		Difference	Signif..
	(g/cm ³)	%		
Autumn pea	1.33	99.5	-0.1	-
Mustard	1.29	96.3	-0.05	o
Rapeseed	1.31	97.5	-0.03	-
Mustard + rapeseed	1.27	94.6	-0.07	oo
Rye	1.26	94.0	-0.08	oo
Control	1.34	100	-	Control
LSD 5%= 0.05; LSD 1%= 0.07; LSD 0.1%= 0.10				

The results confirm that green manures, particularly rye and the rapeseed–mustard mixture, contribute to reducing soil compaction; however, the magnitude of the effect was lower compared with the previous year, suggesting an influence of climatic conditions on the intensity of the soil response.

Green manure influence on penetration resistance

In 2023, soil penetration resistance (Tabel.3) varied depending on the type of green manure applied, compared with the control (31.56 daN/cm²). Most treatments led to a reduction in this parameter, indicating a decrease in soil compaction.

The greatest reduction in penetration resistance was recorded in the mustard treatment (26.11 daN/cm²; 82.7% of the control), with a difference of -5.45, exceeding the LSD 0.1% threshold (2.91), which indicates a highly significant difference. Rye also showed a substantial decrease (28.92 daN/cm²; -2.64), with a distinctly significant difference (p < 0.01). The autumn pea (30.14 daN/cm²; -1.43)

and mustard + rapeseed (30.13 daN/cm²; -1.44) treatments showed moderate reductions, close to the LSD 5% threshold (1.42), and were considered statistically significant.

In contrast, rapeseed showed a slight increase in penetration resistance (31.82 daN/cm²; +0.25), which was not statistically significant, indicating a limited effect on soil loosening.

Table 3. Green manure influence on penetration resistance, 2023

Green manure	Penetration resistance		Difference	Signif..
	(daN/cm ²)	%		
Autumn pea	30.14	95.6	-1.43	o
Mustard	26.11	82.7	-5.45	ooo
Rapeseed	31.82	100.8	0.25	-
Mustard + rapeseed	30.13	95.4	-1.44	o
Rye	28.92	91.6	-2.64	oo
Control	31.56	100	-	Control
LSD 5%= 1.42; LSD 1%= 2.01; LSD 0.1%= 2.91				

The results indicate that green manures contribute to reducing soil compaction, with the strongest effect observed for mustard and rye, which significantly improve soil physical properties.

Soil penetration resistance increased progressively along the 0-45 cm profile in all analyzed treatments (Figure 2), with minimum values in the surface layer (0-10 cm) and maximum values in the 25-35 cm zone. The control consistently showed the highest values, indicating a higher degree of compaction.

The application of green manures reduced penetration resistance throughout the soil profile, with the effect being more pronounced in the 20-35 cm interval, where the differences compared with the control were greatest. This zone corresponds to a compacted layer whose intensity was diminished in the presence of green manure crops. The lowest penetration resistance values were recorded in the mustard, rye, and mustard + rapeseed treatments, which showed a higher capacity to loosen the soil. In contrast, the differences among treatments decreased slightly in the deeper layer (35-45 cm), where the influence of the root system is more limited.

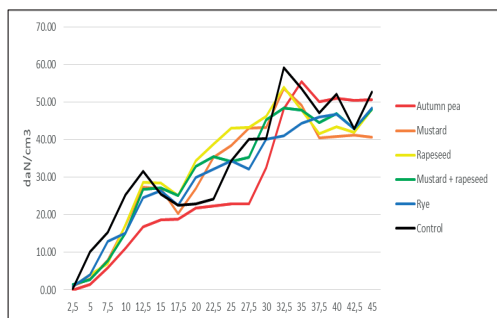


Figure 2. Soil penetration resistance along the 0-45 cm profile under the influence of green manures, 2023

In 2024, soil penetration resistance (Tabel 4) was reduced in all green manure treatments compared with the control (32.32 daN/cm²), indicating a clear improvement in soil physical condition and a decrease in compaction.

Table 4. Green manure influence on penetration resistance, 2024

Green manure	Penetration resistance		Difference	Signif..
	(daN/cm ³)	%		
Autumn pea	28.93	89.5	-3.39	ooo
Mustard	25.01	77.4	-7.31	ooo
Rapeseed	27.93	86.4	-4.39	ooo
Mustard + rapeseed	25.69	79.5	-6.63	ooo
Rye	25.30	78.3	-7.02	ooo
Control	32.32	100	-	Control
LSD 5%= 0.66; LSD 1%= 0.94; LSD 0.1%= 1.36				

The recorded values ranged from 25.01 to 28.93 daN/cm², corresponding to 77.4-89.5% of the control. The greatest reductions were observed in mustard (25.01 daN/cm²; -7.31), rye (25.30 daN/cm²; -7.02), and the mustard + rapeseed mixture (25.69 daN/cm²; -6.63). These differences significantly exceed the LSD 0.1% threshold (1.36) and are therefore considered highly significant.

Rapeseed (27.93 daN/cm²; -4.39) and autumn pea (28.93 daN/cm²; -3.39) also resulted in notable reductions, although of lower magnitude compared with the other treatments, yet still exceeding the statistical significance threshold. Statistical analysis shows that all green manure treatments had a highly significant effect on

reducing penetration resistance, confirming their effectiveness in improving soil structure.

The results demonstrate that green manures consistently contribute to reducing soil compaction, with a more pronounced effect in 2024 compared with the previous year, suggesting a favorable interaction between crop type and climatic conditions on the soil's physical response.

In 2024, soil penetration resistance increased gradually along the 0-45 cm profile in all analyzed treatments (Figure 3), with low values in the surface layer (0-10 cm) and maximum values in the 30-35 cm interval. The control consistently recorded the highest values, reaching a maximum of approximately 58-60 daN/cm², indicating a high degree of compaction.

The application of green manures led to a reduction in penetration resistance throughout the soil profile, with the differences compared with the control being most evident in the 20-40 cm layer. This zone corresponds to a compacted layer whose intensity was reduced in all treated variants.

The lowest penetration resistance values were observed in the mustard, rye, and mustard + rapeseed treatments, which limited the excessive increase in resistance in the critical zone of the profile. Rapeseed and autumn pea showed intermediate values, while still remaining below the control.

In the deeper layer (35-45 cm), resistance values remained high, but the differences among treatments decreased, suggesting a weaker influence of the root system at these depths.

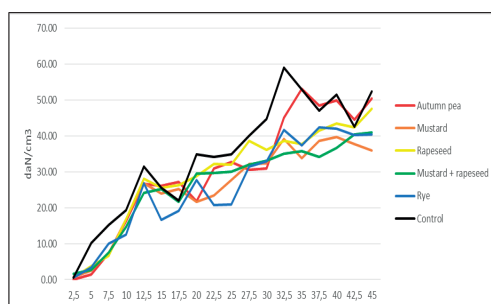


Figure 3. Soil penetration resistance along the 0-45 cm profile under the influence of green manures, 2024

The results highlight the effectiveness of green manures in reducing soil compaction across the entire analyzed profile, with a more pronounced effect in 2024, particularly in the zone of maximum compaction.

CONCLUSIONS

The results obtained over the two experimental years demonstrate that the incorporation of green manure crops led to a consistent improvement in soil physical properties under the pedo-climatic conditions of Brăila.

All green manure treatments contributed to a reduction in soil bulk density compared with the control, indicating improved soil structure and increased porosity. The most pronounced effects were observed for mustard, rye, and the mustard + rapeseed mixture, which showed a higher capacity to reduce soil compaction.

Soil penetration resistance was also significantly reduced in the presence of green manures, particularly in the 20-40 cm soil layer, where compaction was most evident. The strongest effects were recorded for mustard and rye, which consistently showed the lowest resistance values and the highest efficiency in loosening the soil.

The magnitude of the improvements varied between years, with more pronounced effects observed in 2024, suggesting that climatic conditions play an important role in modulating the response of soil physical properties to green manure application.

Overall, the results confirm that green manures represent an effective agronomic practice for reducing soil compaction and improving soil physical quality, with positive implications for root development, water infiltration, and the sustainability of cropping systems.

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HUMIC FERTILIZATION: SOIL REGENERATIVE CONSIDERATIONS

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Abstract

Based on a synthetic analysis of our own and other research, the opportunity to use humic fertilization in order to stop the degradative trend characteristic of arable chernozems and regenerate the chernozem process is argued. Through the perspective of the biophysical concept of the chernozem process of pedogenesis in the present research, the regeneration of the humification process, which constitutes the essence of the chernozem process of solification, is examined based on the direct and indirect action of humic fertilizers on the functionality of the "plant-microbiome" system in the rhizosphere. It has been established that the use of humic fertilizers, as biologically and physiologically active substances, contributes to the biostimulation of the "plant-microbiome" system and the catalysis of the humification processes that occur in the rhizosphere under their interconnected action. The evaluation of the pedoregenerative efficiency based on the cellulolytic activity, the microbial biomass content, the fractional composition of humus and the agrochemical fertility indices highlighted the intensification of the cellulolytic activity in the humuso-accumulative horizon by 10-14%, the increase by 3-12% of the microbial biomass in the 0-40 cm layer, manifested in the intensification of the humification process. It was established that the regeneration of the humification process involves two distinct stages: a) primary humification (the active biological period April-June) with the formation of "young" humic substances of easily mineralizable humic nature and b) secondary humification with the formation and stabilization of stable humic substances of humic nature. The regeneration of the humification process led to the optimization of the soil nutrient regime.

Key words: fertilization, chernozem pedogenesis, humificatic fertilization, rhizosphere, plant-microbiome system, soil regenerative processes.

INTRODUCTION

The synthetic analysis of the effects induced by the current technologies promoted, including by FAO, for the purpose of regenerating typogenetic processes in arable chernozems has shown that they can only ensure a slight restoration of humus reserves (about 10% of the lost quantities) and of the structural aggregate state related to the humification process (Jigău et al., 2022). In this context, we consider that the technologies practiced, although they have sufficient bioenergetic potential for the initiation and unidirectional development of chernozem typogenetic processes, ensure only partial achievement of this due to the advanced degree of physical degradation (Jigău, 2023). Through this prism of ideas, we consider that in the arable chernozems of the region, in a relatively short period of time (about 100 years), very profound changes have occurred in their physical state, which have entailed such significant changes in the soil biota that it is no longer able to support the humification of fresh organic matter and the

accumulation of biophilic elements in the arable horizon. This implies the conclusion that within the natural chain of substance transformations, some links of it, for which certain groups of living organisms are responsible, have formed "weak spots" that limit the intensity of pedogenetic/typogenetic processes (Larionov, 2013). In this regard, research has shown that in the arable layer of arable chernozems, the number and mass of microorganisms increases and the number and mass of pedomesofauna significantly reduces (Larionov et al., 2013). As a result, in arable chernozems, the processes of transformation of plant residues are intensified and the mineralization energy increases approximately twice.

This leads to the disruption of the natural balance between the mineralization and humification processes characteristic of natural ecosystems, manifested in the increase in the share of mineralization processes (Kokovina, 1988). This being the main factor that led to the direction of the evolutionary trend of chernozems in a degradative sense (Jigău et al.,

2023). As a result, the intensification of the degree of suppression of some components of the pedocenosis led to the transfer of the soil ecosystem to a lower ecological-energetic level within which pedogenetic processes are carried out in narrower intervals of the values of physical and chemical parameters. This entails an increase in the vulnerability of arable chernozems to climate change (drought, excessive temperatures) and a unidirectional reduction in the self-reproduction process of chernozem pedogenesis (Jigău & Lesanu, 2021). In addition, the regeneration of the pedogenetic process within such technologies requires a long period of time. At the same time, both in our research and in other research, it has been established that the practice of technologies based on the use of liquid humic substances as biostimulators of processes occurring in the soil-plant system and as catalysts of the humification process contributes to the acceleration of biological and biochemical processes in arable chernozems and the regeneration of the pedogenetic process.

Conceptual-methodological considerations

In the literature, humus-based processes are considered as carbon-rich organic amendments in the form of humic substances extracted from manure, compost, crude and extracted forms of lignite, coal and peat (Lumactud et al., 2022). Humic substances are widely used with a market value exceeding 532.7 million dollars in 2021 and a projected growth rate of approximately 11.8% between 2022 and 2028 (Pulidindi & Prakash, 2021). In addition, for several decades, humic substances have been widely reported to improve soil health and crop productivity (Guo et al., 2019; Nardi et al., 2021). At the same time, numerous studies have demonstrated positive responses of soils and crops to humic substances from various sources in protected areas, open field and controlled environment studies (Rose et al., 2014; Yang et al., 2021; Bezuglova & Klimenko 2022). Ampong et al., recently provided a comprehensive analysis of the significant positive impact of humic acids on crop plant growth, yields and soil physical and chemical properties. Plants are more stress tolerant, more productive, healthier and produce better quality crops in soils with a high content of humic acids (Ampong et al., 2022).

Despite the above, the concept of humic fertilization, to date, faces a number of ambiguities. In this chapter, we mention, in particular, that, in most cases, in the literature in the field, the action of humic substances on the soil is confused with the action of humic substances formed within the continuum process of humification of fresh organic matter in the soil. In this context, in our research, humic substances used in modern agriculture are divided into two groups: a) humic fertilizers and b) humic fertilizers.

Within these, humic fertilizers are considered to be substances that represent the solid fraction of humic nature obtained within an accelerated humification technology of an organomineral substrate, made up of various zootechnical and vegetable wastes, with additions of industrial wastes from the sugar and thermoelectric industries and nitrogen-fixing cyanophyte algae *Chlorella* based on the natural humification model. Humic fertilizers are considered liquid humic substances resulting from accelerated humification within the same technology separated from the solid fraction by periodic decantation without the use of alkaline materials for extraction. In this paper, the principles of using humic fertilizers are fundamental and the effects induced by them on the functioning of the soil-plant system and the processes that occur in the rhizosphere are evaluated.

From the perspective of the biophysical concept of the pedogenetic process, their use is based on their action, as biologically and physiologically active substances that involve biostimulation of the processes that occur in the "plant-microbiome" system and catalysis of the processes that occur in the rhizosphere under their interconnected action.

Through this prism of ideas, the theoretical conceptual framework of humic fertilization is provided by the concept of the unity of the "soil-plant" system (Popov, 2012; Zelenskaya & Kerzhentsev, 2013).

The structural-functional unity of the plant and the soil within the ecosystem is based on metabolism, which includes the cyclical alternation of two processes: the synthesis of organic matter through photosynthesis with the assimilation of mineral elements from the soil by autotrophic organisms and their destruction by heterotrophs. In this sense, the soil, as a

component of the ecosystem, performs a very specific function within it and has its own unique mechanism for such a function, which is part of the mechanism of functioning of the ecosystem. Through this prism of ideas, the ecosystem is a symbiotic unit of biocenoses, specialized in types of nutrition (phytocenosis, zoocenosis, pedocenosis) integrated through a common closed metabolic circuit. Within it, the soil is considered a functional component of the ecosystem-saprotrophic biological community (pedocenosis) that functions synchronously with the phytocenosis, providing it with the necessary conditions (corresponding to landscape conditions) for the production of biomass. In response, plants return organic matter to the soil, ensuring the unidirectional implementation of the pedogenetic process (Zelenskaya & Kerzhentsev, 2013). In this sense, plants and soils form a unique trophic system and the basic functional mechanism of the "soil-plant" system involves quantitatively and qualitatively balanced reciprocal bilateral relationships between soil and plants, within which the basic functional link is the soil organic matter (Popov, 2012).

According to more recent research, the mechanisms of action of humic substances through plant-microbiome associations on pedogenetic processes that occur in the active root layer can be divided into two conventional groups: a) direct - direct stimulation of plant development and growth through the synthesis of growth stimulants (phytohormones and other metabolites) and improvement of plant nutrition; b) indirect stimulation of plant development and growth by suppressing the development of microorganisms (microscopic fungi and bacteria, phytopathogens) in the active root layer (Rajkumar et al., 2009; Fahad et al., 2014).

Direct stimulation of plant development and growth

The formation of phytohormones, vitamins and other biologically active substances by bacteria is an extremely important mechanism of interactions within bacterial-plant associations. It is known that plant development and growth is regulated by phytohormones, especially auxins, cytokinins, gibberellins, etc. Auxins stimulate the development of the root system, regulate organ differentiation, etc. Cytokinins induce cell division, increase seed germination, have a

beneficial effect on plants in unfavorable conditions for their growth and development (salt content in quantities exceeding the toxic threshold, pesticides, negative temperatures, drought, heat waves, etc.). Gibberellins are synthesized, predominantly, in leaves and systematize plant growth, accelerate seed germination.

The production of phytohormones is characteristic of practically all rhizobacteria, providing them with conditions for their own development as well as the establishment of relationships with plants and other microorganisms in the soil (Morgun et al., 2009; Shaposhnikov et al., 2011; Lankina et al., 2013; Afzal et al., 2014).

The intensification of humification processes under the catalytic action of humic fertilizers is accompanied by the formation of physiologically active humic substances that are characterized by a structural composition similar to natural phytohormones in the process of lignin transformation. In this regard, through the concept of staged humification of organic matter (Александрова, 1980; Komarov et al., 2017).

The sustainability of the functioning of the [plant]↔[microbiome] system supports the humification process by intensifying the biological nitrogen cycle. In this context, it is considered that rhizobacteria compensate for the losses of photosynthesis products (3-50%) consumed in the formation of root exudates and rhizodeposits (Umarov et al., 2007; Umarov, 1986).

According to the authors cited in the rhizosphere, in any pedoclimatic conditions, in the soil, processes of associative nitrogen fixation are carried out with the participation of several genera of *Arospirillum*, *Azotobacter*, *Acetobacter*, *Enterobacter*, *Pseudomonas*, *Bacillus*, *Clostridium*, etc. which form with plants integrated multicomponent systems with new features determined by the interactions between "partners" (plant-microorganisms) (Bukharin et al., 2007). At the same time, it has been established that the process of photosynthesis and nitrogen fixation are functionally interconnected (Isobe et al., 2014). In this regard, it has been established that the beneficial action of associative nitrogen-fixing rhizosphere bacteria on plants is based on a complex of interacting properties: nitrogen

fixation, secretion of physiologically active substances that stimulate growth, antagonism for phytopathogenic fungi and bacteria (Klopper et al., 1989).

According to more recent research, about 80-90% of soil bacteria have the ability to fix nitrogen (Turner et al., 2013). At the same time, it has been established that mixed populations of microorganisms develop in the rhizosphere, in which, along with nitrogen-fixing species, pectin- and cellulolytic species develop, forming an integral polycomponent system in which nitrogen-fixing species use (products from the decomposition of polymers, providing them with the nitrogen necessary for their decomposition. As a result, the interactive realization of the intercalated decomposition process of organic polymers (source of organic carbon) and that of nitrogen fixation (source of biological nitrogen) materialized in the intensification of the humification process is intensified. The continuous formation of fresh humic substances contributes to the intensification of the structural aggregation process by ensuring an optimal framework for the functioning of the soil-plant-biota-soil system. In this context, we mention that research has shown that during vegetation, as plants develop, the composition of root exudates and rhizodeposits changes, which is reflected in the formation of bacterial associations (Dobrovol'skaja et al., 2002).

At the same time, the amount of microorganisms also changes - the rhizosphere effect increases starting from the "seedling" phase to the maximum in the flowering and fruit formation phase (primary humification stage) in the subsequent phases (secondary humification stage) the rhizosphere effect decreases.

At the same time, the distribution of rhizobacteria in the rhizosphere is not homogeneous: in larger quantities they are contained in the econiches of young roots where maximum quantities of soluble root exudates are secreted. In the spaces related to mature roots, hydrolytic bacteria have a greater development (Dobrovol'skaja, et al., 2002). This implies the conclusion that under conditions of permanent plant coverage in the rhizosphere, humification processes materialized in aggregation (sequestration of organic carbon) and structuring (stabilization of organic carbon) are

constantly taking place. Through this prism of ideas, the interdependent and interdetermined development of the process of formation-accumulation of humus and of aggregation-structuring represents a continuum of rejuvenation of the humus state and the structural-aggregate state, materialized in the constitution and functioning of the genetic-evolutionary system [humic system]↔[aggregate system] responsible for the reproduction of the chernozem process of solification.

Humification is also favored by the intensive mobilization in the rhizosphere of phosphorus but also of other biophilic elements related to the organic carbon cycle as well as the humus formation process.

In this sense, we consider that the efficiency of humification depends not only on the amount of fresh organic matter in the soil but also on the content of other biophilic elements (about 32 elements), especially the C:N:P:S ratio. In the case of chernozems, phosphorus is one of the factors that limits the efficiency of the humification process.

The microflora of humic preparations influences the phosphorus cycle by modifying the absorption of phosphorus by plants: 1) providing a source of organic phosphorus; 2) intensification of the microbial activity of the soil biota that solubilizes phosphorus; 3) local increase in soil acidity in the rhizosphere as a result of the intensification of the transformation process of organic acids (malic, acetic, citric, etc.) and humic acids (which have pH values < 3.8); 4) increased availability in the soil through the formation of insoluble organo-mineral compounds with metal ions (Al^{3+} , Fe^{3+} , Ca^{2+} , Mg^{2+} , etc.) (Jindo et al., 2020).

Indirect stimulation of the functioning of the "plant-microbiome" system involves the ability of plant growth-stimulating bacteria to suppress phytopathogenic microorganisms - microscopic fungi and bacteria (Chowdhury et al., 2015). Growth-stimulating bacteria suppress the growth and development of pedophytopathogens, thus minimizing their impact and contributing to the normal growth and development of plants. At the same time, they contribute to the aggregation-structuring of the soil mass and the optimization of the pedofunctional environment.

MATERIALS AND METHODS

The research was carried out under production conditions on typical moderately humus-rich clayey-loamy chernozems using the NEOFERT-M humic fertilizer of local origin obtained within a technology based on the natural model of controlled composting-humification of an organomineral substrate made up of various wastes of zootechnical, vegetable, industrial origin (from the sugar, bakery, thermoelectric industries) with additions of green algae (*Chlorella vulgaris*). The technology practiced involves treating the

substrate with a NEOFERT-M+*Chlorella* fertilizer mixture, composting it on the soil surface (4-6 weeks), and subsequently incorporating it into the humus-accumulative horizon by alternating the humification depth over time. During the vegetation period, 3 treatments with the NEOFERT-M+*Chlorella* mixture were carried out in the key phases of crop development.

The soil regenerative efficiency was assessed based on agrochemical fertility indices, fractional composition of humus, microbial biomass content and cellulolytic activity.

Table 1. Dynamics of agrochemical fertility indices of moderately humiferous chernozems under conditions of composting-humification of organic matter in the upper soil horizon (layer 0-30 cm)

Date of soil sample collection	Culture	Agrochemical fertility indices				
		pH	Humus, %	mg/100 g of soil		
				N-NO ₃	P ₂ O ₅	K ₂ O
13.06.25	Control	7.40	3.38	0.63	2.39	25.16
	Sunflower	7.21	3.84	0.94	4.89	26.68
	Winter Peas	7.21	3.89	0.77	4.28	21.38
	Corn	7.18	3.92	0.97	4.43	24.44
10.09.25	Control	7.52	3.24	0.58	2.47	24.31
	Sunflower	6.53	4.32	0.67	3.88	18.74
	Winter Peas	6.39	4.21	1.15	3.78	26.27
	Corn	6.29	4.21	1.15	6.22	22.12

In the initial phase, the soils in the experimental plots are characterized as weakly basic (pH = 7.47-7.53) and differ insignificantly from the control variant (pH = 7.48). During the active biology period of the following year (13.06.2025) the pH values are reduced insignificantly, presenting values within the neutral range, detaching insignificantly from those characteristic of the control variant (Table 1). During the second half of the vegetation period (June-September) the pH values in the soils in the experimental plots are reduced and fall within the weakly-acid range (pH < 6.50). At the same time, in the control variant, the soil reaction is weakly basic (pH = 7.52). This allows us to consider that the dynamics of the soil reaction is determined by organic acids (malic, citric, acetic, etc.) and humic (humitic and fulvic) originating from the humification process. Indispensable for these processes is also the increase in the content of N-NO₃

contained in the newly formed humic substances. Acidification of the soil solution contributed to the establishment in the soils of a stable trend of increasing the content of mobile phosphorus. At the beginning of the 2025 vegetation period, its content exceeded the content in the control variant by about 1.8-2.0 times. At the end of the vegetation period, although part of the phosphorus was used in the formation of crops, its content was about 1.5-2.5 times higher than in the control variant. The potassium content remains relatively stable, establishing itself in the range of relatively optimal values.

The evaluation of the action of humic fertilization on the soil microbiome based on microbial biomass shows that, already in the first year of fertilizer application, it contributes to an increase in its quantity compared to the control and to the relative stabilization of biomass during the vegetation period (Table 2).

Table 2. Microbial biomass of typical chernozem under controlled composting-humification technology conditions during the active biology period (13.06.2025)

Culture	Depth, Cm	Microbial biomass (BM) mg C/g soil	C _{BM} % of C _{total}	BM reserves kg/ha	
				in layers	in layers 0-40 cm
Control	0-30	170.2	1.01	1665.3	1884
	30-40	163.5	0.60	209.0	
Sunflower	0-30	270.7	1.17	1884.1	2254.8
	30-40	168.5	0.63	370.7	
Winter peas	0-30	269.4	1.18	1503.3	1940
	30-40	234.9	1.03	436.9	
Corn	0-30	227	1.89	1539.7	2059.8
	30-40	224.2	1.87	520.1	

At the same time, a dependence of the amount of microbial biomass on the cultivated crop is clearly outlined.

In this sense, it is known that all crops have a direct impact on microbial biomass.

This action is determined by their ability to secrete root exudates that favor the formation of an optimal environment for the functioning of the "plant-microbiome" system.

In this sense, the action of foliar humic fertilization is achieved by intensifying the development processes of the root system on which the amount of exudates and their composition depend, indispensably. Through this prism of ideas, we find that among the crops cultivated in the experimental fields, sunflower and corn, due to their strongly developed root system throughout the thickness of the 0-40 cm layer, most significantly influence the amount of biomass produced in the soil during the vegetation period, this being reproduced continuously throughout the entire period of active functioning of the "plant-microbiome" system.

Pea culture produces a smaller amount of biomass, at the same time, but it is more qualitative since it is richer in biological nitrogen that contributes to the formation of more qualitative humic substances.

According to our previous research, foliar fertilization with humic substances is a factor that catalyzes the meaning and intensity of the humification process, being at the same time a carrier of the "genetic code of humic humification".

Monitoring the fractional composition of soil humus within the experimental plots showed that the humification process under controlled humification conditions under the action of humic fertilizers involves the achievement of the

stages of humification characteristic of uncultivated chernozems.

In this sense, the present research has shown that within the controlled humification process under the action of humic fertilization, two stages characteristic of the natural model of chernozem humification are clearly outlined: a) primary humification (April-June) characterized by the intensive development of the humification process with the formation of humic substances, predominantly of a "young" humic nature with a lower molecular mass manifested in the values of the Cah:Caf ratio >2 ; b) secondary humification that involves the polymerization condensation of newly formed humic substances and the stabilization of "mature" humic substances materialized in Cah: Caf values of about 2.

Primary humification is favored by the favorable temperature and humidity regime + characteristic of the April-June period.

The data in (Table 3) show that primary humification is influenced by the cultivated crop. More favorable conditions are provided by the autumn pea crop, which ensures the formation of a quantity of humic acids that exceeds by about 2.59 times the quantity of fulvic acids. In our opinion, this is due to the enrichment of plant biomass, especially root biomass, with biological nitrogen.

The formation of humic substances also occurs intensively in the case of corn, which, in our opinion, is due to higher amounts of carbohydrates (polysaccharides) in the composition of the exudates of this crop.

Secondary humification is favored by the hydrothermal regime specific to the dry period (July-August) which contributes to the dehydration of humic substances manifested in the polymerization-condensation of molecules

of "young" humic substances with the formation of stable substances with high molecular weight. Probably, the denitrogenation of humic substances also contributes to the realization of this process. The values of the Cah:Caf ratio at the end of the vegetation period testify in favor of this statement. From the data in (Table 3) we find

that its values are significantly reduced and make up 1.91 in the case of spring pea crop (reduction by 0.68) and 2.0 in the case of corn crop (reduction by 0.40). On the other hand, in the case of sunflower crop, the Cah:Caf values do not change, which allows us to assume that in its case more stable humic substances are formed from the start.

Table 3. Fractional composition of humus in the arable layer of typical moderately humiferous chernozem under controlled composting-humification technology in the upper part of the profile (layer 0-30 cm)

Date of soil sample collection	Culture	Humus content, %	Total carbon content (C _t), %	Carbon content, esterified with pyrophosphate+NaOH, %	Humic acid carbon content, %	Fulvic acid carbon content, %	Hydrolyzed carbon content, %	Cah: Caf
13.06.25	Control	3.36	1.95	<u>1.06</u> 54.35	<u>0.68</u> 34.87	<u>0.38</u> 19.49	<u>0.85</u> 51.64	1.78
	Sunflower	3.98	2.20	<u>1.36</u> 59.38	<u>0.91</u> 39.73	<u>1.45</u> 19.65	<u>0.93</u> 40.62	2.02
	Winter Peas	3.84	2.21	<u>1.22</u> 55.20	<u>0.88</u> 39.82	<u>0.34</u> 15.36	<u>0.99</u> 44.20	2.59
	Corn	2.06	1.18	<u>0.68</u> 57.62	<u>0.48</u> 40.68	<u>0.20</u> 16.95	<u>0.50</u> 43.68	2.40
10.09.25	Control	3.26	1.90	<u>1.00</u> 52.63	<u>0.61</u> 32.11	<u>0.39</u> 20.53	<u>0.90</u> 47.37	1.57
	Sunflower	4.21	2.42	<u>1.27</u> 52.48	<u>0.85</u> 35.12	<u>0.42</u> 17.35	<u>1.15</u> 47.51	2.02
	Winter Peas	4.32	2.48	<u>1.26</u> 50.80	<u>0.82</u> 33.03	<u>0.43</u> 17.39	<u>1.22</u> 49.39	1.91
	Corn	2.34	1.34	<u>0.71</u> 52.99	<u>0.62</u> 46.21	<u>0.31</u> 23.13	<u>1.00</u> 47.01	2.00

The administration of humic fertilizer contributes to the intensification of cellulose decomposition throughout the entire thickness of the humus-accumulative horizon by 10-14%, thus contributing to the intensification of the humification process (Table 4).

Table 4. Cellulolytic activity % of typical moderately humic clayey-loamy chernozems under controlled composting-humification technology conditions (13.08.2025)

Culture	Depth, cm	Variants	
		Control	Experimental
Sunflower	0-15	59.7	70.1
	15-25	67.1	70.3
Winter Peas	0-15	64.1	70.2
	15-25	59.6	71.0
Corn	0-15	62.2	71.7
	15-25	62.2	70.5

CONCLUSIONS

The unidirectional degradative trend of arable chernozems implies a specific approach to the regeneration of the chernozem soil formation process within the framework of controlled

humification technology by practicing humic fertilizers as biologically and physiologically active substances for the purpose of biostimulation and catalysis of processes that occur in the rhizosphere under the action of the interconnected functioning of the "plant-microbiome" system.

The action of humic fertilizers on processes through biostimulation of the "plant-microbiome" system depends on the cultivated crop: As a result, depending on the crop, cellulolytic activity increases by 10-14% and microbial biomass by a minimum of 3% (spring peas) and a maximum of 12% (sunflower). As a result, the intensification of the controlled humification process is observed in the upper soil horizon.

In the regeneration of the controlled humification process, two stages are clearly distinguished: a) primary humification (biologically active period April-June) with the formation of humic substances of "young" humic nature and low molecular weight and b) secondary humification with the formation and stabilization of humic substances.

The regeneration of the humification process led to an increase in the humus content in the soil, the optimization and balancing of the nutrient regime (NPK). At the same time, the reduction of pH values determined by the formation of organic acids (malic, citric, acetic) and humic as a result of the controlled humification process is attested.

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ANALYSIS OF THE EVOLUTION OF SOIL FERTILITY UNDER THE INFLUENCE OF DIFFERENT TYPES OF WORKS, IN THE PEDOCLIMATIC CONDITIONS OF THE CENTRAL, SOUTHERN AND SOUTH-EASTERN REGIONS OF ROMANIA

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Abstract

This paper presents the results of research conducted by INMA Bucharest, within a complex project funded by the National Recovery and Resilience Plan, in support of the EU Mission – “A Soil Deal for Europe”. The sustainable soil cultivation technology adapted to the pedoclimatic conditions in the Central, Southern and South-Eastern regions of Romania was developed for the purpose of remediation and restoration of degraded lands. The technology involved performing the following types of soil work: basic work with a plough with bodies equipped with ploughshares to break up the hardpan, in the Central region; scarification and incorporation of biofertilizers into the arable substrate in the South-East region and soil cultivation in narrow strips for sowing of weedy plants with a strip-till type equipment, in the South region. Soil samples were taken before and after applying different soil tillage options and their impact on the soil was evaluated by analyzing the following parameters: macroelements (Ca, Mg, Na, K); chemical forms of nutrients (water-soluble, exchangeable, total); other parameters (Cl^- , SO_4^{2-} , NO_2^- , NO_3^- , NH_4^+ , pH).

Key words: macroelements, mineral nitrogen, region, soil fertility.

INTRODUCTION

Soil fertility is one of the most important factors that influence grains yields and the crop quality. Nowadays, soil degradation is a global phenomenon caused by natural land erosion, human activities and climate change. Soil fertility can be characterized by a series of agrochemical indicators: soil reaction, ion exchange capacity, degree of base saturation, nutrient content.

Of particular importance is cationic adsorption or physico-chemical retention (non-specific sorption or cation exchange), which represents the capacity of cations in the diffuse layer of soil colloids to be exchanged under equivalence conditions by cations in the soil solution. The cations of Ca, Mg, K, Na, NH_4 , etc. are adsorbed and protected from leaching. From the complex, the cations gradually pass into the soil solution according to the laws of equivalent proportions, equilibrium, reversibility and retention energy (Jitareanu et al., 2020).

Agriculture methods include monoculture, synthetic inputs, bare soil, excess of tillage, due to soil degraded, unhealthy plants, animals and humans. All these methods have a negative

impact on soil properties, compaction, crusted, hardpan, excess of synthetic inputs leaching in ground water, beneficial fungi and bacteria are suppressed (Hirt, 2020). Soil structure depends on the balance between calcium, magnesium and sodium. Calcium is opening the soil, makes it more friable, while magnesium and sodium close it, creating hardness that restricts, water, air and roots penetration. Carbon and nitrogen are the basic elements that provide food source for soil life (Wuddivira & Camps-Roach, 2007). New environmentally friendly farming technologies are not possible without new tillage techniques, which also require higher soil and environmental protection requirements, the most important of which are to not deplete the soil, stop the decline of humus and soil degradation, reduce the leaching of nutrients and the most fertile soil particles, protect the soil from erosion and the breakdown of its structure, and to promote natural biological processes in the soil (Romanekas, 2022).

Few years ago, INMA Bucharest, started a new strategy in developing new tillage implements and drilling machinery that are doing more operation on one pass to reduce fuel

consumption, soil compaction and increase soil fertility (Popescu et al., 2022).

In this context, the partners within the CeSoh complex project funded by the PNRR (National Recovery and Resilience Plan) in support of the EU Mission – “A Soil Deal for Europe” have developed a sustainable soil cultivation technology adapted to the pedoclimatic conditions in the Central, Southern and South-Eastern regions of Romania, for remediation and restoration of degraded lands (www.cesoh.ro).

MATERIALS AND METHODS

The study was conducted in four experimental sites representative of three development regions (Center, South and South-East) and pedoclimatic conditions in Romania, during the period 2023-2025. The locations were selected to cover a diversity of soil types and climatic regimes, allowing for the comparative assessment of the efficiency of innovative sustainable technologies in contrasting agroecological contexts.

The sites were located in: Dumbrăvioara (Mureș County), Gologanu (Vrancea County), Dracea (Teleorman County) and Murfatlar (Constanța County). For each location, the general soil characteristics, the history of agricultural use and the specific climatic conditions were taken into account.

The intervention carried out in the experimental site in Dumbrăvioara (Central Region) aimed to improve the physical properties of the soil affected by compaction in the sub-arable horizon, by applying a plowing technology with deep loosening and breaking of the compacted layer (hardpan). The technology aimed to improve the aerohydric regime of the soil and create favorable conditions for the development of the root system of agricultural crops. The work was carried out with a reversible plough, with bodies equipped with ploughshares (Vlăduțoiu et al., 2025a; 2005b).

The configuration of the machine and the way it works in the field are shown in Figure 1. The plow cuts, lifts, crumbles and turns the furrow, and the diggers penetrate 50-100 mm below the furrow level, fracturing the compacted layer. The hydraulic reversal allows continuous plowing, without the formation of furrows or ridges.



Figure 1. Application of basic soil tillage technology with reversible plough with bodies equipped with ploughshares, in the Dumbrăvioara experimental site (Mureș County)

The intervention at the Gologanu experimental site (South-East Region) aimed at the biological regeneration of degraded soil and the increase in chemical fertility, through the controlled application of a liquid biofertilizer formulated based on beneficial soil microorganisms.

The technology aimed to stimulate microbiological activity and improve structural aggregation under conditions of seasonal water deficit. For the implementation of the technology, innovative technical equipment was used for soil processing and inoculation of biofertilizer into the arable layer, developed within the Healthysoil Project (Marin et al., 2023; Marin & Manea, 2024). The general configuration of the machine is shown in Figure 2.



Figure 2. Application of technology with innovative technical equipment for soil processing and biofertilizer inoculation in the Gologanu experimental site (Vrancea County)

The technology allowed for the implementation, in a single pass, of deep loosening (up to 55 cm), fragmentation and leveling of the soil, simultaneously with the application of biofertilizer in the superficial layer (10-20 cm).

The controlled application of the biological solution was carried out directly in the mobilized area, to favor the contact of microorganisms with the soil matrix.

A biofertilizer produced by INMA Bucharest was used through a controlled process, whereby beneficial microorganisms and soluble organic compounds from mature, stabilized compost obtained from plant raw materials were transferred and multiplied into a liquid solution. The biofertilizer was filtered to remove coarse particles and prevent clogging of the application equipment.

The equipment was aggregated with a 220-240 HP tractor, with a 500 l tank for biofertilizer and a recommended working speed of approximately 10 km/h.

The intervention carried out in the experimental site Dracea (South Region) aimed to conserve water in the soil and prepare the seedbed for pea crops in a localized manner, under conditions of poor rainfall and increased frequency of soil drought episodes. The strip-till technology allowed soil mobilization only on the sowing strips, keeping the rest of the surface covered with plant debris. The equipment configuration is shown in Figure 3.



Figure 3. Application of technology with narrow strip tillage equipment, in the Dracea experimental site (Teleorman County)

Each working section is made up of notched discs for stubble removal, a reversible chisel knife for localized loosening and spherical discs for reforming the seedbed. The work process included uncovering a strip approximately 20 cm wide, indenting and localized loosening of the soil, forming the sowing channel and

reforming the working strip, ensuring a uniform germination bed and optimal soil-seed contact.

The equipment was aggregated with an 80 HP tractor, and the tests were carried out on an experimental plot of 1 ha, under conditions of average soil moisture of 20.2% and penetration resistance between 702 and 1720 kPa. The working depth was maintained constant by the deformable parallelogram system and adjustment springs.

The intervention carried out in the Murfatlar experimental site (South-East Region), aimed at reducing the pressure of weeds, cryptogamic diseases and pests specific to viticulture, by applying biological solutions based on the use of beneficial microorganisms and biocontrol agents. The technology aimed to limit the use of synthetic phytosanitary products and maintain the biological balance of the viticultural agroecosystem, in the context of increasing climatic stress and the increased sensitivity of plantations to pathogens.

To apply the technology, a portable spraying equipment was used for phytosanitary treatments in vineyard plantations, equipped with nozzles with a fine spray spectrum and a pressure regulation system.

The intervention consisted of the repeated application, during the vegetation period, of biological treatments, adapted to the phenophases of the vine and the phytosanitary pressure observed in the field. The applications were made locally on the vine rows, respecting the doses recommended by the producers of biological solutions and alternating biocontrol products, to avoid the emergence of biological resistance. Weed control was carried out primarily through preventive and biological methods, correlated with superficial mechanical work between the rows, to limit water and nutrient competition without the use of chemical herbicides.

Experimental design and sampling scheme

To assess the impact of innovative sustainable technologies on soil fertility, a "before-after" experimental design was applied, with soil samples taken before the implementation of the interventions and after their application. In each experimental site, representative areas were delimited, homogeneous in terms of soil and agricultural use history. Soil samples were collected from two depths:

- 0-20 cm - the arable layer, of major importance for plant nutrition;
- 20-40 cm - the sub-arable layer, relevant for the assessment of leaching and nutrient mobility processes.

Composite samples were taken in each plot, consisting of 5 subsamples randomly collected from the study area, using the zigzag method, to reduce spatial variability.

Sampling points were marked and georeferenced using the Garmin GPSMAP 64s portable device, coordinates were recorded and archived to ensure traceability and comparability between sampling campaigns.

Sampling was carried out with soil probes and manual drills, respecting national and European standards regarding agricultural soil sampling.

The samples were uniformly labeled (location code, depth, sampling time, date), packaged in clean, chemically inert containers and transported under conditions that prevent contamination and modification of chemical properties.

Within the Chemistry Laboratory of the National Institute for Research and Development for Cryogenic and Isotopic Technologies I.C.S.I. Râmnicu Vâlcea, the chemical properties of the soil were determined before the application of the technologies, as well as after the implementation of the technologies proposed in the project.

The analyses targeted the following parameters: soluble and exchangeable minerals (Ca, Mg, Na, K), anion (Cl^- , SO_4^{2-}), mineral forms of nitrogen (N-NO_2 , N-NO_3 , N-NH_4^+) and soil pH.

The analytical data were organized in databases structured by: locations; soil depths; sampling time (before/after intervention); type of technology applied. The analysis included: comparison of "before-after" values; descriptive analysis (means, absolute and relative differences); interpretation of variations in relation to local pedoclimatic conditions; comparative analysis between technologies, to highlight solutions with agronomic efficiency and favorable ecological impact.

RESULTS AND DISCUSSIONS

Analysis of the evolution of water-soluble elements (Ca, Mg, Na)

The evolution of water-soluble elements (Ca, Mg, Na) represents an essential indicator of the

dynamics of nutrients in the soil solution and of the impact of applied technologies on ionic balance and fertility. Monitoring these forms allows the evaluation of mobilization, leaching and vertical redistribution processes, as well as the appreciation of the sustainability of agronomic interventions.

The evolution of water-soluble elements was evaluated by calculating the absolute difference between the values determined after applying the technologies and the initial ones.

To calculate the absolute difference (ΔCa , ΔMg , ΔNa), formula (1) was used.

$$\Delta X = X_{\text{after}} - X_{\text{before}} \quad (1)$$

where $X = \text{Ca, Mg or Na}$, in mg/kg.

Interpretation:

- $\Delta\text{Ca}, \Delta\text{Mg}, \Delta\text{Na} > 0 \rightarrow$ increase in the soluble fraction (increased mobilization/solubilization);
- $\Delta\text{Ca}, \Delta\text{Mg}, \Delta\text{Na} < 0 \rightarrow$ decrease in the soluble fraction (consumption, leaching or immobilization);
- $\Delta\text{Ca}, \Delta\text{Mg}, \Delta\text{Na} \approx 0 \rightarrow$ relative chemical stability.

Figures 4 and 5 graphically present the evolution of water-soluble elements (Ca, Mg, Na), which show significant differences between the two analyzed depths.

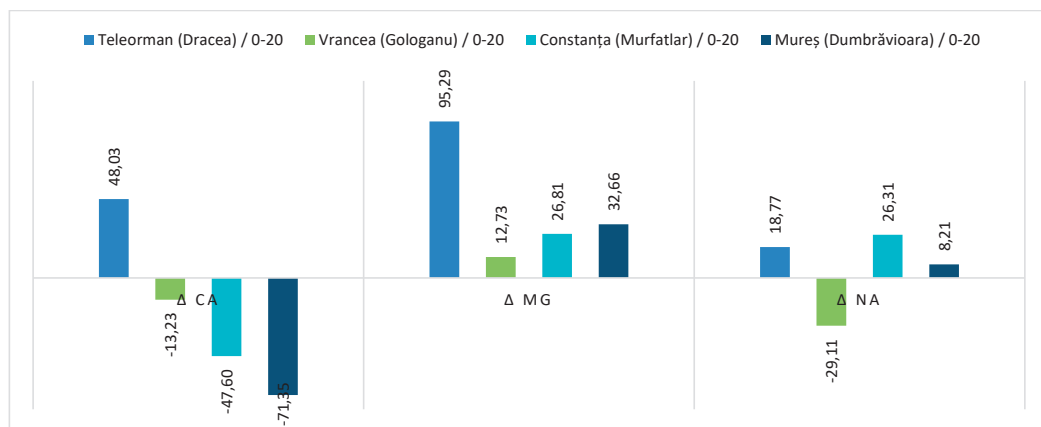
In Teleorman County (Dracea), in the 0-20 cm layer, significant increases are recorded for all the analyzed elements ($\Delta\text{Ca} = +48.03$; $\Delta\text{Mg} = +95.29$; $\Delta\text{Na} = +18.77$). This evolution indicates a sharp mobilization of cations in the arable layer, possibly as an effect of the applied technologies. The pronounced increase in Mg suggests an increased availability for plants, with a potentially positive impact on physiological processes.

In the 20-40 cm layer, all analyzed elements maintain a positive trend ($\Delta\text{Ca} = +37.75$; $\Delta\text{Mg} = +56.29$; $\Delta\text{Na} = +14.90$), but the amplitude of the increases is lower compared to the upper layer. This evolution suggests a relatively uniform distribution of cations in the soil profile. In Vrancea County (Gologanu), in the superficial layer, a decrease in Ca (-13.23) and Na (-29.11) is observed, concomitant with a moderate increase in Mg ($+12.73$). This dynamics may indicate selective leaching or vertical redistribution processes, especially for Ca and Na.

In the 20-40 cm layer, the decrease in Ca (-19.67) and Na (-30.56) is accentuated, while

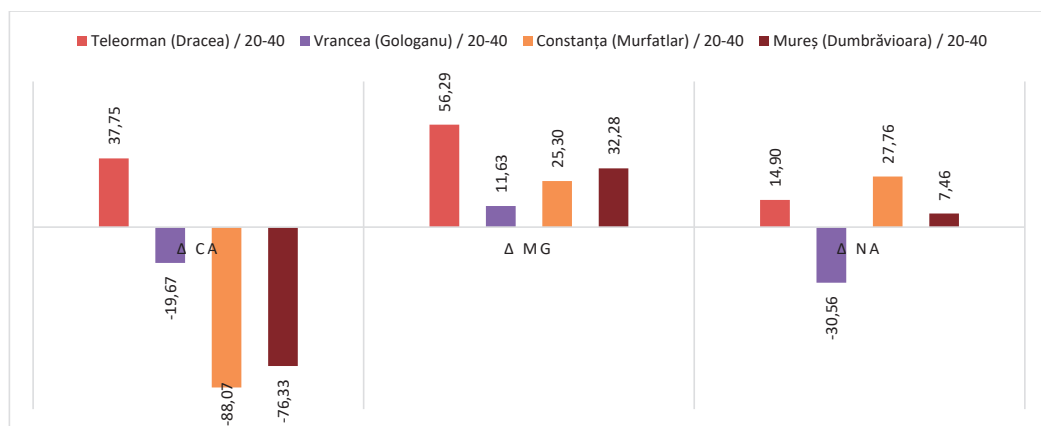
Mg continues to increase moderately (+11.63). These results may indicate a higher mobility of

Ca and Na and a possible migration to deeper horizons.



Note: Values represent concentrations determined in aqueous extract, expressed in mg/kg dry soil

Figure 4. Evolution of water-soluble elements (Ca, Mg, Na) at a depth of 0-20 cm, before and after the application of technologies



Note: Values represent concentrations determined in aqueous extract, expressed in mg/kg dry soil

Figure 5. Evolution of water-soluble elements (Ca, Mg, Na) at a depth of 20-40 cm, before and after the application of technologies

In Constanța County (Murfatlar), in the 0–20 cm layer, a significant decrease in Ca (–47.60) is highlighted, while Mg (+26.81) and Na (+26.31) register increases. The differentiated evolution of cations can be associated with their specific mobility in pedoclimatic conditions characteristic of the area.

In the 20–40 cm layer, the largest decrease in Ca within the study is recorded (–88.07), concomitantly with increases for Mg (+25.30) and Na (+27.76). This evolution suggests

intense processes of Ca redistribution in the soil profile.

In Mureș County (Dumbrăvioara), in the superficial layer, Ca shows a sharp decrease (–71.35), while Mg (+32.66) and Na (+8.21) register moderate increases. The downward trend of Ca may suggest processes of biological consumption or migration in the profile.

In the 20–40 cm layer, the downward trend of Ca (–76.33) is maintained, while Mg (+32.28) and Na (+7.46) show moderate variations. The

relative stability of Mg between the two depths indicates a balanced distribution in the profile. Depth analysis revealed a general trend of increasing soluble Mg in all locations, while soluble Ca recorded significant decreases in most locations, more pronounced in the 20–40 cm layer. Soluble Na showed differentiated evolutions, with potential implications for ionic balance and soil structural stability.

Analysis of the evolution of exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+)

The analysis of the evolution of exchangeable cations is essential for characterizing the chemical state of the soil, as they represent the fraction electrostatically adsorbed on the colloidal (clay-humic) complex, in dynamic equilibrium with the soil solution. This fraction constitutes the active component of the cation exchange capacity and reflects both the potentially available nutrient reserve and the stability of the ionic balance.

The evolution of exchangeable cations was evaluated by calculating the absolute difference

between the values determined after applying the technologies and the initial ones. To calculate the absolute difference (ΔCa^{2+} , ΔMg^{2+} , ΔNa^+ , ΔK^+), which represents the difference between the final value (after applying the technologies) and the initial value (before application), formula (2) was used.

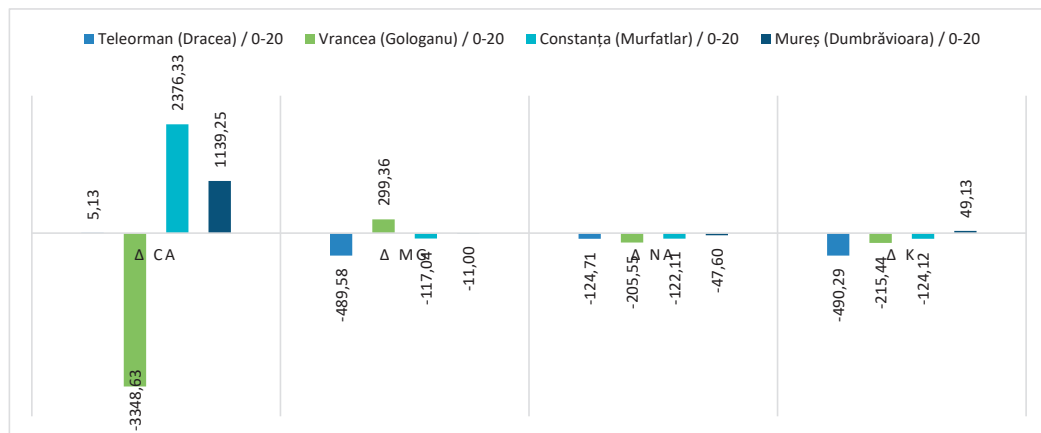
$$\Delta Y = Y_{\text{after}} - Y_{\text{before}} \quad (2)$$

where: $Y = \text{Ca}^{2+}$, Mg^{2+} , Na^+ , K^+ exchangeable, in mg/kg.

Interpretation:

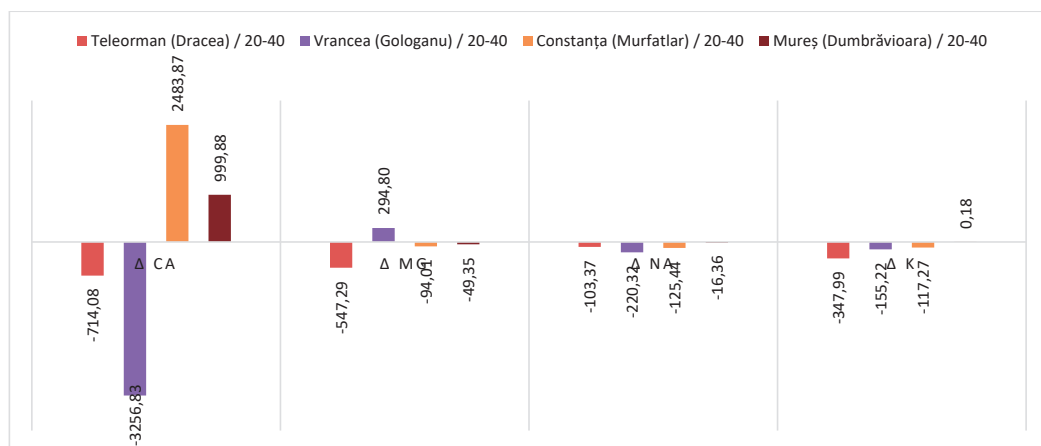
- $\Delta Y > 0 \rightarrow$ increase in exchangeable content (chemical enhancement / accumulation on the colloidal complex);
- $\Delta Y < 0 \rightarrow$ decrease in exchangeable reserve (biological consumption, leaching or ion exchange);
- $\Delta Y \approx 0 \rightarrow$ stability of cation exchange capacity.

Figures 6 and 7 graphically represent the evolution of exchangeable cations at the two analyzed depths.



Note: Values represent concentrations expressed in mg/kg dry soil, determined by specific extraction for the exchangeable fraction

Figure 6. Evolution of exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ , K^+) at a depth of 0-20 cm, before and after the application of technologies



Note: Values represent concentrations expressed in mg/kg dry soil, determined by specific extraction for the exchangeable fraction

Figure 7. Evolution of exchangeable cations (Ca^{2+} , Mg^{2+} , Na^{+} , K^{+}) at a depth of 20–40 cm, before and after the application of technologies

Analysis of the evolution of anions and mineral nitrogen forms (Cl^{-} , SO_4^{2-} , NO_2^{-} , NO_3^{-} , NH_4^{+})

The evolution of anions and mineral nitrogen forms is an indicator of the dynamics of nutrients in the soil solution and of active microbiological processes. These forms are directly influenced by fertilization, climatic conditions and soil biological activity.

The evolution was evaluated by calculating the absolute difference between the values determined after the application of the technologies and the initial ones.

To calculate the absolute difference (ΔCl^{-} , ΔSO_4^{2-} , ΔNO_2^{-} , ΔNO_3^{-} , ΔNH_4^{+}) formula (3) was used.

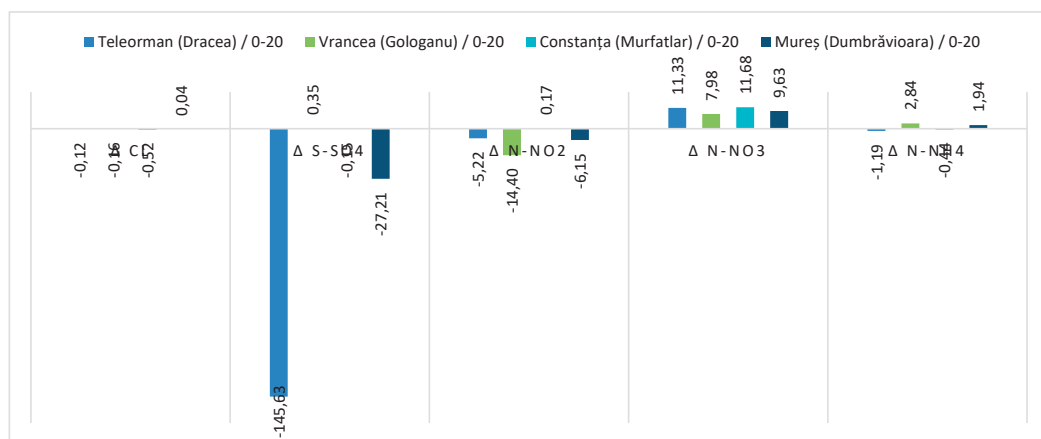
$$\Delta V = V_{\text{after}} - V_{\text{before}} \quad (3)$$

where: V = concentration of the anion or form of mineral nitrogen, in mg/kg.

Interpretation:

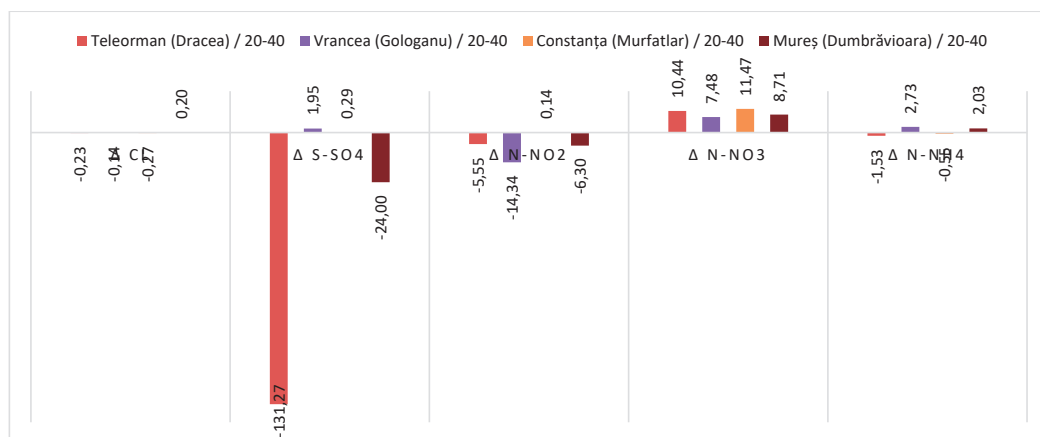
- $\Delta V > 0 \rightarrow$ accumulation in the soil solution;
- $\Delta V < 0 \rightarrow$ biological consumption, leaching or microbiological transformation;
- $\Delta V \approx 0 \rightarrow$ relative stability.

Figures 8 and 9 show the evolution of anions and mineral nitrogen forms at the two analyzed depths.



Note: Values represent concentrations determined in aqueous extract, expressed in mg/kg dry soil

Figure 8. Evolution of anions and mineral nitrogen forms in the 0–20 cm layer



Note: Values represent concentrations determined in aqueous extract, expressed in mg/kg dry soil

Figure 9. Evolution of anions and mineral nitrogen forms in the 20-40 cm layer

Depth analysis allows the identification of leaching risk, especially for NO_3^- , and the evaluation of the efficiency of fertilization management. The evolution of anions and mineral nitrogen forms is a sensitive indicator of the dynamics of the soil solution and the intensity of active biogeochemical processes in the edaphic system. These ions are predominantly found in the liquid phase of the soil, being characterized by high mobility and rapid reaction to changes in the water regime, fertilization and microbiological activity.

Analysis of soil pH evolution

The evolution of soil pH is a central element in the evaluation of changes in chemical fertility following the application of agricultural technologies. Soil reaction directly influences nutrient availability, mineral element mobility and the intensity of biological processes, being considered an integrative parameter of the soil chemical balance.

Changes in pH can cause significant changes in the accessibility of macro- and microelements to plants. Under acidification conditions, some elements can become excessively mobile, generating risks of toxicity or losses through leaching. Conversely, a tendency to alkalize can reduce the availability of some essential microelements, affecting plant nutrition. Thus, the evolution of pH indirectly reflects the efficiency of fertilization and the capacity of the soil to support the growth and development processes of crops.

In the context of assessing the sustainability of applied technologies, the analysis of pH evolution allows the identification of chemical degradation trends and the substantiation of corrective measures necessary to maintain fertility in the long term. Thus, monitoring this parameter contributes to assessing the impact of agronomic interventions on soil quality and ensuring sustainable management of soil resources.

The evolution of pH was evaluated by calculating the absolute difference between the value determined after applying the technologies and the initial value. For the calculation of the absolute difference (ΔpH) the formula (4) was used.

$$\Delta \text{pH} = \text{pH}_{\text{after}} - \text{pH}_{\text{before}} \quad (4)$$

Interpretation:

- $\Delta \text{pH} > 0 \rightarrow$ alkalization tendency;
- $\Delta \text{pH} < 0 \rightarrow$ acidification tendency;
- $\Delta \text{pH} \approx 0 \rightarrow$ stability of the soil reaction.

Figures 10 and 11 illustrate the evolution of pH at the two analyzed depths.

pH variations after the application of technologies may reflect changes in the ionic balance, determined by the input of fertilizers, nitrification processes (which generate acidity by forming H^+ ions), mineralization of organic matter or leaching of exchangeable bases (Ca^{2+} , Mg^{2+} , K^+). An increase in pH may indicate an input of basic cations or a reduction in potential acidity, contributing to the improvement of the soil reaction and the optimization of the conditions for nutrient absorption. Conversely, a

decrease in pH may signal acidification processes associated with nitrogen fertilization, sulfur oxidation or increased microbiological activity.

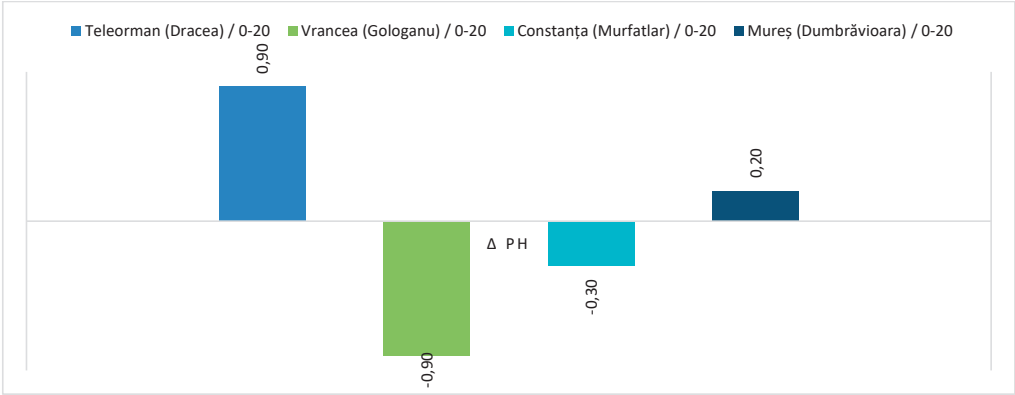


Figure 10. pH evolution in the 0–20 cm layer, before and after the application of technologies

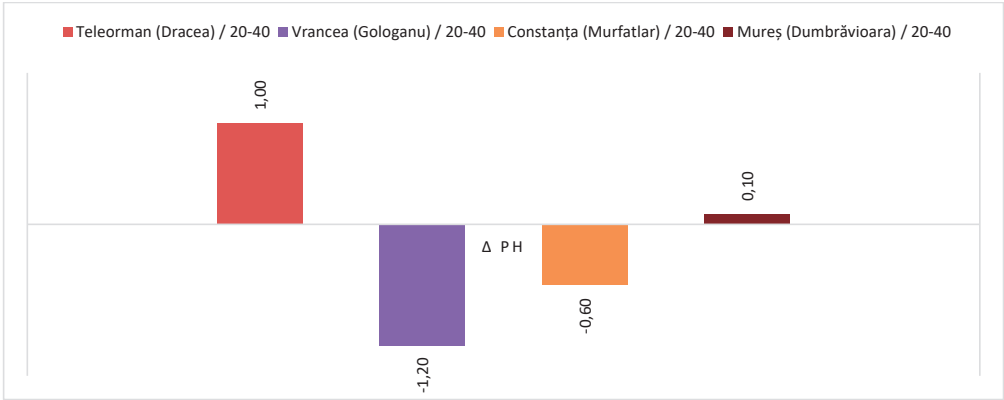


Figure 11. pH evolution in the 20–40 cm layer, before and after the application of technologies

The analysis of the evolution of pH at depth allows highlighting possible processes of acidification or alkalization differentiated in the profile, providing information on the chemical stability of the soil and the sustainability of the applied technologies. Thus, monitoring this parameter is essential for substantiating agronomic management measures and for maintaining an optimal balance between productivity and environmental protection.

Analysis of evolution by region

A comparative analysis was conducted to evaluate the effectiveness of sustainable technologies applied in different regions, by observing soil chemical changes. The use of Δ values (the difference between element concentrations after and before the application

of the technologies) allowed for a clear identification of the effects of each intervention on soil nutrients.

The main goal was to highlight which elements were stimulated, which decreased, and how they differed depending on the technology applied and local pedoclimatic conditions. Changes in the concentrations of the main elements in the soil after the application of sustainable technologies, highlighted by Δ values, are presented comparatively by region in Figure 12. The graph clearly shows the increases and decreases of the key elements (Ca, Mg, Na, K, Zn, N-NO₃⁻) depending on the technology applied and the pedoclimatic context specific to each locality.

The comparative analysis by region, which highlights the elements most affected by each

technology applied, is summarized in Table 1, which shows the increases and decreases of the main elements (Ca, Mg, K, Zn, N-NO₃⁻)

depending on the region and type of intervention.

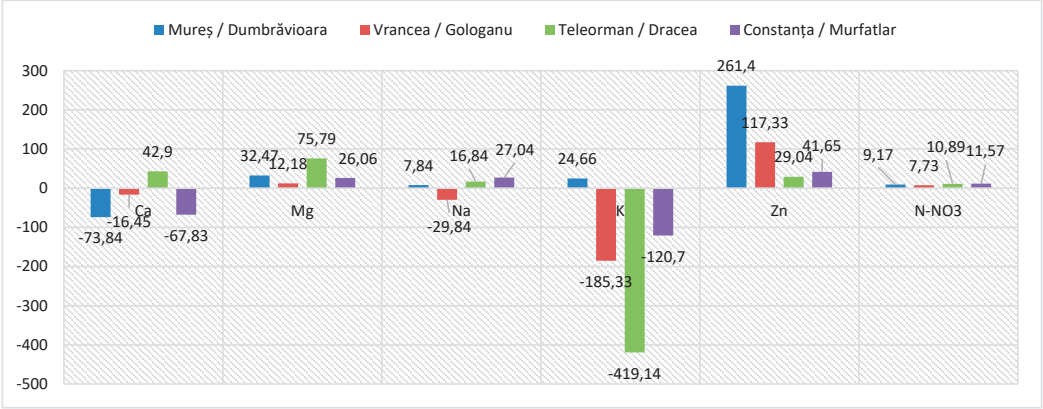


Figure 12. The effect of technologies on soil fertility: Δ variation by region

Table. 1 Regional analysis of elements influenced by sustainable technologies

Region, county	Most affected elements	Observations
Center, Mureș	Mg ↑, Zn ↑, K ↑	Deep soil loosening increased nutrient mobility and biological activity.
South-East, Vrancea	Zn ↑, K ↓, Mg ↑, N-NO ₃ ⁻ ↑	The biofertilizer boosted micronutrients and mineral nitrogen, but K probably decreased through plant uptake and leaching.
South, Teleorman	Ca ↑, Mg ↑, K ↓	Strip-till increased Ca and Mg, but significantly decreased K, reflecting plant uptake and water conservation.
South-East, Constanța	Mg ↑, Na ↑, K ↓, Zn ↑	Ecological interventions boosted Mg, Na and Zn, but K and Ca decreased, with minor impacts on pH and S-SO ₄ .

The analysis by regions shows that the applied sustainable technologies consistently boosted Mg and N-NO₃⁻, while K decreased in all localities. Increases or decreases of other elements (Ca, Na) depend on the type of technology and local pedoclimatic conditions. Overall, the data highlight that each technology has a specific impact on soil fertility and requires adaptation to the regional context.

CONCLUSIONS

The comparative analysis of soil characteristics before and after the application of innovative technologies highlighted positive effects on fertility and nutrient availability in the seedbed, depending on the type of intervention and the pedoclimatic conditions of each region. Mobilization of macroelements: mechanical interventions (deep loosening, strip-till) increased the solubility of Ca and Mg,

facilitating their access for plants; biofertilization stimulated the biological activity of the soil, resulting in notable increases in mineral nitrogen NO₃⁻ and NH₄⁺). Nitrogen regime and soil pH: all applied technologies positively influenced the soluble nitrogen content, favoring mineralization and nutrient availability. Soil pH underwent moderate variations, but sufficient to influence ion balance and micronutrient solubility. Efficiency of technologies in pedoclimatic context: in compact soils and at risk of subsidence (Dumbrăvioara, Dracea), loosening and strip-till improved aeration, water infiltration and nutrient redistribution; in soils with low organic matter content and unstable structure (Gologanu), biofertilization stimulated biological activity, contributing to the mobilization of nutrients; in areas with increased water stress (Murfatlar), ecological interventions supported the increase in the

availability of nitrogen, increasing crop resilience.

The applied technologies had positive effects on soil fertility, demonstrating that combined mechanical and biological interventions can increase soil nutritional efficiency, even under variable and stressful climatic conditions.

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FORMATION AND EVOLUTION OF SOILS IN THE LOW PLAINS OF WESTERN ROMANIA

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Abstract

The paper examines the evolution and pedological importance of soils in the low plains of western Romania, focusing on the Mureș-Galațca Plain and the Bega-Timiș-Bârzava Canal. The main objective is to investigate the natural factors influencing the formation, evolution, and characteristics of these soils, such as morphological and hydrographic variability, lithological aspects, and tectonic activities, including subsidence. A detailed analysis of geological processes, such as sediment deposition, will enhance understanding of their impact on soil fertility. The paper will also highlight the effects of human activity on land resources and the challenges faced, such as salinization and soil compaction. These issues are often worsened by groundwater conditions that can adversely affect agricultural productivity. Proposed strategies for managing soil resources include emphasizing conservation measures and adopting sustainable agricultural practices to improve soil health. By addressing these aspects, the paper underscores the vital connection between pedological characteristics and local agricultural development, emphasizing the need for effective land resource management to ensure long-term agricultural sustainability in these regions. A strategic reevaluation of agricultural policies is essential in light of current ecological challenges.

Key words: *pedology, low plain, soil evolution, resource management, sustainable agriculture.*

INTRODUCTION

The low plains of western Romania, particularly those in Banat, constitute a geographically complex region in which the interplay among subsidence, hydrographic networks, lithology, and anthropogenic interventions has produced a mosaic of pedo-hydrological conditions with direct implications for agricultural productivity. Along a North-South gradient in morpho-hydrographic and lithological settings, the area can be subdivided into units such as the Aranca, Galațca, Jimbolia, Cenei, Timiș, and Banloc plains, which are further grouped into three major sectors: the Mureș-Galațca Plain, the Bega Canal-Galațca Plain, and the Bega Canal-Timiș-Bârzava Plain. Although the Mureș River forms the northern boundary of the study area, the region functions predominantly within the south-western hydrographic systems (Timiș-Bega and Bârzava), characterized by a dense network of drainage canals and regulated channels (Ujvári, 1972; Blaga et al., 2008).

The origin and evolution of the plain are closely linked to the Pannonian Basin, where the

crystalline basement (Tisia) was fragmented into blocks that experienced differentiated vertical movements. The penetration of the Tethys Sea waters along corridors between these blocks promoted substantial sediment accumulation, while neotectonic activity (initiated in the Pontian and continuing through the Pliocene, with limited but detectable present-day expression) supported the development of subsidence areas that exert strong control on drainage and morphology. Lithologically, the plains are composed of heterogeneous fluvio-lacustrine successions (silts, clays, marly clays, and sands), locally interbedded with loess-like materials; the parent material may include swelling clays and soluble salts (especially sodic salts), conditions conducive to the development of halomorphic soils.

The present-day hydrographic structure is strongly anthropogenically modified. Channelization of the Bega River and the embankment/drainage works initiated after 1717 aimed to reclaim marshy areas and improve land use, leading to a reorganization of

runoff pathways and modifications in groundwater levels (Neacşa et al., 1979; Blaga et al., 2008). Nevertheless, in depressional areas the evacuation of pluvial waters often remains incomplete, and surface water stagnation is frequent during wet seasons. Shallow groundwater depths vary with microrelief (shallower in depressions and deeper on natural levees/ridges), the waters are commonly highly mineralized (chlorides, sulphates, bicarbonates), and overall drainage remains imperfect. These conditions sustain a problematic seasonal alternation: excess soil moisture during the cold season and water deficit during the warm season (Dumitru et al., 2000; Niță et al., 2018).

Under these constraints, a highly diverse soil cover has developed. According to the Romanian System of Soil Taxonomy (SRTS 2012), the analysed area includes multiple soil classes and types (e.g., Protisols, Cernisols, Vertisols, Hydrisols, Salsodisols, and Anthrosols), as well as soil complexes with a fine-scale mosaic distribution that is difficult to delineate precisely. The principal limitations to agricultural productivity are associated with stagnant and groundwater-induced excess moisture, salinization/sodification, acidification, compaction, and, seasonally, soil water deficit (Florea et al., 2012; David et al., 2018; Niță et al., 2024). Therefore, the design of agricultural technologies and pedo-hydro-ameliorative measures (e.g., drainage and dewatering systems, deep loosening, traffic/compaction prevention, amendments and corrective fertilization, and rotations including ameliorative crops) should be grounded in a detailed understanding of local conditions and ecological suitability, with the overarching goal of enhancing resilience and ensuring the sustainable use of agricultural land in the low plains (Florea et al., 2012; Țărau et al., 2019).

MATERIALS AND METHODS

The present study addresses the lowland plains of western Romania (the Banat sector of the Pannonian Plain), a region characterized by a very weakly dissected relief, low elevations, gentle slopes, and a hydrographic network strongly conditioned by lithological controls and long-term anthropogenic interventions (embankments, drainage works, and canals).

Along the north–south gradient in morpho-hydrographic and lithological conditions, we analysed the following subunits: the Aranca Plain, Galațca Plain, Jimbolia Plain, Cenei Plain, Timiș Plain, and Banloc Plain. These were further grouped into functional sectors linked to the major hydrographic systems (e.g., the Mureș system and the Timiș-Bega river/canal system). To provide an integrated characterization of the physical-geographical setting and hydrological functioning, we used the following data categories. A digital elevation model with a spatial resolution suitable for low-relief plains ($\geq 10\text{--}30\text{ m}$) was employed to derive slope, curvature, drainage networks, and microdepressions; topographic maps and base plans were used for verification and cartographic control. Geological, lithological, and pedological information was compiled from thematic maps (Cheval et al., 2014; Ioniță et al., 2016). Geological/lithological maps supported the delineation of deposits (e.g., loess-like, alluvial, clay-rich units) and the interpretation of lithological controls on drainage. Soil maps (texture, permeability, hydromorphy) were used to assess susceptibility to excess moisture conditions. Where available, daily and/or monthly discharge and water-level time series were obtained from the hydrometric station network associated with the relevant basins (Mureș, Aranca, and the Timiș-Bega/canal system). Information on hydraulic engineering works (canals, river training/regularization, embankments) was incorporated to interpret departures from natural flow regimes (Kopecký et al., 2021). Climatic and agrometeorological datasets included daily/monthly precipitation and air temperature from representative lowland meteorological stations, together with the variables required to estimate reference evapotranspiration (ETP) and water balance components (radiation, wind, humidity), depending on data availability) (Dumitrescu et al., 2015; Bîrsan et al., 2019). Multispectral satellite imagery (e.g., Sentinel, Landsat) was additionally used to identify temporary wet areas, detect land-use/land-cover changes, and assess vegetation condition (Kopecký et al., 2021).

Subunits (Aranca, Galațca, Jimbolia, Cenei, Timiș, Banloc) were delineated by overlaying morphometric criteria with hydrographic

boundaries and lithological/pedological transitions, resulting in spatially coherent units suitable for comparative analysis. For each subunit, we calculated standardized metrics relevant to lowland plains, including: (i) mean, minimum and maximum elevation, and hypsometric range; (ii) mean slope and slope-class distribution; (iii) drainage density (total channel length per unit area) and stream frequency; (iv) the proportion of areas with high potential for excess moisture derived from hydromorphic soil classes; and (v) characterization of the precipitation and thermal regimes using long-term means, seasonality, and interannual variability (Mihuț et al., 2018; Niță et al., 2018).

For the preparation of this study, we strictly complied with the methodological standards for the development of pedological and agrochemical surveys (ICPA, 1987, Volumes I-III).

RESULTS AND DISCUSSIONS

1) Spatial organization of the lowland plains and the genetic-structural framework

The analysis confirms that the north-south variability in morpho-hydrographic and lithological conditions, superimposed on neotectonic controls (differential subsidence of crystalline blocks and sediment-accumulation corridors), produces a hydrologically heterogeneous landscape despite the overall weakly dissected relief. The investigated subunits (Aranca, Galațca, Jimbolia, Cenei, Timiș, and Banloc) can be operationally grouped into three sectors—the Mureș-Galațca Plain, the Bega Canal-Galațca Plain, and the Bega Canal-Timiș-Bârzava Plain—each displaying distinct drainage characteristics, groundwater-table (pedofreatic) depth patterns, and soil assemblages (Țărău et al., 2019; Niță et al., 2024).

At the process scale, the combined influence of (i) fine-grained fluvio-lacustrine deposits (silts, clays, marly clays), (ii) depressional microrelief and interrridge/interfluvial low-lying surfaces, (iii) a shallow phreatic aquifer, and (iv) long-standing hydro-ameliorative interventions (channelization, embankment construction, and drainage works) explains the emergence of a dual stress regime in agroecosystems. This

regime is characterized by excess soil moisture during the cold and transitional seasons versus soil water deficit during the warm season, with direct consequences for soil fertility, field trafficability/accessibility, and overall agricultural productivity.

2) Soil types: differences among the three sectors (Tables 1-3)

Across the 42 analysed administrative-territorial units, we identified 11 soil types and soil associations that can be assigned to eight soil classes according to the Romanian System of Soil Taxonomy (SRTS, 2012), with marked sectoral contrasts in their spatial distribution, Tables 1-3.

(a) *The Mureș-Galațca Plain sector* (103,531 ha of agricultural land) is dominated by:

- Chernozems (CZ): 46.07%;
- Vertisols (VS): 19.51%;
- soil associations/complexes: 16.15%;
- followed by Fluvisols (AS): 10.24%.

This soil pattern indicates a substantial agricultural resource base, primarily supported by the widespread occurrence of Chernozems. However, the relatively high share of heavy-textured and/or mosaic soils (notably Vertisols and soil complexes) suggests an increased susceptibility to unfavourable aeration–water regime conditions and to physical degradation processes.

(b) *The Bega Canal-Galațca Plain sector* (75,766 ha of agricultural land) exhibits the highest degree of pedological “concentration”, being characterized by:

- Chernozems (CZ): 62.19%;
- Vertisols (VS): 11.99%;
- soil associations: 9.69%;
- Eutric Cambisols (EC): 5.30%.

The clear predominance of Chernozems accounts for the generally high agricultural potential of this sector. Nevertheless, the presence of Vertisols and soil associations indicates the occurrence of micro-areas with fine-textured materials and/or hydromorphic features, as well as abrupt soil transitions over short distances, reflecting a pronounced pedological mosaic. Such small-scale heterogeneity is particularly relevant in lowland settings, where local variations in microrelief and drainage efficiency can strongly influence soil moisture regimes, trafficability, and the spatial stability of crop performance.

(c) *The Bega Canal-Timiș-Bârzava Plain sector* (118,448 ha of agricultural land) is the most heterogeneous, with the following dominant soil groups:

- Eutric Cambisols (EC): 25.08%;
- Chernozems (CZ): 22.78%;
- Vertisols (VS): 12.96%;
- Phaeozems (FZ): 9.32%, and soil associations: 9.04%, as well as notable shares of Gleysols (GS): 5.77% and Solonetz (SN): 5.14%.

This composition is consistent with a sector in which neotectonic control (subsidence), fine-grained parent materials, and the interaction between canal systems and the shallow groundwater table promote a wide range of pedogenetic processes. These include hydromorphism, sodification, and alternating redox conditions, which can lead to strong spatial contrasts in soil physical and chemical

properties and, consequently, in land-use suitability and yield stability at local scales.

Discussions. The high frequency of soil complexes (associations) and fine-textured soils (e.g., Vertisols/pelosols) supports the interpretation that microtopography and near-surface hydrology-together with engineered drainage-are key controls of pedological organization. In subsiding lowland plains, pedological boundaries are often expressed at spatial scales smaller than those captured by conventional mapping, resulting in sharp transitions over short distances. Consequently, effective agricultural management should be implemented at the level of micro-areas (field and sub-field scale), rather than relying solely on administrative units, in order to adequately address local variability in moisture regimes, trafficability, and soil constraints.

Table 1. Main soil types and soil associations in the Mureș-Galațca interfluvial plain*

No.	Administrative-territorial unit	Agricultural, ha	1. PS	2. AS	3. CZ	4. EC	5. VS	6. GS	7. SN	8. AT-TT	9. Asoc.
1	Beba Veche	8817	0	528	3880	0	3263	160	104	0	882
2	Cenad	7383	0	2732	517	0	3175	15	59	0	885
3	Comloșu Mare	8724	646	61	6273	0	1291	436	17	0	0
4	Dudeștii Vechi	13113	0	2402	2096	199	4411	133	40	13	3819
5	Gottlob	4114	66	0	3896	30	0	43	0	19	60
6	Lovrin	3807	27	0	3124	190	120	170	56	20	100
7	Periam	5886	47	265	3905	805	12	132	39	0	681
8	Pesac	3147	94	87	2055	338	6	188	20	0	359
9	Saravale	9620	0	1176	3859	0	2299	48	358	0	1880
10	Sânpetru Mare	9176	0	1155	3678	0	2099	46	338	0	1860
11	Sănnicolau Mare	12689	0	1015	5329	2030	1016	634	127	0	2538
12	Teremia M.	7748	85	138	4804	101	504	39	77	7	1993
13	Tomnatic	3547	48	0	3367	60	32	30	3	7	0
14	Valcani	5760	0	1044	912	87	1975	58	17	6	1661
	Mureș-Galațca	103531	1013	10603	47695	3840	20203	2132	1255	72	16718
	%	100	0.98	10.24	46.07	3.71	19.51	2.06	1.21	0.07	16.15

*Office for Pedological and Agrochemical Studies from Timiș/Arad; Țărău et al., 2019.

Table 2. Main soil types and soil associations in the Bega Canal-Galațca interfluvial plain*

No.	Administrative-territorial unit	Agricultural, ha	AS	CZ	FZ	EC	EL	VS	PE	GS	SN	AT-TT	Asoc.
1	Becicherecu Mic	4217	70	2846	0	640	104	96	64	25	130	18	224
2	Biled	4946	0	4543	0	0	0	10	0	194	49	0	150
3	Cărpiniș	4115	0	4029	0	0	0	51	0	35	0	0	0
4	Cenei	6364	46	2725	0	0	0	3106	0	51	53	70	313
5	Checca	5332	38	2276	0	0	0	2598	0	42	53	64	261
6	Dudeștii Noi	4776	0	3310	296	250	94	113	145	37	125	35	371
7	Ieccea Mare	3273	0	2927	0	0	0	300	0	46	0	0	0
8	Jimbolia	9725	0	6529	0	0	0	58	0	545	29	20	2544
9	Lenauheim	10521	0	8681	0	0	0	210	0	399	0	84	1147
10	Săcălaz	10622	985	3719	617	2951	0	478	1320	249	303	0	0
11	Sânmihailu Român	6841	1446	774	0	177	0	2023	0	132	65	0	2224
12	Șandra	5034		4761	0	0	0	39	0	120	6	0	108
	Bega-Galațca Canal	75766	2585	47120	913	4018	198	9082	1529	1875	813	291	7342
	%	100	3.41	62.19	1.21	5.30	0.26	11.99	2.02	2.47	1.07	0.38	9.69

*Office for Pedological and Agrochemical Studies from Timiș/Arad; Țărău et al., 2019.

Table 3. Main soil types and soil associations in the Bega Canal-Timiș-Bârzava interfluvial plain*

No.	Administrative-territorial unit	Agricultural, ha	AS	CZ	FZ	EC	VS	PE	GS	SN	AT-TT	Asoc.
1	Banloc	10252	437	720	0	2979	2850	358	274	28	0	2606
2	Ciacova	12506	328	2701	129	3512	1914	1932	1380	610	0	0
3	Foeni	5816	64	3390	0	29	20	0	744	378	58	1133
4	Ghilad	10486	762	3035	132	1211	1511	173	1912	954	0	796
5	Giera	8556	0	668	201	3025	2521	421	75	187	0	1458
6	Giroc	3903	458	0	0	2378	53	0	493	0	0	521
7	Giulvăz	8908	0	3285	1719	1052	282	358	0	1514	0	698
8	Jebel	6225	320	626	2275	2198	214	0	280	312	0	0
9	Livezile	5231	27	1133	821	2070	334	154	175	517	0	0
10	Otelec	7746	396	2432	664	1038	1792	603	299	115	3	404
11	Parța	5560	1024	407	638	2973	14	112	0	8	8	376
12	Pădureni	3740	189	368	1338	1357	126	0	179	183	0	0
13	Peciu Nou	12188	115	3983	1938	1885	864	1203	73	1103	11	1013
14	Șag	2751	513	203	319	1461	7	55	0	2	4	187
15	Timișoara	4341	343	864	0	1190	703	0	561	50	0	630
16	Uivar	10239	516	3162	864	1350	2147	785	389	131	5	890
	Bega-Timiș-Bârzava Canal	118448	5492	26977	11038	29708	15352	6154	6834	6092	89	10712
	%	100	4.64	22.78	9.32	25.08	12.96	5.20	5.77	5.14	0.08	9.04

*Office for Pedological and Agrochemical Studies from Timiș/Arad; Țărău et al., 2019.

3) Excess soil moisture: predominance of the groundwater component and sectoral differentiation

(a) Mureș-Galațca Plain

The data indicate that excess soil moisture has two main components: surface waterlogging (ponding following precipitation) and groundwater-induced excess moisture (a shallow groundwater table). Because these components may overlap spatially, percentages are interpreted separately (i.e., they should not be summed).

Area affected by surface waterlogging: 32.72% (slight 20.49% + moderate 7.13% + strong/very strong 5.10%).

Area affected by groundwater-induced excess moisture: 41.06% (moderate 19.13% + strong 15.46% + very strong/extreme 6.47%).

Hotspots (absolute values, from the table) include Sănnicolau Mare (very high surface waterlogging), Beba Veche and Valcani (strong to very strong groundwater influence), and Cenad (strong groundwater influence).

(b) Bega Canal-Galațca Plain

Surface waterlogging: 47.12% (the highest among the three sectors).

Groundwater-induced excess moisture: 36.02%. This pattern suggests that, although shallow groundwater remains an important driver, post-precipitation ponding is more dominant in this sector, likely due to the combined effects of microdepressions, fine-textured materials, and incomplete drainage during wet seasons.

Localities with high values include Cenei, Checea, Jimbolia, and Săcălaz.

(c) Bega Canal-Timiș-Bârzava Plain

Surface waterlogging: 36.06%.

Groundwater-induced excess moisture: 61.52% (the highest among the three sectors).

Here, the major role of the shallow groundwater table is evident (commonly 0.5-1.0 m in depressional areas), together with the influence of supply-drainage canal systems and regional subsidence, which collectively produce slow external drainage and imperfect internal drainage.

Discussions. The mechanism described in the source material-fine-grained deposits combined with a shallow aquifer and internal drainage constraints imposed by clay layers and soluble salts-provides a robust explanation for why groundwater-driven excess moisture remains the dominant control even after large-scale hydro-ameliorative works. In the broader literature, the interaction between shallow groundwater, insufficient drainage, and salt accumulation is widely recognized as a key pathway leading to saline/sodic soil development and associated forms of land degradation.

4) Salinization and soil acidification: a strong regional signal of salinity/sodicity

Salinization shows a very large spatial extent across all three sectors:

- Mureș-Galațca: 68.09% of agricultural land affected (from slight to extreme);
- Bega Canal-Galațca: 56.04%;

- Bega Canal-Timiș-Bârzava: 55.75%.

Importantly, the pattern is not limited to slight-moderate forms; a substantial share of strong and extreme salinization is also present (e.g., in Mureș-Galați: 5.23% strong and 15.36% extreme). This finding is consistent with the described parent materials (carbonate-rich silts, swelling clays, and soluble-particularly sodic-salts), the high mineralization of shallow groundwater (chlorides, sulphates, bicarbonates), and the occurrence of alkaline/alkaline-sodic areas near the listed localities (e.g., Cenad, Sânnicolau Mare, Cenei, Săcălaz, Timișoara, Uivar, Banloc, Moravița). By contrast, *soil acidification* is comparatively limited:

- Mureș-Galați: 1.88% (slight);

- Bega Canal-Galați: 1.10% (slight);

- Bega Canal-Timiș-Bârzava: 3.42% (slight) plus only localized traces (0.01%).

Overall, the dominant chemical constraint on these agroecosystems is therefore salinity/sodicity, rather than acidification.

Discussions. Globally, salt-affected soils (saline, sodic, and saline-sodic) are widely recognized as a major land-degradation issue. In 2021, FAO released a global map of salt-affected soils, highlighting the scale and relevance of the problem. In lowland plains in particular, groundwater-driven salinization and salinity development under insufficient drainage are considered key mechanisms-fully consistent with the process-based interpretation supported by the present dataset.

5) Soil compaction and soil moisture deficit: expression of physical and climatic stress

Soil compaction (traffic-induced densification) reaches very high levels, particularly in the Bega Canal-Galați sector:

- Mureș-Galați: 69.87% (slight to strong);

- Bega Canal-Galați: 90.50%;

- Bega Canal-Timiș-Bârzava: 71.80%.

This pattern is consistent with: (i) fine textures and high clay contents (soils with a short “workability window”); (ii) mechanical operations frequently carried out under suboptimal moisture conditions; and (iii) low bearing capacity over extended periods of the year, especially in areas affected by excess moisture.

Soil moisture deficit is also substantial, with the highest share in the Mureș-Galați sector:

- Mureș-Galați: 69.18% (very low/low + moderate + high deficit);

- Bega Canal-Galați: 38.39%;

- Bega Canal-Timiș-Bârzava: 30.78%.

Discussions. The literature indicates that compaction caused by heavy machinery traffic can reduce plant access to water and nutrients and can negatively affect productivity, while structural recovery may be slow-sometimes on the order of years to decades-depending on soil properties and hydrological regime. In the analysed lowland plains, these impacts are amplified by seasonal alternation: soils are often wet (and therefore highly susceptible to compaction) during the cold season and spring, whereas water deficit typically prevails during the warm season, when compaction further constrains infiltration and root exploration, compounding drought stress and yield instability.

6) Integration of results: functional typologies and pedo-hydro-ameliorative implications

By overlaying soil distribution patterns with the main limiting factors, we delineated three functional typologies that are relevant for interpretation and management:

Areas at high risk of groundwater-induced excess moisture combined with salinity/sodicity. This typology is particularly characteristic of the Bega Canal-Timiș-Bârzava sector, where groundwater-related excess moisture reaches 61.52%, and of depressional/interridge settings where the groundwater table may rise to near-surface levels in wet years. Management priorities include ensuring effective drainage functionality, regular maintenance of canal networks, controlling water circulation within supply-drainage systems, and implementing targeted chemical and biological measures (e.g., sodicity amelioration and rotations with ameliorative crops).

High agricultural potential (Chernozem-dominated) areas affected by post-precipitation waterlogging and severe compaction

This typology is typical of the Bega Canal-Galați sector, which is dominated by Chernozems (62.19%) but also exhibits the highest surface waterlogging (47.12%) and very high compaction (90.50%). Here, the critical issue is not merely the presence of canals, but their effective performance during wet seasons,

combined with strict technological discipline: field operations at optimal soil moisture, reduced machinery traffic, and controlled deep loosening where appropriate.

Pedological mosaic areas with comparatively higher soil moisture deficit

This typology is most evident in the Mureş–Galaţca sector, where soil moisture deficit (69.18%) emerges as a major limitation alongside a very high extent of salinization (68.09%). Optimal management requires both measures aimed at improving the soil aeration–water regime (localized drainage where necessary) and strategies for soil-water conservation (reduced tillage intensity, vegetation cover, and crop rotations), complemented by chemical amelioration and corrective fertilization in areas affected by sodicity.

CONCLUSIONS

The analysed lowland plains are jointly controlled by neotectonic subsidence, fine-grained fluvio-lacustrine lithology, and the hydraulic engineering network, which together explain the pronounced functional (hydrological and pedological) heterogeneity despite the overall weakly dissected relief. The subdivision into three sectors–Mureş–Galaţca, Bega Canal–Galaţca, and Bega Canal–Timiş–Bârzava–is therefore warranted by consistent differences in drainage behaviour, groundwater-table (pedofreatic) levels, and soil assemblages.

The soil cover displays a marked mosaic structure, comprising eight SRTS (2012) soil classes and 11 soil types/associations, with clear pedogenetic contrasts among sectors:

- Mureş-Galaţca: dominated by Chernozems (46.07%), followed by Vertisols (19.51%) and soil associations (16.15%);

- Bega Canal–Galaţca: strongly dominated by Chernozems (62.19%), with secondary shares of Vertisols (11.99%) and soil associations (9.69%);

- Bega Canal–Timiş–Bârzava: the most heterogeneous sector, with Eutric Cambisols (25.08%), Chernozems (22.78%), Vertisols (12.96%), Phaeozems (9.32%), together with notable proportions of Gleysols (5.77%) and Solonetz (5.14%), indicating widespread

hydromorphism and locally enhanced sodification processes.

Excess soil moisture is the dominant limiting factor, but with distinct sectoral expression, confirming the combined control exerted by microtopography, texture, shallow groundwater, and canal connectivity:

- Mureş-Galaţca: surface waterlogging 32.72% and groundwater-induced excess moisture 41.06%;

- Bega Canal–Galaţca: maximum surface waterlogging 47.12% and groundwater-induced excess moisture 36.02% (with an emphasis on post-precipitation ponding);

- Bega Canal–Timiş–Bârzava: extremely high groundwater-induced excess moisture 61.52% and surface waterlogging 36.06% (with an emphasis on a shallow groundwater table).

Salinization constitutes a major regional constraint, consistent with the parent materials (clays, carbonate-rich silts, swelling clays) and the high mineralization of shallow groundwater (chlorides, sulphates, bicarbonates). In contrast, soil acidification plays a secondary role (≈ 1.10 –3.43%), indicating that chemical management should prioritize salinity/sodicity rather than acidification.

Soil compaction is widespread and severe, reflecting both heavy textures with a short workability window and the pressure exerted by mechanized agricultural practices:

- Mureş-Galaţca: 69.87%;

- Bega Canal–Galaţca: 90.50% (the most critical situation);

- Bega Canal–Timiş–Bârzava: 71.80%.

Soil moisture deficit co-occurs with excess moisture, confirming an annual “dual stress” regime–excess moisture in the cold season/spring and deficit during the warm season. The share of land affected by deficit is:

- Mureş-Galaţca: 69.18%;

- Bega Canal–Galaţca: 38.39%;

- Bega Canal–Timiş–Bârzava: 30.78%.

This alternation contributes to yield instability and highlights the need for adaptive, sub-field (micro-area) management.

Historical hydro-ameliorative works have altered the pedofreatic regime and locally reduced hydromorphism; however, the results show that surface-water evacuation remains incomplete, particularly in depressional areas. Internal drainage is often imperfect (interbedded

clays, salts), and the groundwater table exhibits strong seasonal fluctuations (typically 0.5-4.0 m, frequently 0.5-1.0 m in depressions), maintaining persistent pressure on soils and crops.

Practical implications and recommendations
Sector-specific management is required, rather than a uniform regional approach:

Bega Canal-Timiș-Bârzava: priority to controlling groundwater-induced excess moisture (effective drainage operation, canal maintenance, pumping stations where applicable), complemented by anti-sodicity measures.

Bega Canal-Galați: priority to mitigating waterlogging and reducing compaction (controlled traffic, field operations at optimal soil moisture).

Mureș-Galați: priority to a mixed strategy combining warm-season soil-water conservation with the management of local excess moisture and salinization.

The recommended technological package focuses on: balancing the soil aeration-water regime (dewatering/drainage where needed), preventing compaction, and applying ameliorative measures and corrective fertilization aimed at restoring soil structure and biological functioning (rotations with ameliorative crops and organic-matter inputs).

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PRELIMINARY RESEARCH CONCERNING THE POTENTIAL USE OF POULTRY MANURE AS AN AGRICULTURAL FERTILIZER

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Abstract

Poultry manure management is a major technological, agronomic, and environmental challenge in intensive poultry production. The amount and characteristics of manure are influenced by feed composition, water quality, housing system, bird age, and microclimatic conditions. In Romania, poultry are mainly reared either on litter-based floor systems or in cage systems, and these systems strongly affect manure moisture, handling, storage, and final use. This study examines the relationship between poultry housing systems and manure flow, with emphasis on the energetic recovery of poultry litter through combustion and the agricultural use of the resulting ash. The experimental part included the chemical characterization of soil, poultry litter, ash obtained by combustion at different temperature, and soil–ash mixtures prepared at different rates. The results indicate that poultry litter ash can be considered a valuable secondary fertilizer resource within a circular economy framework, provided that application rates are adjusted to crop requirements, soil properties, and salinity risk.

Key words: poultry manure, agricultural fertilizer, chemical characteristics, heavy metals contents.

INTRODUCTION

Intensive poultry production generates large quantities of manure, whose amount and composition depend on feed type, digestibility, water quality, bird age, housing system and microclimatic conditions. These factors directly influence manure moisture content, nutrient concentration, handling, storage and environmental impact. In modern poultry complexes, manure management is therefore an essential component of both production efficiency and environmental protection (Williams, 2013; Bernal et al., 2024).

In Romania, poultry are mainly reared in two systems: floor-based systems with permanent litter and cage systems used especially for laying hens. In floor systems, excreta are mixed with litter material and remain inside the house until the end of the production cycle. During this period, fresh manure with high moisture content gradually dries and becomes solid poultry litter.

In cage systems, manure is removed more frequently, usually two times per week and therefore retains a higher moisture content, resulting in a semi-solid material requiring covered storage before final use.

The concentration of poultry production units, the restrictions imposed by environmental regulations, especially those related to nitrate pollution and the increasing need for renewable energy and nutrient recycling have stimulated interest in alternative manure management pathways. Traditionally, poultry manure has been used as an organic fertilizer, either directly or after composting. More recently, it has also been considered a resource for biogas production, thermal energy generation, and combustion for heat and electricity. Combustion is particularly attractive because it significantly reduces manure volume, destroys pathogens, decrease, and produces ash rich in mineral nutrients, especially phosphorus and potassium.

Recent studies have highlighted the potential of poultry litter ash as a fertilizer or soil amendment within the circular economy concept. However, its agronomic value depends on combustion temperature, ash chemistry, nutrient availability, pH, salinity, and heavy metal content. The aim of this study was to analyse manure flow and maintenance systems in poultry complexes and to evaluate the chemical characteristics and agricultural potential of poultry litter ash obtained by combustion.

MATERIALS AND METHODS

The study considered the main poultry categories and management systems used in Romania, including broilers, replacement pullets, laying hens, breeder hens, turkeys, ducks, and geese. Poultry production is organized into cycles that include both the productive phase and sanitary downtime. These cycles vary depending on the species and category of birds.

Fresh poultry excreta generally contain 75-90% water. In floor-based systems, manure is mixed with bedding material and remains in the house until the end of the cycle. During this period, moisture decreases from approximately 75% to 20-30%, depending on the season. In autumn and winter, litter moisture is usually higher, around 30-33%, whereas in summer it may decrease to about 20%. In cage systems, manure is removed twice a week, and its moisture decreases only to approximately 55-60%, resulting in a pasty material (Cempa et al., 2022).

Permanent litter protects birds from direct contact with the floor and manure and facilitates cleaning at the end of the production cycle. It consists of a layer of absorbent material, usually 10-30 cm thick, placed on the floor and not replaced until the end of the cycle. The material used for litter must have high hygroscopic capacity, good gas absorption, softness, porosity, and low moisture content.

Suitable materials include cereal straw, industrial wood shavings, and sunflower husks (Van, 2025).

Based on BAT (Best Available Techniques) recommendations, the average amount of litter material used was estimated at 6.7 g/bird/day for

broiler chickens, 12.98 g/bird/day for turkeys, 73.4 g/bird/day for ducks, and 7.3 g/bird/day for replacement pullets (Santonja et al., 2010).

The soil used in the experiment was a commercial flower-soil substrate analysed in triplicate. The mean values were: pH 7.37, organic carbon 21.15% dry matter, total nitrogen 1.092% dry matter, available phosphorus 140 mg/kg dry matter, available potassium 319 mg/kg dry matter, and electrical conductivity residue 232 mg/100 g. Heavy metal concentrations were also determined, with mean values of 0.16 mg/kg Cd, 39 mg/kg Cu, 37 mg/kg Cr, 689 mg/kg Mn, 28 mg/kg Ni, 49 mg/kg Pb, and 121 mg/kg Zn.

Poultry litter samples were analysed in triplicate. Mean values were: 51.86% moisture, 48.14% dry matter, 85.26% organic matter, pH 6.45, 4.89% total nitrogen, 115 mg/kg dry matter nitrate nitrogen, 11,244 mg/kg dry matter ammonium nitrogen, 1.56% phosphorus, 1.81% potassium, and electrical conductivity residue of 4,369 mg/100 g. Heavy metal concentrations were 0.46 mg/kg Cd, 40 mg/kg Cu, 28 mg/kg Cr, 325 mg/kg Mn, 19 mg/kg Ni, 9.6 mg/kg Pb, and 280 mg/kg Zn.

Poultry litter was incinerated at three temperatures: 400°C, 600°C, and 800°C. The resulting ash samples were analysed for moisture, dry matter, organic matter, pH, organic carbon, total nitrogen, phosphorus, potassium, electrical conductivity residue, and heavy metals.

A pot experiment was established using soil mixed with poultry litter ash at rates of 5, 15, and 25%. Separate mixtures were prepared for ash obtained at 400°C, 600°C and 800°C, resulting in ten experimental treatments, including the untreated control. The mixtures were analysed for pH, available phosphorus, available potassium, electrical conductivity residue, total nitrogen, organic carbon, and heavy metals.

RESULTS AND DISCUSSIONS

Manure flow in poultry complexes is directly influenced by the housing system. In floor-based systems, the poultry house functions both as a production space and as a temporary manure storage unit. During the cycle, fresh excreta are progressively transformed into solid litter through drying and mixing with bedding. This

reduces moisture and facilitates transport, storage, and subsequent use as fertilizer, compost feedstock, briquetting material, or combustion fuel.

In cage systems, manure is removed more frequently and remains wetter, which makes covered storage necessary and increases the need for careful management because of ammonia emissions, and nutrient losses. These differences show that manure handling cannot be separated from the housing technology itself. According to FAO data, manure production is approximately 80 kg/day per 1,000 broilers, 120 kg/day per 1,000 laying hens, 200-350 kg/day per 1,000 turkeys, and 150 kg/day per 1,000 ducks. These large volumes justify the development of efficient recovery pathways, particularly in areas where land available for direct manure application is limited (Tables 1-4). The poultry litter used in this study had a medium moisture content and a high organic matter concentration, indicating good potential both as an organic fertilizer and as an energy source. The concentrations of total nitrogen,

phosphorus, and potassium were substantial, confirming its agronomic value (Popescu et al., 2024; BAT, 2010; Ehler, 2017; Bernal et al., 2024). The moderate acidity and the high ammonium content suggest that unmanaged poultry litter may also pose environmental risks, especially through ammonia volatilization and nitrate losses.

Heavy metal concentrations in poultry litter were below the agricultural acceptance limits. The relatively higher contents of zinc, manganese, and copper may be associated with feed supplementation and bedding material. At the same time, these elements can act as useful micronutrients when litter is used in agriculture. Combustion considerably altered the chemical properties of the material. The ash showed very low moisture content, high dry matter, and alkaline pH. The pH increased with combustion temperature and reached the highest level at 800°C. Phosphorus and potassium were concentrated in the ash due to the removal of organic matter during combustion (Codling et al., 2002; Ervin et al., 2019; Kan et al., 2024).

Table 1. Chemical characteristics of the soil used in the experiment

Experimental soil	pH	Organic C	Nt	P _{AL}	P _{AL} *	K _{AL}	Conductometric residue
	%			mg kg ⁻¹ d.w.			mg/100 g
Mean of three repetitions	7.37	21.15	1.092	184	140	319	232

Table 2. Heavy metals content of the soil used in the experiment compared with heavy metal normal contents and alert threshold according to Order no. 756/1997

Experimental soil	Cd	Cu	Cr	Mn	Ni	Pb	Zn
	mg kg ⁻¹ d.w.						
Mean of three repetitions	0.16	39	37	689	28	49	121
Normal content	< 1.00	< 21	< 31	< 500	< 21	< 21	< 100
Alert threshold	> 3.00	> 100	> 100	> 1500	> 50	> 100	> 300

Table 3. Chemical characteristics of the poultry manure used in the experiment

Poultry manure	Umidity	Dry weight	Organic matter	pH	Nt	N-NO3*	N-NH4*	P	K	Conductometric residue
	%				%	mg kg ⁻¹ d.w.		%		mg/100 g
Mean of three repetitions	51.86	48.14	85.26	6.45	4.89	115	11244	1.56	1.81	4369

Table 4. Heavy metals content in poultry manure used in the experiment compared with heavy metal loading limits to be accepted in agriculture according to Order no. 344/2004

Poultry manure	Cd	Cu	Cr	Mn	Ni	Pb	Zn
	mg/kg d.w.						
Mean of three repetitions	0.46	40	28	325	19	9.6	280
Heavy metal loading limits to be accepted in agriculture	10	500	500	-	100	300	2000

Ash obtained at 800°C showed the highest pH, phosphorus, potassium, and electrical conductivity values. This suggests that higher combustion temperatures increase nutrient concentration but may also raise the risk of excessive alkalinity and salinity when ash is applied to soil. The literature also indicates that poultry litter ash contains significant amounts of phosphorus, potassium, calcium, and magnesium and may therefore act as both a fertilizer and a liming material, especially on acidic soils.

Heavy metal concentrations in the ash increased compared with raw poultry litter because combustion reduced the original mass. However, even after this concentration effect, the values remained below the accepted limits for agricultural use. This is important because heavy metal accumulation is one of the major constraints for waste-derived fertilizers.

The addition of poultry litter ash increased soil pH, available phosphorus, available potassium, and electrical conductivity. These effects were

more pronounced as the application rate increased from 5 to 25% and were strongest for ash obtained at 800°C.

The untreated soil had a pH of 7.42 (Table 5). After ash application, pH values increased up to 8.25 in the treatment with 25% ash obtained at 800°C. Available phosphorus increased from 184 mg/kg in the control to 469 mg/kg in the same treatment, while available potassium increased from 302 mg/kg to 9,400 mg/kg. Electrical conductivity residue also increased markedly, from 233 to 1,523 mg/100 g.

These findings demonstrate that poultry litter ash is highly effective in enriching soil with phosphorus and potassium. However, the strong increase in potassium and electrical conductivity at high application rates indicates a possible risk of salinity and phytotoxicity, particularly for seedlings and salt-sensitive crops. Therefore, ash should be applied in agronomic doses based on crop phosphorus demand, soil fertility status, and the chemical characteristics of the soil.

Table 5. Chemical characteristics of the soil samples from the experimental variants

Experimental variants	pH	P _{AL}	K _{AL}	Conductometric residue	Nt	Corg
		mg/kg d.w.		mg/100 g	%	%
V1 - Control	7.42	184	302	233	1.288	22.27
V2 - soil+5% ash obtained at 400°C	7.38	318	2220	532	1.445	20.43
V3 - soil+15% ash obtained at 400°C	7.46	367	3780	728	1.619	20.33
V4 - soil+25% ash obtained at 400°C	7.74	380	6920	1250	1.651	19.97
V5 - soil+5% ash obtained at 600°C	7.41	215	1460	431	1.398	21.77
V6 - soil+15% ash obtained at 600°C	7.49	254	2600	548	1.293	21.61
V7 - soil+25% ash obtained at 600°C	7.62	398	4240	768	1.625	22.01
V8 - soil+5% ash obtained at 800°C	7.57	299	2420	457	1.246	19.49
V9 - soil+15% ash obtained at 800°C	7.86	403	5960	877	1.397	20.53
V10 - soil+25% ash obtained at 800°C	8.25	469	9400	1523	1.398	21.88

Table 6. The concentration of total heavy metals (cadmium, lead, zinc and copper) in soil samples from the experimental variants

Experimental variants	Cd	Cu	Cr	Ni	Pb	Mn	Zn
mg/kg d.w.							
V1 - Control	0.177	41	37	30	66	900	125
V2 - soil+5% ash obtained at 400°C	0.256	53	34	30	63	830	177
V3 - soil+15% ash obtained at 400°C	0.296	59	35	27	47	758	204
V4 - soil+25% ash obtained at 400°C	0.374	70	33	26	33	742	227
V5 - soil+5% ash obtained at 600°C	0.215	47	37	29	48	825	160
V6 - soil+15% ash obtained at 600°C	0.258	43	34	28	47	822	143
V7 - soil+25% ash obtained at 600°C	0.223	45	35	29	54	843	155
V8 - soil+5% ash obtained at 800°C	0.300	50	35	32	44	800	181
V9 - soil+15% ash obtained at 800°C	0.319	54	34	28	41	769	205
V10 - soil+25% ash obtained at 800°C	0.500	66	35	28	33	765	280

The increase in pH may be beneficial on acidic soils, where ash could function both as a fertilizer and as a liming amendment. On neutral or alkaline soils, however, high or repeated applications may be problematic. This agrees with previous studies showing that poultry litter ash performs best when carefully matched to soil type and crop requirements.

Heavy metal concentrations in the soil-ash mixtures remained below alert and acceptance thresholds. Although Cd, Cu, and Zn values increased with ash rate, the final concentrations did not indicate an immediate pollution risk (Table 6). For example, Cd increased from 0.177 mg/kg in the control to 0.500 mg/kg in the highest ash treatment, while Zn increased from 125 mg/kg to 280 mg/kg. These values are still far below the cited limits for agricultural use.

This result supports the view that poultry litter ash can be safely used as a fertilizer material, provided that the original manure is monitored and the combustion process is controlled. Nevertheless, long-term field experiments are still needed to assess cumulative effects under repeated use.

From an agronomic perspective, poultry litter ash is especially valuable as a phosphorus- and potassium-rich fertilizer. Compared with raw poultry litter, ash has a lower mass, reduced volume, no biological hazard, lower potential, and easier transport and storage. In addition, combustion provides thermal energy or electricity and therefore contributes to both waste reduction and energy recovery.

From an environmental perspective, combustion decreases the biological risks associated with

raw manure and substantially reduces total residue volume. The use of ash in agriculture also supports nutrient recycling and reduces dependence on conventional mineral fertilizers. However, its successful use depends on efficient combustion, appropriate chemical characterization, nutrient-based application planning, and control of salinity, pH effects, and trace element concentrations.

CONCLUSIONS

Poultry manure management is closely linked to housing system and production cycle. In floor-based systems, manure is transformed into solid litter through mixing with bedding and drying during the cycle, whereas in cage systems it remains wetter and requires frequent removal and covered storage.

The poultry litter analysed in this study had high organic matter content and relevant concentrations of nitrogen, phosphorus, and potassium, confirming its dual value as a fertilizer resource and as an energy substrate. Combustion produced ash with low moisture, alkaline pH, and concentrated phosphorus and potassium contents.

Application of poultry litter ash to soil increased pH, available phosphorus, available potassium, and electrical conductivity. These effects demonstrate strong fertilizer potential, particularly on acidic soils and in phosphorus-deficient conditions. Heavy metal concentrations in poultry litter, ash, and soil-ash mixtures remained below the limits accepted for agricultural use.

Overall, poultry litter ash can be considered a valuable secondary fertilizer material within a circular economy approach. Its agricultural use should be based on controlled combustion, chemical characterization of the ash, and application rates adapted to crop nutrient requirements, soil properties, and salinity sensitivity.

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ADAPTATIVE RESPONSE OF FUNCTIONAL MICROBIOMES IN HEAVY METALS SOILS EXPOSED TO A PHYTOREMEDIATION PROCESS

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Abstract

The current context of land restoration and increasing the quality of urban ecosystems represent a challenge in the areas where historical pollution with heavy metals is present. The aim of this paper is to show the presence of adaptative mechanisms in the functional microbiome of 5 urban soils after a phytoremediation process. Total activities revealed that 3-7 times increase in the activity is visible in the first 72 hours of incubation and is almost double after 96 hours. This indicates an adapted microbiome to heavy metals conditions and its ability to develop as a community. An in-depth analysis reveals that carbohydrates and carboxylic acids were the most prominent guilds from the community, but with a distinct functional signature in each of the analyzed sites. Interestingly, polymers, amino acids and amines/amides guilds which represent almost 40% of the activity reported to the entire community, even though these guilds have a reduced functional profile. The results indicate that plant presence in polluted ecosystems represents a factor that enables the recovery of functional microbiome and maintenance of a high activity.

Key words: functional proliferation, heavy metal tolerance, site-specific activity, functional patterns, community assemblage.

INTRODUCTION

The changes in environmental conditions can modify the structure and functioning of soil microbial communities (Raj et al., 2017). In this context, maintaining ecosystem health requires the conservation of biodiversity and the continuous monitoring of changes at all trophic levels, including the microbial functions (Ojuederie et al., 2017). Soil microbes play a key role in element cycles, organic matter decomposition, energy flow, and soil structure maintenance (Ju et al., 2016; Borowik et al., 2018; Lombard et al., 2011).

In the category of environmental pollutants, heavy metals are known to disturb soil biological activity. These metals cause both biological and chemical soil degradation (Masindi et al., 2018), negatively impacting microbial populations and enzyme activity. They affect both microbial diversity, community functions, assemblage and soil biochemical processes (He et al., 2010; Kavamura et al., 2010).

The distribution of heavy metals in the environment is determined by environmental conditions and the specific characteristics of

metals (Khelifi et al., 2010). This type of pollution negatively affects plant growth and development. However, plants have developed various adaptation mechanisms, especially due to their rhizosphere microbiome (Mishra et al., 2017; Agarwal et al., 2024), that allow them to survive under stressful conditions in contaminated environments. Metallophytes are plants capable of tolerating high concentrations of heavy metals, have adapted to mineral substrates, including those rich in metals (Alford et al., 2010; Khan et al., 2009). In contrast, pseudometallophytes are plant species that can grow on both uncontaminated soils and soils polluted with heavy metals (Becerra-Castro et al., 2012).

Due to its safe and environmentally friendly nature, phytoremediation is an increasingly preferred method for cleaning up the environment. Genetic improvement of hyperaccumulator plants has led to an increase in their ability to retain metals, and sustainable approaches allow pollutants to be removed without compromising agricultural activities (Sharma et al., 2025). The accumulation of heavy metals in plants affects cellular processes and development, diminishing the effectiveness

of phytoremediation; therefore, assessing their impact is essential (Rasheed et al., 2020; Raza et al., 2021).

The aim of this research was to assess the functional assemblage of microbial communities from 5 sites supposed to three years of phytoremediation. The objectives were: i) to analyze the assemblage of functional microbiome during a period of 96 hours of incubation; ii) to assess the activity correlation trends within functional groups in 24 hours periods; iii) to explore the general direction of guild activities toward sites conditions.

MATERIALS AND METHODS

The methodological information presented here has been adapted and developed based on a previously published study by Pop et al., 2025. The daily dynamics of the functional soil microbiome were assessed using the Biolog EcoPlate system at five locations in the municipality of Baia Mare. The sites were chosen due to their historical pollution and were supposed to 3 years phytoremediation process. The investigated areas are identified by the following abbreviations: CR (Craica), CT (Colonia Topitorilor), FR (Fernezuiu), ROMP (Romplumb), and URB (Urbis). Soil samples were collected in 2023 from the long-term experiment initiated in 2019 (47°39' N, 23°34' E) in North-Western Romania, covering a total area of approximately 7.3 hectares of degraded industrial land. EcoPlate analyses were performed according to the methodology described by Weber et al. (2009), with substrates grouped according to chemical similarity. The soil suspension was diluted to 10^{-4} , and absorption was measured at 590 nm using a microplate reader. Monitoring was conducted over a period of five days until the plateau phase was reached, characterized by the stabilization of absorption values.

The Biolog EcoPlate™ method provides no details about the genetic composition of microbial communities, but highlights how bacteria use substrates and their metabolic potential in stressful environments, such as contaminated ones. Its simplicity, low cost, speed, and reproducibility make this method suitable for environmental monitoring as well. (Nemeth et al., 2021).

Data analysis was performed in the RStudio environment, and the flow was based on a general to specific range of tests (R Core Team, 2024). Non-Metric Multidimensional Scaling (NMDS) showed the differences between communities at different incubations times (Oksanen et al., 2022). These projections enabled the correlation between community stability, dispersion of data and the orientation of guild vectors.

At each of the 48, 72 and 96 hours of readings were extracted the Pearson correlations between functional groups and the most significant ones were used as a selection tool for the further analysis at site level. Each group that showed significant positive or negative correlation was analysed with ANOVA and LSD test for the assessment of both importance within community and the presence of differences between sites (de Mendiburu., 2023). The extraction of most correlated groups was performed due to their activity within the entire community and the number of links with other groups. This process enables both the analysis of the most important groups within the community and the real differences between the activity performed in one site.

RESULTS AND DISCUSSIONS

NMDS analysis (Figure 1, Table 1) shows how microbial communities at the five sites (CR, CT, FR, ROMP, and URB) are grouped according to how they use carbon sources, highlighting the influence of historical pollution in Baia Mare on soil metabolic activity. The most important factors explaining the differences between sites are AWCD and SUM, indicators of general metabolic activity. Their orientation along the NMDS1 axis indicates that the samples located on the left side of the graph, especially those from FR (Fernezuiu), show more intense metabolic activity and more efficient use of substrates compared to the other sites.

Carbohydrates (CH) and carboxylic (CX) acids are the main sources of energy for microorganisms, especially under conditions of chemical stress. In contrast, the use of amino acids (AA), amines (AM), and polymers is lower and occurs mainly in sites more affected by metal pollution, such as URB and CT. This pattern shows that although heavy metals affect

soil quality, microbial communities have adapted, maintaining certain metabolic functions essential for the circulation of energy and elements.

At 48 hours, most functional guilds show a significant correlation with the whole microbiome structure. The most significant relationships are observed for SUM, as for the AA, CH, and CX guilds, which present highly significant activity. AWCD indicates significant metabolic activity, suggesting a general intensification of substrate utilization. In contrast, the P guild does not show a statistically significant value which indicates a low level of contribution to the functional diversity of the microbiome.

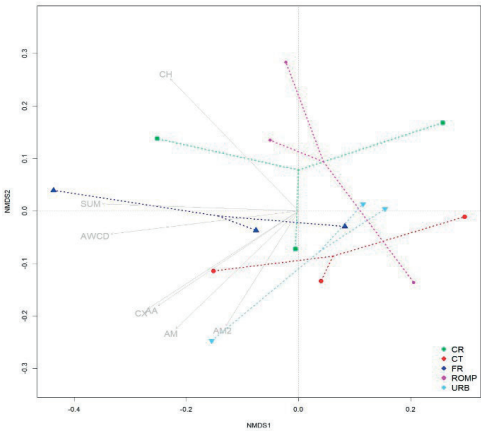


Figure 1. NMDS projection of functional microbiomes in the 5 targeted sites at 48 h

Table 1. Functional guild and groups traits in relation to the entire microbiome (48 h)

	NMDS1	NMDS2	r2	p.val	
AA	-0.9984	0.0566	0.8554	0.001	***
AM	-0.97809	0.20818	0.7059	0.002	**
AWCD	-0.9835	0.18092	0.5598	0.011	*
CH	-0.47266	-0.88124	0.787	0.001	***
CX	-0.97916	0.20308	0.8543	0.001	***
P	-0.72146	-0.69246	0.5008	0.072	.
SUM	-0.93325	-0.35924	0.9769	0.001	***

Note: ns - not significant activity; * - significant activity; ** - distinct significant activity; *** - very significant activity.

At this point of incubation, there is a visible large angle between vectors, with CH positioned toward the -/+ quadrant where points from FR and CR are visible, and CX, AA and AM vectors

that are positioned in the -/- quadrant. The last vectors indicate a strong association in the incipient community, with coupled activities and positive interactions. One interesting case is the CX and AA orientation toward the same direction, indicating a strong positive correlation and a synergic effect in the transformation of the community assemblage. The position of Sum vector is in the middle distance between the marginal vectors, suggesting that each of the guild activities could produce a significant shift toward CH or CX, AA and AM guilds.

The interpretation of the NMDS analysis (Figure 2, Table 2) highlights a clearly differentiated pattern among the sites in Baia Mare based on the metabolic capacity of microbial communities, emphasizing the major role of historical pollution and local environmental conditions. The sites located on the left side of the graphic, in the AWCD and SUM areas, especially the FR site and partially the URB site, show more intense biological activity and more efficient use of substrates, suggesting a functional adaptation of the microbiome to heavy metal-induced stress.

At the same time, carbohydrates and polymers are closely correlated and have a major influence on the functional structure of the communities at the CR site, indicating a metabolism oriented to the degradation of complex organic compounds. For the URB site, carboxylic acids and amines/amides play an important role, reflecting a more diverse metabolic profile, while the ROMP and CT sites are situated on the right side of the graphic, opposite the metabolic activity vectors, indicating an inhibition of enzymatic processes and reduced functional diversity. This distribution confirms that, although the soil microbiome has the ability to adapt, high levels of pollution at industrial sites significantly limit the metabolic and functional potential of microbial communities.

After 72 hours, the metabolic activity of the microbial community is more pronounced, with several variables that are highly significant. The SUM, AWCD, CH, and CX indicators show high r^2 coefficient values and significant levels, which suggests an intense use of the substrates and a metabolic diversification of the microbiome. The AA and AM guilds also

remain significant, which confirms their importance in the functional community structure. As in the previous table, P remains statistically insignificant, indicating a reduced influence on microbiome variation. Both the sum and AWCD vectors are positioned inside the angle formed by CH and CX vectors, indicating a similar impact of microbiome shift of both guilds inside communities. The dispersion of points is moderate, sustaining a future larger dispersion associated with the potential diversification of metabolism within communities, even at site level. Polymer guild vector is positioned outside CH, indicating both the association between those two guilds and a potential increase of this guild in the community.

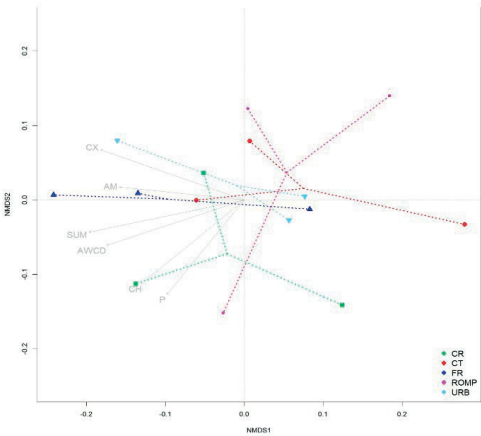


Figure 2. NMDS projection of functional microbiomes in the 5 targeted sites at 72 h

Table 2. Functional guild and groups traits in relation to the entire microbiome (72 h)

	NMDS1	NMDS2	r2	p.val	
AA	-0.81093	-0.58515	0.7284	0.001	***
AM	-0.69876	-0.71536	0.7537	0.002	**
AWCD	-0.99157	-0.12957	0.8725	0.001	***
CH	-0.67167	0.74085	0.8902	0.001	***
CX	-0.82171	-0.5699	0.8125	0.001	***
P	-0.93708	0.34912	0.4039	0.065	.
SUM	-0.99929	0.03768	0.9418	0.001	***

Note: ns – not significant activity; * - significant activity; ** - distinct significant activity; *** - very significant activity.

NMDS analysis (Figure 3, Table 3) based on microbial guilds shows that the distribution of sites in Baia Mare is closely related to the

metabolic capacity of bacterial communities exposed to heavy metal pollution. The FR and, partially, URB sites show the highest biological activity and functional diversity. Carbohydrates and carboxylic acids are the main functional groups that support metabolic activity in all analyzed sites. Guilds associated with amino acids and amines/amides are more strongly represented in sites with relatively stable functional activity, such as URB and CT. In contrast, the ROMP and CT sites are positioned opposite the metabolic activity vectors (SUM/AWCD), suggesting reduced enzymatic activity and limited functional potential in areas significantly affected by heavy metal pollution.

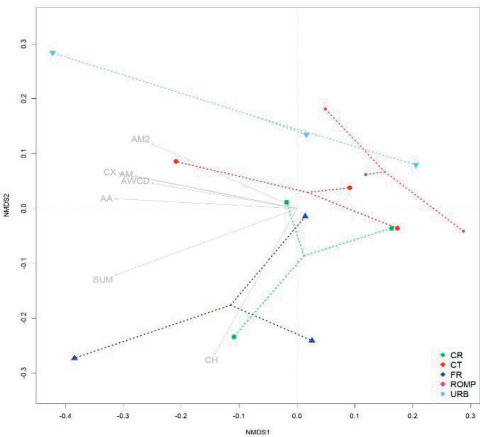


Figure 3. NMDS projection of functional microbiomes in the 5 targeted sites at 96 h

Table 3. Functional guild and groups traits in relation to the entire microbiome (96 h)

	NMDS1	NMDS2	r2	p.val	
AA	-0.9902	0.13965	0.2841	0.142	
AM	-0.99408	0.10864	0.5734	0.003	**
AWCD	-0.94402	-0.32988	0.7707	0.001	***
CH	-0.75725	-0.65312	0.6722	0.003	**
CX	-0.93796	0.34675	0.8508	0.001	***
P	-0.61248	-0.79049	0.5824	0.004	**
SUM	-0.97657	-0.21522	0.9184	0.001	***

Note: ns – not significant activity; * - significant activity; ** - distinct significant activity; *** - very significant activity.

At 96 hours, the highest metabolic activity is observed, with most variables showing significant or very significant correlations. SUM, AWCD, and CX have the highest r² values and very significant levels, which reflects

increased substrate use by the microorganism community. AM, CH, and P also show significant activity. The only insignificant variable is AA, which indicates that this functional group has a lower influence on microbiome diversity.

The entire sum of activities vector is oriented toward FR site, between CH and AA guild vectors, indicating that a change in these guilds activity will modify the entire activity of the microbiome. Most of the sites present dispersed communities, indicating a point-specific activity of the microbiome and a diversity of metabolic potentials within communities.

The values presented indicate how microbial communities at the five sites (CR, CT, FR, ROMP, and URB) utilize different carbon sources from the categories carbohydrates (CH), carboxylic acids (CX), polymers (P), and amino acids (AA) (Table 4). The FR (Ferneziiu) site has the highest values for most of the substrates analysed (CH1, CH3, CH6, CX4, AA5, and AA6), which suggests more intense metabolic activity and a higher capacity of the microbiome to use diverse carbon sources.

Table 4. Site-specific activity at 48 h based on group importance within community

Gro up	+CH1	+CH3	+CH6	+CX4	+P4	+AA 5	+AA6
CR	0.16± 0.02a	0.08± 0a	0.08± 0a	0.07± 0.01a	0.12± 0a	0.08± 0ab	0.08± 0ab
CT	0.15± 0.01a	0.07± 0a	0.07± 0a	0.06± 0a	0.11± 0a	0.07± 0ab	0.08± 0ab
FR	0.2±0. 04a	0.11± 0.03a	0.11± 0.03a	0.09± 0.02a	0.14± 0.03a	0.1±0 .02a	0.11± 0.02a
RO	0.13± 0.01a	0.06± 0a	0.06± 0a	0.05± 0a	0.09± 0a	0.07± 0b	0.07± 0b
MP	0.15± 0.04a	0.06± 0a	0.06± 0a	0.05± 0a	0.07± 0b	0.06± 0b	0.06± 0b
UR							
B							

Note: different letters indicate significant differences at $p < 0.05$ based on LSD test. Groups preceded by + indicate positive interactions.

In contrast, the ROMP and URB sites show lower values, especially for amino acids, which indicates a decrease in microbial activity under stress caused by heavy metals. For carbohydrates and carboxylic acids, the differences between sites are not as pronounced, meaning these substrates are still the main energy sources for microorganisms even when there's contamination. The results show that the metabolic activity of the microbiome is better sustained in the FR site, while in sites more severely affected by pollution, is observed a decline in the use of certain substrates.

Table 5 includes both positive (+) and negative (−) guilds, reflecting substrates that are

positively or negatively associated with microbial metabolic activity. The results indicate that the FR site has the highest values for most substrates in the positive guilds, particularly +CH1, +CH7, and +AA1, which confirms higher metabolic activity and greater functional potential of the microbial communities in this area.

In contrast, the ROMP and URB sites show lower values for some positive guilds, particularly +AA1 and +P4, which may indicate a reduced capacity of microorganisms to utilize specific substrates under severe heavy metal contamination conditions. The lower values observed for +P4 at all sites suggest that the degradation of polymeric substrates is generally limited, most likely due to the higher enzymatic requirements necessary for the decomposition of complex compounds.

Regarding the negative guilds (−AA4 and −CX7), the relatively higher values at some sites may indicate the presence of metabolic limitations or changes in substrate preference under environmental stress conditions. These trends highlight clear functional differences between microbial communities and suggest that the FR site has a higher adaptive capacity, maintaining higher metabolic activity even under conditions of historical contamination

Table 5. Site-specific activity at 72 h based on group importance within the community

Grou p	+CH1	+CH7	+P4	+AA1	−AA4	−CX7
CR	0.16±0.0 2a	0.08±0a	0.12±0a	0.08±0a b	0.75±0.0 1a	0.50±0.13 ab
CT	0.15±0.0 1a	0.07±0a b	0.11±0a b	0.07±0a b	0.70±0.0 6a	0.65±0.29 ab
FR	0.2±0.04 a	0.11±0.0 3a	0.14±0.0 3a	0.1±0.0 2a	0.90±0.2 0a	0.39±0.24 a
ROM	0.13±0.0 1a	0.06±0a	0.09±0a	0.07±0b	0.62±0.1 0a	0.52±0.20 b
P	0.15±0.0 4a	0.06±0a	0.07±0b	0.06±0b	0.62±0.0 2a	0.58±0.14 b
URB						

Note: different letters indicate significant differences at $p < 0.05$ based on LSD test. Groups preceded by + indicate positive interactions; groups preceded by − indicate negative interactions.

Table 6 shows the use of several substrates of the carbohydrate (CH7, CH10), carboxylic acid (CX3, CX4), and amine (AM1) groups by microbial communities. The results show that the FR site has the highest values for most substrates, such as CH7 and CX3, which indicates high metabolic activity and more functional diversity of the soil microbiome at this location.

In contrast, the ROMP site shows lower values for certain substrates, particularly CX3,

suggesting a decrease in microbial metabolic activity that may be associated with a higher level of heavy metal contamination. The lower utilization of carboxylic acids may reflect disruptions in microbial metabolic processes involved in the carbon cycle.

The use of amines (AM1) is more pronounced at the FR and URB sites, which may indicate the presence of microbial groups able to use alternative nitrogen-containing substrates as an adaptive response to environmental stress conditions (Krysenko et al., 2025).

The results suggest that despite heavy metal pollution influencing the metabolic functioning of the soil microbiome, microbial communities maintain to some degree their ability to utilize different carbon sources (Ma et al., 2022). However, the intensity of substrate utilization varies between sites, reflecting different levels of microbe community recovery and adaptation after remediation (Yin et al., 2024).

Table 6. Site-specific activity at 96 h based on group importance within the community

Site	+CH7	+CH10	+CX3	+CX4	+AM1
CR	1.34±0.24a	0.24±0.02a	1.67±0.15ab	0.36±0.15ab	0.34±0.13a
CT	0.99±0.07a	0.18±0.05a	1.90±0.20ab	0.22±0.07b	0.52±0.35a
FR	1.72±0.46a	0.39±0.12a	2.15±0.15a	0.74±0.19a	0.91±0.42a
ROMP	1.16±0.14a	0.30±0.03a	1.43±0.08b	0.25±0.02ab	0.36±0.07a
URB	1.04±0.11a	0.23±0.02a	1.88±0.21ab	0.39±0.17ab	0.72±0.38a
Site	-CH3	-CH9	-CX9	-AA4	-AA5
CR	0.81±0.28a	1.47±0.33a	0.91±0.51a	1.35±0.13a	0.82±0.36a
CT	0.46±0.10a	0.72±0.32b	1.02±0.41a	1.66±0.38a	1.08±0.28a
FR	0.62±0.13a	1.11±0.08ab	1.32±0.43a	1.56±0.33a	0.95±0.09a
ROMP	0.58±0.01a	1.16±0.18ab	1.35±0.60a	1.06±0.05a	0.92±0.27a
URB	0.42±0.16a	1.03±0.17ab	0.81±0.42a	1.54±0.13a	0.63±0.15a

Note: different letters indicate significant differences at $p < 0.05$ based on LSD test. Groups preceded by + indicate positive interactions; groups preceded by – indicate negative interactions.

Phytoremediation is an important example of bioremediation, based on the use of plants to accumulate, remove, or reduce the level of pollutants in the soil (Ali et al., 2013). The method is considered effective due to its direct application on site, the preservation of the biological and structural characteristics of the soil, and its significantly reduced costs, which are up to 100 times lower than those of physical-chemical methods (Stefani et al., 2015).

Improving phytoremediation is essential for limiting the bioavailability and transport of heavy metals to the aerial parts of plants, reducing the risk of biomagnification in the food chain. Plants adapted to heavy metal-contaminated soils exhibit specific survival

strategies and traits-such as tolerance to unfavorable soil conditions, extensive root systems, and high biomass production-that make them suitable for use in phytoremediation (Yang et al., 2005).

These locations have different levels of heavy metal contamination, mainly generated by mining and metallurgical activities, as well as urban development (Pop et al., 2026). The study results highlight that the phytoremediation applied to soils contaminated with heavy metals in Baia Mare affected the functional structure and metabolic activity of soil microbial communities. Analyses conducted with Biolog EcoPlate indicate differences between the sites investigated in the capacity of microorganisms to utilize different carbon sources, which reflects varying levels of adaptation to heavy metal stress.

The Ferneziu site (FR) shows the highest metabolic activity values and the most efficient carbon substrate utilization capacity, which suggests a more advanced functional restoration of the microbiome after the remediation process. This shows that, under specific conditions, microbial communities can develop effective mechanisms of adaptation and maintain active metabolic activity in soils that have historically suffered from pollution.

In contrast, sites such as Romplumb (ROMP) and, Colonia Topitorilor (CT) show lower values for the utilization of specific metabolic substrates, especially amino acids and carboxylic acids, which indicates that these sites are still affected by contamination and that microbiological remediation is less advanced. Limited use of more complex substrates may indicate restrictions in the enzymatic activity and functional diversity of microbial communities (Kandeler et al., 1996).

The results confirm that, in the post-remediation phase, the soil microbiome can maintain important metabolic functions, especially the use of carbohydrates and other simple carbon sources, which are the major energy substrates for microorganisms under environmental stress. Overall, the study highlights that phytoremediation contributes to the gradual restoration of soil microbiome function, but the level of recovery is different from site to site, based on the initial level of contamination and local environmental conditions (Sekhohola-

Dlamini et al., 2022). Functional microbial community analysis is a useful tool in the evaluation of the effectiveness of remediation processes for heavy metal-contaminated soils. Colonia Topitorilor (CT) sites recorded lower values for the use of some substrates, especially amino acids and carboxylic acids, suggesting a slower microbiological remediation.

CONCLUSIONS

Phytoremediation used in soils contaminated with heavy metals in Baia Mare impacted the functional structure and metabolic activity of microbial communities. Biolog EcoPlate analyses highlighted differences between sites in terms of carbon source utilization, which indicated different levels of adaptation to heavy metal stress. The Ferneziu (FR) site showed the highest metabolic activity and the most efficient use of substrates, suggesting a more advanced functional restoration of the soil microbiome. In contrast, the Romplumb (ROMP) and Colonia Topitorilor (CT) sites recorded lower values for the use of some substrates, especially amino acids and carboxylic acids, suggesting a slower microbiological remediation.

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ORGANO-MINERAL FERTILIZATION ON THE MORPHOLOGICAL AND BIOMETRIC TRAITS OF SUGAR BEET (*Beta vulgaris* L.) UNDER THE ECOPEDOLOGICAL CONDITIONS OF COVASNA COUNTY

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Abstract

The application of organo-mineral fertilization in sugar beet cultivation stimulates plant growth and development, reflected by significant changes in their appearance and structural parameters, as a result of optimized nutrient availability and uptake. This study aims to evaluate the effects of different fertilization schemes on the development and biometric performance of three sugar beet hybrids under the ecopedological conditions of Covasna County during the 2022-2023 and 2023-2024 growing seasons. The experimental treatments consisted of three sugar beet hybrids (Darvas, Deseda, and Tatry) combined with different fertilization schemes, including varying doses of manure, nitrogen (N), phosphorus (P), and potassium (K). All applied fertilization schemes increased crown length and diameter, root weight, tuber yield, and sugar content compared to the untreated control, regardless of hybrid or climatic conditions. During the 2022-2023 growing season, the Tatry hybrid recorded the highest yield (60.05 t/ha) with a sugar content of 18.27°S, while in 2023-2024, the Darvas hybrid achieved the highest yield (85.79 t/ha) and a sugar content of 19.02°S across the different fertilization schemes.

Key words: climatic conditions, fertilization, hybrid, plant traits, sugar beet.

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is a strategically important crop for sucrose production in temperate regions, contributing substantially to both agricultural economies and the food industry. Achieving high root yield while maintaining elevated sugar concentration remains a major agronomic objective, as these two traits jointly determine sugar yield and processing efficiency. Crop performance in sugar beet is strongly influenced by genotype, climatic conditions, soil properties, and crop management practices, among which fertilization plays a decisive role (Draycott, 2006; Milford & Watson, 1971; Hoffmann et al., 2020; Kenter & Hoffmann, 2021). Recent studies emphasize that optimized nutrient management is essential for improving productivity while maintaining sugar quality under increasingly variable climatic conditions (Hoffmann et al., 2018; Safina et al., 2022). Nitrogen fertilization is a key factor governing sugar beet growth and yield formation.

Adequate nitrogen supply promotes leaf area development, photosynthetic capacity, and biomass accumulation, thereby enhancing root yield. However, the effect of nitrogen on sugar yield and root quality is strongly dose-dependent. Excessive nitrogen application can negatively affect sugar concentration and technological quality by increasing amino-N and other non-sucrose impurities in the root, ultimately reducing sugar extraction efficiency (Draycott & Christenson, 2003; Milford et al., 1988; Hoffmann & Kluge-Severin, 2011; Zarski et al., 2020). Consequently, optimizing nitrogen rates according to crop demand and soil nitrogen supply is essential to balance yield and quality in sugar beet production systems (Hoffmann et al., 2018; Pulkrábek et al., 2022).

Phosphorus and potassium are equally important macronutrients in sugar beet nutrition. Phosphorus supports early root development, energy transfer, and metabolic activity, while potassium plays a crucial role in carbohydrate transport, osmotic regulation, enzyme activation, and nitrogen utilization. Adequate

potassium availability has been associated with increased root yield, improved sugar accumulation, and enhanced tolerance to abiotic stress, particularly under conditions of intensive nitrogen fertilization (Christenson & Draycott, 2001; Johnston et al., 1999; Koch et al., 2020; Wakeel et al., 2021). Balanced N–P–K fertilization schemes are therefore widely recognized as fundamental for sustaining both yield and quality in sugar beet cultivation (Kenter et al., 2016; Hoffmann et al., 2020).

In recent years, increasing attention has been directed toward the use of organic fertilizers, such as farmyard manure, either alone or in combination with mineral fertilizers. Organic amendments contribute to improved soil structure, increased microbial activity, enhanced nutrient buffering capacity, and improved long-term nutrient availability, which may positively influence crop growth and yield stability (Edmeades, 2003; Diacono & Montemurro, 2010; Lazcano et al., 2018; Diacono et al., 2019). The integration of organic and mineral fertilizers has been shown to provide synergistic effects by combining the immediate nutrient availability of mineral sources with the long-term soil fertility benefits of organic inputs (López-Bellido et al., 2012; Chen et al., 2020; Safina et al., 2022).

Previous studies have demonstrated that organo-mineral fertilization strategies can improve root yield, sugar content, and overall nitrogen use efficiency compared to exclusive mineral or organic fertilization, particularly when nutrient rates are adjusted to crop demand and soil fertility status (Hoffmann & Kenter, 2018; Safina & Abou-Shanab, 2019; Pulkrábek et al., 2022). Nevertheless, the magnitude of these effects varies depending on fertilization rate, nutrient combination, soil conditions, and genotype, highlighting the need for region-specific and hybrid-specific research under field conditions.

Therefore, the objective of the present study was to evaluate the effects of different fertilization schemes, including organic, mineral, and organo-mineral fertilization, on morphological traits, yield components, and sugar content of sugar beet hybrids under field conditions over a two-year study period. The obtained results aim to contribute to the optimization of fertilization

strategies for sustainable and economically efficient sugar beet production.

MATERIALS AND METHODS

The field experiment was conducted in 2023 and 2024 in the locality of Zăbala, Covasna County, Romania, on a moderately acidic soil with a pH of 6.13, which is below the optimal value for sugar beet cultivation.

The field experiment was organized as subdivided plots with 4 replications being of type 3 x 6, with the following experimental factors:

Factor A – hybrids, with 3 graduations:

- a1. Darvas;
- a2. Deseda;
- a3. Tatry.

Factor B – fertilized with organic and mineral fertilizers, with 6 graduations:

- b1. 20 t/ha farmyard manure;
- b2. 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K;
- b3. 10 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K;
- b4. 10 t/ha farmyard manure;
- b5. 150 kg/ha N + 150 kg/ha P + 150 kg/ha K;
- b6. unfertilized control.

The **DARVAS** hybrid is a new, robust sugar beet hybrid characterized by high tolerance to foliar diseases and abiotic stress factors, including drought. The hybrid demonstrates stable performance in yield and root quality, making it suitable for cultivation under variable environmental conditions and ensuring consistent and efficient production.

The **DESEDA** hybrid is a sugar beet hybrid characterized by high sugar content, high yield per hectare, and excellent tolerance to cercospora (*Cercospora beticola*). The hybrid demonstrates stable performance in yield and root quality, making it suitable for cultivation under variable environmental conditions and ensuring consistent and efficient production.

The **TATRY** hybrid is a sugar beet hybrid characterized by high root yield and superior sugar content. It exhibits an increased juice purity coefficient, making it optimal for industrial processing. The hybrid shows high tolerance to cercospora leaf spot (*Cercospora*

beticola) and abiotic stress factors, including drought and temperature fluctuations. Its agronomic characteristics make it suitable for stable and efficient production, even under suboptimal environmental conditions.

The hybrids were sown on 13th of May in 2023 and 2nd of May in 2024 using a precision planter on 6 rows, with a row spacing of 45 cm and a sowing depth of 5 cm, under controlled field conditions.

Following sowing, the first herbicide application was conducted for weed management using Betanal Tandem 390 SC (MCPA 250 g/l + Phenmedipham 140 g/l) at a rate of 1 l/ha, Venzar 500 SC (Bentazone 500 g/l) at a rate of 0.3 l/ha and Safari (Diflufenican 500 g/kg) at a rate of 30 g/ha. The treatment targeted annual dicotyledonous and monocotyledonous weeds, as well as susceptible perennial broadleaf weeds. One week after the initial application, the herbicide treatment was repeated at the same rates to ensure uniform and effective weed control under field conditions.

The experimental plot area for each treatment was 16.2 m², corresponding to six rows of sugar beet, with a row spacing of 45 cm (2.7 m) and a row length of 6 m.

Crown length (cm), crown diameter (cm), and root weight (g) were measured on two representative plants from each treatment and replication

Sugar beet was harvested on 13th of November in 2023 and 24th of November in 2024; the root weight of each plot and replication was determined, and the yield per hectare was subsequently calculated. Sugar content was analyzed on 11th of December in 2023 and 4th of December in 2024 at the Laboratory of Technology and Good Agricultural Practices of the National Institute of Research and Development for Potato and Sugar Beet Braşov (NIRDPSB). Statistical analyses were performed using Microsoft Excel. The experimental data were subjected to analysis of variance (ANOVA), and treatment means were compared with the unfertilized control using the Least Significant Difference (LSD) test at the 5%, 1%, and 0.1% probability levels. The levels of statistical significance are indicated as follows: *significant ($p < 0.05$), **distinctly significant ($p < 0.01$), and ***very significant ($p < 0.001$).

RESULTS AND DISCUSSIONS

Root collar length

The root collar length was determined on 13th November 2023 (Table 1). Thus, the Darvas hybrid recorded very highly significant values compared to the unfertilized control in all treatments fertilized with organic fertilizer or with a combination of organic and mineral fertilizers. In the case of the treatment fertilized exclusively with mineral fertilizer, namely 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a1b5), no significant differences were recorded.

For the Deseda hybrid, the lowest mean value of root collar length was identified in the treatment fertilized with 10 t/ha farmyard manure (a2b4), reaching 2.45 cm, this value being 0.1 cm lower than that of the unfertilized control. All other treatments fertilized with standard organo-mineral fertilizer doses showed no significant differences compared to the control treatment.

Compared to the Deseda hybrid, the Tatry hybrid recorded very highly significant differences in terms of root collar length in the treatment fertilized with 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a3b2), and distinctly significant differences in the treatment fertilized with 20 t/ha farmyard manure (a3b1). All other experimental treatments showed no significant differences compared to the unfertilized control. In contrast to the year 2023, in 2024, on 24th November, very highly significant differences in root collar length values were recorded for all three studied hybrids in the treatments fertilized with 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K and with 20 t/ha farmyard manure (Table 1).

Regarding the treatments fertilized with 10 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, these recorded distinctly significant values in the case of the Darvas hybrid and very highly significant values in the case of the Deseda and Tatry hybrids.

Significant differences compared to the unfertilized control were identified in the treatments fertilized with 10 t/ha farmyard manure in the Darvas (a1b4) and Tatry (a3b4) hybrids, as well as in the treatment fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K in the Darvas hybrid (a1b5). Non-significant differences compared to the unfertilized control

were identified in the treatments fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K in the Deseda (a2b5) and Tatry (a3b5) hybrids.

Table 1. Root collar length (cm)

2023						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	4.48***	2.00	2.68	0.13	3.88**	1.13
b2	4.58***	2.10	3.13	0.58	4.15***	1.40
b3	4.40***	1.93	3.05	0.50	3.40	0.65
b4	3.80***	1.33	2.45***	-0.10	3.08	0.33
b5	2.85	0.38	2.63	0.08	3.58	0.83
b6	2.48	Mt	2.55	Mt	2.75	Mt
DI 5%= 0.661						
DI 1%= 0.882						
DI 0.1%= 1.155						
2024						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	4.98***	1.18	4.85***	1.13	4.83***	1.35
b2	5.35***	1.55	5.10***	1.38	5.15***	1.68
b3	4.53***	0.73	4.68***	0.95	4.43***	0.95
b4	4.35*	0.55	4.65***	0.93	4.15*	0.68
b5	4.30*	0.50	4.15	0.43	3.85	0.38
b6	3.80	Mt	3.73	Mt	3.48	Mt
DI 5%= 0.515						
DI 1%= 0.688						
DI 0.1%= 0.901						
*** - very significant; ** - distinctly significant, * - significant						

Root collar diameter

The root collar diameter recorded the highest value in 2024 in the treatment fertilized with 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K in the Darvas hybrid (a1b2), reaching 7.35 cm. This value was 1.83 cm higher than the maximum value recorded in 2023, namely 5.53 cm, observed in the Tatry hybrid fertilized with the same organo-mineral fertilizer dose. Regarding the minimum value identified for root collar diameter in 2024, it was 5.23 cm, differing by only 0.3 cm from the maximum value recorded in 2023 (Table 2). In contrast, in 2023, the root collar diameter showed very highly significant differences compared to the unfertilized control for each treatment, with two exceptions: the treatment fertilized with 10 t/ha farmyard manure in the Deseda hybrid (a2b4), where the difference was distinctly significant, and the Tatry hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a3b5), where the difference was significant (Table 2).

Analyzing the two study years, these did not show the same uniformity in terms of differences between experimental treatments and their respective controls. Thus, very highly significant differences were evident in treatments fertilized with the maximum dose of organic fertilizer or with combinations of organic and mineral fertilizers. An exception was observed in the Darvas hybrid fertilized with 20 t/ha farmyard manure (a1b1) and in the treatment fertilized with 10 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a1b3), where the differences were distinctly significant and positive.

Significant differences were recorded in the Darvas and Tatry hybrids fertilized with 10 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, as well as in the Deseda hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K.

The Darvas and Tatry hybrids fertilized exclusively with organic fertilizer did not show significant differences.

Table 2. Root collar diameter (cm)

2023						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	5.3***	2.40	4.65***	1.55	4.65***	0.98
b2	5.5***	2.60	4.85***	1.75	5.53***	1.85
b3	5.1***	2.20	4.45***	1.35	4.68***	1.00
b4	4.55***	1.65	3.58**	0.48	4.30***	0.63
b5	3.95***	1.05	3.80***	0.70	4.08*	0.4
b6	2.90	Mt	3.10	Mt	3.68	Mt
DI 5%= 0.323						
DI 1%= 0.431						
DI 0.1%= 0.564						
2024						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	6.78**	1.13	6.63***	1.40	6.85***	1.35
b2	7.35***	1.70	7.35***	2.13	7.20***	1.70
b3	6.65**	1.00	6.60***	1.38	6.90***	1.40
b4	6.40*	0.75	6.13**	0.90	6.35*	0.85
b5	5.93	0.28	6.10*	0.88	5.88	0.38
b6	5.65	Mt	5.23	Mt	5.50	Mt
DI 5%= 0.671						
DI 1%= 0.895						
DI 0.1%= 1.172						
*** - very significant; ** - distinctly significant, * - significant						

Plant weight

From an analytical perspective, across the two study years, the highest mean plant weight was

recorded in the treatment fertilized with 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K for the Darvas hybrid (a1b2). In 2023 (Table 3), Darvas represented the hybrid of extremes, also showing the lowest plant weight in the unfertilized control (302.11 g).

Table 3. Plant weight (g)

2023						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	1193.35 ***	891.2 5	846.37** *	385.97 3	1006.03 ***	467.6 8
b2	1318.20 ***	1016. 1	1063.88 *	603.48 8	1126.59 ***	588.2 4
b3	804.94** *	502.8 3	744.623 *	284.23	896.91** *	358.5 6
b4	732.78** *	430.6 7	523.74 *	63.35	805.56** *	267.2
b5	501.49** *	199.3 8	477.87 *	17.475	701.79** *	163.4 4
b6	302.105	Mt	460.39	Mt	538.35	Mt
DI 5%= 99.205 DI 1%= 132.476 DI 0.1%= 173.391						
2024						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	1445.03 ***	636.7 8	1417.13 ***	625.35	1248.15 ***	431.6 5
b2	1715.9** *	907.6 5	1588.73 ***	796.95	1485.08 ***	668.5 8
b3	1264.45 ***	456.2 *	1196.4** *	404.63	1202.78 ***	386.2 8
b4	1077.18 ***	268.9 3	1020.1** *	228.33	1074.25 ***	257.7 5
b5	973.03** *	164.7 8	906.98** *	115.2	939.65** *	123.1 5
b6	808.25	Mt	791.78	Mt	816.5	Mt
DI 5%= 62.127 DI 1%= 85.027 DI 0.1%= 116.477						
*** - very significant; ** - distinctly significant, * - significant						

From a statistical standpoint, in 2023, all fertilization treatments of the Darvas hybrid showed very highly significant differences compared to the unfertilized control. A similar trend was observed for the Tatry hybrid, except that it presented one treatment with distinctly significant differences relative to the control, specifically the treatment fertilized exclusively with mineral fertilizer (a3b5). The differences recorded for the Darvas hybrid in treatments with 10 t/ha farmyard manure

(a2b4) and 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a2b5) were not significant compared to the unfertilized control.

Regarding 2024, compared to 2023, it was uniform in terms of the statistical significance of differences among the experimental treatments, all of them being very highly significant. The only treatment standing out in terms of plant weight, showing distinctly significant differences compared to the unfertilized control, was the Deseda hybrid fertilized exclusively with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (Table 3).

Storage root yield

The maximum storage root yield reached 85.79 t/ha in 2024 for the Darvas hybrid fertilized with 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a1b2), which was 25.74 t/ha higher than the maximum value recorded in 2023, namely 60.05 t/ha for the Tatry hybrid fertilized with the same organo-mineral fertilizer dose (a3b2).

Regarding the minimum yield values, it can be stated that 2023 was a much more balanced year in terms of storage root production (t/ha), with the minimum value being 49.25 t/ha, resulting in a difference of 10.8 t/ha between the maximum and minimum values (Table 4).

Analyzing 2024, the difference between the minimum value of 2023 and that of 2024 (39.59 t/ha) was 9.67 t/ha, while the difference between the two extreme values in 2024 reached 46.21 t/ha, which highlights the positive influence of the fertilization dose on all yield components, ultimately leading to the achievement of considerably higher total yields.

From a statistical point of view, in 2023, for each hybrid, the treatments fertilized with 20 t/ha farmyard manure, 20 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K, and 10 t/ha farmyard manure + 150 kg/ha N + 150 kg/ha P + 150 kg/ha K recorded very highly significant differences compared to the unfertilized control. Distinctly significant differences were observed in the Darvas hybrid fertilized with 10 t/ha farmyard manure (a1b4) and in the Tatry hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a3b5) (Table 4).

Additionally, significant differences were reported only for the Darvas hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K

(a1b5) and for the Deseda hybrid fertilized with 10 t/ha farmyard manure (a2b4). In 2023, only one treatment showed non-significant differences in storage root yield compared to the unfertilized control, namely the Deseda hybrid fertilized with 150 kg/ha N + 150 kg/ha P + 150 kg/ha K (a2b5).

Table 4. Storage root yield (t/ha)

2023						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	57.88***	8.55	56.80***	6.00	58.78***	9.53
b2	59.43***	10.10	59.68***	8.88	60.05***	10.80
b3	55.23***	5.90	56.90***	6.10	58.18***	8.93
b4	52.70**	3.38	53.58*	2.78	53.88***	4.63
b5	51.88**	2.55	52.18	1.38	52.68**	3.43
b6	49.33	Mt	50.80	Mt	49.25	Mt
DI 5%= 2.161 DI 1%= 2.886 DI 0.1%= 3.778						
2024						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	72.25***	31.84	70.85***	31.27	62.41***	21.58
b2	85.79***	45.38	79.43***	39.85	74.25***	33.43
b3	63.22***	22.81	59.82***	20.23	60.14***	19.31
b4	53.86***	13.45	51.00***	11.41	53.71***	12.89
b5	48.65***	8.24	45.35**	5.76	47.06***	6.23
b6	40.41	Mt	39.59	Mt	40.82	Mt
DI 5%= 3.101 DI 1%= 4.243 DI 0.1%= 5.811						
*** - very significant; ** - distinctly significant, * - significant						

Sugar content

In 2023, the highest mean sugar content at the treatment level was recorded for the Tatry hybrid fertilized with 20 t/ha of farmyard manure combined with 150 kg/ha N, 150 kg/ha P, and 150 kg/ha K (a3b2), reaching a value of 18.27%. All analyzed hybrids under the five fertilization treatments showed very significant differences compared to their corresponding unfertilized control (Table 5).

In 2024, the highest mean sugar content was observed for the Darvas hybrid fertilized with 20 t/ha of farmyard manure combined with 150 kg/ha N, 150 kg/ha P, and 150 kg/ha K (a1b2), with a value of 19.02% (Table 5).

The comparative analysis of the two experimental years (2023 and 2024) revealed that the majority of the differences in sugar

content between the fertilized treatments and the unfertilized control were very highly significant. Nevertheless, two experimental treatments showed non-significant differences compared to the control, namely the Darvas hybrid fertilized with 10 t/ha of farmyard manure (a1b4) and the treatment fertilized with 150 kg/ha N, 150 kg/ha P, and 150 kg/ha K (a1b5).

Table 5. Sugar content (%)

2023						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	17.04***	3.01	18.07***	4.03	17.38***	2.97
b2	17.35***	3.32	17.39***	3.34	18.27***	3.86
b3	16.55***	2.53	16.48***	2.44	17.28***	2.86
b4	16.24***	2.22	15.59***	1.55	16.29***	1.88
b5	15.17***	1.14	15.05***	1.01	15.25***	0.84
b6	14.03	Mt	14.04	Mt	14.41	Mt
DI 5%= 0.161 DI 1%= 0.216 DI 0.1%= 0.282						
2024						
Variant	a1-DARVAS		a2-DESEDA		a3-TATRY	
	Value (cm)	Difference	Value (cm)	Difference	Value (cm)	Difference
b1	18.64***	1.42	17.82***	3.46	18.36***	2.40
b2	19.02***	1.80	18.48***	4.12	18.45***	2.49
b3	18.35***	1.13	17.50***	3.14	18.09***	2.13
b4	17.60	0.38	16.92***	2.56	18.05***	2.09
b5	17.44	0.22	15.86***	1.50	17.33***	1.37
b6	17.22	Mt	14.36	Mt	15.96	Mt
DI 5%= 0.493 DI 1%= 0.658 DI 0.1%= 0.862						
*** - very significant; ** - distinctly significant, * - significant						

CONCLUSIONS

The results demonstrated that organo-mineral fertilization had a positive effect on both the morphological characteristics and productive performance of sugar beet under the ecopedological conditions of Covasna County. The combined application of farmyard manure

and mineral fertilizers improved root collar dimensions, plant weight, storage root yield, and sugar content compared with the unfertilized control.

Among the studied fertilization schemes, the application of 20 t/ha farmyard manure combined with 150 kg/ha N, 150 kg/ha P, and 150 kg/ha K produced the most favorable results. The highest values of the analyzed parameters were consistently obtained by the Darvas hybrid under this fertilization regime, regardless of the study year.

Maximum storage root yield and sugar content were achieved in the Darvas and Tatry hybrids receiving the highest organo-mineral fertilizer dose, confirming the beneficial interaction between genotype and integrated nutrient management.

Overall, the findings highlight the effectiveness of organo-mineral fertilization as a sustainable strategy for improving sugar beet productivity and quality. The study contributes to the optimization of fertilization practices under local environmental conditions and supports the wider adoption of integrated nutrient management in sugar beet cultivation.

The obtained results demonstrate the synergy of organo-mineral fertilization, which led to significant improvements in morphological traits and production parameters compared to exclusive organic or mineral fertilization.

In this context, organo-mineral fertilization represents an agronomically and economically efficient solution for sugar beet cultivation, contributing to increased yield, improved economic efficiency, and enhanced practical relevance for farmers.

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MODERN NITROGEN FERTILIZATION STRATEGIES FOR IMPROVING EFFICIENCY AND GRAIN QUALITY IN WINTER WHEAT

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Abstract

*Efficient nitrogen (N) management is essential for improving yield and grain quality in winter wheat (*Triticum aestivum* L.). This study evaluated the effects of different spring-applied nitrogen fertilization strategies on grain yield, nitrogen use efficiency, and quality parameters of winter wheat. Field experiments compared conventional mineral fertilization based on urea (U46) and ammonium nitrate (AN34) with advanced fertilization systems, including a controlled-release nitrogen fertilizer (NS27) and a liquid nitrogen fertilizer (NS19). Grain yield, partial factor productivity of nitrogen (PFP-N), nitrogen content in grain and straw, and quality traits such as thousand-kernel weight, test weight, protein content, and gluten content were assessed. Controlled-release nitrogen applied at the onset of spring regrowth achieved grain yields comparable to conventional fertilization while significantly increasing partial factor productivity of nitrogen (PFP-N) and reducing nitrogen accumulation in straw. Late application of controlled-release nitrogen enhanced grain protein and gluten content without compromising yield. Liquid nitrogen fertilization showed relatively high nitrogen efficiency but resulted in lower yields when not combined with nitrate-based nitrogen sources.*

Key words: controlled-release fertilizer, liquid nitrogen fertilizer, nitrogen fertilization, nitrogen use efficiency, low nitrogen input.

INTRODUCTION

Nitrogen is one of the most important nutrients for cereal crops, playing a key role in both yield formation and grain quality. The application of nitrogen almost doubles the global average wheat yield (Yokamo et al., 2023; Guo et al., 2022). However, nitrogen use efficiency largely depends on the proportion of applied nitrogen that is effectively taken up by plants and contributes to grain production (Ma et al., 2019; Yokamo et al., 2023).

The type of fertilizer used and the method of its application significantly influence nitrogen uptake and utilization by plants and represent a major concern in modern agriculture (Raus et al., 2016; Allart et al., 2023). Under current conditions, research increasingly focuses on improving the efficiency of fertilizer nitrogen use and reducing nitrogen application rates without compromising crop productivity or economic performance (Hirel et al., 2007; Raus et al., 2025; Walsh, 2018).

The key issue for both farmers and researchers are the capacity of crops to convert available nitrogen into agricultural production, which

represents a fundamental indicator of fertilization system performance (Moll et al., 1982; Raun & Johnson, 1999; Govindasamy et al., 2023). Numerous studies have shown that nitrogen use efficiency tends to decline with increasing nitrogen application rates, as plant capacity to absorb and utilize nitrogen becomes limited once nitrogen supply exceeds crop physiological requirements (Cassman et al., 2002; Yokamo et al., 2023). Consequently, optimizing fertilization strategies is essential for maintaining a balance between yield, nutrient use efficiency, and environmental sustainability. In practice, a substantial proportion of applied nitrogen is not utilized by plants and may be lost through processes such as leaching, denitrification, volatilization, or microbial immobilization (Galloway et al., 2008; Guo et al., 2022; Raus et al., 2025; Govindasamy et al., 2023). These processes reduce fertilization efficiency and may contribute to groundwater contamination and greenhouse gas emissions (Robertson & Vitousek, 2009; Raun & Johnson, 1999; Cassman et al., 2002).

Adjusting fertilizer forms, application timing, and fertilization methods can improve the

synchronization between nitrogen availability and crop demand, allowing reductions in total nitrogen input without affecting yield (Yokamo et al., 2023; Congreves et al., 2021). In addition to the total nitrogen rate applied, nitrogen use efficiency is also strongly influenced by the chemical form of the fertilizer and the timing of application during the crop growth cycle.

Different nitrogen forms exhibit distinct behavior in soil, which determines the rate of transformation and nutrient availability for plants. For example, amide nitrogen from urea must undergo hydrolysis in soil before becoming available to plants, whereas nitrate nitrogen can be readily absorbed by roots (Cui et al., 2022). The timing of nitrogen application is equally important because nutrient availability must coincide with critical stages of crop development.

In winter wheat, nitrogen fertilization is commonly applied in split doses during spring, particularly at the tillering stage, stem elongation, and grain formation stages. This strategy improves nitrogen utilization efficiency and may reduce nutrient losses from the soil-plant system (Ma et al., 2019; Anas et al., 2020; Raus et al., 2016).

The development of modern fertilizers has increasingly focused on improving nitrogen use efficiency and reducing nutrient losses (Ren et al., 2024; Vejan et al., 2021; Raus et al., 2026; Bolohan et al., 2023). Controlled-release fertilizers and liquid fertilizers are currently considered promising alternatives to conventional fertilization systems based on urea or ammonium nitrate (Cui et al., 2022; Jariwala et al., 2022).

Controlled-release fertilizers can ensure a gradual nitrogen release in soil, contributing to improved synchronization between nutrient availability and crop demand throughout the growing season (Shaviv, 2001). Liquid fertilizers, on the other hand, can provide rapid nutrient availability when crop demand is highest and may also be effective under conditions of limited soil moisture (Milyutkin et al., 2022; Raus et al., 2025; Golotov et al., 2024).

However, results reported in the literature regarding the efficiency of these fertilizers are not always consistent. Crop response largely depends on pedoclimatic conditions, soil properties, and fertilization strategies (Raun &

Johnson, 1999; Moll et al., 1982; Raus et al., 2026). Comparative studies evaluating the performance of controlled-release and liquid fertilizers relative to conventional fertilization systems remain limited under many agroecological conditions (Raus et al., 2016).

In this context, evaluating different nitrogen fertilization strategies under field conditions becomes essential for identifying efficient nutrient management solutions.

The main objective of this study was to evaluate the effect of different spring-applied nitrogen fertilization strategies on grain yield, nitrogen use efficiency, and quality parameters of winter wheat. The study aimed to compare conventional mineral fertilization with fertilization systems based on controlled-release and liquid fertilizers in order to identify strategies capable of improving nitrogen use efficiency without compromising grain yield or quality.

The working hypothesis was that the use of controlled-release fertilizers or fertilizers containing both nitrogen and sulfur could improve the synchronization between nitrogen availability and crop physiological demand, leading to more efficient nitrogen utilization and maintaining high yields even under reduced nitrogen application rates. To test this hypothesis, the study evaluated the performance of different spring nitrogen fertilization strategies in winter wheat grown under the excessive continental climatic conditions specific to northeastern Romania by comparing conventional mineral fertilization with fertilization systems based on controlled-release and liquid fertilizers

MATERIALS AND METHODS

a) General conditions and experimental site

The experiment was conducted within the Laboratory of Plant Nutrition and Soil Fertility of the “Ion Ionescu de la Brad” Iași University of Life Sciences, Romania. The experimental field was located at the Ezăreni Research and Student Practice Station (47°07' N, 27°30' E), on a plateau with a slope of 3-5% and a slightly northeastern exposure.

The experimental area is characterized by a temperate continental climate typical of the Moldavian Plain, with relatively warm summers and moderate precipitation. During the 2024-

2025 agricultural year, the rainfall regime was uneven, with total precipitation of approximately 405 mm during the wheat growing season. Autumn conditions favored crop establishment, while spring was characterized by alternating dry periods and episodes of excess rainfall, particularly in May. These climatic conditions were relevant for interpreting nitrogen fertilization efficiency and crop response. The study aimed to evaluate the effect of different spring-applied nitrogen fertilization strategies on grain yield, nitrogen use efficiency (NUE), and selected quality parameters of winter wheat.

b) Soil properties. The main chemical properties of the soil in the 0-20 cm layer are presented in Table 1. The determined values indicate a slightly acidic soil, a moderate content of soil organic carbon, and a relatively high cation exchange capacity. The available phosphorus content falls within the medium range, while available potassium levels are high, reflecting a good fertility status of the experimental soil.

Table 1. Chemical properties of the experimental soil

Depth (cm)	pH (H ₂ O)	SOC (%)	CEC (cmolc kg ⁻¹)	NI	P-AL (mg kg ⁻¹)	K-AL (mg kg ⁻¹)
0-20	6.8	1.68	26.3	2.7	45.1	250

SOC - soil organic carbon; CEC - cation exchange capacity; NI - nitrogen index; P-AL and K-AL.

c) Biological material and crop management Winter wheat (cv. Glosa) was sown on 25 October 2024, with sunflower as the preceding crop, using a seeding rate of 200 kg ha⁻¹. Before sowing, the soil was prepared using conventional tillage practices.

Nitrogen fertilization was applied in spring according to the experimental protocol. During the growing season, plant protection treatments were carried out to control diseases, weeds, and pests using the following products: Doxun (0.8 L ha⁻¹), Prontos (0.12 L ha⁻¹), Systhane C (0.7 L ha⁻¹), and Decis Expert 100 (0.10 and 0.07 L ha⁻¹). The crop was harvested on 15 July 2025.

d) Experimental design and fertilization treatments

The experiment was arranged in a randomized block design with four replications. The area of each experimental plot was 42 m², with a net harvest area of 15 m².

Nitrogen fertilization strategies differed according to fertilizer type and application timing during the main growth stages of winter wheat. The treatment scheme and fertilizer application rates are presented in Table 2.

Table 2. Experimental treatments and nitrogen application rates at different wheat growth stages

Treatment	Fertilizer application rate (kg ha ⁻¹)			Applied nitrogen (kg N ha ⁻¹)			
	N1 (BBCH 21-25)	N2 (BBCH 30-32)	N3 (BBCH 37-41)	N1	N2	N3	Total N
T1	U46 (150)	AN34 (100)	AN34 (100)	69	34	34	137
T2	U46 (150)	AN34 (150)	-	69	51	-	120
T3	NS27 (150)	AN34 (150)	-	40.5	51	-	91.5
T4	U46 (150)	NS27 (150)	-	69	40.5	-	109.5
T5	U46 (150)	AN34 (100)	NS27 (100)	69	34	27	130
T6	U46 (150)	NS19 (150)	-	69	28.5	-	97.5

Fertilizers were applied in three stages during spring, corresponding to the main crop growth stages: N1 - BBCH 21-25 (tillering); N2 - BBCH 30-32 (stem elongation); N3 - BBCH 37-41 (flag leaf emergence)

Total nitrogen rates applied in the experiment ranged from 91.5 to 137 kg N ha⁻¹, depending on the treatment.

e) Characteristics of the fertilizers used

Four nitrogen fertilizers with different chemical forms and nutrient compositions were used in the experiment (Table 3).

Urea (U46) provides nitrogen in amide form and requires hydrolysis in soil before becoming available to plants, resulting in a gradual nitrogen release. Ammonium nitrate (AN34) contains nitrogen in both ammonium and nitrate forms, ensuring rapid nutrient availability after application.

Table 3. Chemical composition and characteristics of nitrogen fertilizers used in the experiment

Code	Fertilizer	Key Components	Type
U46	Urea (46% N)	46% as CO(NH ₂) ₂	Solid
AN34	Ammonium nitrate (34% N)	17% NH ₄ ⁺ + 17% NO ₃ ⁻	Solid
NS27	Controlled-release nitrogen-sulfur fertilizer (27% N)	27% N+ SO ₃	Solid (controlled-release)
NS19	Liquid nitrogen-sulfur fertilizer (19% N)	19%N+ 6% SO ₃ + 3% MgO+0.1% Zn+0.1% B+ PHYSIO PRO	Liquid fertilizer

The experimental fertilizer NS27 represents a solid nitrogen-sulfur source characterized by a more gradual nitrogen release and improved synchronization between nutrient availability and crop demand. The liquid fertilizer NS19 supplies nitrogen together with sulfur, magnesium, and micronutrients. Its liquid form promotes uniform distribution and rapid nutrient availability in the active root zone.

f) Soil and plant analysis methods

Soil chemical analyses were performed using standard pedological and agrochemical methods recommended by the National Research and Development Institute for Pedology, Agrochemistry and Environmental Protection (ICPA), described in Romanian methodological literature and complemented by internationally recognized procedures.

Soil pH was determined potentiometrically in a 1:2.5 soil-water suspension.

Soil organic carbon (SOC) was determined using the modified Walkley–Black method.

Cation exchange capacity (CEC) was calculated as the sum of exchangeable bases (SB) and hydrolytic acidity (Ah), determined by the Kappen method. Base saturation degree was calculated as:

$$V (\%) = (SB / CEC) \times 100.$$

The nitrogen index (NI) was calculated according to Borlan's methodology, using the formula:

$$NI = (\text{Humus} \times V) / 100.$$

Available phosphorus (P-AL) and potassium (K-AL) extracted with ammonium lactate-acetate was determined using the Egner-Riehm method. Phosphorus was measured spectrophotometrically (UV-1700, Shimadzu, Japan), while potassium was determined using flame photometry (Flame Photometer 410, Sherwood, UK).

Grain yield, test weight, and grain moisture at harvest were determined using a Wintersteiger experimental combine equipped with the automated GrainGage HM800 weighing and analysis system. Grain yield was expressed in kg ha⁻¹ and adjusted to a standard grain moisture of 14%.

Thousand-kernel weight (TKW) was determined according to the Romanian standard SR 6123/1999.

Total nitrogen content (Nt) in grain and straw was determined using the Kjeldahl method after sample mineralization. Plant samples were dried, ground, and homogenized prior to analysis.

Grain protein and gluten contents were determined using an automatic analyzer based on near-infrared spectroscopy (NIR) (Infratec 1241 Grain Analyzer, FOSS Analytical, Denmark), a rapid and non-destructive method widely used for evaluating the chemical composition of cereal grains. Analyses were performed on clean and homogenized grain samples, and results were expressed as percentages of dry matter.

g) Calculation of nitrogen use efficiency and quality indicators

To evaluate nitrogen, use efficiency and grain quality, several derived indicators were calculated based on grain yield, nitrogen content, and grain quality parameters.

Partial factor productivity of nitrogen (PFP-N) was calculated as the ratio between grain yield and the total amount of nitrogen applied through fertilization:

$$PFP-N = Y / N_a$$

where: Y represents grain yield (kg ha⁻¹) adjusted to 14% moisture, and N_a represents the total nitrogen applied through fertilization (kg N ha⁻¹).

The amount of nitrogen accumulated in grain (N_{grain}) was calculated using the following equation:

$$N_{\text{grain}} = Y \times (\text{Nt grain} / 100)$$

where: Nt grain represents the total nitrogen content in grain (%).

Protein yield per hectare was calculated based on grain yield and grain protein content:

$$\text{Protein yield} = Y \times (\text{Protein} / 100)$$

To characterize the efficiency of nitrogen translocation to grain, the Nitrogen Harvest Index (NHI) was calculated as:

$$NHI = N_{\text{grain}} / (N_{\text{grain}} + N_{\text{straw}})$$

where: N_{grain} represents nitrogen accumulated in grain (kg ha⁻¹) and N_{straw} represents nitrogen accumulated in straw (kg ha⁻¹).

h) Statistical analysis

Of the experimental data was performed using the mean values obtained from the four replications of each treatment. Differences

between treatments were evaluated using analysis of variance (ANOVA). Mean comparisons were performed using the Duncan multiple range test at a significance level of $p \leq 0.05$. All statistical analyses were carried out using SPSS v.22 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSIONS

a) Grain yield response to nitrogen fertilization strategies

Spring-applied nitrogen fertilization strategies significantly influenced winter wheat grain yield (Table 4). Grain yields obtained in the experiment ranged from 5999 to 6895 kg ha⁻¹. These differences were determined by the total nitrogen rate, the timing of application, and the type of fertilizers used. Previous studies have shown that crop response to nitrogen fertilization largely depends on the synchronization between nitrogen availability and periods of maximum crop demand (Raun & Johnson, 1999; Fageria & Baligar, 2005).

Table 4. Grain yield and nitrogen productivity under different fertilization strategies

Treatment	N rate (kg N ha ⁻¹)	Grain yield (kg ha ⁻¹)	PFP-N (kg grain kg ⁻¹ N)
T1	137	6641 ab	49.2 d
T2	120	6895 a	58.2 c
T3	91.5	6878 a	83.4 a
T4	109.5	6320 bc	62.0 b
T5	130	6692 ab	54.0 c
T6	97.5	5999 c	61.5 b

Values followed by different letters within a column differ significantly at $p \leq 0.05$ according to Duncan's multiple range test.

Grain yield was highest in treatments T2 (6895 kg ha⁻¹) and T3 (6878 kg ha⁻¹), with no significant differences between them. An important observation is that treatment T3 achieved a yield comparable to that of T2, although the total nitrogen rate applied was lower (91.5 kg N ha⁻¹ compared with 120 kg N ha⁻¹). This result may be attributed to the use of the NS27 fertilizer, characterized by controlled nitrogen release and sulfur content. The gradual release of nitrogen can contribute to maintaining a more stable nitrogen availability in the soil, while sulfur plays an important role in nitrogen metabolism, being directly involved in the synthesis of amino acids and proteins (Scherer, 2001; Salvagiotti et al., 2009).

The results also show that increasing nitrogen application rates does not necessarily lead to proportional yield increases. Numerous studies indicate that once a certain nitrogen supply level is reached, nitrogen use efficiency tends to decline (Cassman et al., 2002; Ladha et al., 2005). This aspect is also illustrated by treatment T1, where the application of 137 kg N ha⁻¹ resulted in a grain yield of 6641 kg ha⁻¹, which was close to that obtained in treatment T5 (6692 kg ha⁻¹). Under these conditions, increasing the nitrogen rate or adding an additional fertilization split did not generate significant yield increases.

The lowest grain yields were recorded in treatments T4 (6320 kg ha⁻¹) and T6 (5999 kg ha⁻¹). In treatment T6, the use of the liquid fertilizer NS19, characterized by rapid nitrogen availability in soil, may result in less favorable synchronization between nitrogen availability and crop demand. In such situations, part of the applied nitrogen may be lost through processes such as volatilization or leaching before being efficiently utilized by plants (Galloway et al., 2008; Robertson & Vitousek, 2009).

This dynamic is also reflected in the values of partial factor productivity of nitrogen (PFP-N), which ranged between 49.2 and 83.4 kg grain kg⁻¹ N. The highest nitrogen use efficiency was observed in treatment T3 (83.4 kg grain kg⁻¹ N), significantly higher than in the other treatments. These findings suggest that fertilization strategies based on moderate nitrogen rates combined with controlled-release fertilizers and sulfur supply can substantially improve nitrogen use efficiency. In this respect, the results obtained are consistent with studies highlighting the role of combined N + S fertilization in maintaining high productivity levels even at reduced nitrogen application rates (Fageria & Baligar, 2005; Scherer, 2001).

b) Nitrogen use efficiency and nitrogen accumulation

The fertilization strategies tested influenced the nitrogen content in both grain and straw, having a direct effect on the total amount of nitrogen accumulated in the plant (Table 5).

Treatment T5 recorded the highest nitrogen content in grain (2.56%), a value significantly higher compared with most of the other treatments. At the same time, the relatively high

values observed in treatments T2 (2.49%), T3 (2.46%), and T6 (2.48%) indicate that the applied fertilization strategies ensured adequate nitrogen availability during the grain formation and filling stages. Previous studies have shown that nitrogen availability during these growth stages plays a decisive role in nitrogen accumulation in grain and in determining grain quality (Fageria & Baligar, 2005; Gooding & Davies, 1997).

Table 5. Nitrogen content and nitrogen accumulation in grain and straw

Treat.	Grain N (%)	Straw N (%)	N accumulated in grain (kg ha ⁻¹)	Nitrogen Harvest Index (NHI)
T1	2.36 c	0.537 ab	157 b	0.81 c
T2	2.49 ab	0.547 ab	172 a	0.82 bc
T3	2.46 ab	0.384 d	169 a	0.86 a
T4	2.41 bc	0.459 bc	152 bc	0.84 ab
T5	2.56 a	0.426 c	171 a	0.86 a
T6	2.48 ab	0.566 a	149 c	0.81 c

Values followed by different letters within a column differ significantly at $p \leq 0.05$ according to Duncan's multiple range test.

Regarding the total amount of nitrogen accumulated in grain, the highest values were recorded in treatments T2 (172 kg ha⁻¹), T5 (171 kg ha⁻¹), and T3 (169 kg ha⁻¹), with no statistically significant differences between them. These results reflect both grain yield levels and nitrogen concentration in the grain. In the case of treatments T3 and T5, the higher nitrogen accumulation may be associated with the use of N-S fertilizers, as sulfur plays an important role in nitrogen metabolism and in the synthesis of protein compounds (Scherer, 2001; Salvagiotti et al., 2009).

An interesting aspect was observed in the nitrogen content of straw. The highest value was recorded in treatment T6 (0.566%), while treatment T3 presented the lowest value (0.384%). A higher nitrogen content in straw at harvest may indicate a lower translocation of nitrogen to the grain during the grain-filling period. The efficiency of this process depends on the plant's ability to redistribute nitrogen from vegetative organs to reproductive organs (Hirel et al., 2007).

The efficiency of nitrogen translocation is also reflected in the values of the Nitrogen Harvest Index (NHI), which ranged between 0.81 and 0.86. The highest values were observed in treatments T3 and T5 (0.86), indicating a greater

proportion of accumulated nitrogen being allocated to grain compared with vegetative biomass. This result suggests a more efficient utilization of nitrogen for grain production, a factor considered essential for achieving high yields and good grain quality (Moll et al., 1982).

A particularly relevant aspect is the performance of treatment T3, which combined high grain yield with high nitrogen accumulation in grain and the highest NHI values while using a reduced nitrogen rate. This result suggests that controlled-release N-S fertilizers can significantly improve nitrogen use efficiency in wheat cultivation.

Overall, the results indicate that fertilization strategies based on moderate nitrogen rates combined with controlled-release fertilizers or sulfur supply can promote nitrogen accumulation in grain and improve nitrogen use efficiency in the plant. These observations are consistent with studies highlighting the role of combined N + S fertilization in optimizing nitrogen metabolism and improving nitrogen use efficiency in cereal crops (Scherer, 2001; Salvagiotti et al., 2009).

c) Effect of nitrogen fertilization on grain quality

The results presented in Table 6 highlight a differentiated impact of fertilization strategies on the physical and technological quality parameters of wheat grain. Regarding the physical characteristics, the thousand kernel weight (TKW) varied within a relatively narrow range (49.6-50.8 g), with no statistically significant differences between treatments.

Table 6. Grain physical and technological quality

Treat.	1000-kernel weight (g)	Test weight (kg hl ⁻¹)	Grain protein (%)	Wet gluten (%)	Protein yield (kg ha ⁻¹)
T1	49.6 a	72.3 a	13.7 c	29.9 c	910 b
T2	50.8 a	72.5 a	15.1 a	35.4 a	1041 a
T3	50.0 a	72.3 a	14.7 ab	33.3 b	1011 a
T4	50.0 a	71.2 a	14.6 b	32.4 b	923 b
T5	50.4 a	71.5 a	15.4 a	35.9 a	1031 a
T6	49.8 a	71.4 a	14.3 b	32.0 b	858 c

Values followed by different letters within a column differ significantly at $p \leq 0.05$ according to Duncan's multiple range test.

A similar stability was also observed for hectoliter weight (71.2-72.5 kg hl⁻¹), suggesting that grain bulk density was less influenced by

variations in nitrogen fertilization under the experimental conditions.

In contrast, the chemical parameters showed greater sensitivity to fertilization strategies. The protein content ranged between 13.7% and 15.4%, with the highest values recorded in treatments T5 (15.4%) and T2 (15.1%), significantly higher than in T1 (13.7%). The protein level observed in T3 (14.7%) also indicates that this fertilization strategy ensured adequate nitrogen availability during the period of protein synthesis and accumulation in the grain.

A similar pattern was observed for wet gluten content, which ranged between 29.9% and 35.9%. The highest values were recorded in treatments T5 (35.9%) and T2 (35.4%), exceeding those observed in treatments characterized by lower protein content. The close relationship between protein and gluten content is well documented in the literature, as gluten proteins represent the main structural component of wheat gluten (Gooding & Davies, 1997; Wieser, 2007).

Protein yield per hectare ranged between 858 and 1041 kg ha⁻¹, with the highest values obtained in treatments T2 (1041 kg ha⁻¹), T5 (1031 kg ha⁻¹), and T3 (1011 kg ha⁻¹). The absence of statistically significant differences between these three treatments indicates that total protein production is determined by the interaction between grain yield and grain protein concentration.

Overall, the results show that nitrogen fertilization primarily influences the technological quality parameters of wheat grain, particularly protein and gluten content, while the physical grain characteristics remain relatively stable. These observations are consistent with studies indicating that nitrogen availability during the grain filling period is a key factor determining protein accumulation and the technological quality of wheat (Gooding & Davies, 1997; Triboi et al., 2003).

d) Relationships between nitrogen fertilization, yield and grain quality

The integrated analysis of the experimental data shows that the relationship between nitrogen fertilization, grain yield, and the technological quality of wheat does not depend exclusively on

the total amount of nitrogen applied. Although nitrogen rate remains an important factor, the fertilizer form and the timing of application decisively influence how this element is utilized by the plant.

Under the experimental conditions analyzed, treatments T2 and T3 achieved the highest grain yields. However, the comparison between these two treatments highlights important differences in terms of nitrogen use efficiency. Treatment T3, in which the NS27 fertilizer was applied at the N1 stage, achieved a yield comparable to that of T2, although it used a lower total nitrogen rate. At the same time, this treatment was characterized by high values of efficiency indicators and of the Nitrogen Harvest Index (NHI), suggesting a more efficient redistribution of nitrogen toward the grain.

An important factor that may explain this behavior is the use of controlled-release N–S fertilizers. These fertilizers can ensure a more stable nitrogen availability in the soil and a better synchronization between nutrient supply and crop physiological demand. In addition, the presence of sulfur contributes to improved nitrogen metabolism, as the synthesis of amino acids and proteins depends on the simultaneous availability of nitrogen and sulfur (Scherer, 2001; Salvagiotti et al., 2009).

Regarding grain quality, the highest values of protein and gluten content were recorded in treatments T5 and T2. In particular, treatment T5, in which the NS27 fertilizer was applied at the N3 stage, favored protein accumulation in the grain. This result confirms the important role of nitrogen availability during the grain filling period in determining the technological quality of wheat (Gooding & Davies, 1997; Triboi et al., 2003).

In contrast, treatment T6, based on the use of the liquid fertilizer NS19, showed relatively good nitrogen use efficiency, but grain yield was lower compared with the other treatments. This result suggests that liquid fertilizers characterized by rapid nitrogen availability may require supplementation with other nitrogen sources in order to sustain high yield levels during the later growth stages.

Overall, the results indicate that fertilization strategies including controlled-release N–S fertilizers can improve nitrogen use efficiency

while maintaining high levels of both grain yield and grain quality. At the same time, these strategies may allow a reduction in total nitrogen application rates, which is important for the development of more efficient and sustainable agricultural systems (Raun & Johnson, 1999; Fageria & Baligar, 2005).

CONCLUSIONS

The results of this study show that the response of winter wheat to nitrogen fertilization does not depend only on the total nitrogen rate applied, but also on the fertilizer form and the timing of application during the main growth stages.

The application of the controlled-release fertilizer NS27 at the tillering stage (T3) allowed the achievement of grain yields comparable to those obtained with conventional fertilization based on urea and ammonium nitrate, despite the lower total nitrogen rate applied. In this treatment, the highest values of partial factor productivity of nitrogen (PFP-N) and nitrogen harvest index (NHI) were recorded, indicating a more efficient use of nitrogen.

In contrast, the application of NS27 at the flag leaf stage (T5) was associated with the highest protein and gluten contents, highlighting the important role of nitrogen availability during the grain filling period in determining grain quality. The liquid fertilizer NS19 showed relatively good nitrogen use efficiency; however, grain yield was lower compared with the other fertilization strategies, suggesting that liquid fertilizers characterized by rapid nitrogen availability may require combination with other nitrogen sources in order to sustain high yield levels.

Overall, the results indicate that controlled-release N-S fertilizers may represent an effective strategy for optimizing nitrogen fertilization in winter wheat, contributing to improved nitrogen use efficiency and maintaining high grain yield and grain quality depending on the timing of application.

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CROP SCIENCES

BIOSTIMULATOR INFLUENCE ON THE NUMBER AND WEIGHT OF TUBERS PER HILL AT DIFFERENT CLIMATIC AND TECHNOLOGICAL CONDITIONS

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Abstract

The use of biostimulators in potato crop is a modern agricultural practice aimed at enhancing plant physiological processes, nutrient efficiency, and yield stability under variable environmental conditions. This study evaluated the effects of biostimulator application on the yielding elements of the Romanian potato variety Darilena, considering cultivation location and planting density. Field trials were conducted over three consecutive years (2023-2025) at the National Research and Development Institute for Potato and Sugar Beet Braşov and at a private farm (Lorad Agro SRL, Codlea) in Romania. Two planting densities (45,000 and 53,000 plants/ha) and five treatment variants were tested: control, three biostimulators (Genaktis 1, Irys, Terram Number One), and one foliar fertilizer (Wuxal Macromix). Results indicated that all biostimulants treatments significantly increased the number and weight of tubers per hill, independent of location and annual climatic variations.

Key words: potato, biostimulator, climatic conditions, plant density, number of tubers, weight of tubers, tuber yield.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is widely recognized as a crop of global importance, contributing substantially to food security and agricultural sustainability (Devaux et al., 2020; Birch et al., 2012). Maximizing tuber yield and quality is essential not only for meeting food security goals but also for enhancing the sustainability and profitability of production systems (Barbaś et al., 2025). Recent advances in crop management have identified plant biostimulants as promising agents capable of improving growth, stress tolerance, nutrient use efficiency, and overall yield performance (Moura et al., 2023).

Biostimulants encompass a diverse range of substances and microorganisms - including seaweed extracts, humic and fulvic acids, amino acids, and beneficial microbes - that exert positive effects on plant physiological processes independent of conventional fertilization (Du Jardin, 2015). These agents have been shown to stimulate root system development, enhance nutrient uptake, modulate hormonal balance,

and improve photosynthetic activity, thereby contributing to enhanced biomass production and yield stability (Colla et al., 2015; Di Sario et al., 2025).

In potato cultivation, the use of biostimulants has been widely reported to enhance tuber number, average tuber weight, and yield contributing to improved crop performance under variable environmental conditions (Rouphael et al., 2020; Bulgari et al., 2019).

Environmental conditions play a decisive role in modulating plant responses to biostimulant applications. Climatic and edaphic factors, including temperature regimes, water availability, and soil moisture dynamics, strongly influence nutrient uptake, metabolic activity, and plant stress physiology. Consequently, the effectiveness of biostimulants is highly context-dependent and may vary substantially across environmental conditions. Recent studies emphasize that biostimulant-induced improvements in crop performance are often associated with enhanced tolerance to abiotic stresses such as drought, salinity, and temperature extremes, which are themselves governed by environmental

variability (Bhupenchandra et al., 2022; Di Sario et al., 2025). Furthermore, experimental evidence suggests that soil water status and stress intensity can significantly alter plant responsiveness to biostimulant treatments, ultimately affecting yield formation and biomass allocation (Wadduwage et al., 2024). For example, thermal and drought stresses are well-known constraints on tuber initiation and expansion, whereas biostimulant applications may partially alleviate these limitations through enhanced antioxidant activity, osmotic adjustment, and metabolic regulation (Santi et al., 2018a; Yakhin et al., 2017). Technological factors such as sowing date, fertilizer regime, and crop management practices further influence biostimulant efficacy. Delayed sowing may shorten the active tuber growth period, potentially limiting treatment effectiveness (Kołodziejczyk & Gwóźdź, 2022). Conversely, synchronized biostimulant application strategies integrated with optimized crop management practices have been reported to improve plant physiological performance and yield-related traits, including tuber size distribution and biomass accumulation (Cortiello et al., 2024; Santi et al., 2018b).

Despite the growing body of research on biostimulants in horticultural and field crops, there remains a need for comprehensive studies that examine their effects under diverse climatic and technological scenarios, especially in relation to tuber yield structure and stability across different potato cultivars (Barbaś et al., 2024; Ginter et al., 2023). Elucidating how biostimulants interact with environmental stresses and agronomic practices is critical for optimizing their use in precision agriculture. In this context, the present study investigates the influence of some biostimulant products on tuber number and weight per hill and tuber yield under varying climatic and technological conditions. The objective is to identify strategies to improve potato productivity and quality in temperate cropping systems (Calvo et al., 2014; Van Oosten et al., 2017).

MATERIALS AND METHODS

The research was performed in field experiments carried out during 2023-2025 on the Romanian semi-early potato variety

Darilena, developed by National Research and Development Institute for Potato and Sugar Beet Braşov, Romania (NIRDPSB Braşov). The tubers of Darilena variety are characterized by oval-shaped tubers with yellow skin and light yellow flesh.

The experiment was arranged as a subdivided plot design ($3 \times 2 \times 2 \times 5$) with four replications, including as experimental factors the year of study (2022-2023, 2023-2024 and 2024-2025), the experimental location (NIRDPSB Braşov and the Lorad Agro farm, Codlea), plant density (45,000 and 53,000 plants ha^{-1}), and biostimulator treatments (control, Genaktis 1, Irys, Terram Number One and Wuxal Macromix). Both experimental locations are located in Braşov county, in the center of Romania.

During the vegetation period, two successive harvests were carried out in order to evaluate the main productivity traits, namely the number and weight of tubers formed per hill. For this purpose, two plants were taken from each experimental variant, and the samples were analysed in the Laboratory of Technology and Good Agricultural Practices of NIRDPSB Braşov.

At the first experimental location (NIRDPSB Braşov), the first assessment was conducted on 3rd of July in 2023, 10th of June in 2024 and 2nd of July in 2025, while the second assessment took place on 28th of July in 2023, 17th July in 2024 and 23th of July in 2025.

At the second experimental location (the Lorad Agro farm in Codlea), the first assessment was carried out on 1st of July in 2023, 17th of June in 2024 and 30th of June in 2025, and the second assessment on 26th of July in 2023, 16th July in 2024 and 22th of July 22 in 2025.

At the end of the vegetative period, the final harvest was carried out using a semi-mechanized system on the two middle rows, which had been monitored throughout the growing season for all physiological and morphological traits. At the first experimental location, harvesting took place on 21th of September in 2023, 20th of September in 2024 and 19th of September in 2025, while at the second experimental location harvesting was conducted on 30th of September 2023, 30th of September in 2024 and 20th of September in 2025.

As biostimulator treatments, there were used one foliar biostimulator, two root biostimulators

and one foliar fertilizer with a biostimulating effect (Table 1).

Genaktis 1 is a foliar biostimulator based on seaweed and silica extracts, which stimulates metabolic processes. The product was applied twice during the vegetation period, at the beginning of flowering and 15 days thereafter, at a rate of 3 l/ha/application.

Irys is a root biostimulator based on humic substances and plant extracts, with an effect on the growth and development of the root system. The product was applied at planting and at the three-differentiated-leaf stage, at a rate of 4 l/ha/application.

Terram Number One is a root biostimulator with a high content of carbon, organic substances, amino acids, and polysaccharides. The product was applied during the vegetation period in two applications at 15-day intervals, at a rate of 3 l/ha/application.

Table 1. Chemical and organic profile of studied biostimulators

Element / Substance / Symbol	Genaktis 1	Irys	Terram Nr. One	Wuxal Macromix
Macronutrients				
Total Nitrogen - N (%)	9	7	1.2	16
Phosphorus Pentoxide - P ₂ O ₅ (%)	5	9	0	16
Potassium Oxide - K ₂ O (%)	7	13	7.5	12
Micronutrients				
Boron - B (%)	0.01	0.01	0	0.02
Copper - Cu (%)	0.002	0.002	0	0.05
Iron - Fe (%)	0.02	0.04	0	0.1
Molybdenum - Mo (%)	0.001	0.001	0	0.001
Manganese - Mn (%)	0	0.01	0	0.05
Zinc - Zn (%)	0	0.002	0	0.05
Organic substances				
Organic Carbon - C org (%)	0	0	12	0
Organic Matter <50 kDa, OM<50 kDa(%)	0	0	35.5	0
Alginate Acid - AA (%)	0	0	7	0
Mannitol - Man (%)	0	0	2	0

Wuxal Macromix is a foliar fertilizer with a biostimulating effect, containing a well-balanced nutrient suspension, used to support plant growth under physiological stress conditions and to stabilize plant health. The product was applied at the beginning of tuber formation and again 14 days after the first application, at a rate of 5 l/ha/application.

RESULTS AND DISCUSSIONS

The analysis of the climatic regime over the 2022-2025 interval reveals significant interannual variability, with direct implications for the progression of the phenological stages of the potato crop. Thermal and pluviometric characteristics exerted differentiated influences on the phases of emergence, vegetative growth, tuber initiation, and yield accumulation (Figure 1).

During the 2022-2023 season, the thermal regime was characterized by values close to the multiyear average, ensuring relatively favorable conditions for the early developmental stages, namely emergence and canopy formation. However, summer temperature maxima occasionally reached the upper limit of the optimal range for tuberization, a factor that may reduce the intensity of tuber differentiation and growth processes. The uneven distribution of precipitation led to episodes of water deficit, with potential effects on the critical stages of tuber initiation and formation, which are particularly sensitive to water stress.

The 2023-2024 agricultural season provided favorable agroclimatic conditions throughout the entire growing cycle. The relative stability of the thermal regime supported physiological processes specific to the vegetative growth phase and promoted optimal tuber initiation. Furthermore, the adequate distribution of precipitation during periods corresponding to intensive biomass accumulation and tuberization, particularly in May and June, contributed to maintaining a favorable water balance and reduced the likelihood of abiotic stress occurrence.

The 2024-2025 season was marked by pronounced climatic instability, with potentially disruptive effects on plant developmental dynamics. Rapid temperature increases during the early part of the growing season may accelerate phenological development, potentially shortening critical stages, including tuber initiation. Variability in the precipitation regime, coupled with episodes of excessive rainfall, may favor the occurrence of soil water excess, with negative consequences for root respiration, nutrient uptake, and the incidence of crop-specific diseases.

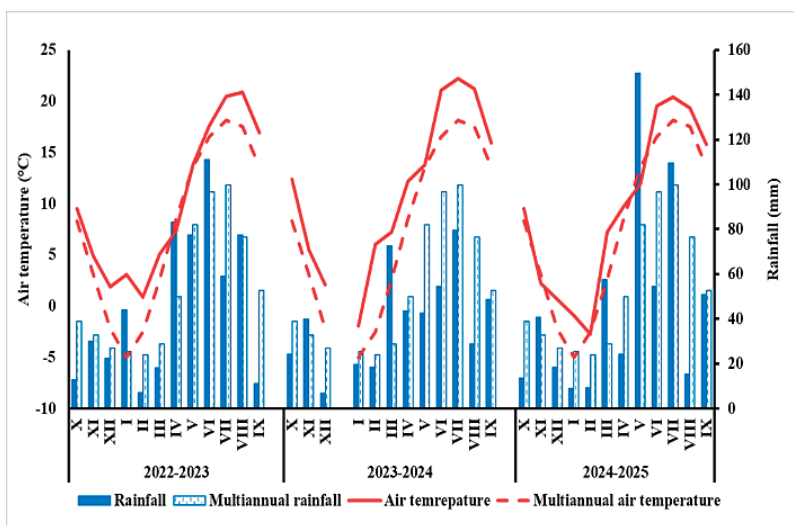


Figure 1. Monthly air temperature and precipitation patterns during the 2022-2025 period in relation to multiyear averages

The multifactorial experiment highlighted differentiated effects of the studied factors on tuber formation dynamics and final yield of the potato cultivar Darilena (Table 2). The experimental year had no significant influence on tuber number and tuber weight at either determination, except the the tuber weight at first determination in the year 2024, where the effect was negativ significant.

The experimental year very strongly influenced final yield. Compared with the multi-year average, the year 2023 resulted in a very significant positive yield increase, whereas in 2024 and 2025 significant negative yield deviations were recorded.

The experimental location mainly influenced the early stages of tuberization, with a significantly higher number of tubers at the first assessment at Codlea, while differences in final yield between locations were not significant relative to the average of both sites.

Planting density did not significantly influence tuber number or tuber weight during the growing season; however, the higher density (53,000 plants ha⁻¹) resulted in a significantly greater yield compared with the other one density (45,000 plants ha⁻¹).

Biostimulator treatments exerted a consistent and highly significant positive effect on all evaluated traits, leading to increases in tuber number, tuber weight at both assessment times, and final yield compared with the untreated

control. Overall, yield formation in the potato cultivar Darilena was predominantly influenced by year-specific conditions and biostimulator application, while experimental location and planting density had a secondary contribution, mainly expressed during the early stages of tuber development.

The stability of tuber yield of the potato cultivar Darilena was analyzed over three experimental years at two locations, NIRDPSB Braşov (BV) and the Lorad Agro farm in Codlea (CDL), under the influence of five experimental variants treated with biostimulants. The average yields recorded in the years 2024 and 2025 were considerably lower compared with those obtained in 2023, a period in which environmental conditions favored the expression of a higher yield potential (Figure 2). In years characterized by less favorable conditions, the NIRDPSB Braşov location consistently recorded higher yields, regardless of the treatment applied. At the Lorad Agro farm in Codlea, similar yield levels were obtained for the variants treated with Terram Number One (V4) and Wuxal Macromix (V5). The beneficial effect of biostimulant application on yield was evident, as the untreated control variant (V1) consistently produced lower yields compared with all treated variants throughout the entire study period.

The experimental variant treated with the biostimulant Genaktis 1 (V2) exhibited the most

pronounced yield response at the Lorad Agro farm in Codlea, particularly in 2023, a year characterized by favorable climatic conditions,

when it outperformed all other experimental variants. Under these conditions, the Darilena cultivar reached a yield level of 32.13 t/ha.

Table 2. Effects of experimental year, site, planting density, and biostimulator treatments on tuber number, tuber weight, and yield of the potato cultivar Darilena

Experimental factor		Number of tubers		Tubers weight (g)		Yield (t/ha)
		First determination	Second determination	First determination	Second determination	
Experimental year (Y)	Average^{Ct}	7.0^{Ct}	7.79^{Ct}	226^{Ct}	465^{Ct}	20.76^{Ct}
	2023	6.59 ^{ns}	8.17 ^{ns}	250 ^{ns}	507 ^{ns}	28.79***
	2024	7.26 ^{ns}	7.93 ^{ns}	159 ⁰	434 ^{ns}	16.04 ⁰⁰⁰
	2025	7.15 ^{ns}	7.26 ^{ns}	270 ^{ns}	455 ^{ns}	17.44 ⁰⁰
	LSD (p 5%)	0.84	0.99	67	83	1.45
	LSD (p 1%)	1.27	1.50	101	125	2.2
	LSD (p 0.1%)	2.04	2.41	162	201	3.53
Site (S)	Average^{Ct}	7.0^{Ct}	7.79^{Ct}	226^{Ct}	465^{Ct}	20.76^{Ct}
	Braşov	6.53 ⁰⁰	7.0 ^{ns}	195 ^{ns}	452 ^{ns}	21.68 ^{ns}
	Codlea	7.47**	8.57 ^{ns}	257 ^{ns}	479 ^{ns}	19.84 ^{ns}
	LSD (p 5%)	0.27	0.88	40	51	1.6
	LSD (p 1%)	0.39	1.27	58	72	2.3
	LSD (p 0.1%)	0.57	1.87	85	107	3.38
Planting Density (D)	45,000^{Ct}	7.12^{Ct}	7.62^{Ct}	226^{Ct}	469^{Ct}	20.32^{Ct}
	53,000	6.88 ^{ns}	7.96 ^{ns}	227 ^{ns}	462 ^{ns}	21.20*
	LSD (p 5%)	0.54	0.59	25	49	0.77
	LSD (p 1%)	0.74	0.81	35	67	1.06
	LSD (p 0.1%)	1.01	1.10	48	91	1.44
Biostimulator treatment (T)	V1- Without treatment^{Ct}	5.85^{Ct}	6.49^{Ct}	176^{Ct}	371^{Ct}	18.69
	V2- „Genaktis”	7.64***	8.01***	238***	503***	21.41***
	V3- „Irys”	6.79**	7.99***	245***	474***	21.33***
	V4- „Number One”	7.32***	8.24***	229***	490***	20.83***
	V5- „Wuxal Macromix”	7.40***	8.21***	243***	489***	21.52***
	LSD (p 5%)	0.65	0.71	26	51	1.13
	LSD (p 1%)	0.86	0.93	34	67	1.49
	LSD (p 0.1%)	1.10	1.20	44	86	1.91
ANOVA (F test)	Y	2.2 ^{ns}	2.7 ^{ns}	9.55*	2.49 ^{ns}	279***
	S	60.3***	15.87**	11.88**	1.51 ^{ns}	6.7*
	D	0.8 ^{ns}	1.48 ^{ns}	0.01 ^{ns}	0.09 ^{ns}	5.74*
	T	9.38***	8.49***	10.07***	8.7***	8.69***

The analysis of potato tuber yield stability over the three experimental years, in relation to planting density, revealed that under unfavorable climatic conditions, a density of 45,000 plants/ha ensured higher yield levels compared with 53,000 plants/ha at both studied locations. In contrast, in 2023, characterized by

favorable conditions, the Darilena cultivar achieved the maximum yield of 30.38 t/ha at the Lorad Agro farm in Codlea when grown at a density of 53,000 plants/ha. Furthermore, this location exhibited the highest yield stability at the lower planting density of 45,000 plants/ha (Figure 3).

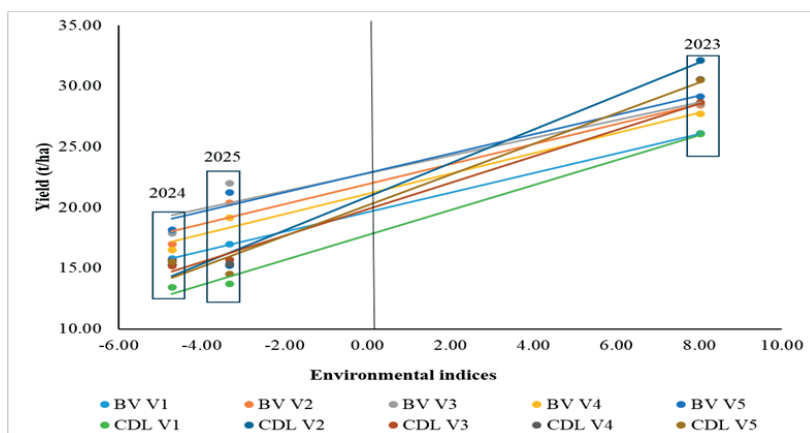


Figure 2. Stability of tuber yield in the potato cultivar Darilena with biostimulant treatments at Braşov and Codlea across three experimental years

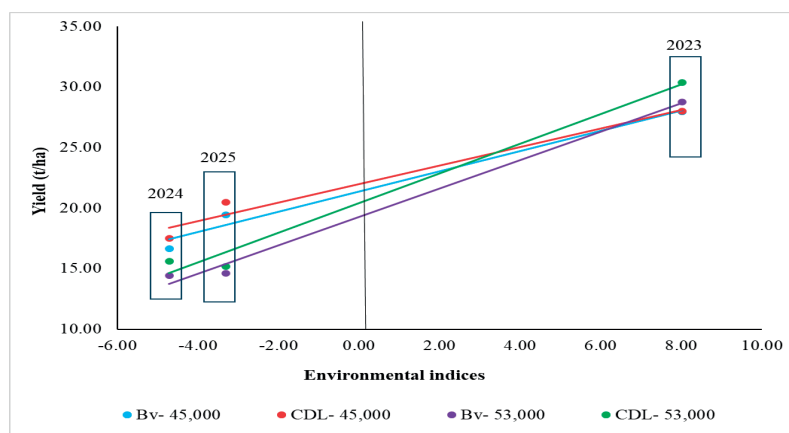


Figure 3. Stability of tuber yield in the potato cultivar Darilena with two plant densities (45,000/ 53,000 plant/ha) at NIRDPSB Braşov and Codlea across three experimental years

The correlation analysis revealed strong positive relationships among the parameters describing tuber development during the growing season. The number of tubers per hill at the first and second determinations was strongly correlated ($r = 0.78$), indicating a stable ranking of variants over time.

Both determinations of the number of tubers per hill showed strong to very strong positive correlations with tuber weight during the growing season, particularly with tuber weight per hill at the first assessment ($r = 0.82$ and $r = 0.91$), suggesting that a higher number of tubers per hill was generally associated with greater early biomass accumulation. Tuber weight at the first and second determinations was also

strongly correlated ($r = 0.76$), reflecting continuity in tuber growth dynamics.

In contrast, correlations between parameters measured during the growing season and final yield were weak or insignificant. The number of tubers at both determinations showed no significant relationship with yield ($r = -0.02$ and $r = -0.03$), while tuber weight at the first assessment was very weakly and negatively correlated with final yield ($r = -0.10$). Only tuber weight per hill at the second determination showed a weak positive correlation with final yield ($r = 0.26$), indicating that biomass accumulation at later stages was more closely related to yield formation than early tuberization (Figure 4).

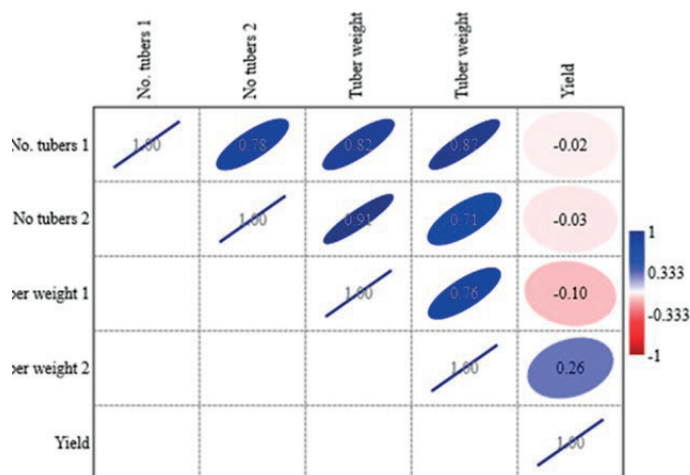


Figure 4. Pearson coefficients for studied parameters

CONCLUSIONS

The climatic regime during the 2022-2025 period exhibited pronounced interannual variability, which significantly influenced potato growth, and development. Thermal and hydric conditions differentially affected phenological stages of potato plants, with tuber initiation and development proving particularly sensitive to climatic stress.

The 2023-2024 growing season may be considered the most favorable in terms of the alignment between climatic factors and the ecological requirements of potato phenological stages, whereas the other seasons exhibited moderate constraints primarily associated with variability in water availability and thermal extremes.

The multifactorial experiment revealed that yield formation in the Darilena cultivar was primarily governed by the experimental year and biostimulant application. While the year had no significant effect on tuber number, it exerted a strong influence on tuber weight and a very highly significant effect on final yield. Biostimulant treatments consistently improved all evaluated traits, highlighting their stabilizing and productivity-enhancing potential under variable climatic conditions.

Planting density exhibited a context-dependent effect: although the higher density increased yield under favorable conditions, the lower density ensured superior yield stability under

less favorable environments. Experimental location had a secondary influence, mainly affecting early tuberization, while its impact on final yield was limited.

Correlation analysis indicated that early tuberization parameters were strongly interrelated but poorly associated with final yield, suggesting that yield determination was more closely linked to later-stage biomass accumulation than to initial tuber formation dynamics.

Overall, the results underline the predominance of climatic variability and biostimulant use as key determinants of yield performance and stability in potato, providing valuable insights for adaptive crop management strategies under increasingly unstable environmental conditions.

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RESEARCH ON THE ANALYSIS OF SOME FACTORS THAT INFLUENCE THE QUANTITY OF POLLEN PRODUCED BY THE INBREED CORN LINES (*Zea Mays* L.)

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Abstract

*This study analyzes factors influencing pollen production in inbred maize (*Zea mays* L.) lines, focusing on genetic material and plant density. Trials in Brăila, Romania (2024), tested six lines across three densities (95k, 135k, and 160k plants/ha). Results indicated that density had no statistically significant effect on flowering time ($p = 0.927$) or tassel branching. However, significant genotype-specific differences were found. The Zeta V43 line exhibited the highest number of tassel branches (up to 13.0), while Epsilon V81 achieved the maximum main axis length (42 cm) and the highest pollen dry weight (76.80 g) at 115,000 plants/ha. Tukey HSD tests confirmed a significant genotype $\times$ density interaction, showing that while Zeta V43 branching improves at lower densities, Epsilon V81 excels in pollen output at medium densities. These findings suggest that genetic selection is the primary driver for tassel development, identifying Epsilon V81 and Zeta V43 as superior candidates for breeding programs aimed at optimizing pollen-producing capacity in hybrid seed production.*

Key words: *Zea mays* L., pollen weight, tassel morphology, plant density, inbred lines.

INTRODUCTION

Maize (*Zea mays* L.) is recognized as the most widely cultivated cereal globally, with the United States of America, China, and Brazil leading production rankings (FAO, 2023). In 2023, the global area dedicated to maize cultivation was estimated to be between 200 and 210 million hectares. This crop's widespread cultivation is attributed to its remarkable productivity and versatility. For example, maize serves as a critical component in diverse industries, including animal feed, staple foods such as flours, hominy, oil, and bread, and even in specialized sectors like brewing, pharmaceuticals, and mining (Strazzi, 2015). In Europe, approximately 16 to 18 million hectares were sown with maize during the 2023 season. This figure varied slightly due to factors such as weather conditions, market dynamics, and crop rotation practices. Among the leading European maize producers are France, Ukraine, Romania, and Hungary, with Romania maintaining a dominant position in maize cultivation. Recent data highlights that Romania consistently cultivates around 2.5 million hectares annually, making it the European Union's largest producer

in terms of cultivated area (FAO, 2023). This leadership can be attributed to Romania's favorable climatic and soil conditions, particularly in its southern and eastern regions. From a personal perspective, Romania's strategic emphasis on maize cultivation reflects its broader agricultural strengths and adaptability. These favorable conditions could position the country as a potential hub for innovation in maize-based product development, which could enhance its competitiveness in the global market. The multiple applications of maize in food production, industrial uses, and beyond further underscore its significance in ensuring both economic stability and food security. Maize holds substantial economic significance, and breeding programs are vital for developing genotypes with agronomic traits that align with producer needs and consumer market expectations. Modern maize genotypes have been optimized for traits such as reduced plant and ear heights, more upright leaf angles, smaller tassels (fewer branches and lower mass), shorter intervals between tasselling and silking, decreased grain protein content, and increased yield potential (Duvick, 2005).

One of the most critical stages in maize development is flowering, which directly impacts yield. This phase typically spans 5 to 14 days (Ogden et al., 1969) and begins with pollen release from the tassel, followed shortly by silk emergence. Remarkably, each maize plant produces approximately 25 million pollen grains, ensuring a robust reproductive capacity with around 25,000 pollen grains available per silk (Kiessebach et al., 1949). This interplay between tassel and silk dynamics underscores the complexity of maize reproductive biology. Tassel size plays a pivotal role in yield determination. Larger tassels can reduce grain yield by shading upper leaves, thereby diminishing photosynthetic efficiency, and by acting as sinks for photo assimilates (Edwards, 2011; Souza et al., 2015). However, in environments with stress factors such as limited water availability, larger tassels may become advantageous by ensuring sufficient pollen production for fertilization (Parvez, 2007). Breeding programs have thus focused on selecting smaller tassels to reduce shading effects, enhance photosynthetic performance, and allow higher planting densities. While this strategy improves yield potential, it has inadvertently reduced pollen production (Duvick & Cassmann, 1999). Consequently, understanding factors influencing pollen production and germination has become a priority to support breeding programs and hybrid seed production. Tassel size, defined by traits such as branch number, branch length, spikelet count, and tassel area index, exhibits a complex relationship with yield. Specifically, a negative correlation with grain yield is often observed, alongside a positive correlation with pollen production (Fonseca et al., 2003). This duality poses challenges in breeding programs aimed at optimizing tassel traits.

The reduction of tassel size has been instrumental in improving planting efficiency and yield under optimal conditions, there is a growing need to develop adaptive strategies for stress-prone environments. Incorporating genetic diversity and exploring innovative breeding techniques could balance the trade-offs between pollen production and yield. This balance is critical to sustaining productivity as environmental conditions continue to fluctuate due to climate change. The main objective of

this study was to evaluate the influence of genetic material and plant density on tassel morphology and flowering synchrony in inbred maize (*Zea mays* L.) lines. By analysing traits such as branch count, main axis length, and pollen dry weight, this research aims to identify optimal genotypes and agronomic conditions for maximizing pollen production in hybrid seed production programs.

MATERIALS AND METHODS

The institutional and administrative framework was provided by the Doctoral School for Plant and Animal Resources Engineering and Management, organized within the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Field trials were conducted on the farm II Olteanu Ștefan SRL, located in the village of Viziru, Brăila County, in the southeastern part of Romania, within the historical region of Muntenia. Geographically, it is situated in the western part of Brăila County, near the border with Galați County, at approximately 45°01' North latitude and 27°43' East longitude. The area has a temperate-continental climate, typical of the lowland regions in eastern Romania, with influences from the Bărăgan Plain. The average annual temperature is 11°C, while the average annual precipitation ranges between 450–500 mm, evenly distributed throughout the year. The Viziru area is highly suitable for intensive agriculture due to its fertile soils (chernozems), hot summers, and the presence of a well-developed irrigation infrastructure in the region. However, the management of climate-related risks (such as drought and strong winds) and soil issues (including salinization and compaction) is essential for ensuring sustainability.

The experiment was designed as a two-factor trial. Factor A consisted of six maize (*Zea mays* L.) inbred lines with varying relative maturities (RM): Alfa R26 and Beta T00 (RM 80), Gamma T13 and Delta A39 (RM 100), and Epsilon V81 and Zeta V43 (RM 120). Factor B involved three plant densities: 95,000, 135,000, and 160,000 plants per hectare, respectively. **Factor A - Genetic material (*Zea mays* L.):**

a1 - Alfa R26 (relative maturity 80);

a2 - Beta T00 (relative maturity 80);

a3 - Gamma T13 (relative maturity 100);

a4 - Delta A39 (relative maturity 100);
a5 - Epsilon V81 (relative maturity 120);
a6 - Zeta V43 (relative maturity 120).
and **Factor B - Plant density:**
b1 - 95.000 plants per hectare;
b2 - 135.000 plants per hectare;
b3 - 160.000 plants per hectare.

The experimental plots were sown in the optimal period characteristic for this region, April 9, 2024, at a sowing depth of 6 centimetres and a row spacing of 60 centimetres. The design used was the completely randomized block design (Table 1), with 3 replications, each plot measuring 5 meters in length and consisting of 4 rows.

Table 1. Completely randomized block design

Plant density 160.000 plants / hectare	Replication 3	a4b3	a5b3	a6b3	Buffer strip
		a1b3	a2b3	a3b3	
	Replication 2	Buffer strip			
		a4b3	a5b3	a6b3	
		a1b3	a2b3	a3b3	
		Buffer strip			
Replication 1	a4b3	a5b3	a6b3		
	a1b3	a2b3	a3b3		
Buffer strip					
Plant density 135.000 plants / hectare	Replication 3	a4b2	a5b2	a6b2	
		a1b2	a2b2	a3b2	
	Replication 2	Buffer strip			
		a4b2	a5b2	a6b2	
		a1b2	a2b2	a3b2	
		Buffer strip			
Replication 1	a4b2	a5b2	a6b2		
	a1b2	a2b2	a3b2		
Buffer strip					
Plant density 95.000 plants / hectare	Replication 3	a4b1	a5b1	a6b1	
		a1b1	a2b1	a3b1	
	Replication 2	Buffer strip			
		a4b1	a5b1	a6b1	
		a1b1	a2b1	a3b1	
		Buffer strip			
Replication 1	a4b1	a5b1	a6b1		
	a1b1	a2b1	a3b1		
Buffer strip					

For the observation and determination of the studies, the following factors were considered:
(1) Sowing date;
(2) The number of days from sowing when 90% of the plants in the plot have visible panicles and anthers releasing pollen;
(3) Number of tassel branches: the number of branches is counted for 5 representative plants/panicles, and the average is calculated (Photo 1);

(4) Size of the main tassel axis: The main panicle axis is measured for 5 representative plants/panicles, and the average is calculated (Photo 1. A);

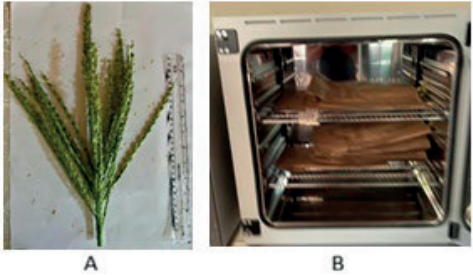


Photo 1. A. Measuring tassel axis; B. Placing the samples in the Binder oven

(5) Tassel weight: 12 panicles are cut at the flowering 90% stage, approximately 5 centimetres below the last branch of the panicle. The sample is weighed and then placed to dry for 72 hours at 55°C in a Binder oven (Foto 1. B). The sample is weighed immediately after being removed from the oven following the 72-hour drying period. This will allow us to determine the amount of pollen released through drying.

RESULTS AND DISCUSSIONS

Flowering

Flowering observations were determined when 90% of the plants in the plot exhibited anthers releasing pollen. Analysing all the genetic material together, we can observe that the average flowering rate decreases slightly as the density increases, but the differences are quite small (Table 2).

Table 2: ANOVA test result (density - P90%)

Density (plants/ha)	Mean P90 (Days to Flowering)	Min	Max	Standard Deviation
75,000	90.5	82	103	~7.26
115,000	89.43	81	102	~7.66
160,000	88.83	82	99	~5.38

Running an Anova analysis results in p-value = 0.927, where is much higher than 0.05, means that there are no statistically significant differences between the 3 densities in terms of flowering day (P90%) (Table 3).

Table 3: ANOVA analysis to determine the influence of density on flowering

Sursa	Sum of Squares	DF	F	p-value (PR(>F))
Densitate	8.78	2	0.077	0.927
Rezidual (eroare)	859.67	15	—	—

After comparing genetic material and density, we observe that P90 values vary more between genetic materials than between densities. Density has a slightly visible effect in some cases (e.g. Delta A39 or Epsilon V81), but it is not a consistent or statistically significant effect (confirmed by ANOVA) according with Figure 1.

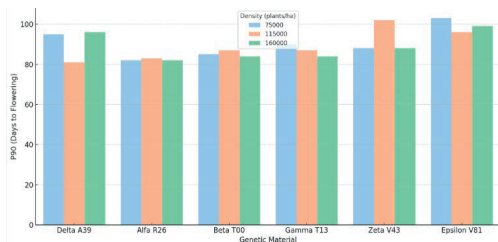


Figure 1. Comparative flowering time (P90%) by material and density

Number of branches per tassel

The number of branches per tassel is determined by counting the number of branches of 5 representative plants in the plot, after which the average of the 5 tassels was calculated. Analysing the graph compared to all genetic materials, we observe that all values are relatively close between densities (Figure 2)

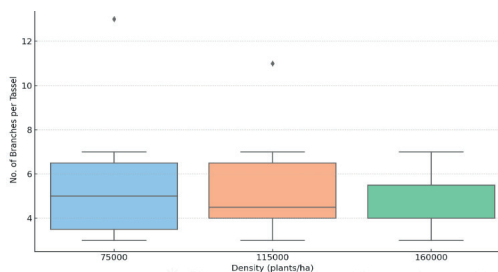


Figure 2. Effect of density on number of branches per tassel

In the comparative graph showing the number of branches per panicle for each genetic material, at the 3 densities tested (Figure 3) density does not seem to significantly influence branching within each material (also confirmed by the previous ANOVA). But it is observed that there

are clear differences between genetic materials – for example, Zeta V43 has many more branches than the others genetics, while some materials (e.g. Gamma T13 or Epsilon V81) are quite constant regardless of density.

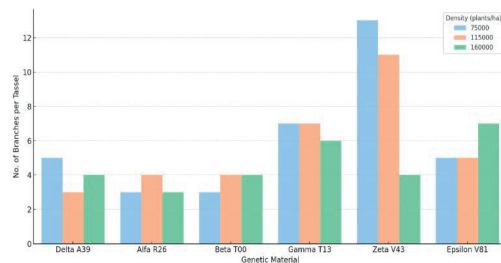


Figure 3. No. of branches per tassel by genetic material and density

Using the Tukey HSD test (Statistical grouping, same letter = not significantly different) to determine if there is a statistical difference between the genetic materials on the number of branches on the tassel, it is found that the Zeta V43 material is statistically significantly different from all other materials, having the highest average number of branches (9,333), according with Table 4. It can be deduced that Zeta V43 is a valuable candidate for selection and improvement, having a significantly higher number of branches than the other materials.

Table 4. Tukey Table with P-Values

	Material	Mean No. of Branches	Group	p-value vs Zeta V43
1	Zeta V43	9.333	a	-
2	Gamma T13	6.667	b	0.0309
3	Epsilon V81	5.667	b	0.001
4	Delta A39	4.0	c	0.0
5	Beta T00	3.667	c	0.0
6	Alfa R26	3.333	c	0.0

Using Tukey HSD test for interpretation of differences between genetic materials and densities (Table 5) show that Zeta V43 have the highest average number of tassel branches (13.0) among all combinations, is statistically significantly different from all other material × density combinations (assigned to group “a”, indicating its superior performance for “b”, and “b” superior performance for “c” etc.). Zeta V43 (115000 density) also performs very well, with an average of 11.0 branches, falls into group “b”, meaning it is significantly different from group “a”, but still better than the rest.

Table 5. Interpretation of differences between genetic materials and densities (Tukey HSD)

Material (Density)	Mean No. of Branches	Group * Significant Diff.
Zeta V43 (75000)	13	a
Zeta V43 (115000)	11	b
Gamma T13 (75000)	7	c
Gamma T13 (115000)	7	c
Epsilon V81 (160000)	7	c
Gamma T13 (160000)	6	d
Delta A39 (75000)	5	e
Epsilon V81 (75000)	5	e
Epsilon V81 (115000)	5	e
Delta A39 (160000)	4	f
Beta T00 (160000)	4	f
Beta T00 (115000)	4	f
Zeta V43 (160000)	4	f
Alfa R26 (115000)	4	f
Alfa R26 (160000)	3	g
Delta A39 (115000)	3	g
Beta T00 (75000)	3	g
Alfa R26 (75000)	3	g

The genetic material Zeta V43 at 75.000 plants/ha shows the best tassel development, with the highest and most statistically distinct number of branches, by reducing the sowing density appears to enhance tassel branching for this genotype. These findings strongly support the use of Zeta V43 at lower densities in breeding programs or agronomic trials where panicle development is a selection criterion.

Size of the main tassel axis

The size of the main axis per tassel is determined by measuring the main axis (in centimetres) of 5 representative plants in the plot, after which the average of the 5 tassels was calculated. In the comparative chart (Figure 4) is observable that Epsilon V81 at high density (160,000 plants/ha) stands out with the highest main axis length (42 cm), other combinations, such as Beta T00 (160000) and Delta A39 / Gamma T13 at 75000, show similarly high values, around 39-40 cm.

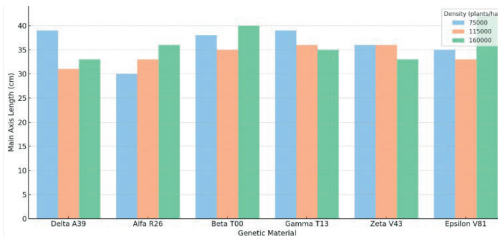


Figure 4. Main axis length (cm) by genetic material and density

There are clear variations between combinations, and an apparent influence of planting density on main axis length for certain genotypes.

Tukey HSD test (Statistical grouping, same letter = not significantly different), indicate that Epsilon V81 (160000) shows the highest axis length (42.0 cm) and is statistically superior to all other combinations (Group a), Beta T00 (160000), with 40.0 cm, falls into group b, significantly different from Epsilon V81 (p = 0.0), Gamma T13 (75000) and Delta A39 (75000) both recorded 39.0 cm, forming group c, also significantly lower than the top performer, while Beta T00 (75000) is in group d, with 38.0 cm, and is significantly lower than all of the above (Table 6).

It is conclusive that Epsilon V81 benefits from higher density (160k) in terms of main axis development, this response highlights its potential under denser planting systems or as a target for breeding selection. The results show clear genotype × density interaction, influencing a key morphological trait for pollen production.

Table 6. Tukey HSD results - main axis length by genetic material × density

Material (Density)	Mean Main Axis Length (cm)	Group * Significant Diff.
Epsilon V81 (160000)	42	a
Beta T00 (160000)	40	b
Gamma T13 (75000)	39	c
Delta A39 (75000)	39	c
Beta T00 (75000)	38	d
Zeta V43 (75000)	36	e
Zeta V43 (115000)	36	e
Alfa R26 (160000)	36	e
Gamma T13 (115000)	36	e
Gamma T13 (160000)	35	f
Beta T00 (115000)	35	f
Epsilon V81 (75000)	35	f
Zeta V43 (160000)	33	g
Alfa R26 (115000)	33	g
Delta A39 (160000)	33	g
Epsilon V81 (115000)	33	g
Delta A39 (115000)	31	h
Alfa R26 (75000)	30	i

Tassel weight

The method used for determined the quantity of pollen is the picking and cutting of 12 consecutive tassels at the flowering 90% stage form each plot, approximately 5 centimetres below the last branch of the tassel. The sample

is weighed and then placed to dry for 72 hours at 55°C in a Binder oven (Photo 1.B). The sample is weighed immediately after being removed from the oven following the 72-hour drying period. This will allow us to determine the amount of pollen released through drying. In the data observation table (Figure 5) it is visible that Epsilon V81 consistently shows the highest pollen dry weight across all densities, with a notable peak at 115,000 plants/ha (76.80 grams), Zeta V43 performs well at 115,000 as well, but its production drops sharply at 160,000 density, while Beta T00 and Gamma T13 exhibit a gradual decline in pollen production as density increases, Alfa R26 and Delta A39 remain among the lowest producers regardless of density.

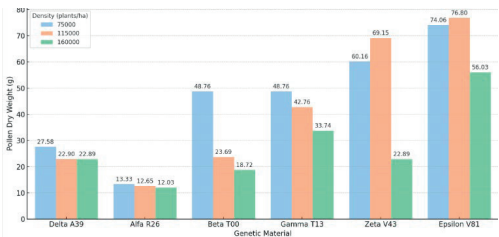


Figure 5. Pollen dry weight by genetic material and density

Putting all pairwise comparisons between every combination of genetic material $\times$ density, Tukey HSD test table for Pollen Dry Weight (Table 7), showing that the combination Epsilon V81 at 115,000 plants/ha yielded the highest pollen dry weight (76.80 g) and belongs to group "a", making it significantly superior to all other combinations ($p < 0.001$). Even Epsilon V81 at 75,000 and Zeta V43 at 115,000, although close in absolute values are statistically distinct, being placed in groups "b" and "c", if we move down the list, Zeta V43 at 75000 and Epsilon V81 at 160000 show clearly lower performances and are grouped separately, indicating a strong statistical drop-off in pollen production. The results confirm a highly significant genotype $\times$ density interaction affecting pollen productivity. Epsilon V81 responds extremely well to medium density (115k), making it an ideal candidate for environments or purposes where maximizing pollen output is critical. The rapid decline in pollen weight at higher densities (160k), especially for genotypes like Zeta V43,

suggests that optimal density management is essential for maintaining reproductive potential.

Table 7. Tukey HSD results - Pollen Dry Weight (g)

Material (Density)	Mean Pollen Dry Weight (g)	Group * Significant Diff.
Epsilon V81 (115000)	76.8	a
Epsilon V81 (75000)	74.06	b
Zeta V43 (115000)	69.15	c
Zeta V43 (75000)	60.16	d
Epsilon V81 (160000)	56.03	e
Beta T00 (75000)	48.76	f
Gamma T13 (75000)	48.76	f
Gamma T13 (115000)	42.76	g
Gamma T13 (160000)	33.74	h
Delta A39 (75000)	27.58	i
Beta T00 (115000)	23.69	j
Delta A39 (115000)	22.9	k
Delta A39 (160000)	22.89	l
Zeta V43 (160000)	22.89	l
Beta T00 (160000)	18.72	m
Alfa R26 (75000)	13.33	n
Alfa R26 (115000)	12.65	o
Alfa R26 (160000)	12.03	p

To correlate tassel characteristics a scatter diagram is used (scatterplot), is a graph that uses dots to represent the relationship between two numerical variables.

Each dot represents one observation, in our case, a specific genetic material at a certain planting density. The X-axis shows the value of one trait. The Y-axis shows the value of another trait. In the diagram which correlate main axis length and number of branches per tassel (Figure 6), no strong linear correlation is visible, some materials (e.g., Zeta V43) combine high branching with moderate axis length.

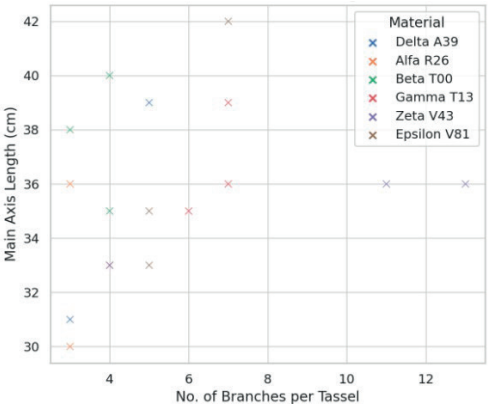


Figure 6. No. of branches vs Main axis length

In the diagram which correlate number of branches per tassel with pollen dry weight (Figure 7) show us a weak positive trend: more branches may slightly associate with higher pollen weight. However, outliers exist (e.g., Epsilon V81) achieves high pollen weight with moderate branching.

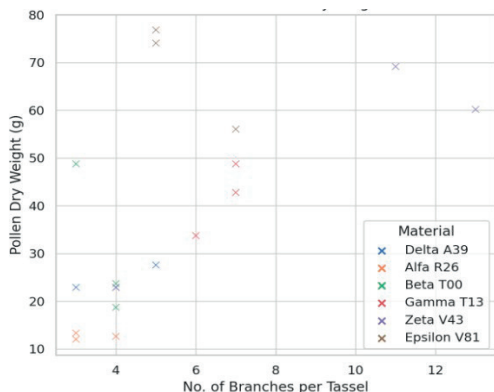


Figure 7. No. of branches vs Pollen dry weight

In the diagram which correlate main axis length with pollen dry weight (Figure 8), this pair shows a stronger positive trend, genetic materials with longer main axes (e.g., Epsilon V81, Gamma T13) tend to have higher pollen productivity.

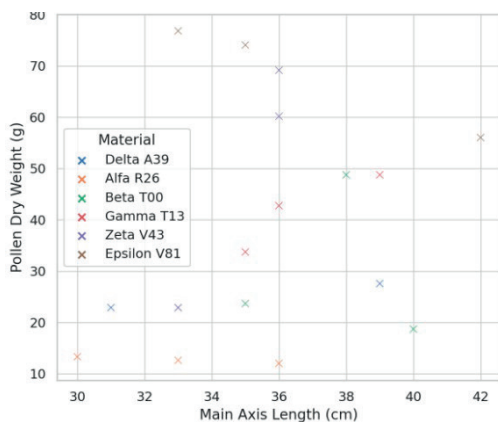


Figure 8. Main axis length vs Pollen dry weight

CONCLUSIONS

Genetic material is the primary driver of trait expression, with significant differences ($p < 0.05$) observed across all studied parameters. Specifically, Zeta V43 demonstrated superior

tassel branching, while Epsilon V81 recorded the highest pollen dry weight and main axis length, particularly at medium or high densities. Planting density modulates trait expression through significant genotype-specific responses. While genotypes like Epsilon V81 benefit from higher densities for pollen productivity, others, such as Zeta V43, optimize branching and reproductive traits at lower densities. This highlights a strong genotype $\times$ density interaction, confirmed by ANOVA and Tukey HSD tests.

Positive correlations between main axis length and pollen dry weight suggest high potential for indirect selection. Conversely, the weak correlation between branch number and other traits indicates that branching is primarily a structural rather than a functional attribute.

A medium density of 115,000 plants/ha appears to optimize both structural and reproductive performance for the top-performing genotypes. However, no universal optimal density was identified, confirming that agronomic management must be tailored to specific genotypes.

Planting density had no statistically significant effect on the time to flowering (P90%), indicating that flowering phenology in these lines is relatively stable across the tested populations.

Future programs should prioritize trait-by-trait optimization, focusing on pollen traits and axis development in density-responsive genotypes. Further multi-environmental testing is recommended to validate these trends.

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INFLUENCE OF DROUGHT ON SEED OIL CONTENT OF SOME SUNFLOWER HYBRIDS IN YEARS 2024 AND 2025, IN SOUTH-EASTERN OF ROMANIA

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Abstract

Drought is most restrictive abiotic factor who affect negatively seed yield and seed oil content of sunflower genotypes. Years 2024 and 2025 are characterized as a drought year and in this paper, we present results about seed oil content of ten sunflower hybrids belonging to NARDI Fundulea, cultivated in Express Sun system, in south eastern of Romania, in Fundulea location. In year 2024 was registered an average seed oil content between 35.2% at sunflower hybrid H8E and 45.8% at sunflower hybrid FD15E27. In year 2025 was registered an average seed oil content between 31.58% at sunflower hybrid H8E and 41.71% at sunflower hybrid FD15E27. In year 2025, was registered a lowest seed oil content at all ten sunflower hybrids analysed in both years. In year 2024 was registered an average seed yield between 623kg/ha at sunflower hybrid H8E and 1155 kg/ha at sunflower hybrid H1E. In year 2025 was registered an average seed yield between 1792 kg/ha at sunflower hybrid H2E and 2334 kg/ha at sunflower hybrid H1E. In year 2024, was registered a lowest seed yield than year 2025.

Key words: sunflower, seed oil content, drought.

INTRODUCTION

At the European Union level, Romania has consistently ranked among the leading sunflower seed producers in recent years (Panzaru et al., 2023; Voicila, 2024). In 2024, Romania occupied the first position within the European Union regarding sunflower cultivated area, accounting for 1,243.25 thousand hectares out of the total 4,791.22 thousand hectares cultivated at the EU level. A similar trend was maintained in 2025, when Romania cultivated 1,205.13 thousand hectares out of the 4,671.16 thousand hectares recorded across the European Union (EUROSTAT, 2026). In 2025, the European Union ranked third globally in sunflower seed production, with a total output of 8.58 million metric tons, following Russia and Ukraine (Table 1) (FAS USDA, 2026). Furthermore, the average sunflower seed yield within the European Union reached 1.7423 t/ha in 2024, representing the lowest value recorded during

the last nine years, while in 2025 the average yield slightly increased to 1.867 t/ha (FAS USDA, 2026).

Agro-meteorological conditions play a major role in determining the potential productivity of agricultural crops through their effects on plant growth and development (Georgieva et al., 2024; Alf et al., 2026). Environmental stress factors, particularly drought and high temperatures, may reduce seed yield, decrease seed oil content, and negatively affect pollen viability (Domenco et al., 2023; Duca et al., 2022; Ma'ali et al., 2024; Momna et al., 2023; Syed et al., 2025).

The novelty of this study consists in the comparative evaluation of 10 experimental ExpressSun sunflower hybrids under the climatic conditions specific to southeastern Romania, with emphasis on flowering dynamics, seed productivity, and oil content. Moreover, the study provides updated information regarding hybrid performance under increasingly variable climatic conditions.

Another important aspect of novelty is the assessment of hybrid adaptability under contrasting agro-meteorological conditions recorded during the experimental period. The selected hybrids differ in maturity group, productivity potential, and adaptability to abiotic stress conditions, allowing a comparative evaluation of their agronomic performance.

Table 1. Sunflower seed production worldwide in 2025

Country	Share of global production (%)	Total production (Metric tons)
Russia	33%	17 Million
Ukraine	20%	10.5 Million
European Union	16%	8.58 Million
Argentina	11%	5.5 Million
Kazakhstan	5%	2.4 Million
China	4%	2.1 Million
Turkey	3%	1.37 Million
United States	2%	1.05 Million
Moldova	2%	850,000
South Africa	1%	720,000

Source: <https://www.fas.usda.gov/data/production/2224000>

The objective of the present study was to evaluate the performance of several ExpressSun sunflower hybrids cultivated under the environmental conditions of southeastern Romania, with particular emphasis on flowering time, seed yield, and oil content. The study also aimed to identify differences among sunflower hybrids in their response to climatic conditions during the vegetation period.

MATERIALS AND METHODS

During the years 2024 and 2025, eleven sunflower hybrids were cultivated, including the check hybrid FD15E27 and ten experimental hybrids (H1E–H10E), developed by the National Agricultural Research and Development Institute Fundulea.

The experiment was arranged in a randomized complete block design with three replications. Each experimental plot consisted of four rows, 6 m in length, spaced 70 cm apart, resulting in a plot area of 16.8 m². The plant density was 55,000 plants/ha under non-irrigated conditions, using the ExpressSun production system, in the southeastern region of Romania, at the Fundulea experimental field on a cambic cernozem soil type. The sowing date was April 5, 2024, and April 25, 2025.

At the four true leaf stage, all sunflower hybrids grown under the ExpressSun system

were sprayed with an herbicide containing tribenuron-methyl at a rate of 35 g/ha for weed control.

Flowering time was recorded when 75% of the plants reached the flowering stage, in order to evaluate differences among the sunflower hybrids.

Seed oil content was determined using an MQC+ Oilseed Analyser employing nuclear magnetic resonance (NMR) technology (Oxford Instruments). Sunflower seed samples were placed in 10 mL glass cylinders, weighed, and subsequently analysed with the MQC+ NMR analyser to determine seed oil content.

Statistical software used for data analysis was MSTAT-C and statistical interpretation was performed according to Snedecor (1956).

RESULTS AND DISCUSSIONS

The total annual rainfall recorded at Fundulea in 2024 (437.0 mm) and 2025 (450.2 mm) was considerably lower than the 60-year average of 584.3 mm, indicating drought conditions during the sunflower growing seasons in Romania (Figure 1).

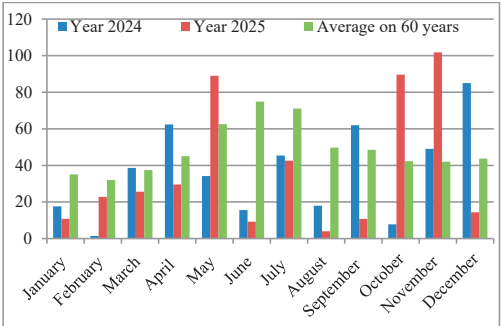


Figure 1. Average monthly rainfalls registered in Fundulea, in 2024 and 2025

For establishing the sunflower crop in April, in Romania, the water reserve accumulated during the October–March period is very important. At the Fundulea location, precipitation accumulated during the October–March period preceding sunflower establishment was below the long term average in both experimental years. Total rainfall reached 196.6 mm during October 2023–March 2024 and 201.0 mm during October 2024–March 2025, compared with the 60 year average of 232.5 mm.

Total rainfall during the sunflower growing season (April-September) was substantially lower in 2024 (237.6 mm) and 2025 (185.2 mm) than the 60-year average of 351.8 mm, confirming the occurrence of severe drought conditions during the vegetation period. Compared with the 60 year average, monthly temperatures in Fundulea during 2024 and 2025 were consistently higher, with the greatest deviations occurring in June and July, coinciding with the flowering stage of sunflower hybrids (Figure 2).

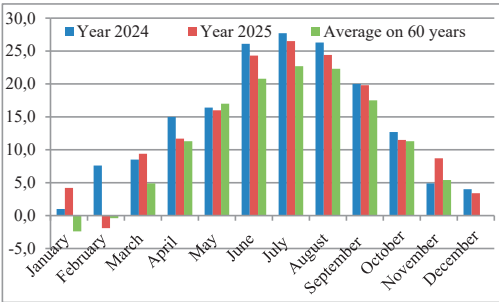


Figure 2. Average monthly temperatures (°C) registered in Fundulea, in 2024 and 2025

The average annual air temperatures recorded in 2024 (14.2°C) and 2025 (13.2°C) were considerably higher than the 60-year average of 10.9°C, reflecting the occurrence of unusually warm climatic conditions during the study period.

The combined occurrence of below average precipitation, elevated temperatures, and reduced relative humidity created drought stress conditions during the critical reproductive stages of sunflower development. Rainfall amounts during both the cold season (October-March), important for soil water reserve accumulation, and the vegetation period (April-September) were substantially lower than the long-term average. Air temperatures exceeded the multiannual average, especially during June and July, corresponding to the flowering and pollination stages of sunflower hybrids.

Average relative air humidity recorded at the Fundulea location during 2024 and 2025 were lower than the 60-year average, especially during the months of June and July (Figure 3).

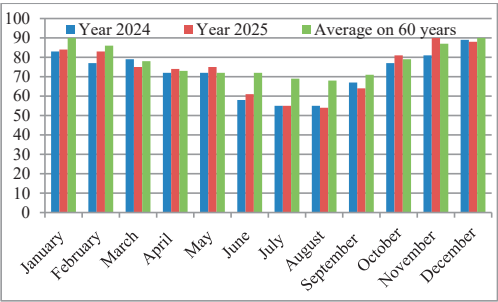


Figure 3. Average relative humidity of the air (%) registered in Fundulea location, in 2024 and 2025

The combination of reduced rainfall, high temperatures, and lower relative air humidity likely intensified evapotranspiration and drought stress conditions, negatively affecting physiological processes involved in flowering, pollination, seed formation, and oil accumulation. These climatic conditions created a favorable framework for evaluating the adaptability and performance of sunflower hybrids under drought and heat stress conditions.

The analysed sunflower hybrids exhibited differences in the number of days from sowing to flowering between the two experimental years (Table 2).

Table 2. Flowering time and number of days from sowing to flowering of sunflower hybrids evaluated at the Fundulea location in 2024 and 2025

Sunflower hybrid	Flowering time in 2024	Days from sowing to flowering in 2024	Flowering time in 2025	Days from sowing to flowering in 2025
FD15E27 check	June 23	79	July 06	72
H1E	June 24	80	July 06	72
H2E	June 24	80	July 05	71
H3E	June 24	80	July 06	72
H4E	June 24	80	July 07	73
H5E	June 25	81	July 05	71
H6E	June 26	82	July 06	72
H7E	June 24	80	July 08	74
H8E	June 23	79	July 07	73
H9E	June 23	79	July 06	72
H10E	June 25	81	July 04	70
Average	-	80	-	72

The earlier flowering observed in 2025 was associated with the higher air temperatures and drought conditions recorded during the vegetation period, which accelerated the phenological development of sunflower plants.

The reduced seed yield recorded during the experimental period (Table 3) was strongly associated with drought and heat stress conditions occurring during the critical phenological stages of sunflower development, particularly flowering and grain filling. Water deficit and elevated temperatures negatively affect photosynthetic activity, assimilate translocation, and pollen viability, leading to reduced fertilization efficiency and lower seed formation.

Table 3. Average seed yield (kg/ ha) of sunflower hybrids evaluated at the Fundulea location in 2024 and 2025

Sunflower hybrid	Average seed yield (kg/ha) in 2024	Average seed yield (kg/ha) in 2025	Average by hybrid
FD15E27	715	2277	1496
H1E	1155	2334	1744
H2E	979	1792	1385
H3E	1069	2138	1603
H4E	1067	2294	1680
H5E	971	2238	1604
H6E	904	2233	1568
H7E	897	2082	1489
H8E	623	1999	1311
H9E	939	2238	1588
H10E	985	2214	1599
Average by year	937	2167	1552

Analysis of variance (ANOVA) for seed yield (kg/ha) of sunflower hybrids cultivated at the Fundulea location during 2024 and 2025 (Table 4), indicated that Factor A (year) significantly

Table 4. Analysis of variance (ANOVA) for seed yield (kg/ ha) of sunflower hybrids cultivated at the Fundulea location during 2024 and 2025

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob	Signification
Factor A (year)	1	52043904	52043904	20.1610	0.0462	*
Error	2	5162838.636	2581419.318	-	-	-
Factor B (Sunflower hybrid)	10	41353007.030	4135300.703	0.8055	> 0.05	NS
A x B interaction	10	52229516	5222951.600	1.0174	0.4463	NS
Error	40	205340977.879	5133524.447	-	-	-

NS = not significant ($p > 0.05$); * $p < 0.05$. Coefficient of Variation: 124.14%

The significant effect of the experimental year highlights the major influence of climatic conditions, particularly drought and high temperatures, on sunflower productivity. Differences observed among sunflower hybrids suggests improved adaptation to drought conditions.

The graphical representation of interannual variation in seed yield among the analysed sunflower hybrids during 2024 and 2025 was generated using Microsoft Excel (Figure 4).

influenced seed yield. Tukey test confirmed significant differences significantly at $p < 0.05$, in seed yield between the two experimental years. Average seed yield in 2025 was substantially higher than in 2024 with 1776 kg/ha. The markedly reduced productivity recorded in 2024 was associated with severe drought and elevated temperatures during flowering and seed filling stages, which negatively affected pollination, assimilate accumulation, and grain filling. These results emphasize the major influence of climatic conditions on sunflower productivity under drought environments. In contrast, Factor B (sunflower hybrid) and the interaction between year and sunflower hybrid ($A \times B$) did not significantly affect seed yield.

The absence of significant differences among hybrids suggests a relatively similar response of the analysed genotypes under the environmental conditions recorded during the study period. Furthermore, the non-significant $A \times B$ interaction indicates that the rank order of hybrids was broadly maintained across the two years despite the marked difference in environmental conditions. The coefficient of variation (Table 4) reflects the strong influence of environmental stress conditions and substantial variability among experimental observations during the study period.

Although genotype effects on seed yield were not statistically significant, several hybrids maintained above average productivity in both years. This consistent performance suggests a lower sensitivity to environmental variation and may indicate more efficient maintenance of reproductive processes under drought and heat stress. Such responses are particularly relevant for breeding programs targeting yield stability under increasingly variable climatic conditions.

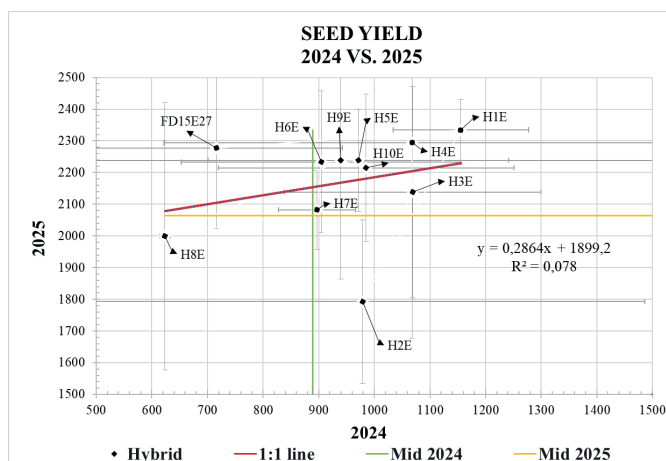


Figure 4. Interannual variation in seed yield of sunflower hybrids between 2024 and 2025

Despite the weak regression relationship between the two experimental years (Figure 4), some sunflower hybrids maintained relatively consistent productivity levels, suggesting lower sensitivity to environmental fluctuations. This observation emphasizes the importance of genotype $\times$ environment interactions in determining sunflower productivity under variable climatic conditions.

Similar reductions in sunflower seed yield under drought and heat stress conditions were reported by Duca et al. (2022), who observed significant decreases in productivity during flowering and grain filling stages. Ma'ali et al. (2024) demonstrated that water deficit negatively affects both seed yield and oil accumulation through reductions in photosynthetic activity and assimilate translocation. The accelerated flowering observed under high temperature conditions in the present study is consistent with findings reported by Domenco et al. (2023), who described shortened phenological phases under heat stress conditions.

Similar genotype $\times$ environment interactions for sunflower productivity were previously described by Syed et al. (2025), emphasizing the importance of hybrid adaptability under climatic variability. Drought stress has important effects on lipid biosynthesis and oil accumulation in sunflower seeds. Under water deficit conditions, the activity of enzymes involved in fatty acid synthesis may be reduced, while accelerated senescence and limited assimilate availability can decrease oil deposition within developing seeds. High temperatures during

grain filling may further intensify these effects by shortening the duration of seed development and reducing the period available for lipid accumulation (Table 5).

Table 5. Average seed oil content (%) of sunflower hybrids cultivated in Fundulea, in 2024 and 2025

Sunflower hybrid	Average seed oil content (%) in 2024	Average seed oil content (%) in 2025	Average by hybrid
FD15E27 check	45.8	41.71	43.75
H1E	36.20	33.68	34.94
H2E	39.95	36.32	38.13
H3E	36.33	35.69	36.01
H4E	35.49	37.47	36.48
H5E	42.49	39.25	40.87
H6E	38.25	35.13	36.69
H7E	40.91	37.61	39.26
H8E	35.20	31.58	33.39
H9E	37.28	35.04	36.16
H10E	41.18	35.80	38.49
Average by year	39.01	36.30	37.65

Analysis of variance (ANOVA) indicated that the experimental year did not significantly influence seed oil content at the 5% significance level, although a clear tendency toward significance was observed. Although the F value for Factor A (year) was relatively high, the corresponding probability value remained non-significant because the denominator error term associated with Factor A had only 2 degrees of freedom. Under these conditions, relatively high F values may still correspond to non-significant probability levels.

Since Factor A included only two levels (2024 and 2025), additional multiple-comparison procedures such as Tukey's test were not

necessary, as the ANOVA is equivalent to a pairwise comparison between years. The observed numerical reduction in seed oil content in 2025 indicates a biological tendency toward reduced oil accumulation under less favorable climatic conditions. Factor B (sunflower hybrid) had a highly significant effect on seed oil content (Table 6). Tukey HSD test (Tukey’s Honestly Significant Difference) for sunflower hybrids (Factor B) has value HSD of 3.25 at $p < 0.05$. The significant genotype effect demonstrates that oil accumulation was more strongly controlled by genetic factors than by annual climatic variation. Sunflower hybrids with consistently higher oil content across years may provide useful germplasm for breeding programs targeting oil production under water limited environments. The interaction between year and sunflower hybrid ($A \times B$) was significant at the threshold

level, suggesting that the response of sunflower hybrids regarding oil accumulation varied depending on the environmental conditions recorded during the two experimental years. The observed genotype dependent response of oil content is consistent with previous studies showing that drought and heat stress reduce lipid accumulation through limitations in carbon assimilation and fatty acid biosynthesis. Similar findings have been reported in previous studies, which demonstrated that drought stress during flowering and grain filling reduces oil accumulation through limitations in carbon assimilation and metabolic activity involved in lipid biosynthesis (Duca et al., 2022; Ma’ali et al., 2024). Other authors also reported that high temperatures during reproductive stages accelerate seed maturation and reduce the duration available for oil deposition in sunflower seeds (Momna et al., 2023).

Table 6. Analysis of variance (ANOVA) for seed oil content of sunflower hybrids in Fundulea location, in 2024 and 2025

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob	Signification
Factor A (year)	1	123.875	123.875	11.7582	0.0755	NS
Error	2	21.070	10.535	-	-	-
Factor B (Sunflower hybrid)	10	497.682	49.768	17.8577	0.000	***
A x B interaction	10	57.760	5.776	2.0725	0.0505	*
Error	40	111.478	2.787	-	-	-

NS = not significant ($p > 0.05$); * $p < 0.05$; *** $p < 0.001$
Coefficient of Variation = (CV) 4.43%

The low coefficient of variation ($CV = 4.43\%$) indicates good experimental precision and relatively low residual variability within the experiment.

The graphical representation of interannual variation in seed oil content among the analysed sunflower hybrids during 2024 and 2025 was generated using Microsoft Excel (Figure 5).

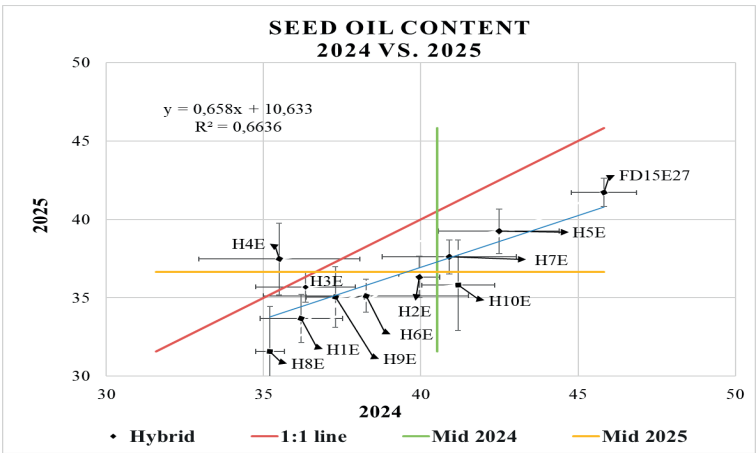


Figure 5. Interannual variation in seed oil content of sunflower hybrids between 2024 and 2025

Several hybrids maintained relatively high oil content in both years, indicating greater stability of oil accumulation under contrasting environmental conditions. This stability is of practical importance because oil yield is a primary breeding objective in sunflower. Drought and high temperature stress occurring during grain filling are known to negatively affect lipid biosynthesis by reducing photosynthetic activity, assimilate availability, and the activity of enzymes involved in fatty acid synthesis. Under severe water deficit conditions, accelerated plant senescence and shortened seed filling duration may further reduce oil accumulation in sunflower seeds. The regression relationship between years ($R^2 = 0.6636$) indicates a moderate positive correlation for seed oil content, demonstrating that hybrids with higher oil content in 2024 generally maintained relatively higher values in 2025. Total oil production per hectare was calculated using the following formula:

$$\text{Total oil} = \frac{\text{Seed yield (kg/ha)} \times \text{Seed oil content (\%)}}{100}$$

Total oil production reflected the combined effects of seed yield and oil content. Sunflower hybrids maintaining favorable values for both traits produced higher oil yields, highlighting the importance of simultaneous selection for productivity and oil accumulation (Table 7). From a breeding perspective, total oil

production may provide a more informative criterion than either trait considered separately

Table 7. Total oil production registered by sunflower hybrids in Fundulea location, in 2024 and 2025

Sunflower hybrid	Total oil (kg/ha) in 2024	Total oil (kg/ha) in 2025	Average by hybrid
FD15E27	327.47	949.73	638.60
Check			
H1E	418.11	786.09	602.10
H2E	391.11	650.85	520.98
H3E	388.36	763.05	575.71
H4E	378.67	859.56	619.12
H5E	412.57	878.41	645.49
H6E	345.78	784.45	565.11
H7E	366.96	783.04	575.00
H8E	219.29	631.28	425.29
H9E	350.05	784.195	567.12
H10E	405.62	792.61	599.11
Average by year	365.52	786.62	576.07

The relationship between productivity and oil content is strongly influenced by environmental conditions. Under severe drought stress, reductions in seed yield are frequently accompanied by lower oil accumulation due to metabolic activity during seed filling. Some sunflower hybrids may maintain relatively stable oil content despite reduced productivity, indicating better physiological adaptation and higher tolerance to abiotic stress. Hectolitre weight of the analysed sunflower hybrids differed between the two experimental years (Table 8).

Table 8. Average hectolitre weight (kg/hl) of sunflower hybrids evaluated at the Fundulea location in 2024 and 2025

Sunflower hybrid	Average hectolitre weight (kg/hl) in 2024	Average hectolitre weight (kg/hl) in 2025	Average by hybrid
FD15E27			
Check	39	37	38
H1E	36.6	33.8	35.2
H2E	38.5	33.6	36.05
H3E	34.9	35.5	35.2
H4E	34.2	32.5	33.35
H5E	36.6	34.7	35.65
H6E	35.8	34.5	35.15
H7E	35.8	33.6	34.7
H8E	36.2	35.2	35.7
H9E	34.6	34	34.3
H10E	37.3	36.3	36.8
Average by year	36.3	34.6	35.45

Analysis of variance (ANOVA) for hectolitre weight (kg/hl) of sunflower hybrids cultivated in Fundulea location during 2024 and 2025 indicated that Factor A (year) did not significantly influence hectolitre weight, although the result was close to the significance

threshold still ecologically meaningful given the drought contrast between 2024 and 2025, but no longer statistically significant at the conventional level (Table 9). Climatic conditions had a strong biological effect but the statistical evidence is marginal with only 2

error degree of freedom for Factor A. This is a power limitation of the design, not absence of an effect.

Factor B (sunflower hybrid) significantly affected hectolitre weight, indicating the existence of genetic variability among the analysed hybrids for this quality trait.

Tukey test for sunflower hybrids (Factor B) has HSD value of 3.69 at $p < 0.05$.

The significant genotype effect indicates that seed density was influenced primarily by genetic factors. Hectolitre weight is closely associated with seed filling efficiency and may therefore serve as an indicator of the ability of genotypes to maintain grain development under drought conditions.

The relatively stable hectolitre weight observed across hybrids suggests that this trait was less affected by environmental variability than seed yield, although drought and elevated temperatures likely contributed to reductions in seed filling and seed density during the experimental period.

The interaction between year and sunflower hybrid ($A \times B$) was not significant, suggesting relatively stable hybrid responses across the two experimental years.

The low coefficient of variation, indicates good experimental precision and relatively low residual variability within the experiment.

Table 9. Analysis of variance (ANOVA) for hectolitre weight (kg/hl) of sunflower hybrids in Fundulea location, in 2024 and 2025

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob	Signification
Factor A (year)	1	47.702	47.702	17.8641	0.0517	NS
Error	2	5.341	2.670	-	-	-
Factor B (Sunflower hybrid)	10	91.460	9.146	2.5516	0.0174	*
A x B interaction	10	28.866	2.887	0.8053	0.81	NS
Error	40	143.375	3.584	-	-	-

NS = not significant ($p > 0.05$); * $p < 0.05$;
Coefficient of Variation (CV) = 5.34%

Interannual variation in hectolitre weight (kg/hl) of the analysed sunflower hybrids during

2024 and 2025 was graphically represented using Microsoft Excel (Figure 6).

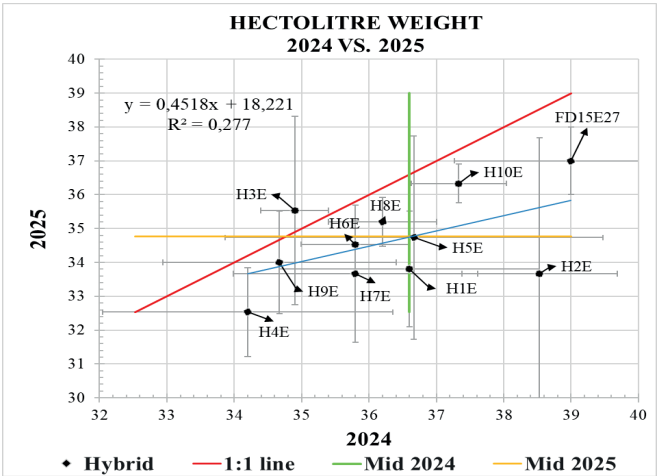


Figure 6. Interannual stability of hectolitre weight (kg/hl) in sunflower hybrids between 2024 and 2025

The regression model, indicates a moderate but relatively weak relationship between the two years.

The moderate relationship observed between years suggests that hectolitre weight was

influenced by both genetic and environmental factors. Compared with seed yield, this trait showed greater stability across years, indicating that seed physical quality was less sensitive to climatic variation than productivity. Sunflower

genotypes combining acceptable hectolitre weight with stable yield and oil content may represent useful breeding material for drought prone environments.

CONCLUSIONS

Drought and high temperatures during the flowering and seed filling periods reduced seed yield, oil content, and hectolitre weight in sunflower under the environmental conditions of Fundulea in 2024 and 2025.

Seed oil content and hectolitre weight were influenced mainly by genotype, whereas seed yield was more strongly affected by annual climatic variation, confirming the importance of genotype $\times$ environment interactions.

Experimental sunflower hybrids H1E, H4E, H5E, H6E, and H10E maintained comparatively stable yield and oil content across the two study years, indicating their potential suitability for cultivation under variable climatic conditions.

These results support the use of stability based selection criteria in sunflower breeding and cultivar evaluation programs targeting drought prone environments.

The evaluated sunflower hybrids and their parental lines represent useful genetic material for breeding programs aimed at improving adaptation to water limited conditions while maintaining acceptable productivity and oil quality.

Additional multi year and multi location studies are required to confirm the consistency of sunflower hybrid performance and their adaptation across a wider range of environments.

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RESEARCH ON THE REACTION OF SOME BEAN GENOTYPES TOWARDS PATHOGENIC AGENTS DETECTED, UNDER IRRIGATED CULTIVATION CONDITIONS, IN THE BALTA-ALBĂ AREA, BUZĂU COUNTY

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Abstract

The aim of this work is to highlight the reaction of bean genotypes to specific pathogens under conditions of natural infection and irrigated cultivation. The observations were carried out under the conditions of 2025, within S.C. BioVitAgro, Balta-Albă, Buzău County. The biological material used was represented by the bean varieties: Elba, Sonesta, Supernano Giallo and Canellino. The diseases detected were: bean common mosaic (Bean common mosaic virus), bacterial blight of bean (*Xanthomonas phaseoli* pv. *phaseoli*), fusarium wilt of bean (*Fusarium oxysporum* f. sp. *phaseoli*) and angular leaf spot of bean (*Pseudocercospora griseola*). Observations were made regarding the incidence and intensity of the attack in the control and fungicide-treated variants. The degree of attack and the effectiveness of the applied treatments were calculated. The highest attack values were determined in the control variant, in which the frequency of attack by *Pseudocercospora griseola* was 59.5%, and the lowest in the attack by *Fusarium oxysporum* f. sp. *phaseoli* (8.25%). The application of treatments had an effectiveness ranging between 63.6% and 96.25% in combating the monitored diseases.

Key words: pathogens, diseases, attack, effectiveness.

INTRODUCTION

Common beans are one of the longest-cultivated plants.

Genetic analyses of the common bean *Phaseolus* show that it originated in Mesoamerica, and subsequently spread southward, along with maize and squash, traditional companion crops (Bitocchi et al., 2012).

Common beans are still an important part of world agriculture and are an essential part of a balanced diet in many countries.

In Romania, beans are cultivated on approximately 35.000 ha. (https://insse.ro/cms/files/GAC/tabele/AUTOREP_8.HTM), of which, in Buzău County in 2023, 307 ha were cultivated (https://buzau.insse.ro/wp-content/uploads/2025/05/Anuar-DJS_BZ-editia-2024.pdf).

The nutritional values of 100 g of *Phaseolus vulgaris* L. - genus *Phaseolus*, family *Fabaceae*, order *Fabales*, class *Angiospermae*, phylum

Magnoliophyta, kingdom *Plantae* (<https://gd.eppo.int/taxon/PHSVX>), dried, boiled, without salt are: carbohydrates - 25.1 g; fats - 0.35 g; proteins - 9.73 g; a series of vitamins: thiamine (B1) - 0.118 mg, riboflavin (B2) - 0.046 mg, niacin (B3) - 0.14 mg, pantothenic acid (B5) - 0.229 mg, vitamin B6 - 0.093 mg, folic acid (B9) - 81 µg, vitamin E - 0.94 mg, vitamin K - 3.5 µg; minerals: Ca - 90 mg, Fe - 3.7 mg, Mg - 63 mg, Mn - 0.636 mg, P - 113 mg, K - 561 mg, Na - 6 mg, Zn - 1.38 mg; choline - 35.1 mg and water - 63.1 g (<https://fdc.nal.usda.gov/food-details/175203/nutrients>).

Consumption of common beans along with cereals, through the supply of essential amino acids and other nutrients, contributes to the prevention of cardiovascular diseases, diabetes and certain types of cancer (Broughton et al., 2003; Thompson et al., 2017; Viguiliouk et al., 2017).

Cultivation peculiarities of the species *Phaseolus vulgaris* L.: altitude: 50-3000 m; daytime temperature: 14-26°C; mean annual precipitation: 400-1600 mm/year; duration of growth cycle from start to end of harvest: 70-330 days; yield potential in tropical areas: 400-5000 kg/ha (<https://hort.purdue.edu/beans.html>).

Biotic factors of phytopathological nature cause the decrease in bean production (Wani et al., 2023).

Of the 20 bean diseases mentioned worldwide (https://en.wikipedia.org/wiki/List_of_diseases_of_the_common_bean), in the present experience the following were detected: bean common mosaic (*Bean common mosaic virus*), bacterial blight of bean (*Xanthomonas phaseoli* pv. *phaseoli*), fusarium wilt of bean (*Fusarium oxysporum* f. sp. *phaseoli*) and angular leaf spot of bean (*Pseudocercospora griseola*).

Common bean (*Phaseolus vulgaris* L.), is susceptible to infection with one of the most widespread and damaging viruses in the *Potyviridae* family: bean common mosaic virus-BCMV (Morales, 2006).

Yield losses as a result of bean common mosaic virus-BCMV and bean common mosaic necrosis virus-BCMNV can reach 100% (Li et al., 2014). Bacterial blight of bean-*Xanthomonas phaseoli* pv. *phaseoli* (Smith) Constantin, Cleenwerck, Maes, Baeyen, Van Malderghem, De Vos, Cottyn, a systemic, seed-transmitted (Aggour et al., 1989), in areas with warm weather, causing yield reductions of up to 40% (Singhet al., 1999).

Fusarium wilt of bean-*Fusarium oxysporum* f. sp. *phaseoli* J.B.Kendrick & W.C.Snyder, first identified in common beans in the USA in 1929 (Xue et al., 2015), leads to the death of infected plants (Batista et al., 2017).

Angular leaf spot of bean-*Pseudocercospora griseola* (Sacc.) Crous & Braun, considered the second most important fungal disease after anthracnose, can cause yield reductions of up to 80% when the disease triangle is favored by all factors (Schwartz et al., 1981; Rava Seijas et al., 1985).

Irrigation is a particularly important link in bean cultivation, for obtaining high yields. Although beans have moderate moisture requirements, the critical phases for water are: seed germination, flowering, grain setting and growth.

Among the mentioned phases, beans are very sensitive to moisture deficits in the flowering and grain formation phenophases, due to the short interval between these phenophases (Coadă et al., 2021).

MATERIALS AND METHODS

The experimental field was established within the BioVitAgro farm, in Balta-Albă commune (45°17'50.1"N, 27°17'5.6"E), Buzău County.

The climate in the experimental fields Balta-Albă, according to the Köppen-Geiger classification, is of the Dfb type (Peel et al., 2007). D: continental climate with hot summers and cold winters; f: wetter summers than winters; b: temperature of the warmest month >22°C and temperature of the coldest month below -4°C.

The main climatic parameters are shown in Figure 1.

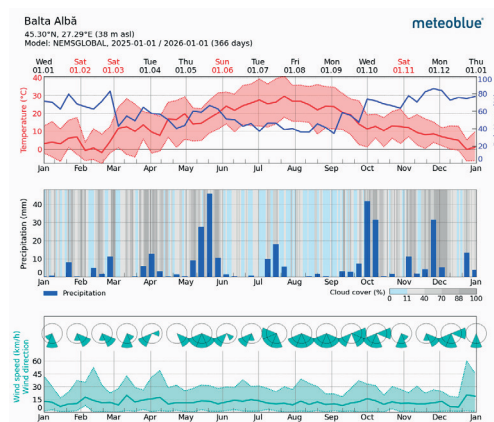


Figure 1. Climatic parameters - Balta-Albă, 2025
(source:https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/balta-alba_romania_685575?fcstlength=1y&year=2025)

Bacterial blight of bean is favored by temperatures of 25-30°C and high humidity (Severin and Iliescu, 2006).

Angular leaf spot of bean is favored by temperatures of 25-30°C and high humidity alternating with dry conditions (<https://www.greenlife.co.ke/bean-angular-leaf-spot/>).

Fusarium wilt of bean is favored by high temperatures and drought (Garcés-Fiallos et al., 2017).

The beans were drip-watered three times: the first to help the emergence (25 mm), the second in the growth stage (25 mm): first flower buds enlarged - BBCH55 (Feller et al., 1995), and the third in the growth stage (25 mm): beginning of pods development - BBCH71.

A uniform agrotechnics was applied in all variants in terms of pre-plant: corn, ploughed to a depth of 25 cm, fertilizing (NPK 15:15:15 - 200 kg/ha), preparation of the germination bed, sowing (April 8, 2025), herbicide with Basagran® SL - 2 l/ha, in the phenophase of weeds with 2-4 leaves (BBCH 12-14); two treatments were applied with the insecticides Faster Delta-0.25 l/ha and Mospilan 20 SG-0.1 kg/ha.

The distance between the rows of common beans was 70.0 cm, and the distance between the beans/row was 8 cm.

Abiotic factors, especially precipitation and temperature, play an important role in the development and pathogenicity of pathogens that cause diseases in agricultural crops (Lipianu et al., 2023; Zală et al., 2023).

The biological material used in the experiments was represented by 4 varieties of common beans: Elba, Sonesta, Supernano Giallo and Canellino.

The experiments were placed using the randomized block method (Săulescu & Săulescu, 1967). There were four repetitions for each of the four varieties of beans (Figure 2.). Area of one variant - 14 m² (4 rows 5 m long).



Figure 2. Aspects from the experimental field with common beans (foto: Cristina Bălașu)

Experimental variants: V1 - control, V2 - 1 treatment applied in the phenophase of the first enlarged flower buds, with Alcupral 50 WP - 2.5 kg/ha; V3 - 1 treatment applied in the phenophase of the beginning of the development of the first pods, with Ortiva Top - 1 l/ha; V4 - 2 treatments, with both fungicides in the same phenophases.

Observations on the efficacy of fungicides were made 10 days after the application of the last treatment.

Visual inspection is the fastest method of identifying diseases based on the typical symptoms presented by infected bean plants.

The attack value is represented by frequency-F% (incidence of the disease), intensity-I% (severity) and attack degree (AD%).

Frequency represents the number of leaves/plants attacked out of 100 leaves/plants examined. Visually estimated intensity (in percent) indicates the degree to which the leaf or plant is attacked.

The attack degree of a disease in bean crop was calculated using frequency and intensity using the formula:

$$AD (\%) = \frac{F (\%) \times I (\%)}{100}$$

The effectiveness of treatments with fungicides was calculated according to the Abbott (1925) formula, based on the recorded attack degrees:

$$E (\%) = \frac{AD \text{ control} - AD \text{ treated}}{AD \text{ control}} \times 100$$

RESULTS AND DISCUSSIONS

Results regarding the symptomatology of the detected diseases

Bean common mosaic virus (BCMV) is of particular importance in warm areas with average rainfall that favors the growth of vector aphid populations, responsible for the spread of the virus.

The virus manifested itself on infected leaves with typical mosaic symptoms (green-yellowish spots alternating with healthy green tissue) and wrinkling of the leaf blade (Figure 3.).

Bacterial blight of bean- *Xanthomonas phaseoli* pv. *phaseoli* starting as small, water-soaked spots on leaves that turn brown with distinctive lemon-yellow halos, leading to large dead areas, and a "burned" look (Figure 4.).



Figure 3. Leaflet with typical symptoms of bean common mosaic virus infection (foto: Cristina Bălașu)

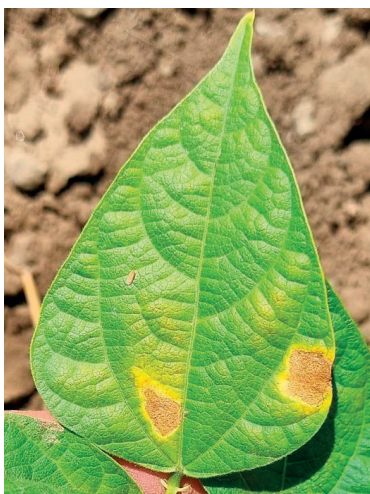


Figure 4. Leaflet with typical symptoms of bacterial blight (foto: Cristina Bălașu)

Fusarium wilt of bean-*Fusarium oxysporum* f. sp. *phaseoli* caused stunting, chlorosis and complete wilting of infected bean plants (Figure 5.).

Angular leaf spot of bean - *Pseudocercospora griseola*, one of the most devastating diseases of common beans in tropical and subtropical production areas (Nay et al., 2019), manifested itself due to the high temperatures recorded in the summer months in the research area.

Angular spot of beans manifested itself on the leaves through small, angular, brown spots, limited by veins (Figure 6.).

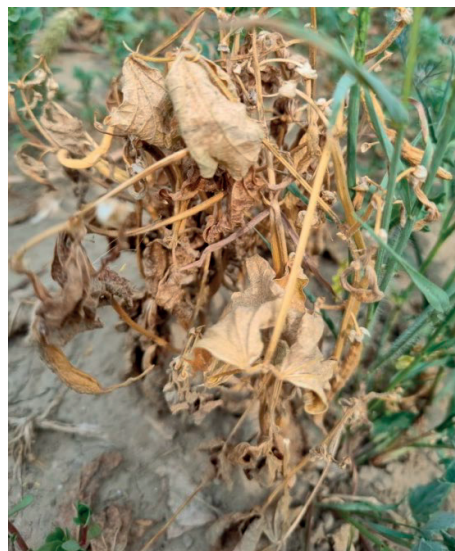


Figure 5. Dried plant as a result of infection with the fungus *Fusarium oxysporum* f.sp. *phaseoli* (foto: Cristina Bălașu)



Figure 6. Leaflets with typical angular leaf spot attack (foto: Cristina Bălașu)

Results regarding attack scoring and treatment efficacy

Bean common mosaic virus was only found in the Supernano Giallo and Canellino varieties. The average frequency was 8% in the Supernano Giallo variety and 9% in the Canellino variety, and the average intensity was 5% in the other varieties.

The frequency of bacterial blight (Table 1) varied in the control variant from 14.75% in the Supernano Giallo variety to 10.5 in the Canellino variety. The application of a treatment with the fungicide Alcupral 50 PU (variant V2) contributed to the reduction of the frequency to 2.55 in the Sonesta and Canellino varieties and to 3.0% in the Supernano Giallo variety. The application of two treatments with Alcupral 50 WP (V4) contributed to the reduction of the frequency to 1.5 in the Sonesta and Canellino varieties and to 1.75% in the Supernano Giallo variety.

The intensity of the bacterial blight attack varied between 2 and 6%. The highest degree of bacterial blight attack, 0.9%, was recorded in the control variant of the Supernano Giallo variety, and the lowest, 0.03%, in the V4 variant of the Sonesta and Canellino varieties.

The Elba variety did not show any bacterial blight attack.

Angular leaf spot of bean only appeared in the Supernano Giallo and Canellino varieties. The highest frequency value, 59.5%, was recorded in V1 of the Supernano Giallo variety, and the lowest, 2.5%, in V4 of the Canellino variety. The intensity varied between 2 and 5%. The degree of attack was the highest, 8.93%, in V1 of the Supernano Giallo variety and the lowest, 0.05%, in v4 of the Canellino variety.

Fusarium wilt of bean only occurred in the Canellino variety. In variants V1 and V2 the attack frequency was 8.25%. The attack intensity was 100% in all experimental variants. The attack degree decreased from 8.25% in V1 and V2 to 3% in V3, respectively to 1.25% in V4.

Regarding the effectiveness of treatments with Alcupral 50 WP, this ranged from 85% in the Canellino variety when one treatment/vegetation was applied (V2), to 96.25% in the Sonesta variety, when two treatments were applied (V4). Ortiva top is not effective against bacterial blight of bean.

Applying a single treatment with Ortiva Top (V3), against leaf scorch, had an effectiveness of 72.7% in the Supenano Giallo variety and 82.1% in the Canellino variety. Applying two treatments with Ortiva Top (V4) had an effectiveness of over 98% on both varieties.

The effectiveness of Ortiva Top fungicide against fusarium wilt was 63.6% in the variants

with one treatment and 84.8% in the variants with two treatments.

Alcupral 50 WP is not effective against angular leaf spot in beans nor against fusarium wilt.

Research on the effectiveness of the treatments applied provides data on the possibility of introducing them into crop management schemes (Chiriac and Cristea, 2021; Toth and Cristea, 2020).

Table 1. Scoring of bacterial blight, angular leaf spot and fusarium wilt attack; calculating the effectiveness of the treatments applied

Variety	Variant	Frequency (%)	Intensity (%)	Attack degree (%)	Effectiveness (%)
Bacterial blight of bean					
SONESTA	V1	14.25	5.5	0.8	-
	V2	3.25	2.5	0.08	90.0
	V3	13.75	5.0	0.7	12.5
	V4	1.5	2.0	0.03	96.25
SUPERNANO GIALLO	V1	14.75	6.0	0.9	-
	V2	3.5	3.0	0.1	88.9
	V3	14.5	5.2	0.8	11.1
	V4	1.75	2.0	0.04	95.6
CANELLINO	V1	10.5	4.1	0.4	-
	V2	2.25	2.5	0.06	85.0
	V3	10.0	4.0	0.4	0.0
	V4	1.5	2.0	0.03	92.5
Angular leaf spot of bean					
SUPERNANO GIALLO	V1	59.5	15.0	8.93	-
	V2	58.25	15.0	8.74	2.13
	V3	30.5	8.0	2.44	72.7
	V4	5.75	2.0	0.12	98.7
CANELLINO	V1	44.75	10.0	4.48	-
	V2	44.75	10.0	4.48	0.0
	V3	20.0	4.0	0.8	82.1
	V4	2.5	2.0	0.05	98.9
Fusarium wilt of bean					
CANELLINO	V1	8.25	100.0	8.25	-
	V2	8.25	100.0	8.25	0.0
	V3	3.0	100.0	3.0	63.6
	V4	1.25	100.0	1.25	84.8

CONCLUSIONS

The common bean mosaic virus only manifested itself on the upper leaves.

Bacterial blight of beans manifested itself only on the lower leaves as a result of the favorable microclimate created by drip irrigation. Symptoms appeared after the first pods formed. Angular leaf spot of bean manifested itself towards the end of the growing season, especially on the leaves in the upper half of the plants.

All four detected diseases manifested only on the leaves.

The application of plant protection products for the management of fungal and bacterial diseases is one of the successful methods in terms of

preventing and combating them and obtaining high and quality bean production.

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STUDY OF YIELD QUANTITY AND ECONOMICAL IMPACT OF MAIZE OBTAINED IN A PRIVATE FARM LOCATED IN MACEA MICROZONE, ARAD COUNTY

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Abstract

This paper investigates the yield efficiency of maize cultivated in a private farm located in Macea, Romania. The study spans two agricultural years, 2024 and 2025, and involves eight commercial maize hybrids developed by three major seed companies. Field experiments were conducted to monitor and compare the performance of these hybrids in terms of grain yield. The average grain yield achieved in 2024 was 3,963 kg/ha, while in 2025 it declined to 2,920 kg/ha, likely reflecting interannual climatic variability and other environmental factors. Despite the lower yield in 2025, the market selling price increased from 0.70 RON/kg in 2024 to 0.80 RON/kg in 2025, partially compensating for the production drop. The study offers a practical assessment of maize productivity in a private farming context and highlights the influence of hybrid selection, seasonal conditions, and market trends on crop efficiency. The results are relevant and aiming to optimize maize production under field conditions in Western Romania and similar agroecological regions. Further investigations integrating input use and cost-efficiency analysis could provide a more comprehensive picture of farm-level maize performance.

Key words: maize, corn hybrids, agriculture technology, impact, economic impact.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, with major roles in food, feed, biofuel production, and industrial processing. Its wide adaptability to different pedoclimatic conditions has enabled its cultivation across diverse agricultural systems, from subsistence farming to highly intensive production systems. In Romania, maize occupies a central position in the cereal economy, accounting for a substantial share of the cultivated cereal area and contributing significantly to national agricultural output (Vili, 2022; Popescu et al., 2021).

The productivity of maize depends on the interaction between genetic potential, environmental conditions, and crop management practices. Among these factors, hybrid selection plays a decisive role because modern maize hybrids combine high yield potential with improved tolerance to abiotic stress, particularly drought and heat stress

(Tollenaar & Lee, 2011). The development and adoption of hybrid maize varieties have represented one of the most important technological advances in cereal production, mainly due to the heterosis effect, which improves plant vigor, biomass accumulation, and grain yield stability compared with open-pollinated varieties (Duvick, 2005; Tollenaar et al., 2017).

Despite favorable pedoclimatic conditions, Romania has often recorded maize yields below its production potential. Previous analyses have indicated that, between 1961 and 2013, the national average yield exceeded 4 t/ha only in a limited number of years, suggesting persistent constraints related to technology adoption, climatic variability, and management practices (Halmajan, 2017). In this context, the evaluation of maize hybrids under local field conditions remains essential for identifying cultivars that combine high productivity with adaptability and yield stability.

Environmental adaptation is particularly important in regions where seasonal variability influences crop performance. Water availability during flowering and grain filling is one of the main factors affecting maize yield, while temperature stress, soil fertility, and nutrient availability further contribute to differences among hybrids (Bänziger et al., 2000; Lobell et al., 2014; Ray et al., 2015). Regional and farm-level trials therefore provide practical information on genotype $\times$ environment interactions and support more accurate hybrid selection for specific agroecological conditions. Western Romania, including Arad County, is one of the country's relevant maize-growing regions due to its fertile soils and generally favorable climatic conditions. However, even in this region, yield variability may occur as a result of annual weather fluctuations and differences in crop management. Farm-level research is especially useful because it evaluates crop performance under real production conditions, where environmental, technological, and economic factors interact simultaneously (Van Es & Woodard, 2017).

In addition to agronomic performance, economic efficiency is increasingly important in maize production. High grain yield does not automatically ensure profitability, as farm outcomes also depend on production costs, selling prices, and market fluctuations. Therefore, integrating yield results with market-based indicators provides a more practical assessment of maize production efficiency at farm level (Kay et al., 2016).

The novelty of this study lies in the farm-level assessment of commercial maize hybrids under real production conditions in Macea, Arad County, Western Romania, by integrating agronomic performance with a basic economic evaluation based on market selling price and gross revenue. Unlike controlled experimental trials, this approach reflects the practical conditions faced by private farms and provides directly applicable information for hybrid selection and production planning.

Therefore, the objectives of this study were to compare the grain yield performance of eight commercial maize hybrids cultivated under private farm conditions in Macea, Western Romania, to assess the influence of interannual variability on maize productivity during the

2024 and 2025 agricultural years; and to evaluate the economic efficiency of maize production using selling price and gross revenue per hectare as practical farm-level indicators.

MATERIALS AND METHODS

Biological Material and Crop Management

The study was conducted under commercial farming conditions on a private farm located in the Macea microzone, Arad County, Western Romania, during the 2024 and 2025 growing seasons. The objective was to evaluate the agronomic performance and economic efficiency of commercial maize hybrids cultivated under the technological conditions specific to the farm.

Eight commercial maize hybrids developed by major seed companies were included in the study. The hybrids were selected based on maturity group, agronomic characteristics, yield potential, and suitability for the pedoclimatic conditions of the western Romanian Plain. The evaluated hybrids belonged to different maturity classes, providing a broad range of genetic backgrounds and adaptation strategies. Their principal agronomic characteristics, including maturity classification, FAO group, and thousand-kernel weight (TKW), are presented in Table 1.

The total maize-cultivated area of the farm was 200 ha in both agricultural years; however, the area allocated to each hybrid varied according to farm management decisions and production planning objectives. Depending on the agricultural season and the production strategy adopted by the farm, the cultivated area assigned to individual hybrids ranged from 15 to 45 ha in 2024 and from 15 to 40 ha in 2025.

All hybrids were cultivated using the same general crop management practices, including soil preparation, fertilization, weed control, and harvesting operations, in order to minimize management-related variability among hybrids. The study was organized as a comparative on-farm field evaluation. Maize hybrid represented the primary comparison variable, while agricultural year represented the temporal component of the study. Because the research was conducted under real commercial farming conditions, it followed a comparative farm-

scale evaluation approach rather than a fully randomized and replicated experimental design. The experimental unit was represented by the commercial field area cultivated with each individual maize hybrid. Grain yield and economic performance were evaluated separately for each hybrid in each agricultural year, allowing comparative assessment of agronomic and economic performance under practical farming conditions. No formal randomization procedure or experimental replications were established, as hybrid placement and cultivated area were determined by operational and management considerations specific to the farm. Consequently, the results should be interpreted as a comparative assessment of hybrid performance under commercial production conditions rather than as inference derived from a strictly controlled experimental trial.

Table 1. Maize hybrid selected for cultivation on the farm

Hybrid	Maturity group	FAO Group	TKW
Pioneer P0217	Semi-early	420	390-420
Pioneer P0710	Late	450	430-450
Pioneer P0450	Semi-late	450	420-445
Pioneer P0412	Semi-late	480	420-445
Pioneer P9889	Semi-late	350	400-425
RAGT Urbanix	Semi-late	420	310-330
Dekalb 5075	Semi-late	400-440	300-350
Dekalb 4611	Semi-early	350-390	300-350

The maize crop was established on a well-prepared seedbed, characterized by loose soil structure, adequate depth, absence of hardpan, good surface crumbling, adequate soil moisture, and low weed infestation. Autumn ploughing was performed at a depth of 28-30 cm. Before sowing, the seedbed was prepared by a single pass with a combinator at a depth of 7-8 cm. This operation ensured seedbed uniformity, preserved soil moisture, and reduced the risk of compaction caused by repeated field passes. Sowing was carried out during the last decade of April, on 24-25 April. Certified and calibrated seed was used, with 99% physical purity and germination exceeding 97%. The seeding rate was 75,000 kernels ha⁻¹, with a sowing depth of 6-7 cm and 70 cm row spacing. Sowing was performed mechanically using precision seed drills.

Before sowing, seeds were treated with fungicidal and insecticidal products to protect seedlings during the early growth stages. Seed treatment included Maxim XL, containing 25 g L⁻¹ fludioxonil and 10 g L⁻¹ metalaxyl-M, for protection against soil-borne pathogens such as *Fusarium* spp. and *Pythium* spp. Seeds were also treated with Seedoprid, containing imidacloprid 600 g L⁻¹, for protection against early-season pests, including *Tanymecus dilaticollis*, *Agriotes lineatus*, and *Diabrotica virgifera virgifera*.

Weed control was carried out chemically through pre-emergence and post-emergence treatments. Immediately after sowing, pre-emergence weed control was applied using Guardian at 2.5 L ha⁻¹ in 2024 and Adengo at 0.4 L ha⁻¹ in 2025.

Post-emergence weed control was performed in both years using Mustang at 0.5 L ha⁻¹ in combination with Gat Motion at 1.5 L ha⁻¹. Mustang was used for the control of thermophilic dicotyledonous weeds, while Gat Motion targeted annual monocotyledonous weeds and perennial grass weeds propagated by rhizomes, particularly *Sorghum halepense* and *Agropyron repens*.

Yield Determination and Economic Relevance

Grain yield was used as the main agronomic indicator for evaluating hybrid performance. Harvesting was performed mechanically, and grain production was recorded automatically by the harvesting machine. The obtained production was expressed as standard grain yield in kg ha⁻¹, adjusted to standard grain moisture, allowing comparison among hybrids and between agricultural years.

For each hybrid and year, yield data were used to calculate mean yield, yield difference between years, relative yield reduction, and performance compared with the annual average. These indicators were used to assess hybrid productivity, yield stability, and adaptation to the pedoclimatic conditions of the study area.

Soil and Climatic Conditions

The experimental field is located in the Macea microzone, Arad County, Western Romania, within the western Romanian Plain, part of the Pannonian Basin. The area is characterized by

fertile soils and climatic conditions generally favorable for maize cultivation. The dominant soils are chernozems, with medium to high humus content, good structure, and favorable water-holding capacity. Their loam to clay-loam texture supports adequate aeration, permeability, root development, and nutrient uptake. These soil characteristics are suitable for maize cultivation because the crop requires deep, well-structured soils with good moisture retention and moderate to high fertility. The climate of the area is temperate-continental, with warm summers and moderate precipitation. Climatic conditions during the maize growing season, from April to

September, were analyzed for the 2024 and 2025 agricultural years and compared with multiannual averages for the Arad region. Temperature conditions during both experimental years were generally close to the regional climatic norms, with average monthly temperatures ranging from approximately 14-15°C in April to 25-26°C in July-August. These values correspond to favorable thermal conditions for maize germination, vegetative growth, flowering, and grain filling. Precipitation distribution during the growing season showed higher rainfall in May and June, when monthly values reached approximately 65-82 mm.

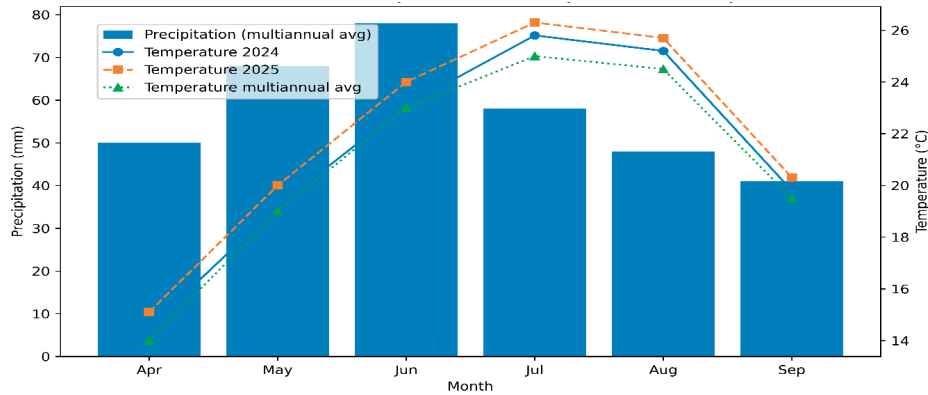


Figure 1. Climatic conditions during the maize growing season

This period coincides with the phase of intensive vegetative growth, when water availability supports biomass accumulation and reproductive organ formation. During July and August, precipitation decreased to approximately 45-60 mm, which is characteristic of the regional climatic pattern. Although these values did not indicate severe drought, reduced rainfall during the reproductive and grain-filling stages may have contributed to differences in hybrid productivity between years. Overall, the pedoclimatic conditions of the Macea microzone correspond well to the ecological requirements of maize. However, interannual variation in temperature and precipitation distribution may influence hybrid performance, especially during the critical stages of tasseling, silking, and grain filling (Figure 1).

Statistical Analysis and Data Processing

The collected data were processed using Microsoft Excel 365. Descriptive statistical indicators, including mean values, year-to-year comparisons, and relative differences among hybrids, were used to characterize agronomic and economic performance under commercial farming conditions. Economic indicators were calculated based on grain yield, input costs, and market prices recorded during each agricultural season. Comparative analyses were performed to evaluate differences in productivity and economic performance among hybrids and between years. Because the study was conducted as a comparative on-farm evaluation under commercial production conditions without formal randomization or experimental replication, the statistical assessment focused primarily on

descriptive and comparative analyses rather than inferential statistical testing.

RESULTS AND DISCUSSIONS

Maize productivity was influenced by the combined effect of hybrid genetics, seasonal climatic conditions, and crop management. The comparison between 2024 and 2025 showed clear differences in grain yield, confirming that interannual variability strongly affected maize performance under the conditions of the western Romanian Plain. The analysis focused on identifying differences in hybrid productivity, examining the influence of

environmental conditions on yield variability, and assessing the economic implications of maize cultivation under farm-level management. Attention was given to the relationship between climatic conditions recorded during the study period, hybrid adaptability, and the resulting economic outcomes of maize production.

The results presented in this section therefore provide an integrated evaluation of agronomic performance and production economics, highlighting the importance of hybrid selection and adaptive management strategies for maintaining productivity and profitability under variable environmental conditions.

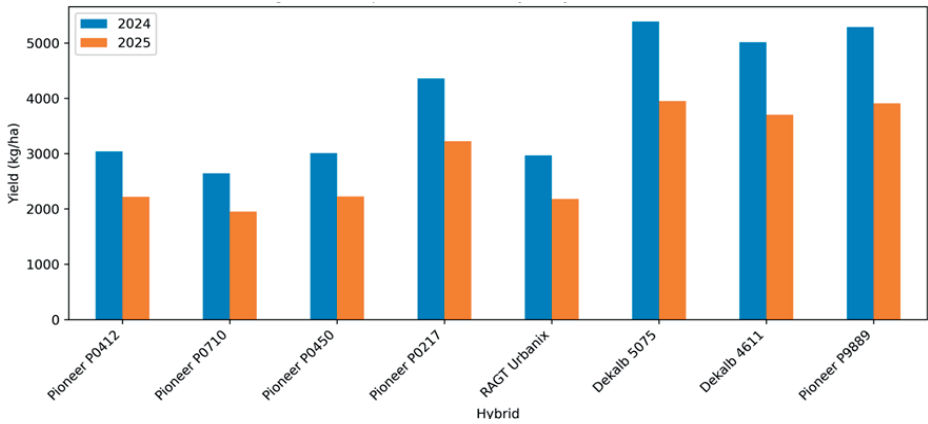


Figure 2. Comparison of maize hybrid yields (2024 vs 2025)

Figure 2 highlights the variability in grain yield among the eight maize hybrids evaluated during the 2024 and 2025 growing seasons. Dekalb 5075 and Pioneer P9889 recorded the highest grain yields in both years, whereas Pioneer P0710 consistently produced lower yields under the environmental conditions of the study area. The observed variation among hybrids confirms the importance of genotype selection for maize production under the pedoclimatic conditions specific to the western Romanian Plain.

The reduction in average grain yield from 3,821 kg ha⁻¹ in 2024 to 2,924 kg ha⁻¹ in 2025 suggests a strong influence of seasonal climatic variability on maize productivity. As presented in the climatic analysis, the second growing season was characterized by less favorable moisture conditions during critical developmental stages, which may have

negatively affected grain formation and kernel filling. Similar responses have been reported by Grassini et al. (2015), who identified water deficits during flowering and grain filling as major determinants of yield reduction in maize production systems.

The comparatively higher productivity observed for Dekalb 5075 and Pioneer P9889 across both seasons suggests better adaptation to the environmental conditions of the study area. Previous studies have shown that hybrid-specific responses to climatic variability may substantially influence final grain yield through differences in phenology, resource-use efficiency, and the ability to maintain productivity under environmental stress (Tollenaar & Lee, 2011; Lobell et al., 2014).

Overall, the results indicate that hybrid choice remains a key agronomic decision for improving productivity and reducing

production risk under increasingly variable climatic conditions.

Table 2. Main input costs for maize cultivation (2024–2025)

Input	2024 cost (RON)	2025 cost (RON)
Fuel	56595	76125
Urea fertilizer	93600	96400
Calcium nitrate	89500	85500

Table 2 summarizes the principal operational costs associated with maize production during the 2024 and 2025 growing seasons. The results indicate that production expenditures were influenced by both seasonal economic conditions and farm management decisions regarding hybrid allocation and input use. Although the overall cost structure remained relatively similar between years, variations in selected input categories contributed to differences in total production costs. These results provide the economic framework necessary for interpreting the relationship between production costs, grain yield, and farm profitability discussed in the following sections.

Table 3. Grain yield of maize hybrids during the 2024–2025 seasons

Hybrid	Yield 2024 (kg/ha)	Yield 2025 (Kg/ha)
Pioneer P0217	4358	3224
Pioneer P0710	2642	1951
Pioneer P0450	3010	2225
Pioneer P0412	3041	2218
Pioneer P9889	5288	3908
RAGT Urbanix	2967	2181
Dekalb 5075	5387	3949
Dekalb 4611	5013	3702

Table 3 presents the grain yield obtained by the evaluated maize hybrids during the 2024 and 2025 growing seasons. Yield performance varied considerably among hybrids and between years, indicating the influence of both genetic background and seasonal growing conditions on maize productivity. Across all hybrids, average grain yield decreased from 3,821 kg ha⁻¹ in 2024 to 2,924 kg ha⁻¹ in 2025, reflecting less favorable environmental conditions during the second experimental season. Dekalb 5075 and Pioneer P9889 recorded the highest grain yields in both years, whereas Pioneer P0710 consistently showed

lower productivity under the conditions of the study area.

The observed variation among hybrids highlights the importance of genotype selection for maintaining productivity under variable growing conditions. A more detailed interpretation of hybrid performance in relation to seasonal climatic conditions is presented in Figure 2.

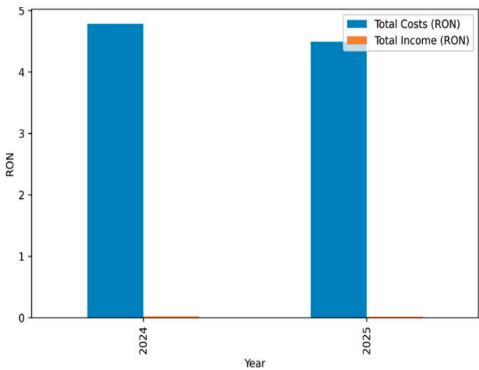


Figure 3. Total production costs vs total income

Figure 3 highlights the economic balance between total production costs and total income for the two seasons. Figure 3 illustrates the distribution of operational costs among the main input categories associated with maize cultivation during the 2024 and 2025 growing seasons. The results indicate that input allocation varied between seasons, mainly due to fluctuations in fuel prices and adjustments in hybrid allocation strategies. The increase in fuel-related expenditures observed during the 2025 season reflects the growing influence of energy prices on the economic sustainability of agricultural production systems. In contrast, fertilizer-related costs remained relatively stable, suggesting consistent nutrient management practices at farm level. Seed-related expenditures represented an important component of the analyzed operational costs due to the comparative evaluation of multiple commercial hybrids under field conditions. This economic structure therefore reflects the experimental and comparative framework of the study rather than the standard cost distribution typically encountered in large-scale commercial maize production systems. These results confirm that yield variability remains one of the most critical factors

influencing farm profitability in maize production systems (Ray et al., 2015).

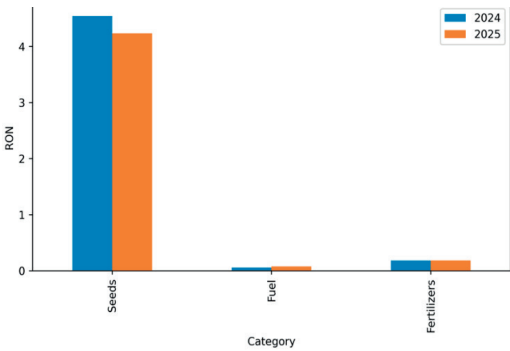


Figure 4. Distribution of maize production costs (2024 vs 2025)

The distribution of operational costs associated with maize cultivation is presented in Figure 4. The results indicate that production expenses varied between the two agricultural seasons mainly due to fluctuations in fuel prices, input

procurement costs, and differences in hybrid allocation strategies.

Fuel-related expenditures increased during the 2025 growing season, reflecting the influence of rising energy prices on the economic sustainability of agricultural production systems. In contrast, fertilizer-related costs remained relatively stable between seasons, suggesting consistent nutrient management practices at farm level.

Seed-related expenditures represented an important component of the analyzed operational costs due to the comparative cultivation of multiple commercial hybrids within the experimental framework adopted in this study. Therefore, the economic structure presented should be interpreted in relation to the comparative hybrid evaluation strategy rather than as the standard cost distribution typical of commercial maize production systems.

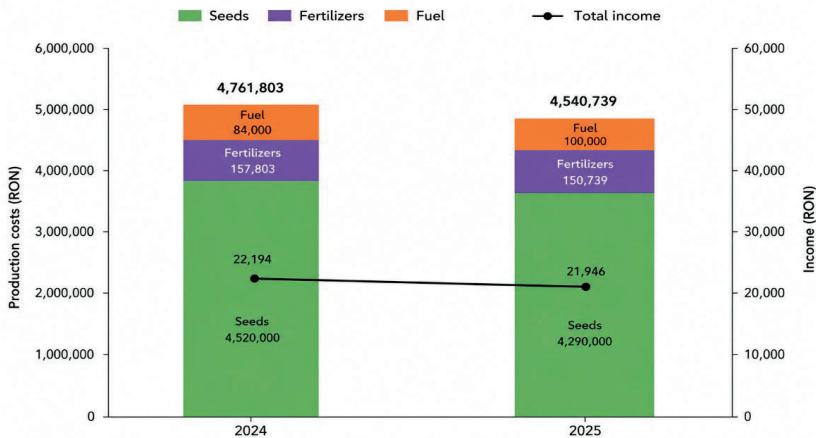


Figure 5. Economic performance and operational costs structure of maize production during 2024 and 2025 growing seasons

Figure 5 integrates the overall economic performance of maize production with the distribution of the principal operational costs recorded during the 2024 and 2025 growing seasons. Total production costs decreased slightly from 4,783,120 RON in 2024 to 4,491,350 RON in 2025, whereas total income declined from 22,194.45 RON to 18,686.80 RON during the same period. This trend suggests that the reduction in grain yield observed during the second growing season

exerted a greater influence on farm income than the modest decrease in production expenditures.

Although the maize selling price increased from 0.70 RON kg⁻¹ in 2024 to 0.80 RON kg⁻¹ in 2025, the economic benefit associated with higher market prices was insufficient to compensate for the decline in productivity. Consequently, lower grain yields were associated with reduced economic returns, highlighting the strong dependence of maize

profitability on production performance under variable environmental conditions.

The analysis of production costs revealed a relatively similar cost structure in both growing seasons. Seed procurement represented the largest share of operational expenditures, accounting for approximately 4.52 million RON in 2024 and 4.29 million RON in 2025. Fuel-related expenses increased from 56,595 RON to 76,125 RON, reflecting the influence of rising energy prices on agricultural production systems, whereas fertilizer costs showed only minor fluctuations between years, suggesting relatively stable nutrient management practices at farm level.

These findings indicate that the economic sustainability of maize production depends not only on input management but also on the ability to maintain productivity under contrasting seasonal conditions. Similar observations were reported by Ray et al. (2015), who emphasized that yield variability remains one of the main determinants of economic performance and production risk in agricultural systems exposed to climatic uncertainty.

From a practical perspective, the results underline the importance of selecting maize hybrids capable of maintaining satisfactory productivity across different environmental conditions. Such an approach may contribute to improving economic resilience, reducing production risk, and supporting the long-term sustainability of maize-based farming systems in regions increasingly affected by climatic variability.

CONCLUSIONS

The present study demonstrated that maize productivity under the pedoclimatic conditions of the Macea microzone was strongly influenced by both hybrid selection and seasonal climatic variability. Across all evaluated hybrids, average grain yield decreased from 3,821 kg ha⁻¹ in 2024 to 2,924 kg ha⁻¹ in 2025, indicating the substantial impact of environmental conditions on crop performance. Among the tested hybrids, Dekalb 5075 and Pioneer P9889 consistently recorded the highest grain yields in both growing seasons, suggesting good suitability

for cultivation under the environmental conditions of the western Romanian Plain.

The economic analysis showed that the increase in maize selling price from 0.70 RON kg⁻¹ to 0.80 RON kg⁻¹ was insufficient to compensate for the reduction in grain yield. Consequently, total income declined from 22,194.45 RON to 18,686.80 RON, indicating that farm profitability was more closely associated with production performance than with short-term market price fluctuations. These findings emphasize the importance of maintaining productivity under variable climatic conditions in order to ensure economic sustainability.

The results further suggest that hybrid selection should be considered not only from a productivity perspective but also from an economic risk-management perspective. Under the conditions of this study, hybrids that maintained comparatively higher yields across contrasting growing seasons contributed to a more favorable economic outcome despite climatic constraints.

The combined agronomic and economic assessment applied in this study provides useful information for hybrid selection under the environmental conditions of the western Romanian Plain. From a practical perspective, the findings indicate that improving yield performance remains one of the most effective strategies for enhancing farm profitability and reducing production risk under increasingly variable climatic conditions.

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ANALYSIS OF PRODUCTIVITY AND CLIMATE-STRESS ADAPTABILITY OF *Lotus corniculatus* L. CULTIVARS IN A COMPARATIVE CULTIVATION SYSTEM

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Abstract

This study examined the agronomic performance of three bird's-foot trefoil (Lotus corniculatus L.) cultivars - Lovrin 25, Dacia, and Doru - within the ISTIS comparative trial network in Romania. The experimental framework assessed dry matter productivity and the stability and adaptability of each cultivar across heterogeneous pedoclimatic environments, thereby enabling rigorous comparisons between newly developed germplasm and established reference cultivars to determine their agronomic and economic value. Across all test sites, the Lovrin 25 cultivar exhibited a remarkably superior dry matter yield compared with the control cultivars Dacia 1 and Doru. This enhanced productivity appears to be associated with improved post-mowing regrowth dynamics and a more homogeneous stand structure, facilitating more efficient resource acquisition and utilisation. The yield advantage observed for Lovrin 25 underscores the potential for targeted genetic improvement to increase biomass accumulation without compromising essential agronomic attributes such as nutritional value, drought tolerance, and persistence. Collectively, the findings indicate that Lovrin 25 integrates high productivity with noticeable resilience to climatic variability, supporting its suitability for sustainable forage production systems.

Key words: *Lotus corniculatus* L., dry matter yield, correlation, regression.

INTRODUCTION

The future development of sustainable feed systems well adapted to climatic conditions is an essential approach to supporting the productive restoration of multi-species ecosystems in which “bird’s-foot trefoil” (BFT), is a component species. Many studies highlight that the natural genetic diversity of bird’s-foot trefoil is key to conserving biodiversity and thus ensuring high agricultural sustainability (Muzzo, 2026; Sultan et al., 2026).

The species is a perennial legume that is particularly important from agronomic and ecological perspectives and is widely cultivated in temperate forage systems across Europe, North America, and other regions with similar climates. This species is valued both for the superior nutritional value of its plant mass and for its ability to contribute to the sustainability of agricultural production systems through

biological nitrogen fixation and reduced chemical inputs (Kaplan et al., 2009; Bostan et al., 2025; Sakariyahu et al., 2025; Plews, 2026). Botanically, BFT is characterised by a semi-erect or prostrate habit, with branched stems and five leaflets per leaf, yellow umbellate inflorescences, and fruits that resemble a “bird's foot”, features that give it a clear morphological identity and make it easy recognisable in the field. Its deeply developed root system enables efficient soil exploration, providing access to water and nutrients even under water stress. It has been demonstrated that increasing nutrient levels, especially through nitrogen fertilisation, is often necessary to meet production targets in intensive agricultural ecosystems. BFT is one of the species that can avoid the use of high doses of nitrogen that are associated with negative environmental effects (Band et al., 2022; Muehleisen et al., 2023; Namuhan et al., 2024).

The importance of BFT is increasing in the context of current climate change because anthropogenic activities have doubled nitrogen flows, with direct consequences for water quality, biodiversity, and adaptation to climate change (Galloway et al., 2008; Sutton et al., 2011). Based on these considerations, intensive and sustainable agriculture will increase productivity while reducing environmental impact through integrated soil-plant management (Galloway et al., 2008; Canfield et al., 2010; Aljazairi et al., 2025). This species can tolerate a wide variety of soil types, including low-fertility or slightly acidic soils, which ensures its high ecological plasticity under variable pedoclimatic conditions (Țiței et al., 2021; Bostan et al., 2025;).

One of the fundamental features of BFT is its ability to fix nitrogen biologically through symbiosis with rhizobia, thereby improving soil fertility and reducing the need for chemical nitrogen fertilisers. This feature offers a significant advantage in sustainable agriculture, leading to lower production costs and reduced environmental impact (Carlsson & Huss-Danell, 2003). In addition to this, the vegetative mass of BFT distinguishes itself for its high crude protein content and favourable digestibility, making it a valuable feed for ruminants, either in the form of hay or when grazed directly (Carlsson & Huss-Danell, 2003; Țiței et al., 2021; Nahursky, 2026).

A distinctive feature of this species is its content of condensed tannins, phenolic compounds with beneficial effects on ruminant health, as they can reduce bloating and increase the efficiency of protein utilisation in the rumen during digestion (Barry, 1985). Thus, BFT combines advantageous nutritional features with no negative side effects, positioning it favourably in comparison with other forage legumes, such as alfalfa or red clover.

BFT, like other legume species, plays an essential role in maintaining the positive relationship between plant diversity and biomass production, but is severely affected by drought, phenomenon also observed in other crops in this study area (maize, hemp, wheat, etc) (Popa et al., 2022; Agapie & Sala, 2022; 2023; Samfira et al., 2025). BFT contributes to the selection effect and nitrogen fixation, directly influencing the stability and

productivity of grassland plant communities under microplastic and water stress conditions. BFT belongs to the legume group, which has played a key role in the relationship between plant diversity and biomass production (Esterhuizen & Kim, 2022). Even under extreme conditions, BFT stands out as an important species for maintaining the productivity and functioning of grassland ecosystems, especially under climate stress and pollution (Man et al., 2025).

Adaptability to abiotic stress, including drought tolerance and the ability to regenerate after repeated mowing, is another feature that supports the intensive use of BFT in modern forage production systems, especially under current climate conditions characterised by intense climate change (Morris et al., 2021). In this regard, selection and genetic improvement efforts have focused on identifying and promoting genotypes with high agronomic potential, stability, and resilience to ensure long-term feed security. The specific adaptability of BFT is ensured by high genetic diversity, which is often a key pillar of the adaptive process that improves stress tolerance and increases biological nitrogen fixation efficiency (King, 2024; Leger et al., 2024).

These findings highlight the importance of ongoing research into genetic variability and the selection of adapted genotypes, with the aim of developing stable and productive crops that are resistant to fluctuating environmental conditions. At a time when modern agriculture must balance economic and ecological requirements, BFT is emerging as a strategic species for ensuring sustainable, high-quality feed resources.

The purpose of the present study is to contribute to deepening knowledge about the agronomic potential of BFT and to support the use of high-performance varieties in modern feed systems. In an ever-changing agricultural context, such research is essential to ensure feed security and to adapt agricultural production to current and future climate challenges by creating new genotypes, better adapted to climate change.

MATERIALS AND METHODS

The experimental fields were located in six administrative-territorial units, distributed

across different geographical areas of Romania: Șimleu Silvaniei, Dej, Satu Mare, Sibiu, Negrești, and Rădăuți. The experiments were conducted on identical agricultural land for each test centre and under the same physical and geographical conditions specific to each centre, during the years 2023-2025.

Three genotypes of *Lotus corniculatus* L. were used as biological material: the Lovrin 25 as a tester and two approved varieties, Dacia 1 and Doru, as controls.

Lovrin 25 is a synthetic variety whose parental clones were extracted from local populations in the Banat plain (Romania), clones whose general combinative capacity was verified using the polycross method. According to Gallais (1990), a synthetic variety is an artificial population resulting from the sexual multiplication over several generations of the offspring through free pollination of a limited number of constituents (clones, families, lines). The research/testing focused on the main quantitative characteristics (dry matter and green mass production) as well as traits such as resistance to unfavourable winter conditions, regeneration capacity, drought resistance, lodging resistance, and disease resistance. In the studies conducted, the Lovrin 25 was compared with the two controls.

The statistical methods used were the t-test, linear regression, and Pearson's correlation, with calculations performed using JASP (2025) and IBM SPSS Statistics (Version 27) software.

RESULTS AND DISCUSSIONS

In accordance with the provisions of Law No. 266/2002 on the production, processing, quality control and certification, marketing of seeds and planting material, as well as the registration of plant varieties, the State Institute for Variety Testing and Registration (ISTIS) technically examines Romanian and foreign varieties for which registration in the Official Catalogue of Plant Varieties in Romania is requested, with a view to marketing them in Romania and the Member States of the European Union (Ivașcu & Ciora, 2008).

The registration of varieties of field crops is based on the distinctiveness, uniformity, and stability (DUS) test and the agronomic value and use (VAU) test. A variety is accepted for

registration only if it is distinct, stable, and sufficiently uniform, and has satisfactory agronomic and use value (Ivașcu & Ciora, 2008).

In Romania, the agronomic value of BFT varieties is assessed through comparative plots within the national testing network. Recent studies have analysed the behaviour of three varieties (Lovrin 25, Dacia, and Doru) to determine dry matter production, stability across different years, and adaptability to various pedoclimatic conditions.

The dry matter yield results (Table 1) indicate that the Lovrin 25 variety had higher dry matter productivity than the control varieties, Dacia 1 and Doru. This highlights its favourable agronomic potential and effective regeneration capacity after harvesting, as noted in other specialised publications (Bostan et al., 2025).

Table 1. Dry matter yield (t/ha) at BFT in the 3 experimental years - descriptive statistics

Year	Variety	Average	St. Dev.	Min.	Max.
I	Dacia 1	4.95	2.74	1.4	9.1
I	Doru	5.15	2.73	1.2	8.2
I	Lovrin 25	5.08	2.65	1.3	8.6
II	Dacia 1	16.78	3.88	10.7	20.5
II	Doru	17.20	2.85	12.6	19.6
II	Lovrin 25	17.40	3.35	12.2	21.1
III	Dacia 1	15.55	4.14	9.7	20.2
III	Doru	16.20	3.46	10.4	19.6
III	Lovrin 25	15.73	3.65	10.0	20.1

Based on the results obtained, the following aspects can be observed regarding the analysed varieties: in the first experimental year, they had similar average values; in the second year, all varieties recorded a strong increase in average production, with Lovrin 25 achieving the highest average dry matter production (17.40 t/ha), slightly exceeding the other varieties; in the third year, the averages decreased slightly compared to the second year for all varieties, but the Lovrin 25 variety remained competitive, recording an average value (15.73 t/ha) close to that of the Doru variety (16.20 t/ha) and higher than that of the Dacia 1 variety (15.55 t/ha).

The comparative analysis of the three BFT varieties highlights several aspects relevant to selecting the best-performing varieties under the experimental conditions analysed. First, it should be noted that in the first year, all three varieties had similar dry matter yields,

averaging around 5 t/ha. This suggests that at the beginning of cultivation or in the early stages of development, the genetic differences between varieties are not noticeable enough to produce significant differences in production. In the second year, the situation changed considerably, with all varieties recording increases in production; this stage marked the moment when the Lovrin 25 variety distinguished itself from the other two with the highest average production (17.40 t/ha). This result confirms the biological potential of the Lovrin 25 variety and highlights its adaptability to specific environmental conditions.

The superiority of the variety Lovrin 25 in the second experimental year makes it a preferred option for farmers seeking high yields. In the third year, the average dry matter yields of the three varieties decreased slightly, possibly due to climatic factors or other less favourable environmental conditions. However, the Lovrin 25 variety remains at a higher production level than the Dacia 1 variety, respectively, one of the controls. This highlights good stability and high performance in the second year of production, demonstrating that the Lovrin 25 variety achieves high yields while maintaining strong performance under varying conditions.

The Shapiro-Wilk test results show that all three varieties, including Lovrin 25, have data compatible with a normal distribution (all p values > 0.05) (Table 2). This allowed us to use parametric tests for further comparisons.

Table 2. Shapiro-Wilk test for dry matter yield results at BFT during 2023-2025

Variety	Year I (p)	Year II (p)	Year III (p)	Conclusion
Dacia 1	0.947	0.310	0.547	Normal
Doru	0.599	0.122	0.414	Normal
Lovrin 25	0.930	0.651	0.515	Normal

Table 3 presents the analysis of the coefficients of variation for the dry matter yield results obtained at BFT in the plots analysed during the period 2023-2025.

Table 3. Coefficients of variation (CV) for dry matter yield results at BFT during the period 2023-2025

Variety	Year I	Year II	Year III
Dacia 1	0.554	0.231	0.266
Doru	0.530	0.166	0.214
Lovrin 25	0.521	0.193	0.232

The results from the first experimental year show that variability is higher across all varieties (CVs of 0.52-0.55), indicating large differences among the analysed samples. In the second year, variability decreases considerably (CVs between 0.16 and 0.23), indicating more uniform production of the analysed samples. The Lovrin 25 variety has an intermediate CV (0.193), indicating greater uniformity than the Dacia 1 variety but slightly lower than that of the Doru variety. In the third year, variability increases slightly but remains moderate (CV 0.21-0.27), and the Lovrin 25 variety is again between the other two varieties with which it was compared. Thus, based on the coefficients of variation obtained, we can say that the Lovrin 25 variety shows a good balance between productivity and stability in dry matter yield. Although Doru variety has the lowest variability in the second and third years of production, the Lovrin 25 variety combines high average dry matter yield values with relatively good stability, making it competitive and reliable for long-term cultivation.

The t-tests between the Lovrin 25 variety and the two other varieties (Doru and Dacia 1) did not reveal any statistically significant differences (all p values > 0.05), which means that within this data set, yields do not differ significantly between varieties (Table 4). However, Lovrin 25 remains of interest due to its stability and high average values, as confirmed by the coefficient of variation calculated above.

Table 4. The t-tests for dry matter yield results at BFT in the period 2023-2025

Comparison	Year I (p)	Year II (p)	Year III (p)
Lovrin 25 vs Doru	0.967	0.914	0.825
Lovrin 25 vs Dacia 1	0.933	0.774	0.937

Pearson's correlations (r) between experimental years were calculated to highlight whether previous years influence dry matter production in subsequent years. The correlation coefficients and corresponding p-values are presented in Table 5.

Regressions were calculated to assess the dependence of dry matter production between experimental years. Table 6 shows the regression coefficients (intercept and slope) for each variety in the experiment.

Table 5. Pearson's correlation coefficients (r) and p-values by year and variety at BFT

Variety	Year I vs Year II	Year II vs Year III	Year I vs Year III
Dacia 1	0.27; p=0.610	0.93; p=0.007	0.23; p=0.656
Doru	0.31; p=0.556	0.98; p=0.001	0.37; p=0.468
Lovrin 25	0.11; p=0.828	0.95; p=0.004	0.34; p=0.510

Table 6. Regression coefficients (intercept and slope) for dry matter production at BFT during 2023-2025

Variety Dacia 1			
Year I -> Year II			
Parameter	Coefficient	Std. Error	p-value
const	14.923	3.776	0.017
Year I	0.376	0.681	0.610
Year II -> Year III			
Parameter	Coefficient	Std. Error	p-value
const	-1.090	3.401	0.765
Year II	0.991	0.198	0.007
Variety Doru			
Year I -> Year II			
Parameter	Coefficient	Std. Error	p-value
const	15.556	2.844	0.005
Year I	0.319	0.497	0.556
Year II -> Year III			
Parameter	Coefficient	Std. Error	p-value
const	-4.193	2.259	0.137
Year II	1.186	0.130	0.001
Variety Lovrin 25			
Year I -> Year II			
Parameter	Coefficient	Std. Error	p-value
const	16.660	3.540	0.009
Year I	0.146	0.629	0.828
Year II -> Year III			
Parameter	Coefficient	Std. Error	p-value
const	-2.168	3.108	0.524
Year II	1.029	0.176	0.004

For the Lovrin 25 variety, the regression indicates a relatively stable relationship between yields across consecutive years, confirming sustained performance. Even if not all relationships are statistically significant, the general pattern suggests that this variety has a predictable and balanced evolution, which increases its attractiveness for practical use.

The analysis of Pearson's correlations and simple linear regressions provides additional insight into the behaviour of the three varieties. The results show a positive link between yields in consecutive years, but the intensity and significance of this relationship differ from one variety to another. Thus, for Dacia 1 variety, the correlations are positive but moderate, suggesting that this variety is more sensitive to environmental variations, leading to differences

from one year to another. For Doru variety, the correlations are stronger and more stable, indicating greater predictability of dry matter yields. The Lovrin 25 variety shows an interesting combination: consistent correlations across years and regressions that confirm the trend of maintaining performance.

This shows that the Lovrin 25 BFT variety has high average dry matter production and predictable production dynamics, making it recommended for cultivation. From a regression perspective, the coefficient values for the Lovrin 25 variety indicate a reasonable linear relationship between yields in successive years. Even though the statistical significance is not strong due to the small sample size, the general trend indicates the variety's stability. Thus, the Lovrin 25 variety combines high productivity with good interannual consistency, giving it greater practical value and a lower risk of large fluctuations between growing seasons.

Figure 1 graphically shows the relationships between dry matter yields per year for each variety analysed. The points represent raw observations, and the lines indicate linear regression models. To highlight the Lovrin 25 variety, its regression lines are coloured in red.

The following section presents the analysis of correlations and regressions, along with significance tests for dry matter production in the BFT varieties analysed during the experiment.

For the Dacia 1 variety, the correlations between years show the general direction of yield evolution (Table 7). The regression results suggest a positive relationship between successive years, but the intensity and statistical significance vary depending on the data pair analysed. Regarding Doru variety, the regression results show a positive relationship across successive years (Table 8), but the intensity and statistical significance vary by pair analysed, as is the case with the Dacia 1 variety. The Lovrin 25 variety shows consistent correlations between years, with regression equations indicating a predictable relationship between dry matter yields in successive years (Table 9). The values of the correlation coefficient r confirm a positive association, and significance tests indicate a low probability that the results are due to chance. Thus, the Lovrin 25 variety stands out for its stability and statistical consistency.

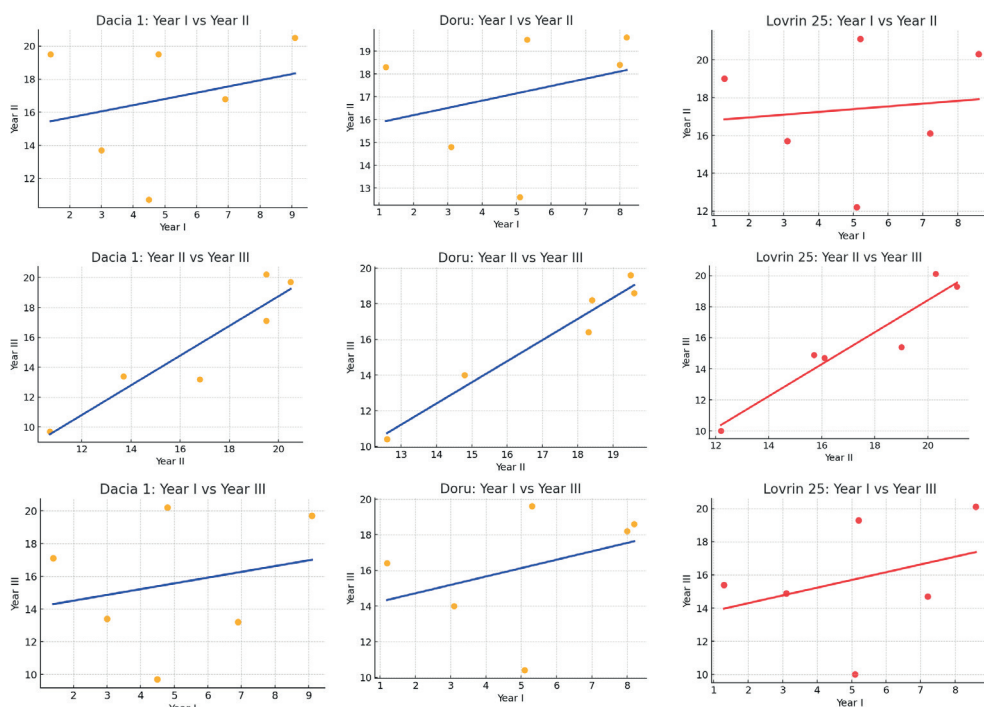


Figure 1. Regression lines of the dry matter yield compared among varieties and pairs of vegetation years

Table 7. Correlation and regression analysis along with significance tests for dry matter production in the Dacia 1 variety of BFT during the period 2023-2025

Relationship	Regression equation	r	p (r)	slope (b)	p (b)
Year I -> Year II	$y = 14.92 + 0.38 \cdot x$	0.27	0.610	0.38	0.610
Year II -> Year III	$y = -1.09 + 0.99 \cdot x$	0.93	0.007	0.99	0.007
Year I -> Year III	$y = 13.81 + 0.35 \cdot x$	0.23	0.656	0.35	0.656

Table 8. Correlation and regression analysis along with significance tests for dry matter production in the Doru variety of BFT during the period 2023-2025

Relationship	Regression equation	r	p (r)	slope (b)	p (b)
Year I -> Year II	$y = 15.56 + 0.32 \cdot x$	0.31	0.556	0.32	0.556
Year II -> Year III	$y = -4.19 + 1.19 \cdot x$	0.98	0.001	1.19	0.001
Year I -> Year III	$y = 13.78 + 0.47 \cdot x$	0.37	0.468	0.47	0.468

Table 9. Correlation and regression analysis along with significance tests for dry matter production in the Lovrin 25 variety of BFT during the period 2023-2025

Relationship	Regression equation	r	p (r)	slope (b)	p (b)
Year I -> Year II	$y = 16.66 + 0.15 \cdot x$	0.11	0.828	0.15	0.828
Year II -> Year III	$y = -2.17 + 1.03 \cdot x$	0.95	0.004	1.03	0.004
Year I -> Year III	$y = 13.36 + 0.47 \cdot x$	0.34	0.510	0.47	0.510

Pearson's correlation analysis shows that Lovrin 25 has positive associations between yields across successive years, confirming consistent performance. Linear regression equations highlight direct relationships between successive yields (Year I–II and Year II–III),

and the correlation coefficients (r) indicate that the data align with a predictable model. Even though some p-values do not indicate strong significance (due to the small sample size), the general direction is clear; the Lovrin 25 variety is stable, predictable, and high-performing.

Summarizing the results obtained for the new BFT Lovrin 25 variety, it stands out for: the highest yield in the second year, confirming its biological potential; good statistical stability, low CV, and consistent correlations between years; resistance to environmental variations, demonstrated by high values even in the third year of production, when yields generally decreased; and predictability proven by regression equations and correlation coefficient values. Considering the above-mentioned aspects, the Lovrin 25 variety ranks first in direct comparison and demonstrates a combination of high dry matter yields, stability, and statistical consistency, providing farmers with a new promising variety option for cultivation. It is a semi-late to traditional variety, characterized by good resistance to diseases, wintering and drought. It has very good resistance to falling and a very good regeneration capacity.

CONCLUSIONS

The results confirm that all BFT varieties analysed show a positive trend in dry matter production over successive years. Among them the Lovrin 25 variety stands out due to its balance high yields and consistent performance, distinguishing it from Dacia 1 and Doru varieties. Lovrin 25 demonstrates both productivity and stability making it a reliable and valuable option for cultivation. Its performance remains relatively consistent across years, even where the statistical differences are not always significant. Overall, Lovrin 25 emerges as the most promising variety analysed. Its combination of yield and stability supports its recommendation for expanding cultivated areas and highlights its suitability for farmers under current agricultural conditions.

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CHEMICAL COMPOSITION, DIGESTIBILITY AND NUTRITIVE VALUE OF SOWN GRASSLANDS OF CEREALS AND LEGUME MEADOW GRASSES

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Abstract

The development of some species of perennial grasses forage crops in double mixtures with red clover was investigated in order to establish the quality and nutritive value of forage under mountain conditions. The dry matter of the mixture of *D. glomerata* L. with *Tr. pratense* L. was found to have the highest content of NDF, ADF and cellulose. The exceeded compared to the mean value of the traits was 6.0%, 8.0% and 12.5%, respectively. The mixture of *Ph. pratense* L. with *Tr. pratense* L. was shown to have the lowest content of fiber structural components (excluding ADL). The biomass had the highest in vitro dry matter digestibility. The values of the parameter were 2.3 to 7.0% higher than those of the other mixtures in the experiment. The plant mass of the two-component mixtures with *L. perenne* L. and *F. rubra* L. contained significantly higher amount of ADL in dry matter and had the highest values of GE.

Key words: perennial grass mixtures, detergent fibers, energy value.

INTRODUCTION

The sustainable agriculture systems in Bulgaria offer new ecological and environmentally friendly methods to improve the quality and feed value of the crop mass.

Perennial grass mixtures with hay or pasture direction are part of good agricultural practices in forage production with balanced composition and low cost (Churkova & Churkova, 2021; Lazarev & Tyazhkorob, 2022; Manevska-Tasevska et al., 2024). Mixed grasslands with high productivity and longevity require selection of species with ecological and combinative ability to maintain dynamic stability in the grassland (Elgersma & Sørensen, 2016). Association and competition (compatibility) between components are tailored to the biological and productive characteristics of species in specific agroecological conditions (Rusinovci et al., 2016; Bozhanska et al., 2024b). They also have significant effects in terms of optimal use of soil resources, water and solar energy (Duchini et al., 2018; Huss et al., 2022).

Mixed grasslands form a long-lasting vegetation mass, with conditions for weed reduction and an

increase in the relative proportion of useful grasses and legume forage crops affecting the evaluation, quality and nutritional value of forage (Naydenova & Vasileva, 2016).

In the mountainous regions of Bulgaria, forage grasses are most exposed to stressful abiotic effects with significant impact on their sustainability and productivity (Bozhanski et al., 2023). Some of the most suitable species for the establishment of temporary grasslands (two-, three- or four-component mixtures) evaluated on the traits of productivity, longevity and quality are the species: *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, *Festuca pratensis*, *Festuca rubra*, *Dactylis glomerata* and *Phleum pratense* (Naydenova & Mitev, 2015; Churkova, 2019; Katova, 2023). The main structurally determining component in the composition of grass associations is perennial meadow grasses, which maintain a high relative share in the total forage mass, and legume species positively affect the stability and yield of both fresh and dry matter (Mitev & Naydenova, 2015; Naydenova et al., 2015). Ecologically, members of the *Fabaceae* family have the ability to supply nitrogen to grasses through symbiotic nitrogen fixation,

respectively increasing crude protein content and improving dry matter digestibility (Pirhofer-Walz et al., 2012; McElroy et al., 2017). The maturity stage is an important factor that influences the nutritional value of the forage plant, as the content of cell walls (including the values of lignified vascular and sclerenchymal tissue in the plant) affects the feed digestibility ratio (Valente et al., 2016). The concentration of those substances is closely related morphological structure of a plant and to habitat conditions (Sosnowski et al., 2017).

In this sense, in the conditions of the Central Balkan Mountain (Bulgaria), the mixed grassland of *Lotus corniculatus* with *Festuca rubra* is distinguished by its biologically determined resistance and suitability to variations in environmental factors (Mitev et al., 2010). The crude protein, crude fiber and nitrogen-free extractable substances in the dry matter of plant mass were 147.87 g kg⁻¹, 372.20 g kg⁻¹ and 306.30 g kg⁻¹, respectively (Churkova & Churkova, 2020). The mixture is highly productive and practically validated for the Central Balkan Mountain region (Bozhanska, 2017b; Churkova, 2019). The two-component mixture of *Trifolium repens* with *Poa pratensis*, grown under mountain conditions, has optimal conversion, low fiber content and high digestibility of dry biomass. The values of neutral-detergent fibers, acid-detergent fibers, cellulose and *in vitro* dry matter digestibility of the grasses were 356.5 g kg⁻¹ (NDF), 226.4 g kg⁻¹ (ADF) and 192.4 g kg⁻¹ (cellulose) and 712.6 g kg⁻¹ (IVDMD), respectively (Bozhanska, 2017a). In the experimental area, good compatibility was also found between the components in mixed grasslands of *Lolium perenne* with *Trifolium repens*, *Lotus corniculatus* L. and *Trifolium pratense* L. The biomass formed was of high nutritional value, and dry matter digestibility ranged from 622.18 g kg⁻¹ to 642.54 g kg⁻¹ (Petkova et al., 2022).

Cell wall components are a factor in forage quality and a major source of energy for animals (Katova & Naydenova, 2017a, 2017b; Ivanova et al., 2019).

The aim of the present study was to determine the fibers cell wall components, *in vitro* dry matter digestibility and energy nutritional value of forage biomass obtained from two-component mixtures of *Trifolium pratense* L.

with *Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L. and *Phleum pratense* L. grown under mountain conditions.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria).

The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

The experimental variants included:

1. *Festuca rubra* L. + *Trifolium pratense* L. (FR + TrP);
2. *Lolium perenne* L. + *Trifolium pratense* L. (LP + TrP);
3. *Phleum pratense* L. + *Trifolium pratense* L. (PP + TrP);
4. *Dactylis glomerata* L. + *Trifolium pratense* L. (DG + TrP).

Sowing was done manually, scattered. The sowing rates of the studied forage species were calculated based on 100% seed germination. Immediately after sowing, the sown areas were rolled for better contact of the seeds with the soil and to ensure simultaneous germination of the plants. The plot size was 5 m², laid out in 4 replications.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

Indicators of study

The fibrous structural elements in the plant cell are analyzed in laboratory: Neutral Detergent Fibers (NDF, g kg⁻¹ DM), Acid detergent fiber (ADF, g kg⁻¹ DM) and Acid detergent lignin (ADL, g kg⁻¹ DM) by the Van Soest & Robertson (1979) detergent assay and *in vitro* dry matter digestibility (IVDMD) according to a two-way pepsin-cellulase method of Aufrere (1982). The polyosides are empirically calculated: Hemicellulose (g kg⁻¹ DM) = NDF -

ADF and Cellulose (g kg⁻¹ DM) = ADF - ADL. The lignification degree is expressed as the percentage of ADL and NDF.

The nutritional value of the feed has been estimated by the Bulgarian system as Feed Unit for Milk (FUM, in kg DM) and Feed units for growth (FUG, in kg DM) and calculated on the basis of equations according to the experimental values of Crude protein (CP), Crude fiber (CF), Crude fat (Cft) and nitrogen-free extract substances (NFE), recalculated by Todorov (2010) digestibility ratios: Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*Cft + 0.0209*CF + 0.017*NFE - 0.0007*Zx and Exchangeable energy (EE, MJ/kg DM) = 0.0152*DP (Digestible protein) + 0.0342*Dft (Digestible fat) + 0.0128*DF (Digestible fibers)

+ 0.0159*DNFE (Digestible Nitrogen-free extractable substances) - 0.0007*Zx.

Soil and climatic characteristics in the experimental area

Temperature, humidity and the amount of macronutrients in the soil indirectly influence forage quality parameters through the amount of nutritions reaching the plant roots (Mariotte et al., 2020).

The soils in the area of experience are one of the poor in the country, making them unfavorable for agricultural crops because of their acidic (pH_{H2O}: 5.2-5.3; pH_{KCL}: 4.3-4.4) reaction and insufficient water-air regime (Table 1). Penkov (1988) found them suitable for growing forage grasses because they provide high productivity.

Table 1. Agrochemical soil analysis (0-40 cm)

Soil characteristics	pH		Σ N - NH ₄ +NO ₃	P ₂ O ₅	K ₂ O	Humus
Soil layer	H ₂ O	KCL	mg/kg ⁻¹	mg/100		%
0-20	5.3	4.4	20.2	2.4	9.9	1.44
20-40	5.2	4.3	8.6	1.2	5.9	0.96

The soils in the experimental area are light gray, pseudopodzolic. The content of the main nutrients in the soil layer was: from 0-20 cm - total N - 20.2 mg/1000 g, P₂O₅ - 2.4 mg/100 g, K₂O - 9.9 mg/100 g, humus - 1.44% and from 20-40 cm - total N - 8.6 mg/1000 g, P₂O₅ - 1.2 mg/100 g, K₂O - 5.9 mg/100 g, humus - 0.96% (Bozhanska, 2017a).

Considering the soil characteristics in the experimental area, we applied in autumn in the year of sowing: 15 kg P₂O₅/da (triple superphosphate) and in spring - 12 kg N/da (NH₄NO₃).

Temperature and precipitation are among the most important factors affecting forage yield and quality because they directly influence plant growth, community composition, nutrient concentration in plant tissues, and forage digestibility (Marshall et al., 2005).

In the years of the experiment, temperature values during the vegetation period (March-October) ranged from 14.6°C (2021) to 16.3°C (2023) for a 30-year average of 14.8°C. Rainfall totals (March-October) in the year of establishing the grasslands were normal for the area (550.2 mm versus 625.6 mm) and spring moisture created optimum conditions for the formation of the first undergrowth. In the second, third, and fourth trial years, growing

season precipitation totals were lower by 102 mm (2022) to 245.7 mm (2023) compared to the 30-year period (625.6 mm). Drought occurred in all seasons of the years of the experiment, which affected physiological processes during different phenophases and stages of individual development of the studied forage crops.

Experimental data were averaged and statistically processed using a variance analysis ANOVA, LSD at P = 0.05.

RESULTS AND DISCUSSIONS

The content of fiber structural components of cell walls is variable and increases with maturity stage in plants (Jung & Deetz, 1993).

In the first experimental year, *Phleum pratense* L. with *Trifolium pratense* L. had the lowest content of neutral-detergent fibers (415.0 g kg⁻¹), acid-detergent fibers (216.4 g kg⁻¹), acid-detergent lignin (28.6 g kg⁻¹) and cellulose (187.8 g kg⁻¹) (Table 2).

This mixture was characterized by the highest relative proportion of legume to wheat (legumes:grasses - 30.3%:13.6%) compared to the other grasslands studied (Bozhanska et al., 2024a) and the highest dry matter digestibility (713.2 g kg⁻¹).

In contrast, the biomass of the two-component mixture *Dactylis glomerata* L. + *Trifolium pratense* L. recorded the highest concentration of ADF (309.9 g kg⁻¹), ADL (37.9 g kg⁻¹) and cellulose (272.0 g kg⁻¹), which correlated positively with the low *in vitro* dry matter digestibility (627.6 g kg⁻¹).

The composition and characteristics of polysaccharides vary greatly among forages and plant cell walls (Li, 2021).

Neutral-detergent fibers is a laboratory indicator to predict the free uptake of feed by animals and the composition of acid-detergent fiber influences dry matter digestibility.

Table 2. Main structural fiber components of cell walls and *in vitro* dry matter digestibility (g kg⁻¹ DM) of mixed perennial grasslands with *Trifolium pratense* L., by year and average over the period

Variants	NDF	ADF	ADL	Hemicell	Cellul	IVDMD of Aufreere
2020						
FR + TrP	448.5	287.8	36.7	160.7	251.1	653.7
LP + TrP	452.4	236.8	32.1	215.6	204.7	698.4
PP + TrP	415.0	216.4	28.6	198.6	187.8	713.2
DG + TrP	433.4	309.9	37.9	123.5	272.0	627.6
2021						
FR + TrP	462.1	296.6	49.7	165.5	246.9	653.4
LP + TrP	472.0	258.3	43.7	213.7	214.6	677.5
PP + TrP	378.1	245.0	32.9	133.1	212.2	685.1
DG + TrP	440.9	296.5	26.6	144.4	269.9	654.2
2022						
FR + TrP	468.2	301.6	50.1	166.6	251.5	643.2
LP + TrP	465.6	271.4	44.8	194.2	226.6	661.4
PP + TrP	398.2	261.1	41.9	137.2	219.1	677.1
DG + TrP	451.6	297.5	31.0	154.1	266.5	651.3
2023						
FR + TrP	471.9	290.2	58.7	181.7	231.5	613.3
LP + TrP	468.4	262.9	49.0	205.5	213.9	633.5
PP + TrP	401.5	271.8	40.2	129.7	231.6	658.9
DG + TrP	586.2	280.2	35.2	306.0	245.0	621.1
2020-2023						
FR + TrP	462.7	294.1	48.8	168.6	245.3	640.9
LP + TrP	464.6	257.4	42.4	207.3	215.0	667.7
PP + TrP	398.2	248.6	35.9	149.6	212.7	683.6
DG + TrP	478.0	296.0	32.7	182.0	263.4	638.6
Mean	450.9	274.0	39.9	176.9	234.1	657.7
LSD_{0.05}	57.6	24.5	8.9	75.4	21.5	21.6
Significance of the differences in the values of the indicator over the years	P < 0.05	P < 0.01	P = 0.01	P > 0.05	P < 0.001	P < 0.01
Significance of differences in indicator values between variants	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P > 0.05	P < 0.01

The results of the analysis showed that in the second and third growing seasons, the mixture of *Phleum pratense* L. with *Trifolium pratense* L. had the lowest contents of NDF (378.1 and 398.2 g kg⁻¹, respectively), ADF (245.0 and 261.1 g kg⁻¹, respectively), hemicellulose (133.1 and 137.2 g kg⁻¹, respectively) and cellulose (212.2 and 219.1 g kg⁻¹, respectively).

The mixtures of *Trifolium pratense* L. with *Lolium perenne* L. (472.0 g kg⁻¹) and *Festuca rubra* L. (468.2 g kg⁻¹) had the highest concentration of total fiber components (NDF). The excess in the values of the indicator is 2.1-19.9% (for 2021) and 0.6-15.0% (for 2022). The low values of ligno-cellulose complexes have a significant effect on the digestibility of

biomass of the grassland. In the years of the experiment, the mixture *Phleum pratense* L. + *Trifolium pratense* L. had the highest *in vitro* dry matter digestibility. On average, the values of the parameter (683.6 g kg^{-1}) were higher by 2.3 to 7.0% compared to the other grasses in the experiment. According to Junga et al. (2012) high digestibility of plant forage had a positive effect on ruminant productivity. A 1% improvement in trait values can increase animal growth by 10% (Naydenova, 2009). Data from the analysis showed a significant difference ($P < 0.01$) in digestibility of two-component mixtures both by year and by variant. Statistical proved the difference in the values of the indicator between the mixtures of *Phleum pratense* L. and *Lolium perenne* L. with those of *Dactylis glomerata* L. and *Festuca rubra* L.

It has been found that as plants mature, the concentration of lignin fraction increases and is associated with decreased cell wall digestibility (Jung & Deetz, 1993). The low levels of the indicator are associated with a biological feature of *Dactylis glomerata* L. and when evaluating the quality of forage with legumes, its values range from 2.98% to 4.00% (Naydenova et al., 2015).

In the years of the experiment, *Dactylis glomerata* L. was the dominant species in the mixed grasslands with *Trifolium pratense* L. (occupying up to 77.7% in the grassland) (Bozhanska et al., 2024), which mainly influenced the percentage of structural components in the dry matter. The acid-detergent lignin content in the biomass of the two-component mixture (*Dactylis glomerata* L. + *Trifolium pratense* L.) ranged from 26.6 g kg^{-1} to 37.9 g kg^{-1} , with an average of 32.7 g kg^{-1} . The values of the indicator compared with the other variants studied were lower in the individual vegetations (the exception being the first vegetation) by 23.7 to 86.8% (for 2021), 35.2 to 61.6% (for 2022) and 14.2 to 66.8% (for 2023), respectively. For the experimental period, the mixed grassland also had the highest cellulose content (a structural polyside that performs a supporting function in plant cells and is partially digestible by ruminants). The obtained results indicate a decrease in the values of the indicator from the first to the fourth growing season, when the age and the proportion of grasses in the grassland increase

(Bozhanska et al., 2024a). The species *Dactylis glomerata* L. was also dominant in the undergrowth of mixed grasslands with *Lotus corniculatus* L., *Onobrychis adans* and *Trifolium repens* L. (Naydenova et al., 2015).

In the later stages of forage crop vegetation, a slow increase in acid-detergent fibers and hemicellulose content occurs, followed by a decrease in the amount of minerals and the relative proportion of digestible cell contents (Carrier et al., 2011). In this regard, according to the analysis of the experimental data, in the second, third and fourth experimental years, *Festuca rubra* L. grasses with *Trifolium pratense* L. showed maximum values concerning the parameters acid-detergent fibers and acid-detergent lignin. The high content of the components negatively affected the digestibility of the grassland. With the increase in plant age, the forage mass in the variant had the lowest *in vitro* dry matter digestibility, namely 653.4 g kg^{-1} (2021), 643.2 g kg^{-1} (2022) and 613.3 g kg^{-1} (2023). The percentage difference in the values of the indicator for the three years, between the studied two-component mixtures ranged from 1.1 to 7.4%.

Forage quality, expressed by the fiber characteristics of the plant cell and digestibility, has a significant influence on animal productivities.

Averaged over the period, the mixture *Dactylis glomerata* L. + *Trifolium pratense* L. had the highest content of structural fiber components, namely: neutral-detergent fibers (478.0 g kg^{-1}), acid-detergent fibers (296.0 g kg^{-1}) and cellulose (263.4 g kg^{-1}). The excess over the mean value of the traits studied was 6.0%, 8.0% and 12.5%, respectively. In contrast, the biomass in the *Phleum pratense* L. + *Trifolium pratense* L. mixture was shown to have lower levels of NDF, ADF and cellulose compared to the mean maximum values of the indicators.

A significant difference in the concentration of acid-detergent lignin was found between the two-component mixtures of *Dactylis glomerata* L. + *Trifolium pratense* L. (32.7 g kg^{-1}) and *Phleum pratense* L. + *Trifolium pratense* L. (35.9 g kg^{-1}) with the mixtures of *Festuca rubra* L. + *Trifolium pratense* L. (48.8 g kg^{-1}) and *Lolium perenne* L. + *Trifolium pratense* L. (42.4 g kg^{-1}). Studies also showed a proven difference in the amount of cellulose between the

grasslands of *Dactylis glomerata* L. + *Trifolium pratense* L. (263.4 g kg⁻¹) and *Festuca rubra* L. + *Trifolium pratense* L. (640.9 g kg⁻¹) with those of *Lolium perenne* L. + *Trifolium pratense* L. (215.0 g kg⁻¹) and *Phleum pratense* L. + *Trifolium pratense* L. (212.7 g kg⁻¹). There was no statistically significant difference (at $P < 0.05$) between the treatments with respect to hemicellulose content. The mean values ranged from 149.6 g kg⁻¹ (*Phleum pratense* L. + *Trifolium pratense* L.) to 207.3 g kg⁻¹ (*Lolium perenne* L. + *Trifolium pratense* L.).

Over the four-year period of the experiment (considering the sources of variation - years and variants), the environmental conditions had a significant effect on the amount of the major fiber structural components of the cell walls

(NDF – $P < 0.05$, ADF – $P < 0.01$, ADL – $P = 0.01$ and cellulose – $P < 0.001$) in the dry matter of the two-component mixtures studied.

The biological process of lignification is estimated to be a major factor limiting the nutritional value of forage and inhibiting its digestibility (Akin & Chesson, 1989; Casler & Jung 2006). In the years of the experiment, the coefficients by degree of lignification in mixed grasslands ranged from 8.2% to 12.4% (2020), from 7.1% to 10.5% (2021), from 6.9% to 10.5% (2022) and from 6.0% to 8.7% (2023), respectively (Figure 1). As the age of the grasslands increased, a decrease in the indicator values was observed only in the two-component mixtures of *Lolium perenne* L. (by 2.7-4.8%) and *Dactylis glomerata* L. (by 4.0-6.4%).

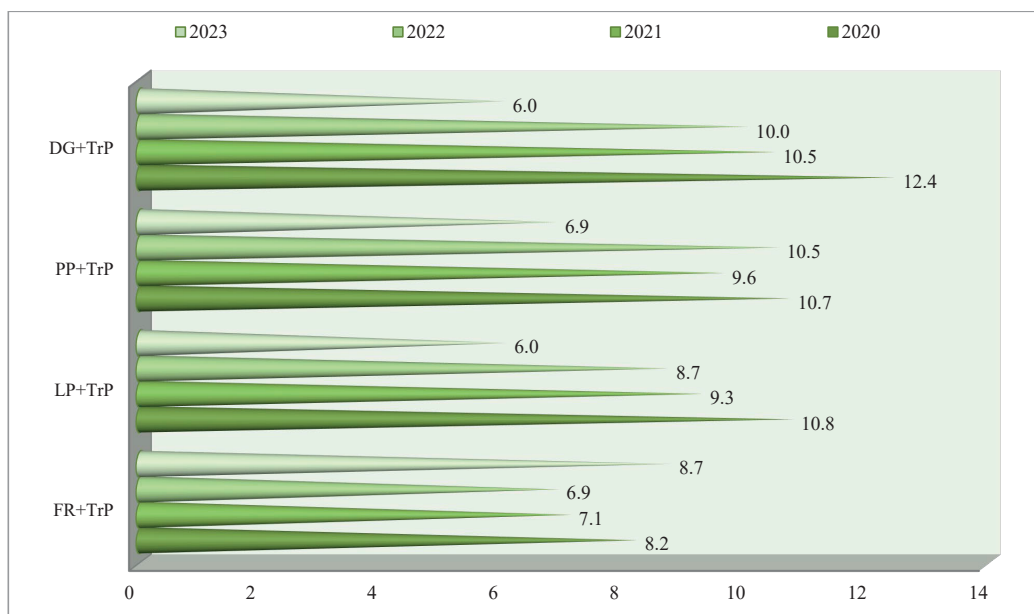


Figure 1. Lignification degree (coefficient) of perennial meadow grasses in mixed grasslands

Efficient utilization and evaluation of mixed grasslands are closely related to the analysis of cell wall components and energy nutritional value of the forage. The degradation of the fiber fraction in the dry matter is a major energy source for ruminants, and an accurate assessment of the calorific value is largely based on the balance between qualitative and

quantitative parameters in the forage of the variants studied.

Average over the period, the higher energy biomass was characterized by mixed grasslands of *Festuca rubra* L. with *Trifolium pratense* L. (19.04 MJ/kg DM) and of *Lolium perenne* L. with *Trifolium pratense* L. (19.10 MJ/kg DM) (Table 3).

Table 3. Potential energy nutrient value of mixed perennial grasslands with *Trifolium pratense* L., by years and average over the period

Variants	GE	EE	FUM	FUG
2020				
FR + TrP	19.02	8.14	0.75	0.67
LP + TrP	19.06	8.28	0.76	0.69
PP + TrP	18.63	8.52	0.79	0.73
DG + TrP	18.92	8.30	0.76	0.70
2021				
FR + TrP	19.08	7.68	0.70	0.62
LP + TrP	19.54	7.88	0.71	0.63
PP + TrP	19.27	7.93	0.72	0.64
DG + TrP	19.48	7.84	0.71	0.63
2022				
FR + TrP	19.31	7.65	0.69	0.61
LP + TrP	19.05	8.07	0.74	0.66
PP + TrP	18.90	8.44	0.78	0.71
DG + TrP	18.86	7.71	0.70	0.62
2023				
FR + TrP	18.74	7.59	0.69	0.61
LP + TrP	18.76	7.77	0.71	0.63
PP + TrP	18.57	7.47	0.68	0.60
DG + TrP	18.25	7.40	0.67	0.60
2020-2023				
FR + TrP	19.04	7.77	0.71	0.63
LP + TrP	19.10	8.00	0.73	0.65
PP + TrP	18.84	8.09	0.74	0.67
DG + TrP	18.88	7.81	0.71	0.64
Mean	18.97	7.92	0.72	0.65
SD	0.12	0.15	0.02	0.02

Legend: GE (MJ/kg DM), EE (MJ/kg DM), FUM (in kg DM), FUG (in kg DM).

The amount of gross energy in the two-component mixtures exceeds the mean by 0.4 and 0.7%, respectively. In the variants of *Trifolium pratense* L. with *Phleum pratense* L. and *Dactylis glomerata* L., the values of the parameter were 18.84 MJ/kg DM and 18.88 MJ/kg DM, respectively.

The plant mass of the mixtures *Phleum pratense* L. + *Trifolium pratense* L. (8.09 MJ/kg DM) and *Lolium perenne* L. + *Trifolium pratense* L. (8.00 MJ/kg DM) was distinguished by higher values in terms of the amount of energy involved in the metabolic processes of the animals.

The exceeded over the mean value of the trait was 1.0-2.1%. The net energy nutrient content of the forage mass, expressed in terms of growth and milk feed units in both treatments, showed a slight excess in the FUM values (by 1.4-2.8%) compared to the mean (0.72 in kg DM).

In the grasslands of *Trifolium pratense* L. with *Festuca rubra* L. (7.77 MJ/kg DM) and *Dactylis glomerata* L. (7.81 MJ/kg DM), the metabolizable energy content had lower values compared to the mean. This trend was also

maintained in terms of the number of straw units (FUM and FUG) in dry matter. The empirically calculated values concerning the number of feed units for growth exceed the average value of the indicator by 3.1% only in the mixture of *Trifolium pratense* L. with *Phleum pratense* L. (0.67 in kg DM).

According to the analysis of the data in the study, there was no significant difference in the values of the indicators determining the energy-nutrient value of the sown perennial cereal grasses with *Trifolium pratense* L. (by year and average over the period). A similar trend was observed in the study of two-component mixtures of *Lotus corniculatus* L. with *Dactylis glomerata* L., *Festuca rubra* L. and *Phleum pratense* L. (Churkova & Churkova, 2020). Perennial grasses and legume grown in mixed grasslands are characterised by a specific morphological and physiological structure (Schaub et al., 2020). Their efficient utilisation and nutritional value are closely related to the analysis of cells walls components.

In the studied mixed grasslands, a correlation was found between the amount of energy regulating metabolic processes in the animal

organism and the number of feed units for growth with the digestibility of the biomass formed (Figure 2).

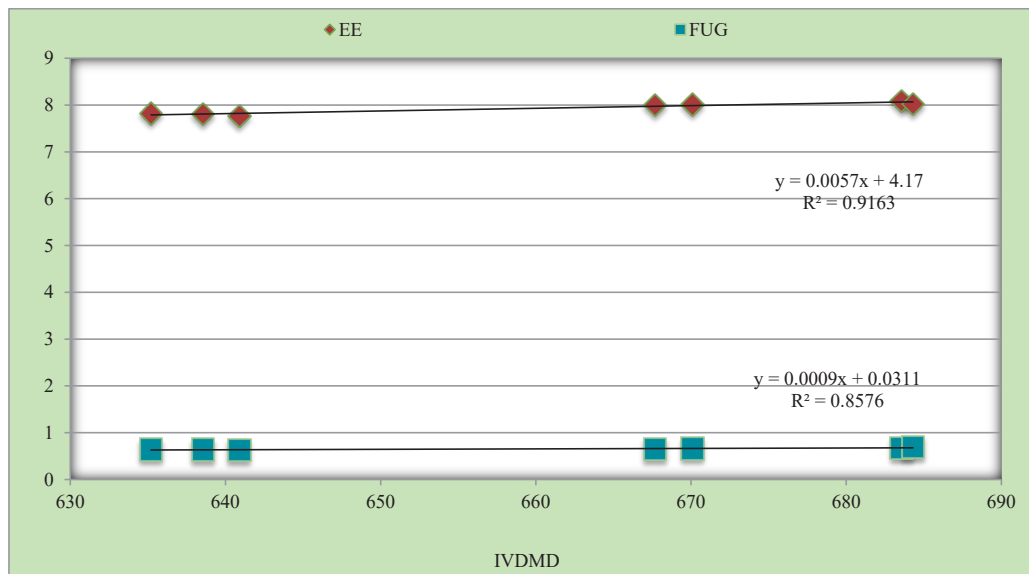


Figure 2. Regression relationship between *in vitro* dry matter digestibility (g kg⁻¹) with the amount of metabolizable energy (MJ/kg DM) and the number of feed units for growth (in kg DM) in mixed stands of perennial species with *Trifolium pratense* L.

The theoretical regression lines and the equations of regression dependence between the indicators EE и FUG с IVDMD are: $y = 0.0057x + 4.17$ (at determination coefficient $R^2 = 0.9163$) and $y = 0.0009x + 0.0311$ (at determination coefficient $R^2 = 0.8576$) with a level of significance $P < 0.05$.

The established regression models reveal a good opportunity for approximate determination of the energy value of forage in mixed grasslands through the digestibility of dry matter.

CONCLUSIONS

The mixture of *Dactylis glomerata* L. with *Trifolium pratense* L. had the highest content of neutral-detergent fibers (478.0 g kg⁻¹), acid-detergent fibers (296.0 g kg⁻¹) and cellulose (263.4 g kg⁻¹). The exceeded compared to the mean value of the traits was 6.0%, 8.0% and 12.5%, respectively. On average over the study period, the two-component mixture had the lowest *in vitro* dry matter digestibility (638.6 g kg⁻¹), and the lowest concentration of acid-detergent lignin (32.7 g kg⁻¹).

In contrast, mixed grasslands of *Phleum pratense* L. + *Trifolium pratense* L. were shown to have a lower detergent fibers content. For the study period, the variant biomass had the highest *in vitro* dry matter digestibility (683.6 g kg⁻¹), and the excess in the values of the parameter ranged from 2.3 to 6.6%. The two-component mixture also had the highest exchangeable and net (FUM and FUG) energy content.

The mixtures of *Trifolium pratense* L. with *Festuca rubra* L. and *Lolium perenne* L. were distinguished by a significantly higher content of acid-detergent lignin in the dry matter and by the highest gross energy values.

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THE IMPACT OF HERBICIDE TREATMENTS IN THE CONTROL OF WEED SPECIES PRESENT IN THE WHEAT CROP

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Abstract

Wheat (Triticum aestivum) is one of the most important cereals worldwide, essential for human and animal nutrition, being a dominant agricultural crop in Romania, with a major economic role. Weed control remains a key component in optimizing wheat crop productivity and the application of herbicide treatments is a fundamental agronomic practice. Weeds are a major factor in reducing wheat production, and the application of herbicides is an effective measure for their control. The present study evaluates the impact of treatments on the control of weed species in wheat crops, under the pedoclimatic conditions of the Fundulea area. The purpose of the research was to identify new technological sequences regarding the control of weeds in wheat crops by using herbicide treatments, having as main objective the broadening of the spectrum of control, synergism, persistence and without negative impact on the environment. The main objective of this work is the study of the degree of selectivity and effectiveness of the application of herbicide treatments in combating weed species existing in wheat crops. The use and application of herbicide treatments must be correlated with the degree of infestation, the spectrum and dominance of weed species and the pedoclimatic conditions of the research area.

Key words: wheat, weeds, herbicide, efficacy, selectivity.

INTRODUCTION

Wheat (*Triticum aestivum*) is the most important plant cultivated in our country and in over 100 countries around the world. Providing abundant food to feed the Earth's population is an essential concern for humanity.

All branches of biological science are working intensively and purposefully in this direction (Shopova and Cholakov, 2014, 2015; Nenova, 2017; Georgiev et al., 2019; Nenova, 2019; Nenova et al., 2019; Petrova et al., 2019)

Weeds also cause indirect damage, as many of the species are hosts to diseases and pests (Kalinova et al., 2012). Weeds are usually characterized by rapid development and abundant seed production, they spread easily.

Weeds have the greatest negative impact, around 37%, compared to insects (18%), fungi and bacteria (16%) and viruses (2%) (Oerke, 2006). Weeds have a negative effect on the level of production and its quality, the presence of a large number of weeds makes harvesting difficult, they also increase drying costs, they can have toxic effects on people and animals, and they can also favor the transmission of

diseases and pests to plants (Chirilă, 2001). Weeds also cause indirect damage, as many of the species are hosts to diseases and pests (Kalinova et al., 2012). Weeds are usually characterized by rapid development and abundant seed production, they spread easily.

Herbicide application must be correlated with the degree of infestation of the crop, the spectrum and dominance of weeds, the application period, the technical potential for effectiveness and the zonal pedoclimatic conditions before and after treatment (Măturaru et al., 2022).

Choosing an appropriate herbicide, the optimal time and application rate is one of the most important and responsible moments in wheat management (Sherawat and Ahmad, 2005; Khalil et al., 2008; Abbas et al., 2009; Petrova and Sabev, 2014; Penchev and Petrova, 2015; Petrova, 2017). To be economically efficient, herbicide treatment must be carried out in accordance with the existing weeds (Klaus, 1992).

The main objective is to reduce the negative impact of weeds on agricultural production, while respecting the principles of sustainability

and environmental protection. Regarding the existing state of research, significant progress has been made in identifying and developing effective methods of weed control. The main objective of this work is the study of the degree of selectivity and effectiveness of the application of herbicide treatments in combating weed species existing in wheat crops. The use and application of herbicide treatments must be correlated with the degree of infestation, the spectrum and dominance of weed species and the pedoclimatic conditions of the research area.

MATERIALS AND METHODS

The research was carried out in the period 2022-2024, at the National Institute for Agricultural Research and Development - Fundulea, being studied the application of the herbicide treatments at the wheat crop. The research was carried out in the experimental field, the experiment being located on a soil of cambic chernozem type (3.2% organic matter, 37% clay, 6.5 pH) using the Glosa created by the institute from NARDI Fundulea. The organization of the experiment was done according to the method of randomized blocks, with a plot area of 25 m², in 3 replications and the amount of water used was 300 l/hectare. In this experiment, we observed the degree of selectivity of plants and the degree of control of weeds by applying herbicide treatments (Table 1): Floramix+ Dasoil 26-N (Adj); Omnera+Foxtrot 69W and Pixxaro Super.

Table. 1. The herbicide treatments applied in the wheat crop. Experimental variants

No var	Herbicides treatments	Active ingredient	Dose g, l/ ha	Time of appli cation
1	Untreated	-	-	
2	Floramix+ Dasoil 26-N(adj)	70.8 g/kg Piroxsulam + 14.2 g/kg Florasulam + 70.8 g/kg Cloquintocet – mexil (safener)	260 g/ha +0.6 l/ha	Poste m BBC H 32
3	Omnera+ Foxtrot 69W	-(fluroxipir 135 g/l + tifensulfuron metil 30 g/l + metsulfuron metil 5 g/l) + -fenoxaprop-P-etil 69 g/l + cloquintocet mexil 34.5 g/l (safener)	1.0 l/ha+ 1.0 l/ha	
4	Pixxaro™	12 g/l halauxifen-metil + 280 g/l fluroxipir meptil + 12 g/l cloquintocet-mexil	0.5 l/ha	

The herbicide treatments were applied in the post-emergence (BBCH 32). After the application of herbicide treatments, observations of selectivity (%) of wheat plants were made at different intervals (7 - 14 - 28 days after the application of treatments) and the degree of control (%) of weeds at different intervals 14 - 28 days from the application of treatments. The climatic conditions (Figure 1) recorded in Fundulea area, during the research period in the 2022-2024 years, were extremely different, especially the amount of precipitation recorded. The average sum of precipitation (mm) for the 2022 year was 258.4 mm, in 2023 year was 423.4 mm and in 2024 was 437.0 mm. The average temperature (°C) in 2022 was 13.3°C, in the year 2023 was 14.3°C and in the year 2023 was 14.2°C

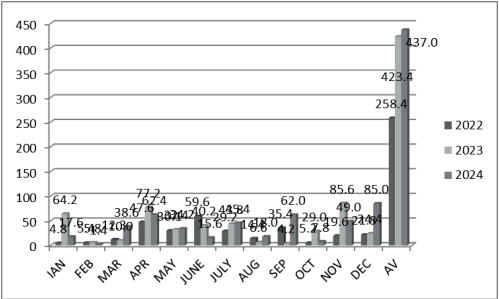


Figure 1. The climatic conditions of 2022-2024 years from NARDI FUNDULEA

RESULTS AND DISCUSSIONS

In the wheat experience carried out in the experimental field, the crop presented an average infestation degree - 89%, with weeds, extremely diversified, depending on the predecessor plant and the local climatic conditions. The most representative (Figure 2) weed species were: *Veronica hederifolia*, *Anthemis arvense*, *Polygonum convolvulus*, *Galium aparine*, *Matricaria inodora*, *Papaver rhoses*, *Apera spica venti* and *Avena fatua*. Weed species are uncultivated plants, harmful, devoid of economic value, adapted to grow and develop alongside agricultural plants. The weed species demonstrate a better adaptation to climate and soil conditions.

Their main cause leads to the compromising of crops, the reduction of production and in some cases to destruction.

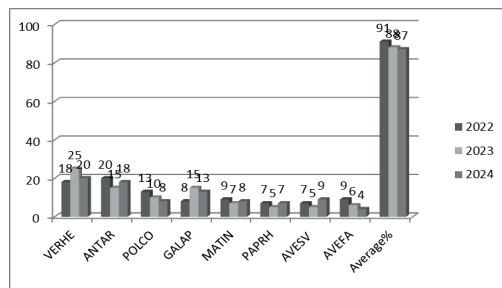


Figure 2. The infestation degree (%) with weeds from the untreated plots

Compared to the pedoclimatic conditions recorded during the research period, the wheat crop presented an average infestation of 80% (2022 - 72% and 2023 - 88%, Figure 3).

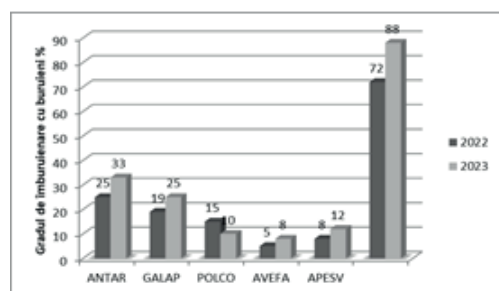


Figure 3. Infestation degree with weeds 2022-2023

In the experiment, the weed species present were diversified: annual dicotyledons *Anthemis arvensis* (ANTAR), *Galium aparine* (GALAP), *Polygonum convolvulus* (POLCO) and monocotyledons *Avena fatua* (AVEFA) and *Apera spica venti* (APSV) (Cergan, 2024)

In the experimental field, all the selectivity observations made for the crop, not recorded phytotoxic phenomena (EWRS scale = 0).

In the wheat crop, the herbicide treatments applied post-emergence (BBCH 32) had a good control effect, highlighting their effectiveness through a single application. By applying the treatment with herbicides, good results were obtained regarding the effect of combating weeds, depending on: the climatic conditions, the degree of infestation, the spectrum and the dominance of the species present in the field from Fundulea area.

The treatment with the herbicide Floramix + Dasoil 26-N (adj) was applied in the dose of 260 g/ha +0.6 l/ha (postemergence - BBCH 32), following the application of this treatment, a very good effectiveness was recorded for the weeds present in the wheat crop, especially for annual dicotyledons *Anthemis arvensis* - 96% and *Matricaria inodora* - 94% (the average of 2022-2024).

The weeds *Papaver rhoses*, *Polygonum convolvulus*, *Galium aparine*, and *Veronica hederifolia* registered a good efficacy 85-75 - 74% (the average of 2022-2024).

The species (Figure 4) *Apera spica venti* and *Avena fatua* have recorded a good control effect following the treatment applied (85% and 89%).

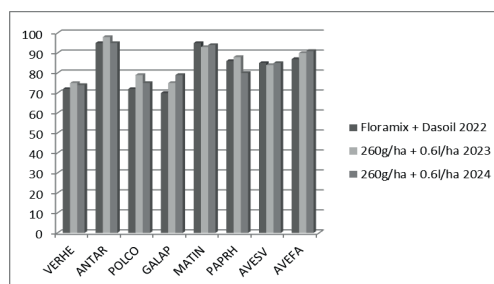


Figure 4. The efficacy (%) of the Floramix+ Dasoil 26-N (adj) applied for the weeds control from the wheat crop (Fundulea, 2022-2024)

Figure 5 shows the average effectiveness results (%) of the Omnera + Foxtrot 69W treatment applied at the dose of 1.0 l/ha + 1.0 l/ha, post-emergence-BBCH 32.

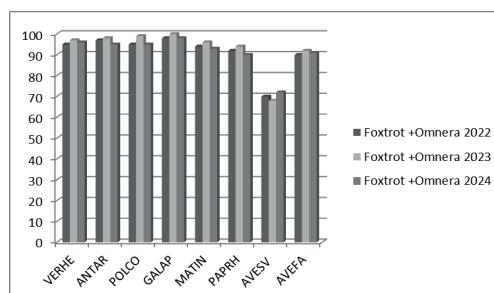


Figure 5. The efficacy (%) of the herbicides Omnera + Foxtrot 69W applied for the weeds control from the wheat crop (Fundulea, 2022-2024)

After applying the Omnera + Foxtrot 69W treatment, the excellent control for annual dicotyledonous weeds was more than 99-96%

Galium aparine, *Anthemis arvensis*, *Polygonum convolvulus* and *Veronica hederifolia*.

The weed species *Matricaria inodora*, *Papaver rh.*, and *Avena fatua* registered a good efficacy 94%, 92% and 91%.

The monocotyledonous species recorded an average control following the treatment applied - *Apera spica venti* - 70%.

The next variant treated was with Pixxaro S. (Figure 6) which ensures very good efficacy on a very large number of problem dicotyledonous weeds in wheat crops. Visible symptoms appear within hours of application and continue until the weed dries out completely within a few weeks.

Following the application of this treatment at a dose of 0.5 l/ha, the control was very well controlled on the species present in the fields: *Polygonum convolvulus* - 98%, *Anthemis arvense*, *Matricaria inodora*, *Galium aparine* - 96%; *Papaver rh.* - 87% and *Veronica h.* - 83%.

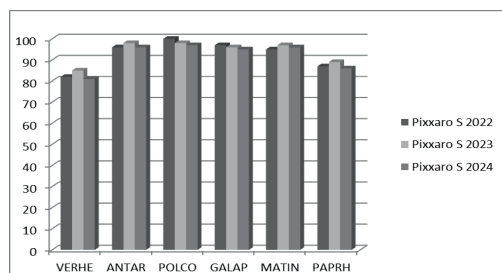


Figure 6. The efficacy (%) of the herbicides Pixxaro S., applied for the weeds control from the wheat crop (Fundulea, 2022-2024).

Following the application of post-emergence herbicide treatments in wheat crops, the average production (Figure 7) obtained during the years 2022-2024 was: Floramix + Adj -6.99 t/ha; Omnera + Foxtrot 69W - 6.65 t/ha and Pixxaro S. -5.07 t/ha.

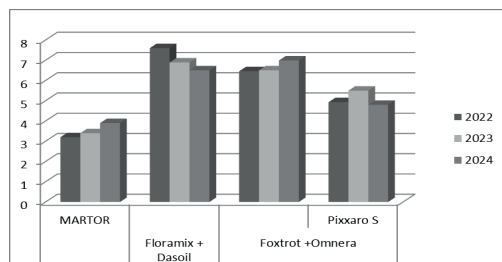


Figure 7. The wheat yields (t/ha) under the 3 experimental treatments

In the uncontrol variants, the yields were much lower (average 3.5 t/ha), due to competition between the crop and existing weeds compared to the variants treated with different products.

Integrated weed management is a system of combined measures that aims to maintain the level of weed infestation of crops below the damage threshold, while minimizing the negative impact on the environment (Cergan, 2025).

In our country, special attention is paid to the control of weed species by using and applying herbicide treatments due to the degree of weeding, dominant and diversified spectrum weeds present in the crop.

Modern agriculture faces multiple challenges generated by climate change that directly influences the dynamics of agricultural ecosystems. Among the biotic factors affecting crop productivity, weeding is one of the most important problems for farmers.

CONCLUSIONS

The herbicide treatments applied post-emergence - BBCH 32, did not record phytotoxic symptoms for the cultivated wheat variety.

The efficacy of herbicide treatments (postemergent- BBCH 32) used for wheat crop depends on certain essential factors: the degree of infestation of the crop with weeds; the spectrum and dominance and the pedoclimatic conditions from Fundulea.

The production obtained was in close correlation with: the biological potential of the cultivated variety, the effectiveness of herbicide treatments and the pedoclimatic conditions in the Fundulea area.

Weed control remains a key component in optimizing wheat crop productivity and the application of herbicide treatments is a fundamental agronomic practice.

Weeds are a major factor in reducing wheat production, and the application of herbicides is an effective measure for their control.

The present study evaluates the impact of treatments on the control of weed species in wheat crops, under the pedoclimatic conditions of the Fundulea area.

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MULTIVARIATE ANALYSIS OF GRAIN QUALITY TRAITS IN OAT (*Avena sativa* L.) CULTIVARS UNDER DIFFERENT NITROGEN FERTILIZATION LEVELS

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Abstract

Understanding relationships among grain quality traits is essential for improving oat performance under varying agronomic conditions. This study used principal component analysis (PCA) to assess the multivariate structure of key physical and chemical grain quality traits in eleven oat cultivars grown under different nitrogen fertilization levels during 2023-2025. A total of 55 cultivar × fertilization combinations were evaluated for hectoliter weight, protein content, fat content, and nitrogen-related traits. The first two principal components explained 93.4% of the total variance. The first component (72.9%) was mainly associated with protein and nitrogen content, indicating the strong influence of nitrogen nutrition on grain composition. The second component (20.5%) was related to hectoliter weight and fat content, reflecting variation in physical grain quality among cultivars. PCA scores clearly differentiated cultivar–fertilization combinations, highlighting distinct cultivar responses to nitrogen supply. These findings confirm that PCA is an effective approach for identifying major determinants of oat grain quality and supports cultivar selection and nitrogen management strategies in oat production systems.

Key words: hectoliter weight, multivariate analysis, nitrogen fertilization, oat cultivars.

INTRODUCTION

Oat (*Avena sativa* L.) is a cereal crop of increasing importance due to its high nutritional value, adaptability to diverse agroecological conditions, and growing demand in human nutrition and functional foods. Oat grain is distinguished from other cereals by its relatively high protein content, favorable lipid composition, and the presence of bioactive compounds such as β -glucans, which contribute to health benefits including cholesterol reduction and glycemic control (Peterson, 1992; Wood, 2007; Ibrahim et al., 2020). As a result, improving oat grain quality has become a major objective in both breeding programs and agronomic management strategies. Grain quality in oat is a complex trait influenced by genetic background, environmental conditions, and crop management practices. Key physical and chemical parameters, such as hectoliter weight, protein content, fat content, and nitrogen concentration, are commonly used to evaluate oat quality for food, feed, and industrial purposes (Mut et al., 2018; Capouchová et al.,

2021; Sitnicki et al., 2024). Understanding the relationships among these traits is essential for identifying superior cultivars and optimizing production systems. Substantial genetic variability exists among oat cultivars with respect to grain composition and physical characteristics. Several studies have demonstrated significant differences in protein, lipid, and mineral content among oat genotypes grown under similar environmental conditions (Sykut-Domańska et al., 2013; Özcan et al., 2017). This variability provides opportunities for breeding programs aimed at improving grain nutritional quality while maintaining agronomic performance. Recent research has applied multivariate and genomic approaches to better understand the genetic basis of quality traits in oat. Carlson et al. (2019) showed that multivariate genome-wide association analyses can effectively identify loci controlling seed fatty acid composition, highlighting the complexity of lipid-related traits. Similarly, Ihsan et al. (2022) reported considerable diversity in nutritional composition among oat accessions, emphasizing the usefulness of

principal component analysis (PCA) and cluster analysis for classifying genotypes based on multiple quality attributes. Nitrogen availability is one of the most important agronomic factors affecting oat grain quality, particularly protein and nitrogen content (Smuleac et al., 2020). Numerous studies have reported strong positive relationships between nitrogen fertilization and grain protein concentration, reflecting the central role of nitrogen in amino acid and protein synthesis (Allwood et al., 2023; Zhang et al., 2025). However, increased nitrogen supply may also influence other quality traits, including lipid content and physical grain characteristics. The response of oat cultivars to nitrogen fertilization is often genotype-dependent, leading to significant cultivar $\times$ nitrogen interactions (Kebede et al., 2023). Excessive nitrogen application can result in diminishing returns or undesirable trade-offs between yield and quality, underscoring the need for integrated evaluations of multiple traits (Köse, 2022). Consequently, assessing how nitrogen-related traits interact with physical grain parameters such as hectoliter weight is critical for developing efficient and sustainable fertilization strategies (Chițu et al., 2024). Hectoliter weight is a widely used physical indicator of grain density and milling quality, while protein and fat content represent major chemical attributes determining nutritional and processing value. Studies have shown that these traits are not always positively correlated, indicating complex physiological relationships during grain development (Mut et al., 2018; Capouchová et al., 2021). Protein and nitrogen content are typically strongly correlated, as nitrogen concentration is a direct determinant of protein synthesis (Ihsan et al., 2021). In contrast, fat content often shows weaker or variable relationships with protein, reflecting differences in carbon and nitrogen allocation within the grain (Ahmet et al., 2019; Banaś & Harasym, 2021). Understanding these interrelationships is essential for identifying cultivar profiles suited to specific end uses, such as food processing or feed formulation. Given the multidimensional nature of grain quality, multivariate statistical techniques have become increasingly important in oat research. Principal component analysis (PCA) is widely used to reduce data dimensionality, identify major sources of

variation, and reveal relationships among traits that may not be evident through univariate analyses (Bose et al., 2014; Ihsan et al., 2022). PCA has been successfully applied to evaluate genotype performance across environments, assess quality trait associations, and discriminate cultivars based on integrated quality profiles (Köse, 2022; Yan et al., 2025). By grouping correlated variables into principal components, PCA facilitates the interpretation of complex datasets and supports decision-making in breeding and agronomic management. In oat studies, PCA has revealed that protein and nitrogen-related traits often dominate the first principal component, while physical traits such as hectoliter weight and lipid content contribute to subsequent components (Ihsan et al., 2022; Yan et al., 2025). These findings highlight the value of PCA for identifying key drivers of grain quality and for selecting cultivars with balanced trait combinations.

MATERIALS AND METHODS

Field experiments were conducted over three consecutive growing seasons (2023-2025) to evaluate the effect of nitrogen fertilization on grain quality traits of oat (*Avena sativa* L.).

The experiment was arranged in a factorial design with two experimental factors.

Factor A - Cultivar included eleven oat cultivars: a1 - Jeremy; a2 - Ovidiu; a3 - Mureșana; a4 - GK; a5 - Pillango; a6 - Prokop; a7 - Effectiv; a8 - Overdrive; a9 - Venafor; a10 - Earl; a11 - Genziana.

Factor B - Nitrogen fertilization level was applied on a uniform agrochemical background of P60K60 and consisted of five levels:

b1 - N0; b2 - N30; b3 - N60; b4 - N90; b5 - N120. The combination of the two factors resulted in a total of 55 treatment variants (11 cultivars $\times$ 5 nitrogen levels).

For the purpose of multivariate analysis, mean values across the three experimental years were calculated to reduce the influence of annual climatic variability. Grain samples were collected at harvest maturity and analyzed for key physical and chemical quality parameters. Hectoliter weight (HW) was determined and expressed in kg hL⁻¹ as an indicator of grain physical quality. Protein content and fat content

were expressed as percentages of dry matter. Nitrogen fertilization level was included as a quantitative explanatory variable reflecting nitrogen input intensity. These parameters were selected due to their agronomic, nutritional, and technological importance in oat production. Prior to multivariate analysis, descriptive statistics were calculated for all measured variables. Principal component analysis (PCA) was performed using the correlation matrix to standardize the data and eliminate scale effects among variables. Eigenvalues, explained variance, and cumulative variance were used to determine the number of significant principal components. The interpretation of PCA results was based on factor loadings, eigenvectors, variable contributions, and factor scores. Biplots were generated to illustrate the relationships among variables and to visualize the distribution of cultivar $\times$ nitrogen fertilization combinations within the space defined by the first two principal components. All statistical analyses were carried out using STATISTICA software.

RESULTS AND DISCUSSIONS

Explained variance and eigenvalue structure of the PCA

Principal component analysis (PCA) applied to the grain quality dataset revealed a strong dimensionality reduction. As shown in Table 1, the first principal component (PC1) had an eigenvalue of 2.916 and explained 72.90% of the total variance, while the second principal component (PC2) had an eigenvalue of 0.820 and explained an additional 20.50%. Together, PC1 and PC2 accounted for 93.41% of the cumulative variance, indicating that these two components captured nearly all the information contained in the original variables.

Table 1. Eigenvalues of correlation matrix, and related statistics

Component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.916188	72.90471	2.916188	72.9047
2	0.820120	20.50301	3.736309	93.4077
3	0.215058	5.37645	3.951367	98.7842
4	0.048633	1.21584	4.000000	100.0000

The scree plot of eigenvalues (Figure 1) displayed a sharp decline after PC1 and a clear

leveling-off after PC2, confirming that the retention of the first two components is statistically justified. The third and fourth components explained only 5.38% and 1.22% of the variance, respectively, and therefore had limited biological and agronomic relevance.

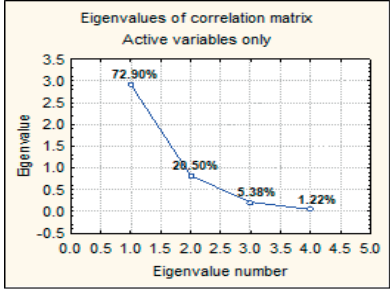


Figure 1. The scree plot of eigenvalues

Factor loadings and variable correlations with principal components

The correlations between the original grain quality variables and the principal components are presented in Table 2.

PC1 showed strong negative correlations with nitrogen (-0.983), protein content (-0.939), fat content (-0.823), and hectoliter weight (-0.625). The magnitude of these loadings indicates that PC1 represents a general grain composition axis, dominated primarily by nitrogen and protein accumulation.

Table 2. Factor-variable correlations, based on correlations

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.625212	0.762461	0.161254	-0.041954
Fat	-0.822974	-0.485186	0.287421	-0.068534
Protein	-0.939381	-0.047444	-0.324583	-0.099793
Nitrogen	-0.982637	-0.033416	-0.033025	0.179493

In contrast, PC2 was strongly and positively correlated with hectoliter weight (0.762) and negatively correlated with fat content (-0.485), while protein and nitrogen exhibited negligible correlations with this component.

This statistical structure suggests that PC2 primarily reflects physical grain quality, largely independent of nitrogen-related chemical traits.

Contribution of variables to principal components

The relative contribution of each variable to the principal components is detailed in Table 3 and

graphically illustrated in Figures 2 and 3. Nitrogen (33.11%) and protein content (30.26%) were the dominant contributors to PC1, followed by fat content (23.23%) and hectoliter weight (13.40%).

These results confirm that PC1 is mainly driven by nitrogen nutrition and its effect on grain protein synthesis.

Table 3.Variable contributions, based on correlations

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	0.134041	0.708856	0.120910	0.036192
Fat	0.232251	0.287038	0.384133	0.096579
Protein	0.302599	0.002745	0.489886	0.204771
Nitrogen	0.331109	0.001362	0.005071	0.662458

For PC2, hectoliter weight accounted for 70.89% of the total contribution, while fat content contributed 28.70%. Protein and nitrogen contributed less than 1% each, reinforcing the interpretation of PC2 as a physical quality component.

The contrasting contribution patterns between PC1 and PC2 highlight the multidimensional nature of oat grain quality.

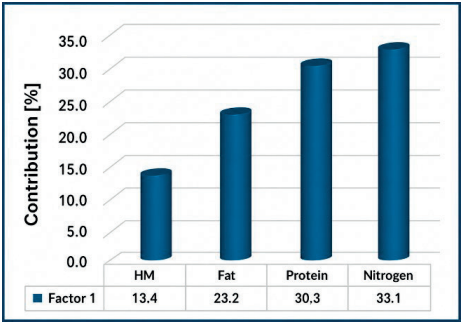


Figure 2. Contribution of variables to CP1

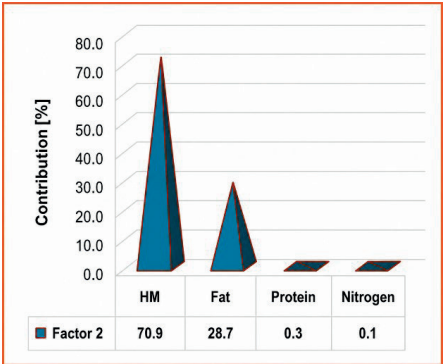


Figure 3. Contribution of variables to CP2

Projection of variables in the factorial plane (PC1 × PC2)

The projection of variables in the factorial plane defined by PC1 and PC2 (Figure 4) provides a visual representation of the relationships among grain quality traits. Nitrogen and protein variables were closely grouped and oriented along the negative direction of PC1, confirming their strong positive association. Fat content was positioned between PC1 and PC2, indicating partial dependence on both chemical composition and physical grain attributes. Hectoliter weight was oriented almost exclusively along the positive direction of PC2, demonstrating its independence from nitrogen and protein accumulation. This spatial separation suggests that increasing nitrogen fertilization enhances protein content but does not necessarily improve physical grain quality, emphasizing a potential trade-off between chemical and physical traits.

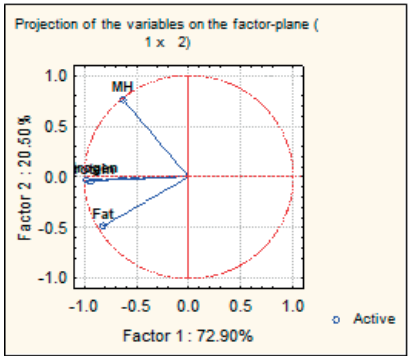


Figure 4. Projection of variables in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2]

Distribution and contribution of cultivar × nitrogen fertilization combinations

The projection of the 55 cultivar × nitrogen fertilization combinations in the PC1–PC2 plane is shown in Figure 5. A wide dispersion of treatments along both principal components was observed, indicating substantial variability in grain quality responses among cultivars and fertilization levels.

The contribution of individual combinations to PC1-PC4 is reported in Table 4 and illustrated in Figures 6 and 7. Treatments with high contributions to PC1 were mainly associated with higher nitrogen fertilization levels,

reflecting increased nitrogen and protein content. In contrast, combinations contributing strongly to PC2 were characterized by higher hectoliter weight, suggesting superior physical grain quality. This clear separation demonstrates that PCA effectively discriminates cultivar × fertilization combinations based on integrated quality profiles rather than individual traits.

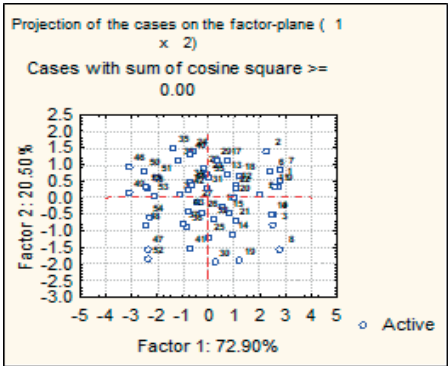


Figure 5. Case projection [combinations of variety x fertilization levels] in the plane of the factorial axes of the two main components [Factor 1/CP1 and Factor 2/CP2]

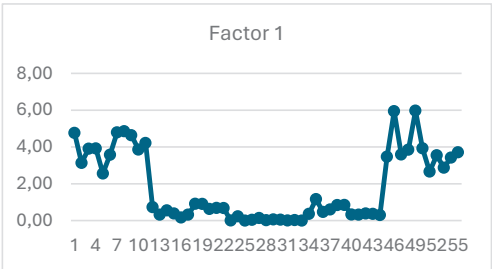


Figure 6. Contribution of combinations [variety x fertilization levels] to CP1

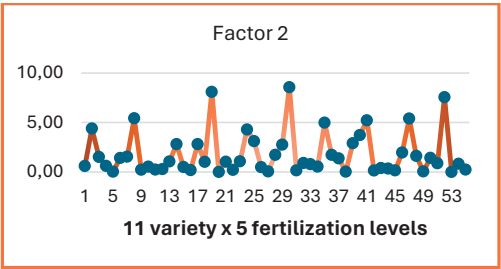


Figure 7. Contribution of combinations [variety x fertilisation levels] to CP2

Table 4. Case contributions, based on correlations

Combination [variety x fertilization levels]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
1	4.7657	0.6028	0.5149	2.5720
2	3.1327	4.3932	0.0016	0.2882
3	3.9086	1.5172	0.1861	0.0120
4	3.9203	0.6108	2.6286	0.1356
5	2.5568	0.0236	4.4303	2.1152
6	3.5778	1.4085	0.1159	0.0122
7	4.7934	1.5444	0.0105	2.4787
8	4.8561	5.4233	0.4197	0.7872
9	4.6398	0.2277	1.3592	2.1656
10	3.8598	0.5384	2.6964	0.0944
11	4.2135	0.2377	5.3510	0.0777
12	0.7315	0.2896	0.0891	0.2252
13	0.3232	1.0605	0.3031	4.4485
14	0.5535	2.8116	0.5045	2.8291
15	0.3866	0.4937	2.2930	3.0988
16	0.1676	0.1895	6.0281	7.2920
17	0.3202	2.8188	0.0635	3.7951
18	0.9090	1.0250	0.0027	0.0075
19	0.9020	8.1030	0.1312	0.5028
20	0.6270	0.0010	2.2766	0.4807
21	0.6866	1.0391	2.5679	1.5885
22	0.6769	0.2125	8.5869	1.5902
23	0.0020	1.0718	1.2090	0.0217
24	0.2320	4.2959	0.0077	4.9943
25	0.0003	3.1109	0.2974	0.1126
26	0.0378	0.4875	4.1048	0.6749
27	0.1354	0.0542	4.3107	2.8093
28	0.0187	1.7345	0.2423	0.3540
29	0.0595	2.7592	0.1420	2.9386
30	0.0433	8.5657	0.0063	0.3358
31	0.0010	0.1608	0.9709	0.0075
32	0.0237	0.9035	3.1568	0.1154
33	0.0001	0.7807	5.3478	0.0444
34	0.3598	0.5332	0.9697	5.5009
35	1.1606	4.9785	0.0855	0.0502
36	0.4669	1.7284	0.2265	1.9996
37	0.6021	1.3772	1.0724	1.0738
38	0.8396	0.0232	3.9048	0.4727
39	0.8501	2.9071	0.1010	0.2740
40	0.3271	3.7329	1.1575	7.5009
41	0.3212	5.2270	0.0073	3.1257
42	0.3869	0.1461	1.1347	4.7258
43	0.3630	0.3884	4.0005	2.3392
44	0.2974	0.3353	3.9321	4.2817
45	3.4688	0.1527	0.3596	0.3733
46	5.9422	1.9695	5.8200	8.4315
47	3.5861	5.3974	0.8594	0.1198
48	3.8477	1.6190	1.9166	0.0094
49	5.9722	0.0517	5.2200	5.2536
50	3.9295	1.4214	0.0224	0.0011
51	2.6603	0.8799	0.2681	3.1478
52	3.5505	7.5675	0.0277	0.0300
53	2.8716	0.0079	0.4830	2.2243
54	3.4221	0.8245	3.1163	0.0000
55	3.711880	0.234755	4.958246	0.058982

Eigenvectors, component equations, and factor scores

The eigenvectors of the correlation matrix are presented in Table 5, providing further insight into the structure of the principal components. The eigenvector coefficients confirm the dominant negative weights of nitrogen and protein in PC1 and the strong positive weight of hectoliter weight in PC2.

Table 5. Eigenvectors of correlation matrix

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.366117	0.841936	0.347722	-0.190243
Fat	-0.481924	-0.535759	0.619785	-0.310771
Protein	-0.550090	-0.052389	-0.699918	-0.452516
Nitrogen	-0.575420	-0.036900	-0.071213	0.813916

Based on these eigenvectors, the component equations (Z1 and Z2) describe the multivariate relationships among traits and quantify their relative importance. The factor score coefficients for variables (Table 6) and for treatment combinations (Table 7) allowed the precise positioning of cultivars and fertilization levels within the multivariate space.

$Z_1 = -0.366117 \times x_1 - 0.481924 \times x_2 - 0.550090 \times x_3 - 0.575420 \times x_4$
 $Z_2 = 0.841936 \times x_1 - 0.535759 \times x_2 - 0.052389 \times x_3 - 0.036900 \times x_4$
 where: x_1 -HW; x_2 -Grasimi; x_3 -protein; x_4 -fertilize N

Table 6. Factor score coefficients, based on correlations for variables

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
HW	-0.214394	0.929694	0.74981	-0.86266
Fat	-0.282209	-0.591604	1.33648	-1.40920
Protein	-0.322126	-0.057850	-1.50928	-2.05195
Nitrogen	-0.336959	-0.040746	-0.15356	3.69073

Table 7. Factor score coefficients, based on correlations for cases [variant]

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
1	1.60421	0.57051	0.52731	1.17850
2	1.30064	1.54023	0.02921	-0.39450
3	1.45280	-0.90516	-0.31704	-0.08044
4	1.45497	-0.57429	1.19141	0.27059
5	1.17502	0.11278	1.54673	-1.06874
6	1.38996	0.87213	-0.25016	-0.08128
7	1.60887	0.91324	-0.07545	1.15693
8	1.61935	-1.71132	-0.47606	0.65197
9	1.58288	0.35063	0.85673	1.08141
10	1.44370	-0.53921	-1.20667	-0.22583
11	1.50841	0.35824	-1.69986	0.20479
12	0.62852	0.39543	0.21933	-0.34869
13	0.41778	0.75676	-0.40454	-1.54990
14	0.54670	-1.23218	-0.52197	-1.23600
15	0.45690	-0.51633	1.11276	-1.29358
16	0.30080	-0.31987	1.80421	-1.98436
17	0.41580	1.23375	-0.18511	-1.43155
18	0.70061	0.74398	-0.03823	0.06348
19	0.69793	-2.09180	-0.26613	-0.52106
20	0.58189	0.02380	1.10876	-0.50950
21	0.60890	-0.74906	-1.17757	-0.92617
22	0.60461	0.33878	-2.15336	-0.92667
23	-0.03324	0.76078	0.80801	0.10834
24	-0.35394	1.52309	0.06465	-1.64222
25	0.01251	-1.29611	-0.40074	-0.24656
26	-0.14286	-0.51309	1.48882	-0.60368
27	-0.27044	-0.17103	1.52570	-1.23167
28	-0.10062	0.96780	-0.36175	-0.43724
29	0.17929	1.22065	0.27687	1.25971
30	0.15289	-2.15070	-0.05837	0.42584
31	-0.02274	0.29468	0.72406	0.06359

Variable [parameter]	Factor 1 [CP1]	Factor 2 [CP2]	Factor 3 [CP3]	Factor 4 [CP4]
32	0.11323	-0.69848	-1.30563	0.24964
33	0.00673	0.64929	-1.69936	-0.15491
34	-0.44077	0.53657	0.72363	1.72352
35	-0.79165	1.63963	-0.21493	-0.16470
36	-0.50212	-0.96608	0.34976	1.03913
37	-0.57018	-0.86237	0.76100	0.76147
38	-0.67335	0.11188	1.45209	0.50523
39	-0.67752	1.25294	-0.23357	0.38466
40	-0.42026	1.41979	0.79059	2.01258
41	-0.41647	-1.68005	-0.06271	1.29919
42	-0.45709	0.28085	0.78279	1.59748
43	-0.44276	-0.45795	-1.46978	1.12392
44	-0.40077	0.42554	-1.45716	1.52057
45	-1.36862	0.28714	0.44067	0.44897
46	-1.79131	1.03127	-1.77279	-2.13378
47	-1.39157	-1.70722	-0.68122	-0.25437
48	-1.44144	-0.93501	1.01735	-0.07109
49	-1.79583	1.67004	1.67892	-1.68433
50	-1.45669	0.87609	-0.11009	-0.02486
51	-1.19856	0.68930	-0.38052	1.30377
52	-1.38466	-2.02150	0.12224	-0.12720
53	-1.24526	0.06544	0.51072	1.09595
54	-1.35938	-0.66727	-1.29724	0.00214
55	-1.41577	0.35604	-1.63629	-0.17847

The factor scores revealed that nitrogen fertilization intensity exerted a stronger influence on PC1, while cultivar-specific characteristics were more influential along PC2. This distinction emphasizes the combined role of agronomic management and genetic background in determining oat grain quality. The integrated interpretation demonstrates that nitrogen fertilization is the main driver of chemical grain quality traits, particularly protein and nitrogen content, while physical quality traits such as hectoliter weight are more strongly influenced by cultivar characteristics. The clear separation of these trait groups suggests that optimizing oat grain quality requires balanced strategies combining appropriate nitrogen inputs with cultivar selection.

Overall, the PCA approach proved to be a powerful tool for identifying key sources of variability in oat grain quality and for supporting decision-making in breeding programs and nitrogen management strategies.

CONCLUSIONS

The multivariate analysis of oat grain quality traits demonstrated that principal component analysis (PCA) is an effective tool for integrating physical and chemical parameters and for discriminating cultivar responses to nitrogen fertilization. The first two principal components explained over 93% of the total variability, confirming that a limited number of

components can adequately describe the complex structure of oat grain quality.

Nitrogen and protein content were the dominant contributors to the first principal component, highlighting the central role of nitrogen fertilization in shaping grain chemical composition. The strong association between these traits confirms that increased nitrogen supply primarily enhances protein accumulation in oat grain. In contrast, hectoliter weight contributed mainly to the second principal component, indicating that physical grain quality is largely independent of nitrogen-driven chemical traits and is more strongly influenced by cultivar characteristics.

The clear separation of grain quality traits into chemical and physical dimensions revealed potential trade-offs between maximizing protein content and maintaining favorable physical grain properties. The dispersion of cultivar $\times$ nitrogen fertilization combinations in the factorial plane demonstrated differential cultivar responses to nitrogen input, emphasizing the importance of genotype-specific nitrogen management.

Overall, the results underline the necessity of integrated agronomic strategies that combine appropriate nitrogen fertilization with cultivar selection to achieve balanced oat grain quality. The PCA-based approach provides valuable support for breeding programs and sustainable nitrogen management in oat production systems.

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AGRONOMIC EVALUATION OF SUNFLOWER HYBRIDS (*Helianthus annuus* L.) BY THE QUANTITY AND QUALITY INDICES

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Abstract

Sunflower is one of the most important oilseed crops on a global scale. The aim of the present study was to determine the productivity of four sunflower hybrids and to establish the influence of the factor hybrid in accordance with the specific climatic conditions of the year on the seed yield, as well as on some qualitative and quantitative indicators. The experiment was conducted during the period 2021-2023 in the region of Targovishte on soil type chernozem and an experimental area of 25 m² in four replications, after predecessor wheat. The following hybrids sunflower were tested: "P64LE136", "LG 50.549 SX", "Subaro" and "P64HE118". The results showed that the tallest plants were formed from P64LE136 hybrid and the lowest one - from LG 50.549 SX. The highest values of head diameter (20.4 cm), number of seeds per head (1513), seed weight of head (240.0 g), seed yield (3593 kg/ha) and 1000 seed weight (204.0 g) was reported for Subaro hybrid. Hybrid LG 50.549 SX was distinguished by the highest values of oil content (48.4%) and oil yield (1548 kg/ha) as well as test weight (43.2 kg).

Key words: sunflower, hybrids, seed yield, quality, oil.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the main oil crops in the world and one of the principal sources of vegetable oil, which is a key component of human nutrition. Sunflower seeds possess a significant oil content, with most sunflower hybrid varieties containing 40-50% (Jocić et al., 2015; Naeem et al., 2019). Sunflower oil is considered a premium-quality oil. Standard sunflower oil is usually composed of polyunsaturated linoleic acid (18:2) and monosaturated oleic acid (18:1) in ratios of 70%:20% (Radanović et al., 2018). The quantitative and qualitative indicators are specific to each sunflower hybrid, but they are influenced by the different growing factors (environmental and technological factors). Soil and climatic conditions are among the environmental factors with a great influence on the sunflower seed yield and the yield attributes of the head (Dalle et al., 2016; Ion et al., 2015; Manhart & Manilov, 2024; Mijić et al., 2017). Sunflower is predominantly cultivated under dry conditions and in rainfed areas worldwide (Kaya, 2020). Under drought conditions, especially during the grain-filling period, oil content is reduced in sunflower seeds (Kaya et al., 2016; Pekcan et al., 2022). In arid and semi-

arid regions, sunflower productivity is significantly reduced by drought stress (Hussain et al., 2018). García-López et al. (2016) reported that the distribution and quality of water have a major impact on sunflower seed and oil yield.

Seed yield and yield components are significantly affected by irrigation treatments applied to sunflower hybrids under arid and semi-arid environments (García-López et al., 2016; Sher et al., 2022; Surucu et al., 2020).

According to González et al. (2013), hybrid yield is conditioned by the capacity of the genotype to efficiently utilize environmental variables during different phenophases, while Hossam (2012) reported that sunflower productivity per unit area is determined by multiple factors, including plant population density and genotype.

Agronomic practices and high-yielding hybrids are the two most important factors for enhancing sunflower crop productivity (Beg et al., 2007).

Selecting the most appropriate hybrid for a specific region based on environmental conditions and cultivation technology facilitates the achievement of desired outcomes and guarantees consistent yields, representing a viable adaptation strategy in agriculture to climate change (Anton et al., 2015; Canavar et al., 2010; Garapova & Kirchev, 2023;

Delibaltova & Dallev, 2018; Drumeva & Yankov, 2020; Tanchev & Georgiev, 2025; Tanchev & Kirchev, 2025).

The aim of the present study was to define the productivity of four sunflower hybrids and to establish the influence of the factor hybrid in accordance with the specific climatic conditions of the year on the seed yield, as well as on some qualitative and quantitative indicators.

MATERIALS AND METHODS

The experiment was conducted in the period 2021-2023 in the region of Targovishte on soil type chernozem and an experimental area of 25 m² in four replications, after predecessor winter wheat. The following hybrids sunflower were tested: “P64LE136”, “LG 50.549 SX”, “Subaro” and “P64HE118”. Deep ploughing, two times disc harrowing, and two times cultivation were done on the trial field before seeding. Basic combination fertilization used 250 kg/ha N:P:K 15:15:15, while spring dressing used 200 kg/ha NH₄NO₃ (Neshev et al., 2020).

The sowing was performed in the second decade of March with planting distance 25 x 70 cm. The herbicide Express 50 SG (containing 500 g/kg tribenuron-methyl) was applied at a rate of 40 g/ha during the 4th-6th true leaf stage of sunflower development (BBCH 14-16). The sunflower crop was grown without irrigation

conditions adopted for region technology. For realizing the aim of the study account was taken following parameters: plant height (cm), head diameter (cm), number of seeds per head, seed weight of head (g), seed yield (kg/ha), 1000 seed weight (g), test weight (kg), oil content (%) and oil yield (kg/ha).

For the purpose of determining the quantitative and qualitative relationships among the studied indicators, the experimental data were processed using analysis of variance (ANOVA). The differences between the variants were determined using Duncan’s Multiple Range Test.

The major climatic factors determining the growth, development, and productivity of sunflower are temperature and rainfall, as well as their interaction and distribution throughout the growing season. The years of the study (2021-2023) differed significantly in terms of the amount and distribution of rainfall during the vegetation period (Figure 1).

The amount of rainfall in the first experimental year was 402.0 mm, i.e. 28.0 mm higher than the long-term average. During the March-September period of the second experimental year, total precipitation amounted to 357.0 mm, which was 45.0 mm lower than in 2021. The lowest amount of precipitation was recorded in the 2022 growing year, with 273.4 mm compared to 374.0 mm for the long-term average.

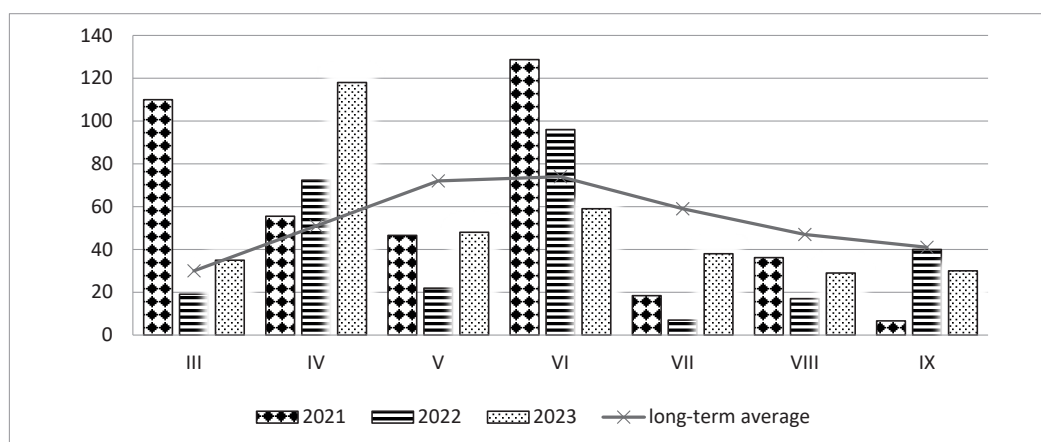


Figure 1. Precipitation, mm

The 2022 growing season was characterized by an uneven distribution of rainfall, which was

insufficient to meet plant water requirements during the critical growth stages. A comparison

of the average monthly air temperatures during the study period showed that temperature values were very similar across all three years and were consistently higher than the long-term averages (Figure 2).

These temperature conditions, in combination with the recorded precipitation during the growing season, influenced the growth and development of the sunflower plants.

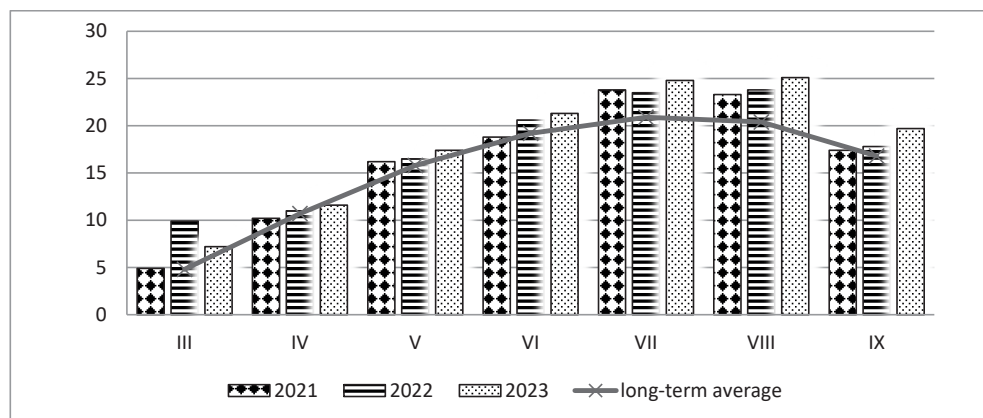


Figure 2. Average monthly air temperature, °C

The first experimental year (2021) was the most favorable for sunflower growth and development, followed by the third (2023), and the second (2022) was the least favorable, all of which had an impact on the productive potential of the sunflower hybrids tested.

RESULTS AND DISCUSSIONS

The seed yield findings (Table 1) reveal that the Subaru hybrid surpassed all other hybrids in the study in terms of seed yield, both by years and on average across the experimental period. The highest yields were recorded in the favorable sunflower year of 2021, when the temperature and precipitation totals fully satisfied the plant requirements throughout the vegetation period, compared to 2022 and 2023. The Subaru hybrid had yields of up to 3730 kg/ha. Referring to yield that hybrid surpassed LG 50.549 SX, P64LE136 and P64HE118 hybrids by 11.7 %, 23.9% and 26.2 %, respectively, the differences are statistically significant.

In the third experimental year (2023), seed yields ranged from 2923 kg/ha in the P64LE136 hybrid to 3640 kg/ha in the Subaru hybrid, or 2.4% lower on average than in 2021. Data analysis revealed that LG 50.549 SX, P64HE118, and P64LE136 hybrids behind

Subaro hybrid by 432, 680, and 717 kg/ha, respectively.

The lowest seed yields during the study period were observed in the second year of the experiment (2022), which was attributable to an insufficiency of moisture during the critical stages of sunflower plant development.

The yields obtained were within the limits of 2786 to 3410 kg/ha. Statistically proven, the lowest were P64HE118 hybrid and the highest were Subaru, with averages of 240 and 164 kg/ha lower than the first and third years, respectively.

During the experimental period (2021-2023), the Subaru hybrid produced an average yield of 3593 kg/ha, beating the hybrids LG 50.549 SX, P64LE136, and P64HE118 by 12.3%, 23.0%, and 23.8%.

The analysis of variance (Anova) shows a strong statistically proven influence on both the tested Hybrid and the Years with their specific climatic conditions on the indicator seed yield. An interaction between Hybrid and Year was proven (Table 2).

Oil yield is an important trait in sunflower which depends on the oil content of the genotype (Ahmed et al., 2021; Pekcan et al., 2022).

Data in Table 1 show that, on average for the experimental period, the LG 50.549 SX hybrid outperformed the other hybrids included in the

experiment. Under the conditions of the first-year oil yield varied from 1037 kg/ha for P64HE118 hybrid to 1647 kg/ha for LG 50.549 SX hybrid. Subaro and P64LE136 hybrids received yields, which were 110 and 359 kg/ha

lower compared to LG 50.549 SX hybrid. Mathematical processing of data showed that the differences between all the studied hybrids were significant (Table 1).

Table 1. Quantitative and morphological traits in sunflower hybrids

Hybrids	Year	Seed yield (kg/ha)	Oil yield (kg/ha)	Seed weight of head (g)	Number of seeds per head	Plant height, (cm)	Head diameter (cm)
P64LE136	2021	3010 ^c	1288 ^c	224.0 ^b	1318 ^c	218.0 ^a	20.0 ^b
	2022	2831 ^c	1110 ^c	207.9 ^c	1240 ^b	196.5 ^a	17.3 ^b
	2023	2923 ^c	1193 ^c	217.3 ^c	1286 ^c	203.6 ^a	18.4 ^b
	Avarage	2921	1197	216.4	1281	206.0	18.6
LG 50,549 SX	2021	3340 ^b	1647 ^a	231.6 ^b	1453 ^b	180.5 ^d	17.0 ^c
	2022	3050 ^b	1452 ^a	218.4 ^b	1212 ^b	164.2 ^c	15.2 ^c
	2023	3208 ^b	1546 ^a	221.2 ^b	1353 ^b	172.0 ^d	16.6 ^c
	Avarage	3199	1548	223.7	1339	172.2	16.3
Subaro	2021	3730 ^a	1537 ^b	257.0 ^a	1600 ^a	195.0 ^b	22.8 ^a
	2022	3410 ^a	1309 ^b	217.0 ^a	1425 ^a	170.0 ^b	18.0 ^a
	2023	3640 ^a	1438 ^b	245.0 ^a	1513 ^a	183.7 ^c	20.5 ^a
	Avarage	3593	1428	240.0	1513	182.9	20.4
P64HE118	2021	2955 ^d	1037 ^d	214.7 ^c	1400 ^b	187.0 ^c	19.6 ^b
	2022	2786 ^d	881 ^d	203.0 ^d	1146 ^c	175.0 ^b	16.8 ^b
	2023	2960 ^c	1002 ^d	208.5 ^d	1279 ^c	190.2 ^b	18.0 ^b
	Avarage	2900	973	208.7	1275	184.1	18.1

*Means within columns followed by different lowercase letters are significantly different ($P < 0.05$), according to the LSD test.

The experimental year 2022 was characterized with less amount of precipitations compared to 2021 during the vegetation period – 273.4 mm, and the obtained oil yield were within the bounds from 881 to 1452 kg/ha. In the last year of the experiment, the oil yield ranged from 1002 to 1546 kg/ha.

On average for the three-year experimental period, the highest yield of oil was obtained from the LG 50.549 SX hybrid - 1548 kg/ha and it surpassed the other hybrids from 8.4 to 59.0%. The results of the analysis of variance for the effects of the factors Hybrid and Year, as well as their interaction, on oil yield (Table 2) show a statistically significant effect of the studied factors and a non-significant effect of their interaction.

Across different years and on average for the studied sunflower hybrids, seed weight per head varied between 203.0 and 257.0 g, depending on the hybrid and climatic conditions (Table 1). The highest values were recorded for the Subaro

hybrid, amounting to 257 g in 2021, 217 g in 2022, and 245 g in 2023. The lowest values of this characteristic were recorded for the hybrid P64HE118 - 208.7 g. On average for the years of the study, this hybrid had 15.3% lower seed weight per head compared to Subaro and from 3.7 to 7.2% lower seed weight per head compared to the hybrids P64LE136 and LG 50.549 SX.

The analysis of variance for the influence of the factors – Hybrid and Year, as well as their interaction on the indicator "seed weight per head" shows a significant influence of the factors on the change of the indicator.

Data on the number of seeds per head of the studied hybrids showed that during the first experimental year the values ranged from 1318 in P64LE136 to 1600 in Subaro, while in 2022, which was unfavorable for sunflower production, the values were approximately 185 seeds lower (Table 1).

On average for the period 2021-2023, the number of seeds per head in the studied hybrids ranged from 1275 to 1513. The lowest values of this characteristic were recorded for the hybrid P64HE118, while the highest were observed for the Subaru hybrid. The hybrids P64LE136 and

LG 50.549 SX had an average number of seeds per head of 1281 and 1339, respectively. The Subaru hybrid exceeded the hybrids LG 50.549 SX, P64LE136, and P64HE118 in this characteristic by 12.9%, 18.1%, and 18.6%, respectively.

Table 2. Analysis of variance ANOVA

Parameter	Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Seed yield (kg/ha)	Hybrid **	3730939.73	3	1243646.58	5485.50	0.00	2.87
	Year**	460959.04	2	230479.52	1016.60	0.00	3.26
	Interactions *	56610.46	6	9435.08	41.62	0.00	2.36
	Within	8161.75	36	226.72			
Oil yield (kg/ha)	Hybrid **	2335949	3	778649.80	263.83	0.00	2,87
	Year*	287879.30	2	143939.60	48.77	0.00	3,26
	Interactions ^{ns}	9650.54	6	1608,42	0.54	0.77	2,36
	Within	106247.50	36	2951.32			
Seed weight of head (g)	Hybrid *	6369.47	3	2123.16	63.93	0.00	2.87
	Year*	2918.65	2	1459.33	43.94	0.00	3.26
	Interactions *	1207.19	6	201.20	6.06	0.00	2.36
	Within	1195.53	36	33.21			
Number of seeds per head	Hybrid *	442148	3	147382.67	100.77	0.00	2.87
	Year*	280568.67	2	140284.33	95.92	0.00	3.26
	Interactions *	39426	6	6571	4.49	0.00	2.36
	Within	52652	36	1462.56			
Plant height, (cm)	Hybrid **	7231.75	3	2410.58	32.87	0.00	2.87
	Year*	2709.29	2	1354.64	18.47	0.00	3.26
	Interactions ^{ns}	400.875	6	66.81	0.91	0.50	2.36
	Within	2640	36	73.33			
Head diameter (cm)	Hybrid *	80.88	3	26.96	7.76	0.00	2.87
	Year*	67.12	2	33.56	9.67	0.00	3.26
	Interactions ^{ns}	8.01	6	1.36	0.38	0.88	2.36
	Within	124.99	36	3.47			

*F-test significant at P<0.05; **F-test significant at P<0.01; ns non-significant

The results of the analysis of variance regarding the effect of the studied factors and their interaction on the number of seeds per head showed a statistically significant effect of both factors, as well as a statistically significant interaction between them (Table 2).

The studied hybrids differed significantly in plant height. The lowest values of this indicator were reported in 2022, when climatic conditions were less favorable for sunflower growth and development compared to 2021 and 2023. The sunflower hybrid P64LE136 had the tallest

plants, while LG 50.549 SX had the shortest plants. Statistical analysis showed that the differences among all studied hybrids were significant. On average for the study period, the plant height of the hybrid P64LE136 was 206.0 cm and exceeded that of P64HE118 by 11.9%, Subaru by 13.2%, and LG 50.549 SX by 19.6% (Table 1).

The analysis of variance (ANOVA) showed that the factors hybrid and year had a statistically significant effect on plant height, whereas their

interaction was not statistically significant (Table 2).

In the studied sunflower hybrids, head diameter varied between 15.2 and 22.8 cm, depending on the hybrid and climatic conditions during the individual agricultural years (Table 1). The highest values were obtained in 2021 and ranged from 17.0 to 22.8 cm, while under less favorable growing conditions in 2022, the values of this characteristic decreased by 11.8 to 26.7%. The lowest values of head diameter were recorded for the hybrid LG 50.549 SX, and the highest for the hybrid Subaru. During the three experimental years, these differences were statistically significant.

On average for the period 2021-2023, the hybrids P64HE118 and P64LE136 exceeded the hybrid LG 50.549 SX by 13.1% and 14.1%, respectively, but lagged behind Subaru by 9.7% and 12.6%.

The results of the analysis of variance regarding the effect of the factors Hybrid and Year, as well as their interaction, on head diameter (Table 2) showed a statistically significant effect of the studied factors and a non-significant effect of their interaction.

Differences in climatic conditions during the years of the experiment were among the reasons for the formation of seeds with different qualitative indices. The 1000-seed weight is one of the most important quality indicators. This trait varies between different hybrids and growing conditions (Radić et al., 2013). Joksimović et al. (2004) reported that 1000-seed weight is a variable trait and, as such, is influenced by both genetic and environmental factors.

According to the results presented in Table 3, the highest 1000-seed weight was obtained in 2021, when there was a good reserve of winter moisture and total rainfall (402 mm), and when its monthly distribution during the growing season was exceptionally favorable. Under these conditions, the values ranged from 181 to 215 g. In 2023, the 1000-seed weight was up to 5.4% lower. In 2022, a less favorable year for sunflower cultivation, the values of this indicator ranged from 163 to 197 g.

On average for the study period, the lowest 1000-seed weight was recorded for the hybrid P64HE118 and the highest was observed for the hybrid Subaru. The hybrids P64LE136 and LG

50.549 SX had values of 189.3 g and 185.3 g, respectively.

The hybrid Subaru exceeded the hybrids P64HE118, LG 50.549 SX, and P64LE136 in 1000-seed weight by 17.0%, 10.1%, and 7.7%, respectively.

The analysis of variance (ANOVA) showed the impact of factors A (year) and B (hybrid), as well as their interaction, on the indicator 1000-seed weight. The results indicated a statistically significant influence of the examined factors and a statistically significant effect of their interaction.

Data on the test weight of the studied hybrids showed that during the years of the experiment, values ranged from 36.0 to 45.3 kg (Table 3). The lowest values of this characteristic were recorded for the hybrid P64HE118, while the highest were observed for the hybrid LG 50.549 SX, and the differences were statistically significant. On average for the period 2021-2023, the hybrid LG 50.549 SX exceeded the hybrids Subaru, P64LE136, and P64HE118 in this characteristic by 4.8%, 12.7%, and 15.5%, respectively. The results of the analysis of variance regarding the effect of the factors Hybrid and Year, as well as their interaction, on test weight (Table 3) showed a statistically significant effect of the studied factors.

The oil content of sunflower is one of the most affected yield traits (Pekcan et al., 2022). The highest oil content was achieved in 2021 (42.1%), which was a favorable year for sunflower cultivation, while the lowest value was recorded in 2022 (39.2%) due to insufficient moisture during the critical stages of sunflower growth and development (Table 3). In the third experimental year (2023), oil content ranged from 31.8% in the hybrid P64HE118 to 48.2% in the hybrid LG 50.549 SX.

On average for the study period, the highest oil content of sunflower seed was recorded for the hybrid LG 50.549 SX (48.4%), followed by P64LE136 and Subaru (40.9% and 39.7%, respectively), while the lowest values were reported for the hybrid P64HE118 (32.8%).

The analysis of variance (ANOVA) shows a strong statistically proven influence on Hybrid and the Year with their specific climatic conditions on the indicator oil content (Table 3). An interaction between Hybrid and Year was unproven.

Table 3. Qualitative components in sunflower hybrids

Variable		1000 seed weight (g)	Test weight (kg)	Oil content (%)
Years (A)	2021	198.0 ^a	42.0 ^a	42.1 ^a
	2022	178.7 ^c	37.7 ^c	39.2 ^c
	2023	188.0 ^b	40.6 ^b	40.1 ^b
Hybrid (B)	P64LE136	189.3	38.3	40.9
	LG 50.549 SX	185.3	43.2	48.4
	Subaro	204.0	41.2	39.7
	P64HE118	174.3	37.4	32.8
2021	P64LE136	200 ^b	39.7 ^c	42.8 ^b
	LG 50.549 SX	196 ^b	45.3 ^a	49.3 ^a
	Subaro	215 ^a	44.2 ^b	41.2 ^b
	P64HE118	181 ^c	38.9 ^c	35.1 ^c
2022	P64LE136	180 ^b	36.5 ^c	39.2 ^b
	LG 50.549 SX	175 ^b	40.7 ^a	47.6 ^a
	Subaro	197 ^a	37.5 ^b	38.4 ^b
	P64HE118	163 ^c	36.0 ^c	31.6 ^c
2023	P64LE136	188 ^b	38.8 ^c	40.8 ^b
	LG 50.549 SX	185 ^b	43.5 ^a	48.2 ^a
	Subaro	200 ^a	42.0 ^b	39.5 ^b
	P64HE118	179 ^c	37.2 ^d	31.8 ^c
Anova	A	*	*	*
	B	*	*	**
	AB	*	*	n.s

*Means within columns followed by different lowercase letters are significantly different ($P < 0.05$) according to the LSD test

*F-test significant at $P < 0.05$; **F-test significant at $P < 0.01$; n.s non-significant.

CONCLUSIONS

The results of the present study demonstrate that the quantitative and qualitative indices of the tested sunflower hybrids are significantly influenced by the genetic characteristics of the hybrid, as well as by the weather conditions of the year, and above all by the the amount and distribution of vegetative rainfall.

The highest seed yield was obtained from the hybrid Subaro (3593 kg/ha), which was associated with higher values of the yield structural components, including head diameter, number of seeds per head, seed weight per head, and 1000-seed weight. Oil content ranged between 32.8% (P64HE118) and 48.4% (LG 50.549 SX). The hybrid LG 50.549 SX exhibited significantly higher mean values of test weight, oil content, and oil yield compared to the other studied hybrids.

The study's findings could be useful in selecting sunflower hybrids for the region. Out of the researched sunflower hybrids for the Targovishte region, it is recommended to cultivate hybrids Subaro and LG 50.549 SX, which have balanced quantity and quality indices under the contrast conditions of the studied years.

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GENETIC DIVERSITY OF HIGH AND LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS IN BULGARIAN ACCESSIONS FROM *Aegilops triuncialis* SPECIES

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Abstract

Thirty-eight samples of the species *Aegilops triuncialis* L. were analyzed for HMW and LMW glutenin subunits. A total of 18 alleles were documented at the Glu-U1 locus, with allele frequencies ranging from 2.63% to 15.79%. A total of ten alleles were identified at the Glu-C1 locus, with the alleles Glu-C1h, Glu-C1i, and Glu-C1j classified as rare. The genotypes under scrutiny exhibited substantial allelic variation with respect to both B-LMW-GS and C-LMW-GS. A total of 41 alleles were identified within the B zone, 20 within the Glu-U3 locus, and 21 within the Glu-C3 locus. A total of 35 alleles were identified in the C zone, 21 in the Glu-U3 locus and 14 in the Glu-C3 locus. The presence of a diverse array of glutenin subunits, encompassing both high and low molecular weight variants, within this species, signifies a valuable genetic resource that has the potential to enhance the quality of wheat.

Key words: *Aegilops triuncialis* L., alleles, glutenins, polymorphism, SDS-PAGE.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops globally, serving as a primary source of calories and protein for over one-third of the world's population (Bayisa et al., 2025; Sharma and Sharma, 2025). Its end-use quality, particularly dough elasticity and baking performance, is largely determined by gluten proteins, which form a viscoelastic network in the presence of water (Ortolan and Steel, 2017; Ye et al., 2023). Gluten is composed of two main protein classes: gliadins and glutenins. Glutenins, in turn, are further classified into high molecular weight (HMW-GS) and low molecular weight (LMW-GS) subunits, encoded by the Glu-1 and Glu-3 loci, respectively, located on the homoeologous group 1 and 6 chromosomes (Sharma et al., 2020; Guzmán et al., 2022; Al-Khayri et al., 2023; Spetsov and Daskalova, 2024).

Among glutenin subunits, HMW-GS are particularly critical for dough strength due to their role in forming intermolecular disulfide bonds that contribute to polymer formation (Nucia et al., 2019; Shewry et al., 2022; Wang

et al., 2022, 2023, 2024). Variability in HMW-GS composition is strongly correlated with differences in bread-making quality, with certain allelic variants (e.g., Glu-D1d) being associated with superior performance (Bouabdellah et al., 2025). LMW-GS, although individually smaller, are more numerous and contribute significantly to gluten polymer size and viscoelasticity (Guzmán et al., 2022; Chen et al., 2025). Thus, understanding the genetic diversity of both HMW-GS and LMW-GS is crucial for wheat improvement.

Wild relatives of wheat, such as those in the genus *Aegilops*, are valuable reservoirs of genetic variation not only for disease resistance and for abiotic stress tolerance but also potentially for grain quality traits (Kishii et al., 2019; Adhikari et al., 2023). *Aegilops triuncialis* L., an allotetraploid species with the CCUU genome constitution ($2n = 28$), is naturally distributed across the Mediterranean Basin, including Bulgaria, where it grows in ruderal and disturbed habitats (Feldman and Levy, 2023). It diverged from the wheat lineage approximately 3-4 million years ago and shares genome homology with the U genome, which is

related to the A genome of wheat (Badaeva et al., 2025). This phylogenetic proximity suggests potential for introgressing U-genome-encoded glutenin subunits into wheat via wide hybridization.

Despite its agronomic potential, *Aegilops triuncialis* L. has been poorly characterized for glutenin variation, especially in Eastern Europe. Bulgarian accessions, in particular, have not been systematically analyzed for HMW-GS and LMW-GS composition. Bulgaria's diverse climate and topography support a wide range of genetic variation in wild cereals, making it an ideal region for exploring underutilized genetic resources (Desheva et al., 2013; Kyosev et al., 2025). Understanding the extent of HMW-GS and LMW-GS diversity is essential to identify superior alleles for introgression into commercial wheat cultivars. Therefore, this study aims to assess the allelic diversity of HMW-GS and LMW-GS in Bulgarian *Aegilops triuncialis* L. accessions using electrophoretic profiling, and to evaluate the implications of this diversity for wheat quality breeding.

MATERIALS AND METHODS

The seeds from thirty-eight accessions of the *Aegilops triuncialis* L. collected from the territory of Bulgaria during 2024 (Table 1) were used to analyse HMW and LMW glutenin subunits. Extraction of HMW-GS and LMW-GS was performed using the method of Wang et al. (2022). SDS-PAGE was conducted with 12% (w/v) running gels and 4% (w/v) stacking gels. The gels were run at 30 mA for 1.50 h (Cytiva SE 260 mini-cell apparatus). The gels were stained with a 1% solution of Coomassie Brilliant Blue R-250 in acetic acid, methanol, and water in a ratio of (1:5:4) overnight. The gel plates were decolorized with a solution of acetic acid, methanol, and distilled water (1:2:7). In order to assign the components of HMW-GS, B-LMW-GS, and C-LMW-GS patterns of the tetraploid species *Aegilops triuncialis* L. to U or C genomes, a comparison of glutenin electrophoretic patterns between *Aegilops triuncialis* L. and the diploid species *Aegilops umbellulata* Zhuk. and *Aegilops caudata* L. was undertaken. The nomenclature of the subunits and alleles of HMW-GS (locus Glu-1) corresponds to the terminology of Rodriguez-

Quijano et al. (2001), with the letter C denoting bands from *Aegilops caudata* L. and the letter U denoting bands from *Aegilops umbellulata* Zhuk. For LMW-GS (B- and C-subunits), the different bands were assigned numerical identifiers according to their order of mobility in SDS-PAGE.

The genetic diversity at each locus was calculated using the formula developed by Nei (1973). The level of variation was estimated by dividing the number of patterns found by the number of accessions. A dendrogram was constructed via the unweighted pair group method with arithmetic mean (UPGMA). The implementation of cluster analysis was conducted utilizing the Past4.03 software.

Table 1. Geographic coordinates of collected accessions from *Aegilops triuncialis* L. included in the study

№	Accession number	Geographical latitude	Geographical longitude	Altitude	Locality
1	C4E0032	42°1'36.462"N	24°42'58.212"E	670	Galabovo, Plovdiv
2	C4E0033	41°39'15.732"N	25°52'16.104"E	199	meandrite na Arda, Haskovo
3	C4E0051	42° 30' 31.8"N	24° 48' 51.0"E	360	Pesnopoy, Plovdiv
4	C4E0052	42°2'443.161"N	27°42'5.513"E	8	Sozopol, Burgas
5	C4E0200	41°47'21.82"N	25°21'55.823"E	402	Pchelarovo, Kardzhali
6	C4E0205	41°25'49.495"N	25°21'40.377"E	285	Samodiva, Kardzhali
7	C4E0207	41°22'51.739"N	25°13'35.430"E	360	Deletz, Kardzhali
8	C4E0209	41°25'26.848"N	25°21'46.488"E	287	Bregovo, Kardzhali
9	C4E0190	43°15'26.323"N	27°49'25.807"E	134	Aksakovo, Varna
10	C4E0061	42°24'39.668"N	24°46'45.351"E	237	Dolino sahrane, Stara Zagora
11	C4E0195	41°52'26.533"N	25°20'36.085"E	249	Susam, Haskovo
12	C4E0044	42° 26' 51.3"N	24° 11' 31.9"E	255	Brestnik, Plovdiv
13	C4E0088	42°44'43.207"N	27°44'31.542"E	100	Kosharitz, Burgas
14	C4E0091	42°46'17.248"N	27°47'35.313"E	805	Banya, Burgas
15	C4E0148	41°57'36.773"N	23°35'52.192"E	805	Daganovo, Blagoevgrad
16	C4E0149	41°57'34.228"N	23°35'45.264"E	805	Daganovo, Blagoevgrad
17	C4E0153	41°52'27.781"N	23°35'30.874"E	725	Eleshtitsa, Blagoevgrad
18	C4E0158	41°39'37.804"N	23°44'4.392"E	551	Gospodintzi, Blagoevgrad
19	C4E0162	41°34'34.388"N	23°44'35.937"E	540	Gotze Delchev, Blagoevgrad
20	C4E0163	41°35'59.904"N	23°40'48.084"E	871	Dobrotino, Blagoevgrad
21	C4E0164	41°31'17.870"N	23°32'36.500"E	773	Pirin, Blagoevgrad
22	C4E0165	41°29'49.373"N	23°29'36.934"E	306	Gorno Spanchevo, Blagoevgrad
23	C4E0168	41°27'13.411"N	23°26'22.104"E	188	Kalimantzi, Blagoevgrad
24	C4E0170	41°27'6.779"N	23°23'37.109"E	248	Harsovo, Blagoevgrad
25	C4E0176	41°27'23.806"N	23°22'28.922"E	230	Kromidovo, Blagoevgrad
26	C4E0178	41°27'31.041"N	23°20'45.984"E	172	Kapatovo, Blagoevgrad
27	C4E0181	41°27'18.600"N	23°19'23.226"E	143	Levanovo, Blagoevgrad
28	C4E0182	41°28'45.584"N	23°15'21.948"E	103	Starchevo, Blagoevgrad
29	C4E0183	41°28'45.584"N	23°15'21.948"E	103	Starchevo, Blagoevgrad
30	C4E0184	41°27'40.311"N	23°15'41.488"E	90	Rupite, Blagoevgrad
31	C4E0185	41°55'31.691"N	23°12'22.29"E	491	Gradevo, Blagoevgrad
32	C4E0197	41°55'45.847"N	25°20'39.434"E	321	Haskovski mineralni bani, Haskovo
33	C4E0218	42°17'31.941"N	22°39'56.116"E	551	Lozno, Kjustendil
34	C4E0014	42°9'54.708"N	24°8'15.162"E	274	Varvara, Pazardzhik
35	C4E0015	42°10'10.956"N	24°6'49.006"E	352	Simenovetz, Pazardzhik
36	C4E0018	42°12'25.344"N	24°30.090"E	313	Belovo, Pazardzhik
37	C4E0023	42°12'1.5328"N	24°42'4.5468"E	784	Tzar Kaloyan, Plovdiv
38	C4E0027	42°2'39.1344"N	24°41'36.355"E	1078	baza Iglika, Plovdiv

RESULTS AND DISCUSSIONS

Glutenin proteins, particularly the high molecular weight (HMW GS) and low molecular weight (LMW GS) subunits, play a decisive role in dough rheology and, consequently, in end use quality. *Aegilops*

species generally exhibit high genetic diversity in both HMW-GS and LMW-GS. This diversity can be harnessed to introduce new alleles into the wheat gene pool, which is often limited in genetic variation (Cuesta et al., 2016; Ahmadi et al., 2018; Tariq et al., 2018; Goldasteh et al., 2019; Mohamed et al., 2022; Jiang et al., 2023). In this study employed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) to characterize the glutenin subunit composition of 38 *Aegilops triuncialis* L. accessions collected from diverse agro ecological zones across Bulgaria.

A total of 28 distinct alleles were identified across the Glu-1 loci, distributed between the Glu-U1 and Glu-C1 regions. In the Glu-U1 locus, 18 alleles were detected, with frequencies ranging from 2.63% to 15.79%. The most common alleles were Glu-U1c and Glu-U1j, each occurring in 15.79% of samples, followed by Glu-U1b and Glu-U1d, each present in 10.53%. Alleles Glu-U1g and Glu-U1a were found in 7.89% of samples, while Glu-U1f appeared in 5.26%. The remaining 11 alleles were each detected at the lowest observed frequency of 2.63% (Tables 2 and 4).

At the Glu-C1 locus, ten alleles were identified. The most prevalent were Glu-C1d (encoding subunit 4) at 21.05% and Glu-C1f (associated with subunits 2 and 7) at 18.42%. Glu-C1g was observed in six genotypes, and Glu-C1b and Glu-C1c each occurred in 13.16% of samples. Alleles Glu-C1h, Glu-C1i, and Glu-C1j were considered rare, each appearing in only a single genotype (Tables 2 and 4).

The genetic diversity indices calculated for the Glu-U1 locus and Glu-C1 locus were high, respectively 0.907 and 0.843 indicating a high level of allelic polymorphism and variability at HMW-GS loci (Table 2).

In accordance with the findings of our study, a high degree of genetic diversity with regard to HMW-GS has also been identified in other *Aegilops* species. Research conducted on *Aegilops tauschii* Coss. has revealed significant variations in HMW-GS. For instance, 14 HMW-GS combinations have been identified in Iranian specimens, suggesting a high degree of genetic diversity (Tahernezhad et al., 2013). In the Sitopsis section of *Aegilops*, six new HMW-GS alleles have been identified in species of this section, with a large molecular weight due to the

insertion of fragments (Jiang et al., 2012). Medouri et al. (2014) have documented the extensive array of high molecular weight glutenin subunits and low molecular weight glutenin subunits in Algerian *Aegilops geniculata* Roth. As demonstrated by Kozub et al. (2012), significant genetic diversity has been identified, with studies identifying numerous alleles for glutenin subunits (Gli-U1 and Gli-M1) across various populations of *Aegilops biuncialis* Vis.

Table 2. Allele frequency in HMW glutenin subunits and genetic diversity index in the Glu-U1 and Glu-C1 loci in samples from *Aegilops triuncialis* L.

Zone	Locus	Allele	Subunits	Frequency
A	Glu-U1	a	1+5	5.26
		b	1+6	10.53
		c	1+7	15.79
		d	1+8	10.53
		e	2+6	2.63
		f	2+7	5.26
		g	2+8	7.89
		h	3+6	2.63
		i	3+7	2.63
		j	3+8	15.79
		k	3+9	5.26
		l	4+8	2.63
		m	6+9	2.63
		n	7+8	2.63
		o	8+9	2.63
		p	9	2.63
		q	2+6+10	2.63
		r	5+7+9	2.63
	H index		0.907	
	Glu-C1	a	1	5.26
		b	2	13.16
		c	3	13.16
		d	4	23.68
		e	1+9	5.26
		f	2+7	18.42
		g	2+9	15.79
		h	4+8	2.63
		i	4+9	2.63
		j	2+7+9	2.63
	H index		0.843	

The Glu3-loci in wheat and its relatives, including *Aegilops* species, encode LMW-GSs, which are essential components of the gluten protein network. LMW-GSs significantly affect dough elasticity, viscosity, and overall bread-making quality (Pourmohammadi et al., 2023; Zhao et al., 2025). In this study set of genotypes of *Aegilops triuncialis* L. showed significant allele variations for B-LMW-GS and C-LMW-GS.

Forty-one alleles were identified in zone B (Tables 3 and 4), 20 in the Glu-U3 locus and 21 in the Glu-C3 locus. In the Glu-U3 locus, sixteen of the alleles were found with the same frequency (2.63%). It was observed that only Glu-U3d (encoding subunits 1 and 9) exhibited a frequency of 28.95%. In the Glu-C3 locus, thirteen distinct alleles were identified in the

analyzed genotypes. Three alleles (Glu-C3c, Glu-C3j, and Glu-C3k) were found with a frequency above 10%. The most prevalent allele (Glu-C3k, encoding subunits 3+12) was identified in six samples (representing 15.79% of the total).

35 alleles were identified in the C-zone (Table 3), 21 of which were located in the Glu-U3 locus and 14 in the Glu-C3 locus. In the Glu-U3 locus, eleven of the alleles were classified as rare and have a frequency of 2.63%. The analysis revealed the presence of Glu-U3b and Glu-U3c (encoding subunits 2 and 3, respectively) in 5 and 4 samples, respectively, with a frequency of 13.16% and 10.53%, respectively. At the Glu-C3 locus, eight alleles were identified in individual genotypes. One allele (Glu-C3f) was identified in six samples, one allele (Glu-C3c) was recorded in another six samples, and frequencies of 13.16% were found for the alleles Glu-C3b, Glu-C3d, and Glu-C3g (Tables 3 and 4).

The Nei diversity index for B-LMW-GS was 0.961 and for C-LMW-GS respectively 0.967 (Table 4). The provided values, represent high genetic diversity for Low Molecular Weight Glutenin subunits in *Aegilops triuncialis* L., with the C-type locus showing slightly greater diversity than the B-type locus, indicating rich allelic variation important for baking quality. These results suggest a complex genetic makeup for glutenins, crucial for dough strength and elasticity in wheat breeding (Wang et al., 2024; Zhao et al., 2025, 2025a). Wang et al. (2018) identify specific LMW-GS from *Aegilops umbellulata* Zhuk., that enhance gluten networks, benefiting traits like dough development time, stability, gluten index, and loaf structure in improved wheat varieties. In the species *Aegilops comosa* Sm. novel LMW-GS genes were isolated, showing significant polymorphisms and evolutionary divergence (Wang et al., 2011; Huang et al., 2018), while in *Aegilops tauschii* Coss. three LMW-GS genes were characterized, showing high homology with bread wheat and related species (Pei et al., 2007).

Based on the HMW- and LMW-glutenin subunit profiles, a UPGMA dendrogram (Figure 1) was constructed to assess genetic relatedness among

the studied genotypes, revealing four major clusters with distinct molecular and geographic patterns.

The first cluster includes four genotypes - 24, 25, 29, and 12 exhibiting high variability in the B and C zones of the LMW-GS at the Glu-C3 locus. Genotypes 24, 25, and 29 originate from the Blagoevgrad region, while genotype 12 was collected in Plovdiv, suggesting possible regional genetic divergence. Cluster II consists of two genotypes - 21 and 26 - both from Blagoevgrad, which share rare alleles in the B-LMW-GS region, highlighting unique genetic features within this group. The largest cluster (Cluster III) comprises 23 accessions, further subdivided into four well-defined subgroups. Subgroup 1 includes genotypes 36 (from Plovdiv region), 32 (from Haskovo region), 33 (from Kardzhali region), and 27 and 30 (from Blagoevgrad region), and is characterized by a strong presence of the Glu-U1d and Glu-C1c alleles. Subgroup 2 features genotypes 1 (from Plovdiv region), 31 (from Blagoevgrad region), 14 (from Burgas region), and 10 (from Stara Zagora region), dominated by the Glu-U3c allele in B-LMW-GS. Subgroup 3 is defined by the prevalent Glu-U3d allele in B-LMW-GS and includes primarily genotypes from Blagoevgrad region (4, 15-20, 22), with only two exceptions: genotype 2 (from Haskovo region) and 13 (from Burgas region). Subgroup 4 contains genotypes 23 and 28 (from Pazardzhik region) and 34 and 35 (from Blagoevgrad region), all carrying rare alleles in the C-LMW-GS region. The fourth cluster groups nine genotypes 3, 37, 38 (from Plovdiv region), 5-8 (from Kardzhali region), 9 (from Varna region), and 11 (from Haskovo region) - distributed across three subgroups and marked by a shared predominance of the Glu-C1d (A-HMW-GS) and Glu-C3f (C-LMW-GS) alleles, suggesting a common genetic background despite geographic dispersion. The dendrogram identifies several genotype pairs with exceptionally high genetic similarity, including 17-18, 13-22, 16-18, 34-35, and 3-7, all exhibiting Dice similarity indices between 0.737 and 0.870 (Figure 1). These close relationships underscore potential duplication, shared ancestry, or limited genetic differentiation within these pairs.

Table 3. Frequency of alleles in LMW glutenin subunits and genetic diversity index in the Glu-U3 and Glu-C3 loci in samples from *Aegilops triuncialis* L.

Zone	Locus	Allele	Subunits	Frequency	Zone	Locus	Allele	Subunits	Frequency
B	Glu-U3	a	1+6	2.63	C	Glu-U3	a	1	5.26
		b	1+7	2.63			b	2	13.16
		c	1+8	13.16			c	3	10.53
		d	1+9	28.95			d	4	7.89
		e	2+10	5.26			e	5	2.63
		f	2+8	2.63			f	6	5.26
		g	2+9	13.16			g	1+11	2.63
		h	3+13	2.63			h	1+8	7.89
		i	4+11	2.63			i	1+9	5.26
		j	4+13	2.63			j	2+10	2.63
		k	4+14	2.63			k	2+12	7.89
		l	1+10+14	2.63			l	2+13	5.26
		m	1+5+14	2.63			m	2+9	5.26
		n	1+7+16	2.63			n	3+10	2.63
		o	1+8+14	2.63			o	3+11	2.63
		p	2+5+15	2.63			p	3+12	2.63
		q	2+6+16	2.63			q	3+13	2.63
		r	2+7+14	2.63			r	3+9	2.63
		s	2+8+15	2.63			s	4+12	2.63
		t	3+7+16	2.63			t	4+13	2.63
	H index	0.868					u	6+13	2.63
	Glu-C3	a	1+10	2.63		Glu-C3	a	3	2.63
		b	1+8	2.63			b	4	13.16
		c	2+10	10.53			c	5	15.79
		d	2+11	2.63			d	6	13.16
		e	2+12	2.63			e	7	10.53
		f	2+13	5.26			f	8	15.79
		g	2+6	2.63			g	9	13.16
		h	2+7	7.89			h	1+8	2.63
		i	3+10	2.63			i	3+11	2.63
		j	3+11	13.16			j	4+11	2.63
		k	3+12	15.79			k	5+11	2.63
		l	3+13	5.26			l	5+9	2.63
		m	3+7	2.63			m	6+10	2.63
		n	3+9	2.63			n	8+11	2.63
		o	4	2.63			H index		0.882
		r	5	2.63					
		s	6	2.63					
		t	8	2.63					
		p	4+10	5.26					
		q	4+12	5.26					
		u	2+9+13	2.63					
	H index	0.920							

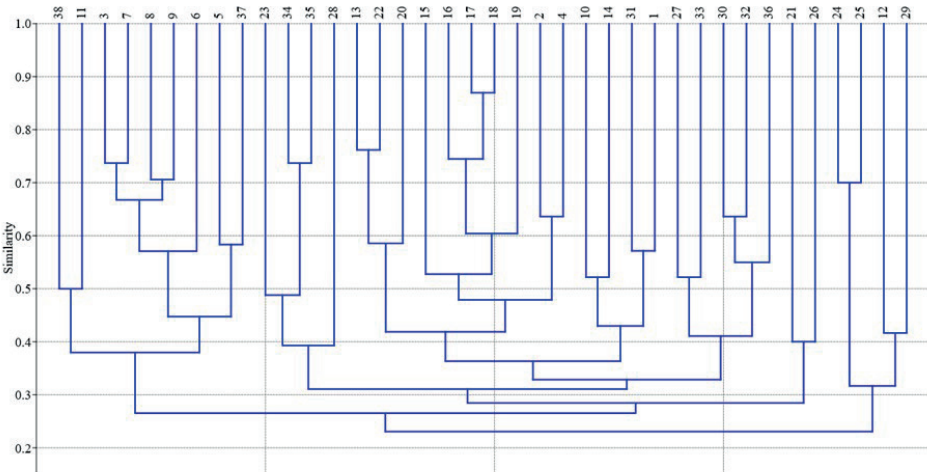


Figure 1. Grouping of 38 genotypes of *Aegilops triuncialis* L. using the UPGMA method (Similarity index-Dice) (The numbers of the genotypes in the dendrogram correspond to the numbers in Table 1)

Table 4. Allele composition of HMW-GS, B-LMW-GS and C-LMW-GS subunits in accessions of the species *Aegilops triuncialis* L

№	Genotype	A subunits				B subunits				C subunits			
		Glu-U1	Allele	Glu-C1	Allele	Glu-U3	Allele	Glu-C3	Allele	Glu-U3	Allele	Glu-C3	Allele
1	CE40032	1+7	c	2	b	1+8	c	2+10	c	1+8	h	5	c
2	CE40033	1+7	c	2+7	f	1+9	d	3+12	k	3+10	n	4+11	j
3	CE40051	3+8	k	2	b	2+9	g	4+12	q	2+10	j	4	b
4	CE40052	4+8	l	2	b	1+9	d	2+12	e	3	c	8	f
5	CE40200	5+7+9	r	2+7+9	j	2+8+15	s	2+7	h	2+12	k	6	f
6	CE40205	6+9	m	4	d	2+6+16	q	2+7	h	6+13	u	8	f
7	CE40207	7+8	n	4	d	2+9	g	4+10	p	2	b	8	f
8	CE40209	8+9	o	4	d	2+9	g	4+12	p	3	c	8	f
9	CE40190	9	p	4	d	2+9	g	3+12	k	2	b	8	f
10	CE40061	2+7	f	2+7	f	1+8	c	3+13	l	6	f	8	f
11	CE40195	3+6	h	2+7	f	2+5+15	p	2+6	g	1	a	5	c
12	CE40044	1+7	c	1	a	1+6	a	1+10	a	4+12	s	8+11	n
13	CE40088	1+6	b	2+7	f	1+5+14	m	1+8	b	4	d	6	d
14	CE40091	2+7	f	2	b	1+8+14	o	2+9+13	u	3+13	q	6	d
15	CE40148	2+8	g	2+9	h	2+9	g	3+12	k	1+8	h	3+11	i
16	CE40149	2+8	g	2+9	g	1+9	d	3+12	k	6	f	7	e
17	CE40153	2+6+10	q	2+7	f	1+9	d	2+13	f	1+9	i	4	b
18	CE40158	2+8	g	2+9	g	1+9	d	2+11	d	1+9	i	4	b
19	CE40162	3+8	j	2+9	g	1+9	d	3+11	j	1	a	9	g
20	CE40163	2+6	e	2+7	f	1+9	d	3+10	i	4	d	9	g
21	CE40164	1+5	a	2+7	f	2+10	e	2+13	f	3	c	9	g
22	CE40165	1+8	d	2	b	1+10+14	l	3+9	n	4	d	9	g
23	CE40168	1+7	c	1	a	1+7+16	n	3+7	m	3+11	o	3	a
24	CE40170	1+6	b	1+9	e	3+7+16	t	4+10	p	2	b	9	g
25	CE40176	1+5	a	1+9	e	3+13	h	5	r	2	b	5	c
26	CE40178	1+6	b	2+9	g	4+11	i	4	o	1+11	g	4	b
27	CE40181	1+8	d	2+9	g	4+14	k	6	s	2+9	m	5+9	l
28	CE40182	1+7	c	3	c	2+7+14	r	2+7	h	3+9	r	6+10	m
29	CE40183	1+6	b	4+8	h	1+7	b	2+10	c	4+13	t	6	d
30	CE40184	1+8	d	3	c	1+8	c	2+10	c	2+13	l	4	b
31	CE40185	3+8	j	4	d	1+8	c	2+10	c	1+8	h	1+8	h
32	CE40197	3+8	j	3	c	1+8	c	3+12	k	2+13	l	7	e
33	CE40218	1+7	c	4+9	i	4+13	j	8	t	2+9	m	5+11	k
34	CE40014	3+8	j	3	c	1+9	d	3+11	j	3	c	5	c
35	CE40015	3+9	k	4	d	1+9	d	3+11	j	3+12	p	5	c
36	CE40018	1+8	d	3	c	1+9	d	3+13	l	2	b	7	e
37	CE40023	3+7	i	4	d	2+10	e	3+12	k	2+12	k	6	d
38	CE40027	3+8	j	4	d	2+8	f	3+11	j	5	e	7	e
H		0.956				0.961				0.967			

CONCLUSIONS

This study presents the first comprehensive SDS-PAGE analysis of high- and low-molecular-weight glutenin subunit (HMW-GS and LMW-GS) diversity in naturally occurring *Aegilops triuncialis* populations from Bulgaria. The findings reveal remarkable diversity in both HMW-GS and LMW-GS profiles, including the detection of several rare subunit variants, underscoring the species' role as an untapped genetic reservoir for enhancing wheat end-use quality. SDS-PAGE emerges as a robust tool for early-generation screening in breeding programs, enabling the rapid identification of desirable subunit combinations. Future research should synergize protein electrophoresis with high-resolution genomic approaches to precisely map functional loci and enable targeted introgression of beneficial alleles into cultivated wheat, thereby accelerating the development of superior grain quality traits.

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EFFICACY AND SELECTIVITY OF HERBICIDES TESTED ALONE AND IN COMBINATION WITH BIOSTIMULANTS IN MAIZE

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Abstract

In 2024 and 2025, a field experiment was conducted with corn, hybrid Adnano (FAO 480). The experiment was conducted in the village of Brest, Gulyantsi municipality, Bulgaria. No-Till technology is applied on the experimental area. The experiment was based on a wheat predecessor. Direct sowing of corn was carried out. The experiment was set up according to a randomized block design. In the phenophase 4-6 leaf (BBCH 14-16) of the crop, treatment was carried out with the herbicides Capreno SC (345 g/l tembotrione + 68 g/l thiencarbazone-methyl + 134 g/l isoxadifen-ethyl) and Mistral Plus OD (220 g/l dicamba + 50 g/l nicosulfuron). Each of the products was applied alone and in a tank mixture with the biostimulants Lebosol Aminosol (115 g/l N - 9.4 %; 15 g/l K₂O - 1.1 %; 0.25 % S; 1.28 % Na; 66.3 % organic substance) and Lebosol Zinc 700 SC (700 g/l Zn - 40 %). The weeds in the experimental field were represented by: *Conyza canadensis* L., *Chenopodium album* L., *Polygonum convolvulus* L., *Abutilon theophrasti* Medic., *Convolvulus arvensis* L. and *Sorghum halepense* (L.) Pers.

Key words: maize; weeds; herbicides; biostimulants.

INTRODUCTION

One of the main problems accompanying the implementation of agricultural production is the fight against weeds. Weeds appear everywhere every year and cause enormous damage to cultivated plants, reducing yields and lowering the quality of the resulting products. (Tonev et al, 2019; Kostadinova et al., 2016; Kalinova and Yanev, 2015; Yanev, 2015; Yanev et al., 2014a; Yanev et al., 2014b). Studies by a number of authors show that depending on the type and degree of weed infestation, corn yield can decrease from 24% to 96.7% (Mukherjee, P. K. and Puspajit Debnath, 2013; Oerke and Dehne, 2004; Khan et al., 2003; Zhalnov and Raykov, 1996). A wide range of broadleaf weeds infest corn fields, including *Amaranthus* spp., *Chenopodium album* L., *Abutilon theophrasti* Medik., *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., etc. За да се избегне негативното влияние на плевелите е необходимо да бъдат контролирани. Pannacci found that application of foramsulfuron at a dose of 20.3 g ai/ha provided 95% efficacy against *Amaranthus retroflexus* L., *Setaria viridis* (L.) Beauv.), *Sinapis arvensis* L. и *Solanum nigrum* L. Against *Abutilon theophrasti* Medik., *Chenopodium album* L. and *Echinochloa crus-*

galli (L.) Beauv. the same percentage of herbicide efficacy was reported, but at doses of 20 to 50 g ai/ha (Pannacci, 2016). For the control of *Sorghum halepense* L., *Convolvulus arvensis* L., *Echinochloa crus-galli* L., *Chenopodium album* L., *Amaranthus retroflexus* L. and *Abutilon theophrasti* L. in corn, the combination of Stomp 33 EC + Mistral 4 SC is recommended at doses of 300 ml/da + 130 ml/da, respectively (Kalinova et al., 2000). Against *Xanthium strumarium*, *Amaranthus retroflexus*, *Datura stramonium* and *Chenopodium album* in maize Damalas et al., 2018 found efficacy of 92-100% after the use of tembotrione alone. Today, high-yield agriculture is heavily dependent on herbicides, as they constitute a vital and integral component of weed control practices. One herbicide-based approach to controlling herbicide-resistant weeds could involve the use of newly registered dual-active herbicides with a novel mechanism of action. (Goranovska and Yanev, 2016; Janak et al., 2016; Koprivlenski et al., 2015; Sevov et al., 2015; Noor Muhammd et al., 2012; Skrzypczak et al., 2011; Pannacci and Covarelli, 2009; Tonev, 1986).

The role of biostimulants applied in a tank mix with chemical weed control agents is also important to study (Alijani et al., 2023). A focus on published studies on the timing of

biostimulant application in field crops confirms that this period is very often close to the herbicide application period and a single spray is sufficient (Li et al., 2022). This may be explained by the need to support the crop at a specific growth stage, due to research on a more effective application period, or possibly due to the convenience of making this application close (sequential) or even together (tank mix) with the main cropping practice for chemical weed control (Neshev et al., 2021; Ronga et al., 2019). For example, Soltani et al. (2015) reported excellent efficacy of combined application of herbicides and biostimulants on *Abutilon theophrasti* Medik., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Chenopodium album* L. and *Setaria viridis* L. and other annual weeds in field trials with maize. The application of biostimulants and herbicides in agricultural practices is a new and very complex objective (Araújo et al., 2023). In general, the combination of biostimulants and herbicides from the research results so far has shown different effects on crops, some of which were positive and others negative or insignificant (Panfili et al., 2019). The huge number of different applications of these combined substances can be considered a difficulty or a challenging opportunity for researchers to explore which of these combinations can be used in agriculture (Sangiorgio et al., 2020; Zarzecka et al., 2020). New biostimulant products are introduced every day, while the availability of herbicides is constantly changing (Borowik et al., 2017). It is up to researchers to find the most appropriate combination and application method to promote crop growth, quality and quantitative characteristics and increase herbicide efficacy, while mitigating their adverse effects on non-target plants, either protecting the crop or helping it overcome damage. (Katsenios et al., 2023).

The aim of the study is to establish the efficacy and selectivity of foliar herbicides applied alone and in combination with biostimulants in corn grown using No-Till technology.

MATERIALS AND METHODS

In 2024 and 2025, a field experiment with corn, hybrid "Adnano" (FAO 480) was conducted in the village of Brest, Gulyantsi municipality,

Bulgaria. The experiment was set up according to a randomized block design in 4 replications with a total plot size of 100 m² for the four replications. Before treatment with herbicides and biostimulants, the species weeding and the density of weeds in the experimental field were recorded. Five dicotyledonous and one monocotyledonous weed were observed in the reporting field. The average density of weeds from the two experimental years per 1 m² is as follows: *Conyza canadensis* L. - 13.25 pieces; *Chenopodium album* L. - 9.5 pieces; *Polygonum convolvulus* L. - 4.25 pieces; *Abutilon theophrasti* Medic. - 4.5 pieces; *Convolvulus arvensis* L. - 4.25 pieces; *Sorghum halepense* (L.) Pers. from rhizomes - 18.5 pieces. The study included the following variants: 1. Untreated control; 2. Capreno SC (345 g/l tembotrione + 68 g/l thienencarbazone-methyl + 134 g/l isoxadifen-ethyl (safener)) - 0.29 l ha⁻¹ + Mero (adjuvant) - 2.00 l ha⁻¹; 3. Mistral Plus OD (220 g/l dicamba + 50 g/l nicosulfuron) - 1.20 l ha⁻¹; 4. Capreno SC - 0.29 l ha⁻¹ + Mero - 2.00 l ha⁻¹ + Lebosol Amimosol (115 g/l N - 9.4 %; 15 g/l K₂O - 1.1 %; 0.25 % S; 1.28 % Na; 66.3 % organic substance) - 2.00 l ha⁻¹; 5. Capreno SC - 0.29 l ha⁻¹ + Mero - 2.00 l ha⁻¹ + Lebosol Zinc 700 SC (700 g/l Zn - 40 %) - 1.00 l ha⁻¹; 6. Mistral Plus OD - 1.20 l ha⁻¹ + Lebosol Amimosol - 2.00 l ha⁻¹; 7. Mistral Plus OD - 1.20 l ha⁻¹ + Lebosol Zinc 700 SC - 1.00 l ha⁻¹. At the time of spraying, the corn had reached the phenophase 4-6 leaves (BBCH 14-16). The treatment with the tested products was carried out with a SOLO backpack battery sprayer, nozzle type: fan-shaped, upwind FLAT FAN, with a working solution consumption of 200 l ha⁻¹. The experimental area is part of a production field, on which the No-Till technology has been applied for eight years. The experiment in both experimental years was established after a wheat predecessor. After harvesting its grain, the field was covered (mulched) with wheat straw. In the post-harvest period, a treatment with a total herbicide (glyphosate) was carried out. Before sowing the corn, a basic nitrogen fertilization was carried out with 60 kg ha⁻¹ active substance nitrogen. Fertilization was carried out with a self-propelled sprayer with special equipment for applying liquid fertilizer UAN 32% at a fertilizer rate of 150 l ha⁻¹. Direct sowing was

carried out under appropriate agrotechnical conditions with a row spacing of 70 cm and a seeding rate of 55,000 germinating seeds per hectare, at a depth of 4.5-5 cm, with a spring row seeder for precise and accurate sowing of seeds "VADERSTAD TEMPO L". All units of the technology for growing corn in no-tillage conditions were observed. Weed control was assessed on days 14, 28 and 56 after application of herbicides and biostimulants. The 10-point EWRS (European Weed Research Society) scale was used. The 9-point EWRS visual scale was used to determine the selectivity of herbicides. The Duncan's multiple range test was used for statistical analyses of the obtained maize yields. Level of significance was at $p < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 shows the dynamics of the efficacy of herbicides applied alone and in a tank mixture with the tested biostimulants on *Conyza canadensis* during the reporting period.

Table 1. Biological efficacy of the tested herbicides (in % on the EWRS visual scale) against *C. canadensis*

Treatments	Doses (l ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	80	70	20
3. Mistral Plus OD	1.2	85	85	70
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	75	75	40
5. Capreno SC+Mero+ Lebosol Zinc	0.29+2 + 1	80	75	45
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	85	75	75
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	80	75	75

At variant 2 (Capreno SC + Mero), as well as in variants 9 and 10, where the same herbicide was applied in combination respectively with Lebosol Aminosol and Lebosol Zinc we report 75-80% efficacy on the 14th day after treatment. On the 28th day, the efficacy decreases by 5 to 10% or remains the same in the same variants of the experiment. On the 56th day after spraying, we observe a very low efficacy of 20% with the single application of Capreno SC + Mero and low after its application with the biostimulants (40-45%). On the first evaluation date, the herbicide Mistral Plus OD controls the weed

relatively well, regardless of whether it is applied alone or in a tank mixture with the tested biostimulants. On the second evaluation date, the same efficacy is maintained only after the use of the herbicide alone. Unsatisfactory efficacy was reported after the application of Mistral Plus OD (70%) and after treatment with the same herbicide, but in combination with the biostimulants Lebosol Aminosol and Lebosol Zinc (75%) on the last evaluation date against *Conyza canadensis*.

Table 2. Biological efficacy of the tested herbicides (in % on the EWRS visual scale) against *Ch. album*

Treatments	Doses (l ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	90	98	98
3. Mistral Plus OD	1.2	90	95	98
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	90	95	98
5. Capreno SC+Mero+ Lebosol Zinc	0.29+2 + 1	90	95	98
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	70	85	90
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	75	85	90

Table 2 shows the dynamics of herbicide efficacy against *Ch. album* reported on the 14th, 28th and 56th day after treatment. At the first evaluation date, we report a high herbicidal effect from the second to the fifth variant inclusive. It is noteworthy that the herbicide Mistral Plus OD, applied alone, has a higher herbicidal efficacy compared to its use in a tank mixture with the biostimulants Lebosol Aminosol (70%) and Lebosol Zinc (75%). This trend persists, but to a lesser extent, until the last evaluation date. On the 28th day after treatment, the highest efficacy (98%) is reported with the single application of Capreno SC + Mero. In the variants treated with the herbicide Mistral Plus OD in combination with the biostimulants, we report a satisfactory efficacy of 85%. Only from Capreno SC + Mero we obtain the same efficacy reported on the 56th day against weeds compared to its combined use with the tested biostimulants. However, at the same evaluation date, we report high efficacy from all treated variants of the experiment, which is from 90 to 98%.

Against *P. convolvulus*, fourteen days after treatment the highest efficacy of 90% was

reported for Capreno SC + Mero, Mistral Plus OD and Capreno SC + Mero + Lebosol Zinc (Table 3). The lowest herbicidal effect of 80% was reported for Mistral Plus OD + Lebosol Zinc. On the 28th day after spraying, we report an increase in efficacy in all treated variants by 5 to 10%. The highest efficacy of 95% was reported with Capreno SC + Mero, as well as from the combination of this herbicide with Lebosol Zinc, Mistral Plus OD applied alone and in a tank mixture with Lebosol Aminosol. A slightly lower herbicidal effect was observed with the application of Capreno SC + Mero + Lebosol Aminosol and Mistral Plus OD + Lebosol Zinc (90%). On the 56th day after treatment, practically all applied products have a very high effect of 95 to 98%.

Table 3. Biological efficacy of the tested herbicides (in % on the EWRS visual scale) against *P. convolvulus*

Treatments	Doses (1 ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	90	95	98
3. Mistral Plus OD	1.2	90	95	98
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	85	90	95
5. Capreno SC+Mero+ Lebosol Zinc	0.29+2 + 1	90	95	98
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	85	95	98
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	80	90	98

Table 4 shows the effect of the tested herbicides, as well as their application with biostimulants against *A. theophrasti*. A high percentage of efficacy is reported already on the 14th day after the application of the products. On the 28th day after the treatment, the percentages of efficacy increase slightly in the variants where the herbicide Capreno SC was applied. After the application of Mistral Plus OD, the herbicidal effect is maintained. In the last reading, the efficacy is again increased only in variants 2, 4 and 5. The herbicide Capreno SC, regardless of whether it is applied alone or in a tank mixture with the tested biostimulants, controls 100% of weed. The herbicide Mistral Plus OD, applied alone, again retains its efficacy of 95%. When used in combination with the biostimulants Lebosol Aminosol and Lebosol Zinc, the effect decreases slightly and reaches 90%.

Table 4. Biological efficacy of the tested herbicides (in % on the EWRS visual scale) against *A. theophrasti*

Treatments	Doses (1 ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	95	98	100
3. Mistral Plus OD	1.2	95	95	95
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	95	98	100
5. Capreno SC+Mero+ Lebosol Zinc	0.29+2 + 1	95	98	100
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	95	95	90
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	95	95	90

Against *C. arvensis*, the results of the herbicides and biostimulants used are reflected in Table 5. On the 14th day, the highest biological efficacy of 85% was reported from the application of Mistral Plus OD. A lower percentage of efficacy was noted from the herbicide applied in a tank mixture with the tested biostimulants. Even lower efficacy was reported in variant 2 treated with Capreno SC + Mero (70%). It is noteworthy that when applying the same herbicide tested in combination with Lebosol Aminosol and Lebosol Zinc, respectively, the effect is increased and reaches 80%. With the exception of variants 3, 6 and 7, in the rest of the experiment, we report a decrease in the herbicidal effect, reported on the next assessment date. On the 28th day after treatment with the products, we report a relatively good herbicidal effect only from the independent application of Mistral Plus OD (85%). This trend is also maintained on the last reporting date regarding weed control.

Table 5. Biological efficacy of the tested herbicides (in % on the EWRS visual scale) against *C. arvensis*

Treatments	Doses (1 ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	70	65	65
3. Mistral Plus OD	1.2	85	85	85
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	80	75	75
5. Capreno SC+Mero+ Lebosol Zinc	0.29+2 + 1	80	75	75
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	75	75	75
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	75	75	70

Table 6. Biological efficacy of the tested herbicides
(in % on the EWRS visual scale)
against *S. halepense* from rhizomes

Treatments	Doses (l ha ⁻¹)	Days after application		
		14	28	56
1. Untreated control	-	-	-	-
2. Capreno SC+Mero	0.29+2	50	25	20
3. Mistral Plus OD	1.2	65	80	85
4. Capreno SC+Mero+ Lebosol Aminosol	0.29+2 + 2	50	25	20
5. Capreno SC+Mero + Lebosol Zinc	0.29+2 + 1	50	25	20
6. Mistral Plus OD + Lebosol Aminosol	1.20 + 2	70	80	90
7. Mistral Plus OD + Lebosol Zinc	1.20 + 1	70	80	90

Table 6 shows that *S. halepense* from rhizomes is not controlled by Capreno SC + Mero, regardless of whether the herbicide is applied alone or in a tank mixture with the biostimulants Lebosol Aminosol and Lebosol Zinc. Only on the 14th day after treatment is there a suppression of weed development, which is very quickly overcome and at the next evaluation date we have an extremely low herbicidal effect from variants 2, 4 and 5. In contrast, after the application of Mistral Plus OD and the biostimulants used, we have an efficacy of 65-70% from the first reading. This herbicidal effect increases and on the 28th day after the application of the same products reaches 80%. We also report an increase in the percentage of efficacy on the 56th day after the application of the herbicide, and in the variants where it is applied together with Lebosol Aminosol and Lebosol Zinc we have the highest 90% control. During the experiment, for both experimental years, no visual symptoms of phytotoxicity were detected in any of the variants treated with herbicides and in their combined use with foliar fertilizers on the 7th, 14th and 28th days after application of the products, which means complete selectivity - no symptoms of damage to the cultivated plants.

Table 7 presents the results of the yields obtained on average for each variant of the experiment. The natural background of weeding with highly competitive weed species on average for the period leads to a low average yield from the untreated control (4.06 t ha⁻¹). By the degree of statistical evidence on average for the period, 3 separate groups of variants are

distinguished here (a, b, c). It is also noticeable here that the variants treated with the herbicide Mistral Plus OD, applied both alone with the Mero adjuvant and in a tank mixture with the tested biostimulants, are from group "c", the most distant from the untreated control group, that is, with the highest yields. Lower yields are reported from the variants in which the herbicide Capreno SC is tested, but nevertheless they also differ significantly from the yield from the untreated control.

Table 7. Corn grain seed yield, t ha⁻¹

Treatments	Doses (l ha ⁻¹)	Yields
1. Untreated control	-	4.06a
2. Capreno SC + Mero	0.29+2	7.68*b
3. Mistral Plus OD	1.2	8.52*c
4. Capreno SC+Mero + Lebosol Aminosol	0.29+2 + 2	7.78*b
5. Capreno SC+Mero + Lebosol Zinc	0.29+2 + 1	7.82*b
6. Mistral Plus OD + Lebosol Aminosol	1.20+ 2	8.66*c
7. Mistral Plus OD + Lebosol Zinc	1.20+ 1	8.65*c

Legend: All variants with * are significantly different from the untreated control. Values followed by different letters (a, b and c) are significantly different according to Duncan's test at $p < 0.05$.

CONCLUSIONS

The herbicides Capreno SC and Mistral Plus OD are very effective in *Ch. album*, *P. convolvulus* and *A. theophrasti*.

Against *A. theophrasti* the highest herbicidal effect is obtained from Capreno SC, applied both alone and in combination with Lebosol Aminosol and Lebosol Zinc.

Mistral Plus OD is superior to Capreno SC in its activity against the weeds *C. canadensis* and *S. halepense* from rhizomes. The herbicide Capreno SC cannot control *C. canadensis* and *S. halepense* from rhizomes.

Mistral Plus OD applied alone controls *C. arvensis* better than its combined use with Lebosol Aminosol and Lebosol Zinc, and with Capreno SC an inverse relationship is observed. Throughout the entire vegetation period of the corn, hybrid Adnano, no visible signs of phytotoxicity were recorded in any of the trial variants.

Compared to the untreated control, mathematically proven differences in corn grain yield were recorded in favor of all treated variants.

The highest average yield was obtained from the variant treated with Mistral Plus OD, applied in a tank mixture with Lebosol Aminosol (8.66 t ha⁻¹).

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EVALUATION OF IMAZAMOX-CONTAINING HERBICIDES FOR WEED CONTROL IN COMMON BEANS (*Phaseolus vulgaris* L.)

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Abstract

In 2024 and 2025, a field experiment was conducted with common beans, variety 'Elexir'. The study was conducted on the agricultural land in the village of Madrets, Stara Zagora region, Bulgaria. The variants of the experiment were: 1. Untreated control - without the use of herbicides and without hoeing; 2. Economic control - removal of weeds by hoeing, without the application of herbicides; 3. Pulsar 40 (0.70 l ha⁻¹); 4. Pulsar 40 (1.00 l ha⁻¹); 5. Pulsar 40 (1.30 l ha⁻¹); 6. Pulsar Plus (1.12 l ha⁻¹); 7. Pulsar Plus (1.60 l ha⁻¹); 8. Pulsar Plus (2.08 l ha⁻¹). The herbicides were applied during the second lateral branching phenophase of the crop (BBCH 22). The highest herbicidal control against *Setaria viridis* L., *Xanthium strumarium* L., *Chenopodium album* L. and *Abutilon theophrasti* Medik. was found after the application of Pulsar Plus (2.08 l/ha) and Pulsar 40 (1.30 l/ha). *Convolvulus arvensis* L. is the most difficult to control of the herbicides studied. The highest bean yield was obtained with Economic control, Pulsar Plus (2.08 l ha⁻¹) and Pulsar 40 (1.30 l ha⁻¹).

Key words: common bean, weeds, herbicides, efficacy, yield.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) in Bulgaria was grown on an area of 1944 ha in 2024 with an average yield of 614 kg/ha (Ministry of Agriculture and Food, 2025). It is a major source of cheap protein food (Duranti and Gius, 1997), and is therefore an important crop in many parts of the world, including the USA (Urwin et al., 1996), Canada (Blackshaw et al., 2000), the Mediterranean (Önder and Babaoglu, 2001) and Africa (Maingi et al., 2001). According to Pavlovic et al. (2024) in the USA *Ph. vulgaris* L. is an important crop for both fresh consumption and processing. In Europe, beans are traditionally grown in countries such as Bulgaria, Greece, Italy and Spain. (Pavlovic et al., 2024).

Successful weed control is an important component of sustainable bean production. Since beans are characterized by limited above-ground development, they are considered to be weak competitors to weeds. High levels of weed density can lead to stunted crop growth and reduced leaf area, as well as fewer pods per plant (Ghamari & Ahmadvand, 2012). Studies conducted in the USA and Canada have reported yield losses of around 80% at high weed densities. Stagnari & Pisante (2011) found that the presence of weeds throughout the growing

season of *Ph. vulgaris* L. resulted in a 59 to 65% yield reduction, and Goudarzi et al. (2023) reported that uncontrolled annual weeds caused a 70% yield reduction in common bean. Other authors have shown that weed associations, in addition to reducing crop yield by 53.3%, have a negative effect on phenological development and delay flowering by 6 days (Kebede et al., 2016). For this reason, successful weed control is important for profitable bean production, with herbicide application currently being the most common practice (Malik et al., 1993; Soltani et al., 2018; Taziar et al., 2017).

Weed control depends on correctly determining the critical period of weed competition. According to Bolaños Espinoza et al. (2009) this is the phenophase after the formation of the first triple leaf, before the flowering of the crop. Stagnari & Pisante (2011) found that weeds are most dangerous for beans from the 11th to the 28th day after crop emergence. Mehrpoyan (2010) reported that the critical period is from the 21st to the 43rd day after emergence of *Ph. vulgaris* L. Bean yield decreases with increasing duration of weed coexistence with the crop (Mehrpoyan, 2010).

The most common weeds in common bean fields are representatives of the dicot species such as *Amaranthus retroflexus* L., *Chenopodium album* L., *Portulaca oleracea* L.,

Datura stramonium L., *Convolvulus arvensis* L., the reed weed species *Cyperus esculentus* L., *Cyperus rotundus* L. and the monocot species *Cynodon dactylon* L., *Sorghum halepense* L., *Echinochloa crus-galli* L., *Eleusine indica* L., *Setaria viridis* L., *Digitaria sanguinalis* L. (Sadeghi et al., 2013; da Costa et al., 2013; Feizollah et al., 2016; Singh et al., 2018).

Chemical control is the most popular method for weed management in common bean, with trifluralin, bentazone, pendimethalin, fomesafen, fluazifop-p-butyl and quizalofop-p-ethyl being among the most commonly used herbicides (da Costa et al., 2013; Głowacka, A. 2013; Singh et al., 2018; Ayonoadu et al., 1974). According to Singh et al. (2018), pendimethalin and quizalofop-p-ethyl significantly reduced weed biomass and density, while pendimethalin provided high efficacy against *Chenopodium album* L., leading to a 72% increase in seed yield compared to the untreated control (Srivastava et al., 2013).

Vegetative treatments are most effective against weeds less than 5 to 7 cm tall, and crop development stage should be considered to avoid plant injury and yield reduction (Bailey et al. 2003). Imazamox, fomesafen, and bentazone – used in beans during the vegetative stage – should be applied only after the first trifoliate leaf is fully developed (Wyenandt et al. 2024). It has been found that the combination of bentazone with fomesafen damages plants up to 34%, while the individual application of these herbicides leads to moderate damage – 12-20%. Treatments in the cotyledon and simple leaf phase are the riskiest, and application in the first three-leaf stage is the safest for the plant. Yields are reduced the most with bentazone + fomesafen and imazamox – 76-77% compared to the control, which confirms that vegetative herbicides should be applied after the full development of the first three-leaf stage in order to minimize phytotoxicity and not compromise yield (Sosnoskie et al., 2025).

Imazamox is an imidazolinone herbicide that inhibits the enzyme acetolactate synthase (ALS). It is absorbed by the leaves and roots and readily translocates into the plant to the point of growth, inhibiting the activity of ALS. This results in the blocking of branched-chain amino acid production and a reduction in protein synthesis and cell division in plants, causing

weed death. Imazamox has been reported to provide effective control against broadleaf weeds (ADAMA, 2021). The first symptoms may appear 2 days after herbicide application, but plant death may take 2-3 weeks (ADAMA, 2021). Soltani et al. (2010) reported that recommended doses of imazethapyr in dry beans can be reduced by combining with trifluralin. The objective of this study was to evaluate imazamox-containing herbicides for weed control in common bean.

MATERIALS AND METHODS

In 2024 and 2025, a field experiment was conducted with beans, variety 'Elexir' grown in the land of the village of Madrets, Stara Zagora region, Republic of Bulgaria. The experiment was set up using a block method in 4 replications with an experimental area of 20 m². Against a natural background of weeding, the efficacy and selectivity of two imazamox containing herbicides - Pulsar 40 (containing 40 g/l imazamox) and Pulsar Plus (containing 25 g/l imazamox and adjuvants) was studied. The experiment variants are: 1. Untreated control – without herbicides and without hoeing; 2. Economic control – hoeing without herbicides; 3. Pulsar 40 – 0.70 l/ha; 4. Pulsar 40 – 1.00 l/ha; 5. Pulsar 40 – 1.30 l/ha; 6. Pulsar Plus – 1.12 l/ha; 7. Pulsar Plus – 1.60 l/ha; 8. Pulsar Plus – 2.08 l/ha. The herbicide was applied in the phenophase second lateral branch BBCH – 22 with a backpack sprayer and a working solution of 300 l/ha, the dates of herbicide application are indicated in Table 1.

Table 1. Term of taking into account the biological efficacy and selectivity of imazamox-containing herbicides

Date of treatment	Bio efficiency reporting		Selectivity reporting			
	20 th	40 th	3 rd	7 th	14 th	28 th
28.05.2024	17.06	07.07	31.05	04.06	11.06	25.06
04.06.2025	24.06	14.07	07.06	11.06	18.06	02.07

In both experimental years, the predecessor of the beans was winter soft wheat. For field preparation in both experimental years, the following treatments were carried out: vertical tillage to a depth of 30 cm after the field was cleared of the predecessor (July) and cultivation to a depth of 15 cm in March and 10 cm in April.

The beans were sown on April 18, 2024 and April 22, 2025 with a sowing rate of 13.5 germinating seeds per linear meter and a row spacing of 75 cm. Immediately afterwards, rolling was carried out with a grapple roller.

To overcome the adverse weather conditions in 2025, the beans were irrigated by sprinkling. In 2024, the beans were not irrigated despite the need, which had an adverse effect on the yield. The biological efficacy of the tested herbicides was recorded on the 20th and 40th day after treatment using the quantitative method pcs/m² (Table 1.). The natural weed vegetation was represented by: common cocklebur (*Xanthium strumarium* L.), lambsquarters (*Chenopodium album* L.), common purslane (*Portulaca oleracea* L.), venice mallow (*Hibiscus trionum* L.), velvetleaf (*Abutilon theophrasti* Medik), green foxtail (*Setaria viridis* L.) and field bindweed (*Convolvulus arvensis* L.). During the two experimental years, single plants of redroot pigweed (*Amaranthus retroflexus* L.) and prickly lettuce (*Lactuca serriola* L.) were also found.

The selectivity of the studied herbicides was determined on the 3rd, 7th, 14th and 28th day after their application (Table 1.) according to the 9-point EWRS scale, where at a score of 1 no damage is observed, and at a score of 9 – the crop is completely destroyed.

In addition to the biological efficacy and selectivity of the studied herbicides, grain yield (t/ha) was also determined. Duncan's method was used for statistical data processing with the

SPSS 19 program (Duncan, 1955). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

On the 20th day after treatment, the highest average efficacy against common cocklebur (*Xanthium strumarium* L.) was reported in Pulsar Plus – 2.08 l/ha – 84% and Pulsar 40 – 1.30 l/ha – 81%. These two options also show high efficiency when considering the results on the 40th day, when they reach almost excellent values – 91% for Pulsar Plus – 2.08 l/ha and 90% for Pulsar 40 – 1.30 l/ha. On this reporting date, good efficacy was also reported in variants Pulsar Plus – 1.60 l/ha – 80% and Pulsar 40 – 1.00 l/ha – 75%. Satisfactory control (55-58%) against the available weeds was reported in the variants with the lowest imazamox content (Table 2). The good efficacy against *Xa. strumarium* L. after the use of imazamox has been confirmed by other studies. In a vessel trial to determine the minimum doses of imazamox for common cocklebur control in green beans, Gurbuz & Yentur, (2022) found that 46.18 g ai/da of imazamox provided 90% weed control when applied at the 2-4 true leaf stage. The same efficacy was achieved when imazamox was applied at a dose of 36.11 g ai/da at the 6-8 true leaf stage. The authors suggest that the efficacy of the herbicide depends not only on the dose, but also on the weed stage. With increasing leaf area, respectively, and with the higher degree of herbicide retention in the 6-8 leaf stage of *Xa. strumarium* L., very good control was reported.

Table 2. Biological efficacy against common cocklebur (*Xanthium strumarium* L.) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	9.50	-	11.00	-	10.25	-	13.00	-	11.75	-	12.38	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	5.25	45	5.50	50	5.38	47	6.25	52	5.00	57	5.63	55
4. Pulsar 40 - 1.00 l/ha	3.50	63	5.00	55	4.25	59	3.50	73	2.75	77	3.13	75
5. Pulsar 40 - 1.30 l/ha	1.25	87	2.75	75	2.00	81	1.00	92	1.50	87	1.25	90
6. Pulsar Plus - 1.12 l/ha	5.00	47	4.50	59	4.75	53	6.25	52	4.25	64	5.25	58
7. Pulsar Plus - 1.60 l/ha	2.75	71	3.50	68	3.13	70	3.00	77	2.00	83	2.50	80
8. Pulsar Plus - 2.08 l/ha	1.25	87	2.00	82	1.63	84	1.00	92	1.25	89	1.13	91

Under the conditions of the experiment on the 20th day after treatment, the best control against lambsquarters (*Chenopodium album* L.) was recorded with Pulsar Plus – 2.08 l/ha – 72%. Satisfactory efficacy was registered in variants

4, 5 and 7 – ranging from 53 to 69%. On the 40th day after treatment, the control against *Chenopodium album* L. increased and reached 80% in variant 8. It is worth noting that the efficacy after the application of Pulsar 40 – 1.30

l/ha – 78% is close to this. In variants 4 and 7, the efficacy is 58% and 65%, respectively. Variants 3 and 6, which have the lowest dose of the herbicide, show limited control against lambsquarters (Table 3). Studies by other authors related to the efficacy of imazamox against *Chenopodium album* L. show the following. Kousta et al. (2024) found that treatment of

lambsquarters with Pulsar 4 SL – 375 g ai ha⁻¹ + Dash 225 g ai ha⁻¹ was effective between 70% and 73.3%. In a study by Neshev et al. (2020) it was found that Pulsar 40 at a dose of 1.25 l/ha on the 56th day after treatment destroyed 80% of lambsquarters, and in the variant with Pulsar Plus – 1.20 l/ha on the 28th day 100% weed control was reported.

Table 3. Biological efficacy against lambsquarters (*Chenopodium album* L.) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	6.00	-	10.50	-	8.25	-	10.00	-	12.00	-	11.00	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	3.50	42	7.25	31	5.38	36	5.50	45	7.00	42	6.25	43
4. Pulsar 40 - 1.00 l/ha	2.50	58	5.25	50	3.88	54	4.00	60	5.25	56	4.63	58
5. Pulsar 40 - 1.30 l/ha	1.75	71	3.50	67	2.63	69	2.00	80	3.00	75	2.50	78
6. Pulsar Plus - 1.12 l/ha	3.75	38	7.00	33	5.38	35	5.50	45	6.00	50	5.75	48
7. Pulsar Plus - 1.60 l/ha	2.75	54	5.00	52	3.88	53	3.50	65	4.25	65	3.88	65
8. Pulsar Plus - 2.08 l/ha	1.50	75	3.25	69	2.38	72	1.50	85	3.00	75	2.25	80

Under the experimental conditions, the herbicides studied in both years showed unsatisfactory efficacy against common purslane (*Portulaca oleracea* L.). On the 40th day after treatment, an average efficacy of only 32% was recorded for the period (Table 4). However, some authors reported a higher

efficacy of Pulsar 40 against common purslane (Kristó et al., 2019). These differences in the data on the efficacy of imazamox against common purslane may be due to differences in agrometeorological conditions, in the different phenophases of the weed at the time of treatment, and others.

Table 4. Biological efficacy against common purslane (*Portulaca oleracea* L.) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	5.00	-	12.50	-	8.75	-	8.00	-	13.00	-	10.50	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	4.50	10	12.25	2	8.38	6	7.00	13	12.25	6	9.63	9
4. Pulsar 40 - 1.00 l/ha	4.00	20	10.50	16	7.25	18	6.00	25	10.25	21	8.13	23
5. Pulsar 40 - 1.30 l/ha	3.25	35	10.00	20	6.63	28	5.00	38	9.50	27	7.25	32
6. Pulsar Plus - 1.12 l/ha	4.25	15	12.00	4	8.13	10	6.75	16	11.75	10	9.25	13
7. Pulsar Plus - 1.60 l/ha	4.00	20	10.25	18	7.13	19	6.00	25	10.00	23	8.00	24
8. Pulsar Plus - 2.08 l/ha	3.50	30	10.00	20	6.75	25	5.00	38	9.75	25	7.38	31

Under the experimental conditions, the studied herbicides exhibited very good control against green foxtail (*Setaria viridis* L.). High average efficacy (>70%) was reported on the 40th day after the application of the herbicides. Excellent efficacy was recorded with Pulsar Plus – 2.08 l/ha – 97% and Pulsar 40 – 1.30 l/ha – 96%. Very good efficacy was recorded with the other two variants – Pulsar 40 - 1.00 l/ha and Pulsar Plus – 1.60 l/ha, respectively 88% and 89%. Despite having the lowest active ingredient content, variants 3 and 6 also provided good control

against green foxtail (Table 5). High efficacy from the application of imazamox against *Setaria viridis* L. was also established by Neshev et al. (2020). The authors found that on the 28th day after treatment with Pulsar 40 – 1.25 l/ha and Pulsar Plus – 1.20 l/ha, green foxtail control was 90%.

In addition to common purslane, limited control of venice mallow (*Hibiscus trionum* L.) was also observed after application of the tested herbicide. On the 40th day after treatment, the average efficacy reported in variant 5 (Pulsar 40

– 1.30 l/ha) was only 39%, and in variant 8 (Pulsar Plus – 2.08 l/ha) – 38% (Table 6). In the other variants under the conditions of the experiment, the efficacy of imazamox against venice mallow was even lower.

Regarding the control of velvetleaf (*Abutilon theophrasti* Medik) on the 20th day after treatment, the highest efficacy was recorded for variant 5 (Pulsar 40 – 1.30 l/ha) – 68% and variant 8 (Pulsar Plus – 2.08 l/ha) – 65%. In the remaining variants, the efficacy was lower (<54%). On average for the period of the 40th day after treatment, the efficacy against *A. theophrasti* Medik increased, reaching 78% for variant 8 (Pulsar Plus – 2.08 l/ha) and 74% for variant 5 (Pulsar 40 – 1.30 l/ha). Satisfactory

control was recorded for variants 4 (Pulsar 40 – 1.00 l/ha) and variant 7 (Pulsar Plus – 1.60 l/ha), respectively 57% and 66% (Table 7).

In both years of study, the control of field bindweed (*Convolvulus arvensis* L.) proved to be the most difficult. On the 40th day after the application of Pulsar 40 at a dose of 1.30 l/ha and Pulsar Plus at a dose of 2.08 l/ha, 3% and 6% of field bindweed were destroyed, respectively. Zero efficacy was recorded for the other variants (Table 8). Limited herbicidal control against *Convolvulus arvensis* L., after the use of imazamox, but in chickpeas was also reported by Kristó et al. (2019). The authors found only 10% control of field bindweed after the use of Pulsar 40 SL – 1.00 l/ha.

Table 5. Biological efficacy against green foxtail (*Setaria viridis* L.) in pcs/m² and % efficiency

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	7.00	-	12.50	-	9.75	-	12.00	-	16.00	-	14.00	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	3.00	57	4.00	68	3.50	63	3.75	69	3.25	80	3.50	74
4. Pulsar 40 - 1.00 l/ha	2.00	71	3.75	70	2.88	71	2.25	81	1.00	94	1.63	88
5. Pulsar 40 - 1.30 l/ha	1.00	86	2.50	80	1.75	83	0.50	96	0.75	95	0.63	96
6. Pulsar Plus - 1.12 l/ha	3.00	57	3.75	70	3.38	64	3.75	69	3.00	81	3.38	75
7. Pulsar Plus - 1.60 l/ha	1.75	75	2.25	82	2.00	79	2.00	83	0.75	95	1.38	89
8. Pulsar Plus - 2.08 l/ha	0.75	89	1.75	86	1.25	88	0.25	98	0.50	97	0.38	97

Table 6. Biological efficacy against venice mallow (*Hibiscus trionum* L.) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	8.00	-	4.75	-	6.38	-	11.00	-	5.50	-	8.25	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	7.50	6	4.25	11	5.88	8	10.00	9	4.00	27	7.00	18
4. Pulsar 40 - 1.00 l/ha	6.75	16	3.50	26	5.13	21	8.75	20	3.25	41	6.00	31
5. Pulsar 40 - 1.30 l/ha	5.50	31	3.25	32	4.38	31	7.00	36	3.25	41	5.13	39
6. Pulsar Plus - 1.12 l/ha	7.25	9	4.50	5	5.88	7	10.00	9	4.25	23	7.13	16
7. Pulsar Plus - 1.60 l/ha	6.50	19	3.75	21	5.13	20	8.25	25	3.50	36	5.88	31
8. Pulsar Plus - 2.08 l/ha	5.75	28	3.00	37	4.38	32	7.25	34	3.25	41	5.25	38

Table 7. Biological efficacy against velvetleaf (*Abutilon theophrasti* Medik) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Средно	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	7.00	-	5.25	-	6.13	-	9.00	-	6.00	-	7.50	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	4.00	43	3.50	33	3.75	38	5.25	42	3.00	50	4.13	46
4. Pulsar 40 - 1.00 l/ha	3.50	50	3.00	43	3.25	46	4.00	56	2.50	58	3.25	57
5. Pulsar 40 - 1.30 l/ha	2.50	64	1.50	71	2.00	68	2.75	69	1.25	79	2.00	74
6. Pulsar Plus - 1.12 l/ha	4.00	43	3.25	38	3.63	40	5.00	44	2.75	54	3.88	49
7. Pulsar Plus - 1.60 l/ha	3.25	54	2.50	52	2.88	53	3.50	61	1.75	71	2.63	66
8. Pulsar Plus - 2.08 l/ha	2.25	68	2.00	62	2.13	65	2.50	72	1.00	83	1.75	78

Table 8. Biological efficacy against field bindweed (*Convolvulus arvensis* L.) in pcs/m² and % efficacy

Variants	20 th day after treatment						40 th day after treatment					
	2024		2025		Average		2024		2025		Average	
	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%	pcs/m ²	%
1. Untreated control	5.00	-	8.25	-	6.63	-	7.00	-	9.00	-	8.00	-
2. Economic control	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
3. Pulsar 40 - 0.70 l/ha	5.00	0	8.25	0	6.63	0	7.00	0	9.00	0	8.00	0
4. Pulsar 40 - 1.00 l/ha	5.00	0	8.25	0	6.63	0	7.00	0	9.00	0	8.00	0
5. Pulsar 40 - 1.30 l/ha	4.50	10	8.25	0	6.38	5	6.75	4	8.75	3	7.75	3
6. Pulsar Plus - 1.12 l/ha	5.00	0	8.25	0	6.63	0	7.00	0	9.00	0	8.00	0
7. Pulsar Plus - 1.60 l/ha	4.75	5	8.25	0	6.50	3	7.00	0	9.00	0	8.00	0
8. Pulsar Plus - 2.08 l/ha	4.50	10	8.00	3	6.25	7	6.50	7	8.50	6	7.50	6

Regarding the selectivity of imazamox towards beans under the conditions of the experiment, the following results were obtained. In 2024, on the 3rd day after the application of Pulsar 40 – 1.00 l/ha, Pulsar 40 – 1.30 l/ha, Pulsar Plus – 1.60 l/ha and Pulsar Plus – 2.08 l/ha, phytotoxic manifestations with a score of 2 were detected. The observed damage to the treated plants is expressed in yellowing of the leaf petiole. On the 7th day, phytotoxicity with a score of 2 was also recorded in the variants Pulsar 40 – 0.70 l/ha and Pulsar Plus – 1.12 l/ha. The symptoms are identical to those in the other variants. On the third reporting date, all herbicide-treated variants retained phytotoxicity and were given a score of 2. On the 28th day, the bean variety 'Elixir' overcame herbicide stress and regained normal leaf colour, and was given a score of 1 (Table 9).

Table 9. Selectivity of the studied imazamox-containing herbicides against beans on the EWRS scale

Variants	3 rd day		7 th day		14 th day		28 th day	
	2024	2025	2024	2025	2024	2025	2024	2025
1. Untreated control	1	1	1	1	1	1	1	1
2. Economic control	1	1	1	1	1	1	1	1
3. Pulsar 40 - 0.70 l/ha	1	1	2	2	2	1	1	1
4. Pulsar 40 - 1.00 l/ha	2	1	2	2	2	2	1	1
5. Pulsar 40 - 1.30 l/ha	2	2	2	2	2	2	1	1
6. Pulsar Plus - 1.12 l/ha	1	1	2	2	2	1	1	1
7. Pulsar Plus - 1.60 l/ha	2	1	2	2	2	2	1	1
8. Pulsar Plus - 2.08 l/ha	2	2	2	2	2	2	1	1

Unlike the first year in 2025, on the 3rd day after treatment, only Pulsar 40 at a dose of 1.30 l/ha and Pulsar Plus at a dose of 2.08 l/ha showed phytotoxicity with a score of 2, with the damage being identical to that in 2024. On the 7th day, the same phytotoxic manifestations were observed in all herbicide variants. On the 14th day after treatment, phytotoxicity was overcome

only with the low doses of Pulsar 40 and Pulsar Plus. Twenty-eight days after treatment, all variants had overcome the herbicide stress and regained normal leaf colour.

The phytotoxicity observed in 2024, already on the 3rd day after application of Pulsar 40 – 1.00 and Pulsar Plus – 1.60 l/ha, was most likely due to the extremely high temperatures during the growing season of the crop, combined with the lack of precipitation.

The results of the yield of common beans are presented in Table 10. It is correct to note that as a result of the additional irrigation in the critical periods of crop development in 2025, in all variants the yield obtained was higher compared to 2024. The highest yield of beans, variety 'Elixir' was obtained in economic control (variant 2), as in 2024 it was 0.723 t/ha, and in 2025 it was 1.030 t/ha. This is most likely due to the created optimal conditions for growing the crop, without competition from weeds. High crop yield was also recorded after the application of Pulsar Plus (2.08 l/ha) – an average of 0.844 t/ha and Pulsar 40 (1.30 l/ha) – an average of 0.843 t/ha. The results obtained correspond to the demonstrated biological efficacy of the herbicides against the weeds present in the experiment (Tables 2-8). Close to the yield of variants 8 and 5 was obtained in variants 7 and 4. It is worth noting that according to Duncan's multiple range test ($p < 0.05$) between the variants Pulsar Plus – 2.08 l/ha, Pulsar 40 – 1.30 l/ha, Pulsar Plus – 1.60 l/ha and Pulsar 40 – 1.00 l/ha there is no statistically proven difference (Table 10). Proven lower than the above four variants is the yield of Pulsar Plus – 1.12 l/ha and Pulsar 40 – 0.70 l/ha. However, the yield of variants 3 and 6 is significantly higher than the untreated control. Under the conditions of the experiment, the lowest yield was recorded in the untreated control, where

competition from weeds is the greatest. In 2024, a yield of 0.158 t/ha was obtained, and in 2025 – 0.192 t/ha.

Table 10. Seed yield of beans (t/ha) in 2024 and 2025

Variants	2024	2025	Average
1. Untreated control	0.158 d	0.192 d	0.175
2. Economic control	0.723 a	1.030 a	0.877
3. Pulsar 40 - 0.70 l/ha	0.683 c	0.855 c	0.769
4. Pulsar 40 - 1.00 l/ha	0.702 b	0.969 b	0.836
5. Pulsar 40 - 1.30 l/ha	0.709 b	0.976 b	0.843
6. Pulsar Plus - 1.12 l/ha	0.680 c	0.849 c	0.765
7. Pulsar Plus - 1.60 l/ha	0.698 b	0.976 b	0.837
8. Pulsar Plus - 2.08 l/ha	0.707 b	0.981 b	0.844

Variants with different letters are with proved difference according to Duncan’s multiple range test (p < 0.05).

CONCLUSIONS

During the study period, Pulsar Plus at a dose of 2.08 l/ha and Pulsar 40 at a dose of 1.30 l/ha exhibited the highest efficacy against *Setaria viridis* L., *Xanthium stumarium* L., *Chenopodium album* L. and *Abutilon theophrasti* Medik on the 40th day after their application. Their biological efficacy varied from 74% to 97% for different weed species. The control against *Portulaca oleracea* L. and *Hibiscus trionum* L. was significantly more limited, with the highest efficacy being shown by Pulsar Plus at a dose of 2.08 l/ha and Pulsar 40 at a dose of 1.30 l/ha. *Convolvulus arvensis* L. is the most difficult to control of the herbicides studied. Under the conditions of the experiment, Pulsar Plus – 2.08 l/ha destroyed only 6% of the weeds, and with Pulsar 40 – 1.30 l/ha only 3%.

Under the conditions of the experiment, on the 7th day after the application of the herbicides, phytotoxicity with a score of 2 was recorded in beans, variety ‘Eleksir’. By the 28th day, the crop was fully recovered – score 1.

The highest bean yield was obtained with economic control – 0.877 t/ha. Similar yields were also reported after the application of Pulsar Plus at a dose of 2.08 l/ha – 0.844 t/ha and Pulsar 40 at a dose of 1.30 l/ha – 0.843 t/ha.

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STUDY ON THE IMPACT OF INNOVATIVE BIOFERTILIZERS ON PROSO MILLET (*Panicum miliaceum*) PRODUCTIVITY

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Abstract

During the period 2023-2025, at the Agricultural Institute - Shumen, millet genotype G2 was treated with innovative bioinoculants. Based on the obtained results, it was established that under carbonate chernozem soil conditions, the application of inoculants at different doses and using different methods, led to an increase in the grain yield of millet in all cases of application. The increase in yield also led to an increase in the coefficient of variation. Under the more favorable agrometeorological conditions of 2023, seven of the tested variants showed significantly higher yields. In conditions of water deficit in 2024 and 2025, significantly higher yields were observed only in the variants treated with Fosfatovit 0.5 l/ha, Azotovit 1 l/ha and Fosfatovit 1 l/ha. The aim of the study is to investigate under field conditions the influence of innovative bioinoculants on grain yield in millet.

Key words: fertilization, inoculants, millet, yield.

INTRODUCTION

Millet (*Panicum miliaceum* L.) is one of the oldest cultivated cereals in the world and played a significant role in early agriculture in Eurasia. Although it is now cultivated on a limited scale, mainly in Central and East Asia, archaeobotanical evidence suggests that domestication of this plant occurred more than 10,000 years ago, in northern and northeastern China (Hunt et al., 2008; Crawford, 2009; Liu et al., 2009; Lu et al., 2009; Hunt, 2014). It is an important tetraploid cereal ($2n=4x=36$; Hamoud et al., 1994). It has the shortest growing season of all cereals (60-90 days) and extremely low water requirements (Baltensperger, 1996; Baltensperger, 2002).

The high nutrient concentration of the grain is a prerequisite for its use for human consumption, as well as as poultry feed and ethanol production (Graybosch and Baltensperger, 2009; Habiyaemye et al., 2017).

Compared to other summer crops, millet has remarkable tolerance to drought and high temperatures and could be used to improve crop rotation and biodiversity (Antonacci et al., 2021; Palchetti et al., 2023). This drought tolerance is based on complex interactions

between the rhizosphere microbiome and plant metabolism (Li et al., 2023).

Studies by Gupta et al. (2021) found that the bacterial community in the rhizosphere of two millet cultivars was resistant to drought stress, showing only minor changes in its diversity and composition. Instead, the growth phase of the plant appeared to be the main factor determining the structure of the microbial community. Specifically, bacterial diversity increased significantly from the germination to the tillering stage and decreased at full maturity. These dynamics indicate that the composition of the rhizosphere microbiome is more responsive to the physiological needs and exudate profiles of the plant than to water stress conditions. Despite these studies, the available information on how bacterial communities in the rhizosphere of different millet cultivars respond to drought is limited (Na et al., 2019).

Major bacterial taxa such as *Actinobacteria*, *Proteobacteria* and *Acidobacteria* remained dominant under different irrigation regimes, suggesting functional stability and potential symbiotic support under limited water availability. The results show that pansy (*Panicum miliaceum* L.), even under drought, maintains a relatively stable and functionally resilient microbiome in the rhizosphere, which

likely contributes to its exceptional drought tolerance compared to other cereals (Na et al., 2019).

The rhizosphere plays a key role in water and nutrient uptake and represents a dynamic interface between plant roots and the surrounding soil. Rhizosphere microorganisms, including fungi and bacteria, are an integral part of this zone and have a strong influence on plant growth under stressful environmental conditions. During the flowering phase of millet (*Panicum miliaceum* L.), drought significantly altered the presence of major fungal phyla such as *Ascomycota* and *Basidiomycota*, but the overall fungal diversity remained stable. This indicates that the rhizosphere fungal community functionally adapts to water deficit conditions, possibly supporting plant water balance through improved nutrient cycling, osmolyte synthesis, and signalling between roots and microorganisms. Such microbial adaptations may partially explain the ability of *P. miliaceum* to maintain productivity under prolonged drought (Liu et al., 2023).

Compared to monocultures, the alfalfa-millet intercropping system at different row ratios significantly increased the yield of feathery millet, with the reported yield advantage reaching 28.3%. Intercropping not only improves nitrogen fixation and soil fertility, but also stimulates the uptake of nitrogen, phosphorus and potassium by plants, mainly through increased activity of soil microorganisms (Liu et al., 2022). This can be partly explained by the additional symbiotic microbiome arising between the different symbionts of the two species.

Intercropping significantly alters the structure of rhizosphere microbial communities, often increasing the relative abundance of beneficial taxa such as Actinobacteria, Proteobacteria, and Firmicutes. These shifts are associated with enhanced soil enzyme activities, improved nutrient cycling, and increased crop yield and nutrient use efficiency (Zhang et al., 2019). Therefore, the interaction between legume root exudates and the microbiome in the rhizosphere of feathery millet plays a crucial role in increasing the yield and sustainability of terrestrial agroecosystems (Li et al., 2025).

The combination of organic nutrients and bioinoculants resulted in significantly higher

grain and straw yield of feathery millet under field conditions in a semi-arid climate (Sharma et al., 2022).

The concentration of trace elements such as zinc, iron and manganese in millet grain also increased, indicating that the crop responded very well to microbial inoculation (Šenk, 2023).

Devasena et al. (2024) found that integrated nutrient management practice by applying vermicompost enriched with *Azospirillum* and *Phosphatobacteria* at a dose of 6 t/ha along with 100% recommended dose of nitrogen, phosphorus and potassium significantly increased plant physiological parameters (leaf area index, crop growth rate, chlorophyll index) and yield (grain yield and straw yield) of millet.

Other studies by Sridevi et al. (2020) on the inoculation of *Pseudomonas fluorescens* of millet seeds resulted in significant increase of germination percentage, vigor index and early growth. Bio-primed seeds showed higher chlorophyll content and enzyme activities, especially amylase and peroxidase, which helped in stronger root development and higher yields under field conditions.

Shishehbor et al. (2013) studied the effect of applying biofertilizers with vermicompost on yield components in millet. They reported a higher 100 seed weight, in plants inoculated with *Azotobacter chroococcum*, which was significantly higher than uninoculated plants. In addition, the effect of mycorrhiza and *Azotobacter* in the presence of vermicompost was greater. The biological yield was higher when mycorrhiza was applied together with *Azotobacter* and vermicompost. The grain yield of plants inoculated with *A. chroococcum* was significantly higher than that of uninoculated ones. Also, the effect of *Azotobacter* in the presence of vermicompost was enhanced.

Inoculation with *Rhizopagus fasciculatus* significantly increased the degree of root colonization and helped the absorption of essential nutrients (nitrogen, phosphorus and potassium) in millet (*Panicum miliaceum* L.). Even under fungicide stress, plants in symbiosis with mycorrhizal fungi showed better root morphology, higher chlorophyll content and increased dry matter accumulation compared to uninoculated controls. The study

demonstrated that arbuscular mycorrhizal fungi not only protect roots from chemical stress but also maintain soil enzymatic activity (dehydrogenase, phosphatase) and nutrient cycling, leading to sustainable growth and yield stability. This highlights the key role of microbial symbioses in maintaining millet productivity and soil health under intensive agronomic practices (Channabasava et al., 2015).

The aim of the current study is to investigate the impact of innovative microbial fertilizers on millet productivity in real field conditions.

MATERIALS AND METHODS

The experiments were conducted during the period 2023-2025 in the experimental fields of the Agricultural Institute - Shumen, Northeastern Bulgaria (GPS coordinates: 43°18'51.3"N 27°01'41.8"E). The soil is sandy, clay, leached chernozem. The chemical properties are provided in Table 1.

Table 1. Chemical properties of the soil

pH (H ₂ O)	6.66
Nutrient reserves	Amount (ppm)
NO ₃	15.62
+NH ₄	1.96
N	17.58
P ₂ O ₅	40.94
K ₂ O	227.14
CaO	6,002.82
MgO	692.65
Na ₂ O	33.98
S	7.71
B	0.57
Cu	1.44
Fe	27.31
Mn	6.74
Zn	0.35

The preceding crop was wheat.

The experiments were set up using a block method in four replications with 10 m² of harvest area under non-irrigated conditions. The accepted technology for cultivation with narrow-row sowing was used, carried out at the end of April or the beginning of May, with permanent warming of the soil to 10 cm above 14°C. During the vegetation period, observations were made on morphological and economic indicators. Harvesting was carried out manually when 75% of the plants were fully mature.

For the purposes of the study, the millet genotype G2, developed in the breeding program of the Agricultural Institute - Shumen, was used, which in field tests has been distinguished by very good productivity so far. The applied doses, methods, and timing of inoculant application were in accordance with the instructions of the respective manufacturer. Due to the indication of different doses and methods of application, two of the products (Phosphatovit and Azotovit) were used both for surface treatment of the soil before sowing in different doses, and for seed coating, as well as a combination of them at the maximum recommended dose.

Table 2 presents the microbial fertilizers used, the timing and method of their application.

Meteorological data, listed in Table 3 were collected starting from the post-harvest period of the previous year (2022) in order to assess the accumulation of soil moisture throughout the year. The agrometeorological conditions table shows a moisture deficit in all three years of testing, compared to the average norm for a 50-year period. 2022 had a deficit of 111 mm of precipitation, compared to the norm, whose lack was reflected in the following harvest year (2023). In 2023, the moisture deficit reached 70 mm., which was a prerequisite for a relatively good yield of millet grain. 2024 had a deficit of 173 mm, which adversely affected the yield, but also the amount of accumulated moisture. In 2025, the moisture deficit was very clearly expressed, although the precipitation in the months of April and May compensated to some extent the moisture deficit accumulated during the winter months.

During the first seven months, corresponding to the millet growing period, the moisture deficit reached 121 mm.

Despite the unfavorable values observed during all three years of research, we can note that millet managed to show its potential, under both high temperatures and water deficit conditions.

The data were processed using the method of variation statistics with the software product "Minitab". The significance of differences was determined using Student's *t*-test (Gosset, 1908) at a probability level of P=0.95, 0.99, 0.999.

Table 2. Microbial fertilizers used

Product name	Method of application	Product description
Biosplito	Surface (suspension) soil with incorporation	<i>Trametes versicolor</i> , <i>Pleurotus ostreatus</i> , <i>Cellulomonas uda</i> , <i>Cellulomonas gelida</i> , <i>Aspergillus awamori</i> , and <i>Trichoderma reesei</i> (in equal concentrations, total 1×10^9 ksv/g).
Biostart	Seed coating	<i>Bacillus subtilis</i> , <i>Paenibacillus azotofixans</i> , <i>Bacillus megaterium</i> , <i>Bacillus mucilaginosus</i> , <i>Bacillus mycoides</i> , <i>Trichoderma viride</i> (total more than or 1×10^9 cfu/g); mycorrhizal fungi 1×10^7 cfu/g.
Naturalis	Surface (suspension) soil with incorporation	<i>Beauveria bassiana</i> ; strain ATCC 74040 0.185 g/kg
Microbtima protect	Seed coating	<i>Trichoderma koningii</i> , <i>Trichoderma viride</i> , <i>Trichoderma harzianum</i> , <i>Trichoderma asperillum</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas chlororaphis</i> ; Total CFU- up to 108 cells/ml
Biospectrum	Surface (suspension) soil with incorporation	<i>Paenibacillus azotofixans</i> , <i>Bacillus megaterium</i> , <i>Bacillus mucilaginosus</i> , <i>Bacillus mycoides</i> . Total 1×10^9 CFU*/g. Mycorrhizal fungi – Total 1×10^7 CFU*/g
Phosphatovit	Seed coating / Surface (suspension) soil with incorporation	Spores and living bacteria cells of <i>Paenibacillus mucilaginosus</i>
Azotovit	Seed coating / Surface (suspension) soil with incorporation	Living bacteria cells <i>Beijerinckia fluminensis</i>
Azotohelp	Seed coating	<i>Azotobacter chroococcu</i> ; minimum 1.0×10^9 CFU*/cm ³
Utrisha N	Leaf in water suspension	<i>Methylobacterium symbioticum</i> ; strain SB23 Total. 3×10^7 CFU*/g.

Table 3. Meteorological conditions in the area of the Agricultural Institute - Shumen

2022									
Month	Temperature - °C				Precipitations – mm				
	Ten-day periods			Mean	Ten-day periods			Sum	Norm
	I	II	III		I	II	III		
VI	23.5	22.9	26.1	24.2	0.6	0.0	3.7	4.3	64.0
VIII	25.1	24.4	24.8	24.8	0.0	27.6	3.0	30.6	42.0
IX	20.0	19.0	16.2	18.4	28.9	9.6	16.7	55.2	53.0
X	14.7	12.5	14.2	13.8	11.7	0.5	0.3	12.5	51.0
XI	13.0	10.1	6.1	9.7	0.0	19.4	32.1	51.5	51.0
XII	4.9	4.6	4.2	4.6	13.9	9.2	0.6	23.7	28.0
Total:								177.8	289.0
2023									
Month	Temperature - °C				Precipitations - mm				
	Ten-day periods			Mean	Ten-day periods			Sum	Norm
	I	II	III		I	II	III		
I	7.0	7.1	2.3	5.4	2.1	30.4	7.0	39.5	35.0
II	2.9	7.0	5.1	5.0	12.3	0.0	14.2	26.5	28.0
III	8.2	6.9	8.5	7.9	6.9	11.0	20.9	38.8	31.0
IV	8.5	11.2	11.8	10.5	13.0	45.3	27.2	85.5	41.0
V	13.8	15.0	17.9	15.6	13.3	11.4	44.7	69.4	64.0
VI	19.9	20.4	23.2	21.2	6.9	17.6	1.9	26.4	41.0
VII	23.8	27.5	25.3	25.5	34.6	3.1	0.0	37.7	64.0
VIII	24.0	24.9	20.8	23.1	0.0	6.7	0.6	7.3	42.0
IX	22.0	20.4	21.3	21.2	0.9	47.8	0.0	48.7	53.0
X	16.7	14.3	18.6	16.6	0.5	0.0	0.0	0.5	51.0
XI	13.5	8.1	4.6	8.7	12.3	20.5	46.1	78.9	51.0
XII	4.6	3.8	6.5	4.9	0.0	2.0	1.1	3.3	28.0
Total:								462.5	529.0
2024									
Month	Temperature - °C				Precipitations - mm				
	Ten-day periods			Mean	Ten-day periods			Sum	Norm
	I	II	III		I	II	III		
I	4.1	2.0	0.3	2.1	8.0	0.3	3.7	12.0	35.0
II	8.2	7.3	9.5	8.3	0.0	0.0	2.6	2.6	28.0
III	7.3	8.0	11.5	9.0	1.1	18.9	8.0	28.0	31.0
IV	15.6	16.2	12.9	14.9	4.5	20.9	45.7	71.1	41.0
V	15.0	14.5	18.3	16.0	13.4	13.5	10.3	37.2	64.0
VI	25.3	24.8	25.2	25.1	0.0	33.3	0.0	33.3	75.0
VII	25.2	24.8	24.0	24.6	15.0	20.0	12.6	12.6	60.0
VIII	25.2	26.9	23.7	25.2	5.1	0.0	5.2	10.3	42.0
IX	22.3	17.4	18.7	19.5	0.0	0.5	15.7	16.2	28.0

X	17.1	11.4	9.9	12.7	6.7	8.3	0.3	15.3	53.0
XI	7.0	5.0	4.1	5.4	14.2	21.0	2.4	37.6	51.0
XII	5.2	4.7	2.9	4.2	30.9	0.8	79.0	110.7	51.0
Total:								416.9	559.0
2025									
Month	Temperature - °C				Precipitations - mm				
	Ten-day periods			Mean	Ten-day periods			Sum	Norm
	I	II	III		I	II	III		
I	5.9	1.1	7.3	4.9	1.2	2.4	3.2	6.8	35.0
II	0.1	-1.4	-2.7	-1.2	0.6	11.2	6.0	17.8	28.0
III	7.5	11.3	11.6	10.2	1.0	15.6	9.2	25.8	31.0
IV	6.5	10.4	13.4	10.1	28.4	1.0	5.4	34.8	41.0
V	16.0	13.5	16.3	15.3	15.0	15.2	37.8	68.0	64.0
VI	21.7	21.7	24.5	22.6	8.0	5.2	0.4	13.6	75.0
VII	25.0	22.2	26.5	24.6	0.0	34.6	11.8	46.4	60.0
Total:								213.2	334.0

RESULTS AND DISCUSSIONS

Table 4 shows the results of the test for 2023. The control variant yielded 1,759 kg/ha of grain. The application of Phosphatovit as a seed coating and at the minimum dose of 0.5 L/ha resulted in a statistically significant increase in yield ($P < 0.05$) corresponding to increases of

15% and 12%, respectively. At doses of 1.0 and 1.5 L/ha, the yield increase averaged 600 and 500 kg/ha, respectively, corresponding to 24.4% and 22.4% increases ($P < 0.01$). The application of Azotovit at doses of 0.5 and 1.0 L/ha also resulted in a significant yield increase ($P < 0.05$), of 221 and 407 kg/ha, corresponding to 19% and 23.1%, respectively.

Table 4. Average millet yield t/ha under different microbial fertilizer application options, 2023

2023 average yield of millet, grain - t/ha					
Microbial agent	Method of application	Application dose	$\bar{x}$	SE	CV
Biosplito	soil surface application	1.5 kg / ha	1.761	9.0	8.1
Biostart	seed coating	200 g / 100 kg seeds	1.761	14.7	10.0
Naturalis	soil surface application	21 / ha	1.893	7.4	8.7
Microbtima	seed coating	500 ml / 100 kg seeds	1.767	7.7	9.9
Biospectrum	soil surface application	250 g / ha	1.763	14.1	15.2
Phosphatovit	seed coating	1.5 l / ha	*2.055	9.2	10.0
Phosphatovit	soil surface application	0.5 l / ha	*1.995	6.7	7.9
Phosphatovit	soil surface application	1 l / ha	**2.357	8.8	8.3
Phosphatovit	soil surface application	1.5 l / ha	**2.265	7.7	7.6
Phosphatovit + azotovit	soil surface application	1.5 l / ha + 1.5 l / ha	1.843	18.1	15.0
Azotovit	soil surface application	1.5 l / ha	2.075	14.4	15.5
Azotovit	soil surface application	1 l / ha	*2.166	10.6	10.9
Azotovit	soil surface application	0.5 l / ha	*1.973	8.0	9.1
Azotovit	seed coating	1.5 l / ha	1.987	14.4	16.2
Azotohelp	seed coating	21 / ha	1.888	13.7	9.3
Utrisha N	leaf application (third-fourth leaf)	330 g / ha	*2.046	13.0	14.2
Control	-	-	1.759	3.7	4.7

$\bar{x}$ - Mean; SE - Standard error; CV - Coefficient of variation

*- Statistically significant difference at $p < 0.05$

**-. Statistically highly significant difference at $p < 0.01$

The experimental year 2024 was characterized by temperatures above normal and precipitation well below normal for the region, although the precipitation that fell in the months of April and May accumulated moisture, which helped the initial development of the plants. Logically, as shown in Table 5, the yields obtained from the experimental fields were the lowest of the three years of study. The control yielded 1,514 kg/ha. Due to the unfavorable conditions, the

differences between the control and the experimental plots were small and insignificant. A proven positive difference $P < 0.05$ was observed with the application of Phosphatovit 0.5 l/ha and Azotovit 1 l/ha, respectively by 9% and 19%, and with Phosphatovit 1 l/ha the increase in yield increases with a proven $P < 0.01$, with the difference being 19% and 354 kg/ha in absolute value. With other microbial preparations and

applied doses, although there is a positive difference, it has not been proven due to

significant variation between yields in the experimental plots.

Table 5. Average millet yield t/ha under different microbial fertilizer application options, 2024

2024 average yield of millet, grain - t/ha					
Microbial agent	Method of application	Application dose	$\bar{x}$	SE	CV
Biosplito	soil surface application	1.5 kg / ha	1.534	4.5	5.1
Biostart	seed coating	200 g / 100 kg seeds	1.491	6.4	5.1
Naturalis	soil surface application	21 / ha	1.532	10.1	14.7
Microbtima	seed coating	500 ml / 100 kg seeds	1.497	5.7	8.5
Biospectrum	soil surface application	250 g / ha	1.540	8.7	7.3
Phosphatovit	seed coating	1.5 l / ha	1.722	12.5	16.2
Phosphatovit	soil surface application	0.5 l / ha	*1.655	5.7	7.7
Phosphatovit	soil surface application	1 l / ha	**1.953	8.2	9.4
Phosphatovit	soil surface application	1.5 l / ha	1.892	10.4	12.3
Phosphatovit + azotovit	soil surface application	1.5 l / ha + 1.5 l / ha	1.560	5.5	8.1
Azotovit	soil surface application	1.5 l / ha	1.808	12.6	15.5
Azotovit	soil surface application	1 l / ha	*1.868	10.8	13.0
Azotovit	soil surface application	0.5 l / ha	1.513	6.4	9.5
Azotovit	seed coating	1.5 l / ha	1.773	9.3	11.7
Azotohelp	seed coating	2 l / ha	1.625	5.6	7.7
Utrisha N	leaf application (third-fourth leaf)	330 g / ha	1.794	7.1	10.2
Control	-	-	1.514	5.3	5.8

$\bar{x}$ - Mean; SE - Standard error; CV - Coefficient of variation

*- Statistically significant difference at $p < 0.05$

** - Statistically highly significant difference at $p < 0.01$

Table 6 shows the grain yield of millet for the 2025 harvest year. The lack of precipitation in the first half of the year, compared to the average precipitation for the region, reached 121 mm. The drought, which was observed in the months of June and July, at a time when millet lays reproductive organs and forms seeds, had a particularly adverse effect on grain production. In this situation, 1.557 t/ha of grain was obtained from the control variant. In all

experimental settings, the positive influence of microbial fertilizers was demonstrated, with a proven difference $P < 0.05$ being recorded in the variants with Phosphatovit 0.5 l/ha and Azotovit 1 l/ha, representing 10% and 21%, respectively. The application of Phosphatovit at a dose of 1 l/ha leads to the proven positive difference $P < 0.01$, representing 410 kg/ha or 24.3% compared to the control variant.

Table 6. Average millet yield t/ha under different microbial fertilizer application options, 2025

2025 average yield of millet, grain - t/ha					
Microbial agent	Method of application	Application dose	$\bar{x}$	SE	CV
Biosplito	soil surface application	1.5 kg / ha	1.616	6.7	7.3
Biostart	seed coating	200 g / 100 kg seeds	1.570	6.6	6.9
Naturalis	soil surface application	21 / ha	1.561	9.1	12.8
Microbtima	seed coating	500 ml / 100 kg seeds	1.596	5.8	8.2
Biospectrum	soil surface application	250 g / ha	1.580	5.1	7.2
Phosphatovit	seed coating	1.5 l / ha	1.713	8.1	10.2
Phosphatovit	soil surface application	0.5 l / ha	*1.722	7.0	9.1
Phosphatovit	soil surface application	1 l / ha	**2.056	8.6	6.4
Phosphatovit	soil surface application	1.5 l / ha	1.992	10.9	12.3
Phosphatovit + azotovit	soil surface application	1.5 l / ha + 1.5 l / ha	1.601	6.3	8.7
Azotovit	soil surface application	1.5 l / ha	1.904	13.2	15.5
Azotovit	soil surface application	1 l / ha	*1.967	11.4	13.0
Azotovit	soil surface application	0.5 l / ha	1.610	4.5	6.3
Azotovit	seed coating	1.5 l / ha	1.823	9.2	10.2
Azotohelp	seed coating	2 l / ha	1.696	6.1	8.0
Utrisha N	leaf application (third-fourth leaf)	330 g / ha	1.733	10.5	9.0
Control	-	-	1.557	4.7	6.8

$\bar{x}$ - Mean; SE - Standard error; CV - Coefficient of variation

*- Statistically significant difference at $p < 0.05$

** - Statistically highly significant difference at $p < 0.01$

From the data obtained during the three years of testing of the various biofertilizers, it can be summarized that in conditions of mild to moderate soil moisture deficiency and higher temperatures than the average for the region, a positive effect on grain yield was observed in all test variants. The independent application of bioinoculants has been proven to increase millet yields, and treatment in combination does not accumulate its effect on grain productivity. This may be due to both the specific abiotic conditions and the antagonistic effect between them. It is concluded that the application of biopreparations, in addition to increasing yield, also leads to an increase in variation. This is also confirmed in the studies of Leggett et al. (2015) and Overbeek et al. (2021).

In conditions of water deficit in 2024 and 2025, proven higher yields were observed in the variants treated with Fosfatovit 0.5 l/ha, Azotovit 1 l/ha and Fosfatovit 1 l/ha. During the more favorable agrometeorological conditions of 2023, seven of the tested variants showed significantly higher yields.

CONCLUSIONS

The new economic realities (expensive nitrogen and phosphorus fertilizers, extreme drought and high temperatures) can be overcome with new methods of farming and fertilizing crops.

Under unfavorable agrometeorological conditions - high temperature and water deficit, microbial fertilizers prove their effectiveness and increase the adaptability of plants to adverse abiotic factors of the environment, however, the highest percentage increase in yield is in the year with more precipitation - 2023. During the three years of research, the preparations Azotovit and Fosfatovit have given a proven increase in yield. From our research, we cannot conclude why high doses of application, as well as the combination of the two products, do not give a cumulative effect. When studying the impact of the aforementioned microbial fertilizers, the results obtained showed a positive effect on increasing millet (*Panicum miliaceum* L.) grain yield with up to 24.4%. Regardless of the composition of the microbial inoculants (spores, live bacteria, fungal strains or combinations thereof), they all

show an increase in yields, although in some of the variants the increase is not statistically proven, but nevertheless higher yields have been registered.

The economic justification for the application of the studied biofertilizers is the subject of another study and the recommendation for their application can be made only after a careful economic analysis.

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THE EFFECT OF TEMPERATURE IN THE MILLET SEED GERMINATION AND THE INFLUENCE OF ROW SPACING ON THE PERFORMANCE OF SEVERAL *Panicum miliaceum* L. GENOTYPES UNDER THE PEDOCLIMATIC CONDITIONS OF A.R.D.S. SECUIENI

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Abstract

Millet is a secondary crop in Romania but at the global scale it occupies substantial cultivated areas in arid regions (Asia and Africa), where its grains constitute a staple food for populations. In the context of recent climatic changes, the diversification of cultivated species has become a well-established objective. Consequently, in 2022, *Panicum miliaceum* L. was incorporated into the research portfolio of the A.R.D.S. Secuieni. The investigation focuses on two main objectives: the effect of temperature on millet seed germination and the interaction between genotype and row spacing on millet grain yield. Laboratory experiments demonstrated a directly proportional relationship between temperature and germination percentage, with 50.25% germination recorded at 10°C and 97.75% at 20°C. Field trial results revealed that millet demonstrates pronounced adaptability to the pedoclimatic conditions of the Secuieni area in 2022. Under these conditions, the evaluated genotypes produced average yields ranging from 1984 kg/ha (cultivar Marius - 12.5 cm row spacing) to 1664 kg/ha (BG 1 - 50 cm row spacing), highlighting an inverse relationship between grain yield and row spacing.

Key words: germination percentage, millet, row spacing, yields.

INTRODUCTION

Currently, grain consumption supports about 50% of the world's total calorie intake and is largely provided by wheat, rice, and maize (Awika, 2011; Das, 2019; Palchetti, 2019).

At the same time, climate change accelerates land degradation and desertification, and extreme weather events decrease yields (Habiyaemye, 2017; Kumar, 2017; Ullah, 2017).

Growing and eating millet would not only reduce protein micronutrient malnutrition, but also help diversify crops and diets.

Millet, botanically, is a member of the *Poaceae* family, sub-family *Panicoideae*, tribe *Paniceae*, representing the *milium* of the Romans and the *dokhan* of the Jews.

Panicum miliaceum L. is one of the world's first domesticated crops and was cultivated before the spread of rice, maize and wheat (Crawford,

2006; Sage, 2011). Throughout history, the introduction of corn into cultivation dethroned "the chief cereal of our peasantry, which ate millet polenta (mălai) nutritious but difficult to consume" (Săulescu, 1947).

Ten thousand years ago, it appeared as a staple food in the semi-arid regions of East Asia and later spread throughout the Eurasian region (Lindquist, 2005; Cavers, 2016), millet grains are still an important source of energy and protein for millions of people living in arid and semi-arid areas in emerging countries, while millet biomass is an important source of food in some Asian countries, such as India (Blümme et al., 2003; Brunette et al., 2016).

About 2/5 of the world's cereal production is used in animal nutrition, sometimes complementing traditional fodder and associations between various animal species and specific grains are well known, for example: horses and oats, poultry and millet so the major

share for millet goes to the poultry feed industry. No matter how many health benefits it has, including gluten-free, low glycemic index, high protein and fiber content, millet is still struggling to break into the food industry.

Among the C₄ panicoids, millet (*P. miliaceum* L.) is known to possess morpho-physiological traits that confer tolerance to abiotic stress and show greater adaptability than other cereal crops in different environmental conditions (Bandyopadhyay, 2017; Vetriventhan, 2018), having a fasciculated, very well developed and branched root system

The most popular name of *P. miliaceum* L. is "proso millet" comes from the general Pan-Slavic name for millet (Croatian: proso, Serbian: ррсо) (Enea, 2023).

Millet is considered a low-input ingredient because it requires low production costs (it can be grown on marginal lands) having special agrotechnical advantages: resistance to drought and pest attack, minimum requirements in regarding fertilization (Amadoubr, 2013; Changmei, 2014). Adding to these the short vegetation period, between 60-100 days (Baltensperger, 2002).

Given the current and future scenarios, scientists suggest that an effective strategy could be to replace cereal crops that have high water requirements with others adapted to drought conditions (Ndiku, 2014).

MATERIALS AND METHODS

This paper focuses on the results obtained from 2 distinct research studies, one conducted in a controlled environment, in the laboratory, and one conducted in the experimental field.

In the laboratory, the influence of temperature on the percentage of germinated grains was investigated.

As a working methodology, germination samples were carried out which consisted of determining the percentage of pure seeds, capable of producing normal germs and which, put in favorable cultivation conditions, produce normally developed plants.

From the pure, well-homogenized seed, four samples of 100 seeds were randomly counted, which were placed on the vegetation layer, in Petri dishes.

For a better investigation of the thermophilic character of this species and to highlight its plasticity and adaptability the millet samples were placed in the germination chamber at for different temperatures:

- 10°C;
- 14°C;
- 18°C;
- 20°C.

The result of the germination test was calculated as an average of the four samples of 100 seeds and was expressed in numerical percentages of normal germs.

In the experimental fields of A.R.D.S. Secuieni, in 2022, the millet experiences placed that the aim to:

- establishing the adaptability of some millet genotypes to the pedoclimatic conditions in Central Moldova;
- establishing the optimal row spacing.

The bifactorial experiment, of the 5x3 type, was located according to the method of subdivided plots, consisting in:

- Factor A – genotypes;
- Factor B – row spacing.

In the experiment the rate seed of sowing was 10 kg/ha.

P. miliaceum L., today in the Official List of Cultivated Varieties in Romania, has as its representative only the variety Marius.

The genotypes used in the field experiment consisted of:

- Marius variety (from N.A.R.D.I. Fundulea);
- local population Secuieni (here referred as "Secuieni");
- BG 1 genotype from Mihai Cristea Plant Genetic Resources Bank Suceava;
- BG 2 genotype Mihai Cristea Plant Genetic Resources Bank Suceava;
- BG 3 genotype Mihai Cristea Plant Genetic Resources Bank Suceava.

The row spacing used for these five varieties was:

- 12.5 cm;
- 25 cm;
- 50 cm.

The millet experimental crop was sown in the optimal epoch for this geographic area (Central Moldavian Plateau), the beginning of May (first decade), in a dry land as a result of rainfall deficit recorded in 2022, it was harvested in

mid-August, the vegetation period for millet under the conditions of A.R.D.S. Secuieni was 95 days and the obtained data were processed and statistically interpreted according to the variation analysis method (Jităreanu, 1994).

During the experiment, the harvested area was 10 sqm, the rows of plants had a length of 10 m, and their width was 1 m for the distance between rows of 12.5 cm and 25 cm and 2 m, respectively, when the distance between the rows of plants was 50 cm. Also, the number of rows/experimental plot was 8 (distance of 12.5 cm between rows) and 4 (distances of 25 cm and 50 cm between rows).

The experimental field of A.R.D.S. Secuieni where the bifactorial experience was located has the following characteristics:

- phaeozem (chernozem) typical cambic with a medium texture;
- slightly acidic, having a $\text{pH}_{\text{H}_2\text{O}} = 5.98$;
- poor-medium fertile, with active humus (1.88%);
- low-medium nitrogen supply (16.2 ppm N-NO_3);
- poorly supplied with potassium (124.6 ppm K_2O);
- Well stocked with:
 - phosphorus (77.6 ppm PAL);
 - calcium (13.6 meq/100 g sol);
 - magnesium (1.8 meq/100 g soil) (Enea, 2025).

RESULTS AND DISCUSSIONS

Proso millet, a well known C_4 species, shows a notable preference for higher thermal conditions, which decisively influences its germination process.

It is well known that in order for millet grains to start the germination process in an efficient way, it is essential to have higher ambient temperatures. This aspect was studied under laboratory conditions, analyzing four thermal variables to determine the percentage of millet grain germination over 7 days (Table 1).

The results of this research revealed that high temperatures are a significant factor in stimulating the germination of millet grains.

Studies have shown that temperatures maintained within a specific range generated a higher germination percentage compared to lower temperatures. This information is

particularly useful in helping to understand the specific needs of millet and to develop optimal agricultural practices to ensure healthy germination and robust growth of this plant.

Analyzing the data presented in Table 1, it can be seen that there is a positive correlation between high temperatures and germination percentage.

When temperatures are higher, there is a significant increase in the rate of seed germination.

Table 1. Influence of temperature on the germination of millet grains

Temperature (°C)	Germinated grains (%)
10	50.25
14	70.50
18	87.75
20	97.75

This result suggests that the dynamics of the germination process are favorably influenced by optimal thermal conditions, which can be crucial in optimizing millet cultivation and production. This finding provides valuable insight for farmers and researchers, pointing to the importance of appropriate temperatures in the millet cultivation process to ensure efficient germination and optimal production.

Analyzing the results obtained over 7 days, we see that the temperature of 10°C ensures the germination of 50.25% of millet grains, while the temperature of 20°C has a significantly greater impact, causing an impressive germination rate of 97.75% (Figure 1).

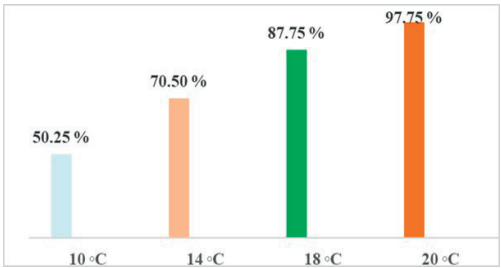


Figure 1. Effect of temperature on the percentage of germinated grains

Data on germination at different temperatures emphasize the importance of thermal influence in the process of millet seed development.

The higher temperature seems to create favorable conditions for rapid and efficient germination, as illustrated in the significant

percentage of 97.75% (Figure 1). This information is very useful in the context of optimizing cultivation conditions, providing farmers and researchers with practical guidelines for improving yield and efficiency in millet production.

The agricultural year 2021/2022 was characterized as:

- hot: +1.60C compared to the multiannual average;
- very dry: deficit: -264.0 mm.

Throughout its period being characterized by an uneven distribution of rainfall and a trend of aridization.

The millet vegetation period (May – August) in 2022 was characterized as:

- hot: deviation of +1.90C;
- very dry: deficit -163.4 mm (Enea Andreea, 2022) (Figures 2 and 3).

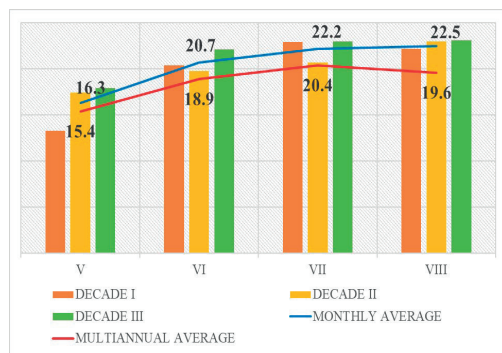


Figure 2. Temperatures (°C) during the millet vegetation period, A.R.D.S. Secuieni, 2022

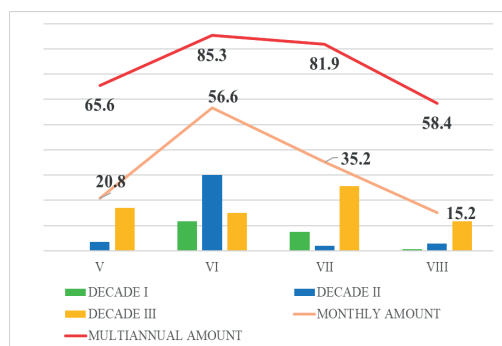


Figure 3. Precipitation (mm) during the millet vegetation period, A.R.D.S. Secuieni, 2022

In May, when the millet crop was sown, the temperatures had a deviation of +0.9°C, and the rainfall deficit was -44.8 mm.

The hottest period was represented by the summer months, the deviation being +1.8 °C (June and July) and 3.1°C (August), aimed rainfall deficits -28.7 mm (June), -46.7 mm (July) and -43.2 mm (August), during this period the growth, filling and maturation of millet grains are achieved, but this species has a pronounced ecological plasticity, proving in these conditions the high resistance it has in drought conditions.

The yield stability in field crops is given by the varieties with a high yield potential, with a superior yield quality and a better adaptability to the biotic and abiotic environmental conditions. Recommending the optimal distance between the rows of plants in the cultivation of millet is an essential aspect in obtaining optimal yields in this crop.

Scientific research abroad has shown that there is an inversely proportional relationship between production and row spacing, which means that increasing this distance can negatively influence millet crop production (Agdag, 2001).

When the row spacing is properly optimized, millet plants benefit from the necessary space to develop their root system and access water and nutrient resources in the soil efficiently.

Also, a properly optimized distance can lead to better absorption of sunlight, an even distribution of the plant in the field, and ultimately higher grain yield.

Since millet is a crop of secondary importance in our country, not being in the attention of farmers, data are limited in terms of its crop technology.

The Romanian literature recommends sowing in rows at a distance of 12.5 cm (Zamfirescu, 1965), subsequently, in more recent studies specialists suggested that the optimal distance should be 25 cm or 50 cm between rows on weeded land (Roman et al., 2011, 2015).

Adjusting the row spacing and proper variety selection can be key-strategies for optimizing proso millet yield crops.

Within this field experiment, compared to the control of the experience, the interaction of the Marius variety x 12.5 cm, which achieved 2984 kg/ha yield, statistically assured differences were obtained:

negatively significant – at the interactions: Marius variety x 25 cm (2423 kg/ha), Marius x

50 cm (2298 kg/ha) and BG 2 x 12.5 cm (2393 kg/ha) and BG 3 x 50 cm (2452 kg/ha);

- distinctly significant negatives - at interactions: BG 2 x 25 cm (2236 kg/ha) and BG 2 x 50 cm (2203 kg/ha);
- very significant negatives – at interactions: BG 1 x 12.5 cm (1878 kg/ha), BG 1 x 25 cm (1720 kg/ha) and BG 1 x 50 cm (1664 kg/ha) (Table 2).

The differences between the studied variants and the control have values ranging between -79 kg/ha (Local population Secuieni x 12.5 cm) and -1320 kg/ha (BG 1 x 50 cm).

These observations provide valuable information for farmers in terms of adapting crop practices to achieve superior yields.

Table 2. Table title example that should be replaced with your information

Genotype	Row distance (cm)	Average yield (kg/ha)	Control difference (%)	Control difference (kg/ha)	Semn
Marius variety	12.5	2984	100	Mt	
	25	2423	81	-561	o
	50	2298	77	-686	o
Local population Secuieni	12.5	2905	97	-79	
	25	2636	88	-348	
	50	2485	83	-499	
BG 1	12.5	1878	63	-1106	ooo
	25	1720	58	-1264	ooo
	50	1664	56	-1320	ooo
BG 2	12.5	2393	80	-591	o
	25	2236	75	-748	oo
	50	2203	74	-781	oo
BG 3	12.5	2738	92	-246	
	25	2623	88	-361	
	50	2452	82	-532	o
Average		2332	78	-652	o
DI A*B 5%				511	
DI A*B 1%				688	
DI A*B 0,1 %				915	

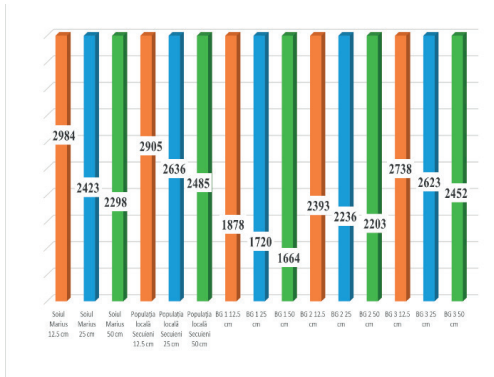


Figure 4. Influence of genotype x row spacing interaction (kg/ha), 2022

The average yields obtained (Figure 4) ranged from 1664 kg/ha (interaction BG 1 x 50 cm between rows) to 2984 kg/ha (interaction Marius variety x 12.5 cm between rows).

We also observe that in all genotypes studied, the yield level decreases with increasing distance between plant rows (Figure 4).

Similar research was carried out in Sidney, Nebraska (1998) which concluded that millet productivity, number of siblings and straw weight decreased as the distance between rows increased (Agdag, 2001).

CONCLUSIONS

Millet is a thermophilic species, which germinates at high temperatures (20°C 98% of the grains germinated).

The agricultural year 2021/2022 was a hot and dry year.

Millet showed a very good adaptation to the pedo-climatic conditions specific to the Central Moldavian Plateau.

The 5 genotypes studied had yields between 2984 kg/ha (Marius variety x 12.5 cm) and 1664 kg/ha (BG 1 x 50 cm).

An inversely proportional relationship was observed between the distance between the rows of plants and the level of production.

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ENHANCING WHEAT RESILIENCE TO HYDRIC STRESS THROUGH DYNAMIZED MINERAL BIOSTIMULANTS: GREENHOUSE TRIAL INSIGHTS

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Abstract

Wheat stands out as one of the most vital crops for global food sovereignty. However, instability due to environmental changes on productive areas, particularly draught, pressure wheat farming systems across the globe. Furthermore, most of the wheat is farmed under heavily petroleum-based agricultural practices that threaten its long-term sustainability. These environmental and economic pressures compromise the stability of global wheat supply chains and undermine the resilience of farming communities. The agroecological transition and its practices offer a promising pathway toward more sustainable wheat production. In the last 30 years, the use of dynamized high dilutions (DHDs) in agriculture has gained recognition as an agroecological method, particularly in Brazil, Ecuador, Mexico, India, Pakistan, Italy, Germany, and Switzerland. The method focuses on using organic low-residual impact biostimulants and a salutogenic approach to treat the agro-ecosystem. However, this field of research is still at its dawn and needs robust studies to comprehensively assess the possibilities and limitations of using DHDs in agriculture. Therefore, the objective of this study is to investigate the biostimulatory impact of mineral DHDs to support wheat plants subjected to hydric stress in greenhouse agronomical trials in 2025 and 2026.

Key words: drought mitigation, health-based solution, homeopathic preparations, plant vitality, *Triticum aestivum*.

INTRODUCTION

Wheat stands out as one of the most vital crops for global food sovereignty and economic stability. A staple food for billions, it provides a significant portion of daily caloric intake worldwide (Velimirović et al., 2021). Its adaptability to diverse climates and its cultural role in various countries have cemented wheat's position as a foundation food for food security (Shewry and Hey, 2015). In many countries, wheat farming supports rural livelihoods, drives economic development, and sustains agricultural communities. However, instability for hydric resources and change in the regimes of rain regimes in the productive regions pressure wheat farming systems (Frison, 2016). Furthermore, most of the wheat is farmed in industrial agricultural systems where wheat farming faces mounting challenges that threaten its long-term sustainability (Kremen et al., 2012 C 2012). The intensive use of chemical fertilizers and

pesticides has led to soil degradation, water pollution, and the decline of biodiversity. Additionally, monoculture practices make wheat crops more susceptible to pests, diseases, and the impacts of climate change, including extreme weather events (Frison, 2016; Dedrasi et al., 2023). These environmental and economic pressures compromise the stability of global wheat supply chains and undermine the resilience of farming communities. The agroecological transition (Gliessman, 2011) offers a promising pathway toward more sustainable wheat production (Tautges et al., 2016). Agroecology integrates ecological principles into agricultural practices, promoting biodiversity, soil health, and resource efficiency. It emphasizes local knowledge, diversified cropping systems, and reduced reliance on synthetic inputs, fostering resilient farming landscapes (Anderson et al., 2021; Wright, 2021).

In the last 30 years, the use of dynamized high dilutions in agriculture has gained recognition

as an agroecological method, particularly in Brazil, Mexico, India, Germany and Switzerland (Faedo et al., 2024). The method focuses on using low-residual impact solutions and applying salutogenic (Antonov, 1996) - health promotion strategies in agroecosystems (Boff et al., 2021). By integrating therapeutic and agronomic disciplines on its framework, the use of dynamized high dilutions (DHDs) is an agroecological technique that involves the application of natural substances (eg. nano solutions of minerals, plant oils and tinctures, soil and insect biopreparations) to the crops, aiming to trigger biostimulation effects promoting plants' natural defences and growth process, ultimately supporting the plant vitality (Faedo et al., 2024). Furthermore, studies suggest the use of DHDs supports the plant natural resilience to pests, diseases, and environmental stressors (Do Prado Mattos et al., 2024).

Its application aligns with agroecological principles and regulations by focusing on ecological balance and plant biostimulation, improving crop quality, and supporting sustainable farming systems (Cordoba Correoso et al., 2022). However, despite the positive results reported, this field of research is still at its dawn and needs robust studies to comprehensively assess the possibilities and limitations of using DHDs in agriculture and in particularly, studies testing DHDs in wheat plants, assessing the biostimulation effects on plant resilience. Therefore, the objective of this study is to develop agronomical trials in greenhouse conditions to test DHDs as an agroecological method to support wheat plants subjected to hydric stress.

MATERIALS AND METHODS

Greenhouse experiments were conducted at the Centre for Agroecology, Water and Resilience (CAWR) in 2025 and 2026. The agronomic trials used a randomized block design (RBD) following a double-blinded treatment application, with 11 treatments and 8 repetitions.

The experimental unit consisted of 5 spring wheat plants (*Triticum aestivum* L. cv KLS Ladum[®]), allocated in a 10-L plastic pot filled with certified organic growing media (UK Soil

Association) (Figures 1-4). In the greenhouse, the pots were placed above ground on metal tables and the pots were spaced 15 cm apart from each other.

The temperature and irrigation were controlled with automated environmental control, with the soil moisture content (% of water in the soil) controlled across the growing season with a moisture meter (HH2 Delta Devices[®], Cambrige, UK). The plants were subjected to hydric stress (Zadok's scale), during phase 7 to 8, during the critical phase between inflorescence to grain filling by keeping the field capacity of the pots at <30%.



Figure 1. Experimental unit at the greenhouse agronomical trials, June 2025. Coventry, UK

The scientific literature on the use of mineral-based DHDs have shown promising results reported on horticultural, ornamental and cereal crops (Kaviraj, 2018; Boff et al., 2021; Faedo et al., 2024). Particularly positive are the effects on morphophysiological development (Do Prado Mattos et al., 2024). Therefore, the DHDs tested in this study were: Calcarea carbonica 30CH (calcium carbonate), Kali carbonicum 30CH (potassium carbonate), Natrum muriaticum 30CH (sodium chloride), Phosphorus 30CH (elemental phosphorus), Sulphur 30CH (elemental sulphur), Silicea terra 30CH (silicon oxide, 30CH, 100CH and 200CH), and distilled deionised water as the control.



Figure 2. Soil moisture content assessment, July 2025, Coventry, UK



Figure 3. DHD treatments coded and prepared for application. April 2025. Centre for Agroecology, Water and Resilience (CAWR), UK

The DHDs were manufactured by Helios Homeopathic Pharmacy® (Royal Tunbridge Wells, UK), according to the standards of the EU homeopathic pharmacopoeia and experiments carried following the recommendations for fundamental research on DHDs (Stock-Schröer et al., 2009).

The DHDs were coded by the CAWR lab technicians and then handed blind to the researcher ready to be used in the experiment. The application of the DHDs and assessment of

crop attributes were performed blind. The treatments were applied by mixing 5 ml of DHD with 995 ml of distilled water, and then 100 ml of the solution treatment was applied in each experimental unit (pot), every 15 days directly to the plants base into the soil, using graduated lab glassware, beakers of 1000 ml and 100 ml, Fisher® (United Kingdom) always after the experiment was evaluated.

The plant morphophysiological development were assessed every 15 days, from the seedling emergence to the harvest period. The agronomic characteristics measured and presented in this preliminary research insights consisted of the number and length of spikes, stem diameter, number of viable tillers, number and weight of seeds per spikelet's (Seidel et al., 2024). The chlorophyll content of the wheat leaves was measured using the equipment SPAD 502 PLUS, Konica Minolta® (Ozaka, Japan), taking randomized measurements from the five (5) topmost leaves from each individual experimental unit.



Figure 4. Assessment of leaf chlorophyll content, July 2025, Coventry, UK

At the time of harvesting, the grains were collected manually and assessed for weight, moisture content and pH. The crop production (yield/pot) was measured using precision digital electronic scale (0.0001 g), model GE1302 Sartorius®. To evaluate productivity (kg ha^{-1}) the flour humidity was corrected to

13% moisture content and hectolitre weight. At the end of the experiment the biomass (fresh and dry) of canopy and roots was assessed. The plants were dried in air-enforced chamber at 70°C for 72 hours. The nutritional content of the plant tissue and flour will be assessed via HPLC. (liquid chromatography) analyses (Seidel et al., 2024).

RESULTS AND DISCUSSIONS

In this paper, the authors present only preliminary insights regarding number of side shoot emissions/plant, leaf chlorophyll content, number of spikes/plants, total fresh and dry biomass production. All the treatments are still coded as the agronomical experiments testing mineral DHDs to support drought stressed wheat plants in 2026 are still being carried out.

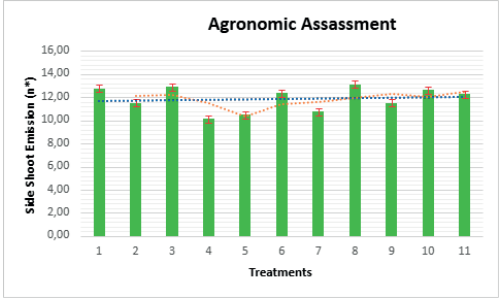


Figure 5. Tillering of wheat plants submitted to drought and treated to DHDs. Coventry, 2025. Treatments means + Standard error

When looking to Figure 5, we observe that during the tillering did differ across treatments with 4, 5, 7, and 9 producing 10 side shoots/experimental unit, 2 less in comparison to treatments 1, 3, 6, 8, 10, and 11 which produced a mean of 12 side shoots/experimental unit. This feature indicates an alteration on the plant’s physiological activity. When we investigate Figure 6, this perception is enhanced by the results of treatments 1, 7, 8, which had 40 and 30 SPAD units respectively. Treatments 2, 3, 4, 5, 6, 9, 10, 11 had all an average of 50 SPAD units, 10 units more than the less concentrated treatments.

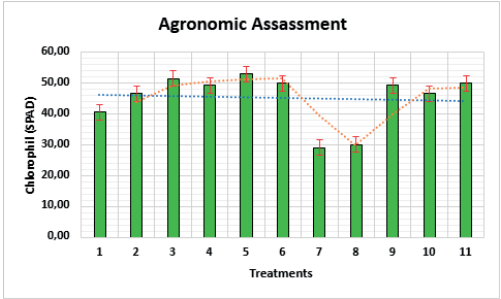


Figure 6. Chlorophyll content of wheat plants submitted to drought and treated with DHDs. Coventry 2025. Treatments means + Standard error

Figure 7 presents the production of spikes/experimental unit, and noticeably the treatments 1, 6, 8, and 10 produced an average of 10 spikes, 4 spikes more when compared to treatments 2 and 11 which produced just about 6 spikes/experimental unit.

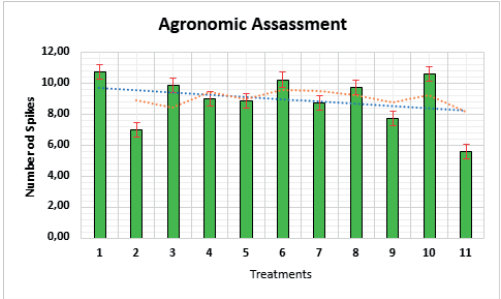


Figure 7. Number of spikes of wheat plants submitted to drought and treated with DHDs. Coventry, 2025. Treatments means + Standard error

Figures 8 and 9 present the results of total biomass production (fresh and dry) and once again is possible to observe difference between the treatments, with treatments 2, 4, 5, 6, 9, and 10 weighing more than 55 g/experimental unit of fresh mass production when compared to treatments 3, 7, 8, and 10 which produced less than 52 g/experimental units. When we look to the dry mass, it’s possible to observe that treatments 2 and 7 weighted an average of 12 g/experimental unit in comparison to all the other treatments which weighted an average of 14 g/experimental unit.

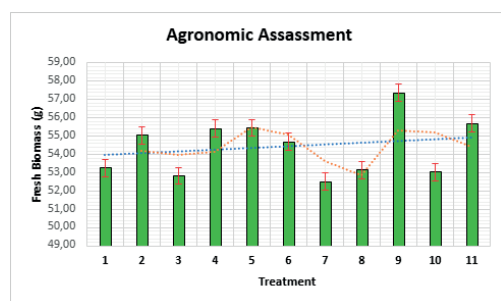


Figure 8. Fresh biomass of wheat plants submitted to drought and treated with DHDs. Coventry, 2025.
Treatments means + Standard error

Overall, the preliminary results for morphophysiological development of wheat plants have indicated potential differences between the treatments, indicating positive biostimulation effect on the plants submitted to environmental stress and treated with the mineral DHDs.

However, no further additional analysis or inferences can be drawn at this phase due to stage where the experiments currently are. It is interesting to mention though that the levels of hydric stress that the plants were submitted were modelled having in mind the harshest of the scenarios (soil moisture <30%).

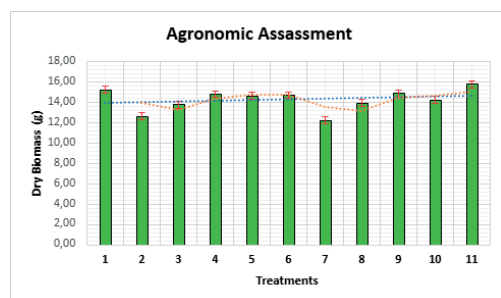


Figure 9. Dry biomass of wheat plants submitted to drought and treated with DHDs. Coventry, 2025.
Treatments means + Standard error

In this way, the morphophysiological results obtained in the controlled environment, namely side shoot emissions, number of spikes, chlorophyll content and fresh and dry biomass give initial insights into the biostimulation effects of the preparations. Taiz (2017) explains that these features are directly related to the stimulation of physiological and metabolic activity of the plants.

CONCLUSIONS

The preliminary results of the agronomical greenhouse trials testing mineral DHDs indicate positive biostimulation effects on wheat plants submitted to drought. However, these results should be considered only as initial insights, as the experiments are not yet fully completed, and data completely analysed. Furthermore, if positive results are confirmed, the treatments should be tested in field conditions so that its applicability can be confirmed for use in wheat cropping systems.

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EVALUATION OF PRODUCTIVE AND QUALITY PARAMETERS IN SPRING BARLEY

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Abstract

*Spring barley (*Hordeum vulgare* L.) ranks fourth globally among cereal crops in terms of cultivated area and production, being used predominantly for feed (~75%), followed by beverage production (~20%) and human consumption (~5%). Starch, which represents ~70% of the grain mass, is highly sensitive to heat stress, as its synthesis depends on the conversion of carbohydrates within the developing kernel. The endosperm, composed of starch granules embedded in a protein matrix, is essential for embryo viability and development. Protein content, which varies between 8-30%, is influenced by environmental conditions, cultivar, and technological factors. For malting barley, an optimal protein content of 10–12% is crucial to ensure uniform germination and high extract yield. This study, conducted at the Agricultural Research and Development Station Turda and involving seven spring barley cultivars, evaluated productivity parameters as well as starch and protein content. Qualitative analyses remain fundamental for cultivar selection and for guiding breeding programs toward more precisely targeted end-use directions.*

Key words: *spring barley, starch, yield parameters, protein, breeding.*

INTRODUCTION

Barley (*Hordeum vulgare* L.) represents a crop of considerable global economic importance, supporting key sectors such as agriculture, the food and beverage industry, and animal nutrition (Bahman & Hossein, 2025). It ranks fourth among cereal crops worldwide and is considered a secondary small-grain cereal, widely cultivated in Europe (Panfilova et al., 2021).

It is widely recognized for its diverse uses, mainly in animal feed and the production of alcoholic beverages such as malt and beer. In some regions, including Tibet and China, it also remains an important food source for humans, commonly consumed as groats and flakes (Sakellariou and Mylona, 2020; Lenartowicz et al., 2024).

A commonly recognized aspect is that spring barley varieties respond differently and produce varying yields depending on growing conditions, as a result of interactions between genotype, environment and cultivation technology (Leistrumaitė and Razbadauskienė, 2008).

Yield components are essential elements in crop breeding programs. For barley, the identification and utilization of genotypes with high yield potential constitute a priority for enhancing crop performance. Simultaneously, these efforts must be integrated into a sustainability-oriented framework to contribute to food security consolidation and the development of genotypes capable of adapting efficiently to current climate conditions and their variability. The evaluation of key yield parameters over a three-year period facilitated a more comprehensive assessment of the analyzed traits within the context of the specific climatic variability of each study year. This approach captured both favorable conditions for crop development and years characterized by less favorable environments, thereby providing a more robust basis for interpreting the results. The main difference between malting and feed barley lies in the quality standards required for acceptance into the malting process (www.bmbri.ca, 2011). Barley protein represents a sustainable, plant-based component with broad potential

applications across the feed, food, nutraceutical, and industrial sectors (Boukid, 2026), making it a crucial target trait in barley breeding programs. Furthermore, barley is the primary cereal grain utilized in the production of brewing malt (Zanamwe and Madzaramba, 2025). The process of starch synthesis during barley growth is intricately influenced by both barley genetics and environmental conditions (Franz et al., 2025). Current studies show that although global barley production could increase over the 2025-2035 period, the balance between supply and demand remains fragile due to climate change and trade uncertainties. To ensure sustainable market development, climate-adapted breeding strategies are required alongside appropriate economic policies and instruments (Kizilcan and Altuntaş, 2025). Within the context of barley's economic relevance and diverse applications, the evaluation of genotypes is essential for identifying potential valuable parents, in order to align breeding objectives toward more focused, sustainable directions adapted to current requirements.

MATERIALS AND METHODS

The research was conducted at the barley breeding field of the Agricultural Research and Development Station Turda (SCDA Turda), Romania. The study included seven two-row spring barley varieties of both Romanian and foreign origin, selected from the station's germplasm collection.

The experiment was established using a linear sowing design, with each genotype manually sown in 1 m rows spaced 25 cm apart, resulting in a harvestable area of 1 m². Biometric data were analyzed using a two-factor design, with year (three levels) and genotype (seven levels) as sources of variation. The evaluated traits included morpho-productive parameters - number of grains per main spike, grain weight per spike and thousand-grain weight (TGW) - as well as quality indicators, namely protein and starch content. Measurements collected over the experimental years, based on 18 spikes per genotype, resulted in a total of 378 spikes analysed. Grain weight measurements were performed using a high-precision electronic balance (Kern 573), and TGW was calculated by dividing the grain weight per spike by the

corresponding number of grains and multiplying the result by 1,000.

Quality traits were evaluated at the Physico-chemical Analysis Laboratory of SCDA Turda over two experimental years. For each genotype, grain samples were divided into three subsamples of approximately 20 g and ground using a Perkin Elmer laboratory mill, resulting in 42 observations. The resulting flour was analyzed with a Tango FT-NIR spectrophotometer to determine protein and starch content. The climatic characterization of the study years was compiled based on data recorded by the Turda regional meteorological station (longitude: 23°47'; latitude: 46°35'; altitude: 427 m).

Five variables - grain number (GN), grain weight (GW), thousand-grain weight (TGW), protein content (PC), and starch content (SC) - were analyzed. For variables following a normal distribution, data were presented as mean and standard deviation (SD). For non-normally distributed variables, the median and interquartile range (25th-75th percentile) were used. The Shapiro-Wilk test was employed to assess the normality of the data distribution.

To compare the seven cultivars, one-way ANOVA was used for data that were normally distributed. For data that were not normal, the Kruskal-Wallis test was applied. When these tests showed significant differences, multiple comparisons were done using Tukey's HSD or Dunn's post-hoc tests, depending on the test used. Statistical significance was considered at $p < 0.05$.

To study how the five variables related to each other within each cultivar, Spearman's rank correlation coefficients (ρ) were calculated. These correlations were shown as heatmaps using the *metan* package in R (Olivoto and Lúcio, 2020). Principal Component Analysis (PCA) was used to explore patterns and relationships between cultivars based on the measured variables. Before running PCA, the suitability of the data was checked using Bartlett's test and the Kaiser-Meyer-Olkin (KMO) measure. PCA was used mainly as a tool to visualize the data, with results shown in a biplot to display how cultivars and variables relate. No specific rules for reducing dimensions or keeping factors were used for further analysis. The PCA plots were made using the *factoextra* package in R (Kassambara & Mundt, 2020). The

statistical analysis was performed using R software (version 4.5.2) (R Core Team, 2025).

RESULTS AND DISCUSSIONS

The analysis of the data recorded at the Turda meteorological station highlights variations in the thermal and rainfall regimes throughout the study years. This context reflects current climatic trends also captured by other authors (Şimon et al., 2025), with direct effects on the development of spring two-row barley, particularly during the grain formation and filling period (Filip, 2022).

In 2017, monthly thermal values were generally close to the 60-year multi-annual averages,

except for March and June, when positive deviations of approximately +4°C and +3°C, respectively, were recorded. The higher temperatures in March, associated with favorable rainfall conditions, led to rapid crop emergence. Precipitation in April and May ensured adequate conditions for the main vegetative phenophases. In June, a rainfall deficit of approximately 50 mm was recorded, generating episodes of water stress, which were partially offset by precipitation in the second decade of the month and higher amounts in July, coinciding with the grain-filling period. Overall, 2017 can be considered favorable for spring crops in the Transylvanian Plain (Figure 1).

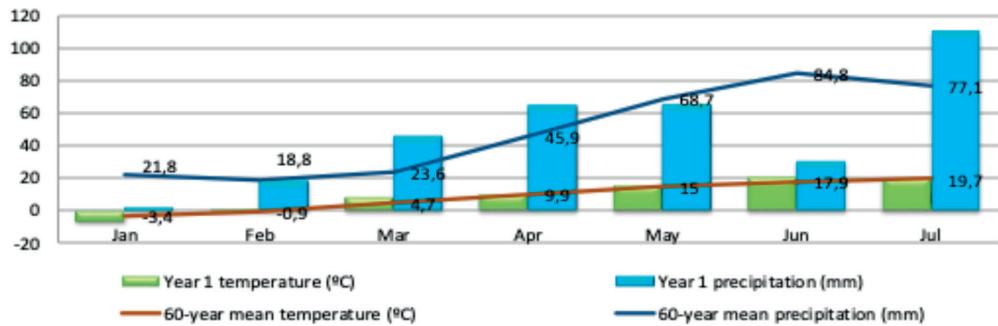


Figure 1. Climatic characteristics of the first crop year, Turda

In 2018, climatic conditions in March, characterized by low temperatures and precipitation, led to a sowing delay of approximately two weeks. Subsequently, high temperatures during the post-sowing period favored the development of specific cereal pests, particularly the cereal

leaf beetle (*Lema melanopa*). During the critical period for grain formation and filling, climatic conditions were close to the seasonal norm, with moderate increases in temperature (+1.5°C) and precipitation (+3.4 mm) compared to the multi-annual averages (Figure 2).

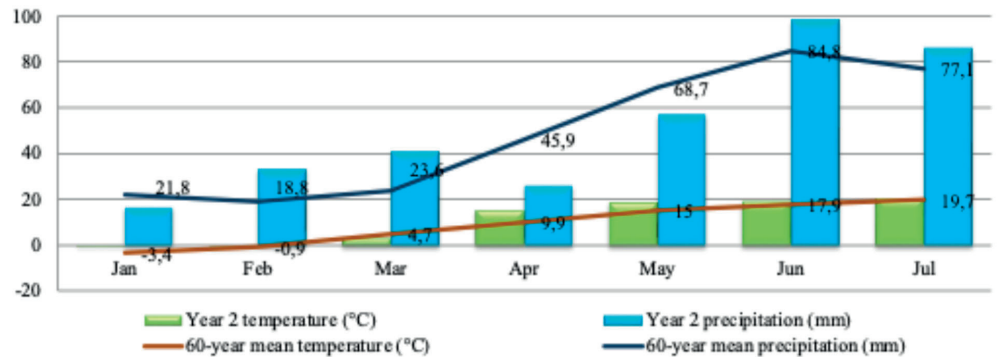


Figure 2. Climatic characteristics of the second crop year, Turda

In 2020, the spring was relatively close to the thermal norm, but the rainfall regime showed marked variations. Abundant precipitation in February and March delayed sowing operations, which was followed by a period of drought in April (-28.1 mm) and May (-24.3 mm). In contrast, significant rainfall in June favored the occurrence of specific cereal diseases (septoria, rusts, fusarium head blight). The succession of

these unfavorable climatic conditions had a negative impact on yield; thus, 2020 can be considered unfavorable for spring crops (Figure 3). This situation was also reported at the national level, with approximately 1.1 million hectares of agricultural crops being affected by extreme climatic conditions (www.agrostandard.ro).

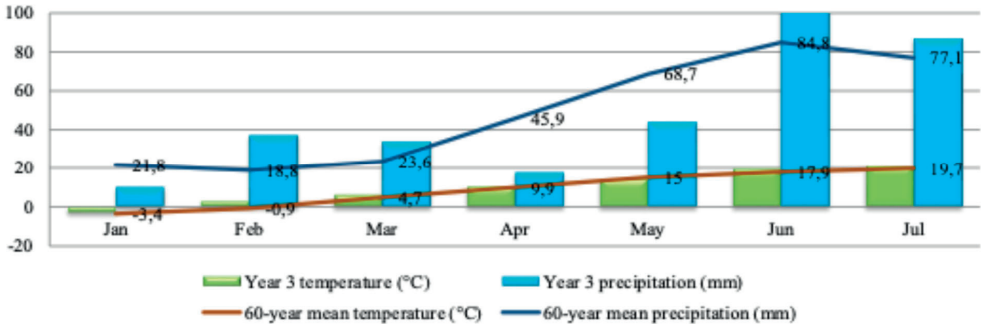


Figure 3. Climatic characteristics of the third crop year, Turda

The analyses revealed significant differences among cultivars for grain weight, thousand-grain weight, and starch content ($p = 0.012$, $p = 0.003$, and $p = 0.002$, respectively). The highest grain weight was recorded in the cultivar

Daciana, while the greatest thousand-grain weight was observed in Romanița. The cultivar Șteffi showed the highest starch content among the studied cultivars (Table 1).

Table 1. Quantitative and qualitative grain performance of selected varieties

Variable	Grain number	Grain weight (g)	Thousand-grain weight (g)	Protein content (%)	Starch content (%)
Romanița	28 [27; 30] a	1.59 [1.41; 1.73] ab	54.99 ± 3.8 c	11.69 ± 1.3 a	55.98 ± 0.7 abc
Daciana	28 [27; 30] a	1.60 [1.37; 1.68] b	54.04 ± 4.6 bc	12.65 ± 0.9 a	55.11 ± 1.0 ab
Jubilant	26 [23; 28] a	1.35 [1.20; 1.39] ab	49.58 ± 2.1 abc	13.06 ± 2.8 a	53.86 ± 1.9 a
Marlen	26 [26;28] a	1.39 [1.30; 1.43] ab	51.19 ± 1.3 abc	13.03 ± 1.5 a	56.48 ± 1.3 abc
Alexis	27 [24; 28] a	1.19 [1.17; 1.57] a	47.05 ± 9.2 ab	13.35 ± 1.7 a	56.64 ± 0.9 abc
Șteffi	27 [25; 28] a	1.11 [1.07; 1.44] a	46.07 ± 6.0 a	12.25 ± 1.9 a	58.16 ± 2.8 c
Germina	28 [21; 28] a	1.44 [0.92; 1.46] ab	49.47 ± 4.5 abc	12.22 ± 2.2 a	57.49 ± 1.7 bc
p-value	0.311	0.012	0.003	0.733	0.002

Note: data as mean ± SD or median (25th-75th percentile); Different superscript letters (a, b, c) within the same column indicate statistically significant differences among means according to post-hoc Tukey's HSD test ($p < 0.05$).

Strong positive correlations were observed between GN and GW ($r = 0.83$, $p < 0.001$), as well as between GW and TGW ($r = 0.91$, $p < 0.001$), indicating that increases in grain number and grain weight are closely associated. TGW also showed a moderate positive correlation with GN ($r = 0.63$, $p < 0.001$).

In contrast, protein content (PC) presented significant negative correlations with GN ($r = -0.61$, $p < 0.001$), GW ($r = -0.52$, $p < 0.001$), and TGW ($r = -0.34$, $p < 0.05$), suggesting that

higher grain number and weight parameters is associated with lower protein content.

Starch content (SC) showed weak negative correlations with GN ($r = -0.34$, $p < 0.05$), GW ($r = -0.35$, $p < 0.05$), and TGW ($r = -0.37$, $p < 0.05$), while its correlation with PC was positive but not statistically significant ($r = 0.14$, $p > 0.05$) (Figure 4).

These results suggest that as grain number and weight increase, protein content tends to decrease, showing a trade-off between yield and

protein. Starch content, however, has weaker and less clear relationships with these traits. Principal Component Analysis (PCA) was performed to explore the relationships among five variables: grain number (GN), grain weight (GW), thousand-grain weight (TGW), protein content (PC), and starch content (SC). Prior to conducting the analysis, assumptions were checked: the Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.651, exceeding the recommended threshold of 0.6, and Bartlett's test of sphericity was significant ($p < 0.001$), indicating that the data were suitable for PCA. The first two principal components explained approximately 84.63% of the total

variance (PC1: 65.03%, PC2: 19.6%), indicating a good representation of the data in the two-dimensional space.

The distribution of points colored by cultivar revealed distinct differences in the profiles of the measured variables. For example, the cultivar Germina was associated with higher protein content values, compared to Șteffi that tended to group in the region characterized by higher starch content.

Other cultivars, such as Daciana and Romanița, grouped predominantly on the negative side of PC1, corresponding to higher values of grain number and grain weight (Figure 5).

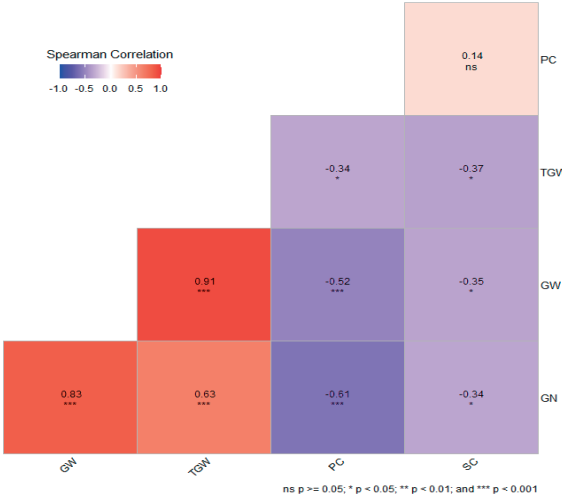


Figure 4. Spearman correlation matrix among yield and quality parameters in selected spring barley varieties. Note: GN = Grain number; GW = Grain weight; TGW = Thousand-grain weight; PC = Protein content and SC = Starch content

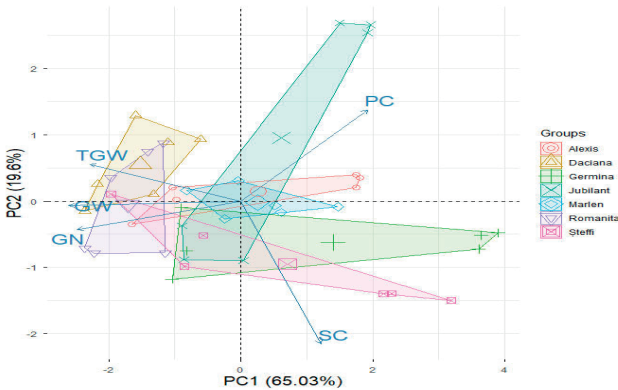


Figure 5. Principal Component Analysis (PCA) biplot of yield and quality parameters in seven spring barley varieties

CONCLUSIONS

The multi-annual evaluation of the seven spring barley genotypes provided a robust framework for analyzing the interactions between yield potential and quality parameters, capturing the plants' response to wide climatic variability. Significant genotypic diversity was observed: the Daciana and Romanița varieties recorded superior performances for yield components (grain weight and thousand-grain weight, respectively), while the Șteffi variety stood out for its superior starch accumulation. Statistical analyses confirmed negative correlations between productivity parameters and protein content. Integrating these results into the requirements of the brewing industry (optimal protein content between 10-12%), the Romanița variety (11.69%) emerges as a suitable option for malting, combining optimal physical indices with an appropriate quality profile. These findings offer clear directions for the breeding programs at SCDA Turda, facilitating the selection of genotypes with high ecological plasticity capable of supporting well-defined capitalization pathways.

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RESEARCH REGARDING EFFICACY OF FENPICOXAMID TREATMENT IN THE CONTROL OF SOME PATHOGENS IN WINTER WHEAT

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Abstract

This study evaluated the efficacy of fenpicoxamid, a novel picolinamide fungicide, for controlling major wheat foliar diseases under Romanian field conditions. Field trials were conducted during 2024/2025 growing season in Buzău and Neamț counties using Anapurna wheat variety. A standardized fungicide programme including SDHI, strobilurin, triazole, picolinamide, and their mixtures was applied across both locations. Disease pressure from *Zymoseptoria tritici*, *Puccinia recondita*, and *Blumeria graminis* was recorded during the season. The combination of fenpicoxamid + prothioconazole (50 g/L + 100 g/L) provided over 90% control of septoria tritici blotch (STB) at both sites. The efficacy against brown rust attack was 86.2% in Buzău location and 84.3% in Neamț location. Efficacy against powdery mildew was higher than 82% in both locations. The results demonstrated a high and constant efficacy under different pedoclimatic conditions. These conclusions confirm fenpicoxamid as an effective tool in integrated management of wheat diseases. Its performance supports its use in diverse production systems.

Key words: efficacy, fenpicoxamid, wheat, foliar diseases, field performance.

INTRODUCTION

Wheat remains one of the most extensively cultivated and strategically important cereal crops worldwide, playing a central role in global food security. At European level, including in Romania, according to Figure 1 and Figure 2 (INS-2026), wheat continued to occupy the largest share of arable land in 2024/2025 season, confirming its agronomic and economic importance within cropping systems.

Monitoring the evolution of cultivated areas and crop production of major crops in Romania represents a continuous concern for agricultural research (Toth and Cristea, 2018; Arghiroiu et al., 2015; Popescu et al., 2018).

Wheat production is increasingly challenged by a complex spectrum of foliar fungal pathogens that can severely reduce yield and grain quality. Among the most economically important diseases are septoria tritici blotch caused by *Zymoseptoria tritici*, leaf rust caused by *Puccinia recondita*, and powdery mildew caused by *Blumeria graminis* f. sp. *tritici*.

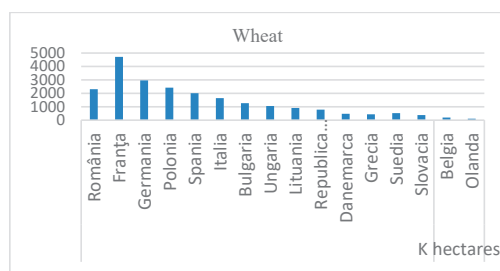


Figure 1. Wheat cultivated area in major European Union countries during the 2024/2025 season (INS - 2026)

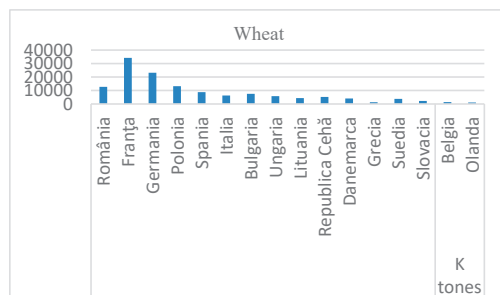


Figure 2. Wheat production in major European Union countries during the 2024/2025 season (INS - 2026)

Leaf septoria frequently occurs in wheat crops in temperate regions and causes losses considered a major issue for wheat cultivation (Fones & Gurr, 2015). Also, brown rust or leaf rust is a fungal disease with high incidence, resulting in significant damage (Chaudhari & Chaudhari, 2013). Powdery mildew causes losses during severe attacks, affecting leaf area and yield (El Shamy et al., 2012; Costamilan, 2005). These pathogens are widely distributed across temperate regions and are responsible for recurrent epidemics, leading to significant yield losses under favorable environmental conditions. In recent years, increased climatic variability, characterized by fluctuating temperatures and prolonged leaf wetness periods, has further enhanced disease pressure and unpredictability.

Effective disease management is therefore essential to sustain wheat productivity. Current strategies rely on an integrated disease management (IDM) framework, combining host resistance, agronomic practices, disease monitoring, and chemical control. However, the durability of these strategies is increasingly threatened by the evolution of pathogen resistance to key fungicide modes of action, particularly within widely used classes such as azoles (DMIs) and SDHIs (AHDB, 2026). The use of synthetic chemicals remains a necessary measure (Esmail & Draz, 2017). This challenge is further intensified by regulatory constraints at the European level, which have reduced the number of available active substances and emphasized the need for more sustainable plant protection solutions. In this context, the introduction of novel fungicide active ingredients with new modes of action is critical. Fenpicoxamid (Inatreq™ active), belonging to the picolinamide chemical group, represents a significant advancement in cereal disease control, offering a unique mode of action and high efficacy against major foliar pathogens, particularly *Zymoseptoria tritici*.

Its use in combination with established triazoles such as prothioconazole provides both curative and protective activity, while also contributing to resistance management strategies. The present study evaluates the efficacy of fenpicoxamid in combination with prothioconazole for the control of key wheat diseases caused by *Zymoseptoria tritici*,

Puccinia recondita, and *Blumeria graminis* f. sp. *tritici*. The findings aim to support the optimization of fungicide programs and to contribute to the development of sustainable disease management strategies in modern wheat production systems.

MATERIALS AND METHODS

Field trials were conducted in Dulbanu (Buzău County) and Zănești (Neamț County) using a foreign wheat genotype (the Anapurna variety) during the 2024-2025 growing season. Both locations were selected based on their historical vulnerability to *Zymoseptoria tritici*, *Puccinia recondita*, and *Blumeria graminis*. The experimental layout followed a randomized block design with four replications per treatment, as illustrated in Figure 3. The minimum size of each experimental plot was 3 × 10 m.

R1	V1	V2	V3	V4	V5	V6
R2	V6	V1	V2	V3	V4	V5
R3	V5	V6	V1	V2	V3	V4
R4	V4	V5	V6	V1	V2	V3

Figure 3. Trial map

The tested fungicides included succinate dehydrogenase inhibitors (SDHIs), strobilurins, triazoles, picolinamides (fenpicoxamid), and their mixtures. Fenpicoxamid was applied at a rate of 1.5 L/ha (50 g/L fenpicoxamid + 100 g/L prothioconazole) as the treatment of interest. Table 1 shows the details of treatment applications at the two trial locations.

Table 1. Fungicide treatment protocol, application doses, solution volumes, and wheat growth stages at application

Trt. No.	Treatment name	Dose rate L/ha	Solution volume	Crop stage
V1	Fenpicoxamid 50 g/l + Prothioconazole 100 g/l	1.5	200 l/ha	BBCH 49
V2	Bezovindiflupir 75 g/l + Prothioconazole 150 g/l	1.0	200 l/ha	BBCH 49
V3	Bixafen 75 g/l + Prothioconazole 150 g/l	1.0	200 l/ha	BBCH 49
V4	Fluxapirroxad 75 g/l + Prothioconazole 150 g/l	1.0	200 l/ha	BBCH 49
V5	Azoxistrobin 250 g/l + Prothioconazole 250 g/l	0.8 +0.6	200 l/ha	BBCH 49
V6	Untreated	-	-	-

All treatments were applied according to trial protocol and following EPPO guidelines.

Fungicides were applied using a hand sprayer calibrated for uniform coverage of each plot (Figure 4). The treatments were applied at BBCH 49 wheat growth stage in accordance with fungicide labels and trial protocol to ensure optimal timing for pathogens control.



Figure 4. Application of protocol in trial location

In Dulbanu (Buzău County), the treatments were applied on May 2, 2025, at 14:00 h. Application conditions were favorable, featuring an air temperature of 20°C, a wind speed of 8 km/h, and a relative humidity of 49%.

In Zănești (Neamț County) the treatments were applied on May 10, 2025, at 13:30 h. Application conditions were also favorable, with an air temperature of 17°C, a wind speed of 6 km/h, and a relative humidity of 56%.

After preparing the spraying solution for each treatment, their physical properties: miscibility, stability, and sedimentation, were analyzed.

All treatments were safe for the crop, with uniform distribution in the solution volume, without sedimentation. Immediately after application, we observed the coverage power of each treatment, where the treatment with fenpicoxamid and prothioconazole stood out for its excellent leaf coverage, due to the innovative IQ4 formulation.

To determine the efficacy (E%) of each treatment in the protocol at each location, a phytosanitary check (assessments) was conducted before application, as well as after application at 7 days, 14 days, and 28 days, identifying the pathogens present in the crop, and calculating the attack frequency (F%) and intensity (I%) to determine the severity of the attack. The following formulas were used:

- $F = (n \times N) / 100$ (%), where: F = attack frequency; n = number of affected plants; N = total number of observed plants;

- $I (\%) = \Sigma (i \times f) / n$, where: I = intensity; i = attack percentage; f = number of plants with that percentage; n = total number of plants with the disease;
- $GA\% = F\% \times I\% / 100$.

Based on the attack severity data, the efficacy of the treatments in the protocol was calculated using Abbott's formula:

$E (\%) = [(Gam - Gav) / Gam] \times 100$, where:

- Gam = attack severity of the untreated control;
- Gav = attack severity of the treated variant.

RESULTS AND DISCUSSIONS

The 2024-2025 growing season (March-July 2025) exhibited distinct agroclimatic patterns across the two experimental sites, Dulbanu (Buzău County) and Zănești (Neamț County), as evidenced by the Granular Link weather data (Figure 5 and Figure 6). In both locations, temperatures followed a progressive upward trend, increasing from approximately 5-10°C in early March to sustained values of 25-32°C during June-July, with occasional peaks exceeding 35°C.

Precipitation was unevenly distributed throughout the season, with notable differences between the two sites. Dulbanu experienced lower cumulative rainfall and longer dry intervals, particularly during April and early June, contributing to moderate drought stress. In contrast, Zănești recorded higher precipitation amounts, including a significant rainfall peak in the second half of May, which temporarily increased relative humidity and created favorable conditions for disease development.

A key observation from both locations was that May 2025 was characterized by lower-than-average temperatures combined with increased rainfall compared to normal seasonal conditions. This combination of cooler and wetter weather created optimal conditions for the development and spread of foliar pathogens, resulting in elevated disease pressure during a critical stage of wheat growth.

These environmental conditions directly influenced disease management decisions. In response to the identified infection risk window, fungicide treatments were applied according to the experimental protocol. The timing of these

applications was strategically aligned with pre-heading stages, ensuring effective protection of the crop and maximizing the efficacy of the treatments under the prevailing climatic conditions.

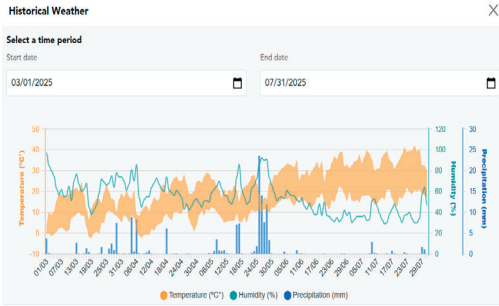


Figure 5. Evolution of temperatures and precipitation from March to July 2025 (Dulbanu, Buzău)

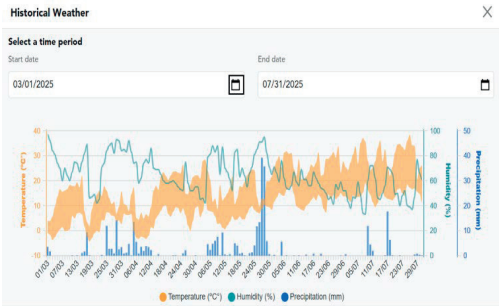


Figure 6. Evolution of temperatures and precipitation from March to July 2025 (Zănești, Neamț)

The treatment with 50 g/L fenpicoxamid + 100 g/L prothioconazole provided significant control over all the monitored pathogens in both Buzău and Neamț counties. The data show a high efficacy against the pathogen *Zymoseptoria tritici* (wheat septoria), *Puccinia recondita* (brown rust), and *Blumeria graminis* f. sp. *tritici* (wheat powdery mildew), with disease attack reductions of over 80%.

Efficacy against *Zymoseptoria tritici* (wheat septoria)

The combined treatment significantly reduced the severity of septoria in both counties, with an overall efficacy of 90.7% (Figure 7) in Buzău and 90.1% (Figure 8) in Neamț. These results demonstrate the robust protective activity of the fenpicoxamid-prothioconazole combination, which performed better than most of other treatments evaluated in the study.

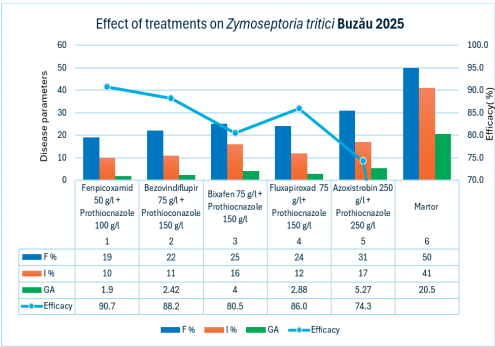


Figure 7. Efficacy of treatments in controlling *Zymoseptoria tritici* (Dulbanu, Buzău)

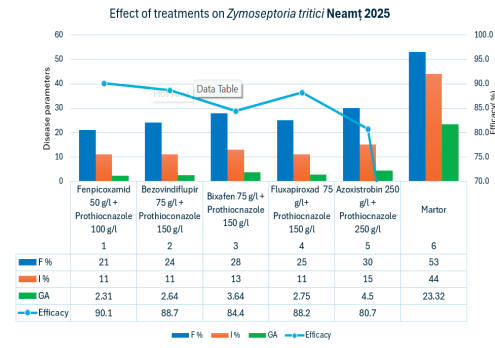


Figure 8. Efficacy of treatments in controlling *Zymoseptoria tritici* (Zănești, Neamț)

Efficacy against *Puccinia recondita* (brown rust)

The treatment was also highly effective in controlling brown rust, showing disease severity reduction rates of 86.2% (Figure 9) in Buzău and 84.3% (Figure 10) in Neamț. This suggests that fenpicoxamid has broad-spectrum activity, providing consistent control across different climate.

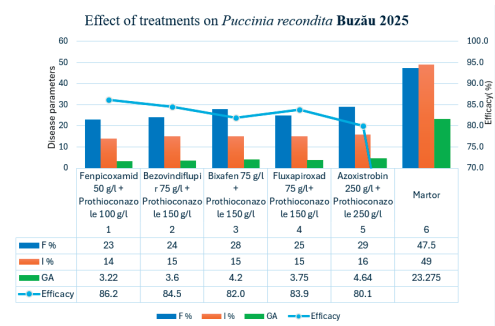


Figure 9. Efficacy of treatments in controlling *Puccinia recondita* (Dulbanu, Buzău)

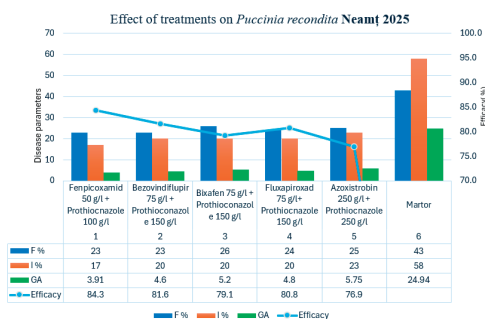


Figure 10. Efficacy of treatments in controlling *Puccinia recondita* (Zănești, Neamț)

Efficacy against *Blumeria graminis* f. sp. *tritici* (wheat powdery mildew)

Although the combined treatment was somewhat less effective against wheat powdery mildew, it still provided a solid reduction in disease incidence. The efficacy rates were 82.7% (Figure 11) in Buzău and 82.4% (Figure 12) in Neamț, indicating that fenpicoxamid is a valuable tool for managing this pathogen.

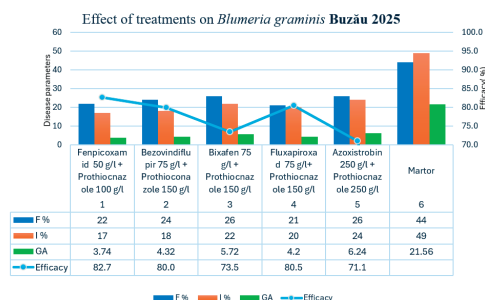


Figure 11. Efficacy of treatments in controlling *Blumeria graminis* (Dulbanu, Buzău)

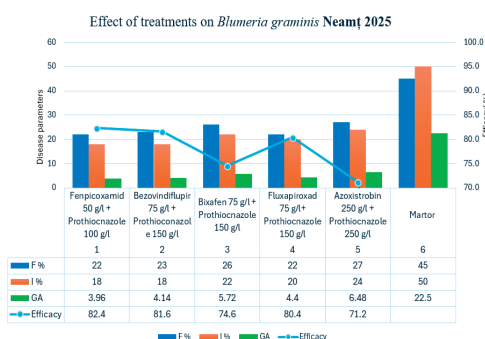


Figure 12. Efficacy of treatments in controlling *Blumeria graminis* (Zănești, Neamț)

Research on the efficacy of treatments in controlling pathogens is a constant concern for establishing phytosanitary interventions and integrating them into plant integrated pest management schemes (Toth and Cristea, 2020; Chiriac and Cristea, 2021), as well as for wheat cultivation (Iosub et al., 2022). Iosub et al. (2022) determined the efficacy values of some molecules recommended for controlling wheat pathogens, including powdery mildew, brown rust, and septoria, ranging between 90% and 82%.

CONCLUSIONS

The novel mode of action of fenpicoxamid, combined with the complementary and synergistic activity of its mixture partner, prothioconazole, demonstrated high efficacy in controlling key wheat pathogens across the experimental sites. In particular, control of *Zymoseptoria tritici* exceeded 90%, highlighting the strong performance of this combination under the prevailing environmental conditions. For *Puccinia recondita*, control efficacy ranged between 84.3% and 86.2%, while *Blumeria graminis* was effectively managed with control levels above 82% at both locations. These results are consistent with current fungicide performance standards and confirm the reliability of this mixture under variable disease pressure.

The use of fenpicoxamid in combination with prothioconazole provides not only effective disease control but also contributes to resistance management by introducing a novel mode of action, thereby reducing the risk of cross-resistance development.

Overall, these findings support the inclusion of this fungicide combination as a valuable component of integrated pest management (IPM) strategies in wheat production. However, further research is required to evaluate its long-term performance and its role in sustainable resistance management under diverse agroecological conditions.

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CHARACTERIZATION OF BLACK POINT DISEASE IN WINTER WHEAT: EPIDEMIOLOGY, SEVERITY SCORING AND FUNGAL COMMUNITY STRUCTURE

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Abstract

Black point is a common fungal disease of wheat caused by several genera, including Alternaria sp., Cochliobolus sp., Cladosporium sp., and Fusarium sp., leading to embryo discoloration and reduced grain quality. This study evaluated winter wheat varieties to characterize disease epidemiology, assess the incidence and severity of kernel darkening, and identify the associated fungal community. Favorable weather conditions during grain filling resulted in variable disease levels among genotypes. Significant differences were observed in both the proportion of affected kernels and the severity of discoloration. Fungal isolations confirmed Alternaria sp. as the dominant pathogen, accompanied by Cochliobolus sp., Cladosporium sp., Fusarium sp., Epicoccum sp., Curvularia sp., and Chaetomium sp.. The results provide insights into varietal responses to black point under Romanian conditions and support breeding and management strategies to mitigate disease impact and maintain grain quality.

Key words: winter wheat, black point, kernel discoloration, fungal disease.

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide. Wheat represents a fundamental component in ensuring global food security (Sharma & Sharma, 2025).

In Romania, autumn wheat is the most widely cultivated crop due to its high economic importance and the favorable environmental conditions for its cultivation. In 2024, approximately 9.3 million tons of wheat were harvested from an area of about 2.3 million hectares (FAO, 2025).

The quality of wheat kernels is a key factor in determining the market value of the crop and in appropriately assessing its suitability for processing, particularly in the milling and baking industries (Awulachew, 2020). However, wheat production is often affected by various diseases, especially fungal ones, which compromise both yield and kernel quality (Duveiller et al., 2007; Aakash Goyal et al., 2010; Rózewicz et al., 2021).

Among the fungal and physiological factors that influence wheat kernel quality, black point is a significant defect (Malaker et al., 2009;

Srivastava et al., 2014). This disorder, manifested by discoloration in the embryo region of the kernels, can considerably reduce both the visual appearance and the technological value of wheat, especially when it is intended for the milling and baking industries (Clarke et al., 2005; Golosna, 2021). Some countries have established limits for the percentage of wheat kernels affected by black point. Globally, these limits vary significantly, as there is no international standard that clearly defines this disorder, and regulation is primarily carried out through national standards. For example, in Australia, the competent authorities have set a maximum limit of 5% for bread wheat and 3% for durum wheat. Exceeding these thresholds results in the classification of wheat lots as downgraded (Rees et al., 1984; Lehmensiek et al., 2004). In Canada, according to the Canadian Grain Commission, black point is a criteria used to classify wheat kernels into different grades. For durum wheat, the tolerance levels are 5%, 10%, and 20% for Grades No.1, No.2, and No.3, respectively, while for common wheat, the tolerances are 10%, 20%, and 35% for the same grades (Canadian Grain Commission, 2025). Another country that has established wheat

kernel quality standards based on black point symptoms is Turkey (Aktaş and Endes, 2025). According to Turkish Standards Institution standards, wheat is considered of low quality if more than 8% of the kernels are affected. The maximum threshold for purchase is 14%, highlighting the restrictions imposed by many countries in international trade (TSE, 2023). In Romania, national standards, aligned with European regulations, do not specify percentage limits exclusively for black point, unlike in the countries mentioned above.

For common wheat, the grading manual includes a factor called “kernels with discolored testa,” without an explicit reference to black point, with a maximum limit of 0.5% for Grades 1 and 2; Grade 3 is not regulated.

For durum wheat, the grading factor “stained kernels” sets maximum limits of 2% for Grade 1, 3% for Grade 2, and 5.8% for Grade 3 (CNGSC, 2017). Given the significant impact of black point on wheat kernel quality, understanding the occurrence of this disease, its

severity, and the associated fungal communities is essential.

This study aims to characterize black point in winter wheat by evaluating the disease’s epidemiology, determining its incidence in the studied kernels using severity scoring, and investigating the structure of the associated fungal communities.

MATERIALS AND METHODS

The winter wheat kernels used in this study were harvested from the wheat experimental platform organized by ACCPT Iași “Grânarii” at the S.C. Agralmixt S.A. farm during the 2024–2025 agricultural year.

The experimental platform was established in Andrieșeni, Iași County (approximate coordinates: 47°29’50”N, 27°16’50”E).

The area is characterized by a temperate continental climate, with moderate precipitation and mean annual temperatures of about 11.8°C (Table 1).

Table 1. Monthly climatic conditions and wheat phenophases during the 2024–2025 growing season

Month	Air temperature (°C)			Precipitation	Phenophase	Average Air temperature(°C)	Precipitation (mm)
	Minimum	Maximum	Average				
September	5.55	41.12	19.86	131.0	Field preparation Sowing Emergence	11.25	214.6
October	-3.81	33.77	10.86	37.0			
November	-7.14	24.92	3.05	46.6			
December	-8.56	17.45	1.79	56.6	Winter reserve	2.69	112.0
January	-9.63	20.98	2.55	13.4			
February	-14.59	15.07	-2.13	5.0			
March	-6.41	28.07	8.55	37.0			
April	-4.74	35.56	12.09	24.8	Booting – Heading – Flowering	13.19	183.2
May	0.45	37.34	14.28	158.4			
June	6.59	39.37	22.75	18.8	Grain filling	22.75	18.8
July	9.30	43.66	24.99	98.2	Full maturity	24.00	98.2
August	3.49	42.21	23.45	5.4	Post-harvest	23.45	5.4
Average (°C) / Sum (mm)		-2.46	31.63	11.84	632.20		

Note: Climatic data were obtained from the weather station 00001CED located within the experimental farm, via the website www.fieldclimate.com

The climatic conditions during the 2024-2025 growing season created favorable environments for the development of black point disease. Consequently, kernels harvested from the 34 wheat cultivars (Table 2) were analyzed for the presence of black point symptoms.

Regarding cultivation practices, a uniform agronomic protocol was applied across all 34 cultivars. Sowing was performed in the autumn

of 2024, during the optimal period for the region (24-25 October 2024). The preceding crop was sunflower. Fertilization was standardized, with 200 kg of NPK 14:28:14 + 5.5 S applied per hectare. Three phytosanitary treatments were applied to control all winter wheat-specific pests, weeds, and pathogens. The crop was harvested on 22 July 2025.

Table 2. Winter wheat cultivars included in the study

No.	Winter wheat cultivar	No.	Winter wheat cultivar
1	ADRIATIC	18	GLIA
2	AGENOR	19	JUNIOR
3	ANAPURNA	20	LEONIDUS
4	APEXUS	21	MIX CEREALE
5	APILCO	22	MUSIK
6	ARTIMUS	23	PASSION
7	ASTERION	24	PG 102
8	AVENUE	25	PIBRAC
9	BASILIO	26	PROMITOR
10	BOROMIR	27	ROCAILLOU
11	CELEBRITY	28	RUBISKO
12	CHEVIGNON	29	SACRAMENTO
13	COMPLICE	30	SPHERE
14	DATINA	31	STK 3
15	EXTREME	32	STK EXP
16	FALADO	33	TOSCADOU
17	FARMEO	34	ULTIM

The evaluation of black point infection was performed on wheat kernels obtained from the studied wheat cultivars. To determine the incidence of black point infection, 100 caryopses per cultivar were analyzed in three replications. The wheat kernels were randomly selected using a sample divider. The caryopses were carefully examined (Figure 1), and the incidence (%) was calculated as the percentage of affected kernels (grade ≥ 1) out of the total number of analyzed kernels.

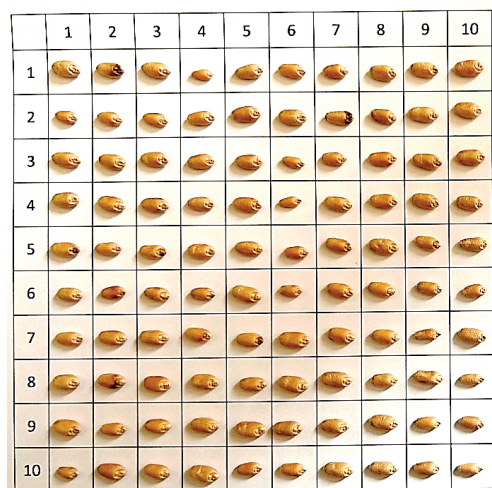


Figure 1. Arrangement of 100 wheat kernels (10×10 grid) used for the visual evaluation of black point incidence. Each kernel was individually examined and classified according to the presence of symptoms

Disease severity was assessed using a six grades rating scale (0-5) adapted for black point in wheat (Table 3), according to the visual

symptoms observed on the kernels. (Gilchrist, 1985).

Tabel 3. Rating scale for black point severity

Rating scale	
Grade 0	Kernel free from any discoloration (apparently healthy)
Grade 1	Tip of the embryo brown to blackish
Grade 2	Discoloration covering the whole embryo
Grade 3	Embryo with $\frac{1}{4}$ of the grain discoloured
Grade 4	Embryo with $\frac{1}{2}$ of the grain discoloured
Grade 5	Embryo with more than $\frac{1}{2}$ of the grain discoloured and shrivelled

A total of 100 kernels per sample were evaluated and classified into the corresponding severity classes. The percentage of kernels in each class was then used to calculate the Disease Severity Index (DSI) as follows (Mishra et al., 2017):

$$DSI(\%) = \left[\frac{\sum(s*n)}{N*S_{max}} \right] * 100,$$

where:

s represents the severity class (0-5);

n is the number of kernels in each class;

N is the total number of analysed kernels;

S_{max} is the maximum score of the rating scale.

To determine the fungal communities associated with wheat kernels affected by black point, 100 kernels from each of the 34 studied cultivars were analysed, of which 75 exhibited visible black point symptoms and 25 were apparently healthy, randomly selected from the harvested samples. The kernels were disinfected by immersion in a 1% sodium hypochlorite solution for 3 minutes, followed by 3–4 rinses with sterile distilled water to remove any residual disinfectant. After drying, the kernels were placed on Petri dishes (9 cm diameter) containing 20 ml of PDA medium (Potato Dextrose Agar) and incubated at 28°C for 5–8 days. Subsequently, emerging fungal colonies were observed and their presence was recorded per plate. Colonies were identified based on their morphological characteristics (Ellis and Ellis, 1985). The aim of this study was to assess the presence of fungi rather than to perform a numeric quantification for each individual kernel.

Statistical analysis of the data was performed using IBM SPSS Statistics 26 software.

Differences among cultivars were evaluated by one-way analysis of variance (ANOVA). Mean comparisons and the identification of homogeneous groups of cultivars were conducted using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p \leq 0.05$. Mean values followed by different letters indicate statistically significant differences among cultivars.

RESULTS AND DISCUSSIONS

Black point was observed in wheat kernels across the studied cultivars, with incidence and severity influenced by environmental conditions during the early grain-filling stage (Conner et al., 1998). Factors such as high humidity, prolonged rainfall, and extreme temperatures are known to promote fungal colonization and accelerate disease progression (Khani et al., 2018). The disease is typically associated with fungal species including *Alternaria* sp., *Fusarium* sp., *Epicoccum* sp., and *Bipolaris* sp., which can colonize the kernels either before or shortly after anthesis (Nuray, 2004; Toklu et al., 2008; Gafencu et al., 2019). Epidemiological studies indicate that both host susceptibility and the timing of environmental stress play crucial roles in determining the extent of infection. High moisture conditions favour spore germination and fungal growth, while temperature fluctuations can further exacerbate the susceptibility of developing kernels.

Under the climatic conditions and using the biological material studied during the 2024-2025 growing season, the following results regarding the presence of winter wheat kernels exhibiting symptoms associated with black point are presented in Table 4.

Symptoms associated with black point disease were observed in the wheat kernels of all cultivated varieties, except for the Sacramento cultivar. The disease occurred at varying intensities across the varieties, highlighting differences in susceptibility among the cultivars examined. The frequency (%) of black point infection ranged from 0.00% in Sacramento to 12.00% in Datina, indicating a significant variation in the proportion of infected kernels among cultivars. The lowest frequency values were also observed in Rubisko (0.67%±0.58%) and Junior (0.67%±0.58%), suggesting that

these varieties exhibited higher tolerance under the experimental conditions. In contrast, the varieties Datina (12.00%±2.00%), Avenue (11.33%±1.53%), and Asterion (11.00%±1.00%) showed the highest frequency values, indicating greater susceptibility to black point occurrence. Intermediate frequency values (approximately 5-7%) were observed in Adriatic, Anapurna, Apexus, and Boromir, indicating moderate susceptibility.

Tabel 4. Frequency and Disease Severity Index of black point in wheat kernels across studied cultivars

Winter wheat cultivar	Frequency (%)	Disease Severity Index (DSI%)
ADRIATIC	5.33% ± 1.53% ^c	1.73% ± 0.42% ^b
AGENOR	6.67% ± 1.53% ^{de}	2.93% ± 0.90% ^h
ANAPURNA	5.67% ± 1.53% ^{cd}	2.40% ± 0.69% ^f
APEXUS	6.67% ± 0.58% ^{de}	2.40% ± 0.60% ^f
APILCO	3.67% ± 1.53% ^{ab}	1.07% ± 0.61% ^c
ARTIMUS	4.33% ± 1.15% ^{bc}	1.07% ± 0.31% ^c
ASTERION	11.00% ± 1.00% ^g	4.80% ± 0.35% ^g
AVENUE	11.33% ± 1.53% ^g	5.47% ± 1.10% ^k
BASILIO	2.00% ± 1.00% ^a	0.73% ± 0.42% ^d
BOROMIR	10.67% ± 2.52% ^{fg}	4.60% ± 0.69% ^g
CELEBRITY	5.33% ± 0.58% ^c	2.67% ± 0.42% ^e
CHEVIGNON	4.33% ± 2.08% ^{bc}	1.13% ± 0.61% ^c
COMPLICE	1.33% ± 1.53% ^a	0.27% ± 0.31% ^a
DATINA	12.00% ± 2.00% ^g	5.53% ± 0.64% ^k
EXTREME	8.00% ± 1.00% ^{ef}	3.53% ± 0.95% ^j
FALADO	3.00% ± 1.00% ^{ab}	1.07% ± 0.31% ^c
FARMEO	1.33% ± 0.58% ^a	0.33% ± 0.12% ^a
GLIA	2.67% ± 0.58% ^{ab}	1.07% ± 0.61% ^c
JUNIOR	0.67% ± 0.58% ^a	0.40% ± 0.40% ^b
LEONIDUS	4.67% ± 0.58% ^{cd}	2.27% ± 0.42% ^d
MIX CEREALE	6.67% ± 0.58% ^{de}	2.87% ± 0.31% ^e
MUSIK	2.33% ± 1.15% ^{ab}	0.87% ± 0.61% ^c
PASSION	2.67% ± 0.58% ^{ab}	0.73% ± 0.31% ^d
PG 102	5.00% ± 1.00% ^c	1.93% ± 0.31% ^d
PIBRAC	5.33% ± 1.53% ^{bc}	1.73% ± 0.42% ^b
PROMITOR	4.00% ± 1.00% ^{bc}	1.67% ± 0.42% ^b
ROCAILLOU	5.33% ± 1.53% ^c	1.73% ± 0.46% ^b
RUBISKO	0.67% ± 0.58% ^a	0.13% ± 0.12% ^a
SACRAMENTO	0.00% ± 0.00% ^a	0.00% ± 0.00% ^a
SPHERE	5.67% ± 1.53% ^{cd}	2.21% ± 0.52% ^d
STK 3	2.33% ± 0.58% ^{ab}	0.87% ± 0.12% ^c
STK EXP	2.33% ± 1.53% ^{ab}	0.73% ± 0.50% ^d
TOSCADOU	5.00% ± 1.00% ^c	1.07% ± 0.12% ^c
ULTIM	4.33% ± 0.58% ^{bc}	1.73% ± 0.31% ^b

Note: The letters (a, b, c, ..., g for Frequency(%); a, b, c, ..., k for DSI%) indicate homogeneous groups according to Tukey's HSD test. Mean values sharing the same letter are not significantly different ($p > 0.05$), while values with different letters are significantly different ($p < 0.05$). Letters for Frequency (%) and DSI (%) were determined separately.

Although frequency (%) provides information on the number of wheat kernels affected by black point, it does not reflect the intensity or degree of symptom severity for each affected kernel. To obtain a more comprehensive assessment of the disease impact, the Disease Severity Index (DSI%) was also calculated for each cultivar. The DSI values ranged from 0.00% in Sacramento to 5.53% in Datina, highlighting both highly tolerant and highly

susceptible cultivars. Cultivars such as Avenue (5.47%±1.10%), Asterion (4.80%±0.35%), and Boromir (4.60%±0.69%) showed high severity, while Complice (0.27%±0.31%), Rubisko (0.13%±0.12%), and Farneo (0.33%±0.12%) exhibited very low severity levels. Intermediate values were recorded for cultivars such as Adriatic, Anapurna, Apexus, Leonidus, and Extreme, indicating a moderate level of infection. Statistical analysis (ANOVA) revealed significant differences among cultivars for both frequency and DSI ($p < 0.001$). Tukey's HSD test allowed the grouping of cultivars into homogeneous subsets (letters a-g for frequency and a-k for DSI). For example, Datina, Avenue,

and Asterion formed the groups with the highest values (letters g or k), whereas Sacramento, Rubisko, and Complice formed the groups with the lowest values (letter a), highlighting significant differences between highly susceptible and highly tolerant cultivars. The mycological analysis of wheat kernels showing black point symptoms revealed the presence of seven fungal genera associated with this disease. Among the fungal genera identified, *Alternaria* sp. was the most frequently detected, being present in the majority of the analysed varieties, confirming its important role in the development of black point symptoms in wheat (Table 5).

Table 5. Fungal species isolated from wheat kernels affected by black point

Winter wheat cultivar	<i>Alternaria</i> sp.	<i>Cladosporium</i> sp.	<i>Fusarium</i> sp.	<i>Epicoccum</i> sp.	<i>Curvularia</i> sp.	<i>Chaetomium</i> sp.	<i>Cochliobolus</i> sp.
ADRIATIC	+	-	+	+	-	-	+
AGENOR	+	-	+	+	-	-	+
ANAPURNA	+	-	+	+	-	-	+
APEXUS	+	-	+	+	-	-	+
APILCO	+	-	-	-	-	-	-
ARTIMUS	+	-	-	-	-	-	-
ASTERION	+	+	+	+	+	+	+
AVENUE	+	+	+	+	+	+	+
BASILIO	+	-	-	-	-	-	-
BOROMIR	+	+	+	+	+	-	+
CELEBRITY	+	-	+	+	-	-	+
CHEVIGNON	+	-	-	-	-	-	+
COMPLICE	+	-	-	-	-	-	-
DATINA	+	+	+	+	-	+	+
EXTREME	+	+	+	+	+	-	+
FALADO	+	-	-	-	-	-	+
FARNEO	+	-	-	-	-	-	-
GLIA	+	-	-	-	-	-	+
JUNIOR	+	-	-	-	-	-	-
LEONIDUS	+	-	+	+	-	-	+
MIX CEREALE	+	+	+	+	-	-	+
MUSIK	+	-	-	-	-	-	-
PASSION	+	-	-	-	-	-	-
PG 102	+	-	+	+	-	-	+
PIBRAC	+	-	+	+	-	-	+
PROMITOR	+	-	-	-	-	-	+
ROCAILLOU	+	-	+	+	-	-	+
RUBISKO	+	-	-	-	-	-	-
SACRAMENTO	-	-	-	-	-	-	-
SPHERE	+	+	+	+	-	-	+
STK 3	+	-	-	-	-	-	-
STK EXP	+	-	-	-	-	-	-
TOSCADOU	+	-	-	-	-	-	+
ULTIM	+	-	+	+	-	-	+

Note: Presence (+) and absence (-) indicate the occurrence of fungal genera associated with wheat kernels affected by black point.

Fusarium sp. was also isolated from several cultivars, particularly from those showing higher values of frequency and DSI, such as Asterion, Avenue, Boromir, and Datina. Fungal genera such as *Cladosporium* sp., *Epicoccum* sp., and *Cochliobolus* sp. were detected sporadically, being more frequent in varieties

with a higher level of infection. In contrast, *Curvularia* sp. and *Chaetomium* sp. were detected only in a few cases, suggesting a limited involvement in the pathogenic complex associated with black point under the conditions of this study. In some varieties with very low disease frequency, such as Sacramento,

Rubisko, and Complice, the presence of fungal genera was limited or absent.

CONCLUSIONS

The results obtained highlight significant differences among wheat cultivars regarding frequency and the severity of black point disease, indicating varying levels of susceptibility. Cultivars exhibiting high frequency and disease severity index proved to be more susceptible, while others showed greater tolerance under the experimental conditions. The fungal community analysis confirmed the complex nature of the disease, with several fungal genera associated with black point symptoms, among which *Alternaria* sp. was the most frequently detected. The types of studies should be conducted regularly, as certain growing seasons and climatic conditions can favor the development of fungal diseases, including the occurrence of black point in wheat.

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THE EFFECT OF BLACK POINT DISEASE ON THE QUALITY OF WHEAT GROWING IN THE SOUTHERN PART OF ROMANIA

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Abstract

Black point is a disease that affects wheat production quality worldwide, affecting the chemical composition. The aim of the study was to evaluate the effect of black point disease on the quality of wheat grown in southern Romania during 2022-2024. The incidence and severity of black point were determined by classical laboratory analyses. Quality analyses were carried out using standard NIR spectroscopy to determine dry matter, hardness, gluten, crude protein, and starch. The incidence of caryopses with black points varied by year and climatic conditions. The year 2023 stands out, with the incidence of the attack ranging from 3.3% (Line 1) to 10.4% (Ursita). The gluten content was low across all 2022 samples, classified as satisfactory only in the Ursita and Miranda varieties (26.6% and 27.7%, respectively). In 2023, gluten exceeded 30% in Trivale, Line 1 and Line 2 being classified as very good. Statistical analysis (Tukey test) shows significant differences among experimental years and varieties, and when the two factors are combined.

Key words: wheat, black point, proteins, hardness, gluten.

INTRODUCTION

Black point is a major disease of wheat because it affects crop quality (Malaker et al., 2009). Unlike common wheat, durum wheat is the most susceptible to black point infection (Davis & Jackson, 2009; Fernandez et al., 2011). An incidence greater than 10% can negatively impact crop quality (Rees et al., 1984; Lorenz, 1986). Due to its effect on the quality and nutritional value of wheat, the selling price decreases, resulting in economic losses (Solanki et al., 2006; Vuckovic et al., 2012; Mishra & Srivstava, 2015; Patel & Minipara, 2015). In severe infections, seed germination, emergence, and weight are negatively affected (Rahman & Islam, 1998; Özer, 2005; Drasz et al., 2016). When incidence and severity are high, the flour will be of poor quality, with changes in dough colour, odour, and taste (King et al., 1981; Prescott et al., 1986). According to Kashem et al. (1999), black point negatively affects the biochemical composition of wheat, as pathogens involved in the disease reduce the levels of starch and soluble carbohydrates. Furthermore, the same authors show that prolamin, gluten,

and phenolic compounds increased in infected caryopses. "Black point" disease is caused by a complex of fungi and typically occurs when climatic conditions are characterised by high humidity and favourable temperatures, as well as rainfall during the flowering and grain-filling stages (Bhowmink, 1969). If the seed-filling period is characterised by low temperatures and heavy rainfall, the severity of the disease increases (Bhowmink, 1969; Williamson, 1997; Wang et al., 2002). Diseased caryopses are easily recognised by their brownish-black colour in the embryonic region as well as in other parts, depending on the severity of the disease. The blackening may be partial and, in severe cases, even total (Khulbe et al., 2011; Srivstava et al., 2014; Patel & Minipara, 2015). Research on the fungi involved in black point has shown that their composition can vary from one agricultural region to another. Most studies indicate that the most commonly isolated fungi are: *Alternaria* species (most frequently *Alternaria alternata* and *Alternaria trititica*), *Curvularia* spp., *Fusarium* spp., *Drechslera sorokiniana*, *Stemphylium* spp., and *Cladosporium* spp. (Adlakha & Joshi, 1974;

Hasan *et al.*, 2009; Srivastava *et al.*, 2014, cited by Patel & Minipara, 2015; Gafencu *et al.*, 2019). Fernandez *et al.* (1994b) highlight that the predominant fungi on black-pointed caryopses were *Alternaria alternata*, *Cochliobolus sativus*, and *Fusarium* spp. Furthermore, the incidence of caryopses infected with *Alternaria* spp. was higher than that of those infected with *Cochliobolus sativus*. Of the two fungi, *Cochliobolus sativus* is considered far more dangerous because it can reduce seed germination. In addition, it infects the roots, potentially killing seedlings immediately after emergence. *Alternaria* spp. does not affect germination (Kilpatrick, 1968; Huguélet *et al.*, 1973; Rees *et al.*, 1984; Fernandez & Conner, 2011).

In western Romania, Cotuna *et al.* (2020) isolated fungi of the genera *Alternaria* and *Fusarium* from black-pointed wheat grains. In the south, in a study of barley and two-row barley, Cristea *et al.* (2024) isolated fungi from the genera *Alternaria*, *Fusarium*, and *Drechslera*. The authors show that the incidence of seeds infected with *Alternaria* spp. was approximately 47% in the analysed varieties. Gafencu *et al.* (2019) isolated the following fungi from black-point wheat in northern Romania: *Alternaria* spp., *Cladosporium* spp., *Fusarium* spp., and *Cochliobolus* spp. The incidence of kernels infected with *Alternaria* spp. did not exceed 14%.

Black point is a disease associated with reduced grain quality, not with reduced wheat yield. Research shows that wheat seeds with black point have a thousand-kernel weight (TKW) that is 10–30% higher than that of seeds without symptoms. Furthermore, the fungus *Alternaria* spp. was predominantly isolated from the microflora growing on seeds with black point. This fungus does not affect plant vigour or yield, unlike the fungi *Fusarium* spp. and *Cochliobolus sativus* (Golosna *et al.*, 2026).

The incidence of the fungus *Cochliobolus sativus* (syn. *Bipolaris sorokiniana*) in European wheat is lower than in Africa and Asia (Hudec *et al.*, 2008; Draz *et al.*, 2016; Sharma *et al.*, 2021).

The aim of this study was to assess black point disease and its impact on the quality of wheat grown in southern Romania. To this end, several

Romanian wheat varieties were monitored over three experimental years.

MATERIALS AND METHODS

In this study, five wheat genotypes developed in Romania (three varieties and two lines) were evaluated for black point disease and grown at the Albota-Pitești Agricultural Research and Development Station in Argeş County. The evaluation of the disease and its effects on wheat quality was conducted between 2022 and 2024. Samples were collected after harvest from each variety (Trivale, Miranda, Ursita) and each line (Line 1 and Line 2). The composite sample consisted of 1 kg for each genotype. From each sample, 50 g seed samples were weighed in three replicates, from which impurities were removed. Over 1,000 caryopses were analysed in each sample to obtain the most accurate result possible.

The incidence was determined by counting the caryopses in each replicate and separating diseased from healthy. All seeds were examined visually and under a stereomicroscope (where necessary) to accurately quantify the diseased seeds. The incidence was determined using the classic formula in plant pathology by dividing the number of caryopses with black spots by the total number of caryopses examined, then multiplying the result by 100.

Severity was classified on a scale of 0-5 (Gilchrist, 1985). Analyses regarding incidence and severity were conducted at the plant pathology laboratory of the Faculty of Agriculture in Timișoara. The quality features (protein, gluten, dry matter, starch, hardness) were determined using near-infrared (NIR) spectroscopy (Norris *et al.*, 1989). These analyses were performed at the Research Platform of the University of Life Sciences “King Mihai I” from Timișoara.



Photo 1. Symptoms caused by black point in the wheat analysed caryopses (discolouration located mainly on the germ end) - original

Climatic data (average temperatures, precipitation) were obtained from the SCDA Albota-Pitești meteorological station and are presented in Figures 1-6.

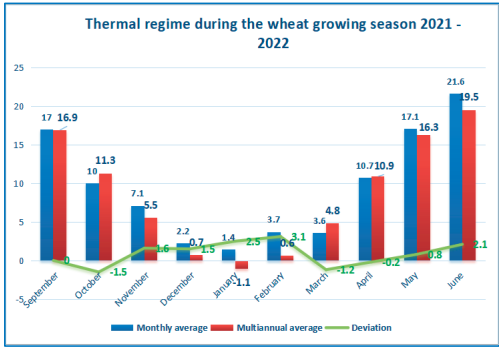


Figure 1. Graphical presentation of the thermal regime from the wheat growing season 2021-2022 from Albota- Pitești (Ghiorghe et al., 2026)

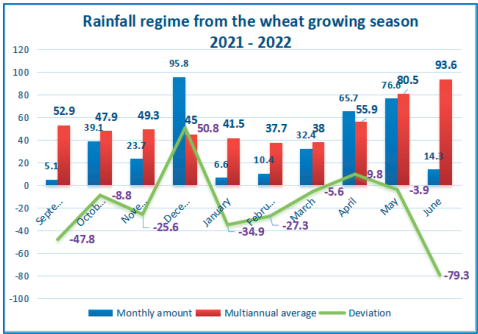


Figure 2. Graphical presentation of the rainfall regime from the wheat growing season 2021-2022 from Albota- Pitești (Ghiorghe et al., 2026)

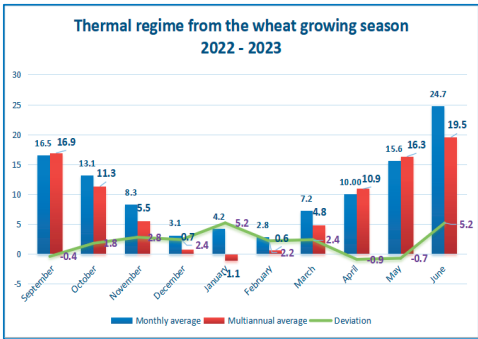


Figure 3. Graphical presentation of the thermal regime from the wheat growing season 2022-2023 from Albota- Pitești (Ghiorghe et al., 2026)

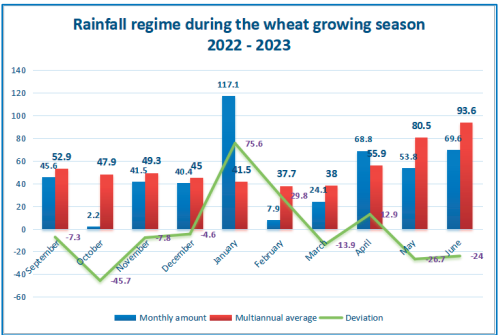


Figure 4. Graphical presentation of the thermal regime from the wheat growing season 2022-2023 from Albota- Pitești (Ghiorghe et al., 2026)

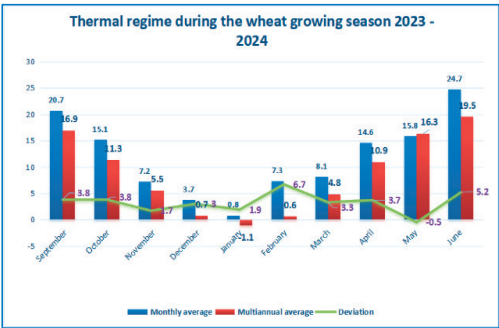


Figure 5. Graphical presentation of the thermal regime from the wheat growing season 2023-2024 from Albota- Pitești (Ghiorghe et al., 2026)

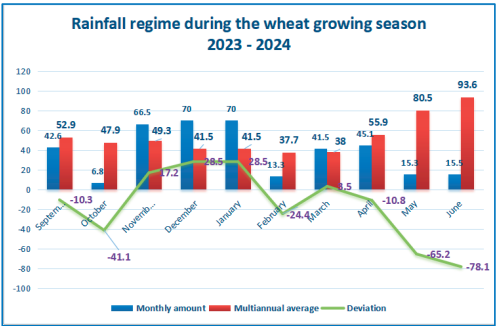


Figure 6. Graphical presentation of the rainfall regime from the wheat growing season 2023-2024 from Albota- Pitești (Ghiorghe et al., 2026)

The statistical analysis was performed with the software JASP (Version 0.95.4) (2025).

RESULTS AND DISCUSSIONS

Assessment of black point disease

The results regarding the incidence of black point disease between 2021 and 2024 for the analysed genotypes are presented in Figure 7. Analysis of the results shows differences between years and genotypes. The year 2023 stands out, with a higher incidence across all genotypes than in 2022 and 2024. The highest incidence was recorded in the Ursita variety (10.4%), followed by Miranda (8.1%) and Line 2 (6%).

In 2022, the incidence of black point was higher in the genotypes Line 2 (8.8%) and Ursita (7.1%). In the other genotypes, the disease did not exceed a 2.8% incidence. The year 2024 was not conducive to black point disease; consequently, the recorded incidence did not exceed 1% (Figure 7).

The severity of the disease was rated 1 (on a scale of 0-5) because all analysed caryopses showed that only the tip of the embryo was coloured brown.

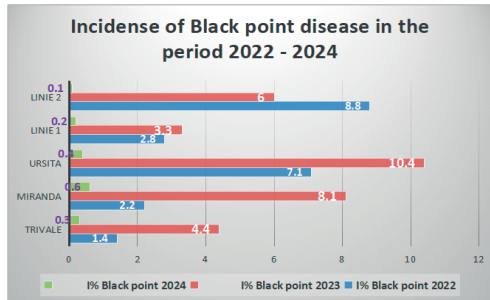


Figure 7. Graphical presentation of the incidence of black point disease in the period 2022-2024 from Albota-Pitești (Ghiorghe et al., 2026)

The data obtained were statistically analysed using analysis of variance (ANOVA). The F-test indicates significant differences among varieties, among experimental years, and between the two factors combined (Table 1).

Table 1. ANOVA for the black point attack frequency (F%) on winter wheat caryopses

Cases	Sum of Squares	df	Mean Square	F	P
Genotype	109.055	4	27.264	28.824	< 0.001
Year	293.534	2	146.767	155.167	< 0.001
Genotype * Year	117.033	8	14.629	15.466	< 0.001
Residuals	28.376	30	0.946		

The coefficient of variation was generally low (Miranda, Trivale and Ursita), indicating a normal distribution of the data, with the exception of two lines: Line 1, 2024 (CV = 0.903) and Line 2, also in 2024 (CV = 0.708) - Table 2. Levene's test rejects the null hypothesis, indicating significant differences among the variants in the incidence of black spots (p = 0.003).

Table 2. Descriptive statistics for the black point attack frequency (F%) on winter wheat caryopses

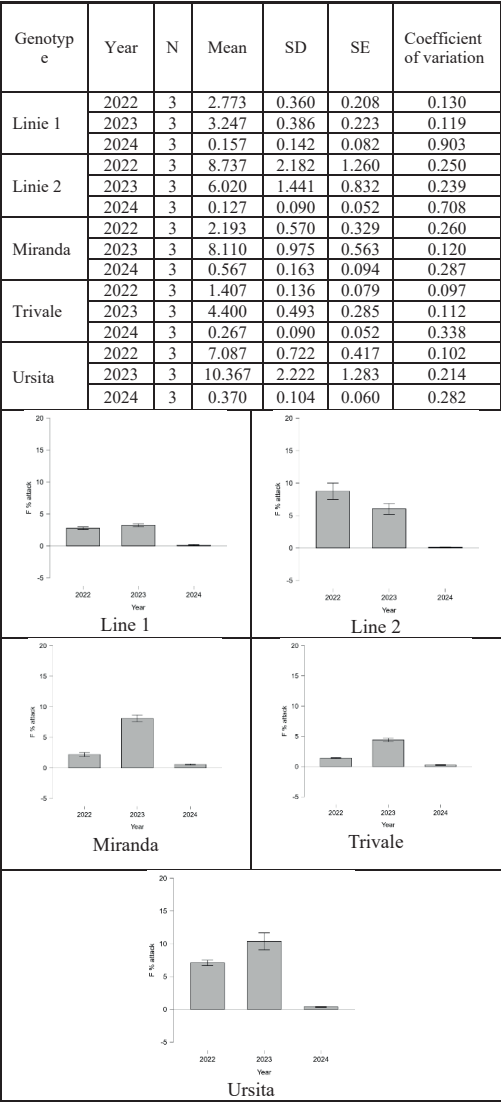


Figure 8. Black point attack frequency (F%) on Line 1. Line 2, Miranda, Trivale and Ursita, during 2022-2024 (SE displayed)

Post hoc comparisons (Tukey's test) regarding the influence of genotype on the incidence of black spot revealed statistically significant differences ($p < 0.001^{***}$) between Line 1 and Line 2, Line 1 and Ursita, Line 2 and Trivale, Miranda and Ursita, and Trivale and Ursita. Significant differences were recorded between Line 1 and Miranda, Line 2 and Miranda, and Miranda and Trivale (Table 3).

Table 3. Post Hoc Comparisons (Tukey test) - Influence of genotype on the black point attack frequency (F%) on winter wheat caryopses

Variety	Variety	Mean Diff.	95% CI for Mean Difference		t	Ptukey
			Lower	Upper		
Linie 1	Linie 2	-2.902	-4.232	-1.572	-6.330	< 0.001***
	Miranda	-1.564	-2.894	-0.235	-3.412	0.015*
	Trivale	0.034	-1.295	1.364	0.075	1.000
	Ursita	-3.882	-5.212	-2.552	-8.468	< 0.001***
Linie 2	Miranda	1.338	0.008	2.668	2.918	0.048*
	Trivale	2.937	1.607	4.267	6.405	< 0.001***
	Ursita	-0.980	-2.310	0.350	-2.138	0.231
	Trivale	1.599	0.269	2.929	3.487	0.012*
Miranda	Ursita	-2.318	-3.648	-0.988	-5.055	< 0.001***
	Trivale	-3.917	-5.247	-2.587	-8.543	< 0.001***

* $p < .05$, *** $p < .001$

Post hoc comparisons (Tukey's test) regarding the effect of year on the incidence of black spot show that the differences are significant ($*** p < 0.001$) across all experimental years (Table 4).

Table 4. Post Hoc Comparisons (Tukey test) - Influence of year on the black point attack frequency (F%) on winter wheat caryopses

Year	Year	Mean Diff.	95% CI for Mean Difference		t	Ptukey
			Lower	Upper		
2022	2023	-1.989	-2.865	-1.114	-5.602	< 0.001***
	2024	4.142	3.267	5.017	11.663	< 0.001***
2023	2024	6.131	5.256	7.007	17.265	< 0.001***

*** $p < 0.001$

Between 2022 and 2024, climatic conditions (precipitation and temperature) varied from year to year. Precipitation was below average in all experimental years during the wheat growing season (Figure 9). In the 2021–2022 and 2023–2024 seasons, the deficit ranged from 172.6 to 155.7 mm, much higher than that of the 2022–2023 season (63.3 mm). In May and June (2022-2024), when grains were forming and developing, precipitation was below average. The highest deficits were recorded in June of 2022 and 2024 (79.3 mm and 78.1 mm, respectively). In June 2023, the deficit was 24 mm (Figure 10), the incidence of black point disease being highest that year (Figure 7).

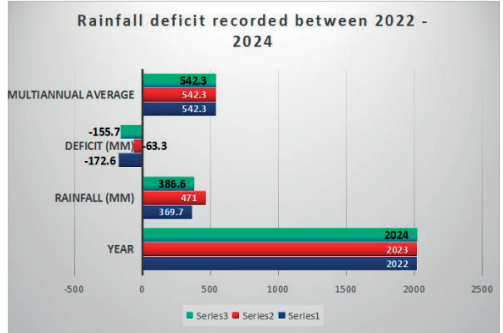


Figure 9. The rainfall deficit from 2022 to 2024 from Albota-Pitești (Ghiorghe et al., 2026)

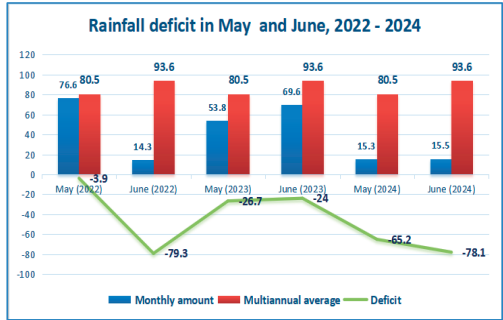


Figure 10. The rainfall deficit in May and June, 2022-2024 from Albota-Pitești (Ghiorghe et al., 2026)

Temperature conditions during the grain-filling stage were characterised by deviations from the long-term monthly averages. June stands out with higher positive deviations from the monthly average, as follows: 2.1 °C in 2022, 5.2 °C in 2023 and 2024 (Figures 1, 3 and 5). Regarding temperature, studies show that wheat exhibited black point symptoms under high-temperature conditions (30°C) in the absence of fungal infection, compared to wheat exposed to temperatures of 10°C. The authors conclude that abiotic stress can cause physiological changes (Ellis et al., 1996). Climatic conditions are decisive in triggering black point disease. It is known that the disease is initiated by both biotic and abiotic factors. In general, rainfall and high humidity during grain development favour the disease, but the most critical period for wheat is considered to be the milk-waxy ripening stage (Petr & Capouchova, 2001; Wang et al., 2002; Moschini et al., 2006).

The effect of black point on wheat quality

Several quality indices of the wheat analysed between 2022 and 2024 were determined in the laboratory: crude protein, gluten, dry matter, starch, hardness, bulk density, and moisture content. The results are presented in Figures 11 and 12.

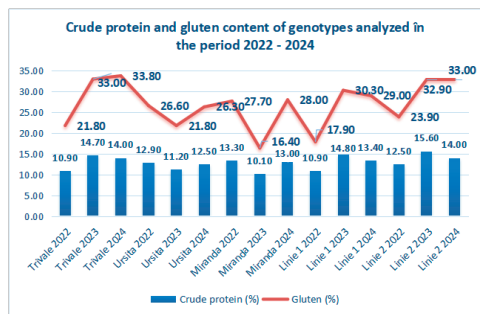


Figure 11. The amount of crude protein and gluten in the wheat genotypes analysed during the period 2022 - 2024 from Albota - Pitești (Ghiorghe *et al.*, 2026)

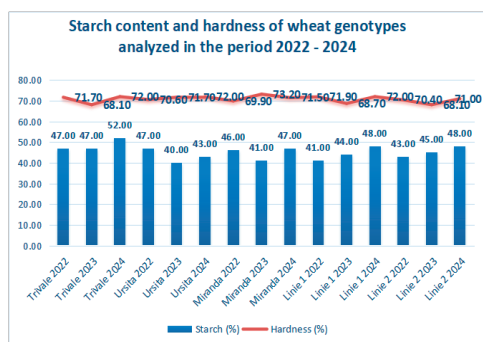


Figure 12. The amount of starch content and hardness in the wheat genotypes analysed during the period 2022- 2024 from Albota-Pitești (Ghiorghe *et al.*, 2026)

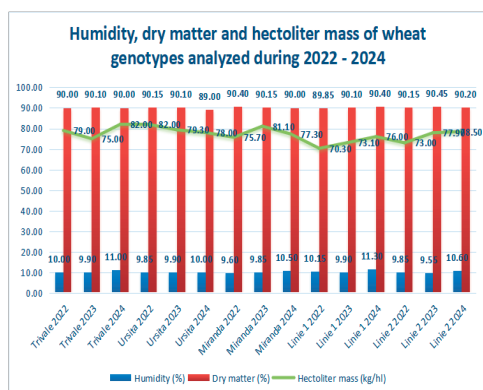


Figure 13. The humidity, dry matter and hectolitre mass in the wheat genotypes analysed during the period 2022- 2024 from Albota-Pitești (Ghiorghe *et al.*, 2026)

The crude protein content ranged from 10.10% to 14.80%, with variations observed between genotypes and years. Higher crude protein levels were observed in the following varieties: Trivale (14.00% in 2023 and 14.70% in 2024), Line 1 (14.80% in 2023 and 13.40% in 2024), and Line 2 (15.60% in 2023 and 14.00% in 2024). In 2022, the crude protein content was lower across nearly all genotypes, ranging from 10.90% (Trivale) to 13.30% (Miranda) - see Figure 11. Generally, the crude protein content in wheat should be between 9% and 14%.

Black point can influence the crude protein content of wheat kernels, along with other factors. Studies show that a severe black point infection (5 on a 0-5 scale) positively influences crude protein accumulation. Malaker *et al.* (2009) show that, in wheat with an infection level rated at 5, the crude protein content was 16.8%, whereas in healthy wheat it was 12.5%.

In the present study, the severity of black point disease was rated as 1, indicating that the embryo tip was brownish-black across all genotypes. The low severity was attributed to unfavourable climatic conditions during the wheat ripening period.

Protein content correlates very well with gluten content. A high protein content generally indicates higher gluten content (Wieser *et al.*, 2023).

The amount of gluten in wheat is an important characteristic for baking and is influenced by several factors: genetic (genotype), climatic (precipitation, temperature), technological, insect infestation (cereal bugs), and fungal pathogens (Torbica *et al.*, 2007).

Based on gluten content, the analysed genotypes were classified as follows: Ursita - satisfactory in 2022 and 2024 and below the minimum threshold in 2023 (below 26%); Trivale and Line 2 - very good quality in 2023 and 2024; Miranda - good quality in 2024, satisfactory in 2022, and below the limit in 2023; Line 1 - very good quality in 2023, good in 2024, and satisfactory in 2022 - Figure 11.

The starch content ranged from 40.00% in the Ursita variety in 2023 to 52.00% in Trivale in 2024. The Ursita variety had a higher incidence of black point in 2022 and 2023 and a lower incidence in 2024. The lower starch content may be correlated with the 10.4% incidence of black point in 2023. The differences between

genotypes and years are very small. Studies show that black point disease affects the composition of wheat kernels, potentially reducing starch content. Seeds with black points have lower starch and protein content than healthy ones (Kashem et al., 1999). Other studies show that a low incidence of black point (up to 5%) has no visible effect on starch content. An incidence greater than 10% negatively affects baking quality (Grădinaru et al., 2017) (Figure 12).

The results obtained regarding the hardness of wheat caryopses show that all genotypes can be classified as hard according to the relative hardness scale measured by the NIR method (Norris et al., 1989) (Figure 12). There are many studies on the influence of black point on hardness, some of which are contradictory. A recent study shows that the disease does not affect hardness as much as other diseases. In general, the effect on caryopsis texture is influenced by the severity of the disease. In severe infections, baking and milling quality may be affected (Golosna et al., 2026). Given that the climatic conditions during the years analysed did not favour the disease, we conclude that these qualities were unaffected in the genotypes studied.

The measured hectolitre weight showed differences between years and genotypes, as follows: high quality - Trivale (2022 and 2024), Ursita (in all years), Miranda (2023 and 2024), Line 2 (2023 and 2024); medium quality - Trivale (2023), Miranda (2022), Line 1 (2024); low quality - Line 1 (2022 and 2023), Line 2 (2022).

The influence of black point on hectolitre weight and dry matter content has been reported in several studies (Malaker et al., 2009; Golosna et al., 2026).

CONCLUSIONS

In this study, the incidence of black point disease in the analysed genotypes was low, as was its effect on the quality indices measured. Identifying more tolerant genotypes was difficult under water-stress conditions, given that the disease is favoured by wet weather during wheat maturation. Statistical analysis revealed significant differences among the

analysed genotypes, experimental years, and between the combined factors (genotype × year). In terms of hectolitre weight, the Ursita variety exhibited high quality in all experimental years. In contrast, Line 2 had lower quality for two consecutive years (2022 and 2023). The lowest crude protein content was recorded in 2022 for all genotypes. For starch, the content did not exceed 52.00%.

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SMART PEST MANAGEMENT IN MAIZE: THE ROLE OF VISUAL DIAGNOSTICS IN CROP DECISION-MAKING

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Abstract

Smart pest management is a key component of resilient maize production systems, particularly under increasing pest pressure and climatic variability. Effective crop management relies on early detection and correct diagnosis of insect pests, yet not all species and developmental stages are equally detectable under field conditions. This study proposes a decision-oriented diagnostic framework for maize pest management, combining visual scouting, high-resolution imaging, digital tools and targeted verification methods, applied across crop phenological stages from emergence to maturity. All maize pests relevant to Western Romania (*Ostrinia nubilalis*, *Helicoverpa armigera*, *Diabrotica virgifera virgifera*, *Oulema melanopus*, *Rhopalosiphum padi*, *Agriotes* spp., *Opatrum* spp. and *Tanymericus* spp.) were evaluated in terms of detectability, diagnostic complexity and management relevance. Species and stages were classified into easy, moderate or difficult to detect categories, based on visibility, symptom expression and habitat. Original high-resolution images were used to illustrate decision-relevant diagnostic features and frequent confusion cases. The proposed approach supports integrated pest management by improving diagnostic accuracy, reducing unnecessary interventions and enhancing smart, field-based decision-making in maize crop management.

Key words: smart pest management, maize, pest diagnostics, IPM, early detection.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide and represents a key component of global food security, animal feed production and bio-based industries (Shiferaw et al., 2011; Pretty et al., 2018). In Europe, maize cultivation occupies extensive agricultural areas, particularly in Central and Eastern European regions where pedoclimatic conditions are favorable for high productivity (Eurostat, 2023). Romania is among the leading maize-producing countries in the European Union, and the western region of the country represents an important agricultural area due to its fertile soils and favorable climatic conditions (Popov et al., 2016; Bujora et al., 2023).

Despite its high yield potential, maize production is strongly affected by numerous insect pests that can cause considerable economic losses if not properly monitored and controlled (Oerke, 2006; Savary et al., 2019). Among the most economically important pests affecting maize crops in Europe are the European corn borer (*Ostrinia nubilalis*

Hübner), the cotton bollworm (*Helicoverpa armigera* Hübner), the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and the cereal leaf beetle (*Oulema melanopus* L.) (Gray et al., 2009; Bažok et al., 2021; EPPO, 2025; CABI, 2021; Grozea, 2015). These species differ considerably in their biology, feeding behavior and damage patterns, affecting different plant organs and crop phenological stages. Their biology and damage symptoms have been extensively documented in previous studies and pest compendia (Pălăgeșiu et al., 2000; Grozea, 2015; CABI, 2021).

In recent decades, climate variability (Mosneang et al., 2015) and changes in agricultural practices have influenced pest population dynamics, increasing the risk of pest outbreaks in many cropping systems (Bebber et al., 2013; Juroszek and von Tiedemann, 2013; Deutsch et al., 2018). In Western Romania, several studies have reported an increasing pressure of maize pests, particularly *Diabrotica virgifera virgifera* and *Ostrinia nubilalis*, whose populations are influenced by climatic conditions and cropping systems (Costea and

Grozea, 2022; Bujora et al., 2023). Previous research conducted in the region has also highlighted the ecological adaptability and population variability of *Diabrotica virgifera virgifera* in maize agroecosystems (Horgoș and Grozea, 2020).

Integrated Pest Management (IPM) has been widely recognized as an effective and sustainable strategy for managing agricultural pests while minimizing environmental and economic impacts (Dhawan and Peshin, 2009; Barzman et al., 2015; Deguine et al., 2021). A key component of IPM is the accurate identification of pest species and the timely detection of infestation levels, which enables targeted interventions and reduces unnecessary pesticide applications (Kogan, 1998; Wearing, 1988). However, pest identification in field conditions may be challenging due to the presence of cryptic developmental stages, concealed feeding habits or morphological similarities among species.

Recent developments in digital agriculture have introduced new opportunities for improving pest monitoring and diagnostics. Image-based insect recognition systems and artificial intelligence tools are increasingly used in agricultural monitoring, enabling rapid pest detection and improving decision-support systems for crop protection (Martineau et al., 2017; Dawei et al., 2019; Li et al., 2021; Chen et al., 2022). Such technologies contribute to the development of smart pest management systems that integrate field observations, digital tools and data-based decision-making (Liu et al., 2016; Ding and Taylor, 2016).

Nevertheless, visual diagnostics supported by high-resolution photography and portable microscopy remains a practical and accessible approach for pest identification under field conditions. These methods allow crop protection specialists to recognize pest species and developmental stages more accurately, detect early infestation symptoms and avoid misidentification that could lead to ineffective control measures (Pălăgeșiu et al., 2000; Grozea, 2015; Bažok et al., 2021).

The present study aims to highlight the role of visual diagnostics and morphological identification as essential components of smart pest management in maize. Using original photographic documentation of several key

maize pests collected from Western Romania, the study focuses on diagnostic morphological traits and symptom patterns relevant for early detection and informed pest management decisions. By integrating field observations, digital imaging and targeted diagnostic approaches, this work contributes to the development of a practical framework for smart pest detection and monitoring in maize agroecosystems.

MATERIALS AND METHODS

Study area and crop establishment

The study was conducted during the 2025 maize growing season in agricultural fields located in Western Romania (Berechiu and Petid localities from Bihor County), a region characterized by favorable pedoclimatic conditions for maize cultivation and the recurrent presence of economically important insect pests.

Maize was sown on 5 May 2025, and pest monitoring was carried out throughout the vegetation period, from early crop emergence to grain development stages. Crop development was recorded using the BBCH phenological scale for maize (*Zea mays* L.), allowing the association of pest occurrence and detectability with specific crop growth stages.

Field observations covered several phenological phases, ranging approximately from BBCH 12 (early vegetative stage) to BBCH 78 (grain filling stage).

Field monitoring design

Field observations were conducted in two maize fields (no insecticides applied), using a standardized scouting protocol designed to capture both pest occurrence and diagnostic detectability.

At each monitoring date, three independent readings (replicates) were performed within each field. Each observation consisted of visual inspection of maize plants in order to record: presence of pest species, pest developmental stage (egg, larva, pupa or adult), visible damage symptoms and damage severity.

Damage intensity was evaluated using a semi-quantitative scale (0-3): (0) no visible damage, (1) minor damage symptoms, (2) moderate damage affecting plant tissues and (3) severe damage affecting plant development.

Additionally, the number of inspected plants and the number of affected plants were recorded in order to estimate pest infestation levels.

The monitoring focused on the main maize pests reported in the region, including: *Ostrinia nubilalis*, *Helicoverpa armigera*, *Diabrotica virgifera virgifera*, *Oulema melanopus*, *Rhopalosiphum padi*, *Agriotes* spp. and *Tanymecus* spp.

These species represent key pest groups affecting maize during different crop developmental stages.

Smart pest detection framework

The study applied a smart pest detection framework integrating field observation, symptom recognition, targeted sampling, digital imaging and laboratory confirmation.

Pest detection was based on two complementary diagnostic components:

1. Recognition of pest developmental stages (eggs, larvae, pupae and adults).
2. Identification of characteristic damage symptoms.

This combined approach allowed the detection of both active stages (larvae and adults) and inactive or cryptic stages (eggs and pupae), which are often overlooked in routine field scouting.

Symptoms recorded during field inspections included leaf feeding damage, window feeding, stem perforations, silk damage, frass deposits, irregular plant stands, and root-associated injury. These symptoms were used as diagnostic indicators for guiding targeted pest verification.

Photographic documentation and imaging equipment

High-resolution photographic documentation was performed in order to record diagnostic morphological traits and characteristic damage symptoms. Images were captured both in field conditions and during laboratory examination of collected specimens.

For photographic documentation, the following imaging systems was used Canon EOS 80D digital camera equipped with a Sigma 105 mm macro lens (1× magnification) and flash units Yongnuo YN14EX II or Yongnuo YN685, with light diffusion accessories.

These configurations allowed the capture of high-resolution macro images of insect morphological structures and plant damage

symptoms, providing detailed visual documentation of diagnostic traits relevant for pest identification.

Laboratory examination of specimens

Selected specimens representing different pest developmental stages were collected during field inspections and transported to the Laboratory of Entomology, University of Life Sciences “King Mihai I” from Timișoara (USVT).

In the laboratory, specimens were examined using a digital binocular microscope with HD touchscreen display, which allowed detailed visualization and documentation of morphological characteristics. Microscopic examination was particularly important for confirming: egg stages, pupal stages, early larval stages and morphologically similar species. Digital microscopic images were used as reference material for documenting developmental stages and supporting the diagnostic process.

Classification of pest detectability

In order to evaluate the practical complexity of pest diagnosis, pest species and developmental stages were classified according to field detectability.

Three diagnostic categories were defined: easy detection-stages easily identifiable through routine visual scouting due to visible insects or highly characteristic damage symptoms; moderate detection-stages detectable through careful plant inspection or targeted scouting of specific plant organs and difficult detection-cryptic stages requiring additional procedures such as soil inspection, plant dissection or laboratory confirmation. This classification allowed the development of a pest detectability matrix, linking pest species, developmental stage and diagnostic complexity.

The diagnostic approach applied in this study integrates field scouting, symptom recognition, detection of pest developmental stages, high-resolution macro imaging and laboratory confirmation. This workflow provides a structured framework for improving pest detection accuracy and supporting decision-oriented pest management in maize cropping systems (Figure 1).

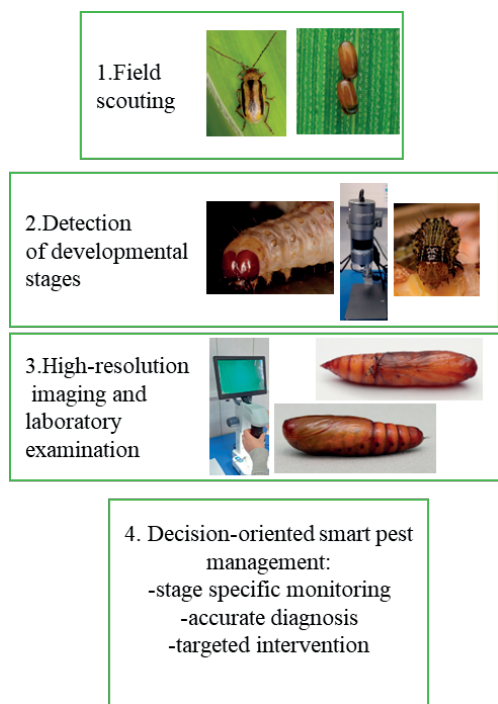


Figure 1. Smart pest detection framework applied for maize pest diagnostics. The framework integrates field scouting preliminary pest identification, detection of pest developmental stages (egg, larva, pupa and adult), high-resolution macro imaging, and laboratory confirmation using a portable digital microscope. All photographs represent original documentation obtained during field monitoring and laboratory examination (made by Veres) and were used as diagnostic references within the smart pest detection approach

Statistical analysis

Descriptive statistics were calculated for pest occurrence and damage intensity.

Differences among pest species were analyzed using one-way analysis of variance (ANOVA) for both damage score and infestation percentage. When significant differences were detected, Tukey's HSD post-hoc test was applied to identify statistically significant pairwise differences.

The relationship between pest occurrence and maize phenological stage was also evaluated using ANOVA, while the association between developmental status (active vs inactive stages) and detection difficulty was analyzed using a Chi-square test.

All statistical analyses were performed using standard statistical procedures commonly applied in agricultural and ecological research.

RESULTS AND DISCUSSIONS

A total of seven major pest taxa were recorded in the two monitored maize fields during the 2025 growing season. Their occurrence differed significantly in terms of infestation rate, damage severity, timing of occurrence, and diagnostic detectability, confirming that maize pest monitoring requires a stage-specific and decision-oriented approach.

Pest occurrence and damage intensity

The summary of pest occurrence and damage intensity is presented in Table 1, while the corresponding graphical representation of mean infestation rate is shown in Figure 2 and mean damage severity in Figure 3. Among the monitored pests, the highest mean infestation levels were recorded for *Ostrinia nubilalis* (52.3%) and *Helicoverpa armigera* (49.7%), followed by *Diabrotica virgifera virgifera* (47.0%) and *Rhopalosiphum padi* (37.3%) (Table 1, Figure 2). Lower infestation values were observed for *Oulema melanopus* (32.8%), while the lowest levels were associated with *Tanymecus* spp. (17.3%) and *Agriotes* spp. (9.3%).

Table 1. Occurrence and damage severity of the monitored maize pest species in the study fields

Pest	n	Mean BBCH	Mean infestation $\pm$ SD (%)	Mean damage $\pm$ SD
<i>Agriotes</i>	6	28,7	7.0 $\pm$ 2.8	0.50 $\pm$ 0.00
<i>Diabrotica</i>	6	75,3	47.0 $\pm$ 3.3	1.83 $\pm$ 0.26
<i>Helicoverpa</i>	6	71	49.7 $\pm$ 12.0	2.33 $\pm$ 0.52
<i>Ostrinia</i>	6	63,3	52.3 $\pm$ 5.3	3.00 $\pm$ 0.00
<i>Oulema</i>	6	23,3	31.3 $\pm$ 8.1	1.25 $\pm$ 0.27
<i>Rhopalosiphum</i>	6	36,3	37.3 $\pm$ 6.5	1.83 $\pm$ 0.26
<i>Tanymecus</i>	6	15	17.3 $\pm$ 5.8	0.92 $\pm$ 0.20

A similar trend was observed for damage severity. The highest mean damage scores were recorded for *O. nubilalis* and *H. armigera* (3.00), whereas intermediate values characterized *D. virgifera virgifera* (1.83), *R. padi* (1.75), and *O. melanopus* (1.35). The lowest damage values were associated with *Tanymecus* spp. (0.92) and *Agriotes* spp. (0.58) (Table 1, Figure 3). These results indicate that the most destructive pest species in the present dataset were also those associated with more

specific and diagnostically informative damage symptoms. The statistical comparison among pest species confirmed that these differences were highly significant. One-way ANOVA showed a strong species effect both for damage score, $F(6.35) = 59.27$, $p < 0.001$, and for infestation percentage, $F(6.35) = 37.42$, $p < 0.001$ (Table 3).

Table 2. Post-hoc comparison of mean damage severity among pest species according to Tukey HSD test

Pest species	n	Mean damage	SD damage	Mean infestation (%)	SD infestation	Tukey group	
Agriotes	6	0.5	0	7	2.75	e*	c**
Diabrotica	6	1.83	0.25	47	3.28	C	a
Helicoverpa	6	2.33	0.51	49.66	12.02	B	a
Ostrinia	6	3	0	52.33	5.27	A	a
Oulema	6	1.25	0.27	31.33	8.06	D	b
Rhopalosiphum	6	1.83	0.25	37.33	6.53	C	b
Tanymecus	6	0.91	0.20	17.33	5.75	De	c

*damage group; **infestation group

ost-hoc comparisons using Tukey’s HSD test grouped *O. nubilalis* and *H. armigera* in the highest damage category, while *Agriotes* spp. and *Tanymecus* spp. formed the lowest group (Table 2, Figure 3). This pattern suggests that pests attacking stems, ears, or reproductive organs produce more visible and agronomically severe symptoms than early-season or soil-associated pests.

Field effect and consistency of observations

The comparison between the two monitored fields showed no significant field effect for either damage score or infestation rate (Table 3). This indicates that the variation observed in the study was determined mainly by pest species and crop phenology rather than by field-specific conditions. From a methodological perspective, this result is important because it supports the consistency of the monitoring framework across the two sites and suggests that the diagnostic approach is reproducible under comparable maize-growing conditions.

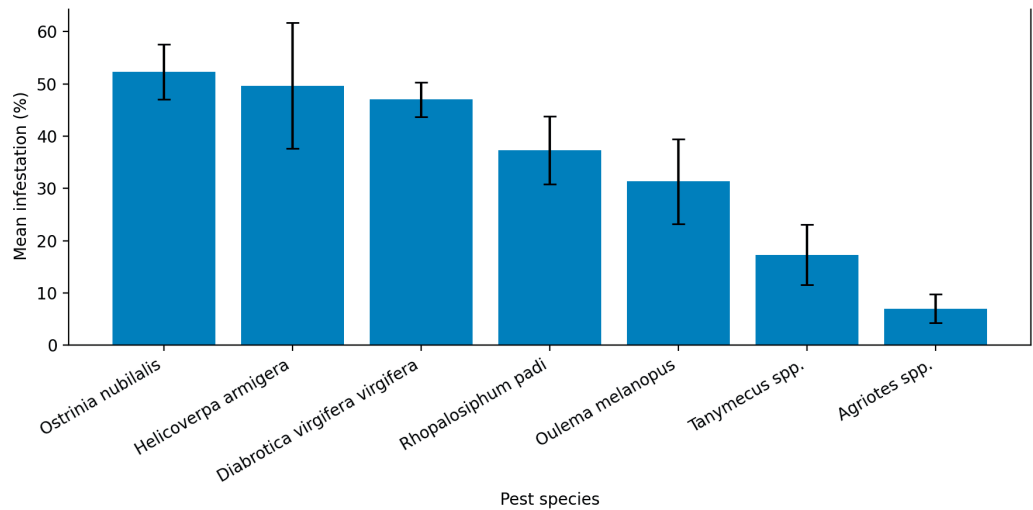


Figure 2. Mean infestation rate (%) recorded for the monitored maize pest

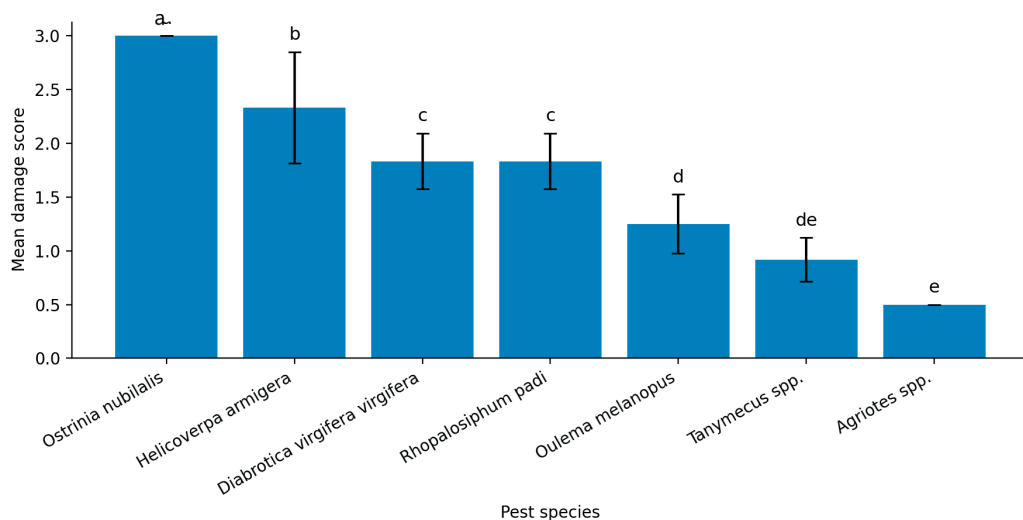


Figure 3. Mean damage severity score for maize pests with Tukey HSD grouping letter

Pest occurrence in relation to maize phenology

The relationship between pest occurrence and crop development stage is summarized in Table 4, while its graphical distribution along BBCH stages is illustrated in Figure 4. Pest detection clearly varied with maize phenology, and the statistical analysis confirmed a highly significant effect of crop developmental phase on both damage score and infestation level. ANOVA showed significant differences among phenological phases for damage, $F(2,39) = 40.45$, $p < 0.001$, and infestation, $F(2,39) = 28.31$, $p < 0.001$ (Table 5).

During early vegetative stages, the most relevant pests were those associated with seedling and early foliar damage, such as *Tanyemecus* spp., *Agriotes* spp., and partly *O. melanopus*. At these stages, detection was frequently based on indirect evidence, including irregular plant stand, leaf notching, or reduced plant vigor. In contrast, during later vegetative and reproductive stages, the pest complex shifted toward species such as *O. nubilalis*, *H. armigera*, and adult *D. virgifera virgifera*, which were associated with more specific and visually recognizable symptoms, including stem

perforation, frass deposits, silk damage, and ear injury (Table 4, Figure 5).

This phenology-dependent pattern is highly relevant for smart pest management because it shows that detection efficiency changes with crop stage. Therefore, a single scouting approach is insufficient; instead, field inspection should be adapted to the expected pest spectrum and symptom expression at each BBCH phase.

Detection of developmental stages and diagnostic complexity

A central objective of the study was to demonstrate that smart pest detection should not rely exclusively on adults or other easily visible stages. The pest detectability matrix, presented in Table 6, shows that the monitored pests differed not only by species but also by developmental stage and diagnostic complexity. Easy-detection cases were mainly represented by visible adult stages, such as *Diabrotica virgifera virgifera* and *Oulema melanopus*, whereas eggs, pupae, and concealed larvae were more frequently assigned to moderate or difficult detection categories.

Table 3. Analysis of variance (ANOVA) results for infestation rate and damage severity among maize pest species

Response	Model	Statistic	p-value	Effect size (η^2)
Damage score	One-way ANOVA (Pest)	F (6.35) = 59.27	<0.001	0.91
Infestation %	One-way ANOVA (Pest)	F (6.35) = 37.42	<0.001	0.865
Damage score	Two-way ANOVA (Pest + Field + Interaction)	Pest: F (6.28) = 52.50; Field: F (1.28) = 0.29; Pest*Field: F(6.28) = 0.45	Pest <0.001; Field = 0.597; Interaction = 0.837	-
Infestation %	Two-way ANOVA (Pest + Field + Interaction)	Pest: F (6.28) = 31.08; Field: F (1.28) = 0.54; Pest*Field: F (6.28) = 0.09	Pest <0.001; Field = 0.468; Interaction = 0.997	-

Table 4. Occurrence of maize pest species across crop phenological stages (BBCH scale)

Phenology phase	n	Mean infestation $\pm$ SD (%)	Mean damage $\pm$ SD
Early vegetative (BBCH 13–34)	20	20.8 $\pm$ 13.1	0.97 $\pm$ 0.44
Mid season (BBCH 35–66)	10	45.6 $\pm$ 10.2	2.55 $\pm$ 0.60
Late season (BBCH 67–78)	12	48.3 $\pm$ 8.5	2.08 $\pm$ 0.47

The representative developmental stages documented during the study are illustrated in Figure 1, which includes original photographic documentation of adult, egg, larval, and pupal stages. These images provide direct support for the diagnostic framework by showing that the study incorporated not only field-visible insects but also less accessible stages confirmed through targeted observation and laboratory examination.

The statistical relationship between biological status and diagnostic detectability is summarized in Table 7 and graphically represented in Figure 5. The Chi-square test showed a significant association between stage status (active vs. inactive) and detection difficulty, $\chi^2(2) = 7.22$, $p = 0.027$ (Table 7). Inactive stages were significantly more represented in the difficult-detection category, while active stages were more frequently associated with easy or moderate detection. This finding strongly supports the main premise of the study: pest diagnosis in maize is stage-dependent, and different biological stages require different detection tools.

Role of macro imaging and digital microscopy in smart detection

The methodological workflow used in this study is summarized in Figure 1, which outlines the sequence from field scouting and preliminary recognition to developmental stage detection, high-resolution imaging, and laboratory confirmation. This framework illustrates how symptom recognition, stage detection, and imaging-based verification were combined into a structured decision-support system.

The inclusion of high-resolution macro photography and portable digital microscopy considerably improved the diagnostic value of the observations.

Table 5. Effect of maize phenological stage on pest infestation and damage severity (ANOVA results)

Response	ANOVA	p-value	Effect size (η^2)
Damage score	F (2.39) = 40.45	<0.001	0.675
Infestation %	F (2.39) = 28.31	<0.001	0.592

While adults of *D. virgifera virgifera* and *O. melanopus* could be recognized directly in the field, stages such as *Oulema* eggs or pupae of *O. nubilalis* and *H. armigera* required closer examination and confirmatory documentation (like in previous Figure 1). In this respect, the use of imaging did not merely serve an illustrative purpose, but functioned as a diagnostic tool within the monitoring framework.

This aspect is particularly important for smart pest management, because it demonstrates that accurate pest recognition depends not only on pest abundance, but also on the observer's ability to detect the biologically relevant stage. In practical terms, the results support a multi-level diagnostic system based on: field symptom recognition, targeted suspicion, selective specimen collection, and image-assisted confirmation.

Symptom-based detection as a decision trigger

The results indicate that symptomatology was an essential component of the diagnostic process, especially for pests with concealed feeding behavior or cryptic developmental stages. Stem galleries, frass deposits, ear injury, silk damage, and irregular crop establishment acted as practical indicators guiding targeted sampling and confirmation. Thus, the first diagnostic signal was often not the pest itself, but the pattern of plant damage.

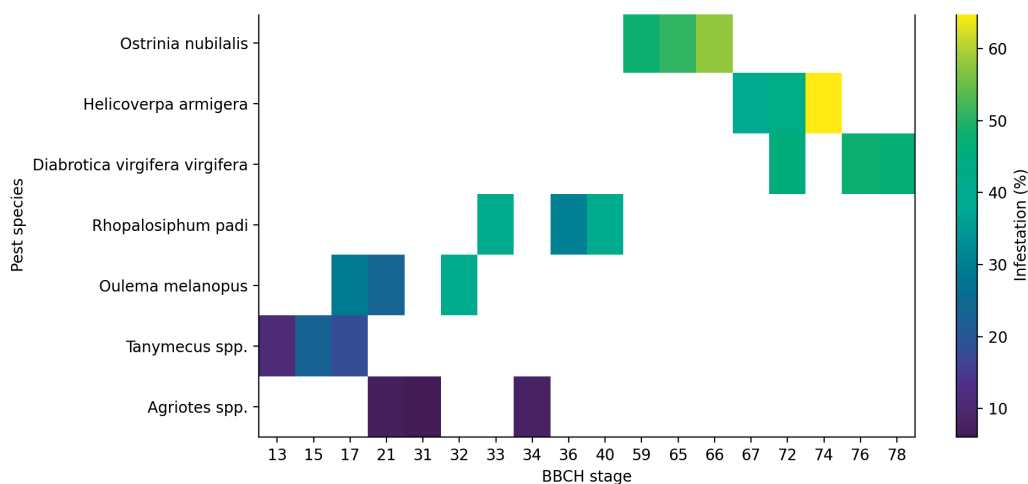


Figure 4. Distribution of pest infestation across maize phenological stages (BBCH scale)

Table 6. Diagnostic detectability of maize pest species based on developmental stages and observation method)

Species	Stage	Status	BBCH	Diagnostic/ symptom location	Detection method
<i>Ostrinia nubilalis</i>	larva	active	66	Panicle / ear zone	Portable digital microscope (Forte)
<i>Ostrinia nubilalis</i>	pupa	inactive	66	Panicle / ear zone	Portable digital microscope (Forte)
<i>Tanymecus spp.</i>	adult	active	17	Aerial plant parts; searched under soil clods near seedlings	Plant inspection and soil surface search within a 0.25 m radius around the plant
<i>Diabrotica virgifera virgifera</i>	adult	active	76	Collected from maize silk	Portable digital microscope (Forte)
<i>Helicoverpa armigera</i>	pupa	inactive	72	Collected from ear zone	Portable digital microscope (Forte)
<i>Helicoverpa armigera</i>	larva	active	72	Identification assisted by portable digital microscope to avoid misidentification	Surface soil inspection around the plant
<i>Oulema melanopus</i>	egg	inactive	21	Eggs located on the upper side of the leaf	Portable digital microscope (Forte) + digital binocular microscope with HD touchscreen
<i>Rhopalosiphum padi</i>	larva	active	40	Colonies on leaves	Inspection of colonies on the underside of leaves
<i>Rhopalosiphum padi</i>	adult	active	40	Colonies on leaves	Inspection of colonies on the underside of leaves
<i>Agriotes spp.</i>	adult	active	34	Direct field observation	Leaf inspection
<i>Agriotes spp.</i>	larva	active	34	Root zone + soil	Inspection of roots and soil within a 0.10 m radius around the root system

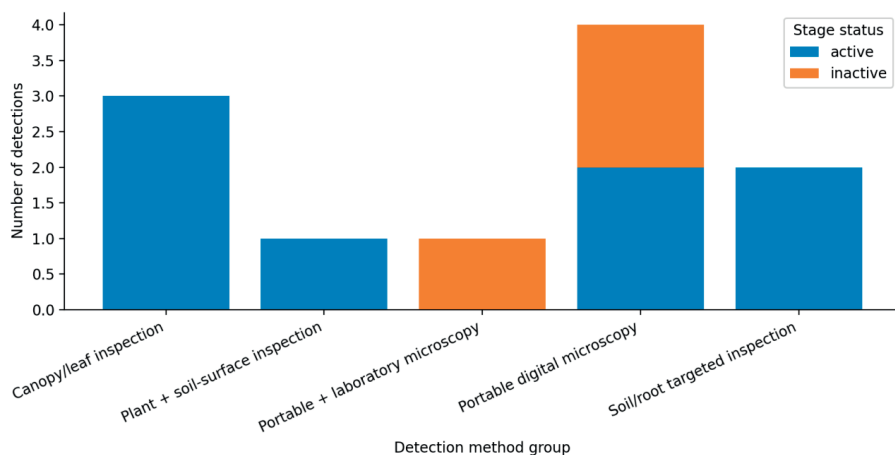


Figure 5. Detection method groups associated with active and inactive pest developmental stages

Table 7. Association between pest developmental stage status (active vs. inactive) and detection method groups.

Test	Statistic	p-value	Interpretation
Status × detectability (chi-square)	$\chi^2(2)=7.2$	0,027	Inactive stages were concentrated in the difficult-detection category.
Status × microscopy-assisted method (Fisher exact)	Odds ratio= ∞	0,061	Microscopy-assisted detection was used more often for inactive stages; trend limited by small n.

This observation has direct management relevance. In field practice, decisions are often made before all developmental stages are directly observed. Therefore, a smart detection system must begin with symptom interpretation and then proceed to species- and stage-specific verification. The present study shows that combining symptom recognition with stage-based diagnostics improves both the speed and accuracy of pest identification, while reducing the risk of misclassification. Taken together, the results presented in Tables 1-7 and Figures 1-5 show that maize pests differ significantly in abundance, damage potential, phenological timing, and detectability. Species such as *O. nubilalis* and *H. armigera* produced the highest damage scores and infestation rates, while *Agriotes spp.* and *Tanymecus spp.* showed lower visible damage but remained agronomically relevant in early stages. More importantly, the significant association between developmental status and detectability demonstrates that the recognition of eggs, pupae, and concealed larvae

is essential for a truly smart pest detection system. Overall, the study supports a decision-oriented framework in which pest diagnosis is based on the integration of crop phenology, symptomatology, developmental stage, and imaging-assisted verification. This approach offers a realistic basis for more precise and resilient pest management in maize.

CONCLUSIONS

The study highlighted significant differences in infestation levels and damage severity among the monitored maize pest species, confirming the major impact of *Ostrinia nubilalis*, *Helicoverpa armigera* and *Diabrotica virgifera virgifera* under the studied conditions. Pest occurrence was closely associated with maize phenological stages, with early-season pests dominating vegetative phases and Lepidopteran and coleopteran pests becoming more prevalent during reproductive stages. The results also demonstrated that pest detectability varies according to developmental stage. Active stages such as adults and exposed larvae were more easily identified in field conditions, while inactive or concealed stages required targeted inspection and microscopic confirmation. The integration of field scouting, symptom recognition and digital imaging proved to be an effective approach for improving pest diagnostics. This smart detection framework can support more accurate monitoring and contribute to better decision-making within

integrated pest management programs for maize.

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EVALUATING THE CHLOROPHYLL CONTENT OF SOME POTATO VARIETIES LEAVES, AGRONOMIC FEATURES AND YIELD USING SPAD METER

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Abstract

Between 2023-2024 at NIRDPSB Brasov, Romania were performed research to determine the leaf greenness index SPAD and selected potato characteristics, i.e. plant height, yields and tubers starch content on twelve varieties. With SPAD 502 Plus meter (Chlorophyll Meter) were carried out 3 determinations on the leaves of the middle level of 3 plants from each variety taken into the study. Values recorded on 22.06.2023 were between 47.9 and 42.5 units, and those on 18.07.2023 between 47.6 and 42.1 units and the yield varied between 14 t/ha and 45.47 t/ha. Values recorded on 18.06.2024 were between 49.9 and 41.4 units, and those on 08.07.2024 between 47.0 and 39.9 units and the yield varied between 25.72 t/ha and 7.58 t/ha. The results showed that threshold SPAD values decrease as the growing season progresses for all varieties and the yield is strongly influenced by climatic conditions.

Key words: leaf greenness index SPAD, potato, plant development, starch, yield.

INTRODUCTION

The potato, because of its adaptability, yielding capacity, and nutrition contribution, and as an important component of diversified cropping systems, has a long history of helping relieve food insecurities (Devaux et al., 2021). Previous studies strongly suggest that SPAD hand-held chlorophyll meter is a promising tool to assess the nitrogen status of potato foliage, and offers opportunities to minimize the influences of other factors on N estimation based on SPAD measurements (Li et al., 2012). In recent years, many destructive and non-destructive methods have been developed to estimate the chlorophyll content of leaves (Liu et al., 2020). With the rapid development of intelligent agriculture, the rapid and non-destructive estimation of chlorophyll content has been realized, which is of great significance for evaluating and managing crop canopy photosynthetic capacity (Shi, 2024).

The first attempt to use the SPAD chlorophyll meter for potato was reported by Vos and Bom (1993). SPAD-502 meter is a hand-held device used for rapid, accurate, and non-destructive measurement of leaf chlorophyll concentrations

(Ling et al., 2011). Effectivity of SPAD-502 meter is tested with various crops such as corn (Bullock and Anderson, 2008), rice, coffee, potato (Guanli and Huachun, 2007) and sugarcane (Jangpromma et al., 2010). It works by emitting two frequencies of light, 660 nm (red) and 970 nm (infrared).

Good development of plants is indicative of their good nutrition, normal course of their life processes and, thus, optimum yielding and nutrient accumulation (Zarzecka et al., 2021).

The chlorophyll content is significantly correlated with the content of nitrogen (Gaurav et al., 2019) which is one of the most important nutrients for the potato plant's healthy growth (Zhou et al., 2018), development (Clevers and Kooistra, 2012) and production (Nigon et al., 2014).

Chlorophyll meter utilization to evaluate plant N status at real time is suitable for precision agriculture and canopy greenness might serve as a useful diagnostic tool to assess plant N demand (Wiesler et al., 2002).

Climate change has emerged as one of the most significant issues over the past two decades. The primary cause of this climate change is known to be the phenomenon of increasing

atmospheric temperature due to the rise in carbon dioxide (CO₂) concentration in the atmosphere (Lobell et al., 2011; Mora et al., 2013). Consequently, the global average temperature is predicted to increase by 2.4-6.4°C by the end of this century. Climate change not only increases the frequency of abiotic stress factors such as droughts, floods, and heavy rainfall, but also has severe impacts on ecosystems, food production, and human health (Calzadilla et al., 2013). Furthermore, climate change in the agricultural sector is expected to cause soil erosion and water scarcity, deteriorating crop cultivation environments, altering suitable cultivation areas, and raising concerns about the emergence of new pests and weeds (Ahn et al., 2017; Park et al., 2025).

Starch is the main carbohydrate found in the potato, and the most important component of the tuber's dry weight (Chakraborty et al., 2022). It is composed of two polysaccharide components: amylose (15-30%) and amylopectin (70-85%) (Brażkiewicz et al., 2025). Its content in potato tubers and quality depend on many factors: genetic and environmental conditions (place of cultivation, temperature and amount of precipitation, time of harvest), as well as applied agrotechnology (Liszka-Skoczylas, 2020).

The purpose of the work was to assess the influence of leaf greenness index SPAD on the potato plant development, tuber yield and starch content.

MATERIALS AND METHODS

Experiments were carried out at the National Institute of Research and Development for Potato and Sugar Beet Braşov, Romania, on a cambic chernozem soil, with 6.7 pH, humus 4.68% and clay 27%. The pre-crop was wheat in both years and for current fertilizer was used 1000 kg/ha N:P:K:15:15:15+S.

Planting distance was 75 cm between rows and 30 cm between plants per row, having 4 rows with 10 plants each one. Planting was done manually in April 28, 2023 and April 10, 2024. During the vegetation period were applied the usual maintenance works (hilling,

herbicides, treatments for late blight and Colorado beetle) for potato crop.

In the two years of study, it was aimed the dynamics of potato plant growth and tuber yield accumulation of twelve Romanian potato varieties (Brasovia, Azaria, Castrum, Marvis, Sarmis, Darilena, Cezarina, Ervant, Asinaria, Foresta, Sevastia, Cosiana). It has been carried out two harvesting in dynamic for each variety. With SPAD 502 Plus meter (Chlorophyll Meter) on the days of 22.06 and 18.07.2023, and respectively, 18.06 and 08.07.2024, three determinations (readings) were carried out on the leaves of the middle layer of 3 plants from each genotype taken into the study.

For interpretation of chlorophyll results was used the formula of nitrogen index (Shapiro et al., 2006): N index = readings average/control average x 100%.

Potato tubers were harvested semi-mechanized on September 13, 2023, respectively September 19, 2024. Brasovia variety was taken as a control variant and all the tubers from two central rows in each repetition/variant were weighed. For the determination of starch content have been taken from tuber mass average samples of one kilo from each variety on 3 replications. Determination of the percentage of starch was done using the Polikeit balance.

Statistical evaluation of the experimental data was performed using Microsoft Excel 2013 and SPSS for Windows 13.0. For the evaluation of the results, analysis of variance and Pearson's correlation analysis were used.

RESULTS AND DISCUSSIONS

To NIRDPSB Braşov, the periods from October 1, 2022, to March 31, 2023, and from October 1, 2023, to March 31, 2024, were characterized by monthly mean air temperatures higher than the long-term averages. In the 2022-2023 agricultural year, the highest mean air temperature relative to the long-term average of -5°C was recorded in January, reaching 3.1°C, whereas in the 2023-2024 agricultural year, the maximum was observed in February, with a mean temperature of 6 °C compared to the long-term average of -2.5°C. At the beginning of November, soil

moisture conditions were favorable for performing autumn plowing. Winter precipitation was generally low; however, in January 2023, 44 mm of rainfall was recorded, exceeding the long-term average of 25.5 mm, while in March 2024, the precipitation reached 72.4 mm, significantly above the long-term average of 28.9 mm. April of the spring of 2023 was cooler than normal, with a mean temperature of 7.3 °C, whereas in April 2024, temperatures were higher than normal, reaching 12.2 °C. Total precipitation in April 2023 amounted to 83.1 mm, while in April 2024 only 43.5 mm was recorded, 6.5 mm below the long-term average. In May, the air temperature was close to the long-term average, but precipitation in 2024 was significantly below normal, amounting to only 42.7 mm compared to 82 mm. The summer months (June-August) were characterized by higher-than-average temperatures. In June 2023, the mean temperature was 17.6°C, increasing substantially to 21.1°C in June 2024, compared to the long-term average of 16.5°C. Precipitation was generally low, although in June 2023 a total of 111.1 mm was recorded, above the long-term average of

96.7 mm. During the summer of 2024, atmospheric and soil drought conditions prevailed throughout the season. Moreover, precipitation distribution was extremely uneven, as exemplified in August 2024, when approximately 90 % of the monthly total fell within only four days (Figure 1).

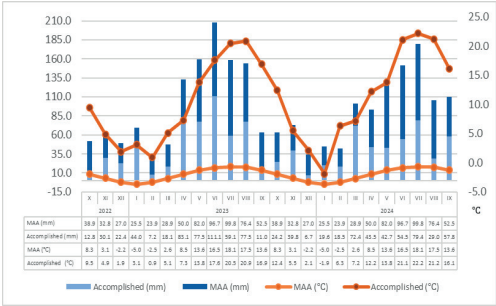


Figure 1. The climatic conditions during the experimental period in Brasov

Drought in the early part of the potato vegetation determines the poor development of the aerial vegetative apparatus, the plants remain smaller, with reduced leaf area, which has a negative influence on the subsequent growth of the tubers.

Table 1. The mean measurements of plant and SPAD-value carried out in dynamics - 2023 Braşov

Variety	Plant height (cm)		Median leaf length (cm)		Total biomass weight (g)		Tuber no./hill		Tuber weight/hill (g)		SPAD values	
	22.06	18.07	22.06	18.07	22.06	18.07	22.06	18.07	22.06	18.07	22.06	18.07
Cosiana	57.00	71.25	24.00	20.00	334.25	520.00	3.25	6.88	41.50	263.38	47.5	47.6
Marvis	44.50	65.13	21.75	24.88	348.75	541.00	10.50	10.25	160.25	875.25	47.6	46.6
Darilena	39.13	50.63	21.38	21.50	182.75	267.88	3.00	5.50	38.00	400.50	47.7	44.3
Asinaria	45.88	58.63	24.88	21.63	240.00	402.75	6.63	7.13	91.75	494.00	45.0	44.7
Sarmis	39.50	51.88	20.13	21.13	231.25	323.25	4.13	6.63	91.00	487.00	47.6	46.2
Cezarina	34.50	55.00	21.63	29.38	140.50	548.00	2.25	9.25	20.75	547.25	47.9	46.4
Foresta	37.00	52.75	23.38	22.25	156.25	426.00	2.88	5.25	40.75	303.50	46.2	47.1
Ervant	61.75	79.00	23.75	23.00	422.00	462.50	10.88	14.00	275.13	1052.25	45.9	44.4
Sevastia	39.25	67.00	22.88	23.75	207.25	479.75	2.63	10.75	26.50	650.75	42.5	42.1
Brasovia	35.63	53.00	19.75	22.13	211.25	423.25	5.63	12.25	66.50	658.25	45.4	43.4
Azaria	50.13	80.63	24.38	26.25	372.25	555.50	11.25	7.63	107.00	632.00	47.4	44.9
Castrum	47.50	81.75	23.38	25.75	364.25	738.50	8.13	8.75	99.00	527.75	46.4	44.2

Plant height varied significantly depending on variety. Cosiana variety produced the tallest plants (57.00 cm, respectively 71.25 cm) to both assessments, whereas Cezarina produced the shortest (34.50 cm) to the first assessment and Sarmis (51.88 cm) to the second assessment. We consider that both genetic and environmental factors influence plant height.

The numbers of tubers per plant are determined by different parameters such as numbers of stolons and stems, varieties grown and status of vegetative growth (Adhikari, 2005; Abeytilakaratna, 2022) (Figure 2). To the first assessment Azaria variety had the highest number of tubers, 11.25, whereas Cezarina had substantially fewer, 2.25. To the second assessment Ervant variety presented the highest

number of tubers, 14.00, and Foresta variety the lowest, 5.25. Between tuber weight there were differences, to the first assessment Ervant variety reach to 275.13 g while Cezarina reach only 20.75 g.

The average weight of tubers increases with temperature, but at temperatures above 25°C, it begins to decrease, and the number of tubers in the hill also decreases. The stems elongate while the leaf area decreases. Too much solar radiation can cause leaves burn.

The SPAD values recorded on the reading of 22.06.2023 were between 42.5 units for the Sevastia variety and 47.9 units for the Cezarina variety, and on the reading of 18.07.2023 between 42.1 units for the Sevastia variety and 47.6 units for the Cosiana variety.

As the vegetation progresses, generally, a decrease in the chlorophyll content value is observed, depending on the leaf mass of the variety and its exposure. Cosiana, variety with later accumulation, continued to provided high

values (47.6 units) and Foresta variety (47.1 units). Chlorophyll content decreased pronounced in the varieties Darilena (44.3 units), Azaria (44.9 units) and Castrum (44.2 units) (Table 1).



Figures 2. Evolutionary elements of sacrificed and analyzed potato plants

Table 2. The mean measurements of plant and SPAD-value carried out in dynamics - 2024 Braşov

Variety	Plant height		Median leaf lenght		Total biomass weight		Tuber no./hill		Tuber weight/hill		SPAD values	
	18.06	8.07	18.06	8.07	18.06	8.07	18.06	8.07	18.06	8.07	18.06	8.07
Cosiana	63.63	66.25	19.00	19.38	469.50	300.75	3.13	9.75	49.75	149.25	41.4	43.8
Marvis	52.63	54.13	19.25	18.13	398.25	284.25	13.75	17.00	292.00	656.75	46.5	42.1
Darilena	49.50	48.75	18.50	16.38	255.25	183.63	7.38	10.25	275.25	337.50	48.9	43.8
Asinaria	58.13	63.75	22.63	21.38	305.00	268.75	9.25	12.00	327.25	512.50	48.4	43.3
Sarmis	52.63	63.63	19.13	17.88	347.25	314.75	8.00	10.50	266.00	579.25	47.2	44.2
Cezarina	50.75	57.75	20.38	21.13	309.75	268.75	13.38	11.88	232.50	423.50	47.9	46.2
Foresta	63.00	61.38	23.00	19.63	288.50	267.25	5.75	9.25	199.50	413.75	49.9	45.7
Ervant	65.38	62.50	21.13	17.00	390.25	173.25	15.00	15.50	579.25	758.00	47.0	41.5
Sevastia	56.88	55.38	21.50	20.50	303.75	208.50	8.13	8.00	186.75	409.50	44.0	42.7
Brasovia	47.13	47.63	17.63	17.75	326.25	166.50	10.25	12.75	215.75	314.25	44.8	39.9
Azaria	62.88	72.88	22.50	22.00	531.75	495.13	12.00	14.25	343.00	549.50	48.2	47.0
Castrum	59.13	64.00	20.25	20.00	476.50	350.25	13.50	16.63	186.75	326.50	47.3	43.4

In 2024 Ervant variety produced the tallest plants (65.38 cm) to the first assessment, whereas Brasovia produced the shortest (47.13 cm). To the second assessment Azaria variety produced the tallest plants (72.88 cm) and Brasovia the shortest (47.63 cm). To the first assessment Ervant variety had the highest number of tubers, 15.00, whereas Cosiana had substantially fewer, 3.13. To the second assessment Marvis variety presented the highest number of tubers, 17.00, and Sevastia variety the lowest, 8.00. Between tuber weight continue to be differences, to the first assessment Ervant variety reach to 579.25 g while Cosiana reach only 49.75 g and to the second assessment

Ervant variety continued to have the highest weight (758.00 g) while Cosiana just had 149.25 g (Table 2).

The SPAD values recorded on the reading of 18.06.2024 were between 49.9 units for the Foresta variety and 41.4 units for the Cosiana variety, and on the reading of 8.07.2024 between 47.0 units for the Azaria variety and 39.9 units for the Braşovia variety.

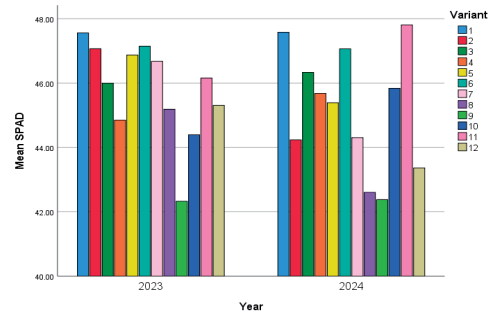
Also in 2024, Cosiana variety recorded an increase in chlorophyll values, compared to its more pronounced decrease in the Marvis, Asinaria, Darilena varieties. Also, the Ervant variety, which accumulates early and has a spreading grow habit, showed a significant

decrease, from 47.0 units to 41.5 units. In contrast, the Cezarina and Azaria varieties continued to show high chlorophyll content, decreasing only 1.7 units (from 47.9 to 46.2 units) and 1.2 units (from 48.2 to 47.0 units), respectively. We consider that the presence of water is essential during tuber formation and growth. If plants are subjected to water stress then the leaves increase stomatal conductance to reduce their temperature.

The experience confirms that the SPAD values of a potato crop decrease as the plants progress in vegetation.

Analyzing the average readings from the two years, we can see the different reaction of some varieties to the influence of climatic conditions. While the Cosiana variety remained approximately at the same level, the Marvis variety recorded a significant decrease in 2024. For the Castrum variety, the situation was similar, recording a pronounced decrease in 2024, while the Azaria variety recorded a spectacular increase in SPAD values. The

varieties Darilena and Asinaria recorded higher values in 2024 compared to 2023, Cezarina and Sevastia varieties performed in the same way in both years, Brasovia variety recorded an insignificant decrease, but Sarmis, Ervant and Foresta varieties recorded stronger decreases in 2024 compared to 2023 (Figure 3).



*Variant codes: 1 = Cosiana, 2 = Marvis, 3 = Darilena, 4 = Asinaria, 5 = Sarmis, 6 = Cezarina, 7 = Foresta, 8 = Ervant, 9 = Sevastia, 10 = Brasovia, 11 = Azaria, 12 = Castrum.

Figure 3. Average SPAD values in the two years of experiment

Table 3. Correlations describing relationship between the years, the plants and SPAD values

		Year	Observation	Variant	Repetition	Plant	Reading	SPAD
Year	Pearson Correlation	1	-.677**	.000	.000	.000	.000	-.067**
	Sig. (2-tailed)		.000	1.000	1.000	1.000	1.000	.006
Observation	Pearson Correlation	-.677**	1	.000	.000	.000	.000	.247**
	Sig. (2-tailed)	.000		1.000	1.000	1.000	1.000	.000
Variant	Pearson Correlation	.000	.000	1	.000	.000	.000	-.149**
	Sig. (2-tailed)	1.000	1.000		1.000	1.000	1.000	.000
Repetition	Pearson Correlation	.000	.000	.000	1	.000	.000	-.014
	Sig. (2-tailed)	1.000	1.000	1.000		1.000	1.000	.552
Plant	Pearson Correlation	.000	.000	.000	.000	1	.000	-.037
	Sig. (2-tailed)	1.000	1.000	1.000	1.000		1.000	.128
Reading	Pearson Correlation	.000	.000	.000	.000	.000	1	-.048*
	Sig. (2-tailed)	1.000	1.000	1.000	1.000	1.000		.047
SPAD	Pearson Correlation	-.067**	.247**	-.149**	-.014	-.037	-.048*	1
	Sig. (2-tailed)	.006	.000	.000	.552	.128	.047	

** . Correlation is significant at the 0.01 level (2-tailed).
* . Correlation is significant at the 0.05 level (2-tailed).

We believe that a negative correlation between SPAD values and the plants on which the readings were taken occurred due to thermo-hydric stress, the relationship being reversed, as

the water content in the leaves decreased, chlorophyll increased, probably in response to stress or other physiological changes (Table 3).

Table 4. Total yield tuber/ha (t/ha) and starch content

Variety	Yield (t/ha)		Starch content (%)	
	13.09.23	19.09.24	09.11.23	05.11.24
Cosiana	21.11	20.61	14.25	13.25
Marvis	35.06	12.03	15.13	14.27
Darilena	21.06	21.11	14.83	14.42
Asinaria	31.64	17.39	15.04	14.63
Sarmis	26.94	14.11	14.44	13.75
Cezarina	26.08	7.58	14.25	13.33
Foresta	14.00	17.39	11.06	13.09
Ervant	45.47	21.11	10.27	10.56
Sevastia	25.69	13.08	12.13	11.58
Brasovia (Mt)	26.11	12.58	16.35	14.67
Azaria	40.69	25.72	15.96	13.33
Castrum	41.31	21.86	13.71	13.69
DL 5% =	4,99 t	3,34 t	Mean:	Mean:
DL 1% =	6,69t	4,47t	13.95	13.38
DL 0,1% =	8,83 t	5,90 t		

The total production in both years was not at the level of the productive potential of most varieties, the decreases being major especially in 2024.

The values recorded in 2023 varied between 14.00 t/ha to Foresta variety and 45.47 t/ha to Ervant variety and in 2024 ranged between 25.72 t/ha to Azaria variety and 7.58 t/ha to Cezarina variety (Table 4).

Comparing the productions obtained in 2024 with those in 2023, which from a climatic point of view was warmer and with less precipitation, it can be observed that the most varieties (Castrum, Azaria, Ervant, Sevastia, Asinaria, Sarmis and Brasovia) had their productions cut in half, the Cosiana and Foresta varieties maintained their production levels, the Marvis and Cezarina varieties recorded dramatic decreases.

The highest level of starch content in 2023 (16.35%) was observed to Brasovia variety, followed by Azaria (15.96%) and Marvis (15.13%). At the lowest level were the varieties Foresta (11.06%) and Ervant (10.27%).

In 2024, the Brasovia variety (14.67%) showed the highest starch content after harvest, followed by Asinaria (14.63%) and Darilena (14.42%) and the lowest values were registered to Sevastia (11.58%) and Ervant (10.56%) varieties (Table 4).

High differences in starch content between the years presented varieties Foresta, Brasovia and Azaria.

The differences in starch content are due to genetic conditions and the agrotechnology used at the time of cultivation. Rymuza et al. (2015), studying different potato varieties, showed that the starch content in tubers depends highly on

precipitation level. The authors also reported on the diversified response of varieties to weather conditions in different growing seasons in terms of starch accumulation in tubers.

CONCLUSIONS

SPAD measurements were performed to study the possibilities of non-invasive assessment of the vegetation status of potato plants in the experimental field compared to measurements on plants sacrificed at the two dynamics.

Values recorded on 22.06.2023 were between 47.9 and 42.5 units, and those on 18.07.2023 between 47.6 and 42.1 units. Values recorded on 18.06.2024 were between 49.9 and 41.4 units, and those on 8.07.2024 between 47.0 and 39.9 units.

The production was significantly influenced by the climatic conditions, being between 45.47 t/ha and 14.00 t/ha in 2023 and between 25.72 t/ha and 7.58 t/ha in 2024. Starch, the main biochemical component of potato tubers, presented average values ranging between 13.95% in 2023 and 13.38% in 2024, the highest average values of starch content in tubers being determined to Brasovia variety in both years (16.35% in 2023 and 14.67% in 2024).

SPAD values of potato varieties grown under current climatic conditions could be useful traits to identify their heat-tolerant and the abilities to resist to higher temperatures.

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EFFECTS OF NITRIC AND AMMONIACAL NITROGEN FERTILIZATION ON THE ZELENY SEDIMENTATION INDEX IN COMMON WHEAT (*Triticum aestivum* L.)

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Abstract

The present study evaluated the effects of nitrogen form and fertilization level on the Zeleny sedimentation quality index of bread wheat (Triticum aestivum L.). A bifactorial experiment was conducted including 27 wheat cultivars and six fertilization regimes combining three nitrogen rates (120, 150, and 170 kg ha⁻¹ N) applied either as nitric or ammoniacal nitrogen, arranged in three replications. Analysis of variance revealed that cultivar, fertilization regime, and their interaction had a highly significant effect ($p < 0.001$) on the Zeleny index. Mean sedimentation values ranged from 44.12 to 60.94 mL among cultivars, while fertilization treatments varied between 48.31 mL under ammoniacal nitrogen and 55.93 mL under nitric nitrogen. Nitric nitrogen consistently produced significantly higher Zeleny index values compared to ammoniacal nitrogen at all fertilization levels. The fertilization regime accounted from the proportion of total variance (2.97%), followed by the cultivar $\times$ fertilization interaction (32.21%) and cultivar effect (28.46%). These results highlight the dominant role of nitrogen form in determining wheat technological quality and emphasize genotype-specific responses to fertilization strategies.

Key words: bread wheat, grain quality, nitrogen form, protein quality, wheat quality, Zeleny sedimentation index.

INTRODUCTION

Wheat mainly uses nitric nitrogen, under the influence of microorganisms, being immediately available and easily absorbed by plants, much more mobile in the soil, which leads to its solubilization in the root zone, especially on sandy soils or in conditions of abundant precipitation. In soil, nitric nitrogen moves mainly by mass flow with water movement and partly by diffusion, while ammonia nitrogen moves mainly by diffusion and less by mass flow (Mooshammer et al., 2014). The rate of movement of soil solution through mass flow depends on plant transpiration rate, soil water content, and soil texture (Barraclough et al., 2014). Absorption of ammoniacal nitrogen occurs specifically through the root plasma membrane, which serves as an ammonium ion transporter. These uptake transporters have a high affinity for ammonium ions, allowing wheat plants to achieve this even in the presence of low external ammonium concentrations. And once used, ammonium can be directly assimilated into

organic compounds through the process of glutamate dehydrogenase (GDH). This process catalyses the formation of glutamate from ammonium and 2-oxoglutarate. However, the efficient uptake mechanism may not have positive effects on wheat plants that are exposed to excessive soil ammonium levels, causing toxicity such as stunted root growth and reduced nutrient uptake efficiency (Ijato et al., 2021). Several studies have tested the molecular mechanisms behind the physiological responses of wheat plants to ammonia nitrogen fertilization. It has been found that NH_4^+ application can decrease proton efflux lipid peroxidation and protein carbonylation in root tissues, consequently causing plant growth suppression (Du et al., 2021). This is associated with the rate of ammonium absorption exceeding the rate of assimilation in the plant tissues, which leads to the disturbance of ion homeostasis, especially in potassium ions (Xiao et al., 2023). This disturbance of potassium ion balance can appear as leaf chlorosis, leaf scorch and lower biomass production (Castro et al., 2023). In addition, ammonium toxicity leads to

damage to the chloroplast ultrastructure, which in turn affects photosynthetic activity and plant growth. Nitrogen, which is essential in the formation of chlorophyll, which is stressed when the plant is exposed to excess ammonium (Kong et al., 2022). However, ammonium toxicity can be ameliorated to some extent by preventive strategies. Studies have revealed how ammonium uptake could be mitigated when nitric nitrogen is included in the fertilizer formulation. Wheat plants innately have the mechanism to store unused nitrate in vacuoles, where it is used for plant development, providing a way to mitigate the effect of ammonium toxicity (Wang et al., 2022). High levels of ammoniacal nitrogen in plants have the effect of significantly altering the oxidative metabolism of wheat roots, as well as most of their enzymatic activities. Research has shown that wheat root activity is negatively affected by ammonia nitrogen through reduced activity of key enzymes such as ascorbic acid, phosphoenolpyruvate carboxylase and mannose pyro phosphorylase (Lyu et al., 2022). In addition, NH_4^+ , through its means of disrupting the electron transport chain, has been shown to negatively influence membrane-bound oxidase and thereby increase cellular production of reactive oxygen, damaging photosynthesis, and organs that enhance uptake electrons inhibits the photosynthesis process (Wang et al., 2020). Nitrogen fertilizers used in agriculture include nitrates (salts of nitric acid), ammonium salts, and various organic nitrogen compounds. Nitric nitrogen and ammoniacal nitrogen fertilizers are mostly found on the market in the form of ammonium sulphate and calcium nitrate (Roman et al., 2011). Ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ is a nitrogen fertilizer containing 21.21% N, characterized by high water solubility and an acidic physiological reaction. Its long-term application may contribute to the acidification of neutral soils. Calcium nitrate $\text{Ca}(\text{NO}_3)_2$ is a readily soluble nitrogen fertilizer with an alkaline physiological reaction, making it suitable for use on acidic soils. It is typically available in hydrated crystalline forms with varying stability and hygroscopic properties. These contrasting properties influence soil reaction and nutrient availability, which are relevant for the present

study (Sala, 2008; Imbrea, 2014; Marschner, 2012).

Moreover, the maximum required amount of plant nutrients occurs in a short period of time from stem elongation to maturity, during which about 80% N, <80% P and <85% K are taken up by plants. Wheat quality indices depend on the variety, climate, and applied agronomic practices. One of the most important factors influencing the baking quality of wheat is nitrogen fertilization, particularly through its effect on protein content and the Zeleny sedimentation index. Zeleny sedimentation values are strongly influenced by the amount of nitrogen applied and its efficient uptake by plants. Numerous studies have shown that bread-making quality indices tend to improve with higher rates of nitrogen fertilization, mainly due to their positive effect on grain protein content and sedimentation values (Ma et al., 2021; Zhong et al., 2018; Kadar et al., 2018; Balla et al., 2019).

Wheat's Zeleny sedimentation index value is directly dependent to a complex of many natural and agrotechnical factors and their mutual connections (cultivated wheat variety, soil type, crop management and weather conditions). Abiotic stress, such as extreme temperatures and water shortages due to climate change, can affect wheat production and wheat baking quality indices worldwide (UNESCO, 2018; FAO, 2024; European Commission, 2024; Mălinaş et al., 2022).

In the context of increasing climatic variability and the occurrence of atypical weather conditions during the wheat growing season, the evaluation of agronomic factors affecting grain technological quality becomes particularly important. Irregular precipitation patterns and temperature fluctuations can influence nitrogen uptake and assimilation, which in turn affects protein accumulation and gluten quality. Under such environmental conditions, the efficiency of nitrogen fertilization and the form in which nitrogen is supplied to the crop may play a crucial role in determining wheat technological quality. However, the response of different wheat cultivars to various nitrogen forms and fertilization levels under atypical climatic conditions remains insufficiently documented. Therefore, the aim of the present study was to evaluate the effect of nitrogen fertilization on

the technological quality of bread wheat, assessed through the Zeleny sedimentation index. Specifically, the study aimed to (i) analyze the influence of different nitrogen forms (nitric and ammoniacal) on sedimentation values, (ii) assess the effect of increasing fertilization levels on gluten quality, and (iii) examine the interaction between wheat cultivars and fertilization regimes under the particular climatic conditions of the experimental year. The results of this research are expected to contribute to a better understanding of the relationship between nitrogen management, genotype response, and environmental conditions, providing useful information for improving wheat quality in modern agricultural systems.

MATERIALS AND METHODS

Site, Soil and Climatic Characteristics

The field experiment was conducted during the 2025–2026 growing seasons at the experimental station in Dudeștii Noi, located in the Western Plain of Romania (45°50'51" N, 21°06'30" E; altitude 87 m). This region represents one of the country's most important agricultural areas and belongs to the forest–steppe climatic zone. The area is characterized by relatively shallow groundwater levels and heterogeneous parent materials, which contribute to a diverse pedological background.

The geological evolution of the region is associated with the retreat of the Pannonian Lake, which resulted in extensive sediment accumulation. These deposits include coarse materials transported by the unstable bed of the Mureș River. The parent materials underlying the soils in the Dudeștii Noi area consist mainly of quartz sands with variable calcium carbonate content and medium-textured loess-like deposits. In certain micro depression areas, the influence of shallow groundwater has promoted salinization and alkalization processes.

The experimental plots were established on a wet phreatic cambic chernozem, slightly leached and characterized by a clay-loam texture, developed on fluvial carbonate deposits of medium to fine granulometry. The soil profile is well developed and shows relatively uniform physical, hydro physical, and chemical properties. Cambic chernozems are generally

characterized by high biological activity and good agricultural productivity. According to Crista (2017), these soils are typically deep and formed from transported and redeposited aeolian materials, such as loess and carbonate loess-like sediments.

The soil profile includes the following horizons: Ap (0–15 cm), Am (15–35 cm), Bv (35–60 cm), and Ck (60–100 cm) (Munteanu, 2012). The upper horizons are dark brown to dark gray brown in colour, with clay-loam to loam textures and well-developed granular structures that favour root penetration and aeration. The Bv horizon exhibits a subangular blocky structure and moderate compaction, whereas the Ck horizon consists of carbonate-rich parent material with a well-developed blocky structure.

Physical and Hydro physical Properties

The soil has a clay-loam texture, with silty-clay characteristics in the upper 70 cm and silty-loam features in the deeper layers. Bulk density values indicate moderate compaction throughout the soil profile, with 1.46 g cm⁻³ in the Ap horizon and 1.58 g cm⁻³ in the Am horizon. Total porosity is moderate in the Ap horizon (48.8%) and higher in the Am horizon (58.2%). Air-filled porosity shows moderate values in the Ap horizon (15.2%), higher values in the Am (23.8%) and Bv horizons (21.7%), and lower values in the Ck horizon (12.2%).

Clay content ranges from 33.4% to 40.3%, with the highest proportion recorded in the Am horizon. Slightly lower values were observed in the deeper Bv (33.8%) and Ck (33.4%) horizons. Hydro physical indices reflect these textural characteristics. The wilting coefficient ranged from 11.7% in the Ap and Am horizons to 13.2% in the Bv horizon, while the field capacity remained relatively uniform across the profile, with values of 23.3% in the Ap, Am, and Bv horizons and 22.4% in the Ck horizon (Obreja, 1964).

Chemical Properties

Soil chemical properties were determined using the Kjeldahl method for total nitrogen and the Olsen method for available phosphorus (Dumitru et al., 2011). Humus content was 3.04% in the Ap horizon, decreasing to 1.78% in the Am horizon and 1.24% in the Bv horizon. Total nitrogen content in the Ap horizon was

0.172%, indicating a moderate nitrogen supply. The soil was very well supplied with available phosphorus (168 ppm in the Am horizon) and well supplied with available potassium (248 ppm).

Soil reaction was measured potentiometrically in a 1:2.5 soil-to-water suspension, indicating a slightly acidic reaction in the Ap horizon (pH 5.58) and a slightly alkaline reaction in deeper horizons (up to pH 7.66). Exchangeable bases showed moderate values (23.2 cmol kg⁻¹ in Ap and 24.8 cmol kg⁻¹ in Am), while the base saturation degree reached approximately 92.6%, increasing slightly toward the Bv horizon. Regarding micronutrients, zinc concentration was relatively high in the Ap horizon (176 ppm) but extremely low in the Bv horizon (0.40 ppm).

Weather conditions during the experimental period

Climatic conditions recorded during the experimental period (October 2025-July 2026) were compared with the long-term climatic averages for the Timișoara meteorological station, located near the experimental field. (Figure 1). The regional climate is characterized as temperate continental with sub-Mediterranean influences, typical for the western part of Romania.

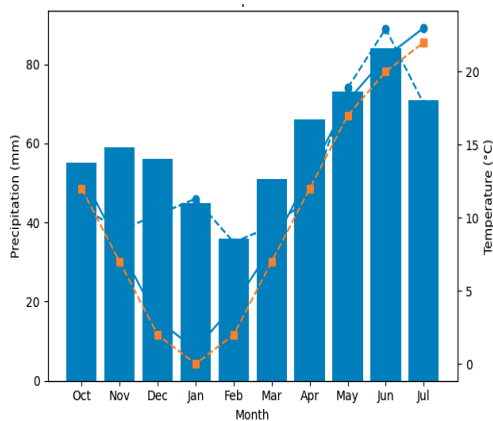


Figure 1. The average monthly climograph temperatures (°C) and rainfall (mm) recorded in Dudeștii Noi, (2025-2026)

The mean air temperature during the study period followed the typical seasonal pattern of the region, with a gradual decrease from autumn toward winter and a subsequent increase during

spring and summer. The lowest temperatures were recorded during January, while the highest values occurred in July. Compared with the multiannual average, temperatures during the experimental period showed slightly higher values during late spring and summer, which are favourable for crop growth and physiological activity.

Precipitation distribution during the experimental period showed moderate seasonal variability. Rainfall amounts were relatively moderate during autumn and winter months, while a significant increase occurred during late spring and early summer. The highest precipitation levels were recorded in June, consistent with the long-term climatic pattern of the region, when convective rainfall events are most frequent.

Overall, the climatic conditions recorded during the study period were close to the multiannual averages for the region, providing favourable moisture and thermal regimes for crop development.

Experimental Design and Plant Material

The biological material consisted of 27 winter wheat (*Triticum aestivum* L.) cultivars originating from breeding programs in Romania, Germany, and France. These cultivars are among the most widely cultivated in the Western Plain of Romania and were selected based on several agronomic criteria, including high yield potential, tolerance to major diseases and pest pressure, and favourable technological characteristics related to milling and baking quality.

The field experiment was arranged using a stratified randomized block design in order to minimize the influence of environmental heterogeneity and to ensure a reliable estimation of nitrogen utilization efficiency. Cultivars and fertilization treatments were factorially combined and distributed in randomized blocks with three replications.

Each experimental plot measured 1.2 m × 7 m, while the distance between neighbouring plots and adjacent blocks was 0.5 m and 1.5 m, respectively. A 2.5 m buffer strip was maintained between experimental units to reduce border effects. Each block contained 27 experimental plots, corresponding to the tested cultivars.

The study was based on a three-factor experiment arranged in subdivided plots, including 27 wheat cultivars and three nitrogen fertilization levels. The experimental factors were defined as follows:

Factor A - Wheat cultivar

Dacic, Miranda, Alex, Litera, Ciprian, Crișana, Biharia, Glosa, Bohemia, Sothys, Sacramento, Rubisko, Certiva, Aurelius, Aspect, Papillon, Activus, Centurion, Tika Taka, Cheignon, Sosthene, Vivendo, Sophie, Solindo, Tiberius, Arrezo, and Apexus.

Factor B - Nitrogen fertilizer form

b1 - ammoniacal nitrogen

b2 - nitric nitrogen

Factor C - Nitrogen fertilization rate

c1 - 120 kg N ha⁻¹

c2 - 150 kg N ha⁻¹

c3 - 170 kg N ha⁻¹

The nitrogen application rates were established considering the maximum allowable nitrogen input of 170 kg N ha⁻¹, according to the regulations of the Romanian Agency for Payments and Intervention in Agriculture (APIA, 2021). The lowest fertilization rate (120 kg N ha⁻¹) was selected based on soil fertility indicators, including humus content and available soil nitrogen, as well as typical nitrogen requirements for winter wheat cultivation.

Nitrogen was supplied in two chemical forms: ammoniacal nitrogen applied as ammonium sulphate (NH₄)₂SO₄ and nitric nitrogen applied as calcium nitrate Ca(NO₃)₂. Fertilizers were applied as a single, non-fractionated dose at the beginning of stem elongation (BBCH growth stage 30-31).

During both years of the study, the seeding rate for each cultivar was calculated based on seed purity, germination capacity, and thousand kernel weight, resulting in a sowing density of 550 viable seeds m⁻². Wheat was sown at a depth of 4 cm, with a row spacing of 12.5 cm.

The fertilization treatments were defined as follows:

V1 - nitric nitrogen, 120 kg N ha⁻¹

V2 - nitric nitrogen, 150 kg N ha⁻¹

V3 - nitric nitrogen, 170 kg N ha⁻¹

V4 - ammoniacal nitrogen, 120 kg N ha⁻¹

V5 - ammoniacal nitrogen, 150 kg N ha⁻¹

V6 - ammoniacal nitrogen, 170 kg N ha⁻¹

The control treatment (Mt) corresponded to the mean value of the experimental field.

Determination of the Zeleny Sedimentation Index

✓ Principle of the Method

The method is based on the formation of a suspension of wheat flour in a lactic acid solution in the presence of bromophenol blue solution. In this acidic environment, the gluten proteins hydrate and swell, forming aggregates that settle during the sedimentation period. The resulting sediment volume is measured after a fixed time interval and is used to estimate the gluten strength and protein quality of the flour. The Zeleny sedimentation value is influenced by several factors, including the total protein content of the flour, the qualitative characteristics of gluten proteins, and the hardness of the wheat kernels. As a result, the test is commonly used in cereal quality evaluation and wheat breeding programs to estimate the technological quality of wheat.

Apparatus

The determination of the sedimentation index was performed using equipment compliant with ISO 5529:2007, including:

- a special four-roll mill designed for preparing wheat samples for the Zeleny sedimentation test (LM 3310 PerkinElmer);
- a metal sieve with a mesh size of 150 μm used for flour standardization;
- a four-position tube shaker equipped with a timer for controlled agitation;
- a sedimentation unit provided with a timer to ensure standardized sedimentation conditions;
- glass sedimentation tubes (flat-bottom cylinders with plastic stoppers), graduated in cm³, with a capacity of 100 cm³;
- an analytical balance with a precision of 0.01 g.

✓ Reagents

The following reagents were used during the analysis:

- bromophenol blue solution, used as a dye indicator to facilitate the visualization of sediment formation;
- lactic acid solution 2% (v/v), which provides the acidic medium necessary for gluten swelling and sediment formation.

All reagents were prepared according to the specifications indicated in the reference standards.

✓ Methodology

Prior to analysis, the wheat sample was cleaned to remove large mechanical impurities and broken kernels. This was carried out by manual

sieving using a sieve with openings of 20 mm × 1 mm. The cleaned sample was subsequently milled using the LM 3310 PerkinElmer mill, ensuring a uniform particle size suitable for the sedimentation test.

The resulting flour was then sieved for 1.5 minutes through a 150 µm metal sieve, in accordance with the requirements of ISO 5529:2007, to obtain a standardized flour fraction for analysis. A sample of 3 g flour was weighed with an analytical balance to an accuracy of 0.01 g and transferred into sedimentation test tubes. Subsequently, 50 mL of bromophenol blue solution were added to each tube. The tubes were sealed with plastic stoppers, and the contents were mixed by manually shaking the tubes horizontally for 5 seconds to ensure homogeneous dispersion of the flour particles.

The tubes were then placed in a mechanical shaker and agitated for 5 minutes. After this initial agitation period, 25 mL of lactic acid solution (2% (v/v)) were added to each tube. The tubes were returned to the shaker and agitated for an additional 5 minutes, ensuring proper interaction between the flour suspension and the acidic medium.

Following agitation, the tubes were placed in the sedimentation unit and allowed to stand undisturbed to permit sediment formation. After a sedimentation period of 15 minutes, the sediment volume was read directly from the graduated scale of the tube and expressed in millilitres (mL). This value represents the Zeleny sedimentation index.

✓ Calculation of Results

For each sample, two parallel determinations were performed under identical experimental conditions in order to ensure the reliability and reproducibility of the results. The final sedimentation index was calculated as the arithmetic mean of the two measurements and expressed as a whole number.

RESULTS AND DISCUSSIONS

The Zeleny sedimentation index is a widely used parameter for assessing the quantity and quality of gluten in wheat flour. It is expressed as the volume (mL) of sediment obtained from a suspension of flour in lactic acid solution after a standardized sedimentation period. The sedimentation value reflects the swelling

capacity of gluten proteins in an acidic medium and is therefore considered an indirect indicator of the baking quality of wheat flour (Hrušková et al., 2003; Kibkalo, 2022).

Higher sedimentation values are generally associated with increased protein content and stronger gluten network formation, leading to improved dough strength and baking performance. Conversely, lower values indicate weaker gluten and reduced baking quality. The Zeleny sedimentation value is positively correlated with grain protein content and gluten composition, particularly glutenin fractions, which are key determinants of bread volume and technological quality (Guerrini et al., 2020).

Thus, the sedimentation test provides an integrated assessment of gluten quantity and quality, including gluten resistance, beyond indices that evaluate these attributes separately (Hrušková et al., 2003; Kibkalo, 2022). From a practical perspective, sedimentation values between 20-34 mL indicate satisfactory flour quality, 35-50 mL indicate good quality, while values above 50 mL are characteristic of excellent baking performance (Jankowski et al., 2015).

The experimental data were statistically analysed using analysis of variance (ANOVA) to evaluate the effects of the studied factors and their interactions. When significant differences among treatments were detected, Student's t-test was applied to compare the means. Statistical significance was considered at $p \leq 0.05$.

F test (Table 1, p column), shows that: A factor (variety), B factor (level of fertilization) and their interaction $A \times B$ had very significant action, meaning: wet gluten content differences between varieties are very significant; there are very significant differences between the 6 fertilization systems; $A \times B$ interaction has very significant action. Hence, the 27 winter wheat varieties reacted differently; within the 6 agrofunds, in terms of wet gluten content, the values obtained differ significantly between them. In conclusion, the null hypothesis H_0 is rejected for factor A (variety), factor B (level of fertilization) and the $A \times B$ interaction. To examine the differences between the different varieties, the differences between the agrofunds, as well as the interaction $A \times B$ (variety $\times$ agrofund) we apply the t-test and we will use as the error variant: $s^2 = 3.6$, degrees of freedom = 324.

Table 1. Analysis of variance

Variation source	SSP (SP)	Degree of Freedom	Weighted Least of Squares WSL (s ²)	F test for s ² error		
				Value	P	Significance
A (variety)	7248	26	279	63,6	0,0000	***
B (agrofund)	18511	5	3702	844,7	0,0000	***
A×B	10867	130	84	19,1	0,0000	***
Error	1420	324	4			
Total	38045					

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$. 0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The Zeleny sedimentation value achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience, the following values of the sedimentation index were obtained: the highest Zeleny sedimentation values were recorded in the varieties Tika Taka (60.94 mL), Tiberius and Ciprian (approximately 58 mL), Aurelius, Cheignon, and Sophie, followed by Crişana,

Biharia, and Arrezo (around 54-55 mL), all exceeding the threshold of 50 mL associated with very good baking quality. In contrast, the lowest values were observed in Papillon (44.12 mL), Centurion (45.57 mL), Rubisko, and Sothys (approximately 46 mL), indicating comparatively weaker gluten strength. The remaining varieties showed intermediate values without significant differences.

Table 2. Influence of variety on winter wheat Zeleny sedimentation value

Measured Parameter	Zeleny sedimentation value / Significance		Difference
Variety	mL		%
a1 – Dacic	54.26/**		1.7
a2 – Miranda	51.18/0		-1.4
a3 – Alex	54.39/**		1.8
a4 – Litera	51.05/0		-1.5
a5 – Ciprian	57.78/**		5.2
a6 – Crişana	55.44/**		2.9
a7 – Biharia	55.29/**		2.7
a8 – Glossa	50.93/00		-1.7
a9 – Boema	52.80/NS		0.2
a10 – Sothys	46.38/000		-6.2
a11 – Sacramento	48.58/000		-4.0
a12 – Rubisko	45.77/000		-6.8
a13 – Certiva	48.97/000		-3.6
a14 – Aurelius	57.18/**		4.6
a15 – Aspekt	53.38/ns		0.8
a16 – Papillon	44.12/000		-8.5
a17 – Activus	53.28/NS		0.7
a18 – Centurion	45.57/000		-7.0
a19 – Tika Taka	60.94/**		8.4
a20 – Cheignon	56.94/**		4.4
a21 – Sosthene	49.26/000		-3.3
a22 – Vivendo	52.95/NS		0.4
a23 – Sophie	56.87/**		4.3
a24 – Solindo	52.84/NS		0.3
a25 – Tiberius	57.85/**		5.3
a26 – Arrezo	54.59/**		2.0
a27 – Apexus	51.38/0		-1.2
Average	52.59	Cv	
LSD 5% = 1.17; LSD 1% = 1.54; LSD 0.1% = 1.96			

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$. 0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

The Zeleny sedimentation value achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control group - the average of the experience, very significant increases were obtained at the all of the 6 treatments. The highest value of this index was obtained at V1 of 55.93 mL, followed by V3 - 55.88 mL and V2 - 55.66 mL. Ammoniacal nitrogen fertilization levels ranked below the experience average.

Relative to the experimental mean (control), the Zeleny sedimentation index showed both positive and negative statistically significant deviations (Table 4), indicating that certain cultivars recorded values either above or below the trial average. This variability highlights the differential response of wheat genotypes to nitrogen fertilization regimes.

Table 3. Influence of nitrogen fertilizers on winter wheat Zeleny sedimentation value

Measured Parameter	Zeleny sedimentation value / Significance	Difference
Variant	mL	%
V1	55.93/***	3.34
V2	55.66/***	3.07
V3	55.88/***	3.29
V4	48.31/000	-4.28
V5	49.18/000	-3.41
V6	50.57/000	-2.02
Average	52.59	Cv
LSD 5% = 0.584; LSD 1% = 0.769; LSD 0.1% = 0.981		

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$. 0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

Table 4. Influence of variety $\times$ nitrogen fertilizers interaction on winter wheat Zeleny sedimentation value

Variety	Zeleny sedimentation value and significations											
	V 1		V 2		V 3		V 4		V 5		V 6	
	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.	SV	Diff.
a1- Dacic	57.05	4.5**	58.80	6.2***	57.80	5.2***	47.60	-5.0 ⁰⁰⁰	49.25	-3.3 ⁰	55.05	2.5
a2- Miranda	53.55	1.0	52.65	0.1	55.35	2.8	47.90	-4.7 ⁰⁰	51.85	-0.7	45.75	-6.8 ⁰⁰⁰
a3- Alex	57.05	4.5**	59.15	6.6***	61.55	9.0***	41.25	-11.3 ⁰⁰⁰	50.95	-1.6	56.40	3.8
a4- Litera	56.80	4.2**	49.50	-3.1 ⁰	51.85	-0.7	42.25	-10.3 ⁰⁰⁰	58.55	6.0***	47.35	-5.2 ⁰⁰⁰
a5- Ciprian	64.35	11.8***	58.05	5.5***	60.95	8.4***	50.55	-2.0	56.70	4.1**	56.05	3.5*
a6- Crišana	60.70	8.1***	54.50	1.9	56.80	4.2**	56.00	3.4*	48.70	-3.9 ⁰⁰	55.95	3.4*
a7- Biharia	59.55	7.0***	65.00	12.4***	56.40	3.8**	47.15	-5.4 ⁰⁰⁰	47.90	-4.7 ⁰⁰	55.75	3.2*
a8- Glossa	55.80	3.2*	53.65	1.1	55.25	2.7	45.20	-7.4 ⁰⁰⁰	47.20	-5.4 ⁰⁰⁰	48.45	-4.1 ⁰⁰
a9- Boema	55.70	3.1*	56.15	3.6*	56.50	3.9**	49.00	-3.6 ⁰	49.65	-2.9 ⁰	49.80	-2.8
a10- Sothys	51.55	-1.0	48.50	-4.1 ⁰⁰	49.15	-3.4 ⁰	44.75	-7.8 ⁰⁰⁰	41.85	-10.7 ⁰⁰⁰	42.45	-10.1 ⁰⁰⁰
a11- Sacramento	53.45	0.9	49.00	-3.6 ⁰	52.05	-0.5	47.50	-5.1 ⁰⁰⁰	44.00	-8.6 ⁰⁰⁰	45.50	-7.1 ⁰⁰⁰
a12- Rubisko	49.10	-3.5 ⁰	50.95	-1.6	49.40	-3.2 ⁰	40.45	-12.1 ⁰⁰⁰	43.90	-8.7 ⁰⁰⁰	40.80	-11.8 ⁰⁰⁰
a13- Certiva	51.75	-0.8	52.50	-0.1	51.10	-1.5	45.55	-7.0 ⁰⁰⁰	44.15	-8.4 ⁰⁰⁰	48.75	-3.8 ⁰⁰
a14- Aurelius	62.00	9.4***	58.35	5.8***	60.30	7.7***	55.75	3.2*	51.20	-1.4	55.45	2.9*
a15- Aspekt	58.55	6.0***	59.00	6.4***	57.05	4.5**	48.65	-3.9 ⁰⁰	46.85	-5.7 ⁰⁰⁰	50.20	-2.4
a16- Papillon	48.65	-3.9 ⁰⁰	47.10	-5.5 ⁰⁰⁰	41.30	-11.3 ⁰⁰	43.30	-9.3 ⁰⁰⁰	41.30	-11.3 ⁰⁰⁰	43.05	-9.5 ⁰⁰⁰
a17- Activus	56.00	3.4*	58.30	5.7***	56.30	3.7*	51.45	-1.1	45.35	-7.2 ⁰⁰⁰	52.25	-0.3
a18- Centurion	49.15	-3.4 ⁰	45.65	-6.9 ⁰⁰⁰	51.25	-1.3	39.30	-13.3 ⁰⁰⁰	44.20	-8.4 ⁰⁰⁰	43.85	-8.7 ⁰⁰⁰
a19- Tika Taka	66.30	13.7***	65.70	13.1***	60.60	8.0***	57.80	5.2***	56.10	3.5*	59.15	6.6***
a20- Chevignon	61.05	8.5***	59.90	7.3***	61.35	8.8***	56.55	4.0**	50.80	-1.8	52.00	-0.6
a21- Sosthene	51.95	-0.6	51.25	-1.3	56.35	3.8**	42.50	-10.1 ⁰⁰⁰	43.60	-9.0 ⁰⁰⁰	49.90	-2.7
a22- Vivendo	52.80	0.2	54.80	2.2	52.30	-0.3	51.75	-0.8	52.45	-0.1	53.60	1.0
a23- Sophie	44.10	-8.5 ⁰⁰⁰	61.05	8.5***	66.95	14.4***	56.55	4.0**	57.60	5.0***	54.95	2.4
a24- Solindo	58.35	5.8***	58.35	5.8***	57.55	5.0***	47.05	-5.5 ⁰⁰⁰	47.25	-5.3 ⁰⁰⁰	48.50	-4.1 ⁰⁰
a25- Tiberius	62.80	10.2***	62.60	10.0***	59.80	7.2***	54.30	1.7	54.20	1.6	53.40	0.8
a26- Arrezo	57.70	5.1***	58.85	6.3***	61.05	8.5***	46.50	-6.1 ⁰⁰⁰	50.65	-1.9	52.80	0.2
a27- Apexus	54.40	1.8	53.45	0.9	52.40	-0.2	47.90	-4.7 ⁰⁰	51.75	-0.8	48.35	-4.2 ⁰⁰
Average	52,59											
DL 5% = 2.86; DL 1% = 3.76; DL 0.1% = 4.80												

*, **, or *** indicate statistically significant differences between sample means and Mt based on t-test, at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively. NS (not significant) indicates the t-test difference between sample means was $p > 0.05$. 0, 00 or 000 also indicate significant differences between sample means and Mt based on t-test at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively, but with negative values.

Under nitric nitrogen fertilization, several cultivars consistently exhibited sedimentation index values exceeding the experimental mean. At the fertilization level of 120 kg N ha⁻¹, the cultivars Dacic, Alex, Litera, Ciprian, Crișana, Biharia, Glossa, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Solindo, Tiberius, and Arrezo recorded values above the experimental average. Increasing the nitrogen rate to 150 kg N ha⁻¹ resulted in above-average sedimentation index values for Dacic, Litera, Ciprian, Biharia, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Sophie, Solindo, Tiberius, and Arrezo. At the highest fertilization level (170 kg N ha⁻¹), the cultivars Dacic, Alex, Crișana, Biharia, Boema, Aurelius, Aspect, Activus, Tika Taka, Chevignon, Sosthene, Sophie, Solindo, Tiberius, and Arrezo maintained sedimentation index values above the experimental mean. In contrast, under ammoniacal nitrogen fertilization, a smaller number of cultivars exceeded the experimental average. At 120 kg N ha⁻¹, the cultivars Crișana, Aurelius, Tika Taka, Chevignon, and Sophie showed higher sedimentation index values than the trial mean. At 150 kg ha⁻¹, only Litera, Ciprian, and Sophie recorded above-average values, while at 170 kg N ha⁻¹ the cultivars Ciprian, Crișana, Biharia, Aurelius, and Tika Taka exceeded the experimental mean. Overall, the results indicate that nitric nitrogen fertilization generally promoted higher Zeleny sedimentation index values compared with ammoniacal nitrogen, suggesting a stronger positive effect on gluten quality and wheat technological performance.

The analysis of variance indicates that the variability of the Zeleny sedimentation index is primarily determined by the combined influence of genetic factors and their interaction with fertilization practices. The relatively high proportion of variance attributed to the genotype × fertilization interaction (32.21%) suggests that the response of wheat cultivars to nitrogen supply is strongly genotype-specific. This highlights the importance of considering cultivar-dependent responses when designing fertilization strategies aimed at improving technological grain quality.

The genotype effect itself accounted for 28.46% of the total variance, confirming the major role of genetic background in controlling gluten

strength and protein quality, which are key determinants of sedimentation capacity.

Although the direct contribution of the fertilization regime was comparatively small (2.97%), its interaction with genotype demonstrates that nitrogen fertilization remains an important agronomic factor capable of enhancing grain quality when applied in combination with well-adapted cultivars. The relatively high proportion of unexplained variance (36.37%) may be associated with environmental variability during the experimental period, particularly under atypical climatic conditions that can influence nitrogen uptake, protein synthesis, and gluten formation (Figure 2).

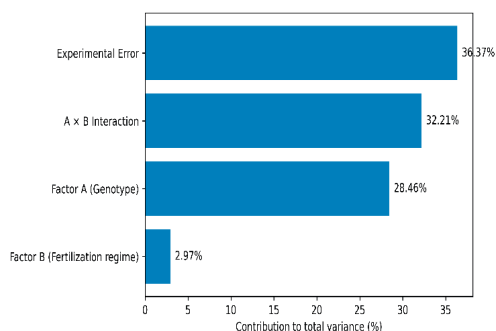


Figure 2. Contribution of experimental factors to total variance of the Zeleny sedimentation index

Overall, these findings emphasize that the technological quality of wheat, expressed through the Zeleny sedimentation index, results from a complex interaction between genetic potential, nitrogen management, and environmental conditions. Consequently, improving wheat quality requires an integrated approach that combines cultivar selection with optimized fertilization practices adapted to specific agroecological conditions.

CONCLUSIONS

The results of the present study highlight the complex interaction between nitrogen fertilization, genetic background, and environmental conditions in determining the technological quality of bread wheat, as expressed through the Zeleny sedimentation index. The experimental data obtained under the agro-climatic conditions of the Western Plain of

Romania revealed considerable variability among cultivars and fertilization treatments.

The analysis of variance showed that the genotype $\times$ fertilization interaction was the main source of variability for the sedimentation index, accounting for 32.21% of the total variance, followed by the genotype factor, which contributed 28.46%. In contrast, the direct effect of fertilization regime was relatively limited, accounting for only 2.97% of the total variance. The relatively high experimental error, 36.37%, suggests that environmental conditions during the experimental year also had a substantial influence on wheat quality expression.

Nitrogen form clearly affected wheat technological quality. Across fertilization levels, nitric nitrogen produced more favorable sedimentation responses than ammoniacal nitrogen, as reflected by the higher number of cultivars exceeding the experimental mean. This effect was particularly evident at the rate of 120 kg ha⁻¹, where sixteen cultivars exceeded the mean under nitric nitrogen, compared with only five under ammoniacal nitrogen. These results indicate that nitric nitrogen had a stronger positive influence on gluten quality under the conditions of this study.

Although increasing nitrogen fertilization generally improved sedimentation values, the magnitude of response differed among cultivars. Several genotypes, including Dacic, Boema, Aurelius, Activus, Tika Taka, Chevignon, Solindo, and Tiberius, consistently recorded values above the experimental mean under multiple fertilization treatments. This behavior indicates stable technological performance and a strong genetic capacity for protein and gluten formation.

These findings are consistent with previous research showing that nitrogen fertilization influences grain protein content and gluten quality, both of which are closely associated with the Zeleny sedimentation index (Zeleny, 1947; Triboi & Triboi-Blondel, 2002; Gooding et al., 2007). The Zeleny test remains a reliable indicator of gluten strength and baking quality and is widely used in wheat quality assessment and breeding programs (Wrigley et al., 2006; Wieser, 2007). The genotype-dependent response to nitrogen fertilization observed in this study has also been reported by other authors (Slafer et al., 2014; Zörb et al., 2018).

Overall, the improvement of wheat technological quality requires an integrated approach that combines appropriate nitrogen management with the selection of cultivars capable of efficiently using nitrogen for protein and gluten synthesis. Under the conditions of this study, nitric nitrogen fertilization proved more effective than ammoniacal nitrogen in enhancing the Zeleny sedimentation index.

The main findings can be summarized as follows:

- The genotype $\times$ fertilization interaction was the strongest determinant of variation in the Zeleny sedimentation index, confirming the importance of genotype-specific responses to nitrogen management.
- Nitric nitrogen was more effective than ammoniacal nitrogen in improving sedimentation values, especially at higher fertilization levels.
- Cultivars such as Dacic, Boema, Aurelius, Activus, Tika Taka, Chevignon, Solindo, and Tiberius showed stable above-average technological performance.
- Environmental conditions contributed substantially to the observed variability, indicating that wheat quality expression is strongly influenced by year-specific agro-climatic factors.
- Optimizing wheat grain quality requires combining cultivar selection with suitable nitrogen fertilization strategies rather than relying on fertilization level alone.

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EFFECTS OF AMMONIUM-BASED FERTILIZERS ON WINTER WHEAT YIELD AND GRAIN QUALITY

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Abstract

This study evaluated the effects of ammonium nitrate and ammonium sulfate fertilization on winter wheat yield and grain quality under field experimental conditions. Grain yield and major quality parameters, including protein content, gluten content, thousand kernel weight, and test weight, were determined. Nitrogen was applied at five fertilization rates: b1 - N0, b2 - N50, b3 - N100, b4 - N150, and b5 - N170 kg N ha⁻¹, to assess crop response to increasing nitrogen supply. The results indicated that both nitrogen source and application rate significantly affected grain yield, with higher nitrogen rates promoting increased productivity. Nitrogen fertilization also exerted a pronounced effect on grain quality traits, leading to higher protein and gluten contents, while thousand kernel weight and test weight showed distinct responses depending on nitrogen form and dose. Overall, the study demonstrates that optimizing nitrogen source and application rate is essential for achieving a balanced improvement in winter wheat yield and grain quality under intensive cropping systems. These findings provide practical guidance for nitrogen management strategies aimed at sustainable wheat production systems.

Key words: ammonium nitrate, ammonium sulfate, grain quality, nitrogen fertilization, protein content.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, providing a major source of calories and protein for the global population. Its strategic role in food security is expected to intensify as climate change, population growth, and dietary shifts increase the pressure on agricultural systems to produce higher yields without compromising grain quality and food safety (Curtis & Halford, 2014; Smuleac et al., 2020; Gherasimescu et al., 2023). In this context, nitrogen (N) nutrition remains a central factor governing wheat productivity and grain quality, yet also represents one of the most complex and controversial aspects of cereal crop management due to its environmental footprint and economic cost (Hawkesford, 2014).

Nitrogen is the primary limiting nutrient for wheat growth and yield formation, exerting strong influence on canopy development, photosynthetic capacity, grain number, and grain filling processes. At the same time, nitrogen availability directly affects the synthesis and accumulation of storage proteins

in the grain, which determine functional quality traits such as protein content, gluten strength, and bread-making performance (Blandino et al., 2020). However, excessive or inefficient N fertilization has negative consequences, including nitrate leaching, greenhouse gas emissions, and an increased risk of reduced nitrogen use efficiency (NUE), all of which challenge the sustainability of intensive wheat production systems (Hawkesford, 2014; Chitu et al., 2024).

The need to balance high yield with acceptable grain quality is increasingly complicated by climate change. Rising temperatures, altered precipitation patterns, and increased frequency of extreme weather events have been shown to affect nitrogen uptake dynamics and protein accumulation in wheat grains (Asseng et al., 2019; Smuleac et al., 2020). Climate-driven changes in crop phenology may shorten grain filling periods or decouple nitrogen uptake from carbohydrate accumulation, leading to reductions or increased variability in grain protein concentration (Asseng et al., 2019). Under these conditions, optimizing fertilizer strategies becomes a crucial adaptation measure

to maintain both yield stability and quality standards.

Grain protein concentration is one of the most important quality parameters in wheat, particularly for bread-making and industrial processing. While higher nitrogen availability generally increases protein content, the relationship between yield and grain protein often shows a dilution effect, whereby increased carbohydrate deposition reduces protein concentration at high yield levels (Curtis & Halford, 2014). This trade-off highlights the need for fertilization strategies that improve nitrogen partitioning to the grain rather than merely stimulating vegetative growth.

In addition to nitrogen, sulfur (S) nutrition has gained increasing attention in wheat production systems. Sulfur is a constituent of essential amino acids such as cysteine and methionine and is directly involved in the formation of gluten proteins that determine dough viscoelastic properties. Several studies have demonstrated that insufficient sulfur supply may limit the effectiveness of nitrogen fertilization, reducing protein quality despite adequate or high nitrogen inputs (Ereku et al., 2012; Hrivna et al., 2015). As atmospheric sulfur deposition has declined significantly due to air pollution control measures, sulfur deficiencies have become more common in agricultural soils, particularly in high-yielding cereal systems (Raffan et al., 2020).

The interaction between nitrogen and sulfur nutrition is therefore critical for wheat grain quality. Research has shown that combined N and S fertilization improves gluten composition, enhances protein functionality, and contributes to more stable bread-making quality under varying environmental conditions (Blandino et al., 2020; Wilson et al., 2020). Moreover, sulfur availability influences nitrogen metabolism in the plant, affecting amino acid synthesis and nitrogen assimilation efficiency (Tabak et al., 2020). These interactions underline the importance of evaluating fertilizer sources that supply both nitrogen and sulfur in an agronomically and environmentally efficient manner.

Among mineral nitrogen fertilizers, ammonium nitrate and ammonium sulfate are widely used due to their high solubility and rapid nitrogen

availability. Ammonium nitrate provides both nitrate and ammonium forms of nitrogen, offering flexibility in plant uptake, while ammonium sulfate supplies nitrogen exclusively in ammonium form along with a substantial amount of sulfur. Comparative studies have demonstrated that the form of nitrogen influences root uptake patterns, soil nitrogen dynamics, and ultimately crop yield and quality (Wang et al., 2015). In dryland and temperate environments, ammonium-based fertilizers may affect nitrate accumulation at different soil depths, with implications for nitrogen losses and plant availability (Wang et al., 2015).

Several authors have reported significant differences in wheat response depending on the nitrogen form applied. Jan et al. (2011) showed that both source and timing of nitrogen application affected wheat productivity, emphasizing the importance of synchronizing nitrogen availability with crop demand. Similarly, Tabak et al. (2019) demonstrated that ammonium nitrate enriched with ammonium sulfate improved both yield and grain quality of winter wheat, while also influencing soil chemical properties. These findings suggest that integrating sulfur into nitrogen fertilization strategies may enhance NUE and quality outcomes.

Grain quality assessment extends beyond protein and gluten concentration to include physical parameters such as thousand kernel weight (TKW) and test weight, which reflect grain filling efficiency and market acceptance. Nitrogen fertilization has been shown to influence these parameters, although responses vary depending on environmental conditions, cultivar, and fertilizer form (Hrivna et al., 2015). Increased nitrogen rates often enhance grain protein while showing variable effects on kernel weight, highlighting the need to optimize fertilizer regimes that do not compromise physical grain quality.

In recent years, food safety considerations have added a new dimension to fertilizer management in wheat. Free asparagine concentration in wheat grain is a precursor for acrylamide formation during high-temperature processing, such as baking and toasting. High nitrogen availability and sulfur deficiency have been associated with increased free asparagine

accumulation, raising concerns about food safety despite improvements in grain protein content (Fritz, 2020; Raffan et al., 2020). Studies have emphasized that balanced N and S nutrition is crucial for minimizing asparagine levels while maintaining desirable quality traits (Wilson et al., 2020; Oddy et al., 2023).

Environmental variability further complicates the relationship between fertilization and grain quality traits. Oddy et al. (2023) highlighted that year-to-year differences in temperature and rainfall significantly influence free asparagine concentration, underscoring the need to account for environmental effects when evaluating fertilizer strategies. Similarly, Asseng et al. (2019) indicated that climate-driven changes may alter protein responses to nitrogen fertilization, reinforcing the importance of adaptive nutrient management under future climatic scenarios.

Late-season nitrogen application has been proposed as a strategy to improve grain protein concentration without excessive yield stimulation. Blandino et al. (2020) demonstrated that late N fertilization significantly affected gluten composition and technological quality in high-protein wheat grown under humid Mediterranean conditions. However, the effectiveness of such strategies depends on nitrogen form, sulfur availability, and environmental context, making it necessary to evaluate their performance under diverse agronomic conditions.

Despite extensive research on nitrogen fertilization, gaps remain regarding the comparative effects of ammonium nitrate and ammonium sulfate across a wide range of nitrogen rates, particularly in relation to combined yield, quality, and food safety parameters. While studies have addressed individual aspects of nitrogen or sulfur fertilization, integrated assessments that consider multiple quality indicators - such as protein content, gluten concentration, thousand kernel weight, and test weight - remain limited. Furthermore, the variability in crop response across nitrogen doses highlights the need for dose-response studies that identify thresholds beyond which additional nitrogen provides diminishing or negative returns (Tabak et al., 2020).

Given the increasing pressure to improve nitrogen use efficiency and reduce environmental impacts, understanding how different ammonium-based fertilizers influence wheat performance is of both scientific and practical importance. The choice of fertilizer source, rate, and nutrient composition must align with agronomic goals, environmental constraints, and quality requirements of the wheat processing industry. As global wheat production systems face mounting challenges from climate change and sustainability demands, research that integrates yield performance, grain quality, and nutrient efficiency remains essential (Curtis & Halford, 2014; Asseng et al., 2019, Sitnicki et al., 2024). In this context, evaluating the effects of ammonium nitrate and ammonium sulfate fertilization across multiple nitrogen rates provides valuable insight into optimized nutrient management strategies for winter wheat. Such studies contribute to the development of balanced fertilization recommendations that enhance productivity while ensuring grain quality and food safety under current and future agricultural conditions.

MATERIALS AND METHODS

The research was carried out at the Didactic and Experimental Station (SDE) Timișoara, Romania, during the agricultural seasons 2023-2025. The area is characterized by a temperate continental climate, favorable for winter wheat cultivation, with moderate rainfall and temperature conditions specific to western Romania.

The soil of the experimental field is classified as cambic chernozem with phreatic moisture influence. This soil type is known for its high fertility, good structure, and adequate water-holding capacity. The soil showed medium to good supply with phosphorus and potassium, while nitrogen availability required fertilization (Crista et al., 2011). The experiment was organized as a bifactorial field trial using a randomized complete block design with three replications. The experimental factors were nitrogen fertilizer type and nitrogen application rate.

Two nitrogen fertilizer sources were studied (Factor A): a₁ - ammonium nitrate (AN); a₂ - ammonium sulfate (AS).

Nitrogen was applied in five different rates (Factor B): b₁ – N₀ (unfertilized control), b₂ – N₅₀, b₃ – N₁₀₀, b₄ – N₁₅₀, b₅ – N₁₇₀ kg N ha⁻¹.

These fertilization levels were chosen to evaluate the response of winter wheat yield and grain quality across a wide range of nitrogen supply.

Winter wheat was sown at the optimal sowing time recommended for the area, using a certified variety adapted to local conditions. The seeding rate ensured uniform plant population in all experimental variants.

Phosphorus and potassium fertilizers were applied uniformly across all plots before sowing, according to local soil recommendations. Crop management practices, including weed control, disease protection, and pest management, were applied equally to all treatments to eliminate non-fertilization effects. Nitrogen fertilizers were applied in two splits: the first application at the beginning of spring vegetation and the second at stem elongation. In ammonium sulfate treatments, sulfur was supplied through the fertilizer source.

At crop maturity, wheat was harvested from the central area of each plot. Grain yield was determined and expressed on a hectare basis at standard moisture content.

Grain samples were collected for laboratory analyses. Grain quality was evaluated through protein content, gluten content, thousand kernel weight, and test weight (hectolitre mass). Standard analytical methods were used for all determinations. Protein content, gluten content, and test weight were determined using a Granomat Pfeuffer analyzer, while thousand kernel weight was determined by counting and weighing the grains using a Kern&Sohn precision balance.

Statistical analyses were performed using STATISTICA 7 software, using one-way and multi-way analysis of variance (ANOVA), as well as cluster analysis. Calculations of marginal differences (LD) and the contribution of experimental factors and their interactions were performed in Microsoft Excel. Comparison of means was performed using Duncan's test using MSTAT-C software.

The effects of nitrogen fertilizer source, nitrogen rate, and year were evaluated, as well as their interactions. Differences between treatment means were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Grain yield of winter wheat was strongly influenced by nitrogen fertilization, both in terms of fertilizer type and application rate (Table 1). In the unfertilized control (N₀), grain yield reached 2308 kg·ha⁻¹, reflecting the baseline productive potential of the cambic chernozem soil at SDE Timișoara. Application of nitrogen resulted in significant yield increases at all fertilization levels, with yields rising progressively from 3042 kg·ha⁻¹ at N₅₀ to 5390 kg·ha⁻¹ at N₂₀₀ (Table 1). All fertilized treatments showed very highly significant differences compared to the control.

Table 1. Grain yield (kg·ha⁻¹) of winter wheat as influenced by nitrogen fertilizer type and nitrogen rate

Nitrogen rate	N0	N50	N100	N150	N200	Mean
Ammonium nitrate	2308	2934	3792	4733	5237	3801
Ammonium sulfate	2308	3150	3933	4915	5543	3970
Relative yield (%)	100	128	164	189	209	–
Yield increase (kg·ha ⁻¹)	–	653	1473	2035	2501	–
Significance	–	***	***	***	***	–
<i>LSD (kg·ha⁻¹): 5% = 149; 1% = 181; 0.1% = 234.</i>						

Significance level: ***highly significant at $p \leq 0.001$

These results confirm the well-documented role of nitrogen as the primary yield-limiting nutrient in wheat production. Similar yield responses to increasing nitrogen rates were reported by Jan et al. (2011) and Hawkesford (2014), who emphasized that adequate nitrogen supply is essential for maximizing grain number and grain weight. The progressive yield increase observed in the present study indicates efficient nitrogen uptake and utilization under the given soil and climatic conditions.

Comparison between fertilizer types revealed a consistent yield advantage for ammonium sulfate over ammonium nitrate at all nitrogen rates (Table 1). The average yield obtained with ammonium sulfate was 3970 kg·ha⁻¹, compared to 3801 kg·ha⁻¹ for ammonium

nitrate, corresponding to a 4% increase. A similar advantage of sulfur-containing nitrogen fertilizers was reported by Tabak et al. (2019) and Tabak et al. (2020), who attributed this effect to improved nitrogen use efficiency and enhanced protein synthesis when sulfur was supplied together with nitrogen. Hrivna et al. (2015) also showed that sulfur fertilization contributes to higher wheat productivity, particularly on soils with moderate sulfur availability.

Thousand kernel weight (TKW) increased steadily with increasing nitrogen rates for both fertilizer types, indicating improved grain filling under enhanced nitrogen availability (Table 2). Under ammonium nitrate fertilization, TKW increased from 37.3 g in the control to 42.7 g at N200, while ammonium sulfate resulted in higher values, reaching 44.4 g at the highest nitrogen rate (Table 2).

Table 2. Thousand kernel weight (TKW, g) as influenced by nitrogen fertilizer type and nitrogen rate

Nitrogen rate	N0	N50	N100	N150	N200	Mean
Ammonium nitrate	37.3	39.6	40.2	42.8	42.7	40.43
Ammonium sulfate	37.3	39.9	41.8	43.4	44.4	41.23
Relative value (%)	100	107	110	116	119	–
Difference (g)	–	2.6	4.5	6.1	7.1	0.85

The observed increase in TKW is consistent with findings reported by Hrivna et al. (2015), who demonstrated that nitrogen fertilization improves kernel development and final grain weight. The superior response under ammonium sulfate fertilization is in agreement with Erekul et al. (2012), who highlighted the positive interaction between nitrogen and sulfur nutrition on grain filling processes. Sulfur plays a key role in amino acid synthesis and enzyme activity, which may explain the improved assimilate accumulation in the grain observed in the present study.

Test weight (hectolitre mass) showed a positive response to nitrogen fertilization, increasing with nitrogen rate regardless of fertilizer type (Table 3). Values increased from 76.7 kg·hl⁻¹ in the unfertilized control to approximately 79.7–79.8 kg·hl⁻¹ at higher nitrogen rates. Ammonium sulfate treatments consistently recorded slightly higher test weight values compared to ammonium nitrate (Table 3).

Table 3. Test weight (hectolitre mass, kg·hl⁻¹) as influenced by nitrogen fertilizer type and nitrogen rate

Nitrogen rate	N0	N50	N100	N150	N200	Mean
Ammonium nitrate	76.7	77.2	77.2	79.2	79.7	76.12
Ammonium sulfate	76.7	78.3	79.1	79.3	79.8	78.52
Relative value (%)	100	102	103	103	104	–
Difference (kg·hl ⁻¹)	–	1.6	2.4	2.6	3.1	0.40

Although absolute differences were relatively small, the average test weight was higher under ammonium sulfate fertilization (78.52 kg hl⁻¹) than under ammonium nitrate (76.12 kg hl⁻¹), indicating improved grain density and uniformity. These results suggest that combined nitrogen and sulfur supply contributed positively to kernel structure and endosperm development.

These results are in line with those reported by Tabak et al. (2019), who observed improvements in test weight following fertilization with ammonium nitrate enriched with ammonium sulfate. Although the absolute differences were relatively small, test weight is an important commercial quality indicator, and even minor increases may improve grain classification and market value.

Grain protein content increased significantly with increasing nitrogen rates for both fertilizer types (Table 4). Protein concentration increased from 10.65% in the unfertilized control to over 13.7% at the highest nitrogen rate.

This response confirms the strong relationship between nitrogen availability and protein accumulation in wheat grain, as widely reported in the literature (Hawkesford, 2014; Curtis & Halford, 2014).

Table 4. Grain protein content (%) as influenced by nitrogen fertilizer type and nitrogen rate

Nitrogen rate	N0	N50	N100	N150	N200	Mean
Ammonium nitrate	10.65	11.05	11.85	13.25	13.75	12.09
Ammonium sulfate	10.65	11.25	12.25	13.75	14.05	12.19
Difference (%)	–	0.60	1.60	3.10	3.40	0.08

At intermediate and high nitrogen rates, ammonium sulfate consistently resulted in slightly higher protein content than ammonium nitrate (Table 4). Mean protein content values were 12.09% for ammonium nitrate and 12.19% for ammonium sulfate, indicating a

modest but consistent advantage of sulfur-containing fertilization in improving grain protein concentration.

Similar findings were reported by Wilson et al. (2020) and Blandino et al. (2020), who demonstrated that sulfur availability enhances protein synthesis and improves gluten-forming protein fractions. Asseng et al. (2019) also emphasized that optimizing nitrogen nutrition is crucial for maintaining grain protein concentration under changing climatic conditions.

Wet gluten content followed a similar trend to protein content, showing a strong positive response to increasing nitrogen rates (Table 5).

Table 5. Wet gluten content (%) as influenced by nitrogen fertilizer type and nitrogen rate

Nitrogen rate	N0	N50	N100	N150	N200	Mean
Ammonium nitrate	20.31	23.21	26.26	28.38	29.43	25.51
Ammonium sulfate	20.31	23.33	26.42	29.84	30.35	26.05
Difference (%)	–	3.02	6.11	9.53	10.04	0.54

Values increased from approximately 20% in the unfertilized control to over 29% at higher nitrogen rates. Ammonium sulfate fertilization resulted in consistently higher gluten content compared to ammonium nitrate at all nitrogen levels.

The mean wet gluten content was 26.05% under ammonium sulfate fertilization, compared to 25.51% under ammonium nitrate. The higher gluten content observed with ammonium sulfate highlights the importance of sulfur availability for the synthesis of sulfur-containing amino acids essential for gluten protein structure and functional quality.

These results are consistent with those reported by Erekul et al. (2012) and Blandino et al. (2020), who showed that sulfur fertilization improves gluten quantity and quality by enhancing the synthesis of sulfur-containing amino acids essential for gluten structure. The positive effect of ammonium sulfate observed in the present study supports the conclusion that balanced nitrogen and sulfur nutrition is critical for achieving high bread-making quality.

The increase in nitrogen application rate led to a marked rise in protein and gluten yield per unit area, as a result of the simultaneous

increase in grain yield and in the concentration of these components (Tables 6 and 7). Compared with the unfertilized control (N0), nitrogen application rates of 150-200 kg N·ha⁻¹ resulted in approximately 2.9-3.0 fold increases in protein yield and more than threefold increases in gluten yield.

At all fertilization levels, ammonium sulfate produced higher protein and gluten yields than ammonium nitrate. The differences between fertilizer types increased with nitrogen rate, reaching maximum values of +58.7 kg·ha⁻¹ for protein yield and +141.1 kg·ha⁻¹ for gluten yield at the N200 rate. This trend indicates superior nitrogen use efficiency when sulfur is supplied together with nitrogen, due to its essential role in the synthesis of sulfur-containing amino acids and in the formation of gluten proteins.

Given that both grain yield and protein and gluten concentrations showed statistically significant differences among treatments, the observed differences in protein and gluten yield can be considered agronomically relevant. These results support the advantage of ammonium sulfate fertilization under the experimental conditions, contributing to higher production of technologically valuable grain components.

Table 6. Protein yield (kg·ha⁻¹) of winter wheat as influenced by nitrogen fertilizer type and rate

Nitrogen rate	Protein yield – AN (kg·ha ⁻¹)	Protein yield – AS (kg·ha ⁻¹)	Difference AS–AN (kg·ha ⁻¹)
N0	245.8	245.8	0.0
N50	324.2	354.4	+30.2
N100	449.4	481.8	+32.4
N150	627.1	675.8	+48.7
N200	720.1	778.8	+58.7
Mean	473.3	507.0	+33.7

Table 7. Wet gluten yield (kg·ha⁻¹) of winter wheat as influenced by nitrogen fertilizer type and rate

Nitrogen rate	Gluten yield – AN (kg·ha ⁻¹)	Gluten yield – AS (kg·ha ⁻¹)	Difference AS–AN (kg·ha ⁻¹)
N0	468.8	468.8	0.0
N50	681.0	734.9	+53.9
N100	995.8	1039.1	+43.3
N150	1343.2	1466.6	+123.4
N200	1541.2	1682.3	+141.1
Mean	1006.0	1078.4	+72.4

The combined interpretation demonstrates that increasing nitrogen rates significantly enhanced winter wheat yield and grain quality, while ammonium sulfate provided consistent advantages over ammonium nitrate across all evaluated parameters. These findings are in agreement with previous studies emphasizing the importance of integrated nitrogen and sulfur fertilization strategies for improving wheat productivity and technological quality (Hrivna et al., 2015; Tabak et al., 2020; Wilson et al., 2020).

The present results confirm and extend existing knowledge by demonstrating that, under cambic chernozem soil conditions and temperate continental climate, ammonium sulfate represents an effective nitrogen source for winter wheat, contributing to both higher yield and improved grain quality.

CONCLUSIONS

The results of this study demonstrate that nitrogen fertilization had a decisive influence on winter wheat yield and grain quality under the agroecological conditions of SDE Timișoara. Grain yield increased significantly with increasing nitrogen rates, regardless of fertilizer type, confirming nitrogen as the primary limiting nutrient for wheat productivity. All nitrogen-fertilized treatments produced very highly significant yield increases compared to the unfertilized control.

Among the two nitrogen fertilizer sources, ammonium sulfate consistently outperformed ammonium nitrate at equivalent nitrogen rates. The average grain yield obtained under ammonium sulfate fertilization was 4% higher than that recorded for ammonium nitrate, indicating improved nitrogen use efficiency when sulfur was supplied together with nitrogen.

Nitrogen fertilization also significantly improved grain quality parameters. Thousand kernel weight and test weight increased with nitrogen rate, reflecting enhanced grain filling and kernel density. Grain protein and wet gluten contents showed a strong positive response to increasing nitrogen supply, with slightly higher values recorded under ammonium sulfate fertilization. These results highlight the beneficial role of sulfur in

supporting protein synthesis and gluten formation.

At all nitrogen levels, ammonium sulfate generated higher protein and gluten yields than ammonium nitrate, highlighting the important role of sulfur in nitrogen assimilation and in the synthesis of gluten-forming proteins.

Overall, the study confirms that combined nitrogen and sulfur fertilization represents an effective strategy for achieving high yield and improved technological quality of winter wheat grown on cambic chernozem soils.

Based on the obtained results, the following practical recommendations can be formulated for winter wheat cultivation under similar soil and climatic conditions:

Nitrogen fertilization is essential for maximizing winter wheat yield and grain quality, with application rates of 100-150 kg N·ha⁻¹ providing a favorable balance between productivity and quality.

Ammonium sulfate is recommended as a nitrogen source, particularly on soils with low or moderate sulfur availability, due to its positive effects on yield, protein content, and gluten quality.

Split application of nitrogen, with doses applied at the resumption of spring vegetation and at stem elongation, is advised to improve nitrogen uptake efficiency and reduce potential losses.

Excessive nitrogen rates should be avoided, as yield increases beyond 150 kg N·ha⁻¹ were relatively smaller, while the risk of reduced nitrogen use efficiency may increase.

Integrating sulfur into nitrogen fertilization strategies is especially important for wheat intended for bread-making, where protein and gluten quality are critical.

These recommendations contribute to more efficient nutrient management and support sustainable winter wheat production systems.

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SURVEY ON INCREASING RATES OF THE HERBICIDE STRATOS ULTRA (100 G/L CYCLOXYDIM) ON YIELD AND GRAIN QUALITY OF DUO SYSTEM MAIZE HYBRIDS

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Abstract

The Duo System technology in maize is extensively used in agricultural practice. It is characterized by the cultivation of cycloxydim-tolerant hybrids, while the herbicide ensures efficient control of monocotyledonous weed species, which may compromise grain yields and quality. Overall, the selectivity of the herbicide primarily depends on the application rate but the hybrids differ in their tolerance levels. A research for evaluating the effects of increasing rates of Stratos Ultra was conducted in the experimental field of the Agricultural University Plovdiv, Bulgaria. Three Duo System hybrids were grown - Zanetixx DUO, Hexxagone DUO, and Elaraxx DUO. Each hybrid was treated five increasing herbicidal rates ranging from 1.00 to 5.00 L ha⁻¹. The obtained results were compared to Untreated (Earthed-up) control. After harvesting, yields and grain quality parameters such as moisture, 1000 grain weight, hectoliter seed mass, protein, oil and starch content were estimated. Due to the increased rates of 4.00 and 5.00 L ha⁻¹ of Stratos Ultra and the reported herbicide phytotoxicity significant decrease in grain yields and its quality was recorded.

Key words: maize, tolerance, cycloxydim, yields, quality.

INTRODUCTION

The area under maize cultivation is increasing significantly, with expectations that it will be the most widely grown crop in the next decade (Erenstein et al., 2022). Advances in maize genetics and good agronomic practices in its cultivation are inextricably linked, and in order to obtain sustainable production, they need to complement each other. This is also one of the main reasons why in recent years, numerous research centers, private investors, and companies have funded and promoted research to improve the genetic characteristics of maize, citing calculations that genetic improvement increases annual maize production by over 50% (Nuccio et al., 2021). One major limitation in maize production is the challenge of weed infestation (Ali et al., 2011; Rai et al., 2018). Maize, as a representative of cereal crops, contains the enzyme acetyl-CoA carboxylase, which makes it highly susceptible to graminicides (Gengenbach et al., 1990). This is the main reason why monocotyledonous weeds significantly reduce yields in fields where weed infestation is high. So far, integrated crop protection has proven to be the most effective

approach for weed control. The use of herbicides, mechanical methods, and biological weed control are essential for increasing the yield potential of maize fields. Herbicide-tolerant crops are compatible with the principles of integrated pest management and, when combined with appropriate weed control measures, can provide sustainable long-term benefits (Knezevic et al., 2003). Duo System is a maize production system based on herbicide-tolerant hybrids. This technology allows treatment against narrow-leaved weeds to be performed using herbicides to which the cultivated hybrids are tolerant. Few studies have addressed the issue of both cycloxydim efficacy and hybrids' tolerance level. Landes et al. (1996) reported excellent selectivity for maize at twice the recommended maximum dose for controlling grass weeds. Pferdmenges et al. (2010) reported studies in which DUO System technology plants were treated with the herbicide Focus Ultra. The results show excellent selectivity of the herbicide even under adverse weather conditions for crop development. A 17% increase in yield was reported for hybrids treated with Focus Ultra compared to the yields

obtained after application of sulfonylureas. Vancetovic et al. (2009) indicate that accomplishment of maize hybrids resistance to cycloxydim at rates up to 4.00 l ha⁻¹ is possible by “the introduction of just one CTM allele (heterozygote) into a single cross maize hybrid”.

According to Szel et al. (2010), cycloxydim-tolerant maize yields are affected by weather conditions during the growing season. This is evidenced by the results of a two-year trial of hybrids treated with cycloxydim. In the first year, yields were higher compared to those in the second year. This is explained by the significantly wetter conditions at the time of treatment with the herbicide in the second year, which may have increased the absorption of the active substance and had a negative effect.

In 2020, Hristova et al. (2021) conducted a field trial with the CT maize hybrid “Blazon Duo” in an experimental field of the Agricultural University - Plovdiv. The efficacy of the herbicide product Starane Gold (100 g/l fluroxypyr + 1 g/l florasulam) was evaluated at increasing rates (1.20, 1.80 and 2.40 l ha⁻¹). The highest efficacy against broadleaf weeds was found at doses of 1.80 and 2.40 l ha⁻¹, but phytotoxic symptoms were assessed for the higher rate. Simultaneously with the decrease in the density of broadleaf weeds, a significant increase in the values of the studied traits such as plant height and number of ears per plant was observed. The absolute and hectoliter seed mass, as well as grain yields, were highest in the treatments with rates of 1.20 and 1.80 l ha⁻¹. During the period 2012-2014 Delchev and Barakova (2016) conducted an experiment with a maize hybrid from the DUO System technology - Ultrafox Duo, treating the plants with various soil-applied and post-emergence herbicides, as well as combinations between them. Analysis of the data collected over the three-year experiment shows that the highest grain yield was obtained from the combination of the soil herbicide Merlin Flex, the herbicide Kalam and Focus Ultra. The technology successfully controlled the perennial grass weeds *Agropyrum repens* (L.) and *Cynodon dactylon* (L.) Pers, as well as all broad-leaved weeds within the boundaries of the experimental field.

Malidža et al. (2020) conducted an experiment by growing cycloxydim-tolerant maize integrated with beans in order to enlarge the monocotyledons weeds control potential. The possibility to use cycloxydim in system with other herbicides results in efficacy over 90% against *S. halepense* from rhizomes.

In Iran, Annabestani et al. (2020) carry out a trial in order to establish effective weed management program. The study showed that treatments with low rates of cycloxydim (75-150 g ha⁻¹) come out with analogous results compared with herbicide treatments including nicosulfuron, foramsulfuron, eradicane, and 2,4-D + MCPA. Nevertheless, increasing the rates of cycloxydim sprayed brings about adequate control of perennial grass weeds without any significant yield reduction.

In practice, the cultivation of herbicide-tolerant crops is widely used and the further improvement of these technologies is important for a number of aspects of agriculture - obtaining high crop yields at lower production costs, environmentally friendly production and a sustainable solution to the weed problem in the future. Trials conducted with hybrids of Duo System technology to date have primarily focused on assessing the weed control efficacy of cycloxydim herbicides, with some experiments using multiple application rates. However, data regarding the effects of increasing rates of cycloxydim on primary compounds of maize grain such as oil, starch and protein (Guo et al., 2013) are insufficient. For practical purposes, more information is needed on whether Duo System hybrids combine high productivity with herbicide tolerance (Simic et al., 2013).

The purpose of the present study is to evaluate the influence of increasing application rates of cycloxydim on the grain yield and quality of three Duo System maize hybrids.

MATERIALS AND METHODS

In 2024 and 2025, a field trial was conducted in the research plot of the Department of “Agriculture and Herbology” at the Agricultural University of Plovdiv, Bulgaria (Latitude 42°7'58"N, Longitude 24°48'19"E). The experimental field underwent soil cultivation and a combination of

fertilizers (500 kg ha⁻¹ N:P:K (15%-15%-15%) + 7% SO₃ + Zn (Borealis – L.A.T Company, Austria) was used. The evaluated hybrids (Zanetixx DUO, Hexxagone DUO and Elaraxx DUO) were planted in the latter part of April with a seeding rate 60 000 seeds per ha⁻¹, marking the beginning of the cultivation period. The experiment was conducted using a randomized complete block design, with each plot measuring 20 m². Stratos Ultra (100 g/l cycloxydim) was sprayed in phenophase 3rd-5th leaves. For broadleaved weeds management a treatment with Casper (50 g/kg prosulfuron + 500 g/kg dicamba) inter-rows only was done. There were no other pesticides used for pests or diseases management. Six experimental variants for every hybrid were included in the study: 1. Untreated control; 2. Stratos Ultra - 1.00 L ha⁻¹; 3. Stratos Ultra - 2.00 L ha⁻¹; 4. Stratos Ultra - 3.00 L ha⁻¹; 5. Stratos Ultra - 4.00 L ha⁻¹; 6. Stratos Ultra - 5.00 L ha⁻¹. Manual harvesting was performed. Moisture content (%) and hectoliter weight (kg hL⁻¹) were determined using a Wile 200 grain moisture meter. In order to monitor grain quality measuring of 1000 grain weight was done. Estimation of crude protein content according to Kjeldahl / Total nitrogen (%),

BDS EN ISO 17025/2006; starch content according to Evers (%), BDS EN ISO 17025/2006 and oil content was done. Statistical analysis of the data was performed using Duncan's Multiple Range Test with SPSS, considering differences significant at a p-value of less than 0.05.

RESULTS AND DISCUSSIONS

A number of studies have been conducted to determine the effects of herbicides on the growth and development of crops and their subsequent yields. However, there is limited information in the scientific literature on their effects on grain quality parameters (Wilkinson & Hardcastle, 1972).

Variations in grain moisture content strongly influence the analysis of machine performance during harvest and post-harvest operations (Mousaviraad et al., 2020).

In order to determine whether there are statistically significant differences in grain moisture content due to increasing rates of cycloxydim mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$ (Table 1).

Table 1. Grain moisture, %

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	12.46 a	12.40 a	12.60 a	12.70 a	12.23 a	12.06 a
	Hexxagone DUO	13.20 a	12.70 ab	12.43 b	12.53 b	12.76 ab	12.60 b
	Elaraxx DUO	12.83 c	13.53 a	13.11 ab	12.86 c	12.96 bc	12.96 bc
2025	Zanetixx DUO	12.63 a	12.80 a	12.80 a	12.90 a	12.96 a	13.06 a
	Hexxagone DUO	13.66 a	13.46 a	13.63 a	13.66 a	13.16 a	13.43 a
	Elaraxx DUO	13.96 a	13.70 a	13.93 a	13.90 a	13.46 a	13.90 a
Average for the period	Zanetixx DUO	12.54	12.60	12.70	12.80	12.59	12.56
	Hexxagone DUO	13.43	13.08	13.03	13.09	12.96	13.01
	Elaraxx DUO	13.39	13.61	13.52	13.38	13.21	13.43

Means followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 1 shows the grain moisture content (%) for the individual treatments of the three hybrids, calculated as the mean of three replications for each treatment. No statistically significant differences in grain moisture content were found for the individual variants of any of the hybrids in the second experimental year. It is necessary to store the maize at a humidity lower than 14%. Maize grain is not a significant source of proteins, and

their content in maize hybrids is a quality indicator that is influenced by numerous external factors, including the application of herbicides (Gołębiowska & Rola, 2010). The qualitative analysis of the production during the two experimental years shows that the percentage of crude protein in the grains decreases in direct proportion to the increase in the dose of the herbicide Stratos Ultra, and this is valid for all three hybrids. Statistically

significant differences are found between the first four variants and variants 5 and 6, in which the values for this indicator are significantly lower. The highest crude protein content was measured in 2025 in the Untreated control of the hybrid Elaraxx DUO - 17.77.

Variant 6 exhibited the lowest mean values over the two-year experimental period, and for the three hybrids the percentage decrease compared to the untreated control is as follows - Zanetixx DUO - 9.34%, Hexxagone DUO - 10.25% and Elaraxx DUO - 8.52% (Table 2).

Table 2. Crude protein content, % in grain

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	16.62 a	16.20 b	16.11 b	15.67 c	15.41 d	14.75 e
	Hexxagone DUO	16.02 a	16.06 a	15.97 a	15.69 b	14.84 c	14.34 d
	Elaraxx DUO	16.95 a	16.87 a	16.54 b	16.21 c	15.83 d	15.66 e
2025	Zanetixx DUO	17.20 a	17.51 a	1675 b	16.60 b	16.12 c	15.91 c
	Hexxagone DUO	17.13 a	17.08 a	16.72 ab	16.35 bc	16.07 c	15.41 d
	Elaraxx DUO	17.77 a	17.46 ab	17.21 bc	16.93 c	16.44 d	16.10 d
Average for the period	Zanetixx DUO	16.91	16.85	16.43	16.13	15.76	15.33
	Hexxagone DUO	16.58	16.57	16.34	16.02	15.45	14.88
	Elaraxx DUO	17.36	17.16	16.88	16.57	16.14	15.88

Means followed by different letters are significantly different according to Duncan’s multiple range test at p ≤ 0.05.

Sugars in plants are stored mainly in the form of starch (Wang et. al., 2023). It is produced as a result of photosynthesis in plants. Despite its tolerance to a particular herbicide, when treating the hybrids with rates higher than the maximum accepted, the production of assimilates during photosynthesis may be reduced. Based on the analysis conducted, in 2024 this indicator decreased most significantly in the Elaraxx DUO hybrid, due to the higher rates applied in variants 5 and 6 of the experiment. In the Hexxagone DUO hybrid, a decrease was also reported, while in the Zanetixx DUO hybrid, the difference in the values of starch in the grains between the

variants was insignificant, although statistically proven according to Duncan's test. However, in the second experimental year, the starch content in the grain decreased significantly as a result of the treatment with increasing rates, with the lowest values measured in variant 6 in all three hybrids. The Elaraxx DUO hybrid showed the highest grain starch content, with the maximum value for the entire experimental period (63.08%) observed in the untreated control. Compared to the variant without treatment, in variant 6 the starch decreased by 4.89%, 4.34% and 8.53% in the Zanetixx DUO, Hexxagone DUO and Elaraxx DUO hybrids, respectively (Table 3).

Table 3. Starch content, % in grain

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	59.81 a	59.69 b	59.64 b	59.62 b	59.51 c	59.3 d
	Hexxagone DUO	60.18 b	60.67 a	60.59 a	59.45 c	59.41 c	58.35 d
	Elaraxx DUO	62.65 a	62.28 b	62.12 c	61.42 d	59.45 e	58.30 f
2025	Zanetixx DUO	61.13 b	62.69 a	60.42 bc	59.54 c	57.87 d	55.80 e
	Hexxagone DUO	61.82 b	61.51 a	60.67 a	59.15 b	58.93 b	58.36 b
	Elaraxx DUO	63.08 a	62.22 ab	62.71 ab	61.20 b	59.15 c	56.71 d
Average for the period	Zanetixx DUO	60.47	61.19	60.03	59.58	58.69	57.51
	Hexxagone DUO	61.00	61.09	60.63	59.30	58.90	58.35
	Elaraxx DUO	62.86	62.25	62.41	61.31	59.30	57.50

Means followed by different letters are significantly different according to Duncan’s multiple range test at p ≤ 0.05.

The oil content in the maize grain is of great importance as an indicator of the nutritional

quality of the product. As a result of the use of herbicides, changes can occur in both the oil

content and its qualitative composition (Wilkinson & Hardcastle, 1973). In their experiment, Guo et al. (2013) reported that treatment with the herbicide atrazine at the maximum recommended rates led to changes in the fatty acid composition of maize oil. However, no significant effect was observed on the total oil content. These findings suggest that, while herbicide application may alter the qualitative profile of maize oil, the overall quantity of oil remains largely unaffected. As it

is a major representative of the group of ACCase inhibitors - a key enzyme for the synthesis of fatty acids in plants, treatment with cycloxydim can negatively affect lipid synthesis, which can affect the formation of membranes and plant growth. In the present study, a significant decrease in oil content was found as a result of increasing rates of herbicide application. The tolerance of the hybrids is lower in variants 5 and 6, which is evident from the results (Table 4).

Table 4. Oil content, % in grain

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	5.03 a	4.96 a	4.86 ab	4.74 b	4.50 c	4.30 d
	Hexxagone DUO	4.12 a	4.07 ab	3.96 b	3.78 c	3.58 d	3.24 e
	Elaraxx DUO	4.12 a	4.11 a	3.99 ab	3.88 b	3.65 c	3.34 d
2025	Zanetixx DUO	4.81 a	4.71 b	4.59 c	4.46 d	4.34 e	4.17 f
	Hexxagone DUO	3.81 a	3.70 b	3.35 c	3.24 d	3.14 e	2.92 f
	Elaraxx DUO	3.85 a	3.79 ab	3.69 b	3.53 c	3.44 c	3.16 d
Average for the period	Zanetixx DUO	4.92	4.84	4.72	4.60	4.42	4.23
	Hexxagone DUO	3.97	3.88	3.66	3.51	3.36	3.08
	Elaraxx DUO	3.99	3.95	3.84	3.71	3.50	3.25

Means followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$

Grain oil content reached its highest value in the untreated control of the Zanetixx DUO hybrid in 2024 - 5.03%, while the lowest value was observed in variant 6 of the Hexxagone DUO hybrid in 2025 - 2.92%. The highest average values for the indicator are recorded in the Zanetixx DUO hybrid, as in all variants, the oil content exceeds 4%. Thousand-seed weight is an important indicator of seed size and overall seed quality. The highest value during

the experimental period was registered in 2024 in the variant without treatment of the hybrid Hexxagone DUO – 336.68g. The value of this indicator largely characterizes the studied hybrids, but can be significantly influenced by the crop management system and other external factors (Petrov, 2013). The analysis shows that the value of this indicator is significantly influenced by the increase in the rate of herbicide application (Table 5).

Table 5. Thousand-seed grain weight, g

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	308.36 a	319.28 a	317.82 a	278.81 ab	256.57 b	213.22 ab
	Hexxagone DUO	336.68 a	314.54 ab	289.80 bc	277.09 c	264.61 d	232.66 cd
	Elaraxx DUO	280.92 ab	318.78 a	324.46 a	299.60 a	282.64 ab	217.42 b
2025	Zanetixx DUO	320.06 a	296.86 ab	281.38 bc	280.86 bc	291.70 ab	249.13 c
	Hexxagone DUO	326.17 a	320.30 a	322.79 a	321.30 a	302.17 b	298.44 b
	Elaraxx DUO	325.62 a	318.42 ab	317.34 ab	309.28 ab	290.25 b	285.75 b
Average for the period	Zanetixx DUO	314.21	308.07	299.60	279.83	274.13	231.17
	Hexxagone DUO	331.42	317.42	306.29	299.19	283.39	265.55
	Elaraxx DUO	303.27	318.60	320.90	304.44	286.44	251.58

Means followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

A significant decrease in the thousand-seed grain weight is clearly observed in variants 5 and 6, treated with the highest rates. However,

in the second growing year, the noted decrease in these variants is relatively small. According to the Duncan test ($p < 0.05$), statistically

significant differences were found between the untreated control and variant 6.

The most severely affected when applying the maximum rate was this indicator in the Zanetixx DUO hybrid, for which a reduction of 31% was reported in 2024, and 22% in 2025, compared to the untreated control. In 2024, the values of the indicator in the Hexxagone DUO and Elaraxx DUO decreased by 31% and 23%, respectively, and by 9 and 12% in 2025.

According to the average values obtained for the two experimental years, as a result of the application of the herbicide Stratos Ultra at a rate of 5 L ha⁻¹ (var. 6), the thousand-seed grain weight parameter decreased most significantly in the Zanetixx DUO hybrid by 26.43%, followed by Hexxagone DUO - 19.88% and Elaraxx DUO - 17.04%.

The hectoliter weight of the grain is an important quality parameter assessed in the grain trade.

A slight variation in the values of this indicator is reported when analyzing the average values for the two experimental years. The decrease in the hectoliter weight in variant 6 compared to the untreated control is only 1.62% for Zanetixx DUO, 2.00% and 0.92% for Hexxagone DUO and Elaraxx DUO. No statistically significant differences were found among the values obtained for this parameter during the second experimental year, as determined by Duncan's multiple range test. Although differences were observed during the first year of the trial, it cannot be concluded that the treatment with Stratos Ultra had an effect on this parameter (Table 6).

Table 6. Hectoliter weight (kg hL⁻¹)

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	79,5 ab	82,1 a	79,43 ab	79,06 ab	78,1 b	77,2 b
	Hexxagone DUO	79,63 ab	79,83 a	79,40 ab	78,5 b	79,02 b	78,43 ab
	Elaraxx DUO	75,73 a	76,53 a	75,90 a	74,13 b	75,93 a	73,46 b
2025	Zanetixx DUO	77,30 a	77,66 a	78,06 a	77,60 a	77,60 a	77,06 a
	Hexxagone DUO	77,90 a	78,46 a	78,23 a	77,96 a	77,63 a	77,90 a
	Elaraxx DUO	75,03 a	75,20 a	75,36 a	75,33 a	75,83 a	75,93 a
Average for the period	Zanetixx DUO	78,40	79,88	78,74	78,33	77,85	77,13
	Hexxagone DUO	79,76	79,14	78,81	78,23	78,32	78,16
	Elaraxx DUO	75,38	75,86	75,63	74,73	75,88	74,69

Means followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Under conditions of severe weed infestation, herbicide application has been shown to significantly improve crop productivity (Stoimenova et. al., 2004; Mahmood and Swenton, 2005; Zhelyazkov, 2007; Tonev et al., 2010; Stoyanova et al., 2021).

The results obtained for this parameter largely support this statement., as well as the results of biometric measurements, although this has not been statistically proven.

In the first experimental year for the Zanetixx DUO hybrid, the highest yield was obtained with variant 3 - 1103.02 kg/da, at an application rate of 200 ml/da of Stratos Ultra, reporting an increase of 16%.

In the Hexxagone DUO hybrid, the highest yield was obtained from the plants from the untreated control - 1144.96 kg/da, and in the Elaraxx DUO hybrid in variant 2, in which the

plants were treated with the indicated minimum effective rate of the herbicide - 1356.02 kg/da, which is 22% higher than the yield obtained from the plants grown without herbicide treatment. In the second experimental year, no statistically significant differences were found between the first three variants.

From the analysis of the average values for the entire experimental period, it was found that the treatment with 50% higher than the recommended maximum rate of the herbicide (variant 4) was accompanied by a slight decrease in the yields of the hybrids.

The values for this indicator in the three hybrids Zanetixx, Hexxagone and Elaraxx DUO decreased by 5%, 14% and 8%, respectively, compared to the variant without treatment (Table 7).

Table 7. Grain yields, t ha⁻¹

Year of the experiment	Hybrids	Variants					
		1.Untreated control	2.Stratos Ultra – 1 L ha ⁻¹	3.Stratos Ultra – 2 L ha ⁻¹	4.Stratos Ultra – 3 L ha ⁻¹	5.Stratos Ultra – 4 L ha ⁻¹	6.Stratos Ultra – 5 L ha ⁻¹
2024	Zanetixx DUO	9,25 bc	10,77 ab	11,03 a	9,08 c	6,17 d	5,60 d
	Hexxagone DUO	11,45 a	10,93 a	10,45 ab	9,04 bc	7,41 cd	7,08 d
	Elaraxx DUO	10,55 bc	13,56 a	11,82 ab	9,28 cd	7,19 de	6,50 e
2025	Zanetixx DUO	11,70 a	10,79 ab	10,71 ab	9,46 bc	8,65 c	6,48 d
	Hexxagone DUO	12,15 a	12,31 a	12,04 a	11,33 a	7,13 b	7,13 b
	Elaraxx DUO	12,50 a	11,26 a	12,11 a	11,82 a	7,16 b	6,90 b
Average for the period	Zanetixx DUO	10,48	10,78	10,87	9,27	7,32	6,04
	Hexxagone DUO	11,80	11,62	11,24	10,19	7,27	7,10
	Elaraxx DUO	11,52	12,41	11,96	10,55	7,18	6,70

Means followed by different letters are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

After applying Stratos Ultra twice at the maximum recommended rate, a yield reduction of 30% was recorded for the Zanetixx DUO hybrid and 38% for the Hexxagone DUO and Elaraxx DUO hybrids. In variant 6 with the highest applied rate used in the experiment, the lowest values for this indicator were recorded, with the percentage yield reduction compared to the untreated control being as follows: 44%, 40% and 42% for the Zanetixx DUO, Hexxagone DUO and Elaraxx DUO hybrids.

CONCLUSIONS

Overall, the findings of the study suggest that the application of Stratos Ultra to the evaluated hybrids should be carried out at the maximum recommended rates (1 L ha⁻¹ and 2 L ha⁻¹) in order to achieve both sufficient level of weed management and optimal crop response. Increasing rates of the herbicide resulted in decrease in yields as well as in grain quality.

The results of the experiment, conducted in 2024 and 2025, indicate that plants' performance was positively affected by the treatment with grass weed herbicide Stratos Ultra, provided that it is applied at the recommended application rates - 1 and 2 L ha⁻¹. The tested herbicide combined with a broadleaf herbicide, resulted in improved maize yields by successfully controlling weeds that had emerged at the time of application in the initial stages of crop development. Although yields in variants 5 and 6 decreased significantly with increasing application rates, none of the hybrids resulted in reduced crop density or total yield loss.

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COMPARATIVE STUDY BETWEEN WINTER WHEAT VARIETIES OF DIFFERENT ORIGINS REGARDING THE VALORIZATION OF WATER ACCUMULATED THROUGH PRECIPITATION DURING THE PERIOD 2023-2025 AT CARACAL

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Abstract

During the period 2023-2025, a wide range of winter wheat varieties of different origins were sown at SCDA Caracal. In addition to the local varieties, were sown varieties from France, Germany, Austria, Serbia, Hungary, marketed or tested in Romania by the companies: Syngenta, KWS, Donau Saat, Limagrain, Saaten Union, RAGT, Lidea, Axereal, Biocrop. In the agricultural year 2022-2023, there was a surplus of precipitation over the entire growing season for wheat (+67.3 mm), the surplus months being November, January, April and May, all against a background of low temperatures. The years 2023-2024 and 2024-2025 recorded a deficit of precipitation over the entire vegetation period but also surplus months - November and May, due to low temperatures, in both years and December only in 2024-2025, due to high temperatures. All these data were reported to the 35-year normal for the area. Distributions of the productions were made depending on the precipitations in certain phenophases and the coefficients of productions variability were calculated in order to identify the productively stable wheat varieties, regardless of climatic conditions.

Key words: wheat, precipitation, production, stability, climatic conditions.

INTRODUCTION

As one of the most important crops worldwide, wheat (*Triticum aestivum* L.) is extensively cultivated in arid and semi-arid regions, where the implementation of irrigation systems becomes indispensable for ensuring yield stability and food security (Yan et al., 2022). Since wheat is a staple cereal frequently grown under drought-prone conditions, proper irrigation management represents a critical factor sustaining both crop productivity and global food supply (Fang et al., 2018; Yuan et al., 2022).

Cultivar selection constitutes a fundamental component of wheat production technology, representing the first step toward achieving high quantitative and qualitative performance. The choice of genotype significantly influences yield potential, grain quality, and adaptability to environmental stressors.

Wheat productivity under water deficit conditions remains one of the major challenges of modern agriculture, particularly in the context

of ongoing climate change. Drought stress directly reduces yield by limiting photosynthetic activity and decreasing the number of kernels per spike, with particularly severe effects during critical phenological stages such as tillering and grain filling.

To mitigate these losses, agricultural research increasingly focuses on the development of genotypes with enhanced genetic tolerance to drought, capable of maintaining high water-use efficiency even under osmotic stress. The success of wheat cultivation in arid environments depends not only on total precipitation but, more importantly, on the cultivar's ability to extract water from deeper soil layers and to regulate evapotranspiration during periods of extreme heat.

Findings reported in the international literature emphasize that water deficit represents the primary limiting factor for global wheat production, causing average yield losses estimated between 50% and 60% in the absence of effective mitigation strategies (Nyaupane et al., 2024).

Improving the efficiency of natural resource use in agriculture is a major challenge of the current decade, with water management in wheat cultivation being a central pillar for ensuring food security. However, the added value of crop production does not stop at harvest, but extends to the livestock sector, where the quality of feed directly influences productive performance (Colă and Colă, 2021, 2024).

From the perspective of yield component dynamics, the impact of water deficit varies according to the timing of stress occurrence. Pre-anthesis stress (before flowering) significantly reduces the number of spikes per square meter and the number of kernels per spike (by approximately 16%). This stage is critical for the determination of yield components. In contrast, post-anthesis stress (during grain filling) predominantly affects thousand-kernel weight (TKW), reducing final grain weight by up to 29% (Miroslavljević et al., 2024). An increase in maximum temperatures around mid-anthesis may further lead to substantial yield reductions.

Water stress occurring during the tillering and stem elongation stages decreases the number of fertile tillers and, consequently, the number of spikes per unit area. Total grain yield may be severely compromised, as the number of kernels per square meter ($\text{spikes/m}^2 \times \text{kernels/spike}$) represents the principal determinant of yield, and this component is largely established before anthesis (Al-Ajlouni et al., 2016). Severe stress during stem elongation may reduce yield by up to 72% compared with optimal growing conditions (Kottmann et al., 2016).

Applying 60-80% of the total crop water requirement can maximize water-use efficiency without drastically penalizing yield. The adoption of drip or micro-sprinkler irrigation systems can reduce water inputs by more than 35% while maintaining harvest stability. Although overall yield may decline under water-limited conditions, drought stress tends to increase grain protein concentration by 9-18%, thereby improving certain bread-making quality parameters (Ma et al., 2025).

Drought-tolerant cultivars employ avoidance strategies (rapid stomatal closure) or escape mechanisms (shortening the period to flowering by up to 12 days) to reduce exposure to summer heat stress (Eser et al., 2024).

Ensuring optimal water supply for wheat crops is a critical challenge in the context of the new climatic realities in Romania, where extreme temperatures forecast for the near future (2021-2040) and variable precipitation regimes directly influence both the quality and quantity of the harvest (Velea et al., 2021). Water stress, not only affects production yield, but also the vulnerability of plants to pathogens, favoring epidemics such as fusarium head blight and the accumulation of mycotoxins (Prioteasa et al., 2025; Cotuna et al., 2022). In this context, understanding the relationship between water availability, the resistance of genetic lines under improvement and the efficiency of alternative organic products with fungicidal effect becomes essential for sustainable management of wheat (Paraschivu et al., 2024; Păunescu et al., 2022). Under Romanian climatic conditions, characterized by frequent pedological drought, several wheat cultivars have been developed and officially registered that combine local genetic resources (for stress resilience) with foreign germplasm (for high yield potential). For soil water conservation in wheat cropping systems, the most efficient technological approaches fall within the framework of conservation agriculture, based on minimizing soil disturbance and maintaining a protective residue layer at the soil surface.

MATERIALS AND METHODS

A collection consisting of 220 winter wheat cultivars of diverse genetic origins was sown over a three-year period (2023-2025) on a chernozem soil at Caracal.

The soil is characterized by a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing by 1.24% in the B horizon. It is moderately supplied with total nitrogen (0.172% in Ap), very well supplied with available phosphorus in the Ap horizon (168 ppm), and well supplied with exchangeable potassium (248 ppm).

Soil reaction along the profile ranges from slightly acidic to slightly alkaline. The sum of exchangeable bases is moderate (23.2 in Ap and 24.8 in Am), while the base saturation degree reaches 92.62%, gradually increasing toward the Cca horizon.

The micronutrient content is high for zinc in the Ap horizon (176 ppm) but extremely low in the B horizon (0.40 ppm).

The soil texture is clay loam to silty clay loam within the first 70 cm and silty loam in the lower part of the profile. As a result, bulk density (BD) is moderate throughout the profile, with values of 1.46 g cm⁻³ in the Am horizon and 1.58 g cm⁻³ in the A/B horizon. Total porosity (TP) is moderate in Ap (48.8%) and high in Am (58.2%). Air-filled porosity (AFP) is moderate in Ap (15.2%), high in Am (23.8%), moderate in B (21.7%), and low in Cca (12.2%). Clay content varies between 33.4% and 40.3%.

Experimental plots were established in the research field, each covering an area of 7 m².

During the 2022-2023 growing season, a precipitation surplus was recorded over the entire wheat vegetation period (+67.3 mm), with excess rainfall occurring in November, January, April, and May, all associated with relatively low temperatures (Table 1). The 2023-2024 and 2024-2025 agricultural years showed similar patterns, characterized by an overall precipitation deficit during the wheat growing season (−32.3 mm and −39.1 mm, respectively). Nevertheless, surplus rainfall months were recorded in November and May in both years under low-temperature conditions, and in December during the 2024-2025 season under comparatively higher temperatures (Tables 2 and 3).

Table 1. Dynamics of the Main Climatic Factors during the Growing Season of Small-Grain Cereal Genotypes at S.C.D.A. Caracal, 2022-2023

Specification		X	XI	XII	I	II	III	IV	V	VI	Total for the Growing Season
Temperature (°C)	Monthly Mean	13.7	8.7	2.8	4.0	3.6	7.7	10.7	15.0	21.4	
	NORMAL (Multiannual Average)	11.7	5.1	0.3	-1.3	0.8	6	12	17.7	21.6	
	Difference (±)	+2.0	+3.6	+2.5	+5.3	+2.8	+1.7	-1.3	-2.7	-0.2	
Average	Monthly Total	15.0	78.8	33.8	103.4	13.2	20.8	68.8	78.6	44.4	456.8
Precipitation (mm)	Multiannual Mean	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	389.5
	Difference (±)	-31.0	+31.8	-5.3	+72.6	-13.1	-13.4	+21.0	+20.0	-25.3	
Relative Air Humidity (%) Mean		75.5	93.0	97.8	96.5	86.0	79.2	84.2	80.5	80.6	

Table 2. Dynamics of the Main Climatic Factors during the Growing Season of Small-Grain Cereal Genotypes at S.C.D.A. Caracal, 2023-2024

Specification		X	XI	XII	I	II	III	IV	V	VI	Total for the Growing Season
Temperature (°C)	Monthly Mean	16.0	8.1	3.9	1.0	8.0	8.9	15.0	17.2	25.9	
	NORMAL (Multiannual Average)	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	
	Difference (±)	+4.3	+3.0	+3.6	+2.3	+7.2	+3.9	+3.0	-0.5	+4.3	
Average	Monthly Total	21.4	124.2	21.4	26.8	11.6	22.4	26.0	71.8	31.6	357.2
Precipitation (mm)	Multiannual Mean	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	389.5
	Difference (±)	-24.6	+87.2	-17.7	-4.0	-14.7	-11.8	-21.8	+13.2	-38.1	-32.3
Relative Air Humidity (%) Mean		66.9	91.1	93.8	95.4	84.7	85.3	70.8	77.8	61.3	

Table 3. Dynamics of the Main Climatic Factors during the Growing Season of Small-Grain Cereal Genotypes at S.C.D.A. Caracal, 2024-2025

Specification		X	XI	XII	I	II	III	IV	V	VI	Total for the Growing Season
Temperature (°C)	Monthly Mean	13.0	4.5	4.0	2.4	-1.3	9.3	12.5	16.0	24.0	9.37
	NORMAL (Multiannual Average)	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	8.21
	Difference (±)	+ 1.3	- 0.6	+3.7	+3.7	-2.1	+3.3	+0.5	-1.7	+2.4	+1.16
Average	Monthly Total	3.4	55.2	113.6	4.4	6.4	19.0	22.0	104.6	21.8	350.4
Precipitation (mm)	Multiannual Mean	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	389.5
	Difference (±)	-42.6	+18.2	+74.5	-26.4	-19.9	-15.2	-25.8	+46.0	-47.9	- 39.1
Relative Air Humidity (%) Mean		74.7	86.4	97.8	93.2	91.8	83.8	76.4	79.1	68.9	

The research was carried out in a randomized block design with 3 replications. Sowing was carried out at the optimal time, in the first decade of October, with a density of 550 germinable grains/m², after peas as a precursor plant. Fertilization was uniform for all variants, applying a dose of N100P40. Statistical analysis of the data was performed using statistical functions and the Data Analysis ToolPak module in Microsoft Excel.

For each tested cultivar, the following parameters were calculated: grain yield standardized to STAS moisture content, the coefficient of variation (CV, %) for yield data across the testing years, and the production index (defined as the ratio between yield under water-deficit conditions and the mean yield of all cultivars under the same conditions).

Cultivar stability was assessed based on the coefficient of variation, as follows: CV < 10% - stable cultivars; CV between 10% and 20% - moderately stable cultivars; CV > 20% - unstable cultivars.

RESULTS AND DISCUSSIONS

The obtained results are presented from multiple analytical perspectives: (i) yield distribution in relation to precipitation accumulated up to the heading stage; (ii) mean yields as influenced by total precipitation recorded throughout the entire growing season; (iii) the coefficient of variation (CV, %) of yields registered during the three experimental years; (iv) the production index, calculated as the ratio between the yield achieved under water-deficit conditions by each tested cultivar and the mean yield of all cultivars evaluated under the same conditions; and (v) graphical classification based on the relationship between the coefficient of variation and the production index.

Yield performance analyzed according to heading date under water-deficit conditions (precipitation-derived drought year) relative to a year with precipitation surplus revealed a marked reduction in yield for very early, early, medium, and late cultivars. In contrast, yield levels tended to converge in the case of very late cultivars (Figure 1).

In the 2022-2023 agricultural year, climatic conditions were characterized by an autumn with a water deficit at wheat emergence, later

compensated by an optimal water supply during the winter and early spring.

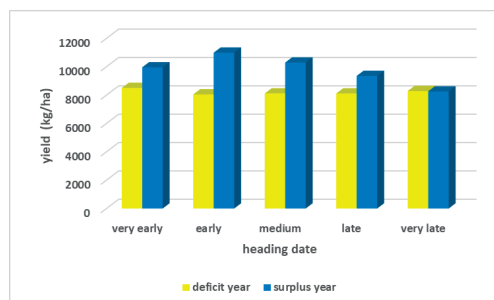


Figure 1. Mean grain yield of winter wheat cultivars in relation to total precipitation accumulated during the entire growing season, classified by earliness group – Caracal, 2023-2025

The grain filling stage took place under favorable thermal conditions, with temperatures in May and June slightly below the multiannual average, against the backdrop of excess precipitation in May (Table 1).

In 2023-2024, the dynamics of climatic factors was marked by a severe deficit at wheat emergence in October, followed by an excess of precipitation during the twinning phase and culminating in a sharp thermal stress during the grain filling period (Table 2).

In the 2024-2025 agricultural year, the wheat emergence period was critical, due to severe drought, while the twinning benefited from a reserve of soil water, accumulated in the warm windows of December. The grain filling stage was marked by a major contrast: optimal moisture conditions in May, followed by thermal and water stress in June (Table 3).

The distribution of yields recorded in relation to precipitation accumulated up to the heading stage in the deficit year (mean of the two dry years = 280 mm, derived from 283.8 mm in 2023-2024 and 276.3 mm in 2024-2025) compared with the surplus year (333.8 mm) revealed a considerably narrower yield range under water-deficit conditions (from below 6,000 up to 10,001-10,500 kg ha⁻¹). In contrast, during the year characterized by higher precipitation, the yield range was substantially broader, reaching values exceeding 13,501 kg ha⁻¹.

The peak of yield distribution in the agricultural years with lower precipitation was positioned within the 8,001-8,500 kg ha⁻¹ class, whereas in

the year with precipitation surplus it shifted toward the 8,501-9,000 kg ha⁻¹ class (Figure 2). Under water-deficit conditions (two-year mean), the highest yield was recorded for the cultivar SY Sanluca (10,299 kg ha⁻¹), while under surplus water conditions registered during the 2024-2025 agricultural year at Caracal, the most productive cultivar was Complice (13,661 kg ha⁻¹). At the opposite end of the productivity spectrum were the cultivars Bezostaia (5,759 kg ha⁻¹) and Viktoria (4,736 kg ha⁻¹), respectively.

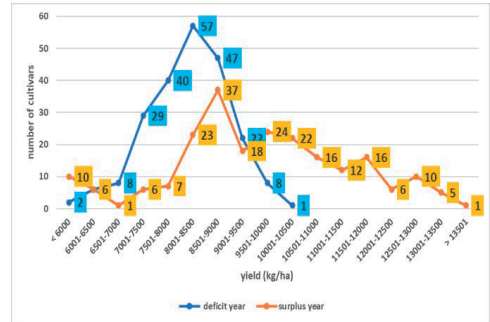


Figure 2. Distribution of winter wheat grain yields in relation to precipitation accumulated up to the heading stage

A low coefficient of variation (CV), corresponding to stable cultivars, highlighted those genotypes for which yield differences between the two contrasting conditions (deficit year versus surplus year) were minimal; however, this indicator did not necessarily emphasize cultivars with outstanding productivity under specific environmental conditions. Several illustrative examples can be provided.

The cultivar Absint, with practically similar yields under contrasting precipitation regimes (6,707 kg ha⁻¹ in the deficit year and 6,611 kg ha⁻¹ in the surplus year), exhibited a coefficient of variation of only 0.9%, thus being classified as highly stable. Likewise, the cultivar Ingenio, with a CV of 5.4% (stable), showed comparable yield performance under both conditions, similar to Absint, but at a considerably higher productivity level (8,250 kg ha⁻¹ in the deficit year versus 8,573 kg ha⁻¹ in the surplus year). In contrast, although the cultivar Irun recorded a yield in the deficit year comparable to that of Absint (6,700 kg ha⁻¹), it reached 10,270 kg ha⁻¹ under excess precipitation conditions, indicating pronounced instability (CV = 30%) (Figure 3).

Figure 3 presents the cultivars with the highest stability among those evaluated. Under the pedo-climatic conditions specific to Caracal and its surrounding area, the following cultivars were distinguished as stable while also maintaining high productivity:

- Novic, Pibrac, Python, RGT Cesario, RGT Palmeo, with yields exceeding 9,000 kg ha⁻¹ under both environmental conditions;
- Ascona, Aurelius, Avenue, Axum, Barba, Centurion, Combin, Consecvent, Dallara, Gabrio, Ingenio, KWS Lazuli, KWS Peplum, KWS Rhum, KWS Usuellum, LG Apilco, Moisson, Montecristo, Omersson, Perkussio, Rubisko, Solehio, Sonathine, SY Exaltation, SY Passion, SY Starlord, all exceeding 8,000 kg ha⁻¹ in both conditions.

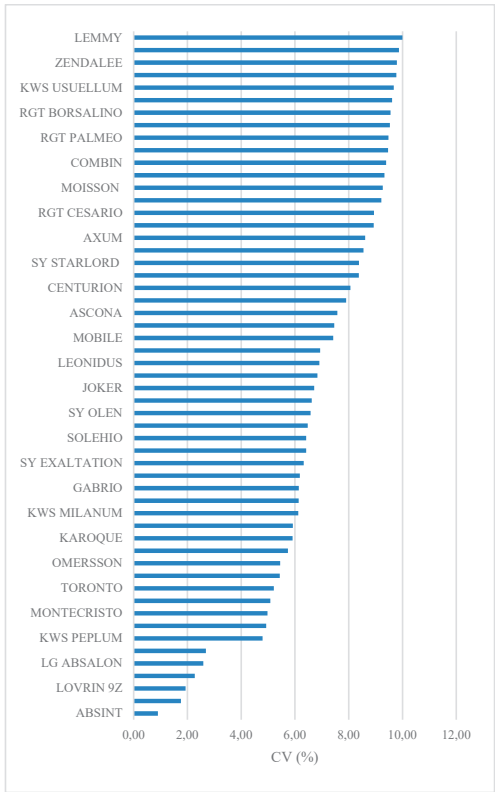


Figure 3. Highly stable winter wheat cultivars identified based on a coefficient of variation (CV) below 10% – Caracal, 2023–2025

Among the unstable cultivars, several genotypes stood out due to their wide yield fluctuations: Bezostaia (3,000-8,000 kg ha⁻¹; CV = 38.94%), Dacic (3,000-10,000 kg ha⁻¹; CV = 38.98%),

Kapitol (5,000-10,000 kg ha⁻¹; CV = 31%), Miranda (5,000-9,000 kg ha⁻¹; CV = 35%), Mv Nador (5,000-9,000 kg ha⁻¹; CV = 32%), and Promitor (6,000-10,000 kg ha⁻¹; CV = 33%). It should be noted that these cultivars were severely affected by yellow rust during the year characterized by excessive precipitation, which led to substantial yield reductions, particularly given that no fungicide treatments were applied in the experimental field (Figure 4).

Numerous studies have investigated various drought-sensitivity indices to facilitate the selection of genotypes according to their productive performance under both favorable and stress conditions (Moosavi et al., 2008; Farshadfar et al., 2013; Mursalova et al., 2015; Ali and El-Sadek, 2016).

Among these indicators, the present study employed the yield index (YI), which belongs to the category of indices most strongly dependent on genotype performance under stress conditions.

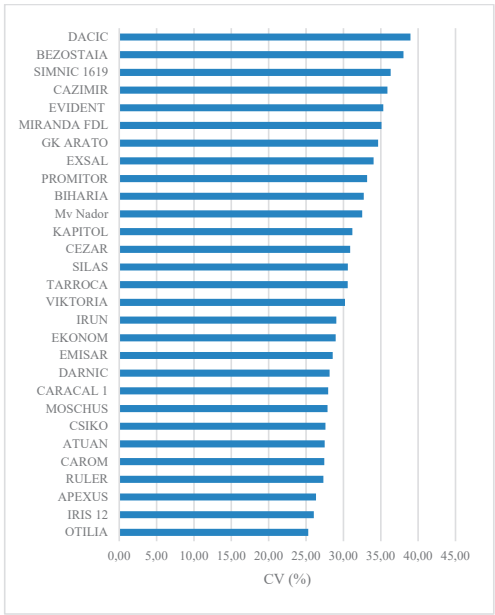


Figure 4. Winter wheat cultivars exhibiting low yield stability (coefficient of variation, CV > 25%) – Caracal, 2023–2025

The graphical representation of the relationship between the yield index (YI) and the coefficient

of variation (CV), together with the division of the scatter plot into four quadrants, allowed a clearer classification of cultivar performance under water-deficit conditions (Figure 5):

Quadrant I - low CV (stable cultivar) and subunitary index (yield below the mean of all cultivars under below-normal water supply conditions);

Quadrant II - CV > 10% (moderately stable or unstable cultivar) and subunitary index (yield below the mean under below-normal water supply conditions);

Quadrant III - CV > 10% (moderately stable or unstable cultivar) and supraunitary index (yield above the mean under below-normal water supply conditions);

Quadrant IV - low CV (stable cultivar) and supraunitary index (yield above the mean of all cultivars under below-normal water supply conditions).

This approach enabled the identification of cultivars belonging to Quadrant IV as the most well-adapted to water-deficit environments. Among these, Palmeo, Milanum, and Scrambler were distinguished as stable cultivars exhibiting the highest yield performance under drought conditions relative to the mean of the experimental assortment.

The yield index calculated for the cultivars tested at Caracal during 2023-2025 ranged from 0.72 for the cultivar Bezostaia to 1.26 for SY Sanluca.

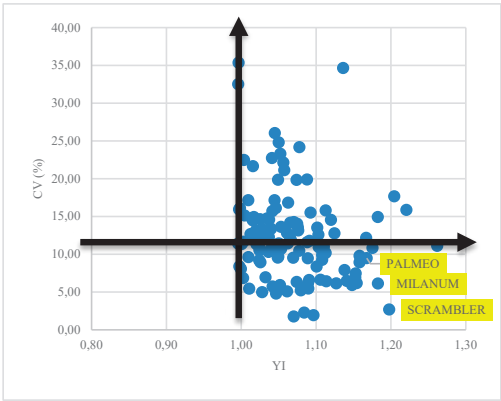


Figure 5. Relationship between yield index (YI) and coefficient of variation (CV) in winter wheat cultivars evaluated at Caracal during 2023-2025

CONCLUSIONS

Under water-deficit conditions (two-year mean), the highest yield was recorded for the cultivar SY Sanluca (10,299 kg ha⁻¹), whereas under surplus water conditions observed during the 2024-2025 agricultural year at Caracal, the most productive cultivar was Complice (13,661 kg ha⁻¹). At the lower end of the productivity spectrum were the cultivars Bezostaia (5,759 kg ha⁻¹) and Viktoria (4,736 kg ha⁻¹).

The cultivars Novic, Pibrac, Python, RGT Cesario, and RGT Palmeo, each exceeding 9,000 kg ha⁻¹ under both environmental conditions, demonstrated high yield stability under the pedo-climatic conditions specific to Caracal.

Based on the analysis of the relationship between the coefficient of variation and the yield index, the cultivars Palmeo, Milanum, and Scrambler were identified as stable genotypes exhibiting superior yield performance under water-deficit conditions relative to the mean of the experimental assortment.

The yield index calculated for the cultivars tested at Caracal during 2023-2025 ranged from 0.72 for Bezostaia to 1.26 for SY Sanluca, reflecting considerable variability in drought adaptation among the evaluated genotypes.

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EFFECTS ON WINTER OILSEED RAPE YIELD OF SOME BIOSTIMULATORS APPLIED IN THE SPRING

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Abstract

In the actual context of climate change which is a global challenge for agriculture, there is a need for new solutions and strategies to minimize the negative impact of stress factors during the growing season of winter oilseed rape. The use of biostimulators is one of the solutions, and therefore, their use has become an important component of crop technology. Starting from these aspects, the aim of the performed research was to identify the effects of the spring application of some biostimulators on grain yield at winter oilseed rape in the specific growing conditions of South Romania. In this respect, research was performed in the years 2023, 2024, and 2025, in field experiments under rainfed conditions having the following experimental variants: v1 - no biostimulator treatment, which was taken as the control variant; v2 - Cropmax biostimulator, applied at a rate of 1 l/ha; v3 - Aminisol biostimulator, applied at a rate of 1 l/ha; v4 - Algamax Fluid biostimulator, applied at a rate of 1.5 l/ha. The obtained results showed that using biostimulators in winter oilseed rape cultivation represents a way to increase the grain yield regardless of the climatic conditions. The yield increase due to the biostimulator application is more consistent the more favorable the year is from a climatic point of view.

Key words: winter oilseed rape, biostimulator, spring application, grain yield.

INTRODUCTION

Oilseed rape (*Brassica napus* L.) is one of the most important oil crops in the world, with an increasing cultivated area and production over the last 60 years (according to FAOSTAT database), ranking third in the world oil production, after palm oil and soybean oil. In Europe, it is widely cultivated in temperate and cool continental regions, this being particularly valued for its healthy, high-quality oil and its high oil content in seeds (Kaya et al., 2026). Oilseed rape was introduced into cultivation in Romania in the 1840s-1850s, the cultivated areas and the obtained yields registering significant variations over time, the cultivated area starting to increase significantly after 1996 (Ion, 2021) reaching 800,000 ha in 2025 (INS, 2026).

Optimal conditions for plant growth and development are key to increasing winter oilseed rape yields (Dubis et al., 2018), these conditions being achievable through cultivation technology. Indeed, agricultural technology is transforming the way we farm, offering

solutions to the many challenges facing the agricultural industry today (Yanog, 2024).

The main problem in the technology of growing winter oilseed rape comes down to overcoming adverse consequences in the early and late development of plants, which leads to the failure of the sown areas or unsatisfactory yields with low quality indicators (Mihaylov, 2024).

It seems highly probable that boosting natural plant defense strategies by applying biostimulators will help to solve an old problem of poor yield in plant cultivation, by provoking their better growth and development even under suboptimal environmental conditions (Posmyk and Szafranska, 2016).

Biostimulators are substances applied to plants, intended to improve their status by helping them to grow and develop well and to go well through periods of stress (Adam et al., 2025). Also, the biostimulator application increase the agronomic effectiveness of fertilization (Nowak et al., 2021). Practically, they contain a bioactive substance that improves plant growth, increases nutritional efficiency and tolerance to abiotic stress, improves plant protection against

pathogens and improves plant traits and yield quality (Bulgari et al., 2019; Calvo et al., 2014; du Jardin, 2015; Hayat et al., 2012; Kolomazník et al., 2012; Kumar & Alope, 2020; Petrova et al., 2023; Ruzzi et al., 2024; Sikorska et al., 2018; van Oosten et al., 2017; Xu and Geelen, 2018).

According to Regulation (EU) 2019/1009 of the European Parliament and of the Council, a plant biostimulator shall be an EU fertilizing product the function of which is to stimulate plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or availability of confined nutrients in the soil or rhizosphere.

Biostimulators are based mainly on the following bioactive substance: humic substances, seaweed extracts, hydrolysed proteins and amino acids, plant extracts, inorganic compounds, beneficial microorganisms, chitosan and other biopolymers (Grammenou et al., 2023).

Many scientific studies have demonstrated the potential of various categories of biostimulants to improve crop production and to ameliorate abiotic stresses such as drought and soil salinity (Calvo et al., 2014). Improving the effects of abiotic stress is the most commonly mentioned benefit of using biostimulants (Rouphael et al., 2017; Yakhin et al., 2017).

The current agricultural system is in search of new strategies to achieve a more sustainable production while keeping or even increasing crop yield and quality, in this scenario, the application of biostimulants being a potent solution (Ancín et al., 2024). Biostimulators have been identified as one of the most important agronomic factors that scientists have been looking for in a range of natural sources over the last few decades (Yaseena and Takacs-Hajosa, 2022). Although biostimulators were initially designed as a helpful tool mainly in organic farming, they are now used in all cropping systems (organic, conventional, and integrated) in the open field and protected cultivation (Ruzzi et al., 2024).

Biostimulators are increasingly integrated into production systems with the goal of modifying physiological processes within the plants

(Yakhin et al., 2017), their application being an important part of intensive agriculture (Petrova et al., 2023). While these products have long been staples of farming systems, the growing demand for sustainable and innovative solutions has created new opportunities for biostimulants (Starr et al., 2026). Indeed, the use of natural growth stimulants is becoming an increasingly common practice in agriculture (Sikorska et al., 2018).

It is clear there is a need for new solutions and strategies to minimize the negative impact of stress factors during the growing season of the winter oilseed rape, the use of biostimulators being one of the solutions. On the other hand, although biostimulants have shown great potential, there is still much to learn about how they work and how to maximize their benefits taking into account their effectiveness depends on crop type, dose, and timing (Carillo et al., 2025). Starting from this idea, the aim of the performed research was to identify the effects of the spring application of some biostimulators on grain yield at winter oilseed rape in the specific growing conditions of South Romania

MATERIALS AND METHODS

Research was performed in the years 2023, 2024, and 2025, in field experiments under rainfed conditions located in South Romania (about 30 km northeast of Bucharest). The experiments were organized as macro-plots with 3 replications, having the following experimental variants:

- V1 - no biostimulator treatment, which was taken as the control variant;
- V2 - Cropmax biostimulator, applied at a rate of 1 l/ha;
- V3 - Aminosol biostimulator, applied at a rate of 1 l/ha;
- V4 - Algamax Fluid biostimulator, applied at a rate of 1.5 l/ha.

Cropmax is a plant biostimulator with a combination of macronutrients, micronutrients, amino acids, vitamins, polysaccharides, enzymes, and other substances.

Aminosol is a plant biostimulator based of amino acids and peptides produced by enzymatic hydrolysis of animal protein.

Algamax Fluid is a plant biostimulator with bioactive compounds (carbohydrates, amino

acids, macronutrients and micronutrients) extracted from *Ascomphyllum nodosum*.

In all experimental years, the biostimulators were applied in the BBCH 31 phase (one visibly extended internode) of the oilseed rape plants.

The preceding crop was winter wheat in all experimental years. Tillage consisted of a passage after harvesting the preceding crop with the cultivator Tiger 3 MT at a depth of 30 cm. The seedbed was prepared before sowing by a passage with a cultivator.

In autumn, before seedbed preparation, the complex fertilizer 20:20:0 + 13S was applied in a rate of 300 kg/ha for all the experimental variants, which provided 60 kg/ha of nitrogen, 60 kg/ha of phosphorus, and 39 kg/ha of sulphur. Early in the spring, 143.5 kg/ha of Urea (46% nitrogen content) was applied, assuring a nitrogen rate of 66 kg/ha. In the BBCH 50 phase (flower buds present, still enclosed by leaves) of the oilseed rape plants, 126 kg/ha of CAN (Calcium Ammonium Nitrate, with 27% nitrogen content, respectively 13.5% NH_4^+ and 13.5% NO_3^- , plus 7% CaO, and 5% MgO) was applied, this providing a nitrogen rate of 34 kg/ha. In total, the nitrogen rate was 160 kg/ha (60 kg in autumn, before seedbed preparation + 66 kg early in the spring + 34 kg in the BBCH 50 phase).

The macro-plots had 0.333 ha for each replication, which means 1 ha for each experimental variant and 4 ha in total for the experimental field.

The oilseed rape hybrid used in the experimental field was PT298 (a mid-early hybrid with high yielding potential). The sowing was performed as follows: on 20th of August in 2022; on 11th of September in 2023; on 4th of September in 2024. In all experimental years, the sowing density was 50 germinating seeds per square meter.

In autumn, one herbicide was applied to control monocotyledonous weeds and one insecticide was applied to control pests. In the years 2023 and 2024, one herbicide was applied also in spring to control monocotyledonous weeds and one herbicide to control dicotyledonous weeds. Pest control was carried out by applying three insecticides in the spring of 2023 and four insecticides in 2024 and 2025.

Disease control was carried out by applying two fungicides in the spring of 2023 and 2024 and three fungicides in 2025.

The harvest of macro-plots on replications for each experimental variant was done using a harvester machine, and after that, the grains were weighed. The seed moisture content was determined using a Portable Moisture Analyser. The grain yield was calculated per hectare and it was expressed in kg reported at 9% moisture content.

The soil where the field experiments were located each year is cambic chernozem. According to the physical plot where the field experiment was located each year, pH varies between 5.77 and 5.96, the organic matter content between 2.25% and 2.65%, and the nitrogen index between 1.82 and 2.15.

From a climatic point of view, the experimental years were characterized as follows for winter oilseed rape: the year 2025 was the most favourable, with good rainfall in the previous autumn and a large amount of precipitation (130.3 mm) in May, during the period of grain setting and filling; the year 2023 comes second as favourability, with high amount of rainfall in April, May, and June, and with not so high temperatures; the year 2024 was the least favourable, with high temperatures in spring and less rainfall.

RESULTS AND DISCUSSIONS

In all the experimental years, all the experimented biostimulators determined higher grain yields compared to control variant (with no biostimulator) (Table 1 and Figure 1). Also in all the experimental years, the highest grain yields were registered for the biostimulator Algamax Fluid, but while the difference compared to control variant was not statistically significant in the year 2023, this was significant in 2024 and very significant in 2025 (Table 1).

In the year 2023, no biostimulator treatment registered differences statistically significant compared to control variant. In the year 2024, only the treatment with the biostimulator Algamax Fluid registered a difference statistically significant compared to control variant, but in the years 2025 all the three experimented biostimulators registered differences statistically as being very significant compared to control variant (Table 1).

Compared to control variant, the experimented biostimulators determined differences from

33 to 186 kg/ha in the year 2023, from 53 to 345 kg/ha in the year 2024, and from 665 to 756 kg/ha in the year 2025 (Table 1).

The biostimulator Algamax Fluid had the smallest coefficient of variation between years (10.21%) of all the three biostimulators taken into experience. The highest coefficient of variation between years (13.3%) was registered for the biostimulator Cropmax. The control variant with no biostimulator applied registered a smaller coefficient of variation (7.63%) compared to the variants treated with biostimulators (Table 1). This suggest that, compared to the untreated variant, the use of biostimulators induces a higher variation of the

grain yield from year to years, according to the climatic favorability of the year.

The highest grain yields, but also the highest differences to control variant were registered in the favorable climatic year 2025. The climatic favorability of the year 2025 made the oilseed rape plants to make maximum use of the biostimulator products. In less favorable climatic conditions of the years 2023 and 2024, the effects determined by the biostimulator products were less consistent. However, it stood out the biostimulator Algamax Fluid based on extracts from *Ascophyllum nodosum* in the worst climate year 2024 with a consistent difference compared to control variant of 345 kg/ha (Table 1).

Table 1. Grain yield and coefficient of variation between years (2023-2025) at oilseed rape treated with biostimulators

Experimental variants	2023		2024		2025		Coefficient of variation between years (%)
	Yield (kg/ha)	Difference to control (kg/ha)	Yield (kg/ha)	Difference to control (kg/ha)	Yield (kg/ha)	Difference to control (kg/ha)	
Control – no biostimulator	3672	0	3107	0	3670	0	7.63
Cropmax	3814	142	3184	77	4424	754 ***	13.30
Aminosol	3705	33	3160	53	4335	665 ***	12.86
Algamax Fluid	3858	186	3452	345 *	4426	756 ***	10.21
<i>DL</i> _{5%}	262.82		279.52		251.92		
<i>DL</i> _{1%}	382.38		406.67		366.53		
<i>DL</i> _{0.1%}	574.55		611.04		550.72		

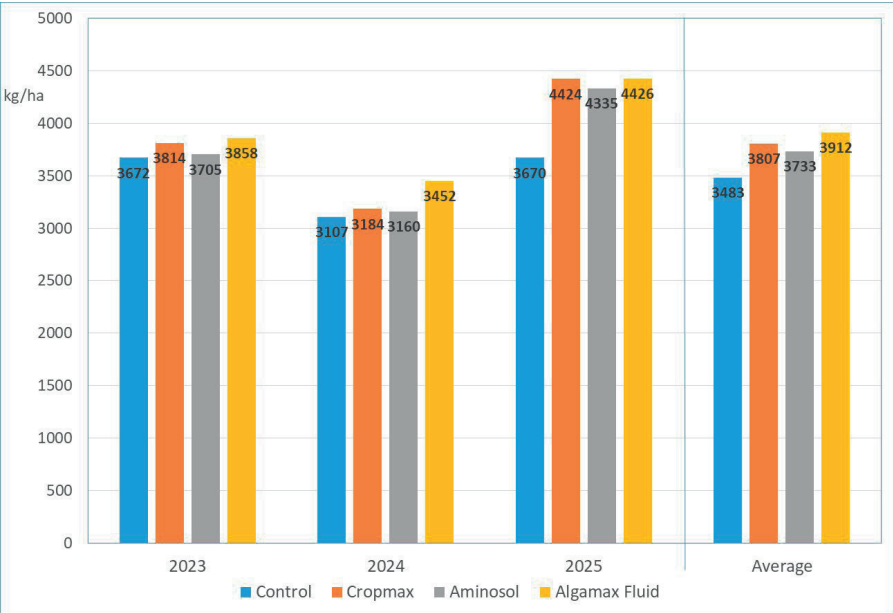


Figure 1. Grain yield at oilseed rape treated with biostimulators, in 2023-2025 period

As average values for the three experimental years, Algamax Fluid determined a difference compared to the control variant of 429 kg/ha, Cropmax determined a difference compared to the control variant of 324 kg/ha, and Aminosol determined a difference compared to the control variant of 250 kg/ha (Figure 1).

CONCLUSIONS

The obtained results showed that using biostimulators in winter oilseed rape cultivation represents a way to increase the grain yield regardless of the climatic conditions.

Compared to the untreated variant, the use of biostimulators induces a higher variation of the grain yield from year to years, according to the climatic favorability of the year. Practically, the yield increase due to the biostimulator application is more consistent the more favorable the year is from a climatic point of view.

Among the experimented biostimulators, it stood out the biostimulator Algamax Fluid based on extracts from *Ascophyllum nodosum* with the highest grain yields and with a consistent difference compared to control variant in the worst climate year 2024.

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EFFECTS OF MINERAL FERTILIZATION AND PLANT SPACING ON *Melissa officinalis* L. YIELD AND PREDICTIVE RELATIONSHIPS UNDER WESTERN ROMANIAN PEDOCLIMATIC CONDITIONS

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Abstract

Melissa officinalis L., commonly known as lemon balm, is a melliferous species from the Lamiaceae family, known and used since antiquity for its medicinal properties. The study investigated the impact of mineral fertilization and planting distance on yield and associated technological relationships in lemon balm cultivation. The bifactorial experiment included three levels of nitrogen fertilization (N0, N30, N60) and two planting densities (50/30 and 60/30), arranged in a randomized block design with four replications. Fresh and dry biomass yields were determined, along with the contribution of each factor to total production. Statistical analysis focused on assessing the significance of differences using ANOVA, as well as on linear correlations among factors and regression coefficients, with the aim of identifying predictive relationships between technological inputs and plant response. Preliminary results highlight a significant influence of mineral fertilization on yield, enhanced by optimal planting densities, with relevant interactions between factors. Calculated correlations indicate direct relationships between fertilization level and biomass performance, while regression models outline predictive trends useful for developing sustainable cultivation technologies.

Key words: lemon balm, mineral fertilization, plant spacing, biomass yield, regression analysis.

INTRODUCTION

The *Lamiaceae* family represents a key taxonomic group in medicinal plant research, as many of its genera are characterized by a high biosynthetic capacity for pharmacologically relevant compounds (Sârbu et al., 2013; Nieto, 2016; Gurita et al., 2019). *Melissa officinalis* L., commonly known as lemon balm, is a perennial herbaceous plant, widely appreciated for its aromatic, medicinal, and melliferous properties, that has long been cultivated across Europe, particularly in regions with temperate climates (Miraj et al., 2016; Beicu et al., 2021; Balasoiu et al., 2024). In recent years, the demand for lemon balm has increased, driven by its extensive use in the pharmaceutical, cosmetic, and food industries, particularly due to its essential oils, flavonoids, and antioxidant compounds (Shakeri et al., 2016; Petrisor et al., 2022; Ciobotaru et al., 2025; Pop et al., 2025). To meet this growing demand and ensure sustainable, high-yield cultivation, it is essential to optimize agronomic practices, notably fertilization and spatial arrangement,

according to specific environmental conditions (Chizzola et al., 2018; Banjaw & Gebre, 2024). Mineral fertilization remains one of the most effective strategies for enhancing plant growth, biomass production, and phytochemical content in medicinal and aromatic species. (Tepecik et al., 2016; Abdellatif et al., 2021; Banjaw & Gebre, 2024; Fallah et al., 2024; Gordanić et al., 2025). Among macroelements, nitrogen is particularly influential in promoting vegetative growth and biomass accumulation, but its optimal dosage can vary significantly depending on species, soil fertility, and climatic conditions (Imbrea et al., 2016; Gurita et al., 2019; Nurzyńska-Wierdak et al., 2023). Excessive or insufficient application can lead not only to reduced yields but also to environmental risks such as nutrient leaching and soil degradation (Sodré et al., 2012; Pinc et al., 2022; Fallah et al., 2024). In addition, the interaction between fertilization and planting, distance is another key agronomic factor that can significantly impact plant morphology, intra-specific competition, and resource availability (light, nutrients, and water).

Planting distance plays an important role in the development of the root system and the branching of the bush, while influencing air circulation and plant access to light. By determining the competition between plants for environmental resources, this technological factor can affect the growth rate and biomass accumulation of lemon balm. Identifying the optimal balance between planting distance and nutrient supply is crucial for achieving both high productivity and plant health (Rahmati, 2024; Gordanić et al., 2025). Given the specific pedoclimatic conditions of western Romania, characterized by cernoziom cambic soils with moderate fertility, a temperate continental climate, and variable rainfall patterns, it is necessary to adapt technological inputs accordingly. This region offers favorable conditions for cultivating *Melissa officinalis*, but scientific data on the combined effects of mineral fertilization and planting density under these local conditions remains limited. Also, the analysis of linear relationships between these factors and production allows for obtaining predictive information, in order to establish precise and sustainable cultivation strategies.

MATERIALS AND METHODS

The research was conducted during the 2023-2024 period at the Medicinal Plants Experimental Platform of the Faculty of Agriculture, within the University of Life Sciences ‘King Mihai I’ from Timișoara, located in western Romania, an area characterized by pedoclimatic conditions specific to the Banat Plain. The biological material used in the experiment was cultivated under open-field conditions following conventional agronomic practices. The experiment was conducted as a bifactorial trial with three replications, arranged in a randomized complete block design. Factor A was planting distance, with two levels (50/30 cm and 60/30 cm), and Factor B was mineral fertilization level, with three treatments: unfertilized control (N₀), N₃₀, and N₆₀. Mineral fertilization was applied according to the experimental protocol, in accordance with the standard cultivation technology for lemon balm.

Harvesting was performed at technological maturity, and fresh biomass yield was determined and expressed in kg/ha. The experimental data were statistically processed using analysis of variance (ANOVA). The statistical significance of the effects of planting distance and fertilization level, as well as the differences among treatment means, was evaluated using the Student’s t test and Duncan’s multiple range test, with the N₀ variant considered as the control. The contribution of each experimental factor to total yield variation was determined based on the analysis of variance, and linear correlation and regression analyses were used to statistically describe the relationships between the studied factors and biomass yield. The production data analyzed refers to fresh material.

RESULTS AND DISCUSSIONS

The results of the influence of planting distance on the production of lemon balm, are presented in Table 1. The analysis of variance showed that planting distance (Factor A) significantly influenced yield in 2023 and in the first harvest of 2024 ($p < 0.001$), while its effect was not statistically significant during the second harvest of 2024. Wider spacing (60/30 cm) consistently resulted in higher mean yields compared to the 50/30 cm distance, indicating improved plant development and reduced intra-specific competition.

Table 1. Effect of planting distance on yield of *Melissa officinalis* L.

Year / Harvest	Planting distance (cm)	Yield (kg/ha)	Standard error
2023	50/30	3917.27	428.52
	60/30	4345.03	403.17
2024 – Harvest I	50/30	5055.36	438.95
	60/30	5430.51	418.13
2024 – Harvest II	50/30	2912.55	432.03
	60/30	3154.10	280.02
2024 – Total	50/30	7967.92	867.87
	60/30	8584.61	692.26

In 2023, mean yield increased from 3917 kg/ha at 50/30 cm to 4345 kg/ha at 60/30 cm. A similar trend was observed in the first harvest of 2024, where yields reached 5431 kg/ha at the wider spacing. However, during the second

harvest, yield differences between planting distances were small and statistically non-significant, suggesting that spatial arrangement becomes less important during regrowth phases, when plants rely more on established root systems.

Overall, planting distance contributed modestly to yield formation, explaining between 1.3% and 3.2% of total variance, depending on year and harvest.

Yield differences were highly significant in 2023 and the first harvest of 2024, but non-significant in the second harvest, indicating that planting distance becomes less influential during regrowth.

The results regarding the influence of mineral fertilization on the production are shown in Table 2.

Table 2. Effect of fertilization level on yield of *Melissa officinalis* L.

Year/Harvest	Fertilization level	Yield (kg/ha)	Standard Error
2023	N ₀	2718.22	174.50
	N ₃₀	4111.29	19.87
	N ₆₀	5563.94	132.32
2024 – Harvest I	N ₀	3793.64	149.55
	N ₃₀	5196.49	10.48
	N ₆₀	6738.68	112.58
2024 – Harvest II	N ₀	1685.45	197.95
	N ₃₀	3319.86	18.72
	N ₆₀	4094.68	17.20
2024 – Total	N ₀	5479.08	347.51
	N ₃₀	8516.36	29.20
	N ₆₀	10833.36	95.39

Mineral fertilization (Factor B) exerted a very highly significant effect on yield in all years and harvests ($p < 0.001$).

Yield increased progressively with nitrogen level, confirming that nutrient availability is the main driver of biomass production in lemon balm under the studied conditions.

In 2023, fertilization accounted for 94.4% of yield variability. Compared to the unfertilized control (N₀), yield increased by 51.2% at N₃₀ and by 104.7% at N₆₀. Similar responses were recorded in 2024, with yield increases ranging from 37–55% at N₃₀ and from 77–98% at N₆₀, depending on harvest.

Duncan and Student tests consistently separated the three fertilization levels into distinct homogeneity groups, indicating that each nitrogen increment resulted in statistically

assured yield gains. These results emphasize the central role of mineral fertilization in optimizing lemon balm productivity in western Romania.

Duncan’s multiple range test separated all fertilization levels into distinct homogeneity groups in all experimental variants, confirming statistically assured differences among N₀, N₃₀, and N₆₀ (Table 3).

Table 3. Effect of fertilization level on yield of *Melissa officinalis* L. (Duncan’s test, $p \leq 0.05$)

Fertilization level	Yield (kg/ha)	Significance group
N ₆₀ (b3)	10830	a
N ₃₀ (b2)	8516	b
N ₀ (b1)	5479	c

The interaction between planting distance and fertilization (A×B) showed a differentiated response depending on year and harvest (Table 4 and Figure 1). While fertilization effects were consistent across planting distances, the magnitude of yield increase varied slightly between spatial arrangements.

Table 4. Interaction effect (A×B) on yield of *Melissa officinalis* L. – mean values (kg/ha)

Planting distance	N ₀	N ₃₀	N ₆₀
50/30 cm	lower	moderate	high
60/30 cm	higher	higher	highest

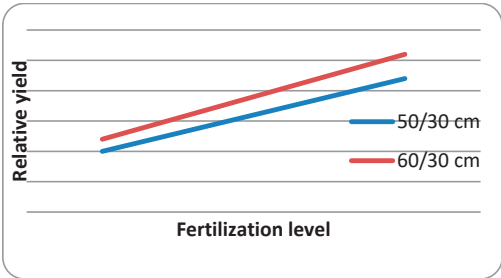


Figure 1. Interaction plot between planting distance and fertilization level for yield of *Melissa officinalis* L.

In years and harvests where planting distance was significant (2023 and first harvest of 2024), nitrogen-induced yield increases were more pronounced at the wider spacing (60/30 cm). This suggests that reduced plant competition enhanced nutrient uptake efficiency and biomass accumulation. In contrast, during the second harvest of 2024, the

interaction effect was weak, reflecting the diminished role of spatial arrangement during regrowth.

Although fertilization remained the dominant factor, the A×B interaction highlights that optimal yield expression occurs when adequate nutrient supply is combined with appropriate plant spacing, particularly during the main growth phase.

The contribution of experimental factors to the production of lemon balm are presented in Table 5 and Figure 2.

Table 5. Contribution of experimental factors to yield variability (%)

Year/Harvest	Planting distance (%)	Fertilization (%)
2023	3.20	94.40
2024 - Harvest I	2.34	96.11
2024 - Harvest II	1.36	93.80
2024 - Total	1.90	95.70

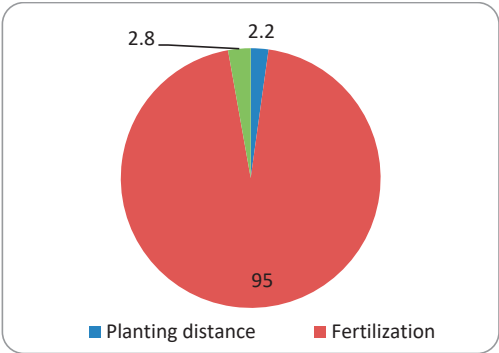


Figure 2. Relative contribution of planting distance and fertilization to yield variation

The contribution analysis clearly demonstrated the predominance of mineral fertilization in yield formation. Across all experimental variants, fertilization explained between 93.8% and 96.1% of total yield variability, whereas planting distance contributed less than 4%.

This disproportionate contribution confirms that, under the pedoclimatic conditions of western Romania, nutrient availability represents the primary limiting factor for *Melissa officinalis* productivity. Planting distance acts mainly as a secondary optimization factor, fine-tuning yield expression rather than driving it.

The variability analysis indicated a low coefficient of variation for planting distance (CV = 9.4%), reflecting the controlled experimental design, while fertilization dose and yield parameters showed high variability (CV > 20%), highlighting their strong influence on production levels (Table 6). Descriptive statistical parameters presented were calculated based on 18 observations.

Yield variability was higher in the second harvest of 2024, reflecting increased sensitivity of regrowth to environmental and nutritional factors.

Simple linear correlation analysis revealed a very strong and statistically significant positive relationship between fertilization level and yield across all years and harvests ($r = 0.95-0.98$, $p < 0.001$). These relationships are illustrated in Figure 3 (a, b), which shows the linear correlation between fertilization level and yield for: (a) first harvest in 2023 and 2024, and (b) second harvest and cumulative yield in 2024.

Table 6. Descriptive statistics of yield by harvest period

Experimental condition	Mean	Minimum	Maximum	Variance	Standard deviation	Standard error	Coefficient of variation
Harvest 2023-I	4131.151	2327.942	5860.13	1514614	1230.697	290.078	29.8
Harvest 2024-I	5242.937	3458.752	6990.63	1593753	1262.439	297.5598	24.1
Harvest 2024-II	3033.328	1242.393	4133.46	113806	1066.799	251.4469	35.2
Total Harvest 2024	8276.265	4701.144	11046.75	532029	2306.575	543.665	27.9
Planting distance	55.000	50.000	60.00	26	5.145	1.2127	9.4
Fertilization level	30.000	0.000	60.00	635	25.205	5.9409	84.0

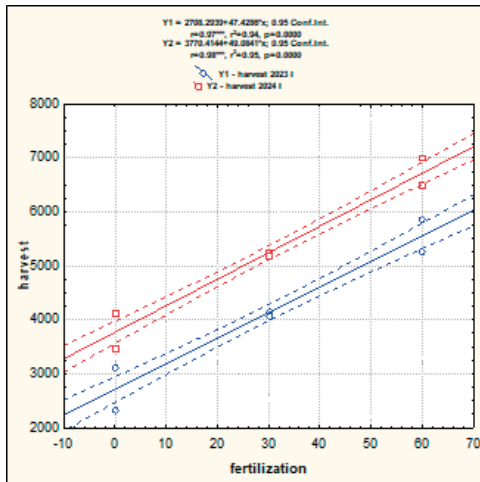


Figure 3a. Correlation between the level of fertilization

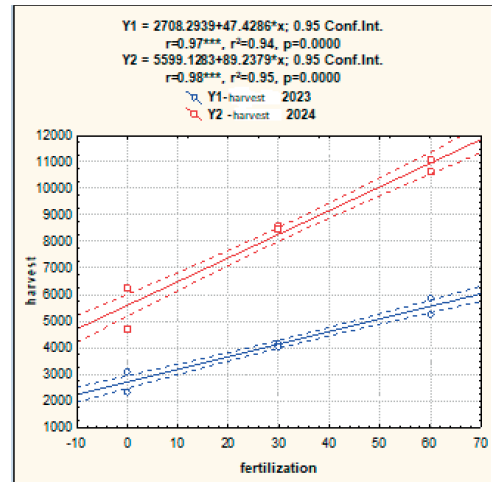


Figure 3b. Correlation between the level of fertilization

Figures 3a and 3b illustrate the correlation between fertilization level and yield, based on a comparative analysis of the first harvest obtained in the two experimental years, as well as on the comparison of total yield recorded in 2023 and 2024.

The three-dimensional response surface illustrates the combined effect of planting distance and fertilization level on the yield of *Melissa officinalis* L. in 2023 (Figure 4).

The relationship between the two experimental factors and yield was described using multiple regression analysis, indicating a positive contribution of both planting distance and fertilization level to yield variation.

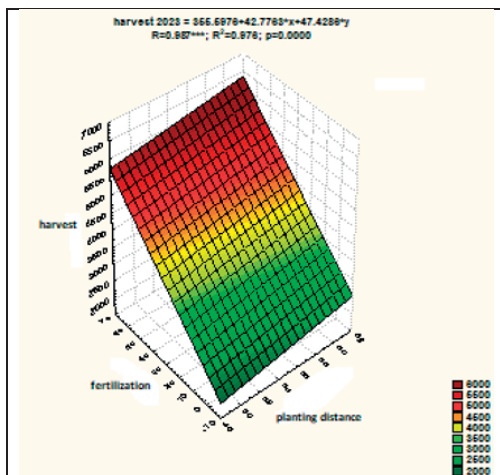


Figure 4. Relationship between planting distance, fertilization and yield in 2023

The high values of the correlation coefficient ($R = 0.987$) and coefficient of determination ($R^2 = 0.97$) indicate an excellent model fit, demonstrating that 97% of yield variability is explained by the combined action of the two experimental factors.

The slope of the response surface shows a much steeper gradient along the fertilization axis compared to the planting distance axis, highlighting fertilization as the dominant factor influencing yield.

An increase in fertilization level leads to substantial yield gains across the entire range of planting distances, confirming the strong positive effect of nitrogen supply on biomass accumulation.

This finding is consistent with the ANOVA and correlation analyses, which identified fertilization as the main determinant of yield. In contrast, planting distance exerts a moderate but positive effect

The three-dimensional response surface illustrates the combined effects of planting distance and fertilization level on the cumulative yield of *Melissa officinalis* L. in 2024 (sum of two harvests) (Figure 5).

The relationship between planting distance, fertilization level, and total yield was described using multiple regression analysis, indicating a positive and additive effect of both experimental factors on total biomass production.

The very high values of the correlation coefficient ($R = 0.98$) and the coefficient of

determination ($R^2 = 0.97$) indicate an excellent model performance, demonstrating that 97% of the variability in total yield is explained by the combined influence of planting distance and fertilization.

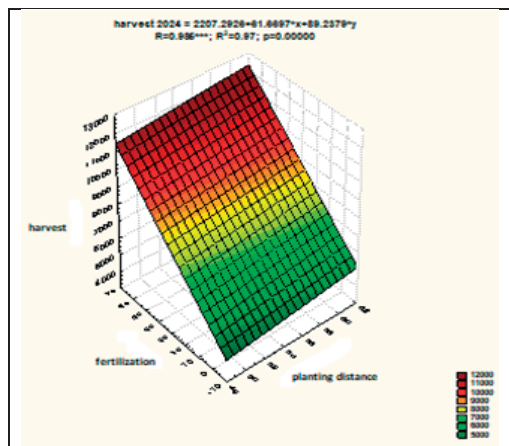


Figure 5. Relationship between planting distance, fertilization level and total yield in 2024

The response surface shows a pronounced and steep gradient along the fertilization axis, confirming fertilization as the dominant factor controlling cumulative biomass production. Increasing fertilization levels resulted in substantial yield gains across the entire range of planting distances, emphasizing the central role of nutrient availability in sustaining regrowth and multiple harvests.

Compared to 2023, the regression coefficient associated with fertilization is even higher, indicating that fertilization becomes increasingly important when total seasonal yield is considered. This reflects the cumulative effect of nitrogen supply on both primary growth and subsequent regrowth after the first harvest.

Planting distance exhibited a positive but secondary effect on total yield, as indicated by the smaller slope of the surface along the distance axis. Wider spacing enhanced yield mainly by improving the efficiency of fertilization, likely through reduced intra-specific competition and improved resource capture during successive harvest cycles.

The near-planar nature of the response surface indicates a weak interaction between planting distance and fertilization, suggesting that

fertilization consistently increased yield regardless of planting distance. This confirms that nutrient supply acts independently and decisively, while planting distance fine-tunes yield expression rather than altering the response pattern.

Overall, the response surface demonstrates that maximum cumulative yields in 2024 were achieved at higher fertilization levels combined with wider planting distances, providing strong quantitative and graphical support for fertilization centred management strategies in lemon balm cultivation under the pedoclimatic conditions of western Romania.

The fresh biomass yields obtained in our study are comparable, in terms of response pattern, with those reported under temperate climate conditions in Poland by Nurzyńska-Wierdak et al. (2023). In their study, fresh herb yield ranged approximately between 11 and 16 t·ha⁻¹, depending on plant spacing and harvest management, with higher values associated with wider spacing and multiple harvests. Although the absolute yield levels recorded in western Romania were lower, the results consistently confirm the dominant role of cultivation technology, particularly fertilization and harvest regime, over planting density in determining lemon balm biomass production (Nurzyńska-Wierdak et al., 2023).

In line with our findings, Gordanic et al. (2025) reported that planting density and cultivation practices significantly influenced agronomic performance and fresh biomass yield of lemon balm under temperate conditions in Serbia, which supports the observed role of planting density and technical management in yield variation (Gordanic et al., 2025).

Comparable agronomic research in Bulgaria by Zorovski et al. (2020) showed that fertilization treatments increased fresh biomass yield of lemon balm up to approximately 9.9 t·ha⁻¹, further emphasizing the positive effect of fertilization on yield performance in similar temperate European environments (Zorovski et al., 2020).

Research results demonstrate the importance of fertilization in optimizing fresh biomass yield in *Melissa officinalis* L., highlighting its dominant role compared to planting distance under field conditions.

CONCLUSIONS

The research results confirm that mineral fertilization is the main technological factor influencing yield in lemon balm under the pedoclimatic conditions of western Romania. Fertilization accounted for more than 95% of yield variability across experimental years and harvests, highlighting its dominant role in biomass accumulation and yield stability.

From an agronomic perspective, the results indicate that the application of moderate to higher nitrogen doses (N₃₀–N₆₀) leads to significant and statistically assured yield increases, irrespective of planting distance. The consistent yield response to fertilization demonstrates that nitrogen management represents a priority component of lemon balm cultivation technology, particularly in systems with multiple harvests per growing season.

Planting distance had a lower influence on yield; however, wider spacing (60/30 cm) proved advantageous by improving the efficiency of fertilization, especially during periods of active vegetative growth. Therefore, wider planting distances can be recommended as a complementary practice to enhance resource use efficiency, reduce intra-specific competition, and promote uniform crop development.

The weak interaction between planting distance and fertilization suggests that fertilization strategies may be applied uniformly across different planting densities, thereby simplifying technological decisions for growers.

The combined application of optimized fertilization regimes and appropriate planting distance represents a sustainable approach for increasing lemon balm productivity, supporting both economic efficiency and rational resource use in medicinal plant cultivation.

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EFFECT OF DIFFERENT TYPES OF MINERAL FERTILIZATION ON YIELDS AND SOME PHYSIOLOGICAL CHARACTERISTICS IN FIELD BEANS

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Abstract

The study was conducted within the Longstanding Stationary Fertilizer Experiment (LSFE) in IASS "Obraztsov chiflik", Rousse with the aim of establishing the influence of different options of mineral fertilization on yield and some physiological characteristics, including resistance to environmental stress in field beans. It was found that the highest yield for the period - 1,070 kg ha⁻¹ - was obtained in the experimental plot with combined N₃K₇ fertilization, represents nearly 70% increase compared to the average yield of the control. The content of photosynthetic pigments in the leaves of field beans varies significantly in different fertilization options, with the highest levels of chlorophyll a+b being found in the variant with nitrogen fertilization (N₃) - 1.520 mg g⁻¹. The variants with combined N₃P₁₂ fertilization stand out as the most resistant to atmospheric drought during the four-year research period, with the reported values - 98.28 μS cm⁻¹, being 9,8% lower, compared to the control. The highest resistance to soil drought was established again in the variants with combined N₃P₁₂ fertilization (500.00 μS cm⁻¹) and the lowest - within the control (711.58 μS cm⁻¹).

Key words: chlorophyll, field beans, mineral fertilizers, physiological characteristics, yield.

INTRODUCTION

The efficient use of resources to improve agricultural production is of utmost importance in view of the continuous growth of the world population. It is expected to reach 9.9 billion by 2050, which requires an increase in food production by over 70% (Sarfraz et al., 2023). The extreme events of abiotic stresses – high temperatures and prolonged periods without precipitation, affects the balance of natural and anthropogenic ecosystems and reduces crop production worldwide (Hossain et al., 2021). This place supporting plant growth and development in conditions of abiotic and biological stress and obtaining higher yields among the main challenges facing agriculture (Reynolds et al., 2011).

Leguminous crops are known for their unique nitrogen fixing ability. Legumes introduced into a cropping system can not only add to the fertility of the soil by their nitrogen fixing ability but also gives additional benefit of soil organic matter improvement, nutrient recycling, improving soil porosity and soil structure, etc.

Because the growing season of legumes is typically short, they fit well into cropping systems, providing flexibility in crop planning (Praharaj & Maitra, 2020).

Fertilization is among the most significant factors influencing the productivity of agricultural crops, making it the subject of numerous scientific studies (Nenova, 2008; Naskova et al, 2019). Particularly valuable information can be obtained in studies conducted within longstanding stationary fertilizer experiments (Samarlieva & Nikolova, 1996; Panayotova, 2007).

Fertilizers improve soil fertility and plant nutrition, making them essential components in agriculture and forestry (Boteva & Cholakov, 2009). Soil fertilization increases nutrient reserves in the root zone of crops, increases pH values and the amount of organic carbon, thus improving the structure of the root zone of the soil (Karcauskiene & Repsiene, 2009).

Macronutrients (N, P and K) are of particular importance for the normal course of physiological processes in plants, for their growth and development. Nitrogen (N) significantly improves and increases the yield and its quality

due to its critical role for the normal course of biochemical and physiological processes (Amancio & Stulen, 2005). It is a key component of enzymes and structural proteins, nucleic acids, pigments and a wide range of secondary metabolites in plants (Leghari et al., 2016). Phosphorus (P) is part of the structure of many enzymes and proteins, DNA and RNA, and plays a significant role in assimilating solar energy and turning it into useful organic compounds. It is a vital component of adenosine triphosphate (ATP), which is formed during photosynthesis, and is actively involved in energy metabolism. Phosphorus increases plant resistance and regulates the physiological response to abiotic stress – high temperatures, salinity, drought, waterlogging, high CO₂ levels and the toxic effects of heavy metals (Hawkesford et al., 2012; Lambers, 2022; Khan et al., 2023). Potassium (K) is among the elements of particular importance for the normal course of a large number of physiological processes, for the growth of agricultural crops, for the quality and quantity of yields, as well as for the resistance of crops to stress (Zörb et al., 2014). It has an important role in limiting water loss from plants at high ambient air temperatures and in drought conditions. In plants under drought conditions, the intensity of photosynthesis is directly related to potassium levels (Chaerle et al., 2007).

Changes in cell membrane organization occur during seed development as seeds reach physiological maturity, as a result of moisture loss prior to harvest and water uptake prior to germination. The electrical conductivity test is based on the measurement of electrolytes released from seeds as a result of changes in cell membrane permeability (Matthews & Powell, 2006). It allows for a rapid and reliable determination of seed quality and viability, in contrast to the standard method for determining germination (Standard Germination Test), which is more species specific and time-consuming (Montenegro et al., 2003).

The bright green chlorophyll is the most widely distributed natural pigment in nature. The chlorophylls are omnipresent in photosynthetic tissue where they are responsible for photoreception (Formaggio et al., 2001). They play a key role in the process of photosynthesis, which allows plants to absorb energy from

sunlight (Willows, 2009; Hörtensteiner & Kräutler, 2011; Filimon et al., 2016). The chlorophylls of higher plants, ferns, mosses and green algae, as well as the prokaryotic organism *Prochloron*, consist of chlorophyll *a* as the main pigment and chlorophyll *b* as an accessory pigment. These two pigments were for a time thought to be the only natural chlorophylls in higher plants, but it has since been shown that a variety of precursors and degradation products are also present in nature (Andersen & Francis, 2009).

Carotenoids are derivatives of isoprene, with a dark red to yellow color found in all higher plants and in many types of microorganisms. Carotenoids are also found in many flowers and fruits where they are valuable in terms of providing signals to other species on the availability of food sources, and in this way ensure pollination and the spread of seeds (Goodwin, 1980; Gross, 1991). Carotenoids perform two main roles in plants – they are functioning as auxiliary pigments: as such, carotenoids participate in the construction of photosystems and participate in the absorption of light in that part of the spectrum (400-500 nm) in which the efficiency of chlorophyll is lower and they protect chlorophyll molecules from photooxidation (Lichtenthaler, 1987; Young and Frank, 1995; Britton, 2008; Cuttriss and Pogson, 2009; Belous et al., 2018).

MATERIALS AND METHODS

Climatic and Soil Characteristics of the Experimental Field

The study covers the four-year period of the 16th rotation (from October 2018 to June 2022) of a Longstanding Stationary Fertilizer Experiment (LSFE), located in the Experimental Field of IASS "Obraztsov chiflik", Rousse, Bulgaria. The northern climatic region of the Danubian rolling plain, in which the Obraztsov chiflik falls (152 m above sea level, 43°48' north latitude, 26°02' east longitude), is characterized by the largest average annual temperature amplitude for Bulgaria – from 24.5 to 26.0°C. In January and February the lowest minimum temperatures here may reach -25°C, while in the hot summer months have been recorded maximum temperatures up to 42-43°C. The rainfall in Obraztsov chiflik follows a continental pattern,

with a maximum in June and a minimum in February. Due to the nature of their distribution (the rainfalls in Obratzov chiflik are most often torrential, interrupted by long periods without precipitation), dry periods occur in the summer. The drought is relatively strong and prolonged not only in August, but also during most of September and even the beginning of October (Lingova, 1965).

Kostov (1981) found that the LSFE was located on a medium sandy-clayey haplic chernozem, uneroded and slightly eroded. According to a new study conducted in 2014, the modern soil classification in Bulgaria defines these soils as leached (Luvic) or degraded (Teoharov et al., 2014). As a result of the multi-year uniform fertilization of the experimental plots, permanent changes in soil acidity and humus content occurred in the different variants. Soil acidity (pH) ranged from 5.25 in plots with nitrogen-potassium fertilization to 5.74 in the no-fertilizer control. The lowest humus content (1.67%) was found in the plots of the control, and the highest (2.65%) – in the variant with full mineral fertilization (N₁₅P₁₂K₇).

Setting Up the Experiment

The longstanding stationary fertilizer experiment was established in 1912 on an area of 1 ha according to the eight-plot scheme of Georg Vile, in two repetitions with an experimental plot of 100 m² and a harvest plot of 60 m². Research was interrupted in 1942 and resumed in the fall of 1958 with a four-pole crop rotation of wheat-maize-barley-field beans (Ermolaev, 1965).

Seven variants of fertilization are being tested with individual and combined application of the macro elements nitrogen, phosphorus and potassium. The eighth variant is the unfertilized control (N₀P₀K₀). The amount of nitrogen fertilizers applied to the individual crops is as follows: for wheat and corn – 150 kg ha⁻¹ a.s. (N₁₅); for barley – 100 kg ha⁻¹ a.s. (N₁₀) and for beans – 50 kg ha⁻¹ a.s. (N₅). The amount of phosphorus and potassium fertilizers applied is the same for all four crops in the crop rotation – 120 kg ha⁻¹ a.s. in the form of P₂O₅ and 70 kg ha⁻¹ a.s. in the form of K₂O. Phosphorous (P₁₂) and potassium (K₇) fertilizers are applied once, before the main tillage, and nitrogen fertilizers – once after crop emergence.

Characteristic of the Variety

The sixteenth, after the restoration of the LSFE in 1958, rotation of the four-pole crop rotation included common wheat "Dunavia" variety, winter barley, "Ahat" variety, field beans, "Obratzov Chiflik 12" variety and maize, hybrid Ps 464.

The "Obratzov Chiflik 12" field beans variety is semi-climbing with erected, tidy bush without lodging. The height of the plants is averagely 48.3 cm with average height of the first formation of pods is 9.9 cm. It does not form pods on stem tendrils. The seeds are semi-flat type, the mass of 1000 seeds – averagely 311 g. The plants ripen at one and the same time and have good resistance to crumbling. It is a high yielding variety with good boiling time and excellent gustatory qualities.

When carrying out the experiment, the agrotechnics adopted for North-Eastern Bulgaria for growing field beans are followed. The obtained data were statistically processed with Microsoft Corporation's *Excel* and TIBCO Software's *Statistica 13* programs.

Sampling and Analysis

- *To determine the electrical conductivity of leaf membranes*, in the flowering stage of field beans a sample of healthy, with normal turgor and without signs of damage, leaves of 10 randomly selected plants in each of the experimental plots was taken. The leaves are placed in clean containers and taken to a laboratory for analysis. Fresh leaves were washed well, rinsed with deionized water and after drying, an average sample was taken from them to measure the electrical conductivity of leaf membranes (Pavlova et al., 2005; Pavlova & Panayotova, 2005; Pavlova & Dochev, 2010). The measurement of the electrical conductivity of leaf membranes is performed with a COND 51 laboratory conductometer after a two-hour incubation of the samples in a thermostat at a temperature of 42°C.

- *The measurement of the electrical conductivity of the seed membranes* makes it possible to quickly and reliably determine the viability of the seeds. The electrical conductivity test is based on measuring electrolytes that are released from the seeds as a result of changes in the permeability of cell membranes (Matthews & Powell, 2006). In our study to determine

electrical conductivity of seed membranes, the methodology described by Avramiuc (2016) was used with some modifications. A month after harvesting 50 seeds of each variant of the LSFE were selected, placed in a suitable container and poured with 50 mL of deionized water. The samples thus prepared were kept in a thermostat at a temperature of 20°C for 24 hours, after which the electrical conductivity of the water was measured using a COND 51 laboratory conductometer.

- Extraction and determination of the content of photosynthetic pigments

The photosynthetic pigments contained in the leaves of the plants in flowering stage were extracted under vacuum, using an 80% acetone solution as a solvent. A sample of the obtained extract was diluted with pure 80% acetone, then photometrically measured. The quantitative determination of the pigments was carried out using a DR 3900 spectrophotometer from HACH LANGE, Germany. The light absorption in the extract was measured at three wavelengths: 663 nm and 644 nm, respectively for chlorophyll *a* and *b*, and 440 nm for carotenoids with a blank sample of pure, 80% acetone solution as a control. The pigments content was calculated according to Berova et al. (2007) in mg g⁻¹ fresh mass, taking into account the concentration of the pigments, the volume of the total extract, the mass of the leaf sample and the proportion of dilution.

The aim of the present research is to study the influence of different nitrogen, phosphorus and potassium fertilization options on the yield of field beans variety "Obraztsov chiflik 12" and to determine the influence of long-term mineral fertilization on its photosynthetic pigments content and resistance to environmental stress.

RESULTS AND DISCUSSIONS

Brief meteorological characteristics

The spring of 2019 is characterized by temperature sums and precipitation close to the climatic norm for the region formed on the basis of the multi-year data obtained from a meteorological cell located in Obraztsov chiflik at the beginning of the 20th century (Table 1a and 1b). The temperature and amounts of precipitation in April and May are close to normal, while June can be defined as hot and

humid with a temperature sum of 672.6°C, significantly exceeding the norm of 604.0°C, and 129.1 mm of total precipitation at a norm of 82.1 mm. The precipitation that fell in July is unevenly distributed over 8 days, with their total amount reaching only 19.5 mm, or slightly over 1/3 of the norm for the period, amounting to 65.9 mm. No significant deviations are observed in terms of the temperature sum.

Table 1a. Temperature sum for the period April-July from 2019 to 2022

Year	Temperature sum, °C			
	IV	V	VI	VII
2019	340.6	527.1	672.6	711.7
2020	366.9	511.6	616.9	746.0
2021	287.4	521.5	599.4	763.5
2022	339.7	542.0	639.4	766.8
Climate norm	341.9	509.5	604.0	699.1

Table 1b. Distribution of precipitation for the period April – July from 2019 to 2022

Year	Precipitation, mm			
	IV	V	VI	VII
2019	56.0	70.4	129.1	19.5
2020	13.6	67.1	126.0	1.1
2021	45.6	73.5	121.0	1.0
2022	74.9	18.0	73.0	11.6
Climate norm	50.6	66.5	82.1	65.9

The temperature sum for the period April – June in 2020 and 2021 is close to normal for the Obraztsov Chiflik region, with the exception of April 2021, which can be defined as cool, with a temperature sum of 287.4°C compared to a norm of 341.9°C. The precipitation in May 2020 and April-May 2021 is close to normal for the period with a significant drought in the early spring of the second year of the 16th LSFE rotation. The total amount of precipitation in April is only 13.6 mm compared to a norm of 50.6 mm. In these meteorological conditions the amount of precipitation in June – distributed mainly in the second ten days of the month – significantly exceeds the norm of 82.1 mm, respectively by 43.9 mm in 2020 and 38.9 mm in 2021. The last month of the crop's vegetation is characterized by serious drought, with notable increase in average daily temperatures. The temperature sum recorded in 2020 exceeds the norm of

699.1°C for the month of July by 46.9°C, in 2021 – by 66.4°C and in 2022 – by 67.7°C.

At these unusually high temperatures, the amount of precipitation is insignificant, with precipitation for the entire month of July in 2020 and 2021 being only 1.1 mm and 1.0 mm, respectively. In conclusion, it can be said that the years from 2019 to 2022 are characterized by a warm spring and hot summer with unevenly distributed precipitation and prolonged periods of drought during the summer months.

Yield

Only the results for the productivity of the crops included in the crop rotation in the 9th, 10th and

12th rotations of LSFE after its restoration in 1952 were analyzed and published (Dimitrov & Beykov, 1995; Stoyanov, 2001; Nenova, 2008; Nenova & Stoyanova, 2011). So far there is just one published study on the influence of different fertilization options on the resistance of some of the crops in crop rotations of LSFE to environmental stress (Ivanova-Kovacheva et al., 2024).

The yields obtained during the fertilization of field beans variety "Obraztsov Chiflik 12" with the macro elements nitrogen (N₅), phosphorus (P₁₂) and potassium (K₇), applied alone and in combination between them, for the period of the 16th rotation of LSFE are presented on Table 2.

Table 2. Yields of field beans variety "Obraztsov Chiflik 12" obtained from LSFE for the period from 2019 to 2022

Variants	Yield, kg ha ⁻¹				Average yield, kg ha ⁻¹	Differences +, - kg ha ⁻¹
	2019	2020	2021	2022		
Control (N ₀ P ₀ K ₀)	540	780	320	890	632.50	0.0
N ₅	980	1,140	780	1,090	997.50	365 +++
P ₁₂	730	820	490	800	710.00	77.5 n.s.
K ₇	720	850	310	740	655.00	22.5 n.s.
N ₅ P ₁₂	1,130	1,260	830	1,050	1,067.50	435 +++
N ₅ K ₇	1,090	1,160	880	930	1,015.00	382.5 +++
P ₁₂ K ₇	600	1,070	420	800	722.50	90 n.s.
N ₅ P ₁₂ K ₇	960	1,100	910	1,050	1,005.00	372.5 +++
Average	843.75	1,022.50	617.50	918.75		
LSD 0.05						150
LSD 0.01						200
LSD 0.001						270

The most favorable year for field beans was 2020, with an average yield of 1,022.5 kg ha⁻¹. The highest yield was reached in the same year – 1,260 kg ha⁻¹, obtained with the two-component fertilization combination (N₅P₁₂), followed by 1,160 kg ha⁻¹ with the N₅K₇ combination.

The data also shows that the highest average yield for the rotation in the variants with combinations of the studied macroelements was achieved in the one with the two macroelements N₅P₁₂ – 1,067.5 kg ha⁻¹, with the increase compared to the unfertilized control being 435 kg ha⁻¹. In the other fertilization combinations in which the macroelement nitrogen participates, a proven increase in yield was also observed, with the N₅K₇ variant being 1,015 kg ha⁻¹ or 382.5 kg ha⁻¹ higher than the unfertilized control. In the case of phosphorus and potassium fertilization alone and in the combination between them, the differences in yield obtained are unproven.

Electrical Conductivity of Leaf Membranes

The measurement of the electrical conductivity of the cell membranes of the leaves during the active vegetation of plants can be used as an indicator of the resistance to atmospheric drought (Pavlova, 2005; 2010). Figure 1 presents the reported values of electrical conductivity of leaf membranes in flowering stage of the field beans for the entire period of the 16th rotation of LSFE.

It can be seen that the lowest measured values vary within very narrow limits – from 70.10 μS cm⁻¹ in the variant with only nitrogen fertilization (N₅) in 2020 to 77.73 μS cm⁻¹ in the variant with combined nitrogen-phosphorus fertilization (N₅P₁₂) in 2021.

The maximum electrical conductivity of leaf membranes during the four years of the study were found in the non-fertilized variant (N₀P₀K₀), with the values falling within significantly wider

margins – from 98.53 $\mu\text{S cm}^{-1}$ in 2020 to 124.60 $\mu\text{S cm}^{-1}$ in 2021.

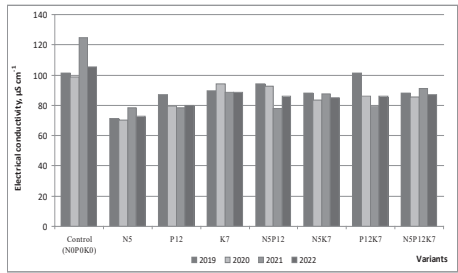


Figure 1. Electrical conductivity of leaf membranes in flowering stage of field beans by year, 16th LSFE rotation

The summarized results show that the leaching of electrolytes through the cell membranes of the leaves in the variants with only N₅ and P₁₂ fertilization is lower, compared to the variants with only K₇ fertilization, as well as the variants with combined fertilization (Table 3).

Table 3. Statistically processed data on electrical conductivity of leaf membranes in flowering stage of field beans, 16th LSFE rotation

Variants	Electrical conductivity of leaf membranes, $\mu\text{S cm}^{-1}$		
	Mean	CV, %	SE, %
Control (N ₀ P ₀ K ₀)	107.44	10.97	5.89
N ₅	73.07	5.04	1.84
P ₁₂	81.22	4.68	1.90
K ₇	90.23	3.17	1.43
N ₅ P ₁₂	87.72	8.56	3.75
N ₅ K ₇	86.12	2.60	1.12
P ₁₂ K ₇	88.16	10.59	4.67
N ₅ P ₁₂ K ₇	88.05	2.80	1.23

The highest values of electrical conductivity of the leaf membranes, averaged over the period of the study, were recorded in the control variant (N₀P₀K₀) – 107.44 $\mu\text{S cm}^{-1}$ against 73.07 $\mu\text{S cm}^{-1}$ for the variant with nitrogen fertilization (N₅). The variants with phosphorus (P₁₂) and two-component nitrogen-potassium (N₅K₇) fertilization are also proven resistant to atmospheric drought, in which the electrical conductivity of the leaf membranes was recorded as 81.22 $\mu\text{S cm}^{-1}$ and 86.12 $\mu\text{S cm}^{-1}$, respectively.

The statistical processing of the data obtained in the four-year period of the study, found that the values of the coefficient of variation (CV, %) for the different variants of field beans fertilization lie within relatively wide range – from 2.60% in

the variant with combined N₅K₇ fertilization to 10.97% in the control variant (N₀P₀K₀). The low values of the coefficient of variation verify the higher accuracy of the results reported during the four years of the study. Low values of the statistical error (SE, %) were established in the variant with combined N₅K₇ and full mineral fertilization (N₅P₁₂K₇) as well as those with applying just one of the three macro elements. The calculated values vary from 1.12% to 1.90%. The largest deviation from the average values of the parameter was reported for the variants without mineral fertilization (N₀P₀K₀) and the combined phosphorus-potassium fertilization (P₁₂K₇), where the SE reached 5.89% and 4.67%, respectively.

Electrical Conductivity of Seed Membranes

It is been established that the higher rate of restoration of the integrity of the cell membrane system during seed moistening will result in weaker extraction of electrolytes in the external environment and therefore the electrical conductivity of the water, which indicates a higher vitality (Baalbaki et al., 2009; Szemruch et al, 2019; Araújo et al., 2022). The relationship between electrical conductivity and field emergence is useful in practical seed technology (Matthews and Powel, 2006). Consequently, it could be assumed that it is possible to use the electrical conductivity of seed membranes as a criterion for determining the resistance of seeds to water stress, including soil drought.

The values of the electrical conductivity of field beans seed membranes varied vastly over the years and between different fertilization options (Figure 2). The seeds with the highest vitality in the individual years were obtained from experimental plots with different variants of fertilization. In the first and third years of the 16th rotation of the LSFE, the lowest extraction of electrolytes was recorded in the variant with phosphorus fertilization (P₁₂), 601.3 $\mu\text{S cm}^{-1}$ and 457.5 $\mu\text{S cm}^{-1}$, respectively, and in the second and fourth years – in the one with full mineral fertilization – 370.3 $\mu\text{S cm}^{-1}$ and 484.0 $\mu\text{S cm}^{-1}$. The analysis of the obtained results shows that the control and the variants with potassium and combined potassium-phosphorus fertilization had the lowest vitality, respectively, the highest amount of extracted electrolytes.

The values of seeds membrane conductivity reported in the first year of the 16th LSFE rotation are significantly higher and vary within wider range. The highest vitality was recorded in the variants with only P₁₂ (601.3 $\mu\text{S cm}^{-1}$) and combined N₅P₁₂ (609.3 $\mu\text{S cm}^{-1}$) fertilization, and the lowest – in the seeds obtained from plants in the plots without fertilization (1045.0 $\mu\text{S cm}^{-1}$) and with only K₇ fertilization. The conductivity in the variants with only phosphorus or combined nitrogen-phosphorus fertilization was higher than that of the control variant by 42.5-41.7%. The greater leaching of electrolytes was found in only one of the four years of the rotation, therefore the data obtained in 2019 cannot claim statistical reliability. The high heterogeneity of the results obtained necessitates the need of further studies to be conducted on the correlation between seed vitality and the different types of fertilization of field beans.

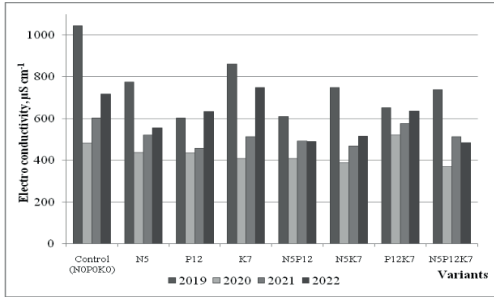


Figure 2. Electrical conductivity of field beans seeds by year, 16th LSFE rotation

The statistical processing of the data (Table 4) confirms the heterogeneity of the results, obtained during the 4-year study.

Table 4. Statistically processed data on electrical conductivity of field beans, 16th LSFE rotation

Variants	Electrical conductivity, $\mu\text{S cm}^{-1}$		
	Mean	CV, %	SE, %
Control (N ₀ P ₀ K ₀)	711.58	34.02	121.03
N ₅	572.75	25.18	72.12
P ₁₂	531.83	18.76	49.89
K ₇	633.03	33.05	104.60
N ₅ P ₁₂	500.00	16.57	41.42
N ₅ K ₇	530.87	29.32	77.83
P ₁₂ K ₇	597.01	10.04	29.98
N ₅ P ₁₂ K ₇	526.53	29.26	77.04

The seeds harvested from the variants with combined nitrogen-phosphorus (N₅P₁₂) and full mineral (N₅P₁₂K₇) fertilization have the highest vitality, on average for the period, 500.0 $\mu\text{S cm}^{-1}$ and 526.53 $\mu\text{S cm}^{-1}$, respectively. High vitality was also found in the variant with combined N₅K₇ (530.87 $\mu\text{S cm}^{-1}$) and with P₁₂ (531.83 $\mu\text{S cm}^{-1}$) fertilization.

The seeds from the control (N₀P₀K₀) have the lowest vitality. The highest electrical conductivity of seed membranes (711.58 $\mu\text{S cm}^{-1}$) was measured in this variant.

Statistical processing of the data obtained during the four-year study period found that the values of the coefficient of variation (CV, %) for the different options for fertilizing field beans fall within very wide limits, but shows satisfactory uniformity of the data by year, regardless. Sampling heterogeneity, i.e., values of CV above 30%, was found in the control and in the variant with potassium fertilization (N₀P₀K₀ – 34.02% and K₇ – 33.05%). The lowest values of the coefficient of variation were established for the variant with combined P₁₂K₇ fertilization (10.04%) with N₅P₁₂ close second (16.57%).

In the variant without fertilization (N₀P₀K₀), a significantly high level of statistical error (121.03%) was observed. According to the formula for calculating the error, in view of the small sample size due to the specific conditions of deriving the LSFE, and the significant coefficient of variation, obtaining such a value is possible. Smaller errors, but again significant in value, were obtained in the variant with potassium (K₇) and nitrogen (N₅) fertilization, 104.6% and 72.12%, respectively. In the variant with two-component fertilization with phosphorus and potassium (P₁₂K₇), the lowest level of statistical error was calculated – 29.98%.

Photosynthetic pigment content

The content of photosynthetic pigments in plant leaves provides important information about their physiological state and gives important information about their resistance to stress (Sims & Gamon, 2002).

The quantitative and qualitative composition of pigments and changes in their ratio in leaves are important characteristics of the physiological state of plants in general and of the photosynthetic apparatus in particular, including

adaptive responses to stress conditions. The total amount of photosynthetic pigments varies significantly depending on the dynamics of their accumulation and the location of plants (Belous et al., 2018). Similar conclusions were reached by Ilieva & Vasileva (2016), whose studies confirm the thesis that the content of plastid pigments changes under the influence of various factors.

Data representing the content of photosynthetic pigments (chlorophylls and carotenoids) during the four-year period covering the 16th rotation of LSFE are presented in Figure 3 and Figure 4.

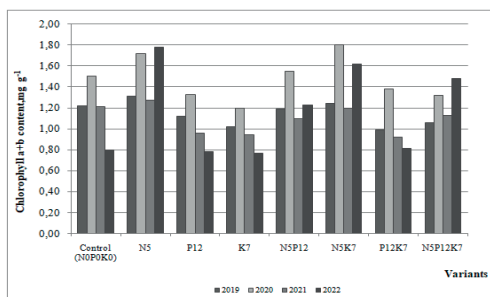


Figure 3. Chlorophyll *a+b* content of field beans in flowering stage, 16th LSFE rotation

It is apparent that the content of chlorophylls (Chl. *a+b*) and carotenoids in the leaves of the studied plants varies significantly between the different fertilization options and by year.

The smallest deviations in the values of the parameters within the studied period are in the variants with full mineral (N₅P₁₂K₇) and two-component fertilization with nitrogen and potassium (N₅K₇).

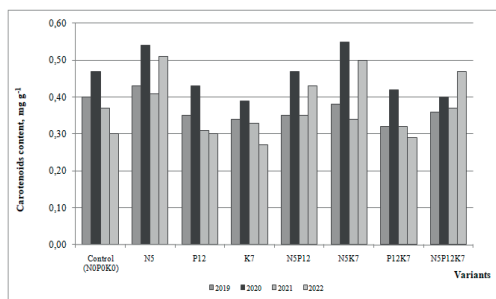


Figure 4. Carotenoid content of field beans in flowering stage, 16th LSFE rotation

The lowest content of chlorophylls (Chl. *a+b*) in the leaves of field beans in 2019 – 0.990 mg g⁻¹,

was found when applying the two-component phosphorus-potassium (P₁₂K₇) fertilization, followed by those with only potassium (K₇) and full mineral (N₅P₁₂K₇) fertilization with 1.020 mg g⁻¹ and 1.060 mg g⁻¹, respectively. The amount of chlorophylls in plants with fertilization including nitrogen is significantly higher. Maximum values were recorded in the variant with only nitrogen fertilization (1.310 mg g⁻¹), followed by those with combined nitrogen-potassium (N₅K₇) fertilization and the control, 1.240 mg g⁻¹ and 1.220 mg g⁻¹, respectively.

The content of carotenoids in the studied plants ranges within narrow limits – the lowest values were recorded in the variant with combined P₁₂K₇ (0.324 mg g⁻¹), followed by those with only potassium (0.343 mg g⁻¹) and phosphorus (0.352 mg g⁻¹) fertilization, and the highest – in the variant with only nitrogen fertilization (0.431 mg g⁻¹).

In the second year of the 16th rotation of the LSFE, the content of chlorophyll *a+b* varied from 1.203 mg g⁻¹ and 1.324 mg g⁻¹ in the variants with potassium (K₇) and full mineral (N₅P₁₂K₇) fertilization to 1.803 mg g⁻¹ and 1.721 mg g⁻¹ in the variants with combined nitrogen-potassium (N₅K₇) and nitrogen (N₅) fertilization. A similar pattern is observed in terms of carotenoid content – the lowest amount was recorded in the variants with potassium (K₇) and with full mineral fertilization (N₅P₁₂K₇) – 0.392 mg g⁻¹ and 0.403 mg g⁻¹, respectively, and the highest – in the combination of nitrogen and potassium (0.553 mg g⁻¹) and in nitrogen (0.541 mg g⁻¹) fertilization.

The lowest chlorophyll content in 2021 – 0.922 mg g⁻¹ was recorded in the variant with combined phosphorus-potassium (P₁₂K₇) fertilization, and the highest – in the variant with nitrogen fertilization (1.273 mg g⁻¹), followed by the control (1.210 mg g⁻¹) and the variant with combined nitrogen-potassium (N₅P₁₂) fertilization – 1.201 mg g⁻¹.

The content of the carotenoids varied from 0.311 mg g⁻¹ and 0.323 mg g⁻¹ in the variants with phosphorus (P₁₂) and two-component phosphorus-potassium (P₁₂K₇) fertilization to 0.412 mg g⁻¹ and 0.371 mg g⁻¹, respectively, in nitrogen (N₅) and full mineral fertilization (N₅P₁₂K₇).

The variants with potassium (K₇) and phosphorus (P₁₂) fertilization had the lowest chlorophyll content in the last year of the 16th rotation of the LSFE, 0.774 mg g⁻¹ and 0.783 mg g⁻¹, respectively. The variant with only nitrogen fertilization (1.782 mg g⁻¹) had the highest chlorophyll content, followed by the one with combined nitrogen-potassium fertilization (N₅K₇) with 1.623 mg g⁻¹.

In terms of carotenoid content, the variants can be divided into two groups – with low and with high carotenoid content. The first group (0.271-0.304 mg g⁻¹) includes the variants with potassium (K₇) and combined phosphorus-potassium (P₁₂K₇) fertilization and the control (N₀P₀K₀), and the second (0.433-0.514 mg g⁻¹) includes all variants with nitrogen – alone or in combination with other macronutrients.

Table 5 presents the results of the statistical processing of the obtained data, averaged over the period of the 16th rotation of LSFE. It can be seen that the content of photosynthetic pigments between the individual fertilization options of field beans varies, within wider limits in terms of chlorophylls and within significantly narrower limits – in terms of carotenoids. The lowest amounts of chlorophyll a+b (Chl. a+b) were found in the variant with potassium fertilization (0.983 mg g⁻¹) and with two-component phosphorus-potassium fertilization (1.025 mg g⁻¹). In plants with nitrogen (N₅) and nitrogen-potassium (N₅K₇) fertilization, the highest chlorophyll content was recorded, 1.520 mg g⁻¹ and 1.465 mg g⁻¹, respectively.

Table 5. Content of photosynthetic pigments in field beans, 16th LSFE rotation

Variants	Chlorophyll a+b, mg g ⁻¹		Carotenoids, mg g ⁻¹		Chl. a/b	Chl. a+b/Car.
	$\bar{X} \pm S_x$	CV, %	$\bar{X} \pm S_x$	CV, %		
Control (N ₀ P ₀ K ₀)	1.183±0.014	24.38	0.385±0.0035	18.31	3.38	3.07
N ₅	1.520±0.013	17.58	0.473±0.0031	13.20	3.14	3.22
P ₁₂	1.048±0.012	22.34	0.348±0.0030	17.00	3.41	3.01
K ₇	0.983±0.009	18.18	0.333±0.0025	14.81	3.42	2.95
N ₅ P ₁₂	1.268±0.010	15.47	0.400±0.0030	15.00	3.12	3.17
N ₅ K ₇	1.465±0.015	19.98	0.443±0.0049	22.32	2.96	3.31
P ₁₂ K ₇	1.025±0.012	24.19	0.338±0.0028	16.83	3.02	3.04
N ₅ P ₁₂ K ₇	1.248±0.009	15.23	0.400±0.0025	12.42	3.09	3.12

Regarding carotenoids (Ccar.), the lowest content of this group of pigments, on average for the 16th rotation of the LSFE, was recorded in the variant with potassium fertilization (0.333 mg g⁻¹), followed by the variant with combined phosphorus-potassium (0.338 mg g⁻¹) and phosphorus fertilization (0.348 mg g⁻¹). In the variant with nitrogen fertilization (N₅), the highest content of carotenoids in the leaves of field beans was found – 0.473 mg g⁻¹.

The statistical processing of the data collected for the four-year period of the study established that the values of the coefficient of variation (CV %) in the different fertilization variants lies within wide margins for both groups of plastid pigments. Nevertheless, the analysis of the obtained results shows good statistical uniformity of the sample. The coefficient of variation of the data on the chlorophyll content

for different variants of fertilization of field beans ranges from 15.23% for full mineral fertilization (N₅P₁₂K₇) to 24.38% for the control (N₀P₀K₀).

The CV values of the data obtained when determining the content of yellow-red photosynthetic pigments in the leaves of field beans show a greater dispersion of the results, compared to that of chlorophylls. The lowest values were calculated for the variant with full mineral fertilization (12.42%), followed by that with nitrogen fertilization (13.20%). The largest deviations from the average values of the carotenoid content were observed for the variant with combined N₅K₇ fertilization (22.32%).

Values from 0.0025% for the determination of carotenoid content of the variants with potassium (K₇) and full mineral fertilization (N₅P₁₂K₇), to 0.0146% for the measurement of

chlorophyll content in the plants with nitrogen-potassium fertilization (N₅K₇), indicate a very high level of statistical reliability of the obtained results.

A number of scientific studies have reported a relationship between the ratio of chlorophyll *a* to chlorophyll *b* (Chl *a/b*) and chlorophyll *a+b* to carotenoids (Chl *a+b/Car*) and the physiological status of plants (Petkova & Poryazov, 2007). The normal ratio of relative amounts of chlorophylls *a* and *b* found in higher plants is about 3:1, although the preponderance of chlorophyll *a* is greater in those grown in bright conditions and less in those grown in shady sites (Gross, 1991; Andersen & Francis, 2009). A lower value of the green/yellow pigment ratio is associated with an increase in protection against chlorophyll photooxidation (Gilmore & Govindjee, 1999; Velinova & Glushkova, 2011).

So, it can be said that there was not observed significant deviations in the ratio of chlorophyll *a* to chlorophyll *b* in the different variants of fertilization of field beans. The lowest values (Chl.*a/b* = 2.96) were found in the variant with combined nitrogen-potassium (N₅K₇) fertilization, and the highest – in those with potassium (K₇) and phosphorus (P₁₂) fertilization, 3.42 and 3.41, respectively.

The ratio of the total amount of green to yellow plastid pigments lies in the range from 2.95 in the potassium (K₇) to 3.31 in the combined nitrogen-potassium (N₅K₇) fertilization. It is noteworthy that the variants with participation of the macroelements potassium and phosphorus had the highest ratio of chlorophyll *a/b* and the lowest of Chl.*a+b/Car.*, and the combination of nitrogen and potassium (N₅K₇) had the lowest ratio of chlorophyll *a/b* and the highest of Chl.*a+b/Car.*

CONCLUSIONS

The results obtained from the research show that, on average, for the period of the 16th rotation of LSFE, fertilization with the combination of the two macroelements (N) and (P) has a decisive influence on increasing the productivity of field beans. The fertilization with only phosphorus and potassium and the combination between them has no proven effect on the yield. The highest efficiency of field

beans fertilization expressed by the obtained additional yield (435 kg ha⁻¹) was achieved when fertilizing with N₅P₁₂.

The highest resistance to atmospheric drought was found in the variant with nitrogen fertilization (N₅), and the lowest – in the variant without fertilization (N₀P₀K₀). The highest vitality (resistance to soil drought) was found in the seeds of field beans obtained from the variants with two-component fertilization with nitrogen (N₅P₁₂ and N₅K₇) and with only phosphorus (P₁₂) fertilization. The seeds of the control (N₀P₀K₀) has shown the lowest vitality. The total content of chlorophylls (Chl.*a+b*) and carotenoids (Ccar.) in the leaves of the studied plants varies significantly between the different fertilization options and by year.

The lowest amounts of chlorophyll *a+b* (Chl.*a+b*) were found in the variant with potassium fertilization (K₇) – 0.983 mg g⁻¹ and with combined phosphorus-potassium (P₁₂K₇) fertilization – 1.025 mg g⁻¹. The highest chlorophyll content was recorded in plants with nitrogen (N₅) fertilization – 1.520 mg g⁻¹.

The lowest carotenoids content (Ccar.) on average for the 16th rotation of the LSFE was found in the variant with potassium fertilization (0.333 mg g⁻¹) and the highest – in the variant with nitrogen fertilization (N₅) – 0.473 mg g⁻¹.

In the variants with applied only potassium and only phosphorus fertilization was found the highest chlorophyll *a* to chlorophyll *b* (Chl.*a/b*) ratio and the lowest chlorophyll *a+b* to carotenoids (Chl.*a+b/Car.*) ratio.

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EFFECTS OF CULTIVAR, LOCATION, AND NITROGEN FERTILIZATION LEVEL ON MORPHOLOGICAL AND YIELD TRAITS OF SOYBEAN (*Glycine max* L.)

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Abstract

*The present study evaluated the effects of cultivar, location, and nitrogen fertilization level on the morphological and yield traits of soybean (*Glycine max* L.). Field experiments were conducted at four locations - Caracal, Crișcior, Jimbolia, and Grabat - under different pedoclimatic conditions. Two soybean cultivars, Amyata and Orakel PZO, were evaluated using three nitrogen fertilization levels (N75, N125, and N150) in a factorial experimental design. The analyzed traits included plant height, number of nodes, height of first pod insertion, number of pods per plant, thousand-seed weight, and hectoliter mass. The results revealed significant differences between cultivars for most of the analyzed traits, reflecting distinct genetic characteristics. Location significantly influenced all morphological and yield traits, highlighting the impact of environmental conditions on soybean development. Nitrogen fertilization level had a significant effect on plant architecture and seed physical characteristics, with variable responses depending on cultivar and location. Significant interactions among cultivar, location, and nitrogen fertilization level emphasize the importance of optimizing nitrogen management according to local conditions in order to improve soybean productivity and seed quality.*

Key words: location, morphological traits, nitrogen fertilization, soybean, yield traits.

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most important grain legumes worldwide due to its high protein and oil content, playing a key role in human nutrition, animal feed, and industrial applications. The increasing demand for soybean products, combined with the challenges posed by climate variability, has intensified the need for production systems that ensure both yield stability and seed quality under diverse environmental conditions (Ray et al., 2015; Smuleac et al., 2020; Cooper & Messina, 2023).

Among field crops, soybean is particularly sensitive to environmental factors, as its growth and yield formation depend strongly on temperature, water availability, and radiation during critical phenological stages. Several studies have shown that interannual climatic variability can induce substantial fluctuations in soybean yield and its components, often exceeding the effects of management practices or genetic differences (Hatfield et al., 2011; Jurcut et al., 2024). Extreme weather events,

such as droughts and heat waves, further exacerbate these effects by disrupting vegetative growth, flowering, and seed filling processes (Lesk et al., 2016).

Soybean yield is determined by the interaction between vegetative growth and reproductive development, with seed number and seed size acting as primary yield components. The balance between these components is highly plastic and responsive to environmental stress. Under favorable conditions, increased vegetative growth often supports a higher number of pods and seeds, whereas under stress conditions, soybean plants tend to reduce seed number while partially compensating through increased seed size (Egli et al., 2017). This compensatory mechanism represents an adaptive response that allows plants to optimize resource allocation under limiting conditions, but it may not fully offset yield losses when stress intensity is high. In addition to climatic variability, site-specific conditions such as soil properties, water-holding capacity, and local microclimate play a crucial role in shaping soybean performance (Setiyono et al., 2010). Spatial variability in yield and

quality parameters has been widely reported, emphasizing the importance of location as a determinant of crop response (Morrison et al., 2008). Differences in soil fertility and moisture availability can influence both vegetative architecture and reproductive success, thereby affecting not only yield but also harvest efficiency and seed quality (Wójcik-Gront et al., 2022).

Genetic factors further modulate soybean responses to environmental and management conditions (Smuleac et al., 2020). Cultivar selection is a key strategy for improving yield stability, particularly under variable or stress-prone environments. Modern breeding efforts increasingly focus on enhancing adaptability and resilience rather than maximizing yield under optimal conditions alone (Morrison et al., 2008; Cooper & Messina et al., 2023). Genotype $\times$ environment interactions have been shown to significantly affect soybean yield components and quality traits, highlighting the need to evaluate cultivars across multiple environments and years (Abebe et al., 2024).

Nitrogen nutrition represents another important aspect of soybean management. Unlike non-leguminous crops, soybean has the ability to meet a large proportion of its nitrogen demand through biological nitrogen fixation, reducing its dependence on mineral nitrogen fertilization. Numerous studies have reported inconsistent or limited yield responses to nitrogen application in soybean, particularly when nodulation and fixation processes are efficient (Salvagiotti et al., 2008; Maluk et al., 2023; Jurcut et al., 2025). However, nitrogen availability can still influence specific physiological processes, including seed composition, especially oil accumulation (Hungria et al., 2015).

Seed quality traits such as protein and oil content, thousand seed weight, and test weight are critical determinants of soybean market value and end-use suitability. These traits are influenced by both genetic and environmental factors, often exhibiting trade-offs that complicate management decisions. Environmental conditions during seed filling, in particular, have been shown to affect protein and oil concentration, sometimes independently of yield responses (Rotundo & Westgate, 2009; Bellaloui et al., 2009). Understanding how management and environmental factors interact

to shape both yield and quality is therefore essential for developing sustainable soybean production system. Despite extensive research on individual factors affecting soybean performance, there remains a need for integrated, multi-year and multi-location studies that simultaneously assess vegetative growth, yield components, and seed quality under contrasting environmental conditions. Such approaches are particularly valuable for disentangling the relative contributions of climate, location, genotype, and management practices to overall crop performance (Sitnicki et al., 2024; Chitu et al., 2024). Moreover, quantifying the relative contribution of these factors provides practical insights for prioritizing management interventions and breeding objectives.

In this context, the present study aimed to evaluate the combined effects of year, location, cultivar, and nitrogen fertilization on soybean vegetative traits, yield components, and seed quality parameters over multiple growing seasons. By integrating analysis of variance with an assessment of factor contributions and compensatory relationships between growth and yield components, this study seeks to clarify the mechanisms underlying soybean performance under variable environmental conditions. The results contribute to a better understanding of how adaptive strategies based on cultivar selection and site-specific management can enhance soybean productivity and quality in the face of increasing climatic variability.

MATERIALS AND METHODS

The objective of the study was to evaluate the effect of the variety, location and level of nitrogen fertilization on the morphological and yield traits of soybean (*Glycine max* L.). Field experiments were carried out in four locations - Caracal, Crișcior, Jimbolia and Grabat - under different pedoclimatic conditions. Two soybean varieties, Amyata and Orakel PZO, were evaluated using three levels of nitrogen fertilization (N75, N125 and N150) in a factorial experimental design.

The study was conducted between 2022 and 2025 in four representative locations for the main agricultural areas of Romania: Caracal (Olt County), Crișcior (Hunedoara County), Jimbolia and Grabat (Timiș County). These

locations were selected to capture a distinct pedoclimatic gradient, from lowland areas with frequent water deficit to hilly areas with better water resources.

The multiannual climate analysis (Table 1) indicates average annual temperatures between 10.9°C (Crișcior) and 13.7°C (Caracal).

Table 1. Multiannual climate characterization of experimental sites (2022-2025)

Location	Average annual temperature (°C)	Average annual rainfall (mm)	Average rainfall during the growing season (Apr.-Sept.) (mm)	The difference in temperature during the growing season compared to the annual average (°C)
Caracal	13.7	492	301	+6.8
Crișcior	10.9	572	356	+6.1
Jimbolia	12.2	447	273	+6.3
Grabaț	12.3	482	296	+6.5

Note: The values represent multi-year averages calculated for the period 2022–2025. The temperature difference expresses the increase in the average temperature during the growing season (April–September) compared to the annual average, indicating the thermal favorability for the soybean crop.

During the growing season of the soybean crop (April–September), the average temperatures exceeded the annual average by 6.1–6.8°C, confirming a favorable thermal regime for growth and development processes. The average annual rainfall varied between 447 mm (Jimbolia) and 572 mm (Crișcior), and those in the vegetation period between 273 mm and

356 mm, highlighting a different water availability between locations. From a pedological point of view (Table 2), the soils are predominantly represented by chernozems and cambic chernozems at Caracal, Jimbolia and Grabaț, characterized by good natural fertility, and by brown clay-alluvial soil at Crișcior, with medium fertility and lower phosphorus content.

Table 2. Synthesis of the main agrochemical indicators of soils in experimental sites

Location	Type of soil	pH (H ₂ O)	Humus (%)	Total N (%)	Mobile P (ppm)	Mobile K (ppm)
Caracal	Chernozems	7.6	2.58	0.189	93	235
Crișcior	brown clay-alluvial soil	6.2	2.51	0.15	33	166
Jimbolia	Chernozems	7.81	4.26	0.27	111.5	192.9
Grabaț	Cambic chernozems	7.5	3.13	0.19	62.3	241.4

Note: Agrochemical analyses were performed in the 0-20 cm layer, before the installation of the experiment.

This pedoclimatic diversity allowed the evaluation of the behavior of the soybean crop in contrasting agroecological conditions and the formulation of conclusions with regional applicability.

RESULTS AND DISCUSSIONS

Analysis of variance for morphological traits

The table synthetically highlights that year was the dominant factor, significantly affecting all analyzed traits (Table 3). Location exerted a strong influence on reproductive and architectural traits, while cultivar showed selective significance.

Table 3. Significance of experimental factors (ANOVA) on soybean morphological traits

Trait	Year (A)	Location (B)	Cultivar (C)	Fertilization (D)
Plant height	***	ns	ns	ns
Pods per plant	***	***	*	ns
Fertile nodes	***	**	ns	ns
First pod height	***	***	**	ns

ns - not significant (p > 0.05); *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

Nitrogen fertilization had no statistically significant effect on the analyzed morphological parameters. Location exerted a strong influence on reproductive and architectural traits, while

cultivar showed selective significance. Nitrogen fertilization had no statistically significant effect on the analyzed morphological parameters.

Effect of year on soybean morphology

The results regarding the effect of the experimental year on soybean morphology are presented in Table 4.

Table 4. Mean values of morphological traits as affected by year

Year	Plant height (cm)	Pods per plant	Fertile nodes	First pod height (cm)
2023	82.38 a	42 a	11 a	14.71 a
2024	82.17 a	40 a	3 c	11.52 b
2025	47.32 b	17 b	8 b	4.22 c

Means followed by different letters differ significantly (Duncan test, p ≤ 0.05).

The drastic reduction observed in 2025 across all traits indicates a strong climatic stress, most likely drought and high temperatures during vegetative and reproductive stages. Similar values recorded in 2023 and 2024 suggest favorable and stable environmental conditions during these growing seasons.

Effect of location

The results regarding the effect of the experimentation site on soybean morphology are presented in Table 5.

Table 5. Mean values of morphological traits as affected by location

Location	Plant height (cm)	Pods per plant	Fertile nodes	First pod height (cm)
Caracal	67.97 a	37 a	6 c	8.64 b
Crișcior	74.18 a	26 b	9 a	12.29 a
Jimbolia	69.71 a	35 a	7 bc	11.26 a
Grabat	70.64 a	33 a	8 ab	8.41 b

Locations with more favorable soil and moisture conditions (Crișcior and Jimbolia) promoted higher insertion of the first pod and increased number of fertile nodes.

Differences among locations confirm the importance of site-specific adaptation in soybean cultivation.

Effect of cultivar

The results regarding the effect of the cultivar on soybean morphology are presented in Table 6.

Table 6. Mean values of morphological traits as affected by cultivar

Cultivar	Plant height (cm)	Pods per plant	Fertile nodes	First pod height (cm)
Amiata	68.88 a	31 b	7 a	9.01 b
Orakel	72.36 a	35 a	8 a	11.29 a

The cultivar Orakel PZO showed superior performance for pods per plant and first pod height, traits directly related to yield potential and harvest efficiency. These results highlight the genetic contribution to yield-forming traits under variable environmental conditions.

Effect of nitrogen fertilization

The results regarding the effect of the nitrogen fertilization on soybean morphology are presented in Table 7.

Table 7. Mean values of morphological traits as affected by nitrogen fertilization

Fertilization	Plant height (cm)	Pods per plant	Fertile nodes	First pod height (cm)
N75	69.77 a	30 b	7 a	10.90 a
N125	70.63 a	33 ab	8 a	9.88 a
N150	71.48 a	35 a	8 a	9.67 a

Although numerical increases were observed with higher nitrogen doses, the lack of statistical significance suggests that nitrogen was not the limiting factor under the experimental conditions, in agreement with soybean’s biological nitrogen fixation capacity.

Thousand-seed weight (TSW) and hectoliter mass

Thousand-seed weight (Table 8) was significantly affected by location, cultivar, and nitrogen fertilization level, highlighting the combined influence of pedoclimatic conditions and technological inputs on seed development. Among locations, the highest thousand-seed weight values were recorded at Jimbolia (149.7 g) and Grabaț (148.1 g), while lower values were observed at Caracal (142.4 g) and Crișcior (151.9 g), with statistically significant differences among sites. These results reflect the more favorable pedoclimatic conditions in the Banat Plain, particularly improved water availability during the seed-filling period, which enhances assimilate accumulation in seeds.

Table 8. Thousand-seed weight (g) as affected by experimental factors

Factor	Level	Mean $\pm$ SE
Location	Caracal	142.4 $\pm$ 3.06 b
	Crușcior	151.9 $\pm$ 2.77 a
	Jimbolia	149.7 $\pm$ 1.84 a
	Grabat	148.1 $\pm$ 2.31 ab
Cultivar	Amiata	146.7 $\pm$ 1.86
	Orakel	149.4 $\pm$ 2.07
Nitrogen Level	N75	144.9 $\pm$ 2.44
	N125	148.2 $\pm$ 2.49
	N150	151.0 $\pm$ 2.07

Regarding the genetic factor, Amyata (146.7 g) showed a significantly higher thousand-seed weight compared with Cypress (149.4 g), indicating cultivar-specific differences in seed filling capacity and reserve accumulation.

Nitrogen fertilization had a positive effect on thousand-seed weight, with values increasing as nitrogen dose increased.

The highest value was obtained at N150 (151.0 g), significantly exceeding those recorded at N75 (144.9 g) and N125 (148.2 g).

This response highlights the role of nitrogen in supporting photosynthetic activity and assimilate translocation during the seed-filling stage. Among locations, the highest hectoliter weight values were recorded at Jimbolia (71.9 kg hL⁻¹) and Grabaț (71.8 kg hL⁻¹), significantly higher than those observed at Caracal (70.3 kg hL⁻¹) and Crișcior (70.9 kg hL⁻¹). These results are associated with more balanced climatic conditions in western Romania, which favor the formation of well-developed and uniform seeds. Hectoliter weight, an important indicator of physical seed quality, was also significantly influenced by all experimental factors (Table 9). Cultivar significantly affected hectoliter weight, with Cypress (71.5 kg hL⁻¹) showing higher values compared with Amyata (70.8 kg hL⁻¹),

suggesting a higher seed density and more compact internal structure.

Nitrogen fertilization had a smaller but consistent effect on hectoliter weight. The highest values were recorded at N125 (71.3 kg·hL⁻¹) and N150 (71.3 kg·hL⁻¹), whereas the lowest value was observed at N75 (70.7 kg·hL⁻¹).

This trend indicates that adequate nitrogen nutrition contributes to improved seed uniformity and density.

Table 9. Hectoliter mass (kg·hL⁻¹) as affected by experimental factors

Factor	Level	Mean $\pm$ SE
Location	Caracal	70.28 $\pm$ 0.16 c
	Crușcior	70.92 $\pm$ 0.19 bc
	Jimbolia	71.81 $\pm$ 0.22 a
	Grabat	71.46 $\pm$ 0.45 ab
Cultivar	Amiata	70.76 $\pm$ 0.22 b
	Orakel	71.48 $\pm$ 0.25 a
Nitrogen Level	N75	70.74 $\pm$ 0.22
	N125	71.28 $\pm$ 0.29
	N150	71.34 $\pm$ 0.39

Relationship between vegetative growth and yield components

A comparative analysis of morphological data (Table 10) shows that the reduction in plant size is not always associated with proportional losses in berry quality. For example, the year 2025, although characterized by shorter plants and a reduced number of pods, recorded the highest values for the mass of 1000 grains (MMB) and hectoliter mass (MH).

This compensation suggests a redistribution of assimilates to a smaller number of grains, a phenomenon frequently reported in soybeans under moderate-severe stress conditions. From an agronomic point of view, this mechanism explains why production decreases are not always proportional to the decrease in vegetative vigor.

Table 10. Integrated relationship between vegetative growth traits and yield components (yearly means)

Year	Plant height (cm)	Fertile nodes (no.)	Pods per plant (no.)	TSW (g)	Test weight (kg·hL ⁻¹)
2023	82.38 a	11 a	42 a	140.4 b	70.45 b
2024	82.17 a	3 c	40 a	140.2 b	70.70 b
2025	47.32 b	8 b	17 b	163.6 a	72.20 a

Different letters indicate significant differences (Duncan test, $p \leq 0.05$).

Contribution of experimental factors to trait variability

The results regarding the contribution of experimental factors (Table 11) confirm that the year of cultivation (factor A) had the greatest contribution to the variation of all the

parameters analyzed, both morphological and quality. For traits related to vegetative growth (plant height, number of pods, number of floors), the year's input frequently exceeded 60-70% of the total variation, indicating a major dependence on interannual climatic conditions.

Table 11. Contribution (%) of experimental factors to the total variance of analyzed traits

Trait	Year (A)	Location (B)	Cultivar (C)	Fertilization (D)	Error
Plant height	~76	~1	~1	<1	~22
Pods per plant	~63	~8	~2	~2	~25
Fertile nodes	~52	~9	~2	~1	~36
First pod height	~60	~9	~4	~1	~26
Thousand seed weight	~53	~6	~1	~3	~37
Test weight	~31	~18	~7	~4	~40

Values derived from sum of squares distribution; percentages rounded for clarity.

The location (factor B) had a secondary but constant contribution, especially for the characteristics of agronomic and technological relevance, such as the height of the first pod, the hectoliter mass and the moisture of the grains.

This contribution reflects the influence of soil conditions and the local microclimate on the stability of the crop.

The variety (factor C) had a relatively low contribution to most of the traits, but its contribution was evident for the parameters associated with grain quality and mechanized harvesting (MH, height of the first pod), confirming the genetic role in optimizing these traits.

Nitrogen fertilization (factor D) showed a limited contribution for most morphological traits. From a practical point of view, the analysis of the contribution of factors demonstrates that the management of the soybean crop must be adapted as a priority to climatic and local conditions, and technological interventions (fertilization) have a complementary role.

The choice of variety becomes essential in particular to reduce the technological risks associated with harvesting and to stabilize the quality of production.

CONCLUSIONS

The multi-year, multi-location analysis demonstrated that environmental conditions were the primary drivers of soybean performance, exerting a dominant influence on

both vegetative growth and seed quality traits. Among the experimental factors, year of cultivation accounted for the largest proportion of total variability, highlighting the strong dependence of soybean development on interannual climatic conditions.

Vegetative growth parameters, including plant height, number of fertile nodes, pod number, and first pod height, were particularly sensitive to climatic stress.

Years characterized by reduced vegetative vigor resulted in pronounced decreases in pod number and first pod height, increasing the risk of harvest losses.

However, these unfavorable conditions also triggered a compensatory response, leading to increased thousand seed weight and test weight, which partially mitigated yield reductions.

Location effects were consistent but secondary to year effects, indicating that site-specific pedoclimatic conditions contribute to stabilizing yield components and seed quality. Locations with more favorable soil and moisture regimes supported improved plant architecture and better technological traits, especially thousand seed weight and test weight.

Cultivar influence was selective but agronomically relevant. The cultivar *Orakel* showed superior performance for traits directly related to harvest efficiency and yield formation, particularly pod number and first pod height, confirming the importance of cultivar selection under variable environmental conditions.

Nitrogen fertilization had a limited impact on vegetative traits, reflecting the capacity of

soybean to rely on biological nitrogen fixation. Its effect was mainly expressed through oil accumulation, suggesting that nitrogen management should be adapted to quality objectives rather than vegetative growth enhancement.

The results emphasize that adaptive strategies in soybean cultivation should prioritize cultivar choice and site-specific management, while acknowledging that climatic variability remains the main limiting factor.

Integrating environmental adaptation with optimized genetic selection represents a sustainable approach to improving soybean productivity and seed quality under increasingly variable climatic conditions.

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IDENTIFICATION OF POTENTIAL PARENTS AND PROMISING CROSSES BASED ON COMBINING EFFECTS FOR IMPROVING PRODUCTIVITY AND FIBER QUALITY IN COTTON

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Abstract

The object of this study was 59 F₁ hybrid populations obtained from three schemes of line × tester crosses. The aim was to find the most promising F₁ crosses for improving productivity, lint percentage and fibre length of new cotton varieties. In 2022 and 2023 the crosses and their parents were included in three trials carried out by the block method design in three replicates. The highest values of total GCA effects of the two parents for each trait were taken as “ideal” and the Euclidean distances between them and the GCA effects of both parents were calculated for each F₁ cross. The crosses with the shortest Euclidean distances were considered as the most reliable for the selection. Some crosses were found to exhibit the highest possible GCAs for the three studied traits and appeared to be the most valuable for the breeding programs. There were identified good general combiners for the three traits under study. Some cross combinations showed high and positive SCA effects and heterosis manifestations. Heterobeltiosis was also registered for the three traits most strongly expressed in productivity per plant.

Key words: cotton, line × tester crosses, combining ability, Euclidean distance.

INTRODUCTION

Cotton breeding in Bulgaria is aimed at increasing seed cotton yield and lint yield, by increasing lint percentage and improving fiber quality, especially its length. According to Chapara and Madugula (2021) the creation of a new cultivar having high yield and high fiber quality is of utmost importance for all cotton breeders.

In our country considerable selection work has been carried out to increase yield and improve fiber quality and relatively good results have been achieved (Dimitrova et al., 2022).

Intra- and interspecific hybridization and experimental mutagenesis are the main breeding methods. A number of new varieties and promising lines have been created, which are valuable starting material for further selection work with cotton. According to Ahsan et al. (2015) the quantity and quality of achievable genetic variability provides sufficient opportunities to upgrade and develop breeding activity into a successful breeding program.

In our country, in cotton breeding the latest achievements are the varieties Tsvetelina, Pirin, Perun (Koleva, Valkova, 2019; 2023), Aida,

Anabel, Tiara, Melani (Dimitrova, 2022a, 2022b, 2023) and Selena (Dimitrova & Nedyalkova, 2024). Pirin, Perun and Anabel were obtained from intraspecific crosses, Aida, Tiara, Melani and Selena were created by remote hybridization and backcrossing. These new varieties are very early. Tiara, Melani and Selena have improved fiber quality, while the others have improved productivity combined with other valuable qualities.

Many authors (Mahmood et al., 2020; Ullah et al., 2020, Sarwar et al., 2021) by applying cluster or PC analyses studied genetic diversity to select the superior genotypes for the future breeding programs as well as the genetic distance with the aim of including in one breeding program genetically dissimilar genotypes to increase genetic diversity or genetically similar to achieve greater breeding progress.

Combining ability and potential of parents involved in crosses are essential to the success of breeding programs. Zhang et al. (2016) concluded that the ability to combine yield with fiber quality in F₁ interspecific *G. hirsutum* L. × *G. barbadense* L. crosses depends on the genetic potential and combining ability of parents.

According to Sivia et al. (2017), Nimbal et al. (2019) combining ability analysis plays an important role in selection of parents to obtained more desirable segregants.

The general combining ability (GCA) of genotypes is of greater importance for synthetic selection (for hybridization and selection programs), while the specific combining ability (SCA) is of greater importance for heterosis selection (for hybrid crop exploitation) (Sajjad et al., 2016).

It is known that the general combining ability is closely related to the additive action of genes and that genotypes with high GCA have more additive genes, while high specific combining ability is determined by the non-additive action of genes - dominance and epistasis. For the selection of cotton in our country the GCA is more important with a view to creating synthetic varieties.

Many authors have reported that additive and non-additive gene effects were important for the inheritance of traits in cotton. Some researchers have pointed greater importance of non-additive gene effects, others of additive gene effects.

Non-additive gene action for most of traits was reported by Ali et al (2016a; 2016b), Karademir et al. (2016), Sajjad et al. (2016), Sivia et al. (2017), Ali et al. (2018), Munir et al. (2018). Additive gene effects were predominant in the inheritance of seed cotton yield, boll weight and lint percentage in studies of Vasconcelos et al. (2018) in *G. hirsutum* diallel crosses, for fiber length in studies of Carvalho et al. (2018), Ekinci (2018) in *G. hirsutum* L. $\times$ *G. barbadense* crosses.

Line $\times$ tester crossing analysis gives very useful information about both maternal and paternal forms (Dabholcar, 1992). According to Sivia et al. (2017) this method is one of the simplest and efficient methods of evaluating large number of inbreds for their combining ability.

In practice, in the synthetic selection (hybridization and selection programs) parents having high GCA for one, two or more traits are wanted, such with high GCA for more traits being more desirable. The importance of individual parental forms with high general combining ability for one or more traits is usually emphasized. Barut et al. (2000) applied selection of parents with the highest possible GCA for all studied traits. The GCA effects of

both parents, for each trait and for each combination, were pooled and ranked and the highest value for each trait was taken as “ideal”. The “ideal” values for each trait were considered to characterize the “ideal” population, and the Euclidean distance between the common GCA effects of both parents and the “ideal” population values for each cross was calculated.

In the selection of cotton in our country this approach for selection of parents with the highest possible GCA for all studied traits was applied by Stoilova (2004).

The aim of this study was to identify promising F_1 crosses of greatest selection value for productivity per plant, lint percentage and fibre length, by applying the indicated approach to select parents with as high as possible GCA for the three traits.

MATERIALS AND METHODS

In 2022-2023 the hybrid populations derived from three line $\times$ tester crosses design mating systems, by applying the experimental method I (without reciprocals) of Savchenko (1984) were studied. In the first crossing scheme (5×3) five advanced lines - 639, 662, 678 (Selena variety), 681 and 682 (Bulgarian selection) used as female parents were crossed with one Bulgarian variety - Sirius and two foreign cultivars - Nazili 954 (Turkish) and FR-H-1002 (Spanish) used as male parents to produced 15 hybrid combinations. In the second scheme of crossing (5×4) the same females were crossed with four other males - line 489, Anabel (line 535), Tiara (line 550) and Melani (line 553) varieties (Bulgarian selection) to produced 20 hybrid combinations. In the third crossing scheme (6×4) Kristal, Perun, Pirin, Tiara, Melani and Anabel varieties (Bulgarian selection), used as female parents, were crossed with Aida (Bulgarian variety) and the foreign cultivars Nazili 954 (Turkish), FR-H-1001 (Spanish) and Bridget (Greek), used as male parents, to produced 24 hybrid combinations.

The trials, two in 2022 and one in 2023, were carried out at the Field Crops Institute in Chirpan, in randomized complete block design with three replications. Each plot with parents and hybrids consisted of two rows 2.4 m long with a distance of 0.60 m between rows and 0.20 m within row. Traditional practices for

cotton growing in our country were applied during the growing season. The traits under study were productivity per plant, lint percentage and mean fiber length. For each genotype ten plants from each replication were observed.

Methodology of Savchenko (1984) was applied to evaluate the general combining ability (GCA) and specific combining ability (SCA). The hybrid sum of squares was subdivided into variation due to females, males and the female $\times$ male interaction. The main effects of females and males are equivalent to GCA, and the female $\times$ male interaction represents SCA (Hallauer and Miranda, 1981).

The common GCA effects of both parents for each trait and each combination are presented in Tables 6 and 7. The highest values were taken as “ideal” (Barut et al., 2000) and the Euclidean distances (Manly, 1995) between them and the total GCA effects of both parents for each F_1 cross were calculated. The smaller are the distances to the ideal population, the more desirable are the crosses.

Euclidean distances were determined by the formula:

$$\text{Squared Euclidean distance} = \sum (x_i - x_j)^2$$

where: x_i – the “ideal” value; x_j – the common of both parents. Data were pre-standardized.

In terms of temperature sum in May-September (the growing season of cotton), 2022 was moderately warm ($P = 34.37\%$) and 2023 was warm ($P = 15.5\%$). The temperature sums during the period April-September (cotton growing season) were 113°C and 145°C , respectively for 2022 and 2023, higher than the multi-year average values (data are not given here). In terms of rainfall in May-August both years were average ($P = 46.8\%$ and 51.5%). The shortage of rainfall in the months of July-August (period of flowering and fruiting) was -14.6 mm in 2022 and -39.2 mm in 2023, which combined with higher temperatures $+77^\circ\text{C}$ and $+150^\circ\text{C}$, respectively for the two years, adversely affected the formation and retention of fruit elements and reflected on the seed cotton yield and fiber quality.

$P\%$ is the coverage factor (coefficient of security) for the temperature sum in May-September and for the rainfall in May-August.

RESULTS AND DISCUSSIONS

Results of combining ability variance analysis are given in Table 1.

In 2022, in the first crossing scheme (trial I), for the three studied traits, the effects of crosses, GCA effects of females and SCA effects of crosses were significant. GCA effects of males were significant for productivity per plant and fiber length, and not significant for lint percentage. Therefore, lines used as females differed in GCA for the three traits, while the cultivars used as males differed in GCA for productivity per plant and fiber length, and did not differ in lint percentage. The crosses differed in SCA for the three traits. In the second crossing scheme (trial II), the effect of crosses, GCA of females and SCA of crosses were also significant for the three traits. GCA effects of males were significant for productivity per plant and lint percentage and insignificant for fiber length. Lines used as females differed in GCA for the three traits, while the males differed in GCA for productivity per plant and for lint percentage and did not differ in GCA for fiber length. Crosses differed in SCA for the three traits.

It can be seen from the Table 1 that in 2022 in the two crossing schemes (trial I and trial II), effects of crosses, GCA effects of females and SCA effects of crosses were significant for the three traits. GCA effects of males for productivity per plant were also significant. GCA effects for lint percentage in crosses included in trial I and GCA effects for fiber length in crosses included in trial II were non-significant. Therefore, the lines used as mothers differed in GCA for the three studied traits in both crossing schemes, while the cultivars used as males, in crosses included in trial I differed in GCA for productivity per plant and for fiber length and did not differ for lint percentage.

In trial II, male parents differed in GCA for productivity per plant and lint percentage and did not differ for fiber length. The crosses in the two crossing schemes differed in SCA for the three traits.

In 2023, in the third crossing scheme, effects of crosses were significant at $P \leq 0.001$ for productivity per plant and lint percentage, and significant at $P \leq 0.05$ for fiber length. GCA effects of females and males were significant for

productivity per plant and lint percentage and non-significant for fiber length, meaning that both females and males did not differ in GCA

for this trait. SCA effects of crosses were significant for the three traits.

Table 1. Analysis of combining ability variance for productivity per plant, lint percentage and fiber length in 5×3 , 5×4 and 6×4 line by tester F_1 crosses

Trial	Sources on variation	Degrees of freedom	Productivity per plant	Mean squares Lint percentage	Fiber length
Trial I - 2022	Crosses	14	91,868*** F_{exp} , 61,545	31,413*** F_{exp} , 80,850	3,591** F_{exp} , 14,988
	Errors	28	1,493	0,388	0,239
	GCA-females	4	5,183** F_{exp} , 10,416	33,281** F_{exp} , 256,971	1,370** F_{exp} , 17,150
	GCA - males	2	5,107** F_{exp} , 10,265	0,197ns F_{exp} , 1,523	1,265** F_{exp} , 15,835
	SCA-females $\times$ males	8	49,724** F_{exp} , 99,934	1,633** F_{exp} , 12,613	1,094** F_{exp} , 13,700
	Errors	28	0,497	0,129	0,080
Trial II - 2022	Crosses	19	89,059*** F_{exp} , 47,105	39,406*** F_{exp} , 110,892	2,076*** F_{exp} , 8,116
	Errors	38	1,890	0,333	0,256
	GCA-females	4	11,164** F_{exp} , 17,662	47,174** F_{exp} , 425,235	1,587** F_{exp} , 18,612
	GCA - males	3	27,00** F_{exp} , 42,716	3,471** F_{exp} , 31,285	0,174ns F_{exp} , 2,042
	SCA-females $\times$ males	12	36,531** F_{exp} , 57,796	2,888** F_{exp} , 26,031	0,523** F_{exp} , 6,133
	Errors	38	0,632	0,111	0,085
Trial III - 2023	Crosses	23	256,342*** F_{exp} , 6,133	5,961*** F_{exp} , 10,196	0,442* F_{exp} , 2,339
	Errors	46	1,090	0,584	0,189
	GCA-females	5	124,33** F_{exp} , 227,982	2,041** F_{exp} , 10,475	0,064ns F_{exp} , 1,026
	GCA - males	3	116,799** F_{exp} , 214,171	6,258** F_{exp} , 32,111	0,052ns F_{exp} , 0,825
	SCA-females $\times$ males	15	66,216** F_{exp} , 121,417	1,116** F_{exp} , 5,725	0,193** F_{exp} , 3,063
	Errors	46	0,545	0,195	0,63

* - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$; n.s. - no significant

Estimates of GCA effects for female and male parents are given in Table 2.

In 2022, of the females, line 622 had the highest and significant positive GCA for productivity per plant in both trials (I and II), while line 682 had significant positive GCA in trial I and significant negative GCA in trial II. Of the males, in trial I, the Turkish variety Nazili 954 had positive and significant GCA for productivity per plant.

The Spanish cultivar FR-H-1002 had positive but insignificant GCA. In trial II, from the male parents, Anabel (line 535) and Melani (line 553) varieties had high and significant GCA for productivity per plant. Melani variety (line 553) had the highest productivity among males. Line 681 of females in both trials and Sirius variety

of males in trial I having the highest productivity per plant exhibited significant negative GCA.

In crosses tested in 2023, from the females, the highest positive GCA for productivity per plant was found for Kristal variety, followed by Tiara (line 550) variety. Pirin variety showed the highest productivity per plant had negative GCA for this trait. Of the males, the Spanish cultivar FR-H-1001 had the highest GCA, followed by the Turkish cultivar Nazili 954. Anabel (line 535), Tiara (line 550) and Melani (line 553) varieties used as males in the crosses tested in 2022 in trial II, and used as females in the crosses tested in 2023, demonstrated different combining ability. Probably the year conditions were also essential for the combining ability manifestation of genotypes.

Table 2. Evaluation of GCA effects for productivity per plant, lint percentage and fiber length in 5×3 , 5×4 and 6×4 line by tester F_1 crosses

Trials		Parents	Productivity per plant		Lint percentage		Fiber length	
			g	GCA	%	GCA	mm	GCA
Trial I 2022	Females	639	43.3	0.203	40.9	1.465	24.5	0.135
		662	44.0	1.525	42.1	3.627	25.4	-0.875
		678	48.0	-1.280	40.3	1.988	26.5	-0.509
		681	52.2	-1.408	35.7	-3.712	26.0	0.591
		682	40.4	0.959	34.7	-3.368	26.9	0.658
	Standard error			0.705		0.360		0.283
	Males	Sirius	56.7	-1.110	39.3	0.135	25.2	-0.313
		Nazili 954	45.8	0.867	41.8	-0.228	25.6	-0.267
		FR-H-1002	34.0	0.243	40.4	0.092	26.9	0.580
		Standard error			0.546		0.279	
Trial II 2022	Females	639	47.3	-0.878	41.6	2.210	25.5	-0.211
		662	48.6	2.947	41.9	2.294	24.7	-0.111
		678	52.6	-0.528	42.0	2.960	25.6	-0.836
		681	52.4	-0.420	37.6	-4.181	27.3	0.297
		682	47.8	-1.120	35.8	-3.283	26.8	0.962
	Standard error			0.687		0.288		0.253
	Males	489	47.7	-1.713	41.9	0.724	25.8	0.020
		Anabel (535)	41.4	2.133	39.2	-0.339	25.1	0.194
		Tiara (550)	46.5	-2.287	41.2	0.635	25.3	-0.255
		Melani (553)	50.0	1.866	40.3	-1.019	25.5	0.040
Standard error			0.615		0.256		0.226	
Trial III 2023	Females	Kristal	29.1	10.807	38.6	-0.042	25.7	-0.215
		Perun	33.9	-2.189	38.2	0.708	25.1	0.031
		Pirin	47.8	-2.743	38.8	0.421	24.8	-0.006
		Tiara (550)	37.8	1.111	40.2	0.412	25.2	0.056
		Melani (553)	34.0	-2.914	39.5	-0.212	25.7	-0.039
		Anabel (535)	27.8	-4.072	38.9	-1.287	25.9	0.173
		Standard error			0.639		0.381	
	Males	Aida	27.6	-2.761	41.8	-0.569	25.9	-0.037
		Nazili 954	25.8	1.105	40.3	-0.778	25.9	0.130
		FR-H-1001	32.8	5.736	39.6	-0.131	26.4	-0.003
		Bridget	22.6	-4.081	43.2	1.478	26.5	-0.090
		Standard error			0.522		0.312	

The foreign cultivars, the Turkish Nazili 954 and the Spanish FR-H-1001 demonstrated very good GCA with the Bulgarian varieties. Both cultivars are highly productive, but under our conditions, in some years they are late in maturing and seed cotton yields are uncertain. SCA effects of crosses for the three traits are presented in Tables 3-5. In 2022, in trial I, 7 crosses exhibited positive and significant SCA effects for productivity per plant. The crosses 681× Nazili 954, 662 × Nazili 954, and 682 × FR-H-1002 were found to have high SCA effects and highest productivity per plant from 52.4 g to 56.9 g. The specified cross combinations manifested mid parent heterosis from 116.1% to 140.9% and heterobeltiosis from 109.0% to 129.7%. Lines 662 and 682 from the females, showed high GCA for this trait, had low SCA variances, while the cultivar

Nazili 954 from the males, showed high GCA, had high SCA variances, which should be taken into account in the selection strategy.

In trial II, 10 crosses showed positive and significant SCA effects. In these crosses the productivity per plant varied from 40.0 g to 59.2 g and mid parent heterosis ranged from 102.1% to 131.2%. Heterobeltiosis was registered in 6 crosses from 101.3% to 122.4%. Crosses 662 × Tiara and 682 × Anabel showed the highest productivity per plant (59.2 g and 58.5 g) and manifested the highest heterobeltiosis (121.8% and 122.4%). Line 662 from the females with high GCA demonstrated high SCA variances, while Anabel and Melani varieties from the males also with high GCA had respectively high and low SCA variances.

In 2023, the productivity per plant was lower compared to 2022, except for the cross

combination Kristal $\times$ FR-H-1001. Seven crosses were found to have positive and significant SCA effects for productivity per plant. In these crosses mid parent heterosis was 109.5-227.0%. Only three crosses manifested heterobeltiosis from 112.9% to 214.0%. Tiara variety (line 550) from the females and the cultivar Nazili 954 from the males, with high GCA effects, had low variances of SCA and are very suitable for synthetic (pedigree) selection. Kristal variety from the females and the cultivar FR-H-1001 from the males with high GCA had high SCA variances and are more suitable for heterosis selection.

Patel et al. (2014) in interspecific *G. hirsutum* $\times$ *G. barbadense* hybridization reported heterobeltiosis (better parent heterosis) up to 35.49% for seed cotton yield. In this hybridization positive mid parent heterosis for this trait was reported by Adzare et al. (2017), Rajeev et al. (2018).

In 2022, 5 crosses in trial I and 7 crosses in trial II demonstrated positive and significant SCA effects for lint percentage. In these crosses, lint percentage ranged from 39.2% to 41.9%. Mid parent heterosis from 102.0 to 106.4% was registered in 9 crosses and heterobeltiosis from 101.4 to 105.9% was found in 6 crosses. Lines 639 and 678 from females in trial I and trial II, and line 489 and Tiara variety (line 550) from males in trial II, with high GCA, had low SCA variances. Line 662 from the females having high GCA demonstrated non-constant SCA variances, high in trial I and low in trial II.

In 2023, only three crosses demonstrated high and significant SCA effects for lint percentage. In these crosses, mid parent heterosis was 102.0-108.7%, while heterobeltiosis 101.1-107.6% was registered in only two crosses. Pirin and Perun varieties from the females, and the cultivar Bridget from the males, with high GCA, had low SCA variances and can be used in synthetic (pedigree) selection. Tiara variety (line 550) displayed high SCA variances. This variety and the Turkish cultivar Nazili 954 demonstrated non-constant SCA variances.

Line 622 with high GCA for productivity/plant and lint percentage showed inconsistent SCA variances for both traits. Tiara variety, used as female parent (in 2023) and male parent (in trial II, 2022), having high GCA in both years, also had inconsistent SCA variances for both traits.

The results for mid parent heterosis for this trait 102.0-108.7% (in 12 crosses) and heterobeltiosis 101.1-107.6% (in 8 crosses) are comparable to those reported by other authors. Karademir & Gençer (2010) reported heterosis of 5.52% in only one cross among studied diallel crosses.

Regarding fiber length, in 2022, 5 crosses in trial I and 3 crosses in trial II showed high SCA effects. In these crosses, fiber length varied from 26.0 mm to 28.3 mm. In all crosses, except one, mid parent heterosis was accounted for from 102.0% to 108.3% and heterobeltiosis was registered from 101.5% to 107.3%. The longest fibers 28.3 mm and 28.1 mm and pronounced heterobeltiosis of 105.2% and 107.3% were found in the crosses 681 $\times$ FR-H-1002 (trial I) and 682 $\times$ Anabel (line 535) (trial II). Line 681 of females, showed high GCA had low SCA variances in both trials, while line 682, also with high GCA for this trait, demonstrated inconsistent low and high SCA variances, respectively.

In 2023, only three crosses displayed significant positive SCA effects for fiber length and manifested mid parent heterosis of 103.1-108.3% and heterobeltiosis of 101.9-105.8%.

Positive heterosis for fiber length was reported by Bölek et al. (2014), Roy et al. (2018) in *G. hirsutum* $\times$ *G. barbadense* hybridization. In this hybridization Çoban et al. (2015) reported that heterosis values for this trait were positive and ranged between 2.05% to 16.9%. Çoban and Ünay (2017) reported mid parent heterosis for fiber length in all cross combinations. Ünay et al. (2018) among the studied *G. hirsutum* $\times$ *G. barbadense* crosses found that heterosis (Ht) values for fiber length varied from -1.77 to 3.81%, the heterobeltiosis (Hb) values varied from -7.87% to -0.03%. Chapara & Madugula (2021) reported heterosis for Upper Half Mean Length (UHML) and mean length in intraspecific line $\times$ tester crosses and the highest values were 21.34% and 25.37%, heterobeltiosis for UHML ranged between 7.53 to 20.73%. In our study heterosis values for mean fiber length were in agreement with those reported in the literature or lower, mid parent heterosis varied from 102.0% to 108.3%, heterobeltiosis varied from 101.5% to 107.3%.

According to Ekinçi & Basbag (2018) estimation of heterotic effects is necessary to

identify new promising cross combinations. In addition, transgressive segregation in F₂ and occurrence of transgressive variability can be expected in pedigree selection. The heterosis results reported in this study for the three traits are comparable to those reported by other

authors or lower (for fiber length), which is understandable given that in interspecific crosses of the tetraploid species *G. hirsutum* and *G. barbadense* the dominance and heterosis manifestations are more strongly expressed than those in intraspecific crosses.

Table 3. Mean values, effects of specific combining ability (below line) and variances (σ^2S_i ; σ^2S_j)
Trial I - 2022

Traits	Females	Males			
		Sirius	Nazili 954	FR-H-1002	σ^2S_i
Productivity per plant, g	639	51.3	49.8	43.7	
		4.127	0.667	-4.793	20.181
	662	50.5	53.3	45.0	
		1.971	2.878	-4.849	17.795
	678	46.5	39.0	54.8	
		0.877	-8.650	7.773	67.964
	681	37.4	56.9	45.7	
		-8.145	9.394	-1.249	78.038
	682	49.1	45.6	52.4	
	σ^2S_j	1.171	-4.289	3.118	14.699
	σ^2S_j	22.307	47.505	29.504	
	SE (SCA)			0.834	
Lint percentage, %	639	40.9	39.0	40.8	
		0.531	-0.972	0.441	0.699
	662	41.1	44.6	41.5	
		-1.447	2.433	-0.987	4.482
	678	40.6	40.0	41.7	
		-0.291	-0.528	0.819	0.505
	681	36.1	34.4	34.7	
		0.909	-0.428	-0.481	0.609
	682	35.8	34.7	35.7	
	σ^2S_j	0.298	-0.505	0.208	0.182
	σ^2S_j	0.832	1.885	0.516	
	SE (SCA)			0.426	
Fiber length, mm	639	25.2	26.3	27.3	
		-0.742	0.278	0.464	0.415
	662	24.2	26.0	25.5	
		-0.698	0.989	-0.291	0.768
	678	26.9	24.6	25.3	
		1.602	-0.711	-0.891	1.926
	681	25.6	26.2	28.3	
		-0.831	-0.211	1.042	0.904
	682	27.1	26.2	27.0	
	σ^2S_j	0.669	-0.344	-0.324	0.329
	σ^2S_j	1.179	0.424	0.564	
	SE (SCA)			0.333	

Table 4. Mean values, effects of specific combining ability (below line) and variances (σ^2S_i ; σ^2S_j)
Trial II - 2022

Traits	Females	Males				σ^2S_i
		489	Anabel (535)	Tiara (550)	Melani (553)	
Productivity per plant, g	639	47.5	50.0	41.6	56.8	20.830
		0.238	1.108	-5.088	5.958	
	662	55.1	47.7	59.2	49.2	57.861
		4.013	-7.233	8.687	-5.467	
	678	50.0	54.2	39.8	53.3	23.290
		2.288	2.742	-7.238	2.108	
	681	38.1	49.5	49.1	52.0	1.897
		0.380	-2.067	0.987	0.700	
Lint percentage, %	639	40.0	58.5	49.1	47.3	41.933
		7.019	7.667	2.653	-3.300	
	662	17.760	30.966	40.376	20.240	
	678					
	681					
Fiber length, mm	639	43.0	42.4	43.2	38.7	1.975
		0.459	0.889	0.748	-2.097	
	662	42.0	40.6	43.0	42.1	0.955
		-0.657	-0.960	0.464	1.153	
	678	43.7	40.1	44.0	42.6	2.148
		0.343	-2.160	0.764	1.052	
	681	38.0	34.4	34.7	34.7	1.959
		1.851	-0.752	-1.360	0.261	
σ ² S _j	639	35.3	39.0	36.5	35.0	4.457
		-1.997	2.983	-0.637	-0.370	
	662	2.032	3.950	0.887	1.749	
	678					
	681					
σ ² S _i	639	26.3	25.6	25.7	26.0	0.176
		0.305	-0.602	0.313	-0.015	
	662	25.5	26.2	26.1	26.9	0.371
		-0.066	-0.302	0.213	0.751	
	678	26.6	25.7	24.2	25.0	0.777
		1.162	0.156	-0.895	-0.424	
	681	26.8	26.6	26.4	26.2	0.066
		0.296	-0.110	0.138	-0.324	
SE (SCA)	639	26.0	28.1	27.1	27.1	0.659
		1.102	0.858	0.232	0.012	
	662	0.788	0.298	0.246	0.204	
	678					
	681					

Table 5. Mean values, effects of specific combining ability (below line) and variances (σ^2S_i ; σ^2S_j)
Trial 2023

Traits	Females	Males				σ^2S_i
		Aida	Nazili 854	FR-H-1001	Bridjet	
Productivity per plant, g	Kristal	27.4	46.3	70.2	40.3	198.16
		-15.91	-0.885	18.385	-1.582	
	Perun	34.2	30.8	34.0	33.3	22.777
		3.878	-3.389	-4.819	4.331	
	Pirin	30.0	40.3	38.3	21.6	30.633
		0.232	6.665	0.001	-6.899	
	Tiara	36.5	42.9	33.9	32.3	34.920
		2.844	5.411	-8.219	-0.036	
	Melani	37.7	29.5	34.1	28.2	32.520
		8.103	-3.997	-4.011	-0.094	
	Anabel	29.3	28.5	35.6	31.4	11.721
		0.861	-3.805	-1.336	4.281	
	σ^2S_j	68.54	23.23	89.275	17.379	
	SE (SCA)			0.977		
Lint percentage, %	Kristal	27.4	46.3	70.2	40.3	198.16
		-15.91	-0.885	18.385	-1.582	
	Perun	34.2	30.8	34.0	33.3	22.777
		3.878	-3.389	-4.819	4.331	
	Pirin	30.0	40.3	38.3	21.6	30.633
		0.232	6.665	0.001	-6.899	
	Tiara	36.5	42.9	33.9	32.3	34.920
		2.844	5.411	-8.219	-0.036	
	Melani	37.7	29.5	34.1	28.2	32.520
		8.103	-3.997	-4.011	-0.094	
	Anabel	29.3	28.5	35.6	31.4	11.721
		0.861	-3.805	-1.336	4.281	
	σ^2S_j	68.54	23.23	89.275	17.379	
	SE (SCA)			0.584		
Fiber length, mm	Kristal	25.6	26.9	26.3	26.3	0.199
		-0.638	0.428	0.062	0.148	
	Perun	26.7	27.0	26.1	26.4	0.101
		0.199	0.299	-0.434	-0.065	
	Pirin	27.4	26.0	26.4	26.3	0.428
		0.903	-0.680	-0.130	-0.094	
	Tiara	26.4	26.7	26.4	26.7	0.020
		-0.109	0.008	-0.126	0.227	
	Melani	26.5	26.3	26.9	26.2	0.099
		0.037	-0.313	0.437	-0.160	
	Anabel	26.3	27.1	26.9	26.5	0.080
		-0.392	0.258	0.191	-0.056	
	σ^2S_j	0.279	0.173	0.084	0.016	
	SE (SCA)			0.332		

Tian et al. (2019) concluded that overdominance is the genetic basis of lint yield heterosis in interspecific hybrids between *G. hirsutum* L. $\times$ *G. barbadense* L., which is confirmed in studies with intraspecific hybrids. Borzan & Güvercin (2021) support that F₁ hybrids can be more superior from mean of parents (mid parent heterosis) and superior parent (heterobeltiosis)

because of the influence of dominant genes. The GCA and SCA of parents, the heterosis manifestations, as well as the SCA variances should be taken into account when choosing the selection strategy and conducting the selection practices.

The common GCA effects (f_i+m_j) of parents for each trait and ranked crosses based on the

Euclidean distances to the “ideal” population are presented in Tables 6 and 7. According to the calculated Euclidean distances the closest to the “ideal” population and the most valuable for cotton breeding programs in trial I were the crosses 639 × FR-H-1002, 662 × FR-H-1002, 639 × Nazili 954 and 682 × FR-H-1002. Of the females, line 639 had high GCA for lint percentage, line 662 had high GCA for productivity per plant and lint percentage, and line 682 had high GCA for productivity per plant and fiber length. Of the males, the cultivar Nazili 954 had high GCA for productivity per plant and the cultivar FR-H-1002 had high GCA for fiber length. These parental components had low

SCA variances for the respective traits, except for the cultivar Nazili 954 for productivity per plant and line 662 for lint percentage. The low variances of SCA indicate that their high GCA was mainly due to additive gene action and desirable segregants for the respective traits can be expected in the earlier hybrid generations. For the cultivar Nazili 954 and line 662, the high SCA variances show that their high GCA, respectively for productivity per plant and lint percentage, was due to additive and non-additive gene action (dominance and epistasis) and the selection for these traits will be more effective in later hybrid generations.

Table 6: Common combining effects of parents for each trait and square Euclidean distance to ideal values, Trial I – 2022

Parents	Productivity per plant, g	Lint percentage	Fiber length, mm	Square Euclidean distance
Trial I - 2022				
639 × FR-H-1002	0.446	1.558	0.715	2.701
662 × FR-H-1002	1.768	3.720	-0.295	4.285
639 × Nazili 954	1.069	1.237	-0.132	4.742
682 × FR-H-1002	1.202	-3.275	1.237	5.833
682 × Nazili 954	1.825	-3.596	0.390	7.078
678 × FR-H-1002	-1.037	2.081	0.072	7.996
639 × Sirius	-0.906	1.6	-0.178	8.92
682 × Sirius	-0.15	-3.233	0.344	9.452
662 × Nazili 954	2.392	3.399	-1.142	9.922
678 × Nazili 954	-0.414	1.76	-0.775	11.072
681 × FR-H-1002	-1.165	-3.619	1.171	11.448
681 × Nazili 954	-0.542	-3.94	0.324	11.577
662 × Sirius	0.417	3.762	-1.188	12.061
678 × Sirius	-2.389	2.123	-0.821	18.045
681 × Sirius	-2.517	-3.577	0.278	18.168
Trial II - 2022				
662 × Anabel	5.08	1.955	0.083	2.903
662 × Melani	4.813	1.274	-0.071	4.077
639 × Anabel	1.255	1.871	-0.017	5.710
662 × 489	1.234	3.018	-0.091	5.917
639 × Melani	0.988	1.19	-0.171	7.289
682 × Anabel	1.013	-3.622	1.056	7.596
662 × Tiara	0.66	2.929	-0.366	8.600
682 × Melani	0.746	-4.303	0.902	8.994
681 × Anabel	1.713	-4.52	0.491	9.021
678 × Anabel	1.605	2.621	-0.642	9.906
681 × Melani	1.446	-5.201	0.337	10.961
678 × Melani	1.338	1.94	-0.796	11.892
639 × 489	-2.591	2.934	-0.191	13.252
682 × 489	-2.833	-2.559	0.882	13.272
681 × 489	-2.133	-3.457	0.317	14.245
682 × Tiara	-3.407	-2.648	0.607	15.276
639 × Tiara	-3.165	2.845	-0.466	16.753
681 × Tiara	-2.707	-3.546	0.042	17.002
678 × 489	-2.241	3.684	-0.816	17.795
678 × Tiara	-2.815	3.595	-1.091	22.173

Table 7. Common combining effects of parents for each trait and square Euclidean distance to ideal values - Trial 2023

Parents	Productivity per plant, g	Lint percentage	Fiber length, mm	Square Euclidean distance
Trial 2023				
Tiara × FR-H-1001	6.85	0.28	0.053	6.847
Perun × FR-H-1001	3.55	0.58	0.028	7.908
Tiara × Nazili 954	2.21	-0.37	0.186	8.772
Perun × Nazili 954	-1.09	-0.07	0.161	9.934
Pirin × FR-H-1001	3.00	0.29	-0.009	10.004
Tiara × Bridget	-2.97	1.89	-0.034	11.759
Pirin × Nazili 954	-1.64	-0.36	0.124	11.983
Melani × FR-H-1001	2.83	-0.34	-0.042	13.323
Tiara × Aida	-1.65	-0.16	0.019	13.518
Kristal × Nazili 954	11.9	-0.82	-0.085	14.348
Anabel × FR-H-1001	1.67	-1.42	0.170	14.377
Perun × Bridget	-6.27	2.19	-0.059	15.324
Melani × Nazili 954	-1.81	-0.99	0.091	15.571
Perun × Aida	-4.95	0.14	-0.006	15.779
Anabel × Bridget	-8.15	0.19	0.083	16.404
Pirin × Bridget	-6.82	1.9	-0.096	17.243
Kristal × FR-H-1001	16.54	-0.17	-0.218	17.505
Pirin × Aida	-5.5	-0.15	-0.043	18.417
Kristal × Bridget	6.72	1.44	-0.305	18.999
Melani × Bridget	-6.99	1.27	-0.129	19.308
Anabel × Nazili 954	-2.97	-2.07	0.303	20.663
Kristal × Aida	8.04	-0.61	-0.252	21.462
Melani × Aida	-5.67	-0.78	-0.076	22.349
Anabel × Aida	-6.83	-1.86	0.136	23.940

Three more crosses 682 × Nazili 954, 678 × FR-H-1002 and 639 × Sirius with relatively short Euclidean distances to the “ideal” population were also valuable for selection programs. Line 678 from the females had high GCA for lint percentage and low SCA variances.

In trial II, the most reliable were the crosses 662 × Anabel, 662 × Tiara, 639 × Anabel and 662 × 489. In the first two crosses, both parents had high GCA for productivity per plant and line 662 had also high GCA for lint percentage. In the other two crosses, one parent (662 and Anabel) had high GCA for productivity per plant, while the other parent (639 and 489) had high GCA for lint percentage. Lines 662 and Anabel showed high GCA for productivity per plant had high SCA variances, which means, that selection for productivity should be conducted in later hybrid generations. Lines 662 and 639 with high GCA for lint percentage had low SCA variances, which means, their high GCA was due to additive gene action and selection for this trait can carried out in the earlier hybrid generations. High efficiency of selection for this trait in early segregating generations can be expected in the cross combination 662 × 489, where both

parents had high GCA and low SCA variances for lint percentage.

In the trial conducted in 2023, the most valuable cross combinations were Tiara × FR-H-1001, Perun × FR-H-1001, Tiara × Nazili 954 and Perun × Nazili 954. Tiara variety and the cultivars FR-H-1001 and Nazili 954 had high GCA for productivity per plant. Tiara variety and Nazili 954 had low SCA variances, while FR-H-1001 had high SCA variances. Consequence, in the crosses Tiara × Nazili 954 and Perun × Nazili 954 selection for productivity per plant can be effective in earlier segregating generations. In the cross Tiara × Nazili 954 both parents had high GCA and low SCA variances and the election by this trait in early segregating generations will be more effective. Perun variety had high GCA and low SCA variances for lint percentage. In the crosses Perun × FR-H-1001 and Perun × Nazili 954 selection for this trait can also be effective in earlier segregating generations.

Barut et al. (2000) concluded that evaluating parents in multiple environments and ranking crosses by the Euclidean distance to the “ideal”

population can be a simple and effective method of selecting desirable crosses.

In the trial I, the cross 681 × Nazili 954 had the highest productivity per plant, the cross 662 × Nazili 954 had the highest lint percentage, and the cross 681 × FR-H-1002 had the longest fiber, all these crosses showed positive SCA effects and heterosis to the better parent (heterobeltiosis) for the respective traits (109.0 %, 105.9 % and 105.2 %, respectively). In the first two crosses, only one of the two parents exhibited high GCA for the respective traits, while in the third cross both parents had high GCA effects and low variances for fibre length, which means the selection can be made in early segregating generations. These crosses were at an average distance from the “ideal” population. Sivia et al. (2017), Vasconcelos (2018) in line × tester and in diallel cotton crosses (*Gossypium hirsutum* L.) also reported crosses exhibited significant SCA have involved at least one parent with high or average GCA for a particular trait.

In the trial II, the cross 662 × Tiara showed the highest productivity, the cross 678 × 550 had the highest lint percentage and the cross 682 × 535 was with the longest fiber, all crosses exhibited high SCA and heterobeltiosis of 121.0 %, 104.8 and 107.3 %, respectively. In the first and third crosses, only one of the two parents expressed high GCA and high SCA variances for the respective traits, while in the second cross, both parents had high GCA and low SCA variances for lint percentage. These crosses were also at an average distance from the “ideal” population. In 2023 (trial III), the cross Kristal × FR-H-1001 showed the highest productivity per plant, the cross Perun × Bridget had the highest lint percentage, and the cross Pirin × Aida formed the longest fiber, all demonstrated high SCA for the respective traits. In the first cross, both parents had high GCA and high SCA variances, in the second cross, both parents had high GCA and low SCA variances and appeared to be very valuable for pedigree selection. These crosses were relatively more distant from the “ideal” population.

The crosses 662 × Nazili 954, 662 × Melani and Kristal × FR-H-1001, respectively in trial I, trial II and trial III, had the highest GCA for the productivity per plant. The first two crosses exhibited positive GCA for lint percentage and

negative one for fiber length. In these two crosses, the increase in productivity can lead to strong reduction in fiber length. The third cross had negative GCA for lint percentage and fiber length, and increasing productivity can result in reduction of both traits.

The crosses 662 × FR-H-1002, 662 × Nazili 954, 662 × Sirius (trial I), 662 × 489, 662 × Tiara, 639 × 489, 678 × 489, 678 × Tiara (trial II), Tiara × Bridget and Perun × Bridget (trial III) had the highest GCA for lint percentage. All these crosses showed negative GCA for fiber length, some also for productivity per plant. Therefore, in these crosses increasing the yield can lead to strong shortening of fiber length and in crosses with negative GCA for productivity per plant can lead to decrease in productivity.

Crosses 682 × FR-H-1002, 681 × FR-H-1002 (trial I), 682 × Anabel, 682 × Melani and 682 × 489 (trial II) having high GCA for fiber length, had negative GCA effects for lint percentage. In these crosses, with fiber length increase decrease in lint percentage and in some cases also in productivity, in crosses with negative GCA for this characteristic, can be expected.

Analysis of the research results gives very useful information about the selection of parental components on the basis of their general or specific combining abilities, as well as to select the desired F₁ crosses depending on the selection objectives for improvement of one or more characters. The selection of parental forms based on the common GCA effects of the two parents for all studied traits and selection of F₁ crosses based on the shortest Euclidean distances to the “ideal” population can help to better optimize the existing negative correlations between the traits in cotton.

CONCLUSIONS

Crosses with relatively short Euclidean distances to the “ideal” population in which desirable segregants are expected to occur were selected.

The crosses 639 × FR-H-1002, 662 × FR-H-1002, 639 × Nazili 954, 682 × FR-H-1002, 662 × Anabel, 662 × Melani, Tiara × FR-H-1001 and Perun × FR-H-1001 exhibited as high as possible general combining ability for the three studied traits and appeared to be most valuable for the breeding programs.

The approach to select the parental components having the highest possible general combining ability for all studied traits gives very useful information about their breeding value and helps to select most promising F₁ crosses, in which substantial improvement in one or more characters is expected as well as optimizing the existing negative correlations in cotton.

Some crosses showed high positive specific combining ability effects and heterosis manifestations for one or two traits. Heterobeltiosis values for productivity per plant ranged from 101.3 % to 214.0 %, for lint percentage - from 101.1 % to 107.6 % and for fiber length - from 101.5 % to 107.5 %.

Lines 662, 682, Kristal and Tiara varieties from the females, the cultivars Nazili 954, FR-H-1001 and Anabel and Melani varieties from the males, were identified as very good general combiners for productivity per plant. Line 682, Melani and Tiara varieties had low SCA variances and appear to be very valuable for the recombinant breeding programs, while the others (Nazili 954 and line 662) had high or unstable SCA variances and are more suitable for the heterosis selection.

Lines 639, 662, 678 and Perun, Pirin and Tiara varieties from females, and lines 489 and the cultivar Bredget from males were identified as very good general combiners for lint percentage. Lines 639, 678, 489, Tiara variety and the cultivar Bridget had low SCA variances and are very suitable for the synthetic selection in creating of new cotton varieties. The Greek cultivar Bridget having the highest lint percentage appears to be especially valuable for the selection of this trait.

Lines 681 and 682 from the females and the cultivar FR-H-1002 from the males were very good combiners for fiber length all with low SCA variances.

Reliable F₁ cross combinations, having high general combining ability for the three studied traits or for individual traits as productivity per plant, lint percentage and fibre length, were selected.

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AGRO-BIOLOGICAL RESPONSE OF GRAIN SORGHUM TO FOLIAR MINERAL FERTILIZATION AND BIOSTIMULANT APPLICATION

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Abstract

Grain sorghum (Sorghum bicolor L.) is a cereal crop of significant importance in agriculture, valued for its adaptability to diverse pedoclimatic conditions and drought resistance, and is used both for forage and human consumption. The experiment was conducted at the Agricultural Research and Development Station Secuieni, Romania (26°5' E; 46°5' N), during 2022-2024, aiming to evaluate the effect of foliar application of three products, Basfoliar 36 Extra, Terra Sorb, and Aminisol®, on the Albanus hybrid. The results highlighted a positive agro-biological response of the hybrid to foliar treatments, reflected in improved plant growth and development, as well as higher grain yield compared to the untreated control. The study emphasizes the importance of efficient use of foliar fertilizers and biostimulants in optimizing plant nutrition, contributing to increased yield and improved quality of grain sorghum.

Key words: biostimulants, drought, foliar fertilizers, sorghum, yield.

INTRODUCTION

The most widely distributed phytotechnical group worldwide, including in Romania, is represented by cereals (Roman et al., 2011). Among these, sorghum has gained increasing importance in recent years. This crop is particularly valuable in arid and semi-arid regions, where it serves as a viable alternative to maize cultivation. Sorghum's significance lies in its strong tolerance to high summer temperatures and its ability to produce high yields even under water stress conditions. Although sorghum has relatively high nutrient requirements, it does not always respond positively to the application of chemical or organic fertilizers. This behavior is attributed to its highly efficient root system, which enables effective nutrient uptake directly from the soil (Maranville et al., 1980).

Sorghum is grown in tropical, semitropical, arid and semi-arid areas, in over 120 countries across Africa, Asia, Australia and Europe (Balole and Legwaila, 2006; Shewale and Pandit, 2011; Matei et al., 2022), between the North latitudes of 50° (North America and Russia) and South

latitudes of 40° S in Argentina (Smith and Frederiksen, 2000).

This climate negatively affects the yield of agricultural crops, and agricultural research must find solutions to diversify the assortment of crops that in tougher environmental conditions guarantee the stability and profitability of agriculture. One of the safest solutions is the cultivation of sorghum, whether for grain or biomass, which due to good drought resistance, the ability to withstand high temperatures and capitalize on poorly fertile land is superior by the yields of all crops and especially, cereals and fodder plants (Isticioaia et al., 2020).

Sorghum (*Sorghum bicolor* L. Moench.) is an important crop for food and feed in several regions of the globe, being the fifth most important cereal in the world in terms of volume of yield after wheat, rice, corn, and barley (FAOSTAT, 2020). The popularity of sorghum occurs due to its drought resistance (Batista et al., 2019; Reddy, 2019; Isticioaia et al., 2020; Abreha et al., 2021), high yield potential (Clarke et al., 2019; Thomas et al., 2019), and various industrial applications.

Known as a culture specific to areas with arid climate and poorly productive soils, grain sorghum (*Sorghum bicolor* (L.) Moench., var. eusorghum) has become, in the context of increasingly obvious climate change, an alternative to maize, the cultivated area of sorghum increasing annually due to the stability of the yields and their nutritional quality (Matei et al., 2020).

MATERIALS AND METHODS

The experience was conducted between 2022 and 2024 at the Agricultural Research and Development Station Secuieni (A.R.D.S. Secuieni), on a typical cambic faeoziom (chernozem) soil with medium texture.

The soil is well supplied in phosphorus (77.6 ppm PAL), calcium ($13.6 \text{ meq} \cdot 100 \cdot \text{g}^{-1} \text{ soil Ca}$), magnesium ($1.8 \text{ meq} \cdot 100 \cdot \text{g}^{-1} \text{ soil Mg}$), medium supplied in nitrogen (16.2 ppm N-NO_3) and active humus (1.88%) and poorly supplied in potassium (124.6 ppm K_2O) with a slightly acidic pH (pH in H_2O - 5.98) (Naie et. al., 2024). The applied cultivation technology was adapted to the pedoclimatic conditions specific to the central Moldova region. Sowing was carried out at a density of 200,000 viable seeds per hectare, with a row spacing of 70 cm, and the preceding crop was sunflower. For the establishment of the experiment, a reduced agrotechnical input adapted to sorghum cultivation was used, consisting of the application of 40 kg active substance/ha of nitrogen (N) and phosphorus (P). The crop did not receive any additional fertilization.

The experiment was monofactorial, with the studied factor being sustainable foliar fertilization, applied exclusively using environmentally friendly products to a grain sorghum crop. The field experiment was laid out using a randomized block design with three replications, and the biological material used was the hybrid Albanus.

The data obtained were statistically analyzed using analysis of variance (Leonte & Simioniuc, 2018).

Foliar treatments were applied during the growing season. Basfoliar 36 Extra is a foliar fertilizer and it was applied at a rate of 8 l/ha at the 3-5 leaf stage. The foliar fertilizer Aminosol was applied at a total rate of 6 l/ha, split into two

applications of 3 l/ha at the 3-5 leaf stage and at the panicle initiation stage. Additionally, foliar biostimulant Terra Sorb was applied at a rate of 3 l/ha at the 3-5 leaf stage.

The analysis of temperature and precipitation trends during the grain sorghum growing period in the Secuieni area indicates a shift towards increasingly hot and dry conditions. Over the three years included in the study, the analysis of the thermal regime during the May-October period highlights variations in mean monthly air temperatures compared to the multi-annual average. In May, a slight negative deviation was recorded (0.5°C), indicating cooler than normal conditions. In contrast, during the June until October interval, increases in mean monthly temperatures were observed, ranging from 1.5°C to 2.7°C . The highest temperature increase occurred in August (2.7°C), followed by July and September (1.9°C) and October (1.8°C). These results reflect a warmer thermal regime during the main vegetative stages of grain sorghum (Figure 1).

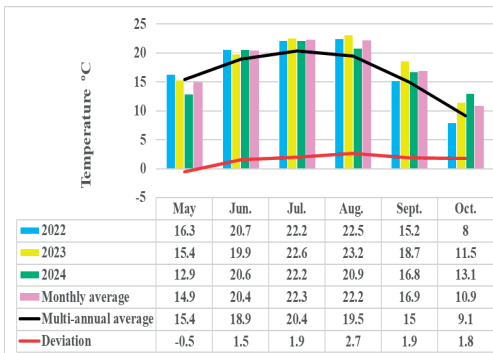


Figure 1. Temperature registered at A.R.D.S. Secuieni during grain sorghum vegetation period

The rainfall regime during the May-September period was characterized by a pronounced precipitation deficit compared to the multi-annual average. Mean monthly precipitation values showed negative deviations in all analyzed months, ranging from 11.6 mm in May to 53.1 mm in June. The most severe rainfall deficits were recorded in June, July, and August (53.1 mm, 21.7 mm, and 22.1 mm), a period corresponding to the critical growth stages of sorghum, including stem elongation, panicle formation, and grain filling. In September and October, precipitation levels remained below

the multi-annual average, with deficits of 14.4 mm and 26.4 mm, respectively (Figure 2).

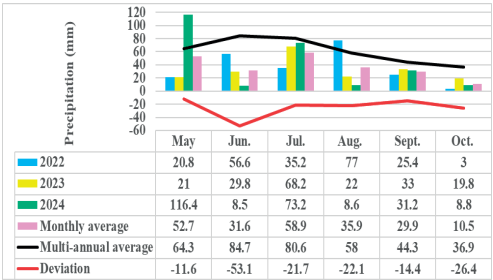


Figure 2. Precipitation registered at A.R.D.S. Secuieni during grain sorghum vegetation period

RESULTS AND DISCUSSIONS

Climatic conditions during the growing season are the primary factor influencing crop yield, particularly in regions where rainfall is the only source of water. In this context, grain sorghum represents a suitable crop due to its high tolerance to drought and high temperatures, maintaining relatively stable yields even under water limited conditions.

During the growing season, agro-biological measurements were carried out on the grain sorghum crop to assess emergence density, panicle emergence date, and plant height, in order to evaluate the effects of foliar fertilization and biostimulants.

From the emergence stage, the Albanus hybrid in the control variant (unfertilized) showed a plant density of 136,000 plants/ha, with panicle emergence observed on July 17. In the fertilized variants, emergence density ranged from 129,000 plants/ha the minimum value recorded with Aminosol application, to 142,000 plants/ha, the maximum value obtained in the variant treated with Basfoliar (8 l/ha). In all variants receiving fertilizers or biostimulants, panicle emergence was recorded on July 20, three days later than in the control variant (unfertilized).

Foliar fertilization and biostimulant applications increased grain sorghum plant height, ranging from 116.9 cm in the unfertilized to 131.4 cm in the Terra Sorb-treated variant (3 l/ha) (Table 1).

Table 1. Measurements taken during the growing season of grain sorghum, average 2022-2024

Fertilisation	Dose (l/ha)	Density at emergence (thousand plants/ha)	Heading date	Plant height (cm)
Unfertilized-Ct.	0	136	17. jul	116.9
Basfoliar	8	142	20. jul	124.5
Aminosol	6	129	20. jul	128.2
Terra Sorb	3	132	20. jul	131.4

Water and heat stress, frequently encountered in grain sorghum cultivation, especially during the early developmental stages, led to plant losses and, consequently, a reduction in crop density at harvest. However, the highest total number of harvested plants was recorded in the Basfoliar treated variant, with 238,000 plants/ha, due to a high number of tillers (128,000/ha), suggesting a significant stimulation of secondary branching. In contrast, the Terra Sorb treated variant had the highest number of main plants (117,000/ha) but a lower number of tillers (106,000/ha), resulting in a total of 223,000 plants/ha. The Aminosol treated variant showed intermediate values, totaling 217,000 plants/ha, while the unfertilized (control variant) had the lowest values, totaling 192,000 plants/ha, clearly indicating the role of foliar fertilization in stimulating tillering (Table 2). Regarding the average number of tillers per plant, these ranged from 0.78 tillers/plant in the control variant to 1.16 shoots/plant in the variant where Basfoliar was applied.

Table 2. Number of harvested plants depending on the applied fertilization under the pedoclimatic conditions at S.C.D.A. Secuieni, average 2022-2024

Fertilisation	Dose (l/ha)	Thousand harvested plants/ha			Number of tillers per plant
		Main plants	Tillers	Total plants	
Unfertilized-Ct.	0	108	84	192	0.78
Basfoliar	8	110	128	238	1.16
Aminosol	6	105	112	217	1.07
Terra Sorb	3	117	106	223	0.91

Analyzing the thousand grain weight (TGW) and hectoliter weight (HW), it was observed that foliar fertilization and biostimulants influenced the seed quality of sorghum. In the unfertilized variant, the TGW was 29.79 grams, representing the lowest value recorded. The application of Basfoliar (8 l/ha) increased the TGW to 32.21 grams, the highest value obtained in the experiment.

Regarding the hectoliter weight, values ranged from 72.9 kg/hl in the Basfoliar, treated variant to 74.2 kg/hl in the Aminosol (6 l/ha) variant (Table 3).

Table 3. Values of grain sorghum physical indices depending on the applied fertilization, average 2022-2024

Fertilisation	Dose (l/ha)	TGW (g.)	HW (kg/hl)
Unfertilized-Ct.	0	29.79	73.2
Basfoliar	8	32.21	72.9
Aminosol	6	31.25	74.2
Terra Sorb	3	30.45	73.6

Over the three years of experimentation, the Albanos variety, in the unfertilized variant, achieved an average yield of 6,256 kg·ha⁻¹, while the maximum yield of 7,780 kg·ha⁻¹ was recorded in the Aminosol treated variant. Relative yields ranged from 10% to 24% in the fertilized variants, with the highest values observed in the Aminosol treatment, followed by Basfoliar, compared to the control. The Basfoliar treatment resulted in a yield of 7,545 kg·ha⁻¹, representing an increase of 1,289 kg·ha⁻¹ and a relative yield of 21%, compared to the control variant. The highest increase compared to the unfertilized was obtained with Aminosol (7,780 kg·ha⁻¹), corresponding to a gain of 1,524 kg·ha⁻¹. In the Terra Sorb variant, the yield was 6,891 kg·ha⁻¹, which was statistically highly significant compared to the control, but lower than the yields obtained with Basfoliar and Aminosol (Table 4).

Table 4. Results on the effect of fertilization on grain sorghum yield under the pedoclimatic conditions at A.R.D.S. Secuieni, average 2022-2024

Fertilization	Yield kg·ha ⁻¹	Relative yield (%)	Dif.	Sem.
Unfertilizat-Ct.	6,256	100	-	
Basfoliar	7,545	121	1,289	***
Aminosol®	7,780	124	1,524	***
Terra Sorb	6,891	110	635	***
LSD	5%	220		
	1%	299		
	0.1%	402		

Data analysis revealed the existence of a direct correlation between the level of sorghum yield obtained under the influence of fertilizer application and the protein content of the harvest. The values of the correlation

coefficients indicate a strong relationship between the two variables, this relationship being confirmed as statistically significant and classified as highly significant ($r = 0.99^{***}$) (Figure 3).

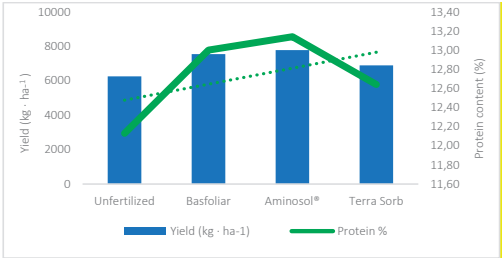


Figure 3. Correlation between grain sorghum yield (kg·ha⁻¹) and protein content (%), average 2022-2024

The application of biostimulators in sorghum cultivation led to a significant increase in grain yield, accompanied by changes in the chemical composition of the seeds. Correlation analysis revealed a negative relationship between sorghum yield and starch content ($r = -0.98^{000}$) (Figure 4), suggesting that stimulation of physiological processes by biostimulators directed the plant's metabolism toward protein synthesis and vegetative growth, at the expense of starch accumulation. This observation is supported by Zhang et al. (2023), who reported that carbon and nitrogen metabolism share common resources, and enhanced protein synthesis results in a relative decrease in starch content in the grains. The results suggest that foliar fertilization and biostimulation not only increase yield but also influence the allocation of assimilates, favoring the accumulation of structural and protein compounds, with direct effects on seed quality.

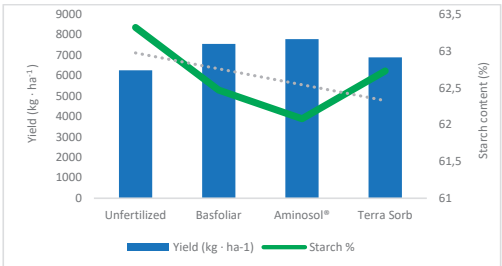


Figure 4. Correlation between grain sorghum yield (kg·ha⁻¹) and starch content (%), average 2022-2024

CONCLUSIONS

The Albanos hybrid in the control (unfertilized) treatment showed an initial density of 136,000 plants per hectare and reached panicle emergence on 17 July. In contrast, all foliar-fertilized treatments delayed this phenological stage by approximately three days, emphasizing the effect of foliar fertilization on sorghum development.

Foliar fertilizer application significantly increased plant height in grain sorghum, from 116.9 cm in the control to 131.4 cm in the treatment receiving Terra Sorb (3 L/ha), confirming the growth-promoting effect of biostimulants on vegetative development.

Grain yield was strongly influenced by foliar fertilization. The highest yields were obtained in the treatments with Aminosol (7,780 kg·ha⁻¹) and Basfoliar (7,545 kg·ha⁻¹), representing yield increases of 24% and 21%, respectively, compared with the control. These differences were statistically highly significant.

Statistical analysis also indicated a highly significant positive correlation between grain yield and protein content, suggesting that foliar fertilization enhances not only production levels but also the nutritional quality of sorghum grain.

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CORN RESILIENCE: INFLUENCE OF TEMPERATURE CHANGES AND BIOREGULATORS ON GERMINATION CHARACTERISTICS AND YIELD

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Abstract

Based on research data and practical observations, the effect of temperature changes on the characteristics of corn germination was analyzed and the role of JS bioregulator, isolated from Juniperus sabina L. plants and used in increasing corn resistance to abiotic stress was assessed. The studies were conducted on corn hybrids Porumbeni 465 (P465) and Porumbeni 398 (P398) with dent (P465) and flint (P398) grain endosperm in laboratory and field conditions. The modification of root/ seedling length and vigor, as well as metabolic efficiency of corn germination under influence of low (10°C) and high (50°C) temperatures and impact of natural growth regulator JS were also established. Comparison of germination characteristics in laboratory studies with corn emergence in the field and its yield made it possible to establish their correlation relationships, some of which were quite strong (≥ 0.65). In conclusion, it should be noted that the differences between the studied hybrids in physical structure, chemical composition of grain and maturity time are reflected in their resilience to temperature stress and response to treatment with a bioregulator.

Key words: corn seed, non optimal temperatures, bioregulator, growth characteristics, correlation.

INTRODUCTION

The negative impacts of climate change, caused by increasing average annual temperatures, changing precipitation patterns (more frequent droughts and floods), increasing atmospheric CO₂ concentrations and more frequent extreme weather events, significantly affect the growth, development, yield and quality of agricultural crops (Xue et al., 2021; Kanjevas et al., 2022; Del Buono et al., 2023; Hultgren et al., 2025).

Corn (*Zea mays* L.), one of the world's major food and forage crops, is particularly susceptible to extreme temperatures. Its productivity is critically dependent on climatic conditions, and the optimal range of daily temperatures for growth is approximately 22-25°C. Both high and low temperatures have a significant negative impact on the growth, development and yield of this crop. According to official data of National Bureau of Statistics, in 2024, the Republic of Moldova suffered a decrease in both the area under corn cultivation and the yield compared to previous years,

which is directly related to unfavorable weather conditions, in particular soil and atmospheric drought and excessively high temperatures from May to July. According to the National Bureau of Statistics, the area under corn cultivation for 2024 amounted to 414.7 thousand hectares, which is 17% lower than in 2021. The average corn yield in 2024 was 1.5-3 tons/ha, which was 40% lower than in 2021. In the southern regions of the country, crop losses reached 70-90%. Corn production in 2024 is estimated at 717 thousand tons, which is significantly below the five-year average. For comparison, in relatively favorable years (for example, 2021), the total corn harvest exceeded these data by 1.5 times. This fact clearly demonstrates the high vulnerability of Moldovan agriculture to climate change, which requires the implementation of adaptation strategies, such as the use of drought-tolerant and temperature stress resistant hybrids.

Although average annual temperatures are rising, weather instability leads to more frequent and abrupt temperature fluctuations, as

well as late spring cold snaps and even frosts, increasing the risk of damage to corn crops during the early stages of development. Therefore, studying the mechanisms of cold tolerance and developing methods to enhance the adaptive potential of agricultural crops remain priorities in modern plant physiology and breeding.

Maximum crop productivity under abiotic stress conditions can be maximal achieved through the exogenous application of biologically active substances, including growth regulators. The use of plant-based bioregulators represents a promising and environmentally friendly approach to enhancing plant adaptive potential and minimizing corn yield losses under climate change. Bioregulators not only improve plant metabolic activity but also actively participate in the fine regulation of all vital processes, including complex responses to abiotic stress, providing effective protection against negative environmental factors (García-García et al., 2020; Del Buono et al., 2023). Research and practical experience demonstrate that the use of bioregulators, particularly pre-sowing seed treatment, has a multifactorial positive effect on plant stability and development. Thus, seed treatment before exposure to low or high temperatures helps improve the mobilization and efficient use of seed reserve substances, ensuring a strong start to seedling growth even after heat stress (Borovskaia et al., 2023; Lutcan et al., 2024; Ivanova et al., 2024). Bioregulators stimulate the activity of key antioxidant enzymes (such as superoxide dismutase, catalase, and peroxidase), which effectively neutralize excess reactive oxygen species (ROS) that form in excess under stressful conditions and damage cellular structures (Zulfiqar & Ashraf, 2021; Khan et al., 2022; Navruzov et al., 2025; Rao et al., 2025). Plant growth bioregulators have some common features: they are synthesized by plants, cause metabolic changes, their effects are present even at very low concentrations, and some of them has also positive effect (antioxidant, antibacterial, anticancer) on mammals (Garban & Ilia, 2024).

The comprehensive analysis of the effects of hypo- and hyperthermia and role of natural bioregulator on key physiological growth

characteristics and final productivity of corn (*Zea mays* L.) was conducted in this study. The relevance of the study lies in the fact that the correlation between the morpho-biological characteristics of seed germination under normal conditions and non-optimal temperatures serves as a reliable criterion for selecting stress-resistant and high-yielding genotypes.

MATERIALS AND METHODS

The laboratory studies were carried out in 2025 year in the Institute of Genetics, Physiology and Plant Protection (IGPPP), Moldova State University (MSU), Republic of Moldova.

Corn seeds. The National Center of Research and Seed Production (“Porumbeni” sector) generously contributed the seeds of corn hybrids:

- Porumbeni 398 (P398), a mid-early maturing flint corn hybrid, belongs to the popcorn group, FAO 400. The kernel has a flinty consistency, characterized by a high specific gravity of vitreous endosperm. The kernel density is high and difficult to grind. The starchy kernels are tightly packed and firmly connected. The floury portion of the endosperm is present only near the embryo. The endosperm has a high protein content (16%), containing a starch by mixture of amylose (20-30%) and amylopectin (70-80%).

- Porumbeni 465 (P465), a late-ripening dent corn hybrid, FAO 460. The grain is dented with a floury endosperm. The grain density is low and easily crushed. The starch grains are loosely arranged, weakly bound by a protein matrix. They are composed primarily of amylopectin (over 90-95%). The grain is easily processed into fine flour and is used in the food and starch-molasses industries.

Natural bioregulator. The concentrated extract from aboveground parts of *Juniperus sabina* (bioregulator JS), was obtained and tested in laboratory as plant growth regulator (Elisovetcaia et al., 2024; Lutcan et al., 2024). Bioregulator JS diluted to a concentration of 0.01% was used to pre-germination or pre-sowing treatment of corn seeds.

The corn seeds were exposed to non-optimal temperatures by two tests.

Cold test. The seeds were germinated for 7 days at +10°C, after which they were transferred to optimal conditions of germination for 4 days (Milivojević et al., 2021).

Heat test. The seeds were kept in a water bath at +50°C for 30 minutes. Then they were germinated under optimal conditions for 7 days.

Germination under optimal conditions of untreated seeds (control) and seeds after cold or heat test was carried out in accordance with the provisions of international rules (ISTA, 2017). After germination, the overall seed germination, root and seedling length, root and seedling biomass, root and seedling vigor were assessed (Kerecki et al., 2021; Milivojević et al., 2021; Khan et al., 2022). The biomass of reserve substances that was spent for energetic support of physiological processes and respiration during seed germination as well as metabolic efficiency were calculated (Sikder et al., 2009; Borovskaia et al., 2023).

Field experiments were conducted during the 2025 growing season at the National Center of Research and Seed Production, “Porumbeni” sector. Control seeds and seeds treated with JS bioregulator were sown in the first 10 days of May, in a randomized manner in four replicates, two rows and 50 seeds on replicate. The characteristics of field emergence and corn yield were determined.

Statistical analysis. The obtained experimental data were processed by the statistical methods using the software package Statgraphics Plus 5.0. The ANOVA test was applied for variance analysis of characteristics, Student test in assessment of statistically significant differences between plots, and correlation was determined by Pearson coefficients.

Abbreviations used in figure.

Control – untreated seeds; **Control_JS** – seeds treated with solution of JS bioregulator; **Cold** – seeds exposed to cold test; **Cold_JS** – seeds pre-treated with JS bioregulator and exposed to cold test; **Thermo** - seeds exposed to heat test; **Thermo_JS** - seeds pre-treated with JS bioregulator and exposed to heat test.

RESULTS AND DISCUSSIONS

The overall germination of control seeds of the studied hybrids varied. The hybrid P398 had a lower initial germination rate (87.9%), with 48.7% of the mobilized reserve substances used

for root and seedling growth and 51.3% for energy and respiration maintenance during germination. This means that the seeds of this hybrid expended more reserve resources simply to maintain their vital functions. Metabolic efficiency, a measure of how effective corn seeds utilize reserve substances to support their vital functions, was 0.9493 (Figure 1).

The control seeds of the P465 hybrid demonstrated high overall germination (98.0%) under normal conditions. Its physiological processes were more coordinated and required less energy to support germination. During germination, this hybrid demonstrated a more favorable ratio of reserve substances spent on growth and energy maintenance. Most of the mobilized reserve substances were used directly for growth of roots and seedlings (50.8%), while a smaller portion was spent on energy and respiration (49.2%). This ensured rapid and vigorous germination and, consequently, higher metabolic efficiency of control seeds, which amounted to 0.9705 (Figure 1).

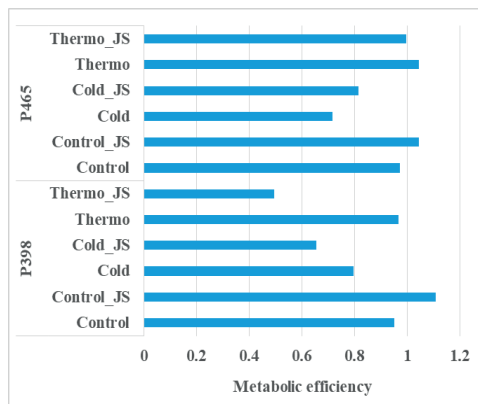


Figure 1. Metabolic efficiency of corn hybrids germinated under different conditions

Metabolic efficiency reflects how optimally and economically the seed embryo utilizes its internal nutrient reserves for two key processes: the construction of new structures - the biomass accumulation of seedlings and roots; and respiration, energy (ATP) production to support all life processes (El-Maarouf-Bouteau, 2022; Kanjevas et al., 2022). A comprehensive analysis of metabolic efficiency during germination confirms that the P465 dent corn hybrid is a slightly more economical and

physiologically vigorous hybrid. It converts internal resources into usable biomass more quickly and efficiently. Thus, the difference in metabolic efficiency between the two hybrids studied is due to their different physiological capabilities and genetically determined vigor, which directly influence how effectively they can mobilize and utilize their reserves for successful germination.

The study of corn resistance to non-optimal temperatures by cold- and heat tests also revealed significant hybrid differences.

The low temperatures cause profound metabolic depression to the P398 hybrid, despite maintaining high germination rate, which was comparable to control seeds. An increase in the consumption of reserve substances for respiration (4.45% higher than in seeds germinating under optimal conditions) indicates a redistribution of energy resources: a significant portion of endosperm reserves is expended on maintaining homeostasis (basic vital functions) rather than on structural processes (root and seedling growth). This leads to a 16.37% decrease in their metabolic efficiency (Figure 1).

Temperature stress acts as a limiting factor for the enzymatic activity responsible for the mobilization of reserve substances. Severe energy deficiency leads to a significant ($p \leq 0.001$) slowdown in morphogenesis: a reduction in the length of roots and seedlings by an average of 2.12-2.59 cm (Figure 2), and a decrease in root and seedling vigor by 43.48% and 53.40%, respectively (Figure 3). The flinty endosperm of the P398 hybrid is characterized by slow mobilization of reserve substances, especially at low temperatures due to the dense matrix. A diminution in root and seedling vigor while maintaining germination rate that the P398 hybrid enters a "passive survival" strategy (Kotak, 2007; Wang, et al, 2019; Sun at al., 2025). The resulting complex energy deficiency and metabolic disturbances pose risks for subsequent development in the field, requiring the use of bioregulators to optimize the use of reserve substances in seeds.

The high sensitivity of the P398 hybrid to temperature of 50°C was also established. This temperature is lethal or critically damaging for a significant amount (30%) of seeds, which is the primary indicator of severe heat stress and a

reduction in yield potential. Furthermore, it was showed the 60.88-63.78% reduction in vigor for roots and seedlings, compared to the same values obtained under optimal germination conditions (Figure 3). This is the most important characteristic, indicating a critical weakening of the surviving seedlings. Although the lengths of roots and seedlings increase by 18.02% and 15.32%, respectively, relative to the control (Figure 2). The observed increase in the linear size of organs is likely due to a disruption of homeostasis under stress.

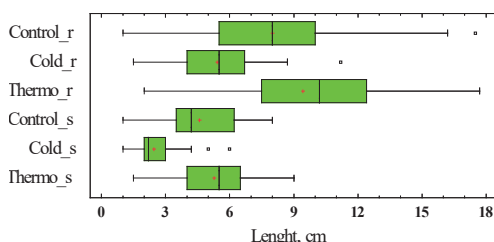


Figure 2. Root (r) and seedlings (s) length of P398 corn hybrid germinated under different conditions

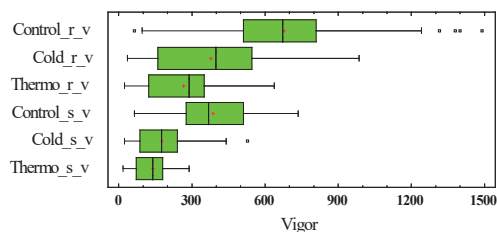


Figure 3. Root and seedlings vigor of P398 corn hybrid germinated under different conditions

Under abiotic stress, plants can rapidly elongate their organs, inefficiently utilize internal resources and form weak, nonviable structures (Rankenberg et al., 2021). Thus, the flint hybrid P398 exhibits low tolerance to heat stress (50°C), although surviving seeds exhibit some metabolic adaptation, with the change in their metabolic efficiency being insignificant ($p \leq 0.05$). However, the reduction in overall germination and the critical drop in vigor make this hybrid extremely vulnerable to the effects of such temperature at the germination stage, and its ability to withstand further stress and develop normally in field conditions is sharply reduced.

The pre-germination treatment of corn seeds with the JS bioregulator led to powerful stimulation of the initial growth and

development of roots and seedlings, as well as proved particularly effective for the genetically weaker hybrid P398. Metabolic efficiency increased by 17.04% compared to the seeds untreated with JS bioregulator, which reflecting more efficient mobilization of reserve substances such as starch, proteins, and fats for growth of roots and seedlings (Figure 1). The length of roots and seedlings of the P398 hybrid seeds, as well as their vigor was 18 - 19% higher than the control, indicating accelerated cell division and growth of the plant's organs. A well-developed root system and longer seedlings are critical for rapid plant establishment in the soil and access to moisture and nutrients during the early stages of vegetation. Vigor combines the speed of seedling emergence, their uniformity and ability to withstand stress. Seeds with high vigor produce stronger, more resilient plants. The use of the JS bioregulator in combination with temperature stress resulted in a significant ($p \leq 0.001$) suppression of root and seedling growth in the P398 hybrid in the cold test and an insignificant ($p \leq 0.05$) increase in root and seedling length in the heat test (Figure 4). However, root and seedling vigor decreased by 2.2-4.2 times in both the cold and heat tests (Figure 5), which was highly significant ($p \leq 0.001$).

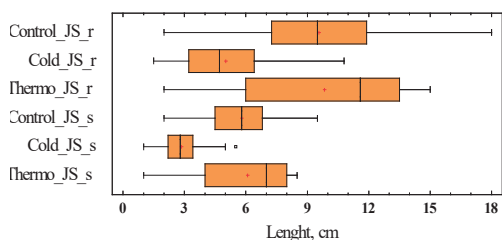


Figure 4. Root (r) and seedlings (s) length of P398 corn hybrid pre-treated with bioregulator JS and germinated under different conditions

The bioregulator is a powerful tool for managing the ontogenesis of intact seeds. However, using the JS bioregulator to treat seeds of a genetically unstable P398 hybrid exposed to non-optimal temperatures introduces an additional stress factor, which also led to a decrease in the metabolic efficiency of seed germination by 1.6-2.2 times. Thus, pre-germination treatment of P398 hybrid seeds with the JS bioregulator increased the negative impact

of non-optimal temperatures, which led to a phytotoxic effect manifested by a significant decrease in overall germination and critical metabolic disturbances.

The response to cold test in hybrid P465 was not as substantial as that of hybrid P398. Statistically insignificant changes in the lengths of embryonic roots and seedlings (Figure 6), as well as their vigor (Figure 7), were observed.

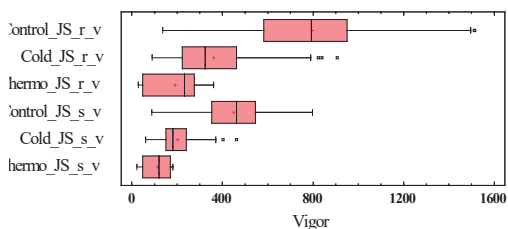


Figure 5. Root (r) and seedlings (s) vigor (v) of P398 corn hybrid pre-treated with bioregulator JS and germinated under different conditions

However, metabolic efficiency decreased by 26.12% relative to optimal conditions due to the redistribution of used reserve substances, the majority of which (58.24%) was used to provide energy for physiological processes and respiration.

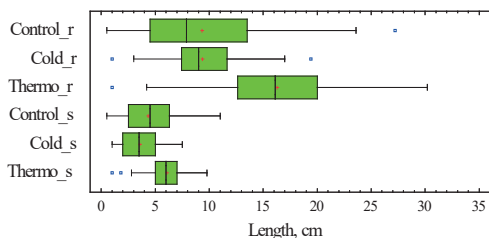


Figure 6. Root (r) and seedlings (s) length of P465 corn hybrid germinated under different conditions

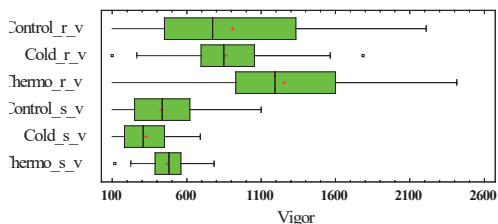


Figure 7. Root (r) and seedlings (s) vigor (v) of P465 corn hybrid germinated under different conditions

An analysis of data on the effect of elevated temperatures on the morpho-biological

characteristics of the dent hybrid P465 demonstrates its pronounced resistance to thermal stress (+50°C) compared to the flint hybrid P398. Hybrid P465 was shown to adapt and maintain basic life functions under heat stress. Better seed survival of P465 at high temperatures is confirmed by a moderate decrease in overall germination by 18% compared to optimal conditions. A key characteristic of the hybrid's high resistance is the length of roots and seedlings, which, on average, exceeded significantly ($p \leq 0.001$) the control seeds by 1.74-6.97 cm (Figure 6). In addition, it was noted that root and seedling vigor was 38.55% and 10.17% higher than in the control, respectively (Figure 7). This confirms the conclusion that intensive linear growth is a consequence of stress exposure. Furthermore, the dent hybrid demonstrated high metabolic plasticity, which enabled resource redistribution, reducing ineffective respiration (by 3.55%) and increasing overall metabolic efficiency (by 7.46%). The seeds rapidly and efficiently mobilize their reserve substances during germination, especially under stress. This made it possible to use reserve substances more effectively and purposefully for the development of a powerful root system.

Thus, the P465 hybrid demonstrated significant resistance to heat stress. It maintained high germination, increased root and seedling vigor, and effectively managed its metabolic processes, demonstrating an adaptive response. Compared to the flint hybrid P398, the dent P465 hybrid is significantly better suited for cultivation in both low and high temperature conditions.

Seeds of the P465 hybrid pre-treated with the bioregulator JS demonstrated metabolic efficiency 7.0% higher than in the control seeds (Figure 1). However, changes in root and seedling length and vigor were not significant. The increase in metabolic efficiency was due to increased root and seedling biomass.

The use of the JS bioregulator for pre-germination treatment of seeds of the dent hybrid P465 under non-optimal temperatures had a positive effect on the restoration of physiological potential after abiotic stress (Figures 8, 9).

The bioregulator stimulated root growth and its vigor, which were significantly greater ($p \leq 0.001$) than those of control seeds. Seedling

length and vigor also tended to increase in cold test, and was significant ($p \leq 0.05$) in heat test. These data indicate that the combined effect of non-optimal temperatures and the JS bioregulator on the seeds of the P465 hybrid had a synergistic effect on the utilization of reserve substances to develop a strong root system without affecting seedling growth.

Hybrid P465 demonstrates significantly improved tolerance to non-optimal temperatures and the combined effects of non-optimal temperatures and a bioregulator. Its ability to adapt and maintain vital functions under abiotic stress includes improved germination, dynamic root growth, and increased root vigor. This hybrid demonstrates high metabolic plasticity, effectively redistributing resources for survival. It is well known, that plants alter numerous phenotypic traits in response to environmental conditions, from the metabolic and cellular levels to the plant architecture level. This phenotypic plasticity is mediated by a combination of genetic and environmental factors and their interactions (Fu & Wang, 2023; Georgieva, 2025).

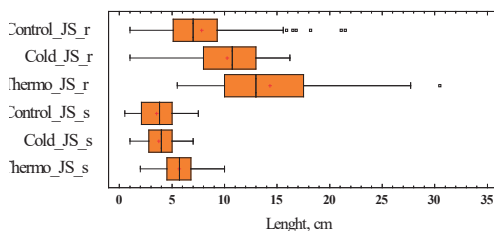


Figure 8. Root (r) and seedlings (s) length of P465 corn hybrid pre-treated with bioregulator JS and germinated under different conditions

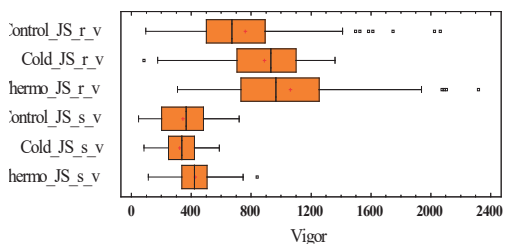


Figure 9. Root (r) and seedlings (s) vigor (v) of P465 corn hybrid pre-treated with bioregulator JS and germinated under different conditions

Thus, our results support the conclusion that the different hybrid responses to the application of non-optimal temperatures and JS

bioregulator were predetermined by the phenotypic plasticity of the corn hybrid.

One of the most important questions in plant stress biology is why the vast amount of knowledge about physiological and molecular responses to abiotic stresses has led to the development of so few climate-tolerant crop varieties (Verslues et al., 2023). Controlled stress experiments typically involve altering temperature, humidity, irrigation, light, or adding a chemical to modify the response to abiotic stress. Ideally, other environmental parameters are kept as stable as possible, so that only one or a combination of a few abiotic stresses occur simultaneously. Field trials do not represent a typical growing season that can be reproduced under standardized conditions. Even when temperature and precipitation are close to long-term averages, periods of extreme weather occur. In the field, some stressors are constantly present, such as rising night temperatures, nutrient deficiencies, salinity and heavy metals in the soil, but other stressors accumulate more gradually, such as drought, and can occur at any stage of development, with varying impacts on yield (Deng et al., 2016; Khalid et al, 2021; Verbraeken et al., 2021; Khaeim et al., 2022).

It should be noted that the 2025 growing season in the Republic of Moldova, when the field experiments were conducted, was characterized by a hot and dry August. These weather conditions resulted in a 9% reduction in corn yield compared to the decade average (Statistical yearbook..., 2025).

In this regard, we try to conduct a comprehensive analysis of the correlations between morpho-biological characteristics of corn growth under hypo- and hyperthermia and productivity. As noted above, the relevance of the study lies in the fact that the correlation between corn yield and its morpho-biological traits under normal and temperature stress conditions serves as a reliable criterion for selecting stress-resistant and high-yielding genotypes.

Data on the P398 hybrid confirm that in early stage of growth under non-optimal temperature the root and seedling lengths are not reliable predictors of final productivity (Table 1). Low correlation coefficients ($r=0.3634$ and $r=0.2295$) indicate that the genetic mechanisms of heat

and cold resistance at early stages and drought resistance during the flowering/filling period are regulated by different groups of genes.

According to the coefficient of determination (R^2), only 5.2-13.2% of the variability in field emergence is due to the potential for linear tissue growth under extreme temperature conditions. This indicates that the survival of the P398 hybrid in field conditions is determined not by the speed of growth processes, but probably by the biochemical stability of cellular structures or the efficiency of antioxidant systems (Pushkina & Kurchenko, 2016; Rao et al., 2025).

The established moderate-strength correlation ($r=0.5185$) between field emergence and embryonic root biomass at +10°C demonstrates that the survival of this genotype is determined not by the speed of linear cell elongation (Table 1), but by the intensity of morphogenesis and the formation of structural elements of the root system (the initiation of lateral roots and the thickening of axial roots).

Table 1. Correlation coefficients between laboratory characteristics of the P398 corn germination after temperature stress and field emergence and yield

Characteristics of P398	Temperature stress	Control seeds	
		Field emergence	Yield
Overall germination	Cold-test	-0.1681	0.3159
	Heat test	-0.0576	0.5364
Root length	Cold-test	-0.0298	0.5535
	Heat test	0.3634	0.2550
Seedlings length	Cold-test	0.2295	0.0374
	Heat test	0.0659	-0.3302
Root biomass	Cold-test	0.5185	-0.3949
	Heat test	-0.0450	-0.1157
Seedlings biomass	Cold-test	0.1933	0.4316
	Heat test	0.1367	0.4959

This hybrid demonstrates the high efficiency of mobilization and reutilization of endosperm reserves, aimed at the architecture of a morphologically and anatomically stable and functionally effective root system under limiting temperature conditions.

Therefore, when assessing the seed quality of the P398 hybrid, the key criterion for predicting field emergence and yield should be the weight of root biomass formed during cold testing, rather than their length or heat resistance. Research confirms that this hybrid excels due to the "quality" of its root system, not growth rate.

The significant positive correlation ($r=0.5535$) was found between yield and the length of the primary root system under cold test conditions (Table 1). This correlation indicates that yield potential is depended by the genotype's ability to develop roots at low positive temperature ($+10^{\circ}\text{C}$). Effective development of primary roots in the early stages not only ensures reliable plant anchorage of the plant in the soil, but also optimizes phosphorus absorption during long, cold spring conditions, determining future productivity (Khan et al., 2023).

The correlation ($r=0.5364$) between seed germination after exposure to heat test ($+50^{\circ}\text{C}$) and corn yield characterizes the high thermal stability of the hybrid's cell membranes and enzymatic systems. The ability of seeds to maintain viability after temperature shock serves as a reliable indicator of the organism's overall nonspecific resistance. This is a reliable indicator that the plant will withstand the August elevated temperatures.

The moderate correlation between yield and biomass expended on seedling growth under non-optimal temperatures ($r=0.4316$ and $r=0.4959$) indicates the importance of the metabolic plasticity (Table 1). Yield depends on how efficiently a hybrid converts endosperm reserves into its own tissues, without wasting energy on stress management. Efficient use of seed resources under extreme temperatures is a key criterion for selecting energy-efficient genotypes. Moreover, the weak correlation between yield and root length under hyperthermia ($r=0.2550$) confirms that tissue growth is less important for productivity in hot conditions than maintaining viability and dry matter accumulation (Körner, 2015; Salika & Riffat, 2021; Samat et al., 2025).

The extremely low correlation coefficients obtained in the experiment using seed treatment with the JS bioregulator (0.1453 for cold and 0.0650 for heat test) mean that after seed treatment, field emergence no longer depends on the intensity of seedling biomass accumulation (Table 2). The lack of strong correlation between germination and biomass at elevated temperatures indicates that the bioregulator works at the cellular level (membrane protection, synthesis of heat shock proteins or antioxidant defense system), and

not by stimulating growth processes (Wahid et al., 2007; Huot et al., 2014; Cannea & Padiglia, 2025; Rao et al., 2025; Samat et al., 2025).

After pre-sowing seed treatment with the JS bioregulator (Table 2), the relationship between field productivity and laboratory germination at low temperatures was strong ($r=0.6559$). This indicates that the bioregulator maximizes the genetic potential of the P398 hybrid specifically for cold conditions.

The low correlation between laboratory germination under heat test (50°C) and hybrid yield indicates the presence of a physiological aftereffect of the bioregulator. Probably, the bioregulator initiates mechanisms of pre-adaptation, shifting the metabolic vector toward mitigating the stress factors of early ontogenesis (in particular, cold resistance).

Thus, the realization of the crop's biological potential in field conditions is mediated not so much by the seeds' direct thermotolerance to heat stress, but by the induced systemic resistance established during the initial stages of germination. This allows to describe the effect of the studied bioregulator as a factor in the formation of cross-resistance, in which an increase in constitutive cold tolerance correlates with a general desensitization of the genotype to subsequent fluctuations in the hydrothermal regime during the growing season (Kanjevas et al., 2022).

Table 2. Correlation coefficients between laboratory germination characteristics of JS pre-treated the P398 corn after temperature stress and field emergence and yield of corn pre-sowing treated with JS

Characteristics of P398	Temperature stress	Seeds pre-treated with JS bioregulator	
		Field emergence	Yield
Overall germination	Cold-test	-0.4086	0.6559
	Heat test	-0.8283	0.2785
Root length	Cold-test	-0.6230	-0.1103
	Heat test	-0.5820	-0.2230
Seedlings length	Cold-test	-0.0220	-0.2726
	Heat test	-0.1815	-0.4961
Root biomass	Cold-test	-0.5391	-0.4503
	Heat test	-0.3259	-0.5170
Seedlings biomass	Cold-test	0.1453	-0.1622
	Heat test	0.0650	0.0750

Thus, under the influence of the bioregulator, a redistribution of the plant's adaptive resources occurs. Maximum mobilization of the P398

genetic potential shifts toward overcoming the initial temperature deficit, laying the foundation for stable biological productivity, regardless of subsequent extreme effects of hyperthermia. The productivity of the P398 corn hybrid in cold climates is largely determined not only by its genetic potential but also by the effectiveness of agricultural practices aimed at optimizing early development and mitigating the impact of low temperatures, where the use of bioregulators plays a key role.

An analysis of the relationships of morpho-biological characters of the P465 hybrid allowed us to verify the physiological profile of a "highly adaptive" type with a high thermotolerance threshold (Table 3). The deterministic relationship ($r=0.8230$) between laboratory germination under heat test ($+50^{\circ}\text{C}$) and field emergence demonstrates that thermal stability is a key predictor of the viability of this genotype. Such a strong correlation indicates that thermal tolerance mechanisms are the primary limiting factor in realizing the hybrid's biological potential. The relationship between the hybrid's field emergence and laboratory germination at 10°C was also significant ($r=0.6679$). This confirms the P465 hybrid is stress resistance to both temperature extremes, although heat resistance remains the dominant trait.

Unlike the P398 genotype, the P465 hybrid is characterized by a positive correlation between field emergence and biomass accumulation ($r=0.5657$) of seedling under heat test (Table 3). This relationship indicates the maintenance of active metabolism at critical temperatures. The hybrid demonstrates not only high survival at heat test, but also the ability to effectively morphogenesis. The formation of a full-bodied, physiologically vigorous seedling under thermal stress serves as a reliable biomarker of high field emergence and successful adaptation of this hybrid.

Thus, the heat test proved to be a comprehensive assessment method for P465 hybrid. This "energy-intensive" hybrid maintains high metabolic activity and biomass accumulation even under extreme heat. In unstable climates, P465 is the most reliable: its field survival is genetically determined through thermal protection mechanisms. This ensures stable

germination during both recurrent cold spells and abnormal heat waves.

However, despite its high heat resistance, the yield of the P465 corn hybrid is predetermined by completely different factors. A very strong, almost functional, relationship was found between corn yield and seedling length ($r=0.7408$) during germination in cold conditions (Table 3). The intensity of tissue elongation under low temperature is an "indicator of future yield". Seedlings that show a quick start in the cold form the most productive plants. The length of embryonic roots at low temperatures also correlates strongly with yield ($r=0.6389$), confirming that the foundation for P465 productivity is laid through early development of the cold soil. It should be noted that a very weak relationship ($r=0.2329$) was found between yield and overall germination at high temperatures (Table 3). This means that high seed thermotolerance is important only for survival, but it alone does not guarantee a high corn yield.

Table 3. Correlation coefficients between laboratory germination characteristics of the P465 corn after temperature stress and field emergence and yield

Characte- rists of P465	Temperature stress	Control seeds	
		Field emergence	Yield
Overall germination	Cold-test	0.6679	-0.3616
	Heat test	0.8230	0.2392
Root length	Cold-test	-0.1439	0.6389
	Heat test	-0.2661	-0.4558
Seedlings length	Cold-test	0.3431	0.7408
	Heat test	0.2638	-0.5502
Root biomass	Cold-test	-0.2354	-0.1655
	Heat test	-0.4555	-0.1320
Seedlings biomass	Cold-test	-0.6814	0.1519
	Heat test	0.5657	-0.4248

The P465 hybrid yield is formed not during the struggle against overheating, but during the period of effective growth at low temperatures. Thus, the P465 corn hybrid utilizes its exceptional thermotolerance as a preservation mechanism of population, but realizes its productivity potential through growth potential during the cold (starter) period.

The pre-sowing treatment of P465 seeds with the JS bioregulator reveals a clear hierarchy of factors determining sowing success and corn productivity (Table 4). The effect of JS bioregulator on growth and the biomass utilized

for germination drives hybrid survival. This effect results in a highly significant correlation between field emergence and the seedling length ($r=0.8086$). Unlike untreated seeds, emergence of pre-treated seeds directly depends on the ability of the seedlings to rapidly elongate at cold test, allowing the plant to more quickly penetrate the soil layer during cold spring weather. The amount of biomass utilized by the seedlings for germination during cold conditions plays a decisive role in field emergence ($r=0.8142$). It turned out that 66% (R^2) of field emergence is determined by the amount of seedling biomass gained at low temperatures. This means that the bioregulator promotes the mobilization of seed resources for germination. The correlation between field emergence and overall germination at heat test remains maximal ($r=0.8100$), confirming the genetic reliability of the P465 hybrid and its thermal stability, meaning that the seeds are guaranteed to survive when the heat shock has passed (Table 4). The correlation between field emergence and root biomass in the cold test was quite significant ($r=0.5286$), while the correlation with root length was weak ($r=0.2282$). This fact confirms that the bioregulator stimulates root "quality" (thickness, branching, weight), rather than simple root elongation.

Table 4. Correlation coefficients between laboratory germination characteristics of JS pre-treated the P465 corn after temperature stress and field emergence and yield of corn pre-sowing treated with JS

Characteristics of P465	Temperature stress	Seeds pre-treated with JS bioregulator	
		Field emergence	Yield
Overall germination	Cold-test	-0.4770	0.3413
	Heat test	0.8100	-0.6185
Root length	Cold-test	0.2282	0.0159
	Heat test	0.1573	0.1542
Seedlings length	Cold-test	0.8086	-0.3899
	Heat test	0.3778	-0.5352
Root biomass	Cold-test	0.5286	-0.1718
	Heat test	-0.0298	0.3317
Seedlings biomass	Cold-test	0.8142	-0.3798
	Heat test	0.6370	-0.3083

It should be noted, that under hyperthermia, temperature (+50°C) acts as a strict "filter" for viability. Furthermore, the realization of germination potential directly correlates with

the efficiency of mobilization seed reserve substances into seedling biomass ($r=0.6370$). Thus, while growth rate is the limiting factor under cold conditions, under heat conditions, it is overall physiological stability and the energy resource for germination.

Unlike field emergence, the yield of P465 hybrid demonstrates a weak dependence on most laboratory characteristics (Table 4). Weak correlations between productivity and root length and biomass under both non-optimal temperature conditions of germination.

Thus, the use of the JS bioregulator on the P465 corn hybrid transforms the architecture of correlation relationships, shifting the focus of root and seedling quality control. The key predictor of field emergence is the length and biomass of root and seedling under cold test conditions: high initial growth rates guarantee the formation of optimal plant density. A notable decrease in the correlation between laboratory tests and hybrid yield indicates that the JS bioregulator effectively mitigates initial stress risks, resulting in the productivity being determined more by growing conditions and agricultural practices than by the initial stress tolerance of the seeds.

CONCLUSIONS

Both hybrids studied demonstrate the response reaction to influence of low and high temperatures, but exhibit varying degrees of resilience. The hybrid P465 significantly outperformed the P398 hybrid in its ability to survive and growing stronger, more vigorous roots and seedlings, especially under elevated temperature. The bioregulator JS is a powerful modifier of seed germination characteristics and adaptive responses, but its effect depends on genotype and growth conditions. Applying the JS bioregulator to seed of the dent hybrid P465 before germination at non-optimal temperatures had a positive effect on restoring physiological potential after abiotic stress. However, for the flint hybrid P398, the combined effect of non-optimal temperatures and the bioregulator JS significantly reduces ($p \leq 0.001$) the vigor of roots and seedlings.

Analysis of correlations between key morphobiological parameters and productivity allows for differentiated hybrid selection strategies

based on predicted agroclimatic risks and bioregulator use. The use of the bioregulator mitigates the rigid dependence of germination and growth intensity on abiotic factors, making both hybrids more adaptive.

Hybrid Porumben 398 (flint) is characterized by moderate endogenous cold tolerance of the root system and pronounced growth depression under abiotic stress. In this case, treatment with the bioregulator not only mobilizes germination potential in low-temperature environments but also creates resistance at the early stages of development.

Hybrid P465 (dent) exhibits as highly adaptive genotype with pronounced genetic heat resistance and metabolic plasticity. For this hybrid, the bioregulator acts as a resource accumulation engine. The bioregulator switches the hybrid to a high metabolic plasticity mode, with field germination becoming 66% dependent on the amount of biomass accumulated by the seedling at +10°C. The bioregulator promotes not only the hybrid's survival in hot weather, which is genetically determined, but also growth during the cold period through the efficient processing of endosperm reserves.

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THE EFFECT OF PHYTOSANITARY TREATMENTS ON WHEAT CROP (*Triticum aestivum* L.) AGAINST *Zymoseptoria tritici* (Desmazières) Quaedvlieg & Crous AND *Blumeria graminis* F. sp. *tritici* (Decandolle) Speer

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated cereals; however, grain yield can be significantly affected by pathogen attacks, especially *Zymoseptoria tritici* (Desmazières) Quaedvlieg & Crous and *Blumeria graminis* (de Candolle) Speer. These pathogens reduce photosynthesis, affect plant vigor, and cause significant yield losses. To reduce these losses, it is necessary to use specific plant protection products containing active substances with different modes of action. This research was carried out in Iasi County and includes observations on crop phytotoxicity and vigor, the severity and incidence of pathogen attacks, as well as the effectiveness of treatments. The studied variety was FDL Miranda, on which 14 plant protection products were applied. Following the conducted research, it was observed that the wheat plants did not exhibit phytotoxicity to any of the applied products, and the crop vigor was very good. The pathogen *Zymoseptoria tritici* recorded the lowest attack severity of 6.00% when the product Priaxor EC (1.0 L/ha) was applied, while for *Blumeria graminis*, the variant treated with Revyicare (1.5 L/ha) recorded the lowest attack severity of 4.83%.

Key words: diseases management, plant protection product, treatment, winter wheat.

INTRODUCTION

Wheat is the primary crop cultivated globally, spanning diverse geographical regions, environments, and production systems.

It is grown on over 220 million hectares annually, yielding more than 798 million tons with an average productivity of 3,624.99 kg/ha according Food and Agriculture Organization. Despite its importance, wheat productivity is consistently threatened by biotic and abiotic factors, coupled with the slow adoption of advanced agricultural technologies.

Among biotic stressors, *Zymoseptoria tritici* (Desmazières) Quaedvlieg & Crous and *Blumeria graminis* (de Candolle) Speer are two of the most significant pathogens (Ünal et al., 2017).

Annual production losses are estimated at approximately 20.75% (Stukenbrock & Gurr, 2023), a figure that continues to rise due to rapid mutations and the increasing diversity of virulent strains (Singh et al., 2016).

Blumeria graminis is the causative agent of powdery mildew in wheat. As an obligate parasite, it induces yield losses ranging from 5% to 35% (He et al., 2011), with extreme regional impacts recorded in Egypt (22.52%), Russia (33.00%), and Brazil (62.00%) (Kayim et al., 2022). The pathogen affects leaves, stems, and ears throughout the growing season, following a polycyclic epidemic pattern. This cycle is characterized by rapid conidial infections, with new conidia being formed and released within just a few days (Cao et al., 2012). While biological control agents such as *Ampelomyces quisqualis* and *Pseudozyma flocculosa* have been successfully registered for use in viticulture and horticulture (Kiss et al., 2004; Paulitz & Bélanger, 2001), no commercial biological products are currently available for controlling powdery mildew in cereal crops. Similarly, *Zymoseptoria tritici* causes *Septoria tritici* Blotch (STB), a devastating foliar disease that can trigger yield losses of 30% to 50% (Stukenbrock & Gurr, 2023). Particularly

prevalent in temperate climates, the fungus exhibits remarkable evolutionary plasticity, allowing it to bypass modern disease control measures. Under field conditions, *Z. tritici* typically persists for 14 days post-infection, though this period may extend to 28 days in cooler climates (Fones & Gurr, 2015). Even with the use of moderately resistant cultivars and a rigorous three-spray fungicide regime, residual losses of 5% to 10% are often observed. Given that several pathogens pose severe threats to grain quality and yield, effective disease management is vital for European agriculture. This necessity has fostered a robust fungicide market in Europe, valued at over \$4.3 billion as of 2024 (Berraies et al., 2014). Holistic strategies, such as Integrated Pest Management (IPM), combine ecosystem management, crop rotation, and soil health practices to favor beneficial microorganisms and insects (Popescu et al., 2021). However, reducing significant losses still necessitates the use of preventive, systemic, and contact fungicides. These agents primarily function by inhibiting pathogen activity through various modes of action, including the disruption of the cytoskeleton, signal transduction, or membrane sterol biosynthesis (Mueller et al., 2021). Notably, while fungicide use is widespread, Romania reported one of the lowest consumption rates in the EU in 2020, at 0.76 kg/ha (Popescu et al., 2021). In this context, the present work aims to evaluate the efficacy of systemic fungicides in controlling *B. graminis* and *Z. tritici* in wheat, while further elucidating the active role of these chemical agents in modern crop protection.

MATERIALS AND METHODS

The experiment was conducted under the pedo-climatic conditions of Iasi County, Buhăieni locality (47°29'49.4"N - 27°16'44.5"E), during 2024-2025 growth season. Observations were made on the FDL Miranda variety. It has good resistance to lodging and wintering, drought and heat. It is resistant to powdery mildew and moderately susceptible to brown rust, yellow rust, and septoria leaf spot disease.

The size of the experience, the timing and frequency of the evaluations, and the evaluation scales were established in accordance with OEPP/PP1 1/26(4). The organization of the experiment was done according to the method of randomized blocks. The experimental plot size is 30 m² with 4 replications, representing 120 m² per treatment, for a total experimental area of 1800 m². Timing and frequency of the evaluations were determined according to Tables 1 and 2.

Table 1 Time, Frequency and number of post-application assessment during of trial development

No	Moment	Time
1	A	Application T1 BBCH 31-32
2	A1	7 days after application
3	A2	14-21 days after application
4	A3	28- 35 days after application
5	A4	42-48 days after application

Table 2 Post-application assessment made during of trial development

No	Moment	Assessment description
1	A0	Disease identification
2	A0 to A4	Crop Stage
3	A1 to A4	Phytotoxicity and Vigor
4	A2 to A4	Severity and efficacy of treatments

The assessment notes for phytopathogens at the foliar level were recorded as shown in Figures 1 and 2. The leaves were numbered from top to bottom, with leaf 1 being the flag leaf, followed by leaves 2, 3, 4, and 5.

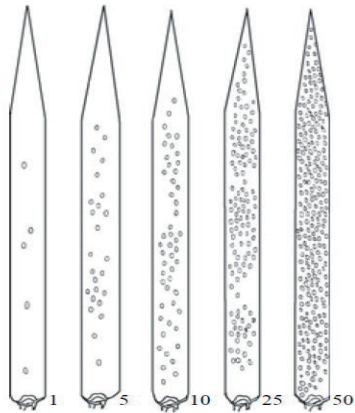


Figure 1. Percentage of leaf affected, *B. graminis* (OEPP/PP 1/26(4)) on wheat



Figure 2. Percentage of leaf affected, *Z. tritici* OEPP/PP 1/26(4) on wheat

Phytotoxicity were assessed according to OEPP/PP 1/135, with observations made on the health status of the plants. These are assigned values from 0-100%, with 100% representing no changes and values from 0-99% indicating discoloration, necrosis, deformation, and anatomical changes. Vigor was defined by assigning scores from 0-100%, with 100% representing no physiological changes and values from 0-99% indicating the presence of changes (e.g., height). The crop's growth stage was described according to the BBCH scale.

A total of 14 treatments were applied to the wheat plants, including 10 single fungicides applied with normal dose and 5 at with reduced dose along with an insecticide.

The experiment variants include: 1. Untreated control - without fungicide applied; 2. Input 460 EC (Prothioconazol 160 g/l + Spiroxamină 300 g/l) at rate of 1.00 l/ha; 3. Phoenix (Folpet 500 g/l) at a rate of 1.50 l/ha; 4. Aviator XPRO (Bixafen 75 g/l + Prothioconazol 150 g/l) at a rate of 0.80 l/ha; 5. Zoxis (Azoxistrobin 250 g/l) at a rate of 1.00 l/ha + Protos (Prothioconazol 150 g/l) at a rate of 0.80 l/ha; 6. Priaxor EC (Fluxapyroxad 75 g/l + Piraclostrobin 150 g/l) at a rate of 1.00 l/ha; 7. Nativo Forte 280 EC (Prothioconazol 93.3 g/l + Spiroxamina 107 g/l + Trifloxistrobin 80 g/l) at a rate of 1.50 l/ha; 8. Revycare (Mefentrifluconazol 100 g/l + Piraclostrobin 100g/l) at a rate of 1.50 l/ha; 9. Elatus ERA (Benzovindiflupir 75 g/l + Prothioconazol 150 g/l) at a rate of 1.00 l/ha; 10. Flexity (Metrafenone 300 g/l) at a rate of 0.50 l/ha; 11. Phoenix (Folpet 500 g/l) at a rate of

1.50 l/ha + Decis Expert 100 EC (Deltametrin 100 g/l) 0.075 l/ha; 12. Flexity (Metrafenone 300 g/l) at a rate of 0.50 l/ha + Wizard (Esfenvalerat 5 %) at a rate of 0.20 l/ha; 13. Priaxor EC (Fluxapyroxad 75 g/l + Piraclostrobin 150 g/l) at a rate of 1.00 l/ha + Karate Zeon (Lamda-cihalotrin 50 g/l) at a rate of 0.15 l/ha; 14. Revycare (Mefentrifluconazol 100 g/l + Piraclostrobin 100g/l) at a rate of 0.50 l/ha + Mospilan (Acetamiprid 200 g/kg) at a rate of 0.10 kg/ha; 15. Zoxis (Azoxistrobin 250 g/l) at a rate of 1.00 l/ha + Sivanto Energy 85EC (Deltametrin 100g/l + Flupiradifuron 75 g/l) at a rate of 0.75 l/ha.

The treatment was applied on BBCH 30-31 - beginning of stem elongation: pseudostem and tillers erect, first internode begins to elongate, top of inflorescence at least 1 cm above tillering node. The experiences were applied using the BOOM SPRAY-SCHACHTNER equipment, and the amount of water used was 300 l/hectare.

Climate data regarding temperature, humidity, and precipitation during the growing season is obtained using the DTN LLC platform.

The data obtained during the research period was verified and processed using the ARM - Agriculture Research Manager software.

RESULTS AND DISCUSSIONS

The treatments were applied during the 2025 growing season, at the BBCH 30-31 vegetation stage, under normal climatic conditions (temperature, humidity, and soil conditions) on April 23, 2025. The data are presented in Table 3.

Table 3. Field condition at application, crop (BBCH), and climate condition (soil, temperature, humidity, and wind), 23.04.2025 data

Crop stage BBCH	T° C Soil 10 cm	T° C Air	Humidity %	Time	Wind
					Mps / Direction
30-31	12.2	14.7	64.5	10:00	0.3 / N

The research period is characterized by low rainfall in April (16.80 mm) and June (4.30 mm) and high rainfall in May (70.50 mm). Thus, due to increased precipitation and high temperatures, optimal conditions for the development of the agents *Z. tritici* and *B. graminis* appeared. *Z. tritici* develops intensely under high humidity and moderate temperatures of 15-20°C (Takele et al., 2015). *B. graminis* f. sp. *tritici* develops at

moderate temperatures (15-22°C) and high atmospheric humidity, without requiring free water on the leaves (Tables 3 and 4).

Table 4. Agrometeorological conditions during the growing seasons of autumn wheat for the trial development of 2025 (source: DTN LLC)

Month	Prep. (mm)	Temperature °C			Humidity %		
		Min.	Max.	Avg.	Min.	Max.	Avg.
April	16.80	7.77	19.13	13.45	41.97	83.23	62.59
May	70.50	11.19	20.07	15.63	56.26	88.81	73.14
June	4.30	18.48	30.58	25.25	53.20	92.17	73.08

During the test development period, the pathogens moved from the basal leaves, leaves 3, 4, and 5, to the upper levels, specifically leaves 1, 2, and 3. *B. graminis* showed a positive trend during the research period, recording a 218.57% increase in A4 compared to A0 in the untreated variant. Isolated in A2 - 14 DAA from *Z. tritici* showed a decrease in development of -16.85% compared to the previous variant A0, but due to favorable climatic conditions, it recorded an increase of 267.10% in A4. On A4 42 - DAA, the *Z. tritici* strain showed a 48.44% increase compared to *B. graminis* (Tables 5 and 6).

Table 5. Development of *Zymoseptoria tritici* on untreated variant during of trial development, at A0 (23.04.2025), A2 (07.05.2025), A3 (24.05.2025), and A4 (04.06.2025) moments (L1-leaf 1, L2-leaf 2, L3-Leaf 3, L4- leaf 4, L5-Leaf 5, Avg.-average)

Moment	L1	L2	L3	L4	L5	Avg.
A0 0-DAA	-	-	1.53	7.67	19.44	9.55
A2 14-DAA	-	2.67	7.17	14.67	-	8.17
A3 28-DAA	2.70	13.20	28.53	-	-	14.81
A4 42-DAA	10.17	23.50	42.83	-	-	25.50

Table 6. Development of *Blumeria graminis* on untreated variant during trial development, at A0 (23.04.2025), A2 (07.05.2025), A3 (24.05.2025), and A4 (04.06.2025) moments; (L1-leaf 1, L2-leaf 2, L3-Leaf 3, L4- leaf 4, L5-Leaf 5, Avg.-Average)

Moment	L1	L2	L3	L4	L5	Avg.
A0 0-DAA	-	-	1.38	5.38	22.64	9.80
A2 14-DAA	-	2.13	7.50	22.13	-	10.59
A3 28-DAA	3.63	11.88	21.38	-	-	12.60
A4 42-DAA	8.25	18.63	37.38	-	-	21.42

After applying the treatments, the severity of the pathogens was low, highlighting the effectiveness of the applied phytosanitary products. Thus, recording control over plant development and disturbances.

During the testing, no symptoms of phytotoxicity or changes in vigor were observed; they recorded 0 phytotoxicity and 100% vigor throughout the experimental period. *Zymoseptoria tritici* is a fungus and causes the fungal disease known as wheat leaf blotch, a particularly important disease (Quaedvlieg et al., 2011; Abeba, 2023), this particularly affects the leaves, where elongated, brownish-gray spots appear, bordered by veins, and within which black dots (pycnidia) can be observed. *Z. tritici* is an apoplastic pathogenic species with a hemibiotrophic life cycle (Ponomarenko et al., 2011; Fones and Gurr, 2015; Savary et al., 2019).

For the control of the pathogen *Z. tritici*, treatments Priaxor EC⁶, Nativo Forte 280 EC⁷ and Revycare⁸ showed a very good response in controlling the pathogen's development, recording low severity ranging from 6.00-6.78% and high efficacy of 77.77-80.36%. Treatments Input 460 EC², Zoxis+Protos⁵ and Elatus ERA⁹ showed a good response, recording low severity of 7.28-9.00% and high efficacy of 68.68-74.59%. An average response was observed in treatments Aviator XPRO⁴, Phoenix³ and Phoenix+Decis Expert 100 EC¹¹, which recorded an average severity of 10.00-11.61% and an efficacy with values ranging between 58.28-64.85%. A poor response was recorded for treatments Phoenix+Decis Expert 100 EC¹¹ and Zoxis+Sivanto Energy 85EC¹⁵, with a severity ranging between 12.33-13.17% and an efficacy of 51.74-55.31%. And a very poor response was recorded for treatments Revycare+Mospilan¹⁴, Flexity+ Wizard¹² și Flexity¹⁰ with a severity ranging between 14.06-15.83 and poor efficacy ranging between 41.08-48.17% (Tables 7 and 8).

Table 7. Severity (%) of *Zymoseptoria tritici* on autumn wheat against at moment A2 (07.05.2025) Leaf 2, 3 and 4, A3 (24.05.2025) Leaf 1,2 and 3, and moment A4 (04.06.2025) Leaf 1, 2 and 3

No	Moment A2			Moment A3			Moment A4		
	L2	L3	L4	L1	L2	L3	L1	L2	L3
1	2.67	7.17	14.67	2.70	13.20	28.53	10.17	23.50	42.83
2	0.00	2.00	8.50	0.00	2.83	8.00	1.83	6.00	14.00
3	0.00	2.67	9.67	0.33	4.33	11.50	2.83	9.50	20.50
4	0.00	2.50	9.67	0.00	3.83	11.00	2.50	8.67	18.83
5	0.00	2.17	8.83	0.00	3.17	9.17	2.00	6.67	16.17
6	0.00	1.50	7.67	0.00	2.17	6.17	1.00	4.83	12.17
7	0.00	1.67	8.00	0.00	2.33	6.33	1.33	5.33	12.67
8	0.00	1.83	8.17	0.00	2.50	7.50	1.50	5.67	13.17
9	0.00	2.33	9.33	0.00	3.67	10.33	2.17	7.67	17.17
10	1.07	4.00	11.83	1.13	6.83	17.33	5.33	13.33	28.83
11	0.67	3.00	10.33	0.67	5.00	13.83	3.67	10.33	23.00
12	1.00	3.67	11.67	1.07	6.33	16.00	4.83	12.67	27.17
13	0.50	2.83	10.17	0.50	4.83	12.17	3.17	10.33	21.33
14	0.83	3.33	11.17	1.00	5.83	15.17	4.50	12.17	25.50
15	0.67	3.17	10.67	0.83	5.33	14.33	4.00	11.67	23.83

Table 8. Efficacy (%) of foliar treatment applied on autumn wheat against *Zymoseptoria tritici* at moment A2 (07.05.2025) Leaf 2, 3 and 4, A3 (24.05.2025) Leaf 1,2 and 3, and moment A4 (04.06.2025) Leaf 1, 2 and 3

No	A2			A3			A4		
	L2	L3	L4	L1	L2	L3	L1	L2	L3
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	100.00	72.31	41.64	100.00	78.56	71.81	82.04	74.44	67.29
3	100.00	63.08	33.42	88.57	66.93	59.53	72.18	59.44	52.06
4	100.00	65.30	33.75	100.00	71.10	61.58	75.36	63.15	56.03
5	100.00	69.74	39.17	100.00	75.99	67.75	80.45	71.62	62.24
6	100.00	78.97	47.67	100.00	83.54	78.33	90.14	79.35	71.59
7	100.00	77.09	45.44	100.00	82.36	77.65	86.97	77.22	70.40
8	100.00	74.53	44.20	100.00	80.97	73.59	85.21	75.83	69.28
9	100.00	67.52	35.32	100.00	72.28	64.03	78.86	67.31	59.88
10	60.00	43.93	19.30	56.70	47.91	38.96	47.37	43.33	32.55
11	75.56	57.95	29.33	75.87	62.15	51.58	63.74	55.97	46.22
12	62.22	49.06	20.53	59.37	52.07	44.14	52.63	46.11	36.52
13	81.11	60.51	30.56	80.63	63.39	57.07	68.67	55.97	50.19
14	68.89	53.50	23.39	61.27	55.78	46.67	55.81	48.33	40.38
15	74.44	55.73	27.09	69.21	59.53	50.07	60.57	50.42	44.23

Blumeria graminis f. sp. *tritici* is the pathogen that causes wheat powdery mildew, a common disease in wheat crops, characterized by the appearance of a whitish, floury coating on leaves, stems, and sometimes on ears, which later turns gray. The attack reduces photosynthesis and weakens the plants (Cowger et al., 2018). For control during the growing season, treatments Revyicare⁸, Input 460 EC², Flexity¹⁰ and Priaxor EC⁶ showed a very good response in controlling the development of pathogens, recording low severity ranging from 4.84-5.80% and high efficacy of 77.94-82.43%. Treatments Nativo Forte 280 EC⁷, Flexity+

Wizard¹², Elatus ERA⁹ and Aviator XPRO⁴ showed a good response, recording low severity of 7.04-8.92% and high efficacy of 63.20-71.89%. A poor response was recorded for treatments Zoxis+Protos⁵, Phoenix³ and Revyicare+ Mospilan¹⁴ with a severity ranging between 9.63-10.71% and an efficacy of 55.04-60.28%. And a very poor response was recorded for treatments Phoenix+Decis Expert 100 EC¹¹, Priaxor EC+Karate Zeon¹³ and Zoxis+Sivanto Energy 85EC¹⁵ with a severity ranging between 11.50-12.71 and a weak efficacy ranging between 43.47-50.58% in controlling the pathogen *Blumeria* (Tables 9 and 10).

Table 9. Severity (%) of *Blumeria graminis* on autumn wheat against at moment A2 (07.05.2025) Leaf 2, 3 and 4, A3 (24.05.2025) Leaf 1,2 and 3, and A4 (04.06.2025) Leaf 1, 2 and 3

No	A2			A3			A4		
	L2	L3	L4	L1	L2	L3	L1	L2	L3
1	2.13	7.50	22.13	3.63	11.88	21.38	8.25	18.63	37.38
2	0.00	1.63	6.00	0.00	1.75	5.25	0.63	3.75	11.38
3	0.63	2.88	11.00	0.88	4.13	9.50	2.63	7.88	19.88
4	0.38	2.50	9.63	0.50	3.13	8.63	2.13	6.88	17.75
5	0.50	2.63	10.25	0.75	3.63	9.25	2.38	7.38	19.13
6	0.00	1.75	6.88	0.00	2.00	5.88	0.88	4.38	12.13
7	0.00	2.00	7.50	0.00	2.38	7.13	1.38	5.25	14.50
8	0.00	1.38	5.38	0.00	1.50	4.63	0.50	3.38	10.63
9	0.00	2.38	8.50	0.38	2.88	8.13	1.88	6.25	16.75
10	0.00	1.88	6.75	0.00	2.13	6.50	1.13	4.88	13.25
11	0.75	3.25	11.75	1.13	5.25	10.63	3.13	9.38	22.00
12	0.00	2.13	7.75	0.25	2.63	7.50	1.63	5.75	15.88
13	0.80	3.38	12.13	1.38	5.50	11.63	3.50	9.88	23.00
14	0.75	3.13	11.63	1.00	4.88	9.88	2.88	8.13	21.13
15	0.85	3.50	13.13	1.50	5.88	12.00	4.13	10.38	23.63

Table 10. Efficacy (%) of foliar treatment applied on autumn wheat against *Blumeria graminis* at moment A2 (07.05.2025) Leaf 2, 3 and 4, A3 (24.05.2025) Leaf 1,2 and 3, and moment A4 (04.06.2025) Leaf 1, 2 and 3

No	A2			A3			A4		
	L2	L3	L4	L1	L2	L3	L1	L2	L3
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	100.00	77.92	72.64	100.00	85.28	75.49	92.46	79.89	69.63
3	70.42	60.56	50.14	75.89	65.26	55.29	67.58	57.85	47.07
4	83.75	65.00	56.19	86.16	73.64	59.27	74.06	62.85	52.69
5	75.42	63.33	53.51	79.46	69.43	56.43	71.42	60.35	49.08
6	100.00	76.53	68.86	100.00	83.11	72.51	89.83	76.33	67.66
7	100.00	72.50	65.74	100.00	79.94	66.53	82.65	71.89	61.14
8	100.00	80.97	75.47	100.00	87.36	78.45	93.78	81.76	71.76
9	100.00	66.67	61.00	89.29	75.77	61.86	77.39	66.06	55.13
10	100.00	74.58	69.38	100.00	82.02	69.50	85.99	73.77	64.65
11	65.42	55.42	46.82	69.20	55.71	50.05	61.10	49.28	41.37
12	100.00	70.42	64.55	92.86	77.85	64.70	80.02	69.39	57.52
13	62.92	53.33	45.11	62.05	53.62	45.53	56.45	46.71	38.55
14	65.42	57.08	47.37	72.32	58.97	53.73	64.95	56.60	43.57
15	60.92	51.67	40.44	58.48	50.50	43.56	49.87	43.80	36.74

Figure 3 shows the efficacy of treatment recorded, treatments Revycare⁸, Priaxor EC⁶, Input 460 EC² and Nativo Forte 280 EC⁷ showed

high efficacy in controlling the pathogens *Z. tritici* and *B. graminis*, recording the highest efficacy values, between 78.7% and 81.30%.

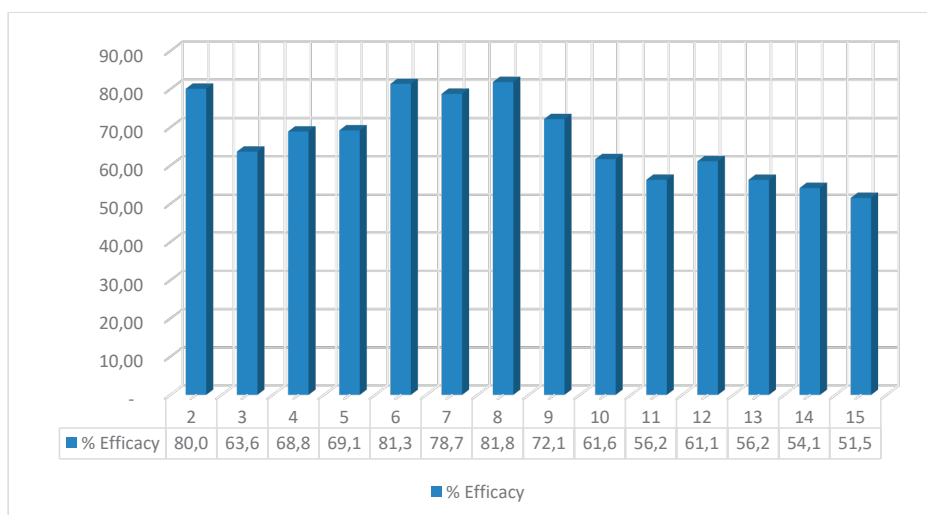


Figure 3. Average efficacy (%) of foliar treatment applied on autumn wheat against *Zymoseptoria tritici* and *Blumeria graminis* at A2 (07.05.2025), A3 (24.05.2025), and A4 (04.06.2025) moments

The timing of fungicide application—specifically determining the optimal moment—is the most critical factor in plant protection. In wheat, fungicide applications are concentrated on specific developmental stages: T1 is applied between the first and second nodes (BBCH 31-32); T2 occurs at flag leaf emergence (BBCH 37-39); and T3 is applied at head emergence (BBCH 49-59) (Borwing et al., 2011).

The T1 application is of particular importance as it significantly reduces disease progression by arresting it at the basal leaves. This protects the upper leaves and extends the time window before they become infected. Data from Tables 6 and 8 demonstrate a notable difference in pathogen severity recorded on the flag leaf and Leaf 2. The most effective treatments yielded control values between 80.02% and 90.14% for *Zymoseptoria tritici*, and 80.45% to 93.78% for *Blumeria graminis*. Conversely, the least effective treatments showed significantly lower control ranges, falling between 47.37% and 60.57% for *Z. tritici* and 49.87% to 61.10% for *B. graminis*.

The T2 application is considered the most critical link in the protection strategy for winter wheat. This intervention directly targets the protection of the flag leaf, which is responsible for approximately 43-45% of the total photosynthesis required for grain filling. Maintaining the green integrity of this leaf by controlling pathogens such as *Z. tritici* and

Puccinia spp. is essential for maximizing yield potential (Loyce et al., 2008).

The importance of these interventions is underscored by the fact that the top three leaves generate nearly 70% of the total grain yield through their photosynthetic contribution. Specifically, the flag leaf contributes 43%, Leaf 2 contributes 23%, and Leaf 3 contributes 7%. Additionally, the green area of the ear (head) contributes a further 22% to the final yield. Consequently, applying fungicides at these key moments of leaf development provides the greatest contribution to overall disease protection and crop productivity (Poole & Arnaudin, 2014).

CONCLUSIONS

The strategic management of foliar diseases in winter wheat depends critically on the precision of fungicide application timing, correlated with the key developmental stages of the crop. The present study demonstrates that the success of phytosanitary protection is ensured by the synergy between the T1 and T2 stages, each playing a decisive role in securing the yield. Applying fungicides at these optimal moments is not merely a biological control measure, but an economic necessity. The significant differences in efficacy recorded between the various treatment options underscore the fact that choosing the correct timing and product

guarantees yield stability and a high return on investment

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IMPACT OF PD-04_DR-02_P4 GREEN COVER CROPS ON THE PRODUCTIVITY OF MAIZE HYBRIDS

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Abstract

*This study assessed the agronomic and environmental effects of pea (*Pisum sativum* L.) green cover crops, implemented under the PD-04_DR-02_P4 agri-environmental scheme of the Romanian Strategic Plan (PNS 2023-2027), on drought-tolerant maize hybrids cultivated in the semi-arid Dobrogea region. Field trials (2024-2025) at Săcele, Constanța County, used two Pioneer AQUAmax® hybrids: P8834 (FAO 360) and P9903 (FAO 390), to evaluate the contribution of legume-based cover crops to soil improvement and yield stability. The 2025 season was extremely dry (124 mm rainfall), resulting in severe water stress. Plots previously cultivated with pea produced 25-30% higher yields (1.10-1.30 t ha⁻¹) than the control, due to improved soil friability, 10-12% higher NDVI, and enhanced biological nitrogen input (≈ 30-40 kg N ha⁻¹). The early hybrid P8834 achieved yields comparable to P9903, confirming its drought-escape advantage. These findings demonstrate that integrating pea cover crops with drought-tolerant maize hybrids strengthens soil structure, increases water retention, and stabilizes yield under extreme drought. The results validate the PD-04 eco-scheme as a cost-effective measure for climate-smart agriculture in southern Romania and similar semi-arid regions.*

Key words: biological nitrogen fixation, cover crops, Dobrogea, drought, maize hybrids, PD-04_DR-02_P4.

INTRODUCTION

Climate change, soil degradation, and water scarcity have become major constraints for sustainable crop production across South-Eastern Europe. In Romania, the Dobrogea region is particularly exposed to these environmental pressures due to its semi-arid continental climate, characterized by high interannual variability of rainfall and frequent summer droughts (Niță et al., 2023). Average annual precipitation rarely exceeds 400 mm, while evapotranspiration often surpasses 800 mm, creating a chronic soil water deficit that severely limits maize (*Zea mays* L.) productivity (Barna & Mănescu, 2025).

The increasing frequency of extreme weather events such as prolonged dry spells, heat waves, and wind erosion, accelerates soil organic matter losses and reduces fertility (Kaye & Quemada, 2017; Scavo et al., 2022). Modern maize cultivation systems increasingly rely on climate-resilient hybrids and adaptive tillage and fertilization strategies to cope with such stress factors (Botoș et al., 2025). According to

Moraru, Ene & Badilă (2020), the decline in soil humus content in southeastern Romania correlates with reduced water-storage capacity and deteriorated soil physical structure, demanding integrated agronomic, ecological and policy interventions.

Recent studies analyzing Romanian crop farms before the implementation of the National Strategic Plan (2023-2027) revealed that crop rotations were highly variable and often limited in legume inclusion, emphasizing the need for integrating environmentally beneficial practices such as cover crops (Stoicea et al., 2023).

Within the framework of the Common Agricultural Policy (CAP 2023-2027), Romania has introduced new eco-schemes under the National Strategic Plan (PNS) to incentivize environmentally friendly practices. One such measure is the PD-04 agri-environmental scheme, which promotes the establishment of green cover crops on arable land during winter to reduce erosion, enhance soil organic carbon, and improve biodiversity. Farmers who implement PD-04 commit to maintaining a living vegetal cover between September and

February, terminating it mechanically before 31 March, and refraining from herbicide or ploughing during this period. This measure is fully aligned with the EU Soil Strategy for 2030, aiming to restore degraded soils and increase climate resilience (European Commission, 2021). Despite the wide promotion of PD-04 and other eco-schemes across Romania, field-level evidence of their agronomic performance under real drought conditions remains limited. However, quantitative field-based assessments combining PD-04-compliant cover crops with drought-tolerant maize hybrids under extreme water limitation are still scarce for semi-arid regions of southeastern Europe. Evaluating such measures in semi-arid regions like Dobrogea is therefore essential for validating their practical relevance and economic viability. This study also contributes to the field of land structuration, emphasizing how green cover crops and reduced tillage practices reshape soil physical properties and support sustainable land use under semi-arid conditions.

Cover crops can mitigate drought stress and stabilize maize yields under limited rainfall conditions (Hunter et al., 2021). Among cover crop species, leguminous plants such as pea (*Pisum sativum* L.) are particularly valuable. Pea (*Pisum sativum* L.) is a major leguminous crop with high agronomic value due to its nitrogen-fixing capacity and adaptability to temperate climates (Podea & Cristea, 2023). They provide biological nitrogen fixation, improve soil porosity, and stimulate microbial activity. In addition, legume residues accelerate nutrient cycling and support carbon sequestration, mitigating greenhouse gas emissions (Kaye & Quemada, 2017; Thorup-Kristensen et al., 2020; Scavo et al., 2022). Legume-based cover crops improve soil fertility, nutrient cycling, and overall plant nutrition in temperate and Mediterranean climates (Scavo et al., 2022). Several studies confirm that pea and vetch cover crops enhance water infiltration and retention capacity in light soils under Mediterranean and continental dry climates (Ward et al., 2012; Gabriel et al., 2012; Krstić et al., 2018; Ziche et al., 2024).

In this context, maize cultivation in Dobrogea faces dual constraints: limited rainfall and declining soil fertility. To maintain profitability, farmers increasingly adopt drought-tolerant

hybrids and conservation agriculture practices such as minimum tillage and cover crops. Among available maize hybrids, Pioneer P8834 (FAO 360) and Pioneer P9903 (FAO 390), both from the AQUAmax® series, are specifically designed for drought and heat tolerance. According to Corteva Agriscience (2024), P8834 exhibits high water-use efficiency and early maturity, allowing partial escape from summer drought, while P9903 combines strong root architecture with excellent grain potential under moderate stress. Recent advances in remote sensing (e.g., UAV-based NDVI monitoring) enable quantitative assessment of crop response to cover-crop management under stress conditions, offering valuable insights for precision agriculture.

Under these conditions, identifying agronomic practices that simultaneously improve soil functionality and yield stability is essential for maintaining maize profitability in drought-prone farming systems. This study therefore aimed to evaluate the effect of a pea green cover crop, managed under PD-04 provisions, on the productivity of two AQUAmax® maize hybrids under semi-arid field conditions in Săcele, Constanța County (Dobrogea region). The research also sought to quantify the potential contribution of cover crops to soil moisture conservation, nitrogen enrichment, and overall yield stability in a drought-prone environment.

MATERIALS AND METHODS

The field experiment was conducted to assess the agronomic effects of pea (*Pisum sativum* L.) green cover crops on maize hybrids under PD-04_DR-02_P4 management conditions in southern Romania. The experiment was carried out in 2024-2025 on a private farm in Săcele, Constanța County (Dobrogea). The area has a semi-arid continental climate (mean $T \approx 11^{\circ}\text{C}$, long-term rainfall ≈ 400 mm). The 2025 season was extremely dry, totaling 124 mm rainfall (Barna & Manescu, 2025). The soil is a calcareous chernozem, medium-textured, slightly alkaline (pH 7.6) (Moise & Lungu, 2013). Similar pedoclimatic conditions and drought-tolerant crop management strategies were previously described for southeastern Romania (Bănică & Marin, 2025).

Consequently, the 2025 season provided a highly restrictive water regime, ideal for evaluating the adaptive response of hybrids and the role of green cover crops in mitigating drought stress. Real-time field data (Figure 1) validated the strong water deficit and justified the low yield potential observed. Such conditions are characteristic of the semi-arid Dobrogea region, where seasonal rainfall frequently falls below 300 mm. Climatic data were retrieved from the local agrometeorological station (ogor.ro platform), confirming the extreme drought conditions (Figure 1).



Figure 1. Drought monitoring data for the maize growing season (April-September 2025) obtained from ogor.ro (Săcele, Constanța County). The total rainfall was 124 mm, with only 1 mm recorded in June

The trial followed a bifactorial randomized block design (Hybrid $\times$ Treatment) with three replications. Each experimental plot had a surface area representative of on-farm conditions, ensuring the applicability of results under practical farming systems:

- Factor A (Hybrid): Pioneer P8834 (FAO 360) and Pioneer P9903 (FAO 390)
 - Factor B (Treatment): Control - no cover crop; Pea (*Pisum sativum* L.) green cover
- Pea was sown at the end of September 2024 (180 kg ha⁻¹) and mechanically incorporated in late February 2025 per PD-04 rules. Maize was sown on mid-April 2025 at 70 000 plants ha⁻¹. Basal

fertilization: NPK 15-15-15 (200 kg ha⁻¹) and ammonium nitrate 33.5 % N (150 kg ha⁻¹) at V6 stage. Minimum tillage and uniform crop protection were applied.

Measured parameters: stand uniformity (V3), SPAD/NDVI (V6–VT), ear length, kernels per ear, 1000-kernel weight, and yield (at 14 % moisture). NDVI and SPAD measurements were used as non-destructive indicators of crop vigor and nitrogen status under drought conditions. Data were analyzed by two-way ANOVA (Hybrid $\times$ Treatment) and LSD test ($p \leq 0.05$). This methodological approach allowed the integrated assessment of crop performance, soil-related effects, and hybrid response under extreme water limitation.

RESULTS AND DISCUSSIONS

The 2025 maize season was characterized by extremely low rainfall (124 mm), with only 1 mm recorded in June, the critical flowering period, leading to severe drought stress and a clear differentiation among treatments and hybrids. The pea green cover improved soil friability, early rooting, and canopy greenness. Soils beneath cover crops retained more surface moisture and showed better aggregation, consistent with observations by Ward et al. (2012), Gabriel et al. (2012), and Krstić et al. (2018).

Beyond the observed yield response, the beneficial effect of the pea (*Pisum sativum* L.) green cover crop can be explained by specific soil physical and biological mechanisms. Leguminous cover crops develop a dense and finely branched root system that penetrates compacted soil layers and creates continuous biopores. After root decay, these biopores remain functional pathways for water infiltration, gas exchange, and early maize root elongation. Under semi-arid conditions and minimum tillage systems, such biologically induced porosity plays a key role in improving root-soil contact and facilitating access to deeper soil moisture reserves, contributing to greater crop stability under drought stress, as also reported for maize systems transitioning from conventional to minimum tillage in Dobrogea (Stroe & Panaitescu, 2025).

In addition to root-related effects, the incorporation of pea biomass supplied residues

characterized by a low C:N ratio, stimulating microbial activity and enhancing soil aggregate stability. Nitrogen-rich residues favor the production of microbial binding agents that increase cohesion between soil particles, reducing surface crusting and improving soil friability. Similar improvements in soil physical condition under reduced tillage and climate

stress were observed in maize cropping systems from southeastern Romania, where biological inputs partially compensated for the reduction of mechanical soil disturbance (Risnoveanu et al., 2023; Stroe et al., 2025). These soil structural improvements translated into measurable differences in maize yield components and grain yield, are presented in Table 1.

Table 1. Effect of pea green cover crop on maize hybrid performance under semi-arid conditions (Săcele, Constanța, 2025)

Hybrid	Treatment	Ear length (cm)	Kernels per ear	1000-kernel weight (g)	Grain yield (t/ha, 14%)
Pioneer P8834	Control	11.8	540	280	0.85
	Pea cover	12.6	580	295	1.10
Pioneer P9903	Control	14.2	610	300	1.00
	Pea cover	15.1	650	310	1.30

On average, the use of pea green cover increased yield by $27 \pm 3\%$, ear length by 6%, and kernel weight by 5%. These improvements are consistent with those reported by Coombs et al. (2017) and Scavo et al. (2022), who found similar yield benefits of legume-based cover crops under Mediterranean drought conditions.

Effect of the cover crop on maize development.

Both hybrids emerged uniformly; however, plots sown after the pea (*Pisum sativum* L.) green cover crop showed visibly better soil tilth, darker surface color, and fewer crusted areas, suggesting improved infiltration and water retention. These qualitative field observations were reflected in the higher grain yields recorded in pea-cover treatments for both hybrids, as illustrated in Figure 2.

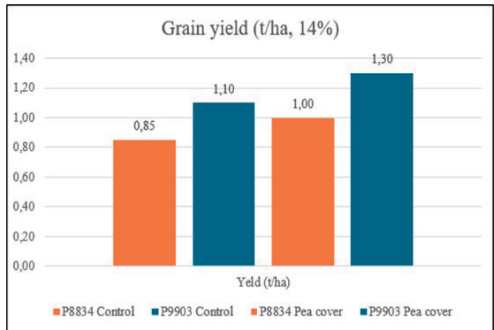


Figure 2. Grain yield response of maize hybrids (Pioneer P8834, P9903) to pea green cover under semi-arid conditions (Săcele, 2025)

The PD-04-compliant cover was incorporated mechanically in late February, allowing partial mineralization of biologically fixed nitrogen before maize sowing.

Hybrid P8834 (FAO 360, AQUAmax®) demonstrated faster early growth and earlier flowering (approx. 5-6 days earlier than P9903), which helped it avoid the peak summer drought. Hybrid P9903 (FAO 390, AQUAmax®) maintained a larger vegetative structure but experienced partial kernel abortion under the prolonged dry period of July-August.

In both cases, the residual nitrogen and improved soil structure from pea biomass contributed to higher SPAD and NDVI values (+8-10%) at the V6 stage compared to the control.

The presence of cover crop residues on the soil surface during early spring further contributed to limiting soil water losses through evaporation, particularly during the V3-V6 maize growth stages. Partial soil shading and reduced temperature fluctuations helped conserve moisture at the soil surface, which is critical for early canopy establishment under semi-arid conditions. Enhanced early vegetative development is known to positively influence kernel formation and thousand kernel weight, especially in short-season hybrids exposed to climatic stress (Mircea et al., 2023). These physiological and developmental responses were reflected in increased ear length, a higher number of kernels per ear, and greater thousand

kernel weight in pea-cover treatments for both hybrids, as shown in Figures 3, 4, and 5.

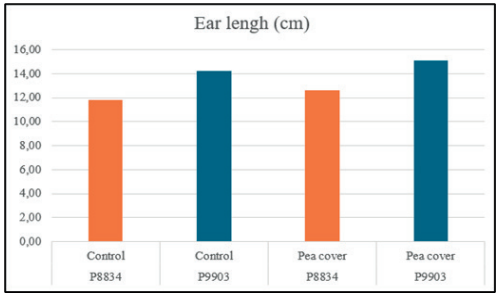


Figure 3. Ear length (cm) of maize hybrids (Pioneer P8834, P9903) (Săcele, 2025)

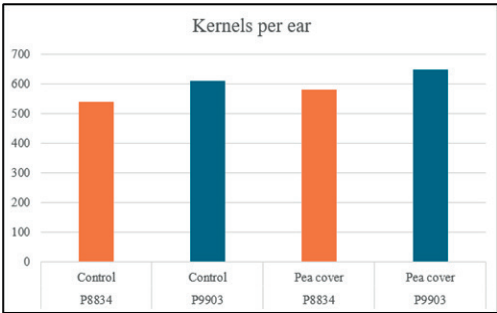


Figure 4. Kernels per ear of maize hybrids (Pioneer P8834, P9903) (Săcele, 2025)

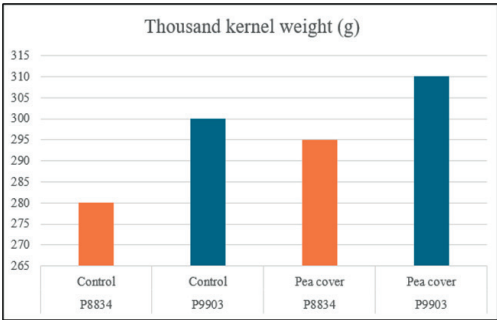


Figure 5. Effect of pea cover crop on 1000-kernel weight

The pea cover increased yields by 25-30%, primarily through higher kernel weight and improved grain filling. Although hybrid P9903 has a higher yield potential under favorable conditions, its advantage was limited under severe drought stress. In contrast, the early-maturing hybrid P8834 achieved comparable yields, confirming its drought-escape efficiency and high water-use performance under restrictive moisture conditions.

The incorporation of pea biomass ($\approx 30\text{--}40 \text{ kg N ha}^{-1}$) enriched the soil with biologically fixed nitrogen, improved soil porosity, and contributed to yield stabilization, in agreement with previous findings reported by Kaye and Quemada (2017), Coombs et al. (2017), and Scavo et al. (2022). Significant positive correlations were observed between grain yield and NDVI ($r = 0.89$), as well as between yield and thousand kernel weight ($r = 0.86$), indicating that canopy greenness and kernel development are reliable indicators of drought resilience and yield potential under semi-arid field conditions. Cover crops thus acted as effective yield stabilizers, improving soil structure and water-retention capacity even under extreme drought conditions. Similar responses have been documented in Mediterranean and continental dryland systems (Ward et al., 2012; Krstić et al., 2018; Ziche et al., 2024). The magnitude of the yield advantage observed in this study ($\approx 0.3 \text{ t ha}^{-1}$) is consistent with results reported for legume-based cover crops under water-limited environments and highlights the capacity of PD-04-compliant practices to deliver agronomic benefits alongside environmental compliance.

These results emphasize the complementary effects of short-season maize hybrids and leguminous cover crops in maintaining yield stability under limited rainfall. The yield advantage can be largely attributed to the nitrogen credit supplied by pea biomass and improved soil moisture conservation during early maize growth stages, when crop sensitivity to water stress is particularly high.

Moreover, PD-04-compliant management practices, maintaining the cover crop over winter and incorporating it mechanically before 31 March, proved fully compatible with the maize cropping calendar in Dobrogea, without delaying sowing or crop establishment.

From a land structuration perspective, the consistent improvements in soil friability, aggregate stability, and biological porosity observed in pea-cover treatments confirm that legume-based green cover crops can partially substitute mechanical soil structuring traditionally achieved through intensive tillage. Biological structuring driven by root development, residue decomposition, and microbial activity enhances soil resilience under repeated drought cycles, supporting sustainable

land management in degraded dryland agroecosystems. The positive effect was particularly evident for the early hybrid P8834, which avoided peak drought periods due to its shorter vegetative cycle. Overall, these findings confirm that the integration of PD-04-compliant green cover crops contributes not only to soil fertility and drought mitigation but also supports the EU CAP 2023-2027 objectives for sustainable agriculture in semi-arid regions.

Under the extreme pedoclimatic conditions recorded in 2025, this study provides field-level evidence that the agronomic benefits of legume-based green cover crops persist even under severe water limitation. By combining drought-tolerant maize hybrids with PD-04-compliant soil management practices, it was possible to achieve measurable yield stabilization and improved soil functionality, reinforcing the role of eco-schemes as practical tools for climate-adaptive agriculture in semi-arid regions.

CONCLUSIONS

The 2025 maize season in Dobrogea was characterized by extreme pedological drought, with only 124 mm of rainfall during the crop cycle. Under such restrictive conditions, the use of pea (*Pisum sativum* L.) green cover crops, managed according to the PD-04 agri-environmental scheme, enhanced soil structure, plant vigor, and grain yield. Beyond yield improvement, pea green covers significantly contributed to land structuration by reinforcing aggregate stability and biological porosity. These effects promote erosion resistance, infiltration efficiency, and long-term soil health under repeated drought cycles. Yield increases of 25-30% were recorded in pea-cover plots, mainly due to improved soil water retention and biological nitrogen input.

Between the tested hybrids, Pioneer P8834 (FAO 360, early-maturing AQUAmax®) demonstrated superior adaptability and yield stability compared with P9903, confirming the advantage of short-season genotypes under severe water stress conditions.

Overall, the results demonstrate that integrating legume-based green cover crops with drought-tolerant maize hybrids represents a viable and cost-effective strategy for stabilizing production in semi-arid regions. The findings provide field-

based evidence that PD-04-compliant practices can deliver both agronomic and environmental benefits under real farm conditions, supporting their wider adoption within the CAP 2023-2027 framework. Further research should focus on quantifying long-term impacts on soil organic carbon dynamics, nutrient cycling efficiency, and farm-level economic performance to strengthen decision-making for sustainable dryland agriculture.

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EFFICACY AND SELECTIVITY OF THE HERBICIDES EXPRESS 50 SG AND PANTERA 40 EC ON SUNFLOWER

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Abstract

In 2024 and 2025, a field trial was conducted with ExpressSun® sunflower, hybrid P64 LE185. The experiment was set up in a production field of the Agricultural University-Plovdiv, Bulgaria. The herbicides Express 50 SG (500 g/kg tribenuron-methyl) for the control of dicotyledonous weeds and Pantera 40 EC (40 g/l quizalofop-P-tefuryl) for the management of monocotyledonous weeds were tested. The herbicides used were applied vegetatively, in the phenophase of the fourth-sixth leaf of the crop (BBCH 14-16). Weeding of the experimental field was represented by: *Sinapis arvensis* L., *Amaranthus blitoides* L., *Datura stramonium* L., *Convolvulus arvensis* L., *Setaria glauca* L. and *Sorghum halepense* (L.) Pers. Weeding with the established weeds is the reason for obtaining a very low average yield from the untreated control (1.05 t ha⁻¹). Herbicidal efficacy was reported on the 10-point EWRS scale. The highest biological grain yield was obtained after the use of Express 50 SG applied in combination with Pantera 40 EC. The 9-point EWRS scale was used to determine the selectivity of the herbicides.

Key words: sunflower, weeds, herbicides, efficacy, selectivity.

INTRODUCTION

Providing the necessary resources for the food sector is one of the key challenges of modern society. In this context, agricultural crops play a leading role as the main source of raw materials. This determines the increased scientific interest and the conduct of numerous studies related to the introduction of new technological solutions aimed at the successful production of agricultural crops (Ahmad et al., 2025; Dulaimy et al., 2025; Thuleau et al., 2025; Tourneur et al., 2025; Panayotov et al., 2024; Panchev & Shopova, 2024; Shevchenko et al., 2024; Balabanova et al., 2023; Önemli & Tetik, 2023; Rankova et al., 2023; Shopova, 2023; Fernández-Aparicio et al., 2022; Komitov et al., 2020; Dimtrova et al., 2019; Marinov-Serafimov et al., 2017; Manolov et al., 2015; Shopova & Cholakov, 2015; Yanev, 2015; López-Bellido et al., 2014; Shopova & Cholakov, 2014; Yanev et al., 2014a). Sunflower is one of the most important crops in Bulgaria and is grown mainly after winter wheat in non-irrigated farming systems. Because sowing is most often done from mid-March to mid-April, this crop is very often weeded by a multispecific weed flora, including dicotyledonous and

monocotyledonous weeds (Fried et al., 2006). Herbicides are a central tool for chemical control in weed management systems. The selection of an appropriate herbicide product is essential as it should meet a number of criteria, including selectivity for the crop, high efficacy against weeds, and safety with respect to crop quality and soil health (Parven et al., 2025; Shopova & Yanev, 2025; Szulc et al., 2025; Yanev, 2025; Yanev, 2024; Yanev & Shopova, 2024; Yanev et al., 2024; Chipomho et al., 2023; Dos Santos et al., 2023; Stefanic et al., 2023; Yanev, 2023; Yakhymuk et al., 2023; Goranovska et al., 2022; Yanev, 2022; Pacanoski & Mehmeti, 2021; Yucel & Saglam, 2021; Yanev, 2021; Mandal et al., 2020; Yanev, 2020; Yanev & Kalinova, 2020; Martinez et al., 2018; Goranovska & Yanev, 2016; Kostadinova et al., 2016; Hristeva et al., 2015; Kalinova & Yanev, 2015; Semerdjieva et al., 2015; Hristeva et al., 2014; Yanev et al., 2014b; Brighenti et al., 2012; Zabaloy et al., 2011; Penescu & Ciontu, 2001). According to research by Pannacci et al. (2007) in sunflower fields the weed flora consisted mainly of *Amaranthus retroflexus* L., *Chenopodium album* L., *Sinapis arvensis* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Polygonum aviculare* L., *Polygonum persicaria* L.,

Heliotropium europaeum L., *Xanthium strumarium* L., and *Lolium multiflorum* L. According to the same author, after treatment with aclonifen and quizalofop-p-ethyl, applied alone, the same types of weeds are not controlled, respectively, while after application of the two herbicides in a tank mixture, good results are reported. Herbicides are part of integrated weed control in sunflower, which is why research is needed on their safe use (Delchev, 2020; Jocić et al., 2011). In recent years, in conditions of increasing drought, serious problems have arisen in the use of soil herbicides, and therefore foliar herbicides are increasingly being used (Delchev, 2021; Zand et al., 2009). Other problems are related to the efficacy and selectivity of foliar herbicides applied under these conditions (Delchev et al., 2021). To reduce the costs of weed control, combined application of herbicides with different mechanisms and spectrum of action is often necessary (Kudsk, 2002). The possibility of growing oilseed sunflower hybrids tolerant to sulfonylurea herbicides contributes to the ease of cultivation of this crop by farmers. Tkalic et al. (2022) investigated the efficacy of the herbicide tribenuron-methyl in combination with the adjuvant Trend 90 in sunflower. They found that the addition of the adjuvant improved the uniformity of herbicide coverage on the plant surface and increased efficacy against sensitive dicotyledonous weeds. Many scientists are studying the efficacy of individual and combined use of herbicides, contributing to the possibility of every farmer to obtain a productive, clean and economical crop (Ciontu et al., 2003; Popescu et al., 1990). Good selectivity towards the hybrid *P64 LE25* was demonstrated when applied in a tank mixture of the herbicides Express 50 SG (500 g/kg tribenuron-methyl) - 40 g ha⁻¹ + Stratos Ultra (100 g/l cycloxydim) - 2.0 l ha⁻¹ (Manilov and Zhalnov, 2018). Regarding selectivity, during foliar treatment of sunflower with aclonifen, slight phytotoxicity can be observed, which is later completely overcome (Pannacci et al., 2007), which has also been found by other scientists such as Mirkovic and Regnault (1996). Although sunflower is relatively more weed-tolerant than most other row crops, yield losses due to weed infestation remain significant (Ivanova &

Neshev, 2023; Neshev, 2022; Ivashchenko et al., 2018; Popescu, 1995).

The aim of the study was to establish the efficacy and selectivity of the herbicide Express 50 SG, applied alone and in a tank mixture with the herbicide Pantera 4 EC to sunflower, hybrid P64 LE185.

MATERIALS AND METHODS

In 2024 and 2025, a field experiment with oilseed sunflower hybrid P64 LE185 (Express Sun®) was conducted at the Agricultural University - Plovdiv, Bulgaria.

The experiment was set up using the block method in 4 replications with a total size of each variant of 112 m².

Before the introduction of herbicides, a survey of the experimental field was carried out. In the reporting field, we identified 7 types of weeds, of which 5 are dicotyledonous and 2 are monocotyledonous. The average density of weeds from the two experimental years per 1 m² is as follows: *Sinapis arvensis* L. - 17.5 pieces; *Amaranthus blitoides* L. - 10 pieces; *Datura stramonium* L. - 5.5 pieces, *Solanum nigrum* L. - 5 pieces; *Convolvulus arvensis* L. - 5.5 pieces; *Setaria glauca* L. - 6 pieces and *Sorghum halepense* (L.) Pers. - 5 pieces.

The experiment included the following variants: 1. Untreated control; 2. Express 50 SG (500 g/kg tribenuron-methyl) - 0.04 kg ha⁻¹ + Trend 90 (adjuvant) - 0.1%; 3. Express 50 SG - 0.04 kg ha⁻¹ + Pantera 40 EC (40 g/l quizalofop-P-tefuryl) - 1.75 l ha⁻¹; 4. Express 50 SG - 0.04 kg ha⁻¹ + Pantera 40 EC - 1.75 l ha⁻¹ + Trend 90 - 0.1%; 5. Two times hoed-up (manual); 6. Express 50 SG - 0.08 kg ha⁻¹ + Trend 90 - 0.2%; 7. Express 50 SG - 0.08 kg ha⁻¹ + Pantera 40 EC - 3.50 l ha⁻¹; 8. Express 50 SG - 0.08 kg ha⁻¹ + Pantera 40 EC - 3.50 l ha⁻¹ + Trend 90 - 0.2%. At lower doses of use of the products, the herbicidal efficacy was determined visually, using the 10-point scale of EWRS (European Weed Research Society). Doubly increased doses were tested to determine possible manifestations of phytotoxicity, using the 9-point scale of EWRS for this purpose.

The herbicides were applied in the 4-6th leaf phase of the sunflower.

The treatment was carried out using a knapsack sprayer with a working solution of 200 l ha⁻¹.

The predecessor of the sunflower experiment was wheat in both years of the study. The area was prepared by deep plowing to a depth of 30 cm, as well as by two cultivations, the first at 12 cm and the second at 8 cm. In spring, pre-sowing fertilization with combined fertilizer (NPK 15:15:15) was carried out with a fertilizer rate of 250 kg ha⁻¹, which was done before the last cultivation. Sowing was carried out at the optimal time of the crop with a "Vaderstad Tempo T6" seeder at a row spacing of 70 cm, with a density of 52000 pcs. germinating seeds per hectare.

Assessment of efficacy against weeds was carried out on the 14th, 28th and 56th days, and to determine the selectivity of herbicides to sunflower on the 7th, 14th and 28th days after their application.

The results for sunflower yields were statistically processed using the Duncan method.

RESULTS AND DISCUSSIONS

In both years of the experiment, on average, the product Express 50 SG had very high to excellent herbicidal efficacy against *Sinapis arvensis* L. (Table 1), reported on the 56th day after its application (95-100%). Here we report a very small difference in the efficacy of the broadleaf herbicide Express 50 SG applied with the adjuvant Trend 90 (100%) compared to its combined use with the product Pantera 40 EC (95%). Despite the lower herbicidal effect (70-75%) reported on the 14th day after treatment, the weed is relatively easily controlled by the herbicide Express 50 SG.

Table 2 shows the dynamics of efficacy against *Amaranthus blitoides* L. For the two years of the experiment, the average percentage of efficacy reported on the fourteenth day after treatment with the herbicide Express 50 SG + Trend 90 is 60%. When applying the same product in a tank mixture with Pantera 40 EC, but without an adjuvant, the efficacy is slightly lower (50%). Although in both variants the herbicidal effect increases on the next two reporting dates, in the combined use of the herbicides, where there is no adjuvant, the efficacy reaches 80%, in the last reporting after

treatment with the products. The percentages of efficacy in the latter variant are close to those reported in the variant treated with Express 50 SG + Trend 90, which showed that when mixing the herbicides and adjuvant, the efficacy does not change significantly.

Table 1. Herbicidal control (%) against *S. arvensis*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	75	95	100
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	70	90	95
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	70	90	100

Table 2. Herbicidal control (%) against *A. blitoides*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	60	80	100
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	50	70	80
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	55	75	95

Table 3. Herbicidal control (%) against *D. stramonium*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	50	70	90
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	40	60	70
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	45	65	85

Table 3 presents the action of the tested herbicides on average for the two years of the experiment against the weed *Datura stramonium* L. The highest percentage of efficacy recorded on the 14th day after the introduction of the products is only 50%. Regarding the increase in the percentages of efficacy at the individual reporting dates on the 56th day, they vary in the range from 70 to 90%. The best result was recorded with the application of Express 50 SG + Trend 90, and the lowest with the variant where we have a combination of the two herbicides Express 50 SG + Pantera 40 EC without adjuvant.

Table 4 shows the dynamics of the herbicidal efficacy against *Solanum nigrum* L. reported on the 14th, 28th and 56th day after treatment. It is noteworthy that with Express 50 SG + Trend 90 and Express 50 SG + Pantera 40 EC + Trend 90 the herbicidal effect on all three reporting dates is almost the same. Only in the variant in which the adjuvant Trend 90 is not applied do we report a relatively lower herbicidal efficacy on all reporting dates. The efficacy reaches only 85% on the 56th day after the application of the herbicides. This proves that the absence of Trend 90 is the reason for the reduction of the herbicidal effect against the weed.

Table 4. Herbicidal control (%) against *S. nigrum*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	65	90	100
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	45	70	85
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	60	85	100

Against *Convolvulus arvensis* L., we report zero efficacy in all three herbicide treatments in the experiment (Table 5).

Table 5. Herbicidal control (%) against *C. arvensis*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	0	0	0
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	0	0	0
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	0	0	0

With the combined use of the herbicides Express 50 SG and Pantera 40 EC on the 14th day after treatment, we report 55% efficacy against the weed *Setaria glauca* L., while with the variant with added adjuvant Trend 90 the herbicidal effect is 65% (Table 6). On the second reporting date, the efficacy in both variants progressively increases and reaches 85% and 95%, respectively. At the last reporting made on the 56th day after the application of the herbicides, the efficacy increases again and reaches 95% in the variant

in which no adjuvant Trend 90 was used. The control over the weed is excellent when we have added adjuvant (100%). From the use of Express 50 SG + Trend 90 we report zero efficacy, since the herbicide has an broad spectrum of action.

Table 6. Herbicidal control (%) against *S. glauca*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	0	0	0
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	55	85	95
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	65	90	100

Table 7. Herbicidal control (%) against *S. halepense*, average for the period

Variants	Days after treatments		
	14	28	56
Untreated control	-	-	-
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	0	0	0
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	60	80	90
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	70	90	100

Against the monocotyledon weed *Sorghum halepense* (L.) Pers. the results of the herbicide used are reflected in Table 7. Only in the variant treated with the herbicides Express 50 SG + Pantera 40 EC + adjuvant Trend 90 we report excellent efficacy of the order of 100% at the last reporting date. The herbicidal effect against this weed is entirely due to the product Pantera 40 EC, which has a completely monocotyledonous spectrum of action. Despite the fact that at the first reporting date in the variants with the application of the herbicide we report from 60 to 70% efficacy against the weed, it progressively increases and reaches from 90 to 100% at the last reporting date. The product Express 50 SG has a completely broadleaf spectrum of action and cannot control the monocotyledon weed.

The tolerance of each plant species to a specific herbicide is within certain limits. When the dose exceeds the recommended limits, negative effects may occur. In the overwhelming number of cases, the presence of a negative effect is judged by the visual manifestations, which may be different, but most often are

chlorosis, necrosis, growth retardation and retardation, and others (Vassilev and Tonev, 2015). No visual manifestations of phytotoxicity were recorded for sunflower, hybrid P64 LE185 (Table 8) from provocatively increased (double) doses of the herbicides Express 75 SG and Pantera 40 EC during the two experimental years in any of the variants.

Table 8. Visual phytotoxicity reading results (score)

Variants	2024	2025
Two times hoed-up (manual)	-	-
Express 50 SG - 0.08 kg ha ⁻¹ + Trend 90 - 0.2%	1	1
Express 50 SG - 0.08 kg ha ⁻¹ + Pantera 40 EC - 3.50 l ha ⁻¹	1	1
Express 50 SG - 0.08 kg ha ⁻¹ + Pantera 40 EC - 3.50 l ha ⁻¹ + Trend 90 - 0.2%	1	1

Table 9. Sunflower grain seed yield, t ha⁻¹

Variants	Yields
Untreated control	1.05a
Express 50 SG - 0.04 kg ha ⁻¹ + Trend 90 - 0.1%	2.31*b
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹	2.40*b
Express 50 SG - 0.04 kg ha ⁻¹ + Pantera 40 EC - 1.75 l ha ⁻¹ + Trend 90 - 0.1%	2.82*c
Two times hoed-up (manual)	2.90*c
Express 50 SG - 0.08 kg ha ⁻¹ + Trend 90 - 0.2%	2.46*b
Express 50 SG - 0.08 kg ha ⁻¹ + Pantera 40 EC - 3.50 l ha ⁻¹	2.81*c
Express 50 SG - 0.08 kg ha ⁻¹ + Pantera 40 EC - 3.50 l ha ⁻¹ + Trend 90 - 0.2%	2.91*c

Legend: All variants with * are significantly different from the untreated control. Values followed by different letters (a, b and c) are significantly different according to Duncan's test at P < 0.05.

Table 9 presents the results of the yields of the individual variants of sunflower grown using the Express Sun technology. The differences in yields are predetermined by the herbicidal efficacy of the products and their ability to control the weeds present in the experiment. The natural background of weeding with highly competitive weed species during the two years of the experiment led to a low average yield of the untreated control (1.05 t ha⁻¹). By the degree of mathematical proof, three separate groups are distinguished here for the different variants (a, b, c). It is also noticeable here that the variants treated with Express 50 SG + Pantera 40 EC + Trend 90, the two times hoed-up control and Express 50 SG - 0.08 kg ha⁻¹ + Pantera 40 EC - 3.50 l ha⁻¹ are from group (c),

the furthest from the untreated control group (a), that is, with the highest yields, followed by the other treated variants.

CONCLUSIONS

The herbicide Express 50 SG applied with the adjuvant Trend 90 has excellent efficacy (100%) against the weeds *S. arvensis*, *A. blitoides* and *S. nigrum*. A relatively high herbicidal effect (90%) is achieved against the weed *D. stramonium*.

Express 50 SG does not control the weed *C. arvensis*, despite the fact that it is applied with Trend 90.

The herbicide Pantera 40 EC has very high (90%) to excellent efficacy (100%) against the weeds *S. glauca* and *S. halepense*.

In the sunflower hybrid *P64 LE185*, no visible signs of phytotoxicity were observed from the combined use of the higher tested double doses of Express 50 SG, Pantera 40 EC and Trend 90. The highest average yield of 2.91 t ha⁻¹ sunflower was obtained with the tank mixture of Express 50 SG - 0.08 kg ha⁻¹ + Pantera 40 EC - 3.50 l ha⁻¹ + Trend 90 - 0.2%.

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RESEARCH ON THE BIOLOGY AND PRODUCTIVITY OF THE *Phacelia tanacetifolia* Benth. SPECIES IN THE CLIMATE CONDITIONS OF CENTRAL MOLDOVA

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Abstract

Phacelia is used in several climatic regions, and this illustrates its adaptability to different climates and soil types. This species is an annual herbaceous, non-leguminous, flowering plant of the Hydrophyllaceae family. It is also a versatile crop, widely used in the USA and Europe, both in crop rotation and as a source of nectar for bees. The main objective of the present research was to study the biology and productivity of the species *Phacelia tanacetifolia* Benth. with the aim of understanding its adaptability to the climatic conditions in Central Moldova in the period 2023-2024. *Phacelia*, depending on the sowing season, reaches maturity between 85-97 days from sowing, and the optimal time for harvesting is when the inflorescences have dried and the seeds have a brown color. Under the conditions at A.R.D.S. Secuieni the highest seed production was obtained with the variant sown in the first decade of April of 415 kg/ha and at a distance of 50 cm between plants/row (395 kg/ha).

Key words: *phacelia*, epoch of sowing, seed.

INTRODUCTION

Lacy phacelia (*Phacelia tanacetifolia* Benth.), a versatile plant of North and Central American origin, is now widely cultivated in many regions around the world (Kirk, 2000).

It is among the world's top twenty melliferous plants, providing monofloral honey with excellent quality for human consumption (Farkas and Zajacz, 2007; Kus et al., 2018; Stanek et al., 2019). It also supports wild pollinators and some biological control agents with pollen and nectar (Williams and Christian, 1991; Hickman and Wraten, 1996; Petanidou, 2003; Sprague et al., 2016; Owayss et al., 2020). As a cover crop and green manure, it is beneficial for soil structure (Bacq-Labreuil et al., 2019) and soil microorganisms (Patkowska and Konopinski, 2013); nevertheless, its effect on weed infestation has been assessed with different outcomes. In Switzerland, the

experiments of Büchi et al. (2020) indicated that previous use of phacelia as a cover crop reduced weed abundances in the subsequent planting of maize; however, in Poland, the trials of Gaweda et al. (2014) and Kolodziejczyk (2015) suggested that phacelia cover crop caused higher weed infestations in the subsequently grown cereals, and that it also resulted in yield loss in potato, when it was sown for living mulch between rows. Phacelia is potentially used in various agricultural practices. It is used as forage and silage in livestock feeding and as a source of pollen and nectar in beekeeping. It has significant contributions to ecosystem balance by improving the habitats of beneficial insects and supporting biological control activities. Phacelia is known as a great nectar source by beekeepers worldwide and is listed among the top 20 nectar plants of the world (Lermi and Palta, 2017). Currently, in many countries, agriculture is required to use non-chemical

methods to control diseases, pests, and weeds (Kubiak et al., 2022). Many studies indicate that phacelia is exceptionally resistant to diseases and pests. Moreover, after harvesting, it constitutes a valuable preceding crop for most crop species. Its species specificity combined with its allelopathic properties, has gained recognition among both proponents of high-volume technologies and those seeking low-volume technologies (Kosolapov et al., 2021).

All these data indicate the high potential of phacelia in both economic and ecological dimensions, particularly in providing various ecosystem services such as pollination support, erosion control, and carbon sequestration. In light of recent assumptions, agricultural production should pursue several goals at the same time. In addition to producing healthy food, sustainable agriculture should mitigate climate change, positively affect soil fertility, and provide renewable energy (Zarczynski et al., 2023; Mackiewicz-Walec et al., 2024; Sienkiewicz et al., 2024). Lacy phacelia is drought-resistant (Schappert et al., 2019) and grows well even in areas with annual precipitations of approximately 200-500 mm. It prefers lighter, well-aerated soils with a good supply of nutrients. The influence of sowing period on plant development has been studied, for example in winter wheat (Fazily, 2021), where a reduction in grain yield was observed with later sowing periods (Ali et al., 2010). The influence of sowing period on the growth and development of agricultural crops has also been studied for soybeans (Bastidas et al., 2008) and cotton (Khan et al., 2017). The effect of sowing period on the vegetation period was also described by Bowie et al. (1995), Thrasyvoulou and Tsirakoglou (1998), or Lermi and Palta (2017). Karadağ and Büyükburç (2001) determined that the highest plant height, fresh and dried hay yield, seed yield and biological yield were obtained at a row spacing of 40 cm. Karadağ and Büyükburç (2003) investigated the effects of four different sowing periods (March 5, March 20, April 5 and April 20) on hay yield in fiddleneck as well as on various other agricultural properties thus showing that sowing periods have significant effects on fresh and dried hay yields while also stating that the highest values were obtained for March 5 sowing period.

This study was conducted to determine the effects of sowing period on the growth parameters of the phacelia plant, taking into account the influence of cultivation techniques on plant development.

The purpose of the research carried out during the 2023-2024 period was to determine the influence of meteorological conditions on the biology, yield and quality of phacelia seeds in the center of Moldova region.

MATERIALS AND METHODS

The Agricultural Research and Development Station (A.R.D.S.) Secuieni is located in the southeastern part of Neamț County, between the geographical coordinates of 26°51'00" east longitude and 46°51'15" north latitude. From an agro-ecosystem point of view, the territory belongs to the Central Moldavian Plateau, and from a morphostructural point of view, most of it is part of the Moldavian Platform. The area where the unit is located has a temperate continental climate (D.f.b.x. Köppen), characterized by short springs, cool summers and harsh winters, with an average annual temperature of 10.1°C and average annual precipitation of 537 mm (Trotuş et al., 2020). Under the conditions of A.R.D.S. Secuieni three sowing periods were tested: V1 (control) - sown in the first decade of April; V2 - sown in the second decade of April; V3 - sown in the third decade of April.

To establish the optimal planting space, a bifactorial experiment was set up using a split-plot design with the following factors: A - row spacing, with the following distances: a1 - 25 cm, a2 - 50 cm and a3 - 70 cm and B - plant spacing with the following distances: b1 - 15 cm, b2 - 25 cm and b3 - 35 cm.

During the phacelia vegetation period, phenological observations were conducted, and the thermal sum requirements for growth and development of the studied species were also determined. From a thermal point of view during the lacy phacelia growing season, in 2023 there were monthly deviations from the multiannual average between -1.5°C (April) and 2.2°C (July). The spring was cool and the summer months were very warm (June and August). In 2024, the lacy phacelia growing season was warm, with monthly deviations from

the multiannual average ranging from 3.6°C (April) to 4.3°C (August) (Figure 1).

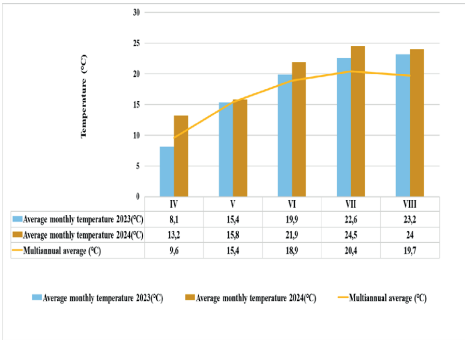


Figure 1. Temperatures recorded during the lacy phacelia growing season in the 2023-2024 period

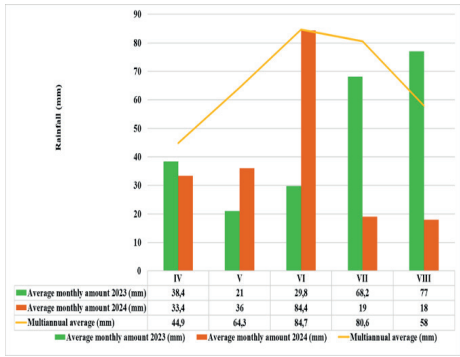


Figure 2. Precipitation recorded during the lacy phacelia growing season in the 2023-2024 period

With regard to the rainfall recorded during the lacy phacelia growing season, in 2023 there were monthly deviations from the multi-annual monthly amount between -54.9 mm (July) and 19.0 mm (August). The lacy phacelia growing season in 2024 was characterized as dry. The recorded precipitation had deviations from the multiannual average between -61.6 mm (July) and -11.5 mm (April). In June, precipitation values close to the multiannual average were recorded (Figure 2). Overall, the precipitation recording in 2023 characterized the phacelia growing season as dry (Naie et al., 2024). Meteorological data were obtained from the unit's own weather station, located in the experimental field of A.R.D.S. Secuieni.

RESULTS AND DISCUSSIONS

In the case of the species *Phacelia tanacetifolia* Benth. sown in first decade of April, on average over two years, the plants emerged after 14 days from sowing, accumulating a thermal sum of 138.5°C and a total precipitation of 15.6 mm. From emergence to the appearance of the flowering stem, 29 days were recorded, requiring a thermal sum of 462.4°C and 26.6 mm of precipitation (Table 1).

From the appearance of the flowering stem to the beginning of flowering, 12 days were required, with a recorded of thermal sum of 554.7°C and 27.2 mm of precipitation (Table 1).



Figure 3. *Phacelia tanacetifolia* in A.R.D.S. field

The seeds were harvested 20 days after the beginning of fruiting, accumulating 798.7°C and 22.4 mm of precipitation (Table 1).

From sowing to harvest, plants sown in the first period required an average of 97 days, with a thermal sum of 2532.6 °C and 146.1 mm of precipitation.

Due to the higher temperatures during the vegetation period, the plants sown in the second and third periods showed a faster development. In the second sowing period, from sowing to seed harvesting, 95 days were required, the thermal sum was 2312.6°C and the precipitation amounted to 139 mm (Table 1).

During the third sowing period, the plants had a vegetation period of 87 days to reach maturity. The thermal sum recorded for this period (2023-2024) was 2268.1°C and the precipitation amounted to 121.4 mm (Table 1).

Table 1. Growth phenophases of the species *Phacelia tanacetifolia* Benth. under the conditions of A.R.D.S. Secuieni during the 2023-2024 period

Phenological observations	Average growing season duration during the 2023-2024 period (days)	Σ average temperature (°C)	Σ average precipitation (mm)
Sowing period I			
Sowing	-	-	-
Emergence	14	138.5	15.6
The appearance of flowering rods	29	462.4	28.6
The beginning of blooming	12	554.7	27.2
The beginning of fructification	22	578.3	52.3
Harvesting for seeds	20	798.7	22.4
TOTAL	97	2532,6	146.1
Sowing period II			
Sowing	-	-	-
Emergence	11	101.3	20.2
The appearance of flowering rods	36	506.6	28.8
The beginning of blooming	8	509.1	36.9
The beginning of fructification	21	601.8	25.4
Harvesting for seeds	17	593.8	26.7
TOTAL	95	2312.6	139.0
Sowing period III			
Sowing	-	-	-
Emergence	10	135.4	13.2
The appearance of flowering rods	33	512.1	30.4
The beginning of blooming	15	477.9	29.4
The beginning of fructification	17	606.1	23.4
Harvesting for seeds	10	536.6	25.0
TOTAL	85	2268.1	121.4

Table 2. Influence of the interaction between row spacing and plant spacing on average seed yield of *Phacelia tanacetifolia* Benth. (phacelia) during the 2023-2024 period

Row spacing (A)	Plant spacing (B)	Yield (kg/ha)	%	Diff.	Significance
a1-25 cm	b1-15 cm	313	90,46	-33	oo
	b2-25 cm	338	97,69	-8	
	b3-35 cm	354	102,31	8	
a2-50 cm	b1-15 cm	341	98,55	-5	
	b2-25 cm	395	114,16	49	***
	b3-35 cm	373	107,80	27	**
a3-70 cm	b1-15 cm	358	103,47	12	
	b2-25 cm	340	98,27	-6	
	b3-35 cm	300	86,71	-46	ooo
Average		346	100	Ct.	
		LSD 1% 16.4 kg/ha LSD 0,1% 26.1 kg/ha LSD 5 % 43.2 kg/ha			

Under the conditions at A.R.D.S. Secuieni, the interaction of the studied factors influenced the average seed yield of the lacy phacelia species. Compared to experimental average (346 kg/ha), yield increases ranging from 27 to 49 kg/ha were recorded, being statistically significant and interpreted as distinctly and highly significant

positive effects. The interaction a2xb2 (50 cm row spacing and 25 cm between plants within the row) with an average yield of 395 kg/ha was noted (Table 2).

The average seed yield ranged from 353 kg/ha for the variant sown in the IIIrd decade of April to 415 kg/ha for the variant sown in the Ist

decade of April (control). Compared to the control variant, yield reductions were statistically confirmed and interpreted as distinctly and highly significant negative effects, and were obtained for the variants sown in the second period (33 kg/ha) and the third period (62 kg/ha) (Table 3).

Tabel 3. Average seed yield of lacy phacelia depending on the sowing period at A.R.D.S. Secuieni during the 2023-2024 period

Variant	Yield			
	kg/ha	%	Diff.	Sign.
V1-I st decade of April	415	100	Ct.	
V2-II nd decade of April	382	92.04	-33	oo
V3-III rd decade of April	353	85.06	-62	ooo
	LSD 5% 12.5 kg/ha LSD 1% 24.6 kg/ha LSD 0.1% 38.7 kg/ha			

CONCLUSIONS

In Central Europe, *Phacelia tanacetifolia* is more appreciated as a honey plant and as a source of valuable biomass for soil regeneration. The above-average nutritional value of phacelia pollen prompts researchers to identify an easy and effective way to support not only the honeybee breeding but also a number of other pollinators. The average yield of lacy phacelia varies widely depending on climatic conditions, the sowing period and the cultivated varieties. It is recommended to sow lacy phacelia in the first decade of April with a row spacing of 50 cm. Ensuring the nutrition space is very important for obtaining high yields in lacy phacelia. Seed yields of lacy phacelia are strongly influenced by the experimental sowing periods.

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CHARACTERISTIC OF MORPHOBIOLOGICAL AND ECONOMICALLY VALUABLE TRAITS OF WINTER TRITICALE VARIETIES IN THE TRANSITIONAL-CONTINENTAL REGION OF BULGARIA

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Abstract

A comparative study of modern Bulgarian and Polish winter triticale varieties over four growing seasons was conducted, after a predecessor of sunflower under unfertilized conditions on Pelic Vertisol. The length of the growing season and the days to heading were characterized based on the number of days with active temperature above 5°C, as well as the variability of productivity and the elements of productivity: plant height, number of grains in a spike and mass of 1000 grains. The results on average for the period showed that the Colorit variety had the shortest growing season (163 days) and the shortest period from sowing to heading (112 days). The duration of the growing season was longest for Casino variety (169 days). The period from sowing to heading was the longest for Avocado and Casino varieties (119 days). Environmental conditions had a dominant effect on yield, number of grains per spike and plant height, while the variety had a dominant effect on thousand-grain mass. The Casino and Avocado varieties demonstrated the best results in terms of thousand-grain mass and grain yield, while the Colorit variety showed the highest number of grains per spike.

Key words: elements of productivity, grain yields, length of the growing season.

INTRODUCTION

Among the winter cereals grown in Bulgaria, triticale is of great interest. In essence, triticale (x *Triticosecale* Wittmack) is an amphiploid hybrid between wheat and rye. According to data from the Ministry of Agriculture for 2024, in the Southeast region, where the Institute of Field Crops is located, the areas of wheat (246,672 ha) dominate, followed by barley (47,678 ha), triticale (5,993 ha) and rye (2,437 ha) (MZH). The area of triticale and rye is the largest in the Southeast region (28.9%), compared to the total area occupied in Bulgaria. Its multifunctional applications characterise it as a valuable crop. The usable parts of triticale are the grain, flour, bran, and green biomass, and it can be fractionated to obtain various components, including starch, dietary fibre, and protein, which can be used for both food and non-food applications (Kamanova et al., 2023). Researchers have found that growing winter triticale is more efficient than wheat in years with anomalous climatic conditions (Lyatamborg et al., 2023). Triticale's grain yield advantage was attributed to its higher grain number per spike and its superior biomass

allocation and reproductive efficiency (Miroslavljević et al., 2025).

The main task in agricultural production is the introduction of high-yielding varieties into production. Obtaining high yields of triticale is directly related to the interaction of the genotypes used with environmental conditions. A study presented a mathematical model for quantitative assessment of the response of individual triticale varieties to the environment and the degree of yield obtained (Stoyanov, 2021). It is believed that the agronomic characteristics of crops (such as grain yield, plant height, thousand-grains mass, flowering date, etc.) are particularly valuable. Other accompanying traits are also useful, including number of grains in spike and length of the growing season. The number of grains in the spike is the main trait that characterizes spike productivity in terms of mass. Grain size, expressed as thousand-grain mass, is a characteristic that determines the fullness of the grain and affects germination and viability, as well as productivity potential. This trait is of fundamental importance for seeding management and is relevant in determining yield in a particular crop year. Grain number and

thousand-grain mass complement each other functionally, ensuring optimal grain productivity (Stoyanov, 2017). Analysis has shown that thousand-grain mass and grain number per spike differ significantly in their stability among the studied varieties and according to different stability indices (Stoyanov, 2025). Therefore, key agronomic traits should be monitored and reported for the benefit of future triticale breeding strategies and to support farming.

The aim of the study: A comparative characteristic of biological and economically valuable traits of modern Bulgarian and Polish winter triticale varieties, grown in transitional-continental region of Bulgaria.

MATERIALS AND METHODS

The work was carried out on the experimental field of the Institute of Field Crops, Chirpan, during the period 2019-2023. Eight Bulgarian triticale varieties included in the Official Variety List of Bulgaria (Colorit, Acord, Dobrudzhanets, Doni-52, Borislav, Blagovest, Irmic and Lovchanetz (IASAS)), and two Polish varieties (Avocado and Casino), admitted to the Polish national list (Coboru), were used. The triticale varieties were grown after a predecessor of sunflower on Pelic Vertisol. The cultivation technology follows the adopted standard technology for winter cereal crops. In order to assess the real potential of the varieties under specific environmental conditions, as well as the natural course of the growing season, the experiment was carried out under unfertilized conditions in three repetitions.

Sowing was carried out using a selection seeder. The seeds were sown in the following periods: November 5, 2019; November 15, 2020; October 10, 2021; and October 18, 2022. The seeding rate was 550 seeds per m². The phenological phases of heading and full ripeness were recorded according to the BBCH scale (Zadoks et al., 1974). The phase of the beginning of ear emergence was noted when approximately 10% of the plants had reached this stage (49-51 BBCH). The duration of the periods is based on the number of days with an active temperature above 5 °C. Laboratory tests to determine the number of grains per spike were carried out according to the accepted methodology. From two harvest plots of each

option, ten spikes were collected at the phase of full maturity, after which the grains were separated and weighed. The determination of thousand-grain mass (g) was performed in accordance with BDS EN ISO 520: 2010. Grain harvest was carried out at full maturity (89-92 BBCH). Yield was collected from the three repetitions and converted to hectares basis. Meteorological data were provided by the field synoptic station located on the territory of Institute of Field Crops.

The results were processed using the software product Biostat (Penchev et al., 1989-1991). The means were compared for significance using the least significant difference (LSD) test at $p = 5.0$, $p = 1.0$, and $p = 0.1\%$.

RESULTS AND DISCUSSIONS

The meteorological conditions during the four growing seasons differed in precipitation and temperature but were generally favorable for the growth and development of triticale (Figure 1). During the 2020-2021 and 2021-2022 growing seasons, precipitation exceeded the multi-year average by 41.7 and 54.7 mm, respectively. Very wet months such as January 2021, October 2021, and December 2021 were recorded, with precipitation exceeding normal levels by 64.2, 103.6, and 38.8 mm, respectively.

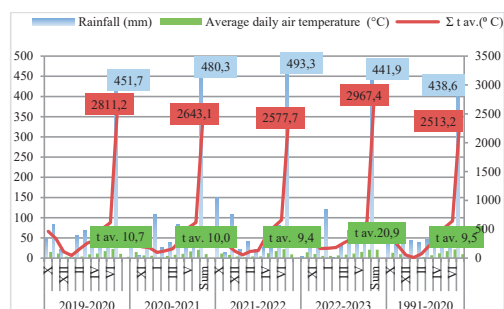


Figure 1. Meteorological data during the growing seasons of triticale

The total air temperature from October to June in 2019-2020 was 297.9°C above the multi-year average, and in 2022-2023 it was 454.2°C. This resulted in an 11.4°C higher average temperature for the last period. Winter periods were warm, due to higher average temperatures in the months of December, January and February. During the fourth period, the average

temperatures these months were 3.1, 4.2, 3.3 and 5.8°C, respectively, which is 1.3, 2.4, 1.5 and 4.0°C higher than the calculated 30-year average (1.8°C).

Table 1 presents the parameters of a descriptive characterization based on four years of data for the varieties. The analysis showed that the coefficient of variation ranged between 11.0 and 12.40% for the length of the growing season. According to this indicator, the varieties did not differ significantly. The shortest period was observed for the Colorit variety (163.3 days), and the longest for Casino (169 days), five days later than the Colorit standard. The Casino variety deviated the most from the others, according to SD = 21.0. For comparison, in Iraq, a growing season of 163–164 days was reported for triticale, and the period from sowing to harvesting was 116 days (Mohammed & Mohammed, 2014).

Heading date is recognized as an important adaptive trait in crop development: a short duration of the pre-anthesis phase in early cultivars and delayed anthesis in late cultivars significantly influence the grain number per spike in different species/spike types, reducing the final grain yields (Miroslavljević et al.,

2018). The date of ear heading significantly affects productivity components contributing to increased grain yield in winter wheat (Tsenov et al., 2020).

The heading phase occurred at different times after sowing, mainly due to the influence of environment temperature. The coefficient of variation indicates average variability and a roughly similar period to heading among the varieties ranging from 19.8 to 22.8% (Table 1). The earliest heading was observed in the Colorit variety (112 days). A comparative study of triticale in the temperate continental zone reported 125 days to heading for Colorit and 128 days for Doni-52, averaged over nine years (Stoyanov et al., 2022). The Polish varieties Casino and Avocado showed a significantly longer period (118.8 and 119 days), seven days later than Colorit, with the difference significant at $p = 0.1$ %. The Bulgarian varieties Acord and Irnik also significantly exceeded the standard for this indicator, with 117.3 and 115.8 days, respectively. A study in Pakistan revealed that the duration from sowing to harvest of triticale varied from 88.8 to 109.3 days, and the total growing season from 120.3 to 142.5 days, depending on sowing time (Khattak et al., 2016).

Table 1. Main variability parameters for the duration from sowing to full ripnesse and from sowing to heading in varieties, with reference to Colorit variety

Varieties	Number of days from sowing to fully ripe			Number of days from sowing to heading		
	lim	$\bar{x}_{av.}$	CV %	lim	$\bar{x}_{av.}$	CV %
Colorit	138–180	163.3	11.0	84–138	112.0	19.8
Avocado	138–185	164.3 ^{ns}	11.9	89–147	118.8 ^{***}	20.1
Acord	138–184	165.0 ^{ns}	12.0	86–151	117.3 ^{**}	22.8
Borislav	138–184	164.0 ^{ns}	11.9	84–144	115.0 ^{ns}	21.3
Blagovest	138–184	164.8 ^{ns}	11.8	84–140	113.5 ^{ns}	20.2
Doni-52	138–185	164.0 ^{ns}	12.1	86–145	114.3 ^{ns}	21.2
Dobrudzhanets	138–184	164.5 ^{ns}	11.8	84–140	113.3 ^{ns}	20.4
Irnik	138–184	164.8 ^{ns}	12.0	86–145	115.8 [*]	20.8
Casino	138–184	169.0 ^{**}	12.4	89–148	119.0 ^{***}	20.3
Lovchanets	138–184	165.0 ^{ns}	11.9	85–140	114.8	19.9
LSD %	$p = 5.0$	3.2		$p = 5.0$	3.4	
	$p = 1.0$	4.3		$p = 1.0$	4.7	
	$p = 0.1$	5.7		$p = 0.1$	6.2	

^{**}, significant at $p = 1\%$; ^{***} significant at $p = 0.1\%$;

A slight to moderate variation in values was also observed for the next trait - plant height (CV = 1.9–12.5%) (Table 2). The data on the average crop height for the tested period showed an increase of 0.4% to 0.8% compared to control, but this difference was not significant. On average over four years, the tallest plants were recorded for Acord and Doni-52 varieties reaching 115.2 cm. A significantly lower value

(86.9% to the control) was recorded for the Casino variety (99.3 cm).

The best results in terms of the thousand-grains mass were obtained for the Avocado, Acord, Blagovest and Casino varieties, with values higher by 12.7, 6.1, 6.7 and 12.6%, respectively, compared to Colorit. For the Blagovest variety, this indicator showed moderate variation over the years, CV = 13.3%.

Table 2. Main variability parameters for plant height and thousand grain mass in varieties, with reference to the Colorit variety

Varieties	Plant height (cm)			Thousand-grains mass (g)		
	lim	$\bar{x}_{av.}$	CV %	lim	$\bar{x}_{av.}$	CV %
Colorit	101.0–134.5	114.3	12.5	35.78–40.37	38.18	5.3
Avocado	102.4–125.3	114.8 ^{ns}	8.3	40.09–46.59	43.04 ^{***}	7.1
Acord	101.1–128.0	115.2 ^{ns}	9.6	38.24–42.34	40.52 ^{***}	4.7
Borislav	101.0–127.3	113.1 ^{ns}	10.6	37.60–40.69	39.43 ^{***}	3.8
Blagovest	103.7–126.7	114.8 ^{ns}	8.2	34.74–46.24	40.73 ^{***}	13.3
Doni-52	105.9–125.7	115.2 ^{ns}	7.1	38.08–40.61	39.20 ^{**}	2.9
Dobrudzhanets	106.5–121.6	113.4 ^{ns}	5.8	37.07–44.28	39.49 ^{***}	8.2
Irnik	105.7–124.7	114.8 ^{ns}	7.6	36.23–40.06	38.05 ^{ns}	4.2
Casino	96.7–101.2	99.3 ⁰⁰⁰	1.9	39.55–47.84	43.00 ^{***}	9.3
Lovchanets	106.9–120.5	112.5 ^{ns}	5.3	34.79–40.20	37.73 ^{ns}	6.1
LSD %	$p = 5.0$	2.3		$p = 5.0$	1.3	
	$p = 1.0$	3.1		$p = 1.0$	1.8	
	$p = 0.1$	4.0		$p = 0.1$	2.6	

^{**}, significant at $p = 5\%$; ^{*}, significant at $p = 1\%$; ^{***} significant at $p = 0.1\%$; ⁰⁰⁰ significant at $p = 0.001$; ^{ns}, non-significant

The most variable trait during the study period was the number of grains per spike (Table 3). Variation in the number of grains per spike indicates a strong genetic influence on the components of grain yield. In addition, the number of grains is strongly influenced by meteorological conditions during the pollination and fertilization of flowers. This statement is supported by the data in Table 4, which shows a high influence of the environment (48.90%). The values of all varieties are significantly lower than the control, with the standart variety Colorit in the first position, averaging 74.1 grains. Dobrudzhanets showed the highest variability among the varieties regarding the number of

grains per spike (27.1%), while the lowest variability was observed for Acord (6.6 %) and Casino (6.9 %). Avocado, Borislav and Doni-52 were approximately homogeneous for this characteristic. High CVs has been reported, on average over the years, for the number of grains per spike and thousand-grains mass - 34.21% and 25.55%, respectively-for another cereal-wheat amphidiploid (Stoyanov, 2017). The results of Triputin et al. (2020) showed average variability for the number of the grains per spike and thousand-grains mass in winter triticale of 19.1% and 14.7%, respectively, whit no such variability reported for plant height.

Table 3. Main variability parameters for grain number per spike and grain yield in varieties, with reference to the Colorit variety

Varieties	Number of grains in spike			Grain yields (kg/ha)		
	lim	$\bar{x}_{av.}$	CV %	lim	$\bar{x}_{av.}$	CV %
Colorit	62.7–52.0	74.1	12.1	4,196.0–5,850.0	4,956.0	17.1
Avocado	48.0–77.4	62.0 ⁰⁰⁰	19.6	4,272.4–7,538.0	5,348.0 ^{**}	27.2
Acord	53.4–62.1	59.0 ⁰⁰⁰	6.6	3,870.0–6,231.3	4,675.0 ^{ns}	23.5
Borislav	51.5–80.1	65.4 ⁰⁰⁰	20.0	2,307.2–6,846.0	4,666.0 ^{ns}	45.0
Blagovest	55.8–68.0	60.6 ⁰⁰⁰	9.6	3,375.0–6,866.0	5,140.0 ^{ns}	28.8
Doni-52	45.6–74.0	61.4 ⁰⁰⁰	20.9	2,921.4–6,852.0	5,001.0 ^{ns}	35.0
Dobrudzhanets	48.8–88.8	64.0 ⁰⁰⁰	27.1	2,453.7–7,110.0	4,983.0 ^{ns}	39.8
Irnik	48.0–74.1	62.9 ⁰⁰⁰	17.3	2,528.6–6,052.0	4,561.0 ^{ns}	32.9
Casino	48.7–67.5	60.2 ⁰⁰⁰	6.9	1,893.1–6,820.0	5,196.0 ^{ns}	44.6
Lovchanets	49.4–78.3	62.5 ⁰⁰⁰	19.1	3,501.7–5,962.1	4,977.0 ^{ns}	24.1
LSD %	$p = 5.0$	3.9		$p = 5.0$	285.3	
	$p = 1.0$	5.3		$p = 1.0$	378.4	
	$p = 0.1$	6.9		$p = 0.1$	490.0	

^{**}, significant at $p = 1\%$; ⁰⁰⁰ significant at $p = 0.001$; ^{ns}, non-significant

The yield characteristics revealed a significance difference in the yield among the varieties Borislav, Doni-52, Dobrudzhanets, Irnik, and Casino, with the coefficient of variation ranging from 32.9 to 45.0%. The yield of the same varieties showed similar average productivity

and approximate uniformity, with the coefficient of variation ranging from 15.06 % to 26.47%, as reported in a study by Stoyanov (2025). In another study, Doni-52 reliably exceeded all standards and the average standard by 11.9% over a 9-year period, indicating the exceptional

productivity and stability of the variety (Stoyanov et al., 2022). The varieties Avocado, Blagovest, and Casino achieved the highest grain yield, exceeding the control by 7.9, 3.7, and 4.9%, respectively. The result for the Avocado variety was statistically significant at $p = 1.0\%$. According to another study, Polish varieties, regardless of environmental conditions, significantly outperformed Bulgarian triticale varieties in terms of productivity, with Blagovest, Avocado, and Casino being note for their high, complex stability (Stoyanov, 2024). Among the Bulgarian varieties, Doni-52 also demonstrated a high grain yield of 0.7%, though this is not confirmed.

According to the data in Table 4, the variety's effect on yield formation was not significant (2.54%), whereas environmental conditions had a substantial influence (80.11%). In contrast, the results of Jańczak-Pieniążek and Kaszuba (2024) showed a significant influence of both genotype and environment on the grain yield of triticale. Other studies have also confirmed that growing conditions affect both the productivity

of winter triticale and the mass of 1000 grains (Kyrylchuk et al., 2023). For example, under sandy soils and irrigation conditions, contrasting with the conditions of the present study, grain yield ranged from 4310 to 4604 kg/ha, with 1000-grain weights ranging from 34.5 to 41.7 g. The vegetation period lasted from 225 to 228 days, the number of grains per spike ranged from 32.6 to 41.3, and the plant height varied from 86.3 to 102.6 cm (Draghici, 2012). The low effect of the variety $\times$ environment interaction (13.52%) indicated a wide variation in yield values over the years. In other environmental conditions, similarly low interactions of environment $\times$ genotype (14.00 %), genotype (11.13 %), and environment (56.43 %) were reported (Stoyanov, 2025).

Other studies confirmed the effect of the variety on yield but found no statistically significant influence on the grain yield and 1000-grain mass due to the strong effect of nitrogen fertilization (Lalević et al., 2022). The impact of environments factors accounted for 92.6% of all variation, compared to 2.3% for genotype and 4.46% for their interaction, as reported in a study by Kendal (2024).

Table 4. Effect of cultivar (G) and environment conditions (E) on key agronomic traits

Source of variation	PH		TGM		NGS		GY	
	df	η (%)	df	η (%)	df	η (%)	df	η (%)
Total	79	100.0	79	100.0	79	100.0	119	100.0
Blocks	1	5.444806E-02	1	0	1	0.62	2	0.25
Options	39	97.16***	39	98.48***	39	93.10***	39	96.17***
G	9	23.51***	9	34.68***	9	13.80***	9	2.54***
E	3	54.71***	3	35.53***	3	48.90***	3	80.11***
G×E	27	18.94***	27	28.28***	27	30.40***	27	13.52***
Error	39	2.79	39	1.52	39	6.29	78	3.58

PH-plant height; TGM-tousand grains mass; NGS-number of grains per spike; GY-grain yield.

Table 5. Effect of cultivar (G) on the duration of phases from sowing to heading and from sowing to full ripeness

Source of variation	Days from sowing to heading				Days from sowing to fully ripe			
	df	SS	η (%)	MS	df	SS	η (%)	MS
Total	39	17311.13	100.0	-	39	11589	100.0	-
Blocks	3	16960.94	97.98***	5653.646	3	11371.38	98.12***	3790.458
Options	9	197.625	1.14**	21.95834	9	87	0.75	9.66667
G	9	197.625	1.14**	21.95834	9	87	0.75	9.66667
Error	27	152.5625	0.88	5.650463	27	130.625	1.13	4.837963

It is evident that the environment exerts a strong dominant influence on the remaining traits – the number of grains per spike and plant height, accounting for 54.71% and 48.90%, respectively. These results contradict the conclusions of Mirosavljević et al. (2018) who reported a very low environmental influence on the number of grains per spike (10.6%) and the

proportion of variance explained by variety across different cereal species (5.3%). In terms of the duration from sowing to harvesting and the growing season, it was found that the environment accounts for the highest portion of the variation (97.98% and 98.12%, respectively), while the genotype only 1.14%), as shown in Table 5.

CONCLUSIONS

The study monitored key agronomic and economic characteristics of ten modern winter triticale varieties over a four-year period, including the duration of the growing season, days from sowing to heading, plant height, number of grains per spike, mass of a thousand-grain mass, and grain yields. The variability of these traits was assessed, and average values were compared with those of the Bulgarian standard variety Colorit. The effects of genotype and environment on each trait were also evaluated.

Varietal differences were primarily observed in thousand-grain mass and days to heading, with Colorit being the earliest to head.

The varieties with the shortest and longest periods from sowing full maturity were identified, as well as those with the best agronomic indicators.

Avocado and Casino demonstrated the highest thousand-grain mass and yields.

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RESEARCH ON THE COMPETITIVENESS OF SOME PERENNIAL GRASS AND LEGUME SPECIES IN SIMPLE MIXTURES, UNDER THE CONDITIONS OF ARDS SECUIENI

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Abstract

The experiment was located within the ARDS Secuieni during the period 2023-2025. The aim of the research was to analyze the competition relationships between the species Phleum pratense L. and Trifolium pratense L. cultivated alone or in simple mixtures, under different fertilization conditions with complex mineral fertilizers. In the second year of vegetation, the RYT (Relative Yield Total) index was calculated, which recorded values >1 except for the unfertilized variant and sown with the mixture of Phleum pratense L. (25%) + Trifolium pratense L. (75%), showing that the species Phleum pratense L. and Trifolium pratense L. compete for the same vegetation factors. and the CR index (Competition Rate) for the species Phleum pratense L. was higher than for the species Trifolium pratense L., resulting in the grass species being more competitive.

Key words: mixtures of species, fertilization, RYT (Relative Yield Total), CR (Competition Rate).

INTRODUCTION

The composition of mixtures of perennial grasses and legumes, in the technology of establishing temporary meadows, represents the most important work on which the production potential of the meadow, the method of use, the fertilization system, as well as the economic duration of exploitation depend. The composition of the mixtures is made according to scientific criteria based on knowledge of the biology of species and varieties, relationships with environmental factors, use value and other economic characteristics.

For the establishment of temporary meadows, simple mixtures consisting of a small number of species are generally recommended.

The formation of mixtures of perennial grasses and legumes should be conditioned by the

biological properties of the species, in accordance with the use and duration of existence of temporary meadows (Maruşca T., 2001; Belesky D.P. et al., 2002; Moisa F. et al., 2018; Bozhanska T. et al., 2024). The ability of each species to compete will also be taken into account when composing mixtures of perennial grasses and legumes. This is a feature of the species, but it is also largely influenced by the pedoclimatic conditions and the mode of exploitation (Skinner R.H. et al., 2006; Lazaridou M., 2008; Saghin et al., 2011; Croitoru A. et al., 2012; Stanciu A.Ş. et al., 2015; Leonte A. et al., 2024).

The production capacity of temporary grasslands is influenced by fertilization, thus obtaining good quality forage (Salis L. et al., 2008; Zou Y. et al., 2010; Yu Y.W. et al., 2011; Ciobanu C., 2014).

Fertilization also leads to a change in the vegetation structure, with grass species usually being favored, and there is also a change in soil properties and soil microbial activity (Kleczek C., 1991; Rotar I., 1993; Rautio, P. et al., 2012; Delevatii L.M. et al., 2019; Simić A. et al., 2020; Mrázková M. et al., 2020).

Through research conducted during 2023-2025, at A.R.D.S. Secuieni, the competitiveness of the species *Phleum pratense* L. and *Trifolium pratense* L., cultivated alone or in simple mixtures, under different fertilization conditions with complex mineral fertilizers, was analyzed.

MATERIALS AND METHODS

The research was carried out during 2023-2025 at the A.R.D.S. Secuieni and aimed to analyze the influence of competition between *Phleum pratense* L. and *Trifolium pratense* L., cultivated in simple mixtures, on the forage production obtained. The biological materials used were the species *Phleum pratense* L. (timothy), the Rarău variety, homologated in 2002, created at the Agricultural Research and Development Station Suceava, and *Trifolium pratense* L. (red clover), the David Liv variety, homologated in 2015, created at the Agricultural Research and Development Station Livada.

In the spring of 2023, a bifactorial experiment, of the A×B type, was established, being placed in the field according to the method of subdivided plots, in three repetitions. The studied factors were: A - species and simple mixtures of species (perennial grasses and legumes) with five graduations: a₁ - 100% grasses (100% *Phleum pratense* L.); a₂ - 75% grasses (75% *Phleum pratense* L.) + 25% legume (25% *Trifolium pratense* L.); a₃ - 50% grasses (50% *Phleum pratense* L.) + 50% legume (50% *Trifolium pratense* L.); a₄ - 25% grasses (25% *Phleum pratense* L.) + 75% legume (75% *Trifolium pratense* L.); a₅ - 100% legume (100% *Trifolium pratense* L.); B - fertilization with three graduations: b₁ - N₀P₀K₀ (unfertilized); b₂ - N₃₀P₃₀K₃₀; b₃ - N₅₀P₅₀K₅₀. Sowing was done with the U650 tractor in combination with the Haldrup experimental field seeder (Photo 1).



Photo 1. Field placement of the experience at A.R.D.S. Secuieni

The soil on which the experiment was located was characterized as being well supplied in active humus (2.33 %), very well supplied in phosphorus (189 mg·kg⁻¹), potassium (304 mg·kg⁻¹), excessively supplied in Mg (253 mg·kg⁻¹) and Mn (369 mg·kg⁻¹), poorly supplied in nitrogen (9.4 mg·kg⁻¹ N -NO₃) and Zn (1 mg·kg⁻¹). The sown area of the experimental plot was 12 m², of which 10 m² was harvested. A complex N₁₅P₁₅K₁₅ fertilizer, was used for fertilization. Fertilization was done manually with the Easy Drop device in the first year when preparing the land, and from the second year, mineral fertilizers were administered when the plants started to grow (Photo 2).



Photo 2. Administration of complex fertilizers in the experimental field

The harvesting of the experiments was done using the BCS 615 lawn mower, at a height of 4-5 centimeters from the ground, and the experiment was of the stationary type.

In order to calculate the competitiveness of the species in the two years of study, the Relative Total Yield (RYT) index was calculated, which characterizes the species used in mixtures in terms of the ecological resources used, one in relation to the other, and was performed using equation (1) (Zuo, Y. et al., 2010). Depending on the result obtained, the following three situations can be encountered: $RYT > 1$, the species occupy different eco-niches; $RYT = 1$, the species use common resources; and $RYT < 1$, the species are in an antagonistic relationship.

$$RYT = Y_{AB} \times Y_{AA} - 1 + Y_{BA} \times Y_{BB} - 1$$

(Equation 1);

where:

Y_{AB} = QGM for species A, mixed culture;

Y_{AA} = QGM for species A, in pure culture;

Y_{BA} = QGM for species B, mixed culture;

Y_{BB} = QGM for species B, in pure culture;

QGM = Green mass production ($Mg \cdot ha^{-1}$).

The calculation of the Competition Rate (CR) index characterizes the species used in mixtures in terms of their mutual competitiveness and was performed using equation (2) (Zuo, Y. et al., 2010). Depending on the result obtained, the following three situations can be encountered: $CR > 1$, species A is more competitive than species B; $CR = 1$, the species are equally competitive; and $CR < 1$, species A is less competitive than species B.

$$CR = (Y_{AB} \times Y_{AA} - 1 \times Z_{AB}) \times (Y_{BA} \times Y_{BB} - 1 \times Z_{BA}) - 1$$

(Equation 2)

where:

Y_{AB} = QGM for species A, mixed culture;

Y_{AA} = QGM for species A, in pure culture;

Z_{AB} = proportion of species A and B in the mixture;

Y_{BA} = QGM for species B, mixed culture;

Y_{BB} = QGM for species B, in pure culture;

Z_{BA} = proportion of species B and A in the mixture;

QGM = Green mass production ($Mg \cdot ha^{-1}$).

RESULTS AND DISCUSSIONS

At the Agricultural Research and Development Secuieni Station in Neamț County, the species *Phleum pratense* L. and *Trifolium pratense* L.,

cultivated individually or in simple mixtures formed between them, under different fertilization conditions with nitrogen and phosphorus-based mineral fertilizers, were analyzed for their competitiveness, one in relation to the other, and the Relative Total Yield (RYT) index was calculated, which characterizes the species used in mixtures in terms of the ecological resources used. The Competition Rate (CR) index was also calculated, which characterizes the species used in mixtures in terms of their mutual competitiveness.

Analyzing the influence of the interaction between the studied factors (mixture $\times$ fertilization) on the interspecific relationships, on average over the two years, but also over the three scythes, it was found that the RYT index recorded values < 1 in all the studied mixture and fertilization variants, with the exception of the variant sown with the of *Phleum pratense* L. (25%) + *Trifolium pratense* L. (75%) mixture, unfertilized.

The value of the RYT index had values ranging from 0.95 in the mixture formed by *Phleum pratense* L. (25%) + *Trifolium pratense* L. (75%) unfertilized and up to 1.88 as recorded in the same mixture but in the variant fertilized with $N_{50}P_{50}K_{50}$. It was found that in all the studied mixtures the RYT index recorded the highest values in the variants that were fertilized with fertilizers of the $N_{50}P_{50}K_{50}$ type. This result indicates that for *Phleum pratense* L. and *Trifolium pratense* L., although they occupy different ecological niches, the resources were insufficient to express the high productive potential. The experience being in the second year of vegetation, the plants of the species *Trifolium pratense* L. have a well-developed underground part, are at their maximum productive potential and are therefore more competitive compared to the plants of the species *Phleum pratense* L.

The CR index was calculated for all the mixtures studied and it was found that it had values ranging from 3.53 to 19.20 for the species *Phleum pratense* L. and from 0.05 to 0.28 for the species *Trifolium pratense* L.. Thus, it is found that this index was higher for the species *Phleum pratense* L. than for the species *Trifolium pratense* L., the grass being more competitive. The variants in which *Phleum pratense* L. had

lower CR index values were those in which the participation percentage was 25%. In this case, *Phleum pratense* L. was considered to be poorly competitive.

As observed, the CR index values for *Trifolium pratense* L. were lower only in the variants with a participation of 25%, which demonstrates a weaker competitiveness compared to *Phleum*

pratense L. due to the lower number of individuals. The variants in which *Trifolium pratense* L. presented high values of the CR index were those in which the participation percentage was 75%. In these mixtures, *Trifolium pratense* L. was considered more competitive (Table 1).

Table 1. Competitive relationships between the species *Phleum pratense* L. and *Trifolium pratense* L.

Variant	1 st harvest			2 nd harvest			3 rd harvest			TOTAL		
	RYT	CR <i>Ph.p.</i>	CR <i>T.p.</i>	RYT	CR <i>Ph.p.</i>	CR <i>T.p.</i>	RYT	CR <i>Ph.p.</i>	CR <i>T.p.</i>	RYT	CR <i>Ph.p.</i>	CR <i>T.p.</i>
a ₂ - <i>Ph.p.</i> b ₁ - N ₀ P ₀ K ₀	2.41	8.52	0.12	2.20	8.82	0.11	2.55	7.67	0.13	1.19	9.27	0.08
(75%) + b ₂ - N ₃₀ P ₃₀ K ₃₀	1.52	2.49	0.40	2.12	3.51	0.29	1.93	2.61	0.38	1.24	19.20	0.05
<i>T.p.</i> (25%) b ₃ - N ₅₀ P ₅₀ K ₅₀	1.34	15.84	0.06	1.10	7.91	0.13	1.55	12.33	0.08	1.64	12.89	0.11
a ₃ - <i>Ph.p.</i> b ₁ - N ₀ P ₀ K ₀	2.45	6.87	0.15	2.69	12.17	0.08	2.63	6.08	0.16	1.25	6.12	0.16
(50%) + b ₂ - N ₃₀ P ₃₀ K ₃₀	2.45	6.85	0.15	2.79	4.64	0.22	2.48	7.58	0.13	1.24	10.78	0.09
<i>T.p.</i> (50%) b ₃ - N ₅₀ P ₅₀ K ₅₀	1.21	9.24	0.11	1.23	8.18	0.12	1.08	8.47	0.12	1.40	10.18	0.10
a ₄ - <i>Ph.p.</i> b ₁ - N ₀ P ₀ K ₀	2.23	4.98	0.20	1.54	8.06	0.12	2.38	4.51	0.22	0.95	3.53	0.28
(25%) + b ₂ - N ₃₀ P ₃₀ K ₃₀	1.66	5.81	0.17	1.55	7.96	0.13	1.86	5.38	0.19	1.53	3.73	0.27
<i>T.p.</i> (75%) b ₃ - N ₅₀ P ₅₀ K ₅₀	1.29	7.32	0.14	0.98	7.34	0.14	1.26	7.12	0.14	1.88	8.23	0.12

The legumes present in the vegetation cover recorded values ranging between 37% in the variant sown with the mixture *Phleum pratense* L. 75% + *Trifolium pratense* L. 25%, fertilized with N₅₀P₅₀K₅₀ and 71% in the variant sown with *Phleum pratense* L. 75% + *Trifolium pratense* L. 25% and fertilized with N₃₀P₃₀K₃₀. The species in the miscellaneous group had values ranging between 1% and 45%.

Analyzing the variants sown with *Phleum pratense* L. 100% in pure culture, it can be seen that the participation percentage remained constant at 100%, and in the case of the variants sown with the species *Trifolium pratense* L. 100%, the lowest percentage was recorded in the unfertilized variant, at 55% (Figure 1).

Regarding the data obtained at the second mowing, it is found that the percentage of grasses was between 36% as recorded in the variant sown with the mixture of *Phleum pratense* (25%) + *Trifolium pratense* (75%) and fertilized with N₅₀P₅₀K₅₀ and 59% as recorded in the variant sown with the mixture of *Phleum pratense* (25%) + *Trifolium pratense* (75%) and fertilized with N₃₀P₃₀K₃₀. The percentage of participation of species from the miscellaneous group was between 1% and 41% (Figure 2).

Analyzing the influence of the species and the cultivated mixture (*Phleum pratense* L. + *Trifolium pratense* L.), on the evolution of the structure of the vegetal carpet, it can be observed that in row I, on average over the two years, the percentage of participation of the species *Phleum pratense* L. recorded values ranging between 28% in the variant sown with the mixture *Phleum pratense* L. 75% + *Trifolium pratense* L. 25% and fertilized with N₃₀P₃₀K₃₀ and 52% in the variant sown with the mixture *Phleum pratense* L. 75% + *Trifolium pratense* L. 25% and fertilized with N₅₀P₅₀K₅₀ (Figure 3).

Analyzing the data obtained in the third mowing, it is found that the percentage of participation of the species *Phleum pratense* L. recorded values ranging from 31% as recorded in the variant sown with the mixture *Phleum pratense* L. 75% + *Trifolium pratense* L. 25%, fertilized with N₃₀P₃₀K₃₀ and 44% as recorded in the variant *Phleum pratense* L. 75% + *Trifolium pratense* L. 25%, unfertilized. The highest percentage of species in the diverse group was 77%, this value being recorded in the variant sown with the species *Trifolium pratense* L., unfertilized variant.

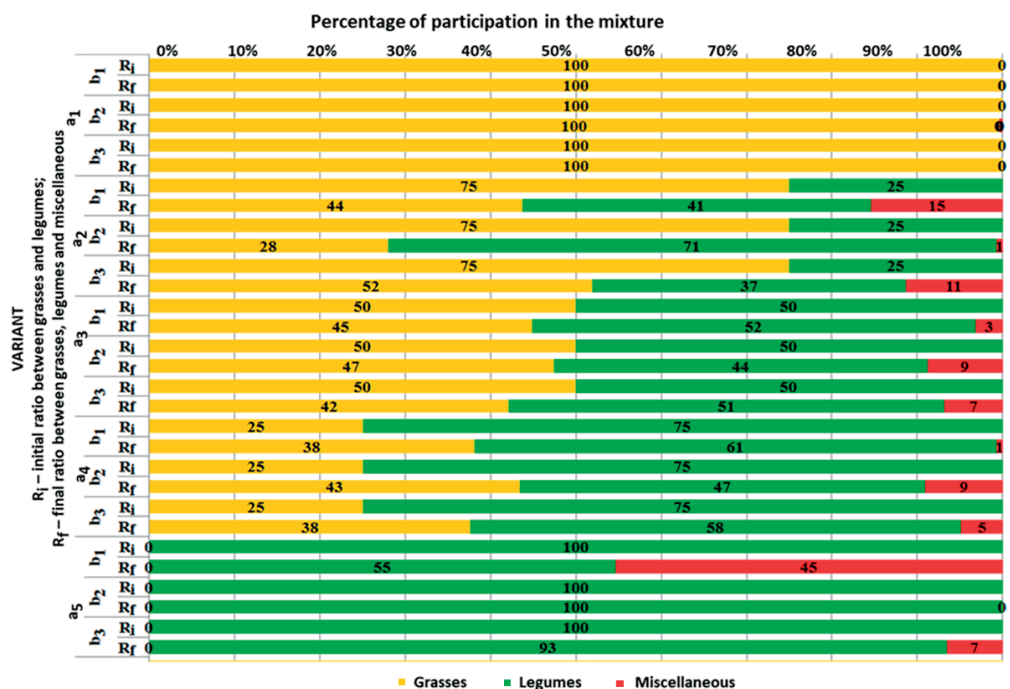


Figure 1. The influence of the cultivated species or mixture on the evolution of the structure of the vegetation cover, at the first harvest

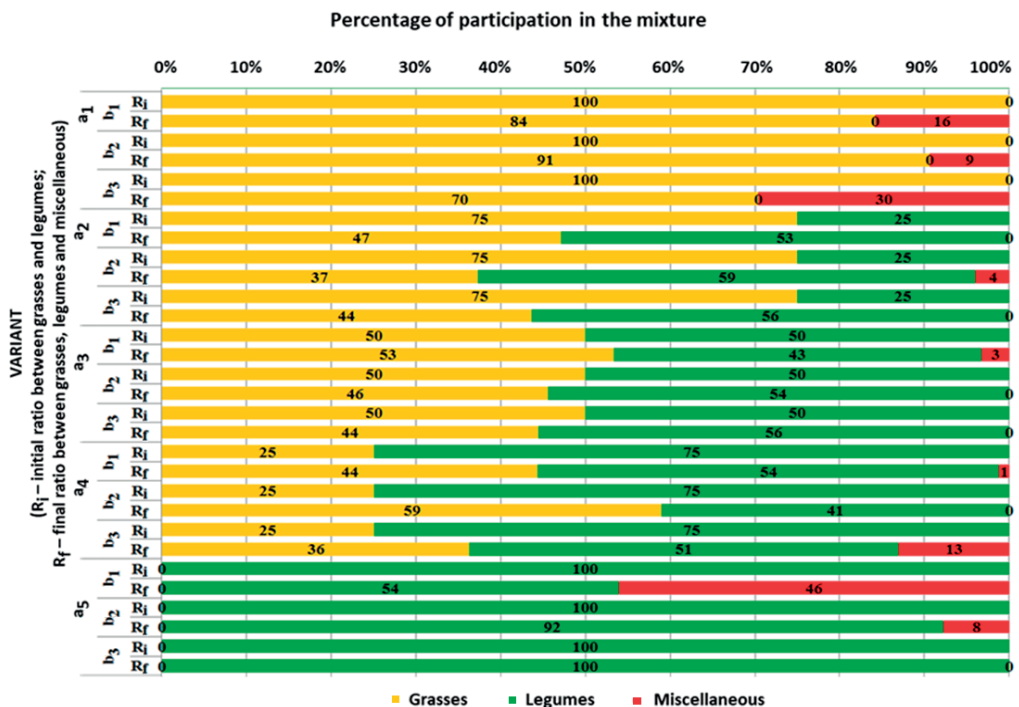


Figure 2. The influence of the cultivated species or mixture on the evolution of the structure of the vegetation cover, at the second harvest



Figure 3. The influence of the cultivated species or mixture on the evolution of the structure of the vegetation cover, at the third harvest

Intraspecific competition occurs between individuals of the same species and is a consequence of the mass effect for space, minerals, water, and light. This mass effect of intraspecific competition is regulated by homeostatic mechanisms or by human intervention, indicating that individuals can maintain a stronger species. Interspecific competition can determine which species and how many species can coexist within the same community but can affect population dynamics by modifying species structure and community structure.

CONCLUSIONS

The analysis of the influence of the interaction between the mixture used and fertilization with mineral fertilizers (nitrogen, phosphorus and potassium) on interspecific relationships in the two years of vegetation showed that the RYT index recorded values > 1 , except for the variant sown with the mixture formed by *Phleum pratense* L. (25%) + *Trifolium pratense* L. (75%) and unfertilized, showing that the species

Phleum pratense L. and *Trifolium pratense* L. compete for the same vegetation factors.

During the analyzed period, the CR index for the species *Phleum pratense* L. was higher than for the species *Trifolium pratense* L., but lower values of the CR index were recorded in the variants in which the percentage of participation in the mixture of the species *Phleum pratense* L. was lower.

The variants in which the species *Phleum pratense* L. recorded low values of the CR index were those in which the participation percentage was 50% and 25%, respectively, in which case the species is considered to be poorly competitive. These aspects were highlighted inversely proportionally in the case of the species *Trifolium pratense* L.

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RESEARCH ON DOWNY MILDEW ATTACK IN *Camelina sativa* UNDER FIELD CONDITIONS IN DRACEA, TELEORMAN COUNTY IN 2025

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Abstract

Camelina sativa has attracted increasing scientific interest due to the valuable composition of its oil and its high adaptability to different pedoclimatic conditions. The main objective of this research was to evaluate the level of downy mildew (*Peronospora camelinae*) attack in camelina crops under field conditions. The research was conducted in 2025, in Dracea, Teleorman County. The experiment included two camelina cultivars, Mădălina and Camelia. Phytosanitary assessment was carried out by determining the frequency, intensity of attack, and degree of attack for each analyzed cultivar. The results highlighted a downy mildew attack value of 21% in the Mădălina cultivar, while the Camelia cultivar recorded a higher attack level of 29%.

Key words: *Camelina sativa*, downy mildew, *Peronospora camelinae*, genotype.

INTRODUCTION

Camelina sativa (L.) Crantz is an oilseed species belonging to the Brassicaceae family, native to the regions of Eastern Europe and Western Asia, and is recognized for its agronomic value and its wide range of industrial applications (Vollmann & Eynck, 2015; Blume et al., 2023). At present, this crop is attracting increasing interest in both research and the economic sector, particularly in Europe and North America, due to its adaptability to diverse ecological conditions, its low cultivation-input requirements, and the potential for utilizing its oil in numerous fields, including biofuels, aviation fuel, and oleochemical compounds (Zanetti et al., 2017; Larsson, 2013; Neupane et al., 2022; Montero-Muñoz et al., 2023; Sydor et al., 2022; Clavijo-Bernal et al., 2024). *Camelina sativa* oil obtained from seeds cultivated in Romania has been characterized by a high fatty acid content, predominantly linolenic acid, while the differences among the analyzed varieties were minimal (Popa et al., 2019). These findings indicate that *Camelina sativa* oil possesses therapeutic and regenerative potential, with possible applications in the pharmaceutical and cosmetic industries (Crăciun et al., 2019).

From an agronomic perspective, camelina is considered a hardy and relatively undemanding species, capable of performing under less favorable growing conditions compared with other oilseed crops (Dobre et al., 2014; Putnam et al., 1993; Jiang et al., 2016). *Camelina sativa* is recognized as a raw material with high potential for biofuel production, which justifies the growing interest in breeding more productive cultivars with increased oil content (Matei et al., 2017; Nadakuduti et al., 2023). However, the success of its cultivation also depends on maintaining an appropriate phytosanitary status, as the occurrence of pathogens may significantly affect plant development and yield level (Podgoreanu et al., 2015; Séguin-Swartz et al., 2009). Although *Camelina sativa* naturally exhibits a high degree of tolerance to numerous diseases and pests specific to other species within the Brassicaceae family (Putnam et al., 1993; Vollmann et al., 2001; Jiang et al., 2016), field-grown crops have nevertheless been reported to be affected by various pathogens. Among these, *Peronospora camelinae*, the causal agent of downy mildew, is considered one of the most important diseases reported in this crop (Vollmann et al., 2001; Séguin-Swartz et al., 2009; Babiker et al., 2012).

Downy mildew is caused by an obligate biotrophic pathogen that develops exclusively on living tissues and depends entirely on the host plant for its survival and multiplication (Turk, 2002, as cited in Jiang et al., 2016). The characteristic symptoms of the disease include the appearance of a grayish-white mycelial growth on the abaxial surface of the leaves, as well as on the internodes and developing siliques; these symptoms may be accompanied by chlorosis or visible discoloration on the upper side of the leaf blade (Séguin-Swartz et al., 2009; Jiang et al., 2016). In cases of severe infection, plants may exhibit pronounced growth abnormalities, including deformation, twisting, or bending of the aerial organs, which can negatively affect both vegetative development and crop productivity (Vollmann et al., 2001; Jiang et al., 2016). The occurrence of *Sclerotinia sclerotiorum* in cultivated camelina also highlights the need for increased attention to crop management practices, breeding strategies, and phytosanitary protection measures (Cristea & Jurcoane, 2016). In this context, the present study was conducted to analyze the effect of *Peronospora camelinae* infection on two *Camelina sativa* cultivars under environmental conditions characterized by a temperate thermal regime and limited water availability. The aim of the study was to evaluate the occurrence and expression of the disease, with particular emphasis on downy mildew as the main disorder identified in the crop, and to highlight the relationship between disease severity and the overall performance of the plants.

MATERIALS AND METHODS

Aim of the research

The objective of this research was to investigate the occurrence and effect of the pathogen *Peronospora camelinae* on *Camelina sativa* crops under the specific conditions of Dracea locality, Teleorman County. In addition, the study aimed to evaluate the influence of row spacing and seeding rate on the severity of downy mildew infection in camelina.

In Romania, the pathogen was reported in camelina crops in 2014 in the Bucharest area, a finding that highlighted the increasing importance of downy mildew for this crop under the conditions of southern Romania (Cristea &

Manole, 2014). In this context, the results obtained in the present study may contribute to the optimization of cultivation technology and to the improvement of phytosanitary management in camelina under the pedoclimatic conditions of southern Romania.

Experimental site and experimental conditions

The research was conducted during the spring of 2025 in the experimental field located in Dracea, Teleorman County. The experiment was established under field conditions using a randomized complete block design (RCBD), with each block measuring 48 m in length.

Two main experimental factors were evaluated in the study: row spacing and seeding rate. Row spacing was tested at two levels, 13 cm and 25 cm. The seeding rate also had two levels: 6 kg/ha, corresponding to 525 germinable seeds/m², and 8 kg/ha, equivalent to 700 germinable seeds/m².

Variant V1 consisted of a seeding rate of 6 kg/ha combined with a row spacing of 13 cm. Variant V2 was established at the same row spacing of 13 cm, but with a higher seeding rate of 8 kg/ha, resulting in increased crop density. In the case of Variant V3, the seeding rate of 8 kg/ha was combined with a row spacing of 25 cm. Variant V4 included a seeding rate of 6 kg/ha at a row spacing of 25 cm, representing the lowest-density configuration.

Biologic material

The biological material used in this study consisted of two Romanian camelina cultivars adapted to the environmental conditions of Romania. The cultivar Mădălina was developed at the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Dobre P. et al., 2014), whereas the cultivar Camelia was developed at the National Agricultural Research and Development Institute Fundulea (Toncea et al., 2013).

Experimental design

The experimental layout was established in a mirrored arrangement for the two cultivars within a randomized complete block design. The selection of this experimental design aimed to reduce the effects generated by field heterogeneity and to increase the precision of comparisons among the studied variants. By

including all factorial combinations within each block and randomizing them inside the blocks, the influence of soil variation, microclimatic conditions, and other local factors on the experimental results was minimized. Consequently, the differences observed among variants can be attributed, to a greater extent, to the factors under investigation rather than to the non-uniformity of the experimental environment.

General characterization of the thermal and pluviometric regime during 2025 growing season

During the period March-June 2025, the climatic conditions were characterized by a progressive increase in both air and soil temperatures, accompanied by a reduced and unevenly distributed precipitation regime. At the beginning of the growing season, in March, minimum air temperatures were low, approaching 0°C and (Figure 1), in isolated cases, slightly below freezing, while mean temperatures remained within the range typical of early spring. Soil temperature at a depth of 10 cm showed a more stable pattern, with values ranging approximately between 8 and 14°C.

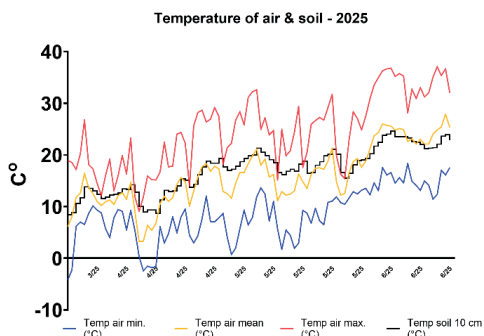


Figure 1. Air and soil temperature

In April and during the first part of May, both air and soil temperatures increased gradually. Mean air temperatures frequently ranged between 10 and 18°C, while soil temperature progressively increased to approximately 15-20°C. This pattern indicates a relatively balanced thermal regime during the first part of the growing season.

In the second half of May, and especially during June, the thermal regime intensified significantly. Maximum air temperatures frequently exceeded 30°C, reaching 35-37°C on some days, while mean air temperature rose above 20°C.

During the same period, soil temperature at a depth of 10 cm generally remained within the range of 20-25°C. Compared with air temperature, soil temperature showed lower variability, maintaining a more uniform upward trend throughout the entire period analyzed.

From a pluviometric standpoint (Figure 2), the growing season was characterized by low precipitation amounts and an uneven distribution of rainfall. Water input from precipitation was episodic, with relatively long intervals lacking significant rainfall, while the most notable precipitation events were recorded during the second half of May. Overall, the climatic pattern of 2025 was marked by a season onset with moderate temperatures, followed by a pronounced increase in temperature toward the end of the analyzed period, under conditions of a modest precipitation regime.

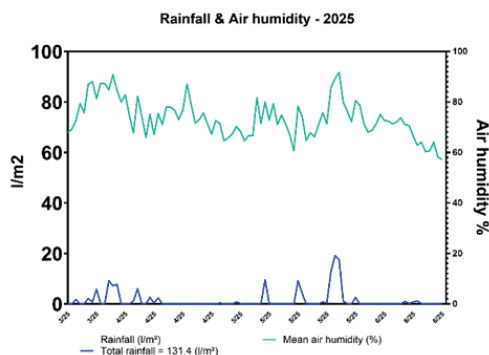


Figure 2. Precipitation and air humidity conditions in 2025

Assessment of downy mildew infection

Downy mildew infection was assessed at two distinct stages during the growing season. The first evaluation was performed before flowering, at BBCH stage 6, corresponding to the maximum development of the leaf canopy, while the second assessment was carried out during seed formation and maturation, at BBCH stage 8. Disease assessment under field conditions was conducted using the “N” sampling pattern method in order to ensure a representative evaluation of each experimental plot. A total of 100 plants were analyzed, with 25 plants collected from each observation point established in the field. This method allowed for a rigorous estimation of both infection frequency and symptom intensity under field conditions.

Phytosanitary indicators analyzed

Attack frequency (F%) was calculated as the ratio between the number of symptomatic plants (n) and the total number of evaluated plants (N), expressed as a percentage, according to the following equation:

$$F\ (\%) = (n \times 100) / N$$

Attack intensity (I%) was determined by estimating the surface area affected by symptoms, using a percentage scale ranging from 0 to 100% of the plant surface. It was calculated according to the following formula:

$$I\ (\%) = \Sigma (i \times f) / n$$

where: i represents the assigned percentage of infection, f the number of plants included in the respective category, and n the total number of infected plants analyzed.

Degree of attack (DA %) was used to express disease severity at the crop level by correlating attack frequency with attack intensity, according to the following equation:

$$DA\ (\%) = (F \times I) / 100$$

Statistical analysis

The experimental data obtained were statistically processed using analysis of variance (ANOVA) in order to evaluate the significance of the differences among the experimental variants. Data processing and statistical analysis were performed using R and GraphPad Prism.

RESULTS AND DISCUSSIONS

Identification of *Peronospora camelinae* infection

The identification of infection caused by *Peronospora camelinae* was based on the

characteristic symptomatology observed on the aerial organs of the plants (Cristea & Manole, 2014) (Figure3).



Figure 3. Downy mildew attack 2025

At the level of the leaves and the upper portion of the stem, discoloration spots were observed on the adaxial surface, which gradually developed into necrotic areas, indicating the progressive deterioration of the tissues. On the abaxial surface of the leaves, corresponding to these lesions, a fine, powdery layer ranging in color from whitish to grayish-white was observed (Figure 3), representing the asexual sporulation of the pathogen. As the intensity of the infection increased, the symptoms became more pronounced and were accompanied by deformation of the vegetative organs. Severely affected plants exhibited the characteristic stem-twisting phenomenon, considered a typical symptom of downy mildew in camelina (Vollmann J., 2001). Overall, the association of chlorotic and necrotic lesions on the upper leaf surface, whitish sporulation on the lower surface, and stem deformation allowed the clear identification of *Peronospora camelinae* infection in *Camelina sativa* plants.

Table 1. The influence of seeding rate per hectare and row spacing on downy mildew incidence in camelina (2025) at Dracea, Teleorman location

Variety	Variant*	BBCH6 / 2025			BBCH8 / 2025		
		F (%)	I (%)	DA (%)	F (%)	I (%)	DA (%)
Mădălina	V1	6	21	1.26	14	36	5.0
	V2	14	38	5.3	28	52	14.6
	V3	9	31	2.8	20	43	8.6
	V4	4	18	0.7	10	28	2.8
Camelia	V1	9	29	2.61	18	42	7.6
	V2	18	44	7.9	34	60	20.4
	V3	12	35	4.2	25	49	12.3
	V4	6	22	1.3	13	33	4.3

*V1 – 6 kg/ha & 13 cm; V2 – 8 kg/ha & 13 cm; V3 – 8 kg/ha & 25 cm; V4 – 6 kg/ha & 25 cm;

Table 2. Results of the analysis of variance (ANOVA)

Source of variation	df	Sum of squares (SS)	Mean square (MS)	F value	p-value
Variety (A)	1	48.27	48.27	9.84	0.015*
Variant (B)	3	212.91	70.97	14.47	0.002**
Phenological stage (C)	1	356.14	356.14	72.65	<0.001***
A × B	3	24.62	8.21	1.67	0.231
A × C	1	19.74	19.74	4.03	0.079
B × C	3	68.38	22.79	4.65	0.034*
A × B × C	3	11.26	3.75	0.76	0.542
Error	16	78.41	4.90	—	—
Total	31	819.73	—	—	—

Note: A = cultivar; B = technological variant; C = phenological stage; df = degrees of freedom; SS = sum of squares; MS = mean square; F = Fisher's test value; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Dynamics of the frequency, intensity, and degree of attack of *Peronospora cameliniae* in 2025

The data obtained in 2025 highlight variations in the frequency (F), intensity (I), and degree of attack (DA) of *Peronospora cameliniae* depending on cultivar, phenological stage, and the applied technological variant. The values of these indicators were lower at the BBCH6 stage and increased significantly by BBCH8, indicating disease progression throughout the growing season.

At the BBCH6 stage, attack frequency ranged from 4% to 14% in the cultivar Mădălina and from 6% to 18% in Camelia, while attack intensity ranged from 18% to 38% in Mădălina and from 22% to 44% in Camelia. Consequently, the degree of attack recorded relatively low values at this stage of crop development, ranging from 0.72% to 5.32% in Mădălina and from 1.32% to 7.92% in Camelia. These results indicate that primary infections were already present in the crop, although symptom severity remained limited during the early stages of plant development.

At the BBCH8 stage, disease severity increased markedly. Attack frequency reached values ranging from 10% to 28% in Mădălina and from 13% to 34% in Camelia. Attack intensity also increased, reaching 28-52% in Mădălina and 33-60% in Camelia. As a result, the degree of attack reached maximum values of 14.56% in Mădălina and 20.40% in Camelia under variant V2. This pattern reflects disease progression as the crop developed a larger leaf canopy, which favored the maintenance of a humid microclimate within the stand. A comparison of the two cultivars shows that Camelia exhibited higher values for all phytosanitary indicators,

suggesting greater susceptibility to *Peronospora cameliniae* infection compared with Mădălina, which displayed relatively higher tolerance under the same conditions.

The technological variant clearly influenced disease severity. The highest values of frequency, intensity, and degree of attack were recorded in variant V2 (8 kg/ha and 13 cm), where the higher plant density and reduced row spacing favored the formation of a humid microclimate within the crop and limited air circulation. Variant V3 (8 kg/ha and 25 cm) showed intermediate levels of infection, indicating that wider row spacing may partially reduce the conditions favorable for disease development, even under high-density conditions. The lowest values of the degree of attack were observed in variant V4 (6 kg/ha and 25 cm), where the combination of lower density and wider row spacing contributed to limiting pathogen development by improving air circulation and reducing the persistence of moisture at the leaf level.

The results of the analysis of variance (ANOVA) confirm these observations. Phenological stage had the strongest influence on degree of attack ($F = 72.65$, $p < 0.001$), indicating a significant increase in disease severity from BBCH6 to BBCH8. The technological variant also had a significant effect on degree of attack ($F = 14.47$, $p = 0.002$), demonstrating that crop density and row spacing can alter the microclimate within the crop canopy and may either favor or limit pathogen development. Cultivar also showed a significant effect ($F = 9.84$, $p = 0.015$), confirming genetic differences in the response to *Peronospora cameliniae* infection.

The interaction between technological variant and phenological stage was significant ($p < 0.05$), indicating that the differences among variants became more pronounced as the crop advanced toward the reproductive stages. In contrast, the interactions between cultivar and variant, as well as the three-way interaction, did not show statistically significant differences (Figure 4).

Overall, the results suggest that the severity of *Peronospora camelinae* infection in 2025 was determined by the interaction between climatic conditions favorable to disease development and crop architecture shaped by the sowing technology. Variants characterized by higher plant density and narrower row spacing favored the development of a humid microclimate within the crop, leading to higher values of attack frequency and severity.

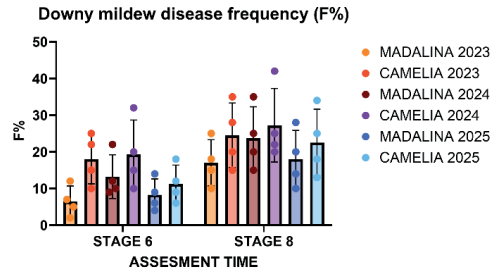


Figure 4. Comparison of the frequency of *Peronospora camelinae* infection over the three experimental years

The graph highlights an increase in the frequency of downy mildew infection from phenophase BBCH 6 to BBCH 8, regardless of cultivar or year, indicating a progressive expansion of the infection throughout the growing season. In all years included in the comparison, the cultivar Camelia exhibited higher frequency values than Mădălina, suggesting greater susceptibility to *Peronospora camelinae* infection. At the same time, 2024 was distinguished by the highest frequency values, particularly in the cultivar Camelia, at both BBCH 6 and BBCH 8, whereas 2023 showed intermediate values. The data for 2023 and 2024 were taken from the study conducted by Năstase et al. (2025), and their comparison with the data obtained in 2025 highlights the persistence of the same general trend, namely the increase in attack frequency at more advanced stages of vegetation and the contrasting behavior of the

two cultivars. Overall, the cultivar Camelia proved to be more susceptible to *Peronospora camelinae* infection than Mădălina across most of the analyzed variants (Năstase et al., 2025).

CONCLUSIONS

The results obtained in the present study on *Peronospora camelinae* infection in Camelia sativa crops highlight the combined influence of climatic conditions and cultivation technology on the occurrence and progression of the disease. The analysis of the phytosanitary indicators, namely attack frequency (F), attack intensity (I), and degree of attack (DA), demonstrated that disease severity increased throughout the growing season, with higher values recorded at phenological stage BBCH8 compared with BBCH6.

The climatic conditions during the 2025 growing season favored the occurrence and development of *Peronospora camelinae* infection. The moderate temperatures recorded during the first part of the growing season, together with rainfall events and high relative air humidity, created favorable conditions for the establishment of primary infections. As the crop developed and the leaf canopy became denser, these conditions allowed the disease to spread further, resulting in increased frequency and severity of symptoms during the reproductive stages of the crop.

The differences observed between the cultivars revealed a higher susceptibility of the cultivar Camelia to *Peronospora camelinae* infection compared with Mădălina, which consistently showed lower values of attack frequency, attack intensity, and degree of attack across all analyzed technological variants. This finding suggests the existence of genetic differences in tolerance to the pathogen between the two camelina genotypes.

Cultivation technology also played an important role in disease development. The variant characterized by high plant density and reduced row spacing (8 kg/ha and 13 cm) recorded the highest values of the phytosanitary indicators, indicating that greater crop density favors the formation of a humid microclimate within the canopy and reduces air circulation, thereby creating favorable conditions for pathogen

development. In contrast, the variant with a lower seeding rate and wider row spacing (6 kg/ha and 25 cm) showed the lowest values of degree of attack, suggesting that a more open crop structure may limit disease development. The results of the statistical analysis (ANOVA) confirmed the significant influence of phenological stage, technological variant, and cultivar on the severity of *Peronospora camelinae* infection. In particular, phenological stage had the strongest effect on disease progression, indicating a significant increase in disease severity from BBCH6 to BBCH8. In addition, the interaction between technological variant and phenological stage showed that the differences among variants became more pronounced during the advanced stages of crop development.

Overall, the results of this study underline the importance of technological crop management in reducing the risk of downy mildew occurrence and development in camelina. Adjusting seeding density and row spacing may represent an effective agronomic strategy for limiting pathogen pressure, especially under climatic conditions favorable to disease development. At the same time, the selection of more tolerant genotypes, such as the cultivar Mădălina, may contribute to reducing the phytosanitary impact of the pathogen and to improving yield stability under the pedoclimatic conditions of southern Romania.

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IDENTIFICATION OF PATHOGENS CAUSING LEAF SPOTS ON DURUM WHEAT VARIETIES IN BULGARIA AND DESCRIPTION OF THEIR SYMPTOMS

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Abstract

In the experimental field of Field Crop Institute - Chirpan, 12 varieties of durum wheat selected by the institute are sown annually. The dynamics of the appearance and development of leaf spots during the growing season were monitored. The main method for determining the species composition of fungal pathogens is the comparative-morphological one. Symptoms are described on naturally infected durum wheat plants and compared with those on common wheat caused by the respective pathogens. The identification was made by direct observation of the structures of the pathogens formed on plant tissue and during cultivation in nutrient media - potato-dextrose agar, water agar, malt extract agar, oat agar, Synthetischer Nährstoffarmer agar, Shear's solution and is based on a set of morphological and cultural characters. The characters with the highest taxonomic value were taken into account: morphology and sizes of asexual and sexual mating, colony morphology. The most widespread on durum wheat are the fungal species that form a mass sexual stage in the conditions of Bulgaria: *Pyrenophora tritici-repentis*, *Parastagonospora avenae* f. sp. *triticea*, *Monographella nivalis*, *Cladosporium herbarum*.

Key words: *Cladosporium herbarum*, fungal pathogens, *Monographella nivalis*, *Parastagonospora avenae* f. sp. *triticea*, *Pyrenophora tritici-repentis*.

INTRODUCTION

Fungal pathogens on wheat significantly limit yields and degrade the quality of the produce. The diseases they cause can lead to 15-20% annual losses, the most important contributors to which are rusts, leaf spots and grade attack (Figueroa et al., 2018). A study presents a gene network analysis of wheat resistance and susceptibility to *Pyrenophora tritici-repentis* (*Ptr*); it includes RNA-seq and WGCNA approaches (Ferreira et al., 2025). A novel virulence genetic locus associated with the pathogenicity of *Ptr* has been discovered (Guo et al., 2025). The increased economic importance in the affected areas is due to certain agrotechnical practices, mainly minimal tillage (Bockus & Claasen, 1992). The main source of primary inoculum is ascospores formed on overwintered wheat straw residues (Ciuffetti & Tuori, 1999; Todorova, 2006; Bankina & Priekule, 2011). The transmission of the pathogen through seeds has also been proven (Schilder & Bergstrom, 1995). By conducting systematic observations and field assessments,

the presence and consequences of foliar pathogens, as well as wheat resistance, were determined. The biodiversity of fungal species involved in the leaf spot complex of durum wheat in Bulgaria was studied and information on the most common pathogens and their specific features was presented (Nedyalkova et al., 2020). In addition to their fundamental significance, the results also have practical applications. Accurate identification is important for several reasons. Often several diseases are similar in their manifestations and therefore must be determined with great precision, also when deciding on methods of control. A statistical analysis of the distribution of *Parastagonospora* in Europe including *Parastagonospora avenae* f. sp. *triticea* (*Pat*) was performed (Bartosiak et al., 2021). It is not always possible to distinguish fungal leaf diseases solely by field assessment of the size and shape of leaf spots. For accurate diagnosis, observation of the reproductive structures of the causative agents and isolations are necessary. In this regard, Salgado and Paul (2016) present a summary of the diseases caused by

Parastagonospora nodorum (Pan) and *Parastagonospora avenae* f. sp. *triticea* on wheat. The range of diseases found in each region varies considerably. Knowledge of the important diseases in a given production area, their identification, modes of spread and response to environmental conditions is of great importance when making decisions for profitable and environmentally friendly wheat production. *Monographella nivalis* (Mn) is described as an ascomycete causing snow mold on cereals and grasses (taxonomic data lists; *Microdochium/Monographella*) an experimental study evaluating the impact of *Monographella nivalis* on winter wheat under snow cover. The results show a significant reduction in viability and yield, which confirms the real phytopathological significance of the species (Sabeckis et al., 2025). *Cladosporium herbarum* (Cl) is often considered a saprophyte/weakly pathogenic in the context of wheat – therefore, specific studies are often lacking in the clinical/field literature, but it is widely described in mycology and ecology. A study by Li Y. et al. (2024) reviewed the taxon (including *C. herbarum*) and its distribution. Different diseases require appropriate strategies for combating them, and their precise diagnosis is of utmost importance.

MATERIALS AND METHODS

In the experimental field of the FCI - Chirpan in the period 2022/2024, 6 Bulgarian and 6 foreign varieties of durum wheat were sown. The study included durum wheat varieties Progres, Predel, Reyadur, Raylidul, Saya and Helix, which are achievements of Bulgarian selection, and varieties Hundur, Makaroni, Aurelio, Cesare, Meridio and Ovidio, foreign selection. The field experiment was set up using a randomized block design. Systematic phenological observations were conducted and the appearance and development of leaf spots during the growing season was monitored in dynamics. After visual assessment of the size and shape of the spots in field conditions, samples were collected for observation of reproductive structures of the

causative agents, isolation and further diagnosis. The collected infectious material was examined in the Phytopathology laboratory at the FCI - Chirpan. Isolations were made directly from sexual and asexual structures of the fungal pathogens (perithecia and pycnidia) or indirectly by cutting small pieces (about 1 cm²) from leaf spots at the border between diseased and healthy tissue. The leaf samples were surface sterilized and washed with sterile distilled water, followed by drying on sterile filter paper and placing in Petri dishes containing culture medium. To obtain monospore cultures, a suspension (conidial or ascospore) was plated on the surface of 2% water agar in Petri dishes. After 24 hours under sterile conditions, single germinated spores were separated using a sterile needle under a microscope and transferred together with the agar block onto PDA (Potato Dextrose Agar) in test tubes or Petri dishes.

RESULTS AND DISCUSSIONS

The occurrence and relative prevalence of individual species varies depending on the year of study. Agrometeorological conditions in some years are extremely favorable for the development of certain diseases. A total of 249 leaf samples were collected at the FCI - Chirpan in the period 2022-2024. Data on the occurrence of foliar pathogens depending on the year of study are presented in (Figure 1). In 2022, 7 fungal species were present in the population, of which *M. nivalis* was dominant, followed by *Pa. avenae*. It is interesting to note that this year, not only *P. tritici-repentis*, one of the most common species on durum wheat, was isolated. In 2024, 5 pathogens were registered, but they were represented in different proportions. During the year, *C. herbarum*, *Pa. nodorum*, *Pa. avenae* and *M. nivalis*. In 2023, 3 pathogens were identified, with *P. tritici-repentis* and *C. herbarum* being the dominant ones. Two of the species (*M. nivalis* and *C. herbarum*) appeared in all years studied, but with different relative frequencies. *P. tr* was recorded in three of the years, but in a significant number of samples.

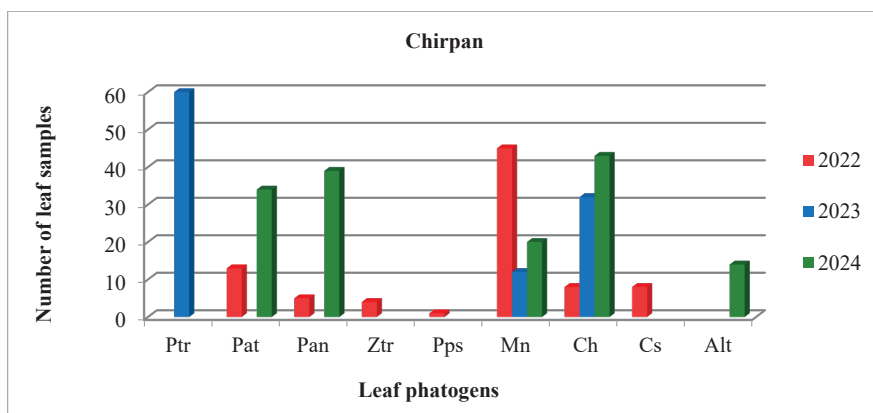


Figure 1. Occurrence of leaf pathogens in 12 varieties of durum wheat in the FCI - Chirpan depending on the year of study: *Ptr* – *Pyrenophora tritici-repentis*; *Pat* – *Parastagonospora avenae* f. sp. *triticea*; *Pan* – *Parastagonospora nodorum*; *Ztr* – *Zymoseptoria tritici*; *Pps* – *Phaeophleospora* sp.; *Mn* – *Monographella nivalis*; *Ch* – *Cladosporium herbarum*; *Cs* – *Cochliobolus sativus*; *Alt* – *Alternaria* sp.

The 12 varieties studied differ in their susceptibility to fungal diseases. Figure 2 presents data on the occurrence of leaf pathogens in the FCI - Chirpan depending on the variety. The pathogens *P. tritici-repentis*, *C. herbarum* and *M. nivalis* were found in all varieties, but with a certain predominance of the first species in Saya and Hundur, the second in Hundur and Progress, and the third in Meridio and Cesare. Characteristic symptoms of the manifested pathogens: The results obtained show that during the studied period, leaf spots on durum wheat (*T. durum*) were most often caused by *Pyrenophora tritici-repentis*. It develops on the upper and lower surfaces of the leaves. Two main manifestations of the disease are observed: necrosis and chlorosis. Necrotic symptoms initially appear as small brown spots with or without a chlorotic halo. They grow into necroses with an irregular elliptical shape and pointed ends, reaching about 15 mm, sometimes with a yellow border. Chlorotic symptoms consist of rapidly expanding yellow streaks around a small dark center. The formation of a small dark brown to black spot in the middle of the necrotic or chlorotic spots is very characteristic of this disease. Lesions can coalesce into large spots and cause premature leaf wilting. In all years of the study, symptoms of snow mold disease caused by the ascomycete fungus *Monographella nivalis* were observed in late winter and early spring. The disease also occurs in the absence of snow cover. In the threshing crop, completely dead plants are

observed, initially covered abundantly with white mycelium. They gradually turn pink due to a change in the color of the mycelium and the development of pink-orange sporodochia on them. In partially affected plants, the lower leaves have died and stuck to the soil surface. A large number of small, round, gray-green to light brown spots form on the upper and lower surfaces of subsequent leaves. They expand rapidly, leaving their center grayish-green and their periphery lighter or darker brown. Well-developed spots are spread out, with irregular outlines, quickly grow and merge into extensive necrosis that occupies the entire width of the leaves. White mycelium and numerous sporodochia form on their surface, which contain the conidia of the fungus. Along with the symptoms caused by the pathogens described so far, unusual spotting was observed during the study period, found mainly on some foreign varieties of durum wheat. A large number of small light brown spots are formed, oblong-oval with rounded edges, partially limited by the veins, with unclear outlines, a weakly pronounced dark border and a chlorotic halo, which quickly grow and merge, giving relief to the attacked leaf surface. In humid conditions, spores of the fungus, which has been identified as *Cladosporium herbarum*, form on the spots. The pathogens *Pa. avenae* and *Pa. nodorum* were isolated from all varieties except Ovidio. During the research period, the predominant species in durum wheat was *Parastagonospora avenae* f. sp. *triticea*. During the growing season

until the end of May, the asexual morph was mainly recorded. Gray-brownish watery spots with a darker brown edge, elongated and irregular in shape, form on the lower leaves. They contain scattered single, spherical, dark brown pycnidia. Typical symptoms are in the form of oval-rhomboid, ovoid, lenticular spots, sometimes surrounded by a dark brown border, which grow and merge into extensive necrosis. The ascomycete fungus *Parastagonospora*

nodorum attacks all green parts of the plant. Relatively small, oval to lenticulate, initially chlorotic, and later during the growing season, merging reddish-brown spots with a fading center appear on the leaves of durum wheat, where pycnidia form. The symptoms are clearly visible on the spikes and spikelets. As the disease progresses, the pycnidia of the fungus are visible in the tissue of the spikelets.

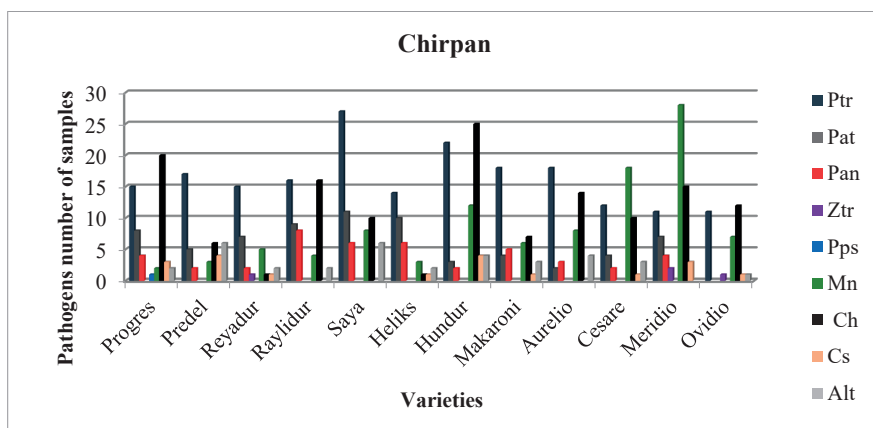


Figure 2. Occurrence of leaf pathogens in 12 varieties of durum wheat in the FCI-Chirpan depending on the variety: *Ptr* – *Pyrenophora tritici-repentis*; *Pat* – *Parastagonospora avenae* f. sp. *triticea*; *Pan* – *Parastagonospora nodorum*; *Ztr* – *Zymoseptoria tritici*; *Pps* – *Phaeophleospora* sp.; *Mn* – *Monographella nivalis*; *Ch* – *Cladosporium herbarum*; *Cs* – *Cochliobolus sativus*; *Alt* – *Alternaria* sp.

Morphological-cultural and pathogenic characteristics of selected isolates - the inoculum for studying the growth and sporulation of the fungi was obtained by cultivating the starting isolates on PDA at 22°C in the dark. Disks with a diameter of 4 mm were cut from the periphery of actively growing colonies and placed in the center of Petri dishes (d = 90 mm), each containing 25 ml of the corresponding culture medium. The inoculated Petri dishes were incubated at 22°C. One replicate consisted of 5 Petri dishes. To assess mycelial growth, two mutually perpendicular diameters of the colonies were measured. Their shape, profile, periphery and color, as well as the formation of pigment, were determined visually; the contours and structure – at low magnification of the binocular, and the presence of sporulation – by microscopy. The ability of the isolates to sporulate on natural substrate and culture medium was monitored. The color of the

colonies was described according to Rayner (1970).

The study of the growth and morphological and cultural characteristics of *P. tritici-repentis* included 4 isolates (*Ptr4*, *Ptr6*, *Ptr7* and *Ptr8*), which originated from single ascospores formed on overwintered durum wheat stems, geographical area - the experimental field of the FCI – Chirpan (Figure 3).

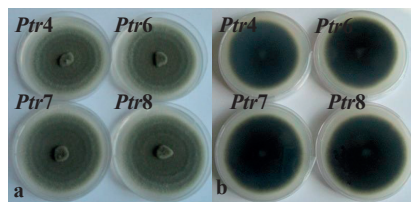


Figure 3. Colonies of *Pyrenophora tritici-repentis* after 7 days of cultivation on potato-dextrose agar (isolates *Ptr4*, *Ptr6*, *Ptr7* and *Ptr8* originate from ascospores): a. from the upper side; b. from the lower side

In the laboratory experiments with *M. nivalis*, isolate *MnTd4* was used, which was obtained from a durum wheat stem substrate, geographical area of the experimental field of the FCI – Chirpan (Figure 4).

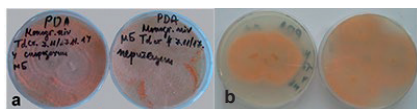


Figure 4. Colonies of *Monographella nivalis* on potato-dextrose agar originating from conidia (left) and ascospores (right): a. from the upper side; b. from the lower side

The isolates of *Pa. nodorum* and *Pa. avenae* f. sp. *triticea* are of geographical origin in the experimental field of the FCI - Chirpan, from a substrate, respectively, of the ear and leaf of the variety Predel (Figures 5 and 6).

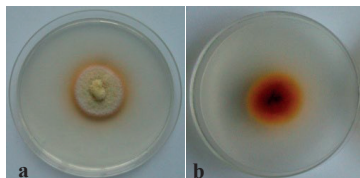


Figure 5. Morphology of colonies of *Parastagonospora avenae* f. sp. *triticea* after 5-day cultivation on potato-dextrose agar: a. from the upper side; b. from the lower side

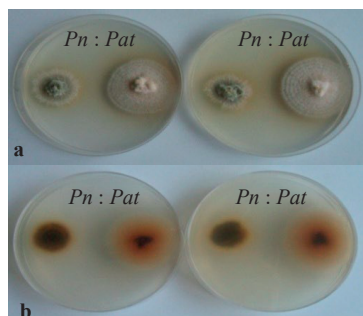


Figure 6. Colonies of *Parastagonospora nodorum* (*Pan*) and *Pa. avena* f. sp. *triticea* (*Pat*) after 10-day cultivation on potato-dextrose agar by the double culture method: a. from the upper side; b. from the lower side

To study the morphological and cultural characteristics of the sexual and asexual morphs of *C. herbarum*, 6 isolates originating from single ascospores (*D1*, *D3* and *D4-2*) and from single conidia (*C1*, *CR* and *C30-2*) were included, which were inoculated on PDA

(potato dextrose agar), MEA (malt extract agar) and OA (oats agar) (Gams et al., 2007). They were cultured for 14 days at 25°C, after which the color of the colonies on the upper and lower sides was assessed using the Rayner (1970) chart. Linear growth was determined on PDA MEA and OA by inoculating 4 Petri dishes for each isolate on each medium and incubating for 14 days at 25°C, after which the diameter of the colonies was measured (Figure 7).

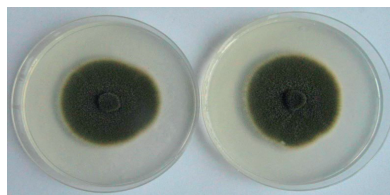


Figure 7. Colonies of *Cladosporium herbarum* after 14 days of cultivation on potato-dextrose agar

Microscopic observations were performed on colonies cultured for 7 days on SNA under continuous UV illumination and 25°C to stimulate sporulation. The preparations were mounted in Shear's solution (Gams et al., 2007). Pathogenicity test of selected isolates - a test to verify the pathogenicity of selected isolates was conducted at the FCI - Chirpan. The 12 varieties described above (*T. durum*) and the Enola variety (*T. aestivum*). The test included 10 isolates from 6 fungal species obtained during the study from *T. durum* (*Td*) and *T. aestivum* (*Ta*): *P. tritici repentis* (*PtTd34-1* and *PtTa26-1*; *Pa. avenae* f.sp. *triticea* (*PatTd114-1* and *PatTa118-1*); *Phaeophleospora* sp. (*Td126-1* and *Ta129-2*); *C. herbarum* (*CTd30-2*); *M. nivale* (*MnTd18-1* and *MnTa15-2*) and *Z. tritici* (*ZtrTaVII-10*).

An inoculum of each isolate was prepared from 10-day-old cultures of the fungi grown on PDA. Plants in the fully developed second leaf phase were sprayed with a spore suspension (concentration of about 10^6 conidia/ml), then placed in a humid chamber for 96 hours. The reporting for *Z. tritici* was done after two weeks, and for all other species after two weeks. Only the spotting on the second leaf of the inoculated plants was evaluated. The leaves with spots were examined under binoculars for the presence of sporulation. Re-insulations have been made.

The sexual morph of *C. herbarum* was identified in the culture, and two isolates of the fungus were selected: *D1* (ascospore origin) and *C1* (conidia origin) to check pathogenicity. Plants of two cultivars (Predel and Hundur) were inoculated by spraying with a spore suspension ($\sim 10^5$ conidia/ml) and placed in a humid chamber for 48 hours. Controls were treated in the same way but sprayed with sterile distilled water only. Re-insulations have been made.

CONCLUSIONS

The conducted study provides a comprehensive characterization of the complex of fungal pathogens causing leaf spot on durum wheat in Bulgarian conditions. In the experimental fields of the FCI - Chirpan, significant species diversity has been established, with the composition and relative prevalence of individual pathogens varying depending on the year and agrometeorological conditions. The most widespread on durum wheat are the fungal species that form the sexual stage under the specific conditions - *Pyrenophora tritici-repentis*, *Parastagonospora avenae* f. sp. *triticea*, *Monographella nivalis* and *Cladosporium herbarum*. Two of them - *M. nivalis* and *C. herbarum* - were recorded in all years of the study, which highlights their ecological plasticity and stable presence in the agroecosystems of durum wheat. *P. tritici-repentis* is characterized by high incidence in individual years, which confirms its importance as one of the leading causes of leaf spots.

Differences in the susceptibility of the studied varieties to individual pathogens were found, which is essential for breeding work and the development of resistant durum wheat varieties. Morphological-cultural analysis and pathogenic tests confirm the reliability of the comparative-morphological method of diagnosis, especially in diseases with similar field symptoms.

The results obtained expand the knowledge about the biodiversity and distribution of pathogens involved in the leaf spot complex and can serve as a scientific basis for optimizing phytosanitary monitoring, making informed decisions for plant protection, and directing selection towards increased resistance in order to prevent future epiphytotia.

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STUDY ON THE AGRONOMIC AND PRODUCTIVE TRAITS OF WINTER CEREALS GROWN SOLE AND AS MIXED CROPS

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Abstract

There is a limited information for the growing of winter cereals in mixed cropping system. Therefore, a field experiment in two growing seasons (2023/2024 and 2024/2025) was conducted. The trial was performed on the lands of the Agricultural University of Plovdiv, Bulgaria. Variants of the experiment were: 1. Winter wheat - variety 'Sofru' (sole crop); 2. Winter wheat - variety 'Solindo' (sole crop); 3. Winter wheat - variety 'Sofru' + winter wheat - variety 'Solindo' (mixed crop); 4. Winter barley - variety 'Odyssey' (sole crop); 5. Winter barley - variety 'Odyssey' + winter wheat - variety 'Sofru' (mixed crop); 6. Winter barley - variety 'Odyssey' + winter wheat - variety 'Solindo' (mixed crop); 7. Rye, variety 'Millennium' (sole crop). Differences were found in the height of the plants and the ear length, as well as the 1000 grain weight and the hectoliter mass of the seeds in mixed crops. The highest yield was recorded when the wheat variety 'Solindo' was sown alone. Regarding barley, a higher yield was recorded when it was grown together with the wheat variety 'Solindo'.

Key words: winter cereals, sole crops, mixed cropping.

INTRODUCTION

Mixed cropping is a cropping system in which different types of crops are grown together in a field so that nutrient requirements are shared. This reduces risk and provides some insurance against the failure of one crop. It is one of the oldest agricultural production systems (Plucknett and Smith, 1986).

Cropping systems based on carefully designed crop mixtures have shown several advantages under different farming conditions (Willey, 1979; Malézieux, 2009).

Some advantages of intercropping are nutrient use efficiency and yield stability (Lizarazo et al., 2020).

As described by Silvertown (1982), in mixed cropping two or more crops are grown together at the same time on the same area. For example, the forage of cereal-legume-cereal intercropping has a higher crude protein content and a higher relative feeding value for the animals (Strydhorst et al., 2008).

According to Lauk and Lauk (2009), the highest yield was found for the combination of pea-oat mixture. After growing pea+barley and pea+oilseed rape as intercropping, the yield was higher than that of single crops (Malhi, 2012).

Sibma (1977) and Spitters and Kramer (1986) have indicated that knowledge of differences in growth characteristics contributes to a better understanding of competitive differences or complementarities between varieties or crop species in mixtures. The growth rate of crop plants is determined by a number of factors, among which are the crop species/varieties grown in a mixed crop (Harris, 1990).

Wang et al. (2021) tested the intercropping of lentil (*Lens culinaris*) with five spring-sown companion crops: (*Hordeum vulgare*), wheat (*Triticum aestivum*), oats (*Avena sativa*), seed flax (*Linum usitatissimum*) and buckwheat (*Fagopyrum esculentum*). The intercropping was compared with single crops of these crops at the Kleinhohenheim Organic Research Station, University of Hohenheim, Germany, in 2009 and 2010. The aim of the study was to identify the most suitable intercropping and intercropping ratio for lentil in terms of yield, weed control and lodging under temperate climatic conditions. Intercropping of wheat and barley with lentil in Central Europe has significant benefits in terms of grain yield, weed control and crop resistance to lodging.

Mixed cultivation of peas together with camelina showed a significant suppressive effect on the weed flora.

Study conducted by Poggio (2005), mixing plant species can also reduce weed diversity. According to Leclère et al. (2019), the co-cultivation of camelina with peas or barley showed a reduction in weed biomass. The weed suppression is probably due to the mutually increased competitiveness of the two crops, indicating a mechanism based on resource allocation (Saucke and Ackermann, 2006).

Liebman and Dyck (1993) found that the composition of two or more crops reduced the weed biomass in the system compared to the crops grown alone.

In the mixed sowing of camelina genotype sown together with pea and vetch the development of the weeds *S. halepense* and *S. viridis* was severely suppressed (Neshev et al. 2023).

The information about the growing winter cereals in mixed cropping systems is still scarce. Therefore, this experiment's goal was to evaluate the systems effect on some agronomic and productive traits of different grain crops sown alone and mixed together.

MATERIALS AND METHODS

The research was conducted at the Experimental Field of Agricultural University – Plovdiv, Bulgaria. The study was performed in two growing seasons of the winter cereals – 2023/2024 and 2024/2025.

For the aim of the trial, the winter wheat varieties “Sofru” and “Solindo” were grown, the barley variety grown was “Odyssey”, and the rye variety “Millennium”. The experiment was based on the “long plots” method.

The following variants were under evaluation:

1. Winter wheat variety “Sofru” (sole crop – 200 kg ha⁻¹ sowing rate);
2. Winter wheat variety “Solindo” (sole crop – 200 kg ha⁻¹ sowing rate);
3. Winter wheat varieties “Sofru” + “Solindo” (mixed crop – 100:100 kg ha⁻¹ sowing rates);
4. Winter barley variety “Odyssey” (sole crop – 180 kg ha⁻¹ sowing rate);
5. Winter barley variety “Odyssey” + wheat variety “Solindo” (mixed crop – 90:100 kg ha⁻¹ sowing rates);
6. Winter barley variety „Odyssey“ + wheat variety “Sofru” (mixed crop – 90:100 kg ha⁻¹ sowing rates);
7. Winter rye variety

“Millennium” (sole crop – 200 kg ha⁻¹ sowing rate).

The preceding crop was maize. The soil preparation performed before sowing was deep plowing, followed by discing and harrowing. Before sowing, fertilization was performed with 300 kg ha⁻¹ of NPK. The spring dressing was done with 300 kg ha⁻¹ of ammonium nitrate.

The following biometrical parameters were evaluated:

- Visual assessment of area coverage by single and mixed crops - according to the Braun-Blanquet (1932) abundance-dominance scale (%). The assessment was done in two stages – first at tillering and second at flowering of the plansts;

- Stem length (cm);

- Ear length (cm).

The Biometric measurements were performed in three replicates for each variant of a given sole crop and mixed cropping system. A replicate was 30 plants (90 plants and ears total were measured).

Grain parameters studied were 1000 grain weight (g) and Hectoliter grain weight (kg).

The evaluation of these parameter was in three replications per variant.

The productivity of the sole and mixed crops (t ha⁻¹) were determined after harvesting the entire experimental plot of each variant. Harvesting was carried out with a combine harvester for plot trials of the company Wintersteiger®.

The agrometeorological data was taken from a weather station Meteobot® Pro.

For the processing and interpretation of the experimental data, Duncan's Multiple Range Test (Duncan, 1955) by SPSS 19 program was used. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

On Table 1 is presented the amount of precipitation and the average minimum and maximum temperatures during the vegetation of the sole and mixed crops. According to the meteorological data, we can determine how agrometeorological conditions affect the plants. In the two research years (2023/2024 and 2024/2025) the amount of precipitation in October, when the sowing of the sole and mixed

crops was carried out, was low. In the two years, the vegetation began in November, when 52.8 mm fell in 2023 and 22.8 mm in 2024. Lower precipitation was recorded in February, 2024 as well – 6.8 mm. The minimum and maximum

temperatures were relatively favourable for plants’ development. Despite the high winter temperatures, no negative impact of the warm winter months on the growth and development of crops was recorded.

Table 1. Average monthly precipitation and minimum and maximum monthly temperatures during the trial seasons

Years / Months	Precipitation, mm	min. t, C°	max. t, C°	Precipitation, mm	min. t, C°	Max. t, C°
	2023/2024	2023/2024	2023/2024	2024/2025	2024/2025	2024/2025
October	2.3	8.6	26.5	4.2	5.3	23.9
November	52.8	3.9	15.8	22.8	-0.4	14.1
December	51.1	-0.2	12.1	150.3	2.12	9.7
January	47.8	-1.9	9.9	22.6	-1.9	10.9
February	6.8	2.6	17.4	25.3	-3.2	8.3
March	39.3	4.9	17.7	67.2	3.3	19.3
April	37.3	8.4	25.8	72.8	5.6	21.1
May	74.5	11.2	24.3	55.1	10.6	25.2
June	5.3	17.5	35.1	11.5	15.4	34.9

In the first observation (in the tillering stage), the percentage of area coverage was the highest in the sole crop of barley – 95% in 2024 and 85% in 2025 (Table 2). The sole crop of the wheat varieties also show a high percentage of area coverage – 90% in 2024 and 80% in 2025. In this assessment, it is observed that in the mixed cropping systems of “Odyssey” + “Sofru”, as well as in that of “Odyssey” + “Solindo”, a predominance of barley was observed over wheat, and this was more severely pronounced in the mixed cropping of barley with the wheat

variety “Solindo”. Rye, in turn, has a lower area coverage – 80% in individual years.

In the second assessment, the highest percentage of area coverage was in the sole crops of barley and the winter wheat varieties (Table 2). Sole crops of wheat varieties increased the percentage of area coverage to 95% in 2024 and up to 90% in 2025. In the mixed crop of the two wheats, a 50:45% coverage was found in 2024 and in 2025 the coverage was 50:50. During this period, the excess of barley over wheat varieties in the mixed crop of these crops increased.

Table 2. Crops’ abundance-dominance by Braun-Blanquet’s scale (%)

Variants	2024	
	1 st – tillering	2 nd – flowering
1. Sofru	90	95
2. Solindo	90	95
3. Sofru + Solindo	40: 45	50: 45
4. Odyssey	95	95
5. Odyssey + Sofru	60: 35	65: 40
6. Odyssey + Solindo	60: 35	70: 25
7. Millennium	85	90
Variants	2025	
	1 st – tillering	2 nd – flowering
1. Sofru	80	90
2. Solindo	80	90
3. Sofru + Solindo	45: 55	50: 50
4. Odyssey	85	95
5. Odyssey + Sofru	50: 40	65: 30
6. Odyssey + Solindo	55: 40	70: 25
7. Millennium	80	95

Table 3 presents the height of the plants at the end of the growing season. Genetically, rye has the tallest plants, which is also confirmed in the current experiment. According to Trenbath (1976) if grown as mixed rather than as a sole crop, the plants may reach an equivalent size or either dominants or suppressed plants in the sole crop's stand. In our study differences in the height of the plants grown in mixed crops were established.

It was observed that when growing the two varieties of wheat ("Sofru" + "Solindo") as a

mixed crop, the height of the plants of the "Sofru" variety decreases, and that of the "Solindo" variety increases. This was less pronounced when wheat is grown in a mixed crop with barley. When barley is grown in a mixed crop with the wheat variety "Sofru", the plants were shorter than those of the barley alone, and the wheat of the "Sofru" variety in the mixed crop increased its height. When barley was mixed with "Solindo", the barley developed better and its height slightly decreased, while the wheat was higher.

Table 3. Plant height at the end of the vegetation (cm)

Variants	2024	2025	Average
1. Sofru	75.42	74.24	74.83
2. Solindo	70.11	77.54	73.83
3. Sofru + Solindo	66.40 + 81.41	70.22 + 85.27	68.31 + 83.34
4. Odyssey	85.15	88.57	86.86
5. Odyssey + Sofru	67.51 + 78.90	74.12 + 79.34	70.82 + 79.12
6. Odyssey + Solindo	85.90 + 81.80	87.78 + 80.09	96.84 + 80.95
7. Millennium	163.98	174.35	169.17

On Table 4 is the ear length. Differences were found in this indicator when the plants were grown sole or as mixed crops. It was observed that when the wheat varieties were grown as a mixed crop, the length of their ears was

decreased. This was more pronounced when the winter wheats were grown in a mixed crop with barley. It was also observed that the barley ears also decreased in length when the wheats were grown mixed crop with barley.

Table 4. Ear length at the end of the vegetation (cm)

Variants	2024	2025	Average
1. Sofru	11.21	12.05	11.63
2. Solindo	10.78	11.27	11.03
3. Sofru + Solindo	10.20 + 10.60	11.41 + 10.47	10.81 + 10.54
4. Odyssey	10.35	11.42	10.86
5. Odyssey + Sofru	9.79 + 9.52	10.27 + 10.23	10.03 + 9.88
6. Odyssey + Solindo	10.20 + 9.31	10.54 + 10.07	10.37 + 9.69
7. Millennium	10.24	11.13	10.66

Table 5 shows the data for the 1000 grain weight. In different years the 1000 grain weight may differ in mixed cropping systems (Xiao et al, 2026). In our research it was established in the sole crops of the wheat variety "Solindo" the indicator was with the highest values. It was noted that in the mixed sowing of the two wheat

varieties, the 1000 grain mass was decreased. It should be noted as well that the 1000 grain weight from the mixed crops of barley with the two wheat varieties slightly decreased compared to the sole crop of barley. The lowest 1000 grain weight was measured for rye, which in turn is again genetically justified.

Table 5. 1000 grain weight (g)

Variants	2024	2025	Average
1. Sofru	37.56 b	38.25 b	37.91
2. Solindo	38.47 a	40.57 a	39.52
3. Sofru + Solindo	37.65 b	38.04 b	37.85
4. Odyssey	36.84 c	38.25 b	37.55
5. Odyssey + Sofru	37.80 b	36.41 c	37.11
6. Odyssey + Solindo	37.57 b	35.51 c	36.54
7. Millennium	29.84 d	31.29 d	30.57

Values with different letters are with proven differences according to Duncan's Multiple Range test ($p < 0.05$).

The hectoliter mass of the grain follows the trend established for the 1000 grain mass of the seeds. The highest hectoliter mass of the seeds was established for the independent sowing of the variety "Solindo" - 80.14 kg. Here it was also noticeable that for the mixed sowing of the two varieties of wheat the hectoliter mass of the

grain slightly decreased to 78.16 kg. It should be noted that the hectoliter mass of the grain from the mixed crops of barley with the two varieties of wheat increases slightly compared to the sole sowing of barley, with the increase being in favor of the mixed crop of "Odyssey" + "Solindo" - 74.61 kg (Table 6).

Table 6. Hectoliter weight (kg)

Variants	2024	2025	Average
1. Sofru	76.48 c	78.56 b	77.52
2. Solindo	79.02 a	81.26 a	80.14
3. Sofru + Solindo	77.12 b	79.19 b	78.16
4. Odyssey	69.77 e	71.09 d	70.43
5. Odyssey + Sofru	73.47 d	75.45 c	74.46
6. Odyssey + Solindo	73.54 d	75.67 c	74.61
7. Millennium	68.23 f	70.78 e	69.51

Values with different letters are with proven differences according to Duncan's Multiple Range test ($p < 0.05$).

Grain yields are genetically justified for each plant species, and are also influenced by growing conditions.

According to Willey (1979), Reddy and Reddy (1981), Kara et al. (2010) and Wang et al. (2021) the yield was significantly increased by mixed cropping compared to the sole crops. Our obtained results for the plants' productivity from the sole and mixed crops are in Table 7.

Overall, the highest yield was recorded with single cropping of wheat variety Solindo – 6.88

t ha⁻¹ average for the period. It should be noted that the average yield from the mixed cropping of the two wheat varieties was 6.68 t ha⁻¹, which in turn was close to that of sole cropping of Sofru variety – 6.55 t ha⁻¹ average for the period of the study.

Regarding barley, a higher yield was recorded when it was grown together with the winter wheat variety Solindo – 5.89 t ha⁻¹.

Rye has a low yield potential, with grain yields of 2.11 t ha⁻¹ obtained from it.

Table 7. Grain yields (t ha⁻¹)

Variants	2024	2025	Average
1. Sofru	6.30 a	6.79 b	6.55
2. Solindo	6.54 a	7.21 a	6.88
3. Sofru + Solindo	6.39 a	6.96 b	6.68
4. Odyssey	5.60 b	5.96 c	5.78
5. Odyssey + Sofru	5.49 b	5.84 c	5.67
6. Odyssey + Solindo	5.75 b	6.04 c	5.89
7. Millennium	1.97 d	2.24 d	2.11

Values with different letters are with proven differences according to Duncan's Multiple Range test ($p < 0.05$)

CONCLUSIONS

The abundance-dominance of the studied crops was in favour of the barley variety "Odyssey" grown as sole crop. In mixed crops the barley prevailed in the covered area while the sowing of the two winter wheat varieties together on the same area showed that both winter wheat varieties cover approximately the same area. When grown together, the plant height of the winter wheat variety "Sofru" decreased in comparison to the height of the plans of the

"Solindo" variety where an increase was found when compared to the sole crops. It was also found for the ear length. Similar results were recorded for barley grown as mixed crop with the winter wheat varieties.

The indicator 1000 grain weight was the highest for the winter wheat variety "" grown as sole crop. In mixed roping with "Sofru" and the barley, the parameter was diminished.

When barley was grown together with winter wheat the 1000 grain weight increased. The

hectolitre seed mass follows the tendency of the 1000 grain weight.

The highest yield was recorded for the sole crop of the winter wheat variety "Solindo". Regarding barley, a higher yield was recorded when it was grown together with "Solindo".

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COMPARATIVE STUDY ON ANALYTICAL VARIABILITY OF PHYSICAL PURITY IN SEED TESTING LABORATORIES

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Abstract

This study presents a comprehensive six-year analysis (2019-2024) of inter-laboratory analytical variability across Romania's 30-laboratory seed testing network through 11 biannual proficiency testing rounds. Following ISTA Standard Proficiency Test (PT-P-01) methodology, the research examined all three purity components across 11 crop species representing diverse ISTA crop groups. Mathematical analysis revealed that while overall physical purity demonstrated exceptional standardization (mean CV 0.36%), constituent components exhibited dramatically different variability patterns due to mathematical amplification effects in constrained compositional systems. Inert matter showed extreme variability (mean CV 70.7% - 197-fold higher than purity), other seeds demonstrated intermediate variability (CV 32.0% - 89-fold higher), while pure seed maintained exceptional consistency. This component-specific analysis reveals that traditional purity-focused proficiency testing masks substantial analytical complexity at component level. Z-score analysis confirmed outstanding network competency (95.45% excellent ratings), validating that observed variability reflects mathematical constraints and biological factors rather than analytical incompetence. The study demonstrates that mechanical separation methodology achieves chemical-procedure precision under quality-assured conditions, while providing new insights into compositional data analysis for seed testing networks.

Key words: physical purity, inter-laboratory variability, seed testing laboratories, components, quality assurance.

INTRODUCTION

Physical purity determination constitutes a foundational requirement in seed certification protocols worldwide. As defined in the International Rules for Seed Testing (ISTA, 2025a), physical purity determination involves the mechanical separation of seed lots into three distinct components: pure seed, other seeds, and inert matter. This analysis provides essential data for gravimetric characterization of seed lots and ensures both commercial and agronomic value. The methodology, which relies on mechanical separation rather than biological evaluation, remains the most practical and economical rapid analysis method available. While ISTA regulations and national standards such as SR 7713:1999 establish standardized analytical procedures, the methodology alone does not guarantee uniform implementation or performance across laboratory networks. Laboratory competency encompassing analyst expertise, equipment calibration, and quality

management systems-represents the critical variable determining analytical reliability.

Traditional proficiency testing evaluation in seed purity determination focuses exclusively on overall purity performance using z-score analysis according to ISO 13528:2022 methodology, potentially overlooking component-specific variability patterns that may provide deeper insights into laboratory competency assessment. Recent advances in understanding multi-component analytical systems reveal that when analytical results are subject to sum-to-constant constraints as in seed purity analysis where components must total exactly 100% measurement uncertainty propagates according to predictable mathematical patterns, with minor components experiencing disproportionately amplified variability relative to major components (Aitchison, 1982; Büchse et al., 2007).

This mathematical amplification phenomenon, well-established in compositional data analysis, offers a new perspective for interpreting

proficiency testing results in seed purity determination, potentially distinguishing between genuine competency issues and mathematically inevitable variability patterns in trace-level components.

As demonstrated by proficiency testing analysis over extended periods, improving laboratory performance requires continuous staff development and supervision of the entire testing process (Kojić et al., 2011). By analyzing the results obtained in testing rounds, laboratories improve the proficiency and competency of their staff and emphasize the necessity of permanent learning, specialization, and comprehensive supervision to obtain reliable results.

The Romanian seed testing laboratory network comprises 30 territorial inspectorates for quality of seed and planting material - ITCSMS and one Central Laboratory for Quality of Seed and Planting Material-LCCSMS, all operating under mandatory authorization per Ministerial Order 141/2012, which requires ISO/IEC 17025:2017 quality management system compliance and biannual proficiency testing participation. This regulatory framework reflects the recognition that analytical standardization alone is insufficient; sustained competency validation through systematic performance evaluation is essential for reliable seed certification and to ensure that all participating laboratories maintain consistent competency thresholds.

The objective of this study was to conduct a six-year longitudinal evaluation (2019-2024) of inter-laboratory competency in physical purity determination within the Romanian laboratory network, incorporating component-specific mathematical analysis to enhance traditional proficiency testing assessment. The analysis encompasses 11 biannual proficiency testing rounds, 11 crop species representing all ISTA crop group classifications, and performance data from all 30 participating laboratories. By quantifying inter-laboratory variability and individual laboratory performance against ISTA international methodology, this study provides empirical assessment of actual analytical variability within the network regarding a standardized, mechanically-based methodology

and validation of whether the national regulatory framework effectively maintains competency thresholds across all participating units.

MATERIALS AND METHODS

This retrospective comparative study analyzed proficiency testing (PT) results from 11 consecutive biannual rounds conducted during 2019-2024 across the Romanian seed testing laboratory network.

All 30 territorial inspection units (ITCSMS) and the central laboratory (LCCSMS) participated under Ministerial Order 141/2012, operating with ISO/IEC 17025:2017 authorization and applying identical SR 7713:1999 analytical procedures for the purity determination. Since all laboratories operated under identical regulatory requirements, standardized methodologies, and synchronized equipment calibration protocols, measured inter-laboratory variability directly reflects differences in laboratory competency, analyst expertise, procedural compliance, and equipment functionality.

Biological material and proficiency test sample preparation

Test material selection was based on the ISTA crop group classification system (International Seed Testing Association, 2025d). The 11 crop species tested across all proficiency testing rounds (Table 1) were selected to encompass diverse botanical families, agricultural relevance, and seed morphologies, including cereal (*Triticum aestivum*, *Hordeum vulgare*), forage grasses (*Festuca pratensis*, *Lolium multiflorum*), legumes (*Medicago sativa*, *Glycine max*), and vegetable and oilseed species (*Allium cepa*, *Lactuca sativa*, *Helianthus annuus*, *Brassica napus*, *Sinapis alba*). The selection ensured representation of both chaffy seeds and non-chaffy seeds thereby enabling evaluation of proficiency testing performance across botanically diverse crop groups. All scientific nomenclature and taxonomic designations were standardized in accordance with International Seed Testing Association (ISTA, 2025c).

Table 1. Crop species tested in ISTA proficiency testing rounds (2019-2024)

No	Species	Family	Crop Group (ISTA)	Year	Round	Chaffiness	PSD
1	<i>Medicago sativa</i> L.	<i>Fabaceae</i>	3 (Small legumes)	2019	1	non chaffy	10
2	<i>Allium cepa</i> L.	<i>Amaryllidaceae</i>	6 (Vegetables, spices, herbs, and medicinal species)	2019	2	non chaffy	10
3	<i>Triticum aestivum</i> L.	<i>Poaceae</i>	2 (Cereals)	2020	1	non chaffy	40
4	<i>Festuca pratensis</i> Huds.	<i>Poaceae</i>	1 (Grasses)	2021	1	chaffy	33
5	<i>Brassica napus</i> L.	<i>Brassicaceae</i>	5 (Other agricultural species)	2021	2	non chaffy	10
6	<i>Hordeum vulgare</i> L.	<i>Poaceae</i>	2 (Cereals)	2022	1	non chaffy	62
7	<i>Lolium multiflorum</i> Lam.	<i>Poaceae</i>	1 (Grasses)	2022	2	chaffy	33
8	<i>Lactuca sativa</i> L.	<i>Asteraceae</i>	6 (Vegetables, spices, herbs, and medicinal species)	2023	1	chaffy	4
9	<i>Helianthus annuus</i> L.	<i>Asteraceae</i>	5 (Other agricultural species)	2023	2	non chaffy	4
10	<i>Sinapis alba</i> L.	<i>Brassicaceae</i>	5 (Other agricultural species)	2024	1	non chaffy	10
11	<i>Glycine max</i> (L.) Merr.	<i>Fabaceae</i>	4 (Pulses)	2024	2	non chaffy	10

Note: Pure seed definitions (PSD) represent the number of intact caryopses (*Poaceae*), seeds (other families), or seed units required for classification as pure seed per SR 7713:1999 and ISTA Rules, Chapter 3: Purity Analysis (ISTA, 2025a). Chaffy species include grasses with lemma and palea; non-chaffy species refer to naked seeds without these structures.

Proficiency test samples were prepared by the Central Laboratory for Quality of Seed and Planting Material (LCCSMS) according to ISTA PT-P-01 Standard Proficiency Test (ISTA, 2025b) and ISO/IEC 17043:2023 requirements. For each biannual testing round, a single seed lot of each species was physically mixed and divided into 30 individual test portions (one per laboratory) plus 10 quality control sub-samples for homogeneity verification. While this single-lot approach differs from multi-lot protocols recommended in ISO 13528:2022, the extended temporal scope across 11 different lots provides comprehensive assessment of laboratory competency across diverse biological materials. This methodological adaptation was necessitated by practical constraints in proficiency test sample preparation within the Romanian network during the study period.

Sample homogeneity verification and quality assurance

Homogeneity verification was performed independently on 10 sub-samples of each proficiency test batch, with each sub-sample analyzed according to SR 7713:1999 pure seed determination procedures. Test samples were sealed in protective packaging bearing unique

laboratory identifiers (PT-LC-01 through PT-LC-30) and distributed to all 30 participating laboratories within 5 working days of batch preparation. Participating laboratories were instructed to store samples at room temperature (15-25°C) and relative humidity (45-75%) prior to analysis, with a maximum storage period of 30 days before testing in accordance with ISTA PT-P-01 stability requirements. All laboratories completed testing and submitted the results within the required three-month reporting window similarly to ISTA proficiency testing protocol.

Purity testing methodology and component classification challenges

All participating laboratories conducted pure seed determination following SR 7713:1999 working procedure. The procedure involved: (1) mechanical and manual separation of seed samples into three components (pure seed, other seeds, and inert matter); (2) species-specific identification of non-target seeds using standardized taxonomic keys consistent with ISTA nomenclature; (3) mass quantification of the pure seed component using calibrated analytical balances (calibrated biannually according to ISO 17025:2017 requirements); and (4) conversion of mass values to

percentages. Pure seed component classification followed species-specific criteria defined in national standard SR 7713:1999, with the critical "half-seed" criterion requiring subjective assessment of fragment size relative to original seed dimensions - a decision point that becomes increasingly challenging for species with seed diameter below 3mm.

Component Interdependence in Proficiency Testing Assessment

The mathematical constraint that pure seed + other seeds + inert matter = 100% creates analytical interdependence where classification uncertainty affecting any component produces compensatory adjustments in others. This constraint has profound implications for proficiency testing evaluation, as identical analytical uncertainty produces disproportionate impacts across components based on their baseline proportions.

In constrained compositional systems, coefficient of variation amplification follows predictable mathematical patterns where components approaching zero exhibit extreme relative variations for identical absolute differences (Aitchison, 1982; Büchse et al., 2007). This phenomenon necessitates component-specific analysis to distinguish mathematical amplification effects from genuine competency differences, particularly for trace-level components subject to equipment sensitivity constraints.

The sum-to-100% constraint creates a "zero-sum" classification system where analytical uncertainty redistributes among components rather than disappearing. When laboratories disagree on borderline particle classification, the dominant component absorbs variations with minimal relative impact, while trace components experience extreme proportional variations. This mathematical behavior requires specialized interpretation when evaluating proficiency testing results across individual components versus overall purity performance.

Quality control and outlier treatment

For each proficiency test round and crop species, submitted results underwent systematic quality assessment using a dual-standard approach combining ISTA proficiency testing protocols with ISO statistical methodology.

The initial assessment was done according to ISTA PT-P-01, where results with individual Z-scores exceeding ± 2.68 were flagged as requiring laboratory investigation, while results in the range ± 2.0 to ± 2.68 were classified as indicating possible analytical problems requiring verification. Following identification of potential outliers, each result was reviewed for transcription errors, calculation errors, or genuine analytical discrepancies.

The statistical treatment based on ISO 13528:2022, following quality assessment, statistical outliers were excluded from consensus calculations using the robust statistical methods specified in ISO 13528:2022 outlier exclusion protocol. This approach ensures that consensus parameters (mean, standard deviation, coefficient of variation) reflect the true analytical performance of competent laboratories while maintaining statistical validity of the proficiency testing evaluation.

Following quality assessment and outlier treatment, the following parameters were calculated for each species-round combination: consensus mean ($\bar{x}$), sample standard deviation (sd), coefficient of variation, minimum and maximum values, and amplitude.

The standard deviation (sd) was calculated according to the formula:

$$sd = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n - 1}}$$

where n = the number of laboratories.

The coefficient of variation (CV) was calculated as follows:

$$CV = \frac{sd}{\bar{x}} * 100$$

These descriptive statistics characterized the distribution and spread of component results across the 30 participating laboratories for each species and testing round.

Laboratory performance evaluation and z-score analysis

Laboratory competency was evaluated using Z-score analysis per ISTA Standard Proficiency Test methodology (ISTA, 2025b). Individual laboratory Z-scores were calculated as:

$$Z_i = \frac{x_i - \bar{x}}{sd}$$

where x_i represents the pure seed percentage reported by the individual laboratory, $\bar{x}$ is the consensus mean across all laboratories, and sd is the standard deviation of the distribution. Individual performance was classified using ISTA criteria: Satisfactory ($|Z| \leq 2.0$), Questionable ($2.0 < |Z| \leq 2.68$), and Unsatisfactory ($|Z| > 2.68$). Aggregate laboratory performance was assessed by summing absolute Z-scores across all species ($\Sigma|Z|$), with ratings of Excellent (≤ 3.5), Good (3.5-5.3), Acceptable (5.3-7.0), and Below Minimum (> 7.0). Component-specific analysis was performed to distinguish mathematical amplification effects from genuine competency differences, particularly for trace-level components subject to equipment sensitivity constraints (Aitchison, 1982; Büchse et al., 2007).

RESULTS AND DISCUSSIONS

Physical purity standardization

This retrospective analysis of 11 consecutive biannual proficiency testing rounds demonstrates exceptional standardization in physical purity determination across the Romanian seed testing laboratory network, with a mean coefficient of variation of only 0.36% across all species examined (Table 2). This level of precision approaches that typically associated

with chemical analytical procedures, validating the effectiveness of mechanical separation methodology under quality-assured conditions. The data presented in Table 2 reveals remarkably consistent performance across botanically diverse crop species, with mean purity values ranging from 97.70% in *Lactuca sativa* to 99.82% in *Helianthus annuus*. Most species maintained exceptionally tight analytical control, as evidenced by standard deviations below 0.20% for the majority of crops tested. Particularly noteworthy are the large-seeded species such as *Helianthus annuus* (CV 0.06%), *Triticum aestivum* (CV 0.08%), and *Glycine max* (CV 0.10%), which achieved minimal inter-laboratory variability due to clear morphological boundaries that enable objective classification even under challenging analytical conditions. The amplitude values shown in Table 2 provide additional insight into the practical range of laboratory performance variation. While most species demonstrated minimal spread between minimum and maximum values, certain crops presented greater analytical challenges. Notably, *Brassica napus* emerged as the sole species with substantially elevated variability (CV 0.51%), reflecting the combined analytical challenges of extremely small seed size (1–2 mm diameter), high-magnification requirements, and intrinsically elevated impurity fractions totaling 7.62% in commercial seed lots.

Table 2. Statistical Summary of Physical Purity (%) Results by Species (2019-2024)

No	Species	Mean (%)	SD (%)	CV (%)	Min. (%)	Max. (%)	Amplitude (%)
1	<i>Medicago sativa</i> L.	99.35	0.42	0.43	98.30	99.90	1.60
2	<i>Allium cepa</i> L.	98.70	0.18	0.18	98.30	99.10	0.80
3	<i>Triticum aestivum</i> L.	99.81	0.08	0.08	99.60	100.00	0.40
4	<i>Festuca pratensis</i> Huds.	98.66	0.12	0.12	98.40	98.90	0.50
5	<i>Brassica napus</i> L.	97.89	0.50	0.51	97.00	98.50	1.50
6	<i>Hordeum vulgare</i> L.	98.11	0.34	0.35	97.30	98.70	1.40
7	<i>Lolium multiflorum</i> Lam.	97.89	0.29	0.30	97.00	98.50	1.50
8	<i>Lactuca sativa</i> L.	97.70	0.17	0.18	97.30	98.00	0.70
9	<i>Helianthus annuus</i> L.	99.82	0.06	0.06	99.70	99.90	0.20
10	<i>Sinapis alba</i> L.	99.73	0.09	0.09	99.60	99.90	0.30
11	<i>Glycine max</i> (L.) Merr.	99.53	0.10	0.10	99.40	99.70	0.30

Note: SD = Standard Deviation; CV = Coefficient of Variation. Statistics calculated using dual-standard approach: initial assessment per ISTA PT-P-01 ($|Z| > 2.68$), followed by statistical exclusion per ISO 13528:2022 protocol. Sample sizes: n=29 for *Festuca pratensis* and *Lolium multiflorum* (1 outlier excluded per species); n=30 for remaining species. Values rounded to 2 decimal places.

The consistency observed across the remaining species validates several critical aspects of the

network's analytical framework. The regulatory uniformity established under Ministerial Order

141/2012, coupled with standardized SR 7713:1999 procedures and synchronized ISO/IEC 17025:2017 calibration protocols, has successfully eliminated methodological variability as a confounding factor. Consequently, the minimal inter-laboratory variations recorded in Table 2 can be attributed to genuine differences in laboratory competency, analyst expertise, and equipment functionality rather than procedural inconsistencies.

Mathematical Amplification Dynamics in Component Analysis

Analysis of the complete dataset reveals systematic relationships between component magnitude and variability, demonstrating the mathematical amplification effects predicted by compositional data theory. The amplification patterns follow a clear hierarchy: the Pure seed (mean 98.62%): CV = 0.36% (baseline reference), followed by the Other seeds (mean 0.68%): CV = 32.0% (89-fold amplification relative to pure seed) and the Inert matter (mean 0.40%): CV = 70.7% (197-fold amplification relative to pure seed).

This amplification follows the mathematical principle that for components approaching zero, small absolute differences translate to extreme relative variations. When a component represents 0.01% (as in *Medicago sativa* inert matter), a single 0.01g particle classification difference produces ±100% CV variation - mathematically inevitable under standard

analytical balance precision. The sum-to-100% constraint creates a compensatory mechanism where analytical uncertainty redistributes among components rather than disappearing, explaining why overall purity remains exceptionally consistent (CV 0.36%) despite substantial component-level variability.

Component-specific mathematical amplification effects

Component analysis revealed differential variability patterns consistent with mathematical amplification effects in constrained compositional systems (Aitchison, 1982; Büchse et al., 2007). While overall physical purity demonstrated exceptional standardization (CV 0.36%), constituent components exhibited substantially elevated variability that is effectively masked in purity totals through compensatory classification mechanisms inherent to the sum-to-100% constraint.

Inert Matter Component: Equipment Precision Limitations

The inert matter component demonstrated substantially higher inter-laboratory variability compared to overall physical purity, with a mean coefficient of variation of 70.7% across all species (Table 3). However, this apparent inconsistency reflects inherent analytical constraints and mathematical amplification effects rather than systematic competency deficiencies across the laboratory network.

Table 3. Statistical Summary of Inert Matter (%) component Results by Species (2019-2024)

No	Species	Mean (%)	SD (%)	CV (%)	Min. (%)	Max. (%)	Amplitude (%)
1	<i>Medicago sativa</i> L.	0.01	0.04	313.88	0.00	0.20	0.20
2	<i>Allium cepa</i> L.	0.00	0.00	0.00	0.00	0.00	0.00
3	<i>Triticum aestivum</i> L.	0.03	0.04	116.31	0.00	0.10	0.10
4	<i>Festuca pratensis</i> Huds.	0.39	0.09	24.27	0.25	0.65	0.40
5	<i>Brassica napus</i> L.	0.89	0.29	32.30	0.60	1.90	1.30
6	<i>Hordeum vulgare</i> L.	1.02	0.29	28.60	0.60	1.90	1.30
7	<i>Lolium multiflorum</i> Lam.	0.93	0.24	25.86	0.60	1.90	1.30
8	<i>Lactuca sativa</i> L.	0.76	0.20	25.86	0.30	1.20	0.90
9	<i>Helianthus annuus</i> L.	0.10	0.00	0.00	0.10	0.10	0.00
10	<i>Sinapis alba</i> L.	0.16	0.07	46.47	0.00	0.30	0.30
11	<i>Glycine max</i> (L.) Merr.	0.26	0.09	32.29	0.10	0.40	0.30

Note: SD = Standard Deviation; CV = Coefficient of Variation. Statistics calculated per ISO 13528:2022 outlier exclusion protocol following ISTA PT-P-01 assessment. Sample sizes: n=28 for *Helianthus annuus* (2 outliers excluded); n=29 for *Festuca pratensis*, *Lolium multiflorum*, and *Medicago sativa* (1 outlier excluded per species); n=30 for remaining species. Extreme CV values reflect mathematical amplification effects in trace-level components rather than analytical deficiencies. Values rounded to 2 decimal places.

Mathematical analysis confirms predictable amplification patterns in trace-level components, with species containing minimal inert matter showing extreme variability despite minimal absolute differences. *Medicago sativa* exemplifies this phenomenon with a CV of 313.88% despite a mean inert matter content of only 0.01%, while *Triticum aestivum* demonstrated similar behavior with a CV of 116.31% at 0.03% mean content. These extreme coefficients of variation become comprehensible when considering the underlying analytical realities: for small-seeded legumes with individual seed masses of 1.8-2.0 mg and typical sample masses of 2.0-3.0 g, trace-level inert matter fractions represent only 0.2-0.3 individual particles within the analytical sample. Under standard analytical balance precision requirements (0.01 g per SR 7713:1999), inter-laboratory variation in classifying borderline particles becomes mechanistically inevitable. The data in Table 3 reveals a clear relationship between component magnitude and analytical

precision. Species with moderate inert matter content ($\geq 0.5\%$) achieved substantially greater consistency, including *Lactuca sativa* (CV 25.86%), *Festuca pratensis* (CV 24.27%), and *Hordeum vulgare* (CV 28.60%), averaging CV 23.4% for this category. This dramatic improvement in precision validates that equipment sensitivity rather than analyst competency represents the primary constraint in trace-level determinations.

Other Seeds Component: Taxonomic Discrimination Challenges

The other seeds component demonstrated intermediate standardization with a mean coefficient of variation of 32.0%, exhibiting species-specific patterns that reflect botanical discrimination complexity rather than systematic analytical deficiencies (Table 4). The variability patterns observed provide valuable insights into the taxonomic challenges faced by analysts across the laboratory network.

Table 4. Statistical Summary of Other Seeds (%) Component Results by Species (2019-2024)

No	Species	Mean (%)	SD (%)	CV (%)	Min. (%)	Max. (%)	Amplitude (%)
1	<i>Medicago sativa</i> L.	0.64	0.40	62.14	0.10	1.70	1.60
2	<i>Allium cepa</i> L.	1.30	0.18	13.96	0.90	1.70	0.80
3	<i>Triticum aestivum</i> L.	0.16	0.08	50.79	0.00	0.30	0.30
4	<i>Festuca pratensis</i> Huds.	0.95	0.10	10.58	0.75	1.15	0.40
5	<i>Brassica napus</i> L.	1.22	0.39	32.30	0.70	2.40	1.70
6	<i>Hordeum vulgare</i> L.	0.87	0.11	12.25	0.60	1.10	0.50
7	<i>Lolium multiflorum</i> Lam.	1.18	0.20	16.95	0.70	1.60	0.90
8	<i>Lactuca sativa</i> L.	1.54	0.19	12.60	1.20	1.90	0.70
9	<i>Helianthus annuus</i> L.	0.08	0.05	58.55	0.00	0.20	0.20
10	<i>Sinapis alba</i> L.	0.11	0.05	45.49	0.00	0.30	0.30
11	<i>Glycine max</i> (L.) Merr.	0.21	0.07	35.79	0.00	0.40	0.40

Note: SD = Standard Deviation; CV = Coefficient of Variation. Statistics calculated per ISO 13528:2022 outlier exclusion protocol following ISTA PT-P-01 assessment. Sample sizes: n=28-30 per species based on statistical outlier exclusion detailed in methodology. Elevated CV values in certain species reflect taxonomic discrimination challenges rather than systematic analytical deficiencies. Values rounded to 2 decimal places.

Species facilitating consistent inter-laboratory identification demonstrated relatively low variability, including *Festuca pratensis* (CV 10.58%), *Hordeum vulgare* (CV 12.25%), and *Allium cepa* (CV 13.96%). These crops benefit from clear taxonomic distinctiveness that enables reliable differentiation between target species and morphologically distinct foreign seed fractions. The amplitude values in Table 4 support this interpretation, with these species showing manageable ranges between minimum and maximum laboratory results. Conversely,

species exhibiting substantial variability included those with morphological ambiguity in distinguishing foreign seeds from atypical pure seed variants, notably *Medicago sativa* (CV 62.14%), *Helianthus annuus* (CV 58.55%), and *Triticum aestivum* (CV 50.79%). The challenges are particularly pronounced in recently domesticated forage species such as *Medicago sativa*, where pronounced intraspecific morphological variation reflects the maintenance of atavistic seed characters. This biological reality introduces diagnostic

complexity in distinguishing damaged conspecific seeds from genuine inert material, and morphologically atypical pure seed variants from genuinely foreign species.

Laboratory competency assessment via z-score analysis

Z-score analysis following ISTA PT-P-01 methodology revealed exceptional network competency maintenance across the six-year study period. Of 330 total individual laboratory assessments (30 laboratories $\times$ 11 species), 315 (95.45%) achieved Excellent ratings ($|Z| \leq 2.0$), with only two isolated exceptions: one laboratory achieved questionable performance for *Lolium multiflorum* ($Z = 3.16$) and one laboratory received unsatisfactory rating for *Festuca pratensis* ($Z = -4.23$), representing analytical edge cases requiring investigation rather than systematic competency failures.

Aggregate performance assessment via summed absolute Z-scores ($\Sigma|Z|$) demonstrated that all 30 laboratories maintained satisfactory competency thresholds: 28 laboratories (93.3%) achieved Excellent aggregate ratings ($\Sigma|Z| \leq 3.5$), while 2 laboratories (6.7%) achieved Good ratings ($3.5 < \Sigma|Z| \leq 5.3$). Zero laboratories fell into Acceptable (5.3–7.0) or Below Minimum (>7.0) categories throughout the entire study period, providing empirical validation that mandatory biannual proficiency testing participation effectively maintains competency thresholds across all network participants.

Component-specific proficiency analysis confirmed that elevated component variability reflects mathematical amplification and equipment constraints rather than systematic competency issues.

Following outlier removal per ISO 13528:2022 protocol, 97.0% of both inert matter and other seeds assessments achieved satisfactory ratings, demonstrating sustained analyst competency even under challenging analytical conditions at component level.

Species-specific analytical hierarchy and morphological determinants

Analysis revealed a clear and interpretable hierarchy of analytical difficulty correlating with seed morphological characteristics. Contrary to conventional assumptions, chaffy versus non-chaffy morphology demonstrated

minimal impact on standardization: chaffy species averaged CV 0.29% compared to 0.42% for non-chaffy species, validating that modern mechanical procedures successfully manage morphological complexity under quality-assured conditions.

Primary morphological determinants of analytical difficulty include seed diameter, taxonomic distinctiveness of associated foreign seeds, and baseline commercial purity levels. The network achieved optimal performance for large-seeded species with clear morphological boundaries and elevated commercial purity, while demonstrating sustained competency even under challenging conditions of extreme seed size reduction and elevated contaminant fractions.

Temporal consistency and species-driven performance variation

Annual performance trajectories demonstrated overall network stability with species-driven fluctuations: 2019 (CV 0.74%), 2020 (CV 0.30%), 2021 (CV 1.43%), 2022 (CV 0.35%), 2023 (CV 0.40%), 2024 (CV 0.15%).

The 2021 elevation to CV 1.43% reflects the simultaneous analytical challenge of testing both *Festuca pratensis* and *Brassica napus* - the two most morphologically challenging species in the dataset - rather than competency degradation.

Critical validation of species-driven interpretation emerges from rapid recovery patterns: the 76% reduction in variability from 2021 to 2022 occurred without systematic corrective action, retraining, or equipment recalibration.

This automatic recovery demonstrates that identical laboratories applying identical procedures achieve predictable consistency when testing species of moderate analytical difficulty, confirming that annual performance variations correlate with intrinsic species-specific analytical complexity rather than competency drift.

CONCLUSIONS

This six-year longitudinal study of inter-laboratory analytical variability across Romania's 30-laboratory seed testing network (2019–2024) demonstrates that standardized

physical purity determination achieves exceptional analytical consistency comparable to chemical procedures.

Regulatory Framework Validation

The Romanian network, operating under Ministerial Order 141/2012 with mandatory ISO/IEC 17025:2017 authorization and biannual proficiency testing, maintained outstanding competency: mean inter-laboratory CV of 0.36% across 11 species, 95.45% of assessments achieving Excellent ratings, and zero laboratories requiring performance improvement throughout the study period.

This validates that standardized methodology (SR 7713:1999), mandatory authorization, and systematic competency verification successfully maintain analytical reliability across geographically dispersed laboratory units.

Mathematical Amplification in Component Analysis

While overall physical purity demonstrated exceptional consensus, constituent components exhibited substantially elevated variability effectively masked through compensatory classification mechanisms. Inert matter variability (mean CV 70.7%) exceeded physical purity variability (CV 0.36%) by 197-fold, while other seeds classification demonstrated 89-fold greater variability (CV 32.0%). This reflects mathematical amplification effects in constrained compositional systems rather than analyst incompetence.

Equipment and Biological Constraints

Component variability reflects two primary factors: equipment precision limitations and intraspecific morphological polymorphism.

For trace-level inert matter (<0.1%), standard analytical balance precision (0.01 g) creates mechanistically inevitable variation when analyzing 0.2-0.3 individual particles per sample.

For forage species (*Medicago sativa*, *Festuca pratensis*, *Lolium multiflorum*), pronounced morphological polymorphism introduces legitimate diagnostic complexity in botanical classification.

Species-Specific Analytical Hierarchy

Analysis revealed morphological determinants of analytical difficulty: large-seeded species (>3 mm) achieved minimal variability (*Helianthus annuus* CV 0.06%), while small-seeded species demonstrated elevated but manageable

variability (*Brassica napus* CV 0.51%). Importantly, chaffy versus non-chaffy morphology showed minimal impact (mean CV 0.29% versus 0.42%), validating modern analytical procedures.

Temporal Stability and Species-Driven Variation

The 2021 CV elevation (1.43%) reflected testing of morphologically challenging species (*Festuca pratensis*, *Brassica napus*) rather than competency degradation. Rapid recovery to 0.35% CV in 2022 without corrective action confirms species-difficulty interpretation and validates regulatory framework effectiveness.

Component Compensation Mechanism

This study documents a previously undescribed phenomenon whereby analyst classification decisions in ambiguous cases result in compensatory adjustments between inert matter and other seeds allocations, maintaining overall purity consensus while masking component-level precision variations.

Recommendations and Future Directions

Enhanced component-specific proficiency monitoring would improve botanical composition reliability, particularly for small-seeded legumes. Investment in higher-precision balances (0.001 g sensitivity) could substantially reduce trace-component variability.

Current regulatory protocols successfully maintain network competency, with future enhancements focusing on component-specific rather than purity-global performance monitoring.

Study Limitations and Future Protocol Enhancements

The demonstrated network stability validates current proficiency testing protocols while highlighting opportunities for methodological enhancement.

Future implementation of multi-lot protocols per ISO 13528:2022 recommendations would further strengthen statistical robustness, complementing the already exceptional competency maintenance documented across the six-year study period.

Final Validation

The Romanian seed testing network demonstrates that mechanical component separation, conducted under quality-assured conditions, represents a highly reliable analytical procedure. Zero below-minimum

proficiency classifications across six years provide empirical validation that systematic competency verification maintains analytical standards across diverse laboratory units, supporting the effectiveness of current regulatory approaches.

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We thank all 30 ITCSMS (Territorial Inspectorates for Seed Quality and Plant Material Testing) laboratories throughout Romania for their consistent participation and demonstrated commitment to maintaining high standards of analytical competency.

The dedication of laboratory personnel across these territorial units was essential to the success of this six-year longitudinal study.

Special recognition is due to ISTA-authorized samplers who conducted formal sample collection and documentation according to standard operating procedures established by LCCSMS for seed sampling, ensuring the representativeness and traceability of all proficiency test materials.

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INVESTIGATION OF PRODUCTIVE AND NUTRITIONAL VARIABILITY IN SUNFLOWER HYBRIDS IN PEDOCLIMATIC CONDITIONS SPECIFIC TO THE WESTERN ROMANIA

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Abstract

This paper aims to analyze the performance of five sunflower hybrids in the agricultural years 2023 and 2024 by evaluating physical and quality indicators. The experimental setup was located in the moderate continental climate with sub-Mediterranean influences, specific to the West of Romania, specifically Banat's microclimate. Average productions recorded significant increases in 2024, with an increase of around 16%, attributable to favorable climatic conditions and technological advances. Hybrids LE99 and SURELI HTS showed the highest yields, while AROMATIC SU showed the most modest results. Harvest humidity ranged between 4.0-5.5%, registering decreases in 2024 for all hybrids, confirming better ripening conditions; DAVERO stood out for its lowest values. Quality analyses indicated differences between hybrids, with LE99 demonstrating superior mineral content (2.99%), crude protein (19.33%) and total fat (35.73%), associated with a higher quality oil and a slightly higher energy value. The carbohydrate content ranged from 38.43% to 41.61%, with AROMATIC SU showing higher values. The results confirm the high agronomic and nutritional potential of the analyzed hybrids focusing on optimization of production and recovery processes.

Key words: sunflower hybrids, productions, quality analyses.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops cultivated worldwide, ranking fourth after palm oil, soybean, rapeseed and sunflower in global vegetable oil production (Eurostat, 2023; FAOSTAT, 2023). In Europe, and particularly in Romania, sunflower represents a strategic crop due to its adaptability to semi-arid conditions and its high oil content, which may exceed 45% depending on genotype and environmental conditions (Skoric et al., 2012; Kaya et al., 2019).

Romania consistently ranks among the leading sunflower producers within the European Union, benefiting from favorable pedoclimatic conditions in regions such as the Western Plain and Banat area (Eurostat, 2023; Imbrea & Mocioi, 2001). However, increasing climatic variability characterized by irregular rainfall distribution, prolonged drought periods, and high summer temperatures has intensified the need for stable and adaptable hybrids (IPCC, 2021; Olesen et al., 2011).

Sunflower productivity is strongly influenced by genotype × environment interaction. Yield performance depends on hybrid genetic potential, soil fertility, crop management practices, and climatic factors during critical phenological stages (Unger & Thompson, 2018; Hussain et al., 2018). Moreover, harvest moisture plays a key role in post-harvest management, influencing storage stability and processing costs.

Beyond yield, the nutritional composition of sunflower seeds is of increasing interest for both the food and feed industries. Sunflower seeds are valued for their high lipid content, favorable fatty acid profile, protein concentration, and mineral composition (Salas et al., 2014; Grompone, 2011). The balance between saturated and unsaturated fatty acids determines oil quality and market value.

Crude protein content generally ranges between 15–20%, while oil concentration may vary depending on hybrid and environmental conditions (Dreccer et al., 2007). Mineral content also contributes to nutritional value and

reflects nutrient uptake efficiency under specific soil conditions (Marschner, 2012).

Given the increasing demand for sustainable oilseed production systems, evaluating both productive and nutritional variability of sunflower hybrids under specific regional conditions becomes essential (Velasco et al., 2016; Zheljazkov et al., 2008). Western Romania, characterized by moderate continental climate with sub-Mediterranean influences, offers a relevant framework for assessing hybrid adaptability and performance stability.

The vegetal fats contain mostly unsaturated fatty acids such as: oleic, linoleic, linolenic, arachidonic, ricinoleic, erucic and saturated fatty acids such as palmitic and others (Panaiteescu et al., 2011). An important index by which vegetal oils can be appreciated and classified is the iodine index, namely the grams of iodine fixed by 100 g of oil (Tabără et al., 2018)

Therefore, the objective of this study was to investigate the productive and qualitative variability of five sunflower hybrids cultivated under Western Romanian pedoclimatic conditions during the agricultural years 2023–2024, focusing on yield performance, harvest moisture, and key nutritional indicators.

MATERIALS AND METHODS

The study was conducted on five sunflower hybrids (*Helianthus annuus* L.): AROMATIC SU, CEYLON SU, DAVERO SU, P64LE99, and SURELI HTS, evaluated during the 2023–2024 experimental cycle. These hybrids were selected to compare agronomic performance, seed quality, and nutritional potential under variable pedoclimatic conditions.

The experiments were monofactorial and arranged in the field using the randomized block method. During the two experimental years, the following hybrids were cultivated:

- P64LE99 (Pioneer);
- SURELI HTS (Syngenta);
- CEYLON SU (Lidea);
- AROMATIC SU (Lidea),
- DAVERO (Saaten Union).

Sunflower hybrids show good plasticity and adaptability and respond well to intensive cultivation conditions. The results obtained for these hybrids are good across all regions of Romania.

The objectives of the research fall within the main goals of sunflower cultivation: selecting the most suitable hybrids for each area and developing economically efficient and environmentally friendly cultivation technologies, correlated with crop requirements and the pedoclimatic characteristics of each region.

In this context, the research presented in this paper aimed to perform comparative trials with five sunflower hybrids and to evaluate the nutritional value of sunflower seeds under the pedoclimatic conditions of the Pecica area. The preceding crop was rapeseed.

In April, two insecticide treatments were carried out using SUMI ALPHA (esfenvalerate 5%), applied at a rate of 0.2 l/ha on 16.04 and 27.04.2024, targeting early-season pest pressure.

During May, post-emergence herbicide applications were implemented in accordance with the herbicide tolerance technology specific to each sunflower hybrid. The experiment was formed with the expresssun hybrids SURELI HTS (Syngenta), P64LE99 (Pioneer), CEYLON SU and AROMATIC SU (Lidea), as well as DAVERO SU (Saaten Union), a tribenuron-methyl-based herbicide FLUENCE was applied 30 g ha⁻¹, in line with their specific tolerance to sulfonylurea herbicides, providing selective control of dicotyledonous weeds.

Additionally, EVOLUTIO (cletodim 140 g/l and quizalofop-P-ethyl 70 g/l, 0.5 l/ha) was applied uniformly across all variants for the control of annual and perennial grass weeds, complementing the broadleaf weed management strategy.

The soil on which the experiment was established is classified as gleic chernozem.

The area where the experiment was conducted falls within a moderate continental climate, at the interference between the climatic province sector with oceanic influences and the sector with sub-Mediterranean influences, specific to the southwestern part of the country, corresponding to the microclimate characteristic of the Banat region.

This area is characterized by a temperate climate with precipitation throughout the year, but with moisture deficits during the summer months. The average annual precipitation would provide favorable conditions for most agricultural crops if it were evenly distributed throughout the year

and appropriately distributed across months. In some years, deviations occur from the multiannual averages of temperature and precipitation, especially regarding their distribution during the crop vegetation period. These deviations can be corrected through appropriate agrotechnical measures (drainage and controlled soil water storage) as well as through careful adaptation of cultivation technologies to climatic parameters.

Compared to the multiannual average of 13.6°C, the spring and summer of 2023 can be considered very warm from a thermal perspective (Figure 1). The year 2024, like the previous one, was also very warm, especially during the summer months.

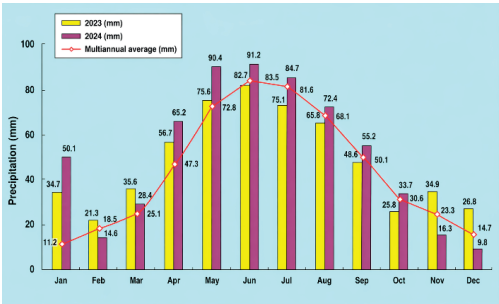


Figure 1. Average monthly temperatures (°C) recorded during the experiment period (Sănnicolau Mare Meteorological Station)

Overall, compared to the multiannual averages, both years can be characterized as very warm throughout the sunflower vegetation period. The multiannual precipitation as well as the precipitation recorded during the experimental period (2023-2024) are shown in Figure 2.

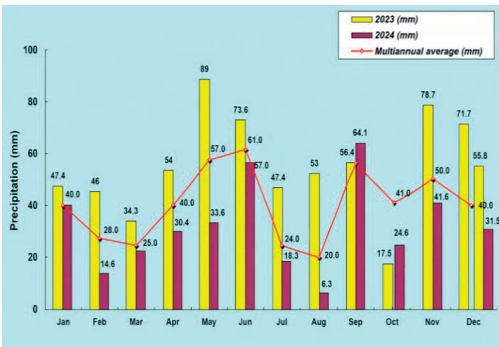


Figure 2. Average monthly rainfall (mm) recorded during the experiment period (Sănnicolau Mare Meteorological Station)

Compared to the multiannual average (430 mm), the year 2024, with a total precipitation of 315 mm, can be considered very dry overall, including during the sunflower vegetation period.

The year 2023, with a total precipitation of 558 mm, although overall relatively dry, provided favorable water supply conditions for sunflower during the vegetation period (except for July, when abundant rainfall created harvesting difficulties). These different conditions visibly influenced the level of weed infestation as well as the sunflower yields obtained.

The main chemical components of the oilseed samples were determined according to ISO standard methods, as follows:

Seed moisture content (%) was determined in accordance with ISO 665. The method is based on drying a known mass of sample in an oven (typically at $103 \pm 2^\circ\text{C}$) until constant weight, the loss in mass being expressed as moisture content.

Mineral content (%) (ash) was determined using ISO 749. The sample is incinerated in a muffle furnace at approximately 550°C until complete combustion of organic matter, and the remaining inorganic residue is weighed.

Crude protein content (%) was determined by the Kjeldahl method according to ISO 5983-1. The method involves digestion of the sample in sulfuric acid, distillation of the released ammonia, and titration; the nitrogen content is converted to protein using an appropriate factor (usually 6.25).

Total fat content (%) was determined in accordance with ISO 659 by solvent extraction (e.g., Soxhlet). The oil is extracted with an organic solvent, the solvent is removed, and the residue is weighed as total fat.

The relatively low fat content values recorded in the present study (34.14-35.73%) can be explained by the combined influence of pedoclimatic conditions and sample characteristics.

Firstly, the experimental years were characterized by high temperatures throughout the growing season and significant water deficit, particularly in 2024, when total precipitation reached only 315 mm compared to the multiannual average of 430 mm. These conditions, especially during the seed filling period, are known to limit lipid biosynthesis and oil accumulation in sunflower seeds.

Secondly, the analysis was performed on intact seeds (whole seed samples), including the hull fraction. This aspect is important, as the presence of the hull reduces the relative proportion of extractable lipids compared to values reported for dehulled kernels, which are typically higher in oil content.

Therefore, the obtained fat content values should be interpreted as representative of whole seeds cultivated under drought-affected conditions, rather than as maximum oil concentrations under optimal conditions.

It is also worth noting that the use of the ISO 659 reference method (Soxhlet extraction) ensures that the reported values reflect the actual lipid content of the analyzed samples and are not underestimated due to methodological limitations.

Fatty acid composition (% saturated) was analyzed by gas chromatography following preparation of fatty acid methyl esters, according to ISO 12966-2. Individual fatty acids are separated and quantified, allowing calculation of the proportion of saturated fatty acids.

Carbohydrate content (%) was calculated by difference, using the relation:

$$\text{Carbohydrates(\%)} = 100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash})$$

This indirect method assumes that carbohydrates represent the remaining fraction after the quantified components.

RESULTS AND DISCUSSIONS

The comparative analysis of sunflower hybrid performance during the agricultural years 2023 and 2024 was based on seed yield (kg/ha) as the main indicator, according to the data presented in Table 1.

Table 1. Analysis of production results in the 2023-2024 experimental cycle

Variant	Yield (kg/ha)		Difference (kg/ha)	Relative difference (%)
	2023	2024		
Field average	2416	2807	391	16
AROMATIC SU	1860	2139	279	15
CEYLON SU	2330	2749	419	18
DAVERO SU	2080	2412	332	16
P64LE99	3020	3503	483	16
SURELI HTS	2790	3236	446	16

The objective of this evaluation was to identify hybrids characterized by high yield potential,

production stability, and adaptability to varying pedoclimatic conditions.

The obtained results, supported by comparative graphical representations and the table of relative differences, indicate a favorable evolution of the analyzed genetic material.

The comparison of seed yields between the two years highlights a general increase in productivity, with relative values ranging between 15% and 18%.

The field average yield increased from 2416 kg/ha in 2023 to 2807 kg/ha in 2024, representing an absolute gain of 391 kg/ha, equivalent to a relative increase of 16%. This positive trend can be attributed to more favorable climatic conditions.

The hybrid AROMATIC SU recorded the lowest yields in both experimental years (1860 kg/ha in 2023 and 2139 kg/ha in 2024). Although the relative increase of 15% is close to the field average, the absolute gain of 279 kg/ha suggests a limited yield potential.

The hybrid CEYLON SU showed an increase of 419 kg/ha (18%), representing the highest relative gain among the analyzed variants, indicating a strong responsiveness to the favorable conditions of 2024.

The hybrid DAVERO SU achieved a yield increase of 332 kg/ha (16%), corresponding exactly to the field average, which reflects good agronomic stability and consistent performance under different growing conditions.

The hybrid P64LE99 recorded the highest absolute yields in both years (3020 kg/ha in 2023 and 3503 kg/ha in 2024), with a gain of 483 kg/ha (16%). This hybrid combines high yield potential with very good production stability.

The hybrid SURELI HTS registered an increase of 446 kg/ha, maintaining the same relative growth rate (16%) as the field average. Its consistently high yield level indicates good agronomic performance and high stability.

Harvest moisture analysis of sunflower hybrids (AROMATIC SU, CEYLON SU, DAVERO SU, P64LE99, and SURELI HTS) during the 2023-2024 growing seasons revealed values ranging between 4.0% and 5.5% (Figure 3). A general decrease in moisture in 2024 compared to 2023 suggests the influence of warmer and drier climatic conditions, promoting natural seed dehydration.

The DAVERO SU hybrid exhibited the lowest and most stable moisture content (4.1% in 2023 and 4.0% in 2024), indicating uniform maturity and superior adaptability for mechanical harvesting and storage without artificial drying. In contrast, AROMATIC SU and P64LE99 showed higher moisture levels (5.5% in 2023 and 5.0% in 2024), still within safe storage limits, while CEYLON SU and SURELI HTS presented intermediate values, with a noticeable decrease in 2024.

The similar curve patterns between the two years highlight the predictable and stable behavior of these hybrids regarding moisture loss. Low moisture values allow direct seed storage, reducing risks of mold, rancidity, and oil quality deterioration, and provide economic advantages in post-harvest handling by lowering drying and handling costs.

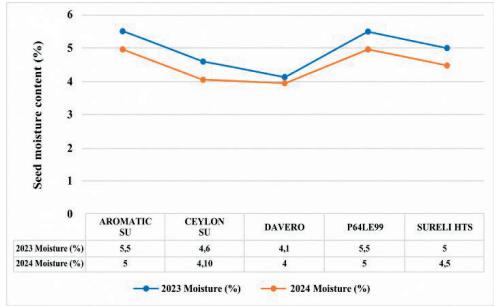


Figure 3. Analysis of moisture content in the five sunflower hybrids in the experimental cycle 2023-2024

Analysis of mineral content in two sunflower hybrids, AROMATIC SU and P64LE99

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in Romanian agriculture due to its ability to adapt to diverse pedoclimatic conditions, as well as its high seed oil content. Crop quality is influenced by genetic, agronomic, and environmental factors, and one of the relevant indicators for assessing seed nutritional value is mineral content. Minerals in sunflower seeds include essential macronutrients (phosphorus, potassium, calcium, magnesium) and micronutrients (zinc, iron, manganese, copper), which play a crucial role in plant metabolism and seed quality. The P64LE99 hybrid showed a mineral content of 2.99%, higher than the AROMATIC SU hybrid, which recorded 2.74% (Figure 4).

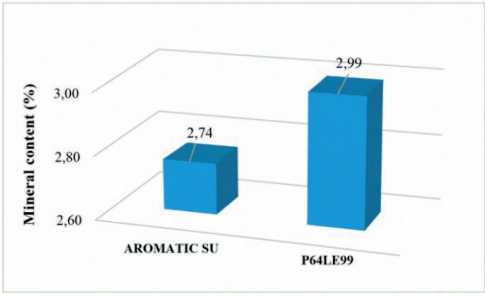


Figure 4. Mineral content (%)

Although the differences are seemingly small, they provide useful information regarding the agronomic behavior of the hybrids in terms of soil mineral uptake and their nutritional potential. A difference of 0.25% may appear minor, but it can have significant effects on the nutritional value of the seeds and the quality of the oil produced.

The values obtained fall within the normal range for sunflower.

Analysis of crude protein content in sunflower hybrids AROMATIC SU and P64LE99

Beyond their high oil content, one of the key indicators for evaluating seed quality is the crude protein content. Crude protein content represents the total percentage of organic nitrogen converted into protein and is essential for assessing the nutritional value of seeds, particularly for use as feed or for protein concentrate extraction.

The P64LE99 hybrid, with a crude protein content of 19.33%, exceeds the AROMATIC SU hybrid by approximately 0.69% (Figure 5). Both values (18.64% and 19.33%) are above average for sunflower, reflecting favorable growing conditions and appropriate cultivation practices.

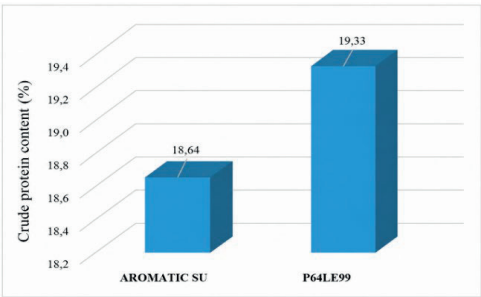


Figure 5. Crude protein content (%)

Analysis of total fat content and saturated fatty acids in sunflower hybrids AROMATIC SU And P64LE99

The total fat content and its composition in fatty acids - particularly the proportion of saturated fatty acids - are essential indicators in assessing seed quality.

The P64LE99 hybrid has a higher fat content (35.73%) compared to Aromatic SU (34.14%), as shown in Figure 6.

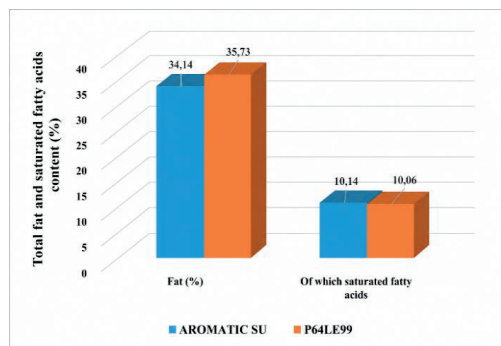


Figure 6. Total fat and saturated fatty acid content

The difference of 1.59 percentage points is significant for the industry, as it translates into greater efficiency in oil extraction. Saturated fatty acids are considered less healthy from a nutritional standpoint, and a lower content is desirable in vegetable oils. P64LE99 has a slightly lower proportion of saturated fatty acids, making it somewhat more advantageous nutritionally.

This suggests that P64LE99 not only produces more oil but also a slightly “healthier” one.

Carbohydrate analysis by difference (%)

Carbohydrates by difference in sunflower seeds represent the amount of carbohydrates estimated indirectly, by subtracting the known components of the product from 100%: water, proteins, lipids (fats), fiber, and ash (minerals). Carbohydrates by difference in sunflower seeds are a moderate source of energy, part of a complex nutritional profile along with healthy fats, proteins, and fiber. These figures (Figure 7) indicate that, in each type of sunflower, approximately 41.61% and 38.43% of the total seed mass is composed of carbohydrates

calculated by difference (i.e., what remains after subtracting water, fats, proteins, fiber, and ash).

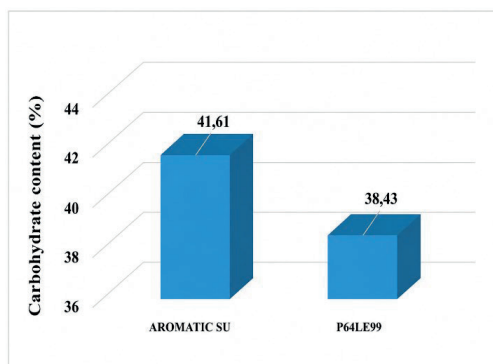


Figure 7. Carbohydrate analysis by difference (%)

Values over 38% are relatively high for sunflower seeds, which means that the AROMATIC SU and P64LE99 hybrids have either fewer fats (which usually dominate sunflower seeds) or more dry matter rich in carbohydrates (likely starch or complex sugars). AROMATIC SU (41.61%) has a slightly higher carbohydrate content compared to P64LE99 (38.43%). The difference of approximately 3.2% could be significant in certain uses (e.g., animal feed, industrial processing, dietary products).

Energy value

The energy value represents the amount of energy that a food provides to the body through digestion and metabolism. Usually expressed in kilocalories (kcal) or kilojoules (kJ), this value is essential both for assessing the nutritional contribution of foods and for rational diet planning, in both human and animal contexts.

The energy value of a food is determined based on its macronutrient content: proteins, lipids (fats), and carbohydrates. From the presented figure (Figure 8), both hybrids have over 540 kcal/100 g, which means they are very calorie-dense. These values are characteristic of foods high in fat. The P64LE99 hybrid has an energy value 4.35 kcal higher than AROMATIC SU.

This difference is small (less than 1%), so it may result from natural variations between batches (differences in fat, moisture, or protein content).

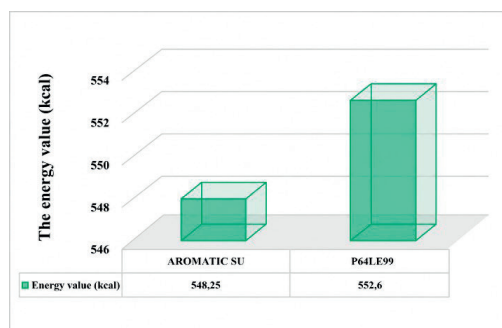


Figure 8. The energy value of the two sunflower hybrids

CONCLUSIONS

- Overall, all variants showed increases in yield in 2024 compared to 2023, indicating favorable agronomic conditions.
- Regarding moisture content, there was an improvement in harvest quality in 2024, with all five analyzed hybrids reaching levels that allow safe and efficient storage without additional treatments. This reflects a successful combination of hybrid selection and favorable harvesting conditions in the 2024 growing season.
- The P64LE99 hybrid exhibits a higher mineral content compared to AROMATIC SU, which may indicate more efficient nutrient uptake and greater agronomic potential.
- P64LE99 stands out with a higher protein content, making it suitable for uses where the nutritional quality of the seeds is a priority.
- P64LE99 surpasses AROMATIC SU in total fat content, showing higher oil-producing potential. The 1.59% difference in favor of P64LE99 can translate into increased economic yield during oil extraction.
- Saturated fatty acid content is nearly identical, with a slight advantage for P64LE99 (0.08% lower). P64LE99 presents itself as a balanced hybrid, offering both high oil productivity and a favorable fat composition.
- AROMATIC SU is richer in carbohydrates than P64LE99, making it potentially more suitable for applications where a higher energy contribution from carbohydrates is desired (e.g., animal feed, certain processed foods). P64LE99's slightly lower carbohydrate content may indicate higher fat content (beneficial for oil extraction) and a more balanced composition for human or industrial use.

- Although both hybrids have high energy content, P64LE99 stands out with a slightly higher energy value, which has direct technological and economic implications.

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EVALUATION OF THE FORAGE QUALITY OF SOME MIXTURES OF AROMATIC MEDICINAL PLANTS AND GRASSLAND GRASS SPECIES WITH A VIEW TO OPTIMIZING THE NUTRITIONAL VALUE

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Abstract

Cultivated grasslands are composed of a diverse mix of plant species with different forage qualities. This study aimed to evaluate the quality of forage resulted from grassland grass species and aromatic medicinal plants, in order to optimize their nutritional value and forage potential. The study was conducted at ICDP Braşov, Romania, within a field experiment of 5 variants of plant mixtures with 20 species of aromatic medicinal plants and grasses. The main quality parameters that were analyzed were: crude protein (CP), total ash (ASH), crude fiber (CF), acid detergent fiber (ADF), lignin (ADL), neutral detergent fiber (NDF), digestibility dry matter (DMD) and digestibility organic matter (DOM). The highest CP content was obtained in variants V3 (20.33%) and V5 (19.50%), compared to the control variant V1 (17.40%). The highest ADF content was recorded in the control variant V1 (34.57%), compared to the other 4 variants, which recorded lower values, with positive implications on the quality of the feed. The correlations between the analyzed parameters showed that crude protein was positively correlated with total ash, dry matter digestibility and organic matter.

Key words: aromatic medicinal plants, fodder quality, grasses, grassland.

INTRODUCTION

Grasslands are the main agricultural land use both at European and global scale (Stypinski, 2011; Török et al., 2021). Forage crops have served as a source of feed of domestic animals and cultivated to produce high yields of feed/fodder, which are also rich in nutrients suitable for animal's requirements (Kumar et al., 2018). The growing of medicinal and aromatic plants can improve the cropping systems, and ultimately improve the fertility status of field. Integrating these crops into livestock diets, alongside other fodder sources, will enhance both performance and health status while protecting animals from major diseases (Kirti and Arya, 2019).

Medicinal plants remain a primary, and sometimes the sole, therapeutic source and a viable option for livestock management. Their use is driven by lower production costs and the ability to replace antibiotics, thereby preventing antibiotic residues in food products. Furthermore, this approach mitigates problems associated with antibiotic-resistant bacteria and,

consequently, enhances public health by improving food safety (Salmerón - Manzano et al., 2020; Oluwafemi et al., 2020).

Sustainable Intercropping forage-medicinal plants plays an important role in farming systems that can improve the light absorption and soil conservation compared to monoculture (Huss et al., 2022). Plant functional types and traits may also influence forage nutritive values (Gardarin et al., 2014; Blaix et al., 2023; Tasset et al., 2019). This study aimed to evaluate the quality of forage resulted from grassland grass species mixtures of aromatic medicinal plants and, in order to optimize their nutritional value.

MATERIALS AND METHODS

The research was established and monitored within the Institute for Research and Development for Grasslands (ICDP) from Braşov, Romania, within the Laboratory of Medicinal and Aromatic Plants.

The experimental field of the ICDP Braşov is located in the Braşov Depression located at 25°45' East longitude and 45°42' North latitude,

and the altitude at which the experimental field is located is 520 m.

The climate of the Braşov Depression falls into the temperate-continental type, representing a transition zone between the influences of the temperate oceanic climate and those of the temperate continental climat.

The present study provides partial results of a three-year internal research initiative (2024-2027) conducted at the Research and Development Institute for Grassland Braşov (ICDP Braşov). This research focuses on improving the nutritional quality of forages from sown grasslands through the use of optimized seed mixtures. The central working hypothesis

aims to increase the floristic diversity of sown phytocenoses by incorporating medicinal and aromatic plant taxa, thereby developing more resilient and functional feed resources for livestock. The research focused on the study of five variants (V1 to V5) of mixtures of grasses and some medicinal plant species. The basic structure, common to all variants, consisted of a mixture of perennial grasses: *Lolium perenne* (9 g), *Dactylis glomerata* (6 g) and *Festuca arundinacea* (5 g), to which variable proportions of medicinal and aromatic plants were added, depending on the Thousand Grain Weight (TGW) and the plant habit are presented in Table 1.

Table 1. Composition of experimental variants

Species	Botanical family	V1	V2	V3	V4	V5
<i>Lolium perenne</i>	Poaceae	9 g	9 g	9 g	9 g	9 g
<i>Dactylis glomerata</i>	Poaceae	6 g	6 g	6 g	6 g	6 g
<i>Festuca arundinacea</i>	Poaceae	5 g	5 g	5 g	5 g	5 g
<i>Ocimum basilicum</i>	Lamiaceae	-	2 g	-	-	1 g
<i>Dracocephalum moldavica</i>	Lamiaceae	-	1.5 g	-	-	-
<i>Agastache foeniculum</i>	Lamiaceae	-	-	1 g	-	-
<i>Sideritis scardica</i>	Lamiaceae	-	-	2 g	-	-
<i>Satureja hortensis</i>	Lamiaceae	-	-	-	1 g	-
<i>Salvia sclarea</i>	Lamiaceae	-	-	-	1 g	-
<i>Hyssopus officinalis</i>	Lamiaceae	-	-	-	1 g	-
<i>Monarda citriodora</i>	Lamiaceae	-	-	-	-	1 g
<i>Calendula officinalis</i>	Asteraceae	-	-	5 g	-	-
<i>Echinacea purpurea</i>	Asteraceae	-	-	2 g	-	-
<i>Leucanthemum vulgare</i>	Asteraceae	-	-	1 g	-	-
<i>Cichorium intybus</i>	Asteraceae	-	-	-	-	1 g
<i>Trigonella foenum-graecum</i>	Fabaceae	-	-	-	-	2 g
<i>Cicer arietinum</i>	Fabaceae	-	50b*	-	-	-
<i>Lupinus albus</i>	Fabaceae	-	-	-	100b*	-
<i>Phacelia tanacetifolia</i>	Boraginaceae	-	4 g	-	-	-
<i>Nigella damascena</i>	Ranunculaceae	-	2 g	-	-	-
<i>Linaria vulgaris</i>	Plantaginaceae	-	-	-	2 g	-
<i>Hypericum perforatum</i>	Hypericaceae	-	-	-	-	1 g
Total seed quantity (g)/6 m ² plot		20 g	29.5 g	31 g	25 g	26 g

b* = seed count; these quantities are excluded from the total gravimetric calculation

The percentage distribution of botanical families (Poaceae, Lamiaceae, Asteraceae, Boraginaceae, Ranunculaceae, Plantaginaceae,

Fabaceae and Hypericaceae) for the five experimental variants is presented in Figure 1.

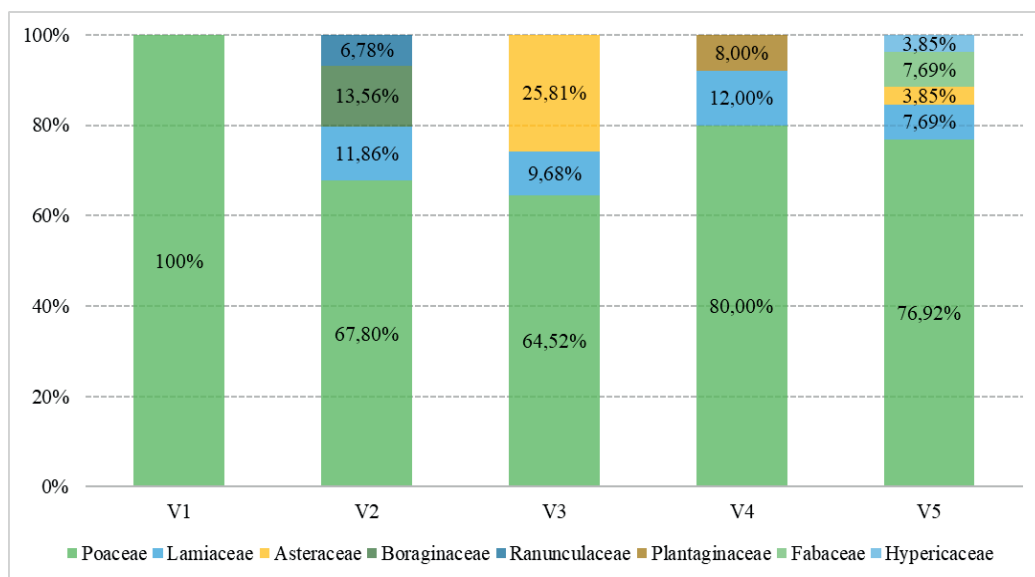


Figure 1. Distribution of botanical families across experimental variants

The experiment was designed on a single-factor model in triplicates, using the randomized block design. The plots were sized at 6 m², with a length of 4 m, and a width of 1.5 m. A 25 cm protection zones was left between variants, and 2 m wide alleys were drawn between the blocks (repeats).

To determine the qualitative parameters, plant mass sampling was conducted chronologically for each experimental variant. Specifically, one representative sample was collected from each of the three biological replicates, resulting in a total of three samples per variant. This procedure ensured statistical consistency and scientific rigor across all experimental plots. The samples were dried at 60°C and then ground with a Grindomix GM 200 mill and prepared for the chemical parameter's analysis.

The chemical parameters analysis was carried out by the Near Infrared Spectroscopy (NIR) method with Perten DA 7200, as follows: crude protein (CP), ash (ASH), crude fiber (CF), acid detergent fiber (ADF), lignin (ADL), neutral fiber detergent (NDF), digestibility of the dry matter (DMD) and the digestibility of the organic matter (DOM). Forage quality was evaluated using two standardized forage grading systems: Relative Feed Value (RFV) and Relative Feed Quality (RFQ). These systems provide a numerical index to rank cool-season legumes, grasses, and mixtures. While the RFV

system (Rohweder et al., 1978) is based on potential digestible dry matter intake calculated from ADF and NDF, the RFQ system (Moore and Undersander, 2002) is a more recent evaluation system that incorporates in vitro fiber digestibility to provide a more accurate prediction of animal performance. All the data was statistically analysed using ANOVA and DUNCAN Test.

RESULTS AND DISCUSSIONS

The results obtained from the analyses performed on the feed samples and the statistical calculations regarding the content of crude protein, crude ash, crude fiber and acid detergent fiber, are presented in Table 2. The variants with the highest average crude CP are V3 (20.33%) and V5 (19.50%), both of which were statistically significantly different from V1, V2 and V4 according to Duncan's test. The increase of CP in variants V3 and V5 can be attributed to the inclusion of nitrogen-fixing species (legumes) and other species, such as *Cichorium intybus* L., with high protein potential, and high forage quality (Iqbal et al., 2022; Nwafor et al., 2017). In terms of forage quality, grasses are often characterized by lower protein levels than those found in leguminous and forb species (Lee, 2018).

Crude ash, which represents the mineral content remaining after calcination, ranged from 9.80%, in V2, to 10.60%, in V3. But, highest average values for ASH were registered in V3 (10.60%) and V5 (10.57%) variants, indicating a significantly higher total mineral content compared to V1 and V2 variants. This suggests an efficient accumulation of minerals from the soil by the diverse species included in the mixtures, possibly due to the varied root systems and intrinsic absorption capacity of plants such as *Cichorium intybus* (adapted to calcareous soils) (Song et al., 2025). Regarding the crude fiber content, the highest value, with negative indications, was obtained in V1 (30.77%), the variant with only grass

species, being more fibrous. The lowest values of CF were obtained in V2 (27.17%), V3 (26.10%) and V5 (26.83%) variants. The presence of aromatic and medicinal plants in V2, V3, V4 and V5 contributes to the reduction of fiber content, compared to V1. A similar pattern was observed for the fiber fractions. Specifically, variant V1 recorded the highest acid detergent fiber (ADF) content (34.57%), being significantly different from variants V2, V3, and V5. In contrast, variants V2, V3 (both 30.70%), and V5 (30.83%) exhibited the lowest ADF values. These lower concentrations indicate a higher energy potential and superior digestibility for these specific variants.

Table 2. Evaluation of CP, ASH, CF and ADF in feed samples

	Variant	CP (%)	ASH (%)	CF (%)	ADF (%)
V1	Mean	17.40 a	9.90 a	30.77 b	34.57 b
	Std. Deviation	0.9644	0.1732	17.786	15.567
	Variance	0.930	0.030	3.163	2.423
V2	Mean	17.73 a	9.80 a	27.17 a	30.70 a
	Std. Deviation	17.926	0.1732	32.005	32.512
	Variance	3.213	0.030	10.243	10.570
V3	Mean	20.33 b	10.60 b	26.10 a	30.70 a
	Std. Deviation	13.317	0.3000	17.000	10.149
	Variance	1.773	0.090	2.890	1.030
V4	Mean	17.77 a	10.17b	29.10 ab	33.10 ab
	Std. Deviation	12.014	0.3215	27.622	31.480
	Variance	1.443	0.103	7.630	9.910
V5	Mean	19.50 ab	10.57 b	26.83 a	30.83 a
	Std. Deviation	0.3000	0.2517	10.504	11.719
	Variance	0.090	0.063	1.103	1.373
Duncan test DL 5%		2.00	0.51	3.39	3.51

The results regarding lignin content, neutral detergent fiber, dry matter digestibility and organic matter digestibility are presented in Table 3. The lowest value of ADL was obtained in variant V5 (2.47%), and the highest value was obtained in variant V4 (3.23%). In the variants V1, V2 and V3 was obtained the same lignin content (2.70%). Regarding the NDF content, the lowest (ideal) value was recorded in variants V2, V3 and V5 (approximately 55-55.5%). These had a significantly lower total structural fiber content, which allows for higher feed intake and more efficient digestion, compared to variant V1, which had the highest value (62.43%) of NDF.

The dry matter digestibility coefficients had the highest value in variant V5 (74.17%). The complex mixture that includes *Monarda citriodora* and *Cichorium intybus*, seems to have generated the best nutritional balance. The highest values of NDF were obtained in V1 (62.43%) and V4 (58.27%) variants, the mixtures being more difficult to digest, either due to the higher accumulation of lignin in variant V4, or the excess of fiber in variant V1. The highest value of organic matter digestibility was obtained in variant V5 (69.13%), ranked first (letter "b" in the Duncan test) indicating a clear superiority. The mixture of medicinal herbs and chicory offers a much "easier" feed digestion.

Table 3. Evaluation of ADL, NDF, DMD and DOM in feed samples

	Variant	ADL (%)	NDF (%)	DMD (%)	DOM (%)
V1	Mean	2.70 ab	62.43 b	67.57 a	63.03 ab
	Std. Deviation	0.1000	26.006	26.407	20.133
	Variance	0.010	6.763	6.973	4.053
V2	Mean	2.70 ab	55.37 a	71.17 a	66.03 ab
	Std. Deviation	0.3606	51.248	61.175	56.323
	Variance	0.130	26.263	37.423	31.723
V3	Mean	2.70 ab	55.20 a	71.40 a	66.13 ab
	Std. Deviation	0.4583	0.3000	25.239	31.005
	Variance	0.210	0.090	6.370	9.613
V4	Mean	3.23 b	58.27 ab	67.40 a	62.70 a
	Std. Deviation	0.3055	61.093	45.530	40.731
	Variance	0.093	37.323	20.730	16.590
V5	Mean	2.47 a	55.53 a	74.17 a	69.13 b
	Std. Deviation	0.2517	32.347	15.011	15.885
	Variance	0.063	10.463	2.253	2.523
Duncan test (DL 5%)		0,50	6,57	6,34	5,89

To provide a comprehensive assessment of the forage quality beyond individual chemical components, the Relative Feed Value (RFV) and Relative Feed Quality (RFQ) were calculated. These integrated indices allow for a standardized ranking of the experimental variants, reflecting their potential for animal intake and energy availability. The performance of the experimental variants regarding these quality indicators is illustrated in Figure 2.

The RFV values ranged from 92.49 to 110.52. Based on the quality scales established by Undersander and Moore (2002) and Jeranyama (2024), variants V2, V3, and V5 are classified as having “fair quality” (103-124), whereas V1 and

V4 fall into the “low quality” category (below 102).

A more dynamic pattern was observed for the RFQ index, with values fluctuating between 99.61 and 117.88. Notably, variant V2 achieved the highest RFQ score (117.88). This superior performance confirms the high nutritional value of this specific mixture, likely due to the inclusion of *Cicer arietinum*. The presence of this legume enhances the overall digestibility and protein profile of the forage, leading to a more favorable RFQ compared to the grass-dominant variant V1, which recorded the lowest value (99.61)

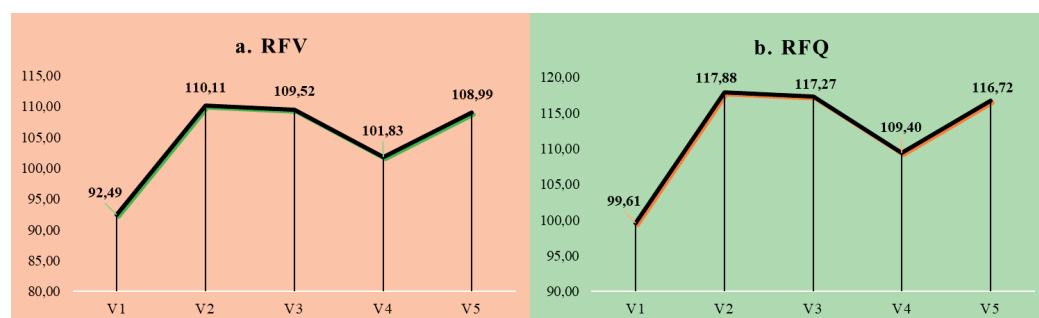


Figure 2. Index variation Relative Feed Value (RFV) and Relative Forage Quality (RFQ) for variants

Correlation analysis is an important source for understanding the degree of association between two or more variables (Andreoiu and Gruia, 2023, Chandra et al., 2012), providing critical insights into the internal dynamics of forage

quality. In the context of forage science, identifying strong associations between protein and digestibility is a standard approach to predict animal performance (Van Soest, 1994).

The Pearson correlation coefficients obtained in this study are detailed in Table 4.

There was a statistically significant positive correlation ($p \leq 0.01$) between crude protein content (CP) and crude ash (ASH), dry matter digestibility (DMD) and digestibility organic matter (DOM) (0.812** and 0.808**), suggesting that feeds from mixtures with more crude protein tend to have better overall digestibility. On the other hand, the correlations between crude protein (CP) and crude fibre (CF), acid detergent fiber (ADF), neutral detergent fiber

(NDF) were negative ($p \leq 0.01$). There was a very strong positive correlation (0.980**) between ADF and NDF, which is logical since ADF is a sub-fraction of NDF (Saha et al., 2010). Strong negative correlations (between -0.61* and -0.92**) existed between ADF, NDF and ADL and digestibility dry matter (DMD) and organic matter (DOM), confirming that higher fiber and lignin content significantly reduces the digestibility of the feed by the animal (Moore and Jung 2001).

Table 4. Correlations between the main indicators analyzed

Characters	CP	ASH	CF	ADF	ADL	NDF	DMD
ASH	0.862**						
CF	-0.857**	-0.610*					
ADF	-0.0780**	-0.525*	0.980**				
ADL	-.0555*	-0.409	0.441	0.422			
NDF	-0.705**	-0.500	0.928**	0.969**	0.224		
DMD	0.812**	0.609*	-0.899**	-0.921**	-0.663**	-0.847**	
DOM	0.808**	0.615*	-0.878**	-0.889**	-0.716**	-0.792**	0.99**

Significance levels: * $P < 0.05$; ** $P < 0.01$.

CONCLUSIONS

Overall, diversifying the floristic composition within our experiment by including different species of aromatic/medicinal plants species, as well as other species from different botanical families in the mixtures made for V2, V3, V4 and V5 variants, led to an improvement in the nutritional quality of the forage compared to the mixture of pure grass stand, made in variant V1. Variants V3 and V5 stood out due to their superior nutritional profiles, having the highest protein content and the lowest fiber levels, indicating increased digestibility and forage value.

Variant V1, the mixture composed exclusively of grasses (*Lolium perenne*, *Dactylis glomerata* and *Festuca arundinacea*), showed the highest fiber and ADF (Acid Detergent Fiber) content, classifying it as the most fibrous and difficult-to-digest variant.

Variant V2 achieved the highest RFQ score (117.88), being the forage mixture with the best potential for rapid consumption and utilization by livestock.

The superior quality of V2 is attributed to the

inclusion of *Cicer arietinum*, which significantly bolstered the protein profile and digestibility. Furthermore, the diversification of the grassland with Lamiaceae species, such as *Ocimum basilicum* and *Dracocephalum moldavica*, alongside *Phacelia tanacetifolia* and *Nigella damascena*, contributed to a more balanced and functional forage.

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WINTER TWO-ROW BARLEY YIELD RESPONSE TO SOWING DENSITY IN SOUTHEASTERN ROMANIA

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Abstract

*This study evaluated the performance of grain yield and its components of 19 winter two-row barley genotypes under two sowing densities: a reduced density of 350 germinable seeds/m² (D1) and a conventional density of 500 germinable seeds/m² (D2). Field experiments were conducted over three consecutive growing seasons (2020-2022) at the National Agricultural Research and Development Institute Fundulea, Romania, on chernozem soil. Total rainfall varied among years (310.4 mm in 2020, 523.2 mm in 2021, and 316.8 mm in 2022), while mean air temperature was ~2°C above the long-term average. Grain yield was significantly higher at D1, averaging 6426 kg/ha, compared with 6012 kg/ha at D2, a 6.9% increase. Correlation analysis showed strong positive associations between thousand-kernel weight and seed weight per spike ($r = 0.844^{***}$) and a significant negative correlation between yield and spike number ($r = -0.347^{*}$), reflecting compensatory effects among yield components. These results indicate that reduced sowing density enhances individual plant performance and is an effective strategy to increase barley grain yield under Southeastern Romania conditions.*

Key words: winter two-row barley, sowing density, yield components, climate variability.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated cereal crops in the world, with archaeological evidence of domestication dating back over 10,000 years in the Fertile Crescent region of the Middle East (Harlan, 1979). It ranks fourth globally among cereal crops in terms of production, after wheat, maize, and rice (FAO, 2023).

Barley grain is a high-energy feed ingredient for livestock, particularly poultry, swine, and ruminants. Its digestible energy content and moderate protein level (10-12%) make it a cost-effective alternative to maize in feed rations (McDonald et al., 2010).

Two-row and six-row malting varieties are extensively used in the beer and whisky industries. The malting process exploits barley's enzymatic activity (particularly α - and β -amylase) to convert starch into fermentable sugars (MacGregor & Bhatti, 1993).

Barley flour, flakes, and pearl barley are used in bread, soups, and health foods. Barley is notably rich in β -glucan, a soluble dietary fibre shown to reduce LDL cholesterol and improve glycemic

response (Brennan & Cleary, 2005; EFSA, 2011). Lodging is a major concern for barley growers and can reduce cereal yield by reducing grain size and number, or by reducing the amount of crop that can be recovered by the combine harvester (Yadav et al., 2024). There have been reports of yield reductions from this kind of lodging: 31% (Weibel & Pendleton, 1964) to 80% (Easson et al., 1993) for wheat, 28-65% (Sisler & Olsen, 1951; Stanca & Jenkins, 1979; Jedel & Helm, 1991) for barley, and 37% (Pendleton, 1954) for oats.

High plant stands can contribute to lodging risk by intensifying competition for resources such as light, water, and nutrients. High sowing densities promote elongated, weak stems that are more susceptible to bending or breaking (Berry et al., 2003; Spink et al., 2003).

The bulk density of barley grain typically ranges between 560-680 kg/m³ (test weight: 56-68 kg/hl) for commercial and feed grades, while malting barley is generally required to reach higher values, with the European Brewery Convention (EBC) specifying a minimum test weight of 65-75 kg/hl for brewing barley - a key

quality parameter for both trading and storage (Rani et al., 2021).

Field experiments conducted in 2006, 2007 and 2008 at eight locations in western Canada (24 site-years) to evaluate the effects of seeding rate (100, 200, 300, 400 and 500 seeds m⁻²) revealed that yields obtained at densities beyond 350 g.s/m⁻² tended to level off or even decrease (O'Donovan et al., 2012).

For Romanian pedoclimatic conditions, Moga et al. (1996) recommend a seeding density of 400-500 germinating seeds/m⁻², equivalent to approximately 180 to 220 kg/ha depending on the thousand-grain weight (TGW) of the variety used. In conditions of late sowing or drought stress, densities may be increased by 10 to 15% to compensate for reduced tillering capacity (Borleanu et al., 1982).

After a relatively flat and even declining trend between 2015 and 2022 (dipping to a low of 423,500 ha in 2018), barley cultivation area in Romania increased sharply thereafter, reaching 638,04 ha in 2026 (Eurostat, 2026).

MATERIALS AND METHODS

A field experiment was conducted using conventional tillage production practices in Southeastern Romania. The experimental site was located at the National Agricultural Research and Development Institute Fundulea (44.4547°N, 26.5155°E), as shown in Figure 1.

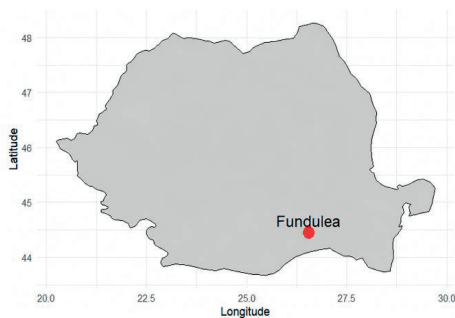


Figure 1. Experimental site over the three consecutive growing seasons

Soil preparation consisted of disc tillage followed by harrowing for seedbed refinement. The study was conducted over three growing seasons (2020, 2021, and 2022). The soil at the experimental site is classified as cambic chernozem, and the climatic conditions recorded

during the experimental period are presented in Table 2.

The experimental design was a randomised split-plot with three replications. Sowing density comprised two treatments: a reduced density of 350 germinable seeds/m⁻² (D1) and a conventional density of 500 germinable seeds/m⁻² (D2).

The comparative trial included 19 two-row winter barley genotypes: five registered varieties and 14 experimental lines developed through the doubled-haploid method. Among the five registered varieties, one produces naked grain (the Ileana variety) (Table 1).

Plot size was 4.5 m² for all genotypes and both density treatments. Sowing depth was 2-3 cm. A standard agronomic management program was applied uniformly across all plots. A complex fertiliser of 200 kg/ha⁻¹ commercial product (18:46, N:P) was applied at sowing in autumn, followed by a rate of 100 kg ha⁻¹ urea (commercial product) in spring. During the growing season, registered herbicides were applied for weed control, and two fungicide treatments were carried out to manage foliar diseases.

Table 1. Studied biological material

No	Variety/line	Registering year	Breeding method
1.	Andreea	1992	Pedigree
2.	Artemis	2021	Pedigree
3.	Gabriela	2017	Pedigree
4.	Diana	2019	Pedigree
5.	Ileana	2021	<i>Bulbosum</i>
6.	DH 381-1	Perspective line	<i>Bulbosum</i>
7.	DH 425-4	Perspective line	<i>Bulbosum</i>
8.	DH 315-10	Perspective line	<i>Bulbosum</i>
9.	F8-114-10	Perspective line	<i>Bulbosum</i>
10.	DH 432-6	Perspective line	<i>Bulbosum</i>
11.	DH 431-1	Perspective line	<i>Bulbosum</i>
12.	DH 425-3	Perspective line	<i>Bulbosum</i>
13.	DH 417-12	Perspective line	<i>Bulbosum</i>
14.	DH 417-13	Perspective line	<i>Bulbosum</i>
15.	DH 437-2	Perspective line	<i>Bulbosum</i>
16.	DH 439-3	Perspective line	<i>Bulbosum</i>
17.	DH 439-8	Perspective line	<i>Bulbosum</i>
18.	DH 403-12	Perspective line	<i>Bulbosum</i>
19.	DH 406-1	Perspective line	<i>Bulbosum</i>

The following yield components and agronomic traits were determined for each sowing density treatment.

Spike number/m⁻² was assessed at full maturity by counting all spikes within a metric frame placed in each plot.

Before harvest, five representative spikes per replicate were sampled from each of the three replicates, threshed individually, and the number of seeds per spike was recorded. The seeds from each spike were then weighed, and the mean value was calculated across the three replicates, yielding the seed weight per spike (g spike⁻¹). Grain yield was determined by harvesting each experimental plot with a Wintersteiger plot combine harvester and was expressed in kg/ha⁻¹ at the standard moisture content of 14%, in accordance with STAS requirements. Thousand-grain weight (TGW) was determined using an automatic seed counter (Contador) and

a precision electronic balance accurate to two decimal places (g). Statistical analyses were performed using SPSS Statistics (version 26.0; IBM Corp., Armonk, NY, USA), while graphical representations were generated in RStudio using the ggplot2 and corplot packages. Analysis of the monthly mean temperatures recorded during the three experimental growing seasons reveals a consistent and notable positive deviation from the 60-year multiannual average of 7.5°C (Table 2). The 2019-2020 growing season recorded the highest seasonal mean temperature of 11.9°C, exceeding the long-term average by 4.4°C - the largest temperature deviation among the three experimental years.

Table 2. Monthly average temperature (°C) and registered rainfall (mm) during the barley growing period (October–June) at Fundulea Meteorological Station (2020–2022)

AVERAGE TEMPERATURE (°C)											
Growing Season	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total / Mean
Multiannual average (60 years)	11.3	5.4	0.0	-2.4	-0.4	4.9	11.3	17.0	20.8	22.7	7.5
2019-2020	12.8 (+1.5)	10.2 (+4.8)	4.0 (+4.0)	0.9 (+3.3)	5.2 (+5.6)	8.3 (+3.4)	12.3 (+1.0)	17.0	21.7 (+0.9)	25.1 (+2.4)	11.9 (+4.4)
2020-2021	14.7 (+3.4)	6.1 (+0.7)	3.9 (+3.9)	1.6 (+4.0)	3.2 (+3.6)	5.1 (+0.2)	9.7 (-1.6)	17.2 (+0.2)	21.1 (+0.3)	25.3 (+2.6)	10.7 (+3.2)
2021-2022	10.2 (-1.1)	7.7 (+2.3)	2.6 (+2.6)	2.1 (+4.5)	4.7 (+5.1)	4.4 (-0.5)	12.1 (+0.8)	17.9 (+0.9)	22.6 (+1.8)	25 (+2.3)	11.1 (+3.6)
RAINFALL (mm)											
Growing Season	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total / Mean
Multiannual average (60 years)	43.0	42.0	43.7	35.1	32.0	37.4	45.1	62.5	74.9	71.1	486.1
2019-2020	38.0 (-5.0)	33.2 (-8.8)	16.2 (-27.5)	2.0 (-33.1)	16.6 (-15.4)	29.8 (-7.6)	14.0 (-31.1)	58.0 (-4.5)	68.4 (-6.5)	34.2 (-36.9)	310.4 (-175.7)
2020-2021	28.6 (-14.4)	20.0 (-22.0)	77.6 (+33.9)	77.0 (+41.9)	16.2 (-15.8)	59.0 (+21.6)	31.0 (-14.1)	57.6 (-4.9)	135.0 (+60.1)	21.2 (-49.9)	523.2 (+37.1)
2021-2022	56.4 (+13.4)	33.8 (-8.2)	37.6 (-6.1)	4.8 (-30.3)	5.4 (-26.6)	12.3 (-25.1)	47.6 (+2.5)	30.1 (-32.4)	59.6 (-15.3)	29.2 (-41.9)	316.8 (-169.3)

*Values in parentheses indicate deviation from the multiannual average (1960-2019).

The 2020-2021 season recorded a mean of 10.7°C (+3.2°C), with a more uniform distribution of positive deviations throughout the season; the only below-average month was April (-1.6°C), which coincides with a critical phase of tillering and stem elongation in two-row barley. The 2021-2022 season also exhibited a mean temperature of 11.1°C (+3.6°C above the multiannual average). Overall, all three experimental seasons were characterised by mild winters and warm growing periods, which may have influenced both crop development rates and winter hardiness. The above-average temperatures recorded throughout the experimental period must be considered a significant environmental

factor when interpreting the agronomic results obtained. The rainfall regime during the experimental period was highly variable between seasons, contrasting markedly with the 60-year multiannual total of 486.1 mm (Table 2). The 2019-2020 growing season was characterised by a severe rainfall deficit, with a total of only 310.4 mm, representing a shortfall of 175.7 mm. In contrast, the 2020-2021 season was the only year to exceed the multiannual rainfall average, recording a total of 523.2 mm (+37.1 mm). The 2021-2022 season returned to conditions of severe water deficit, with a seasonal total of 316.8 mm (-169.3 mm). Consequently, two of

the three experimental years were affected by pronounced drought conditions.

RESULTS AND DISCUSSIONS

The results of the three-way analysis of variance (ANOVA) revealed highly significant effects of the main factors - genotype (G), year (Y), and sowing density (D) - on grain yield and the main yield components analysed (Table 3). All three main factors exerted a statistically significant influence ($p \leq 0.05$) on grain yield (kg/ha^{-1}), number of spikes per square meter, seeds per spike, and seed weight per spike, indicating that genetic differences between

genotypes, environmental variation across growing seasons, and sowing density all played a decisive role in the yield performance of two-row barley. Regarding thousand-grain weight (TGW), the genotype and year factors were both highly significant ($p = .000$), confirming that grain size is strongly determined by genotype characteristics and by seasonal environmental conditions. However, sowing density did not significantly affect TGW ($p = .066$, ns), suggesting that, within the range of densities tested, plant competition had no substantial impact on individual grain filling.

Table 3. Three-way ANOVA for studied parameters

Source	Grain yield (kg ha^{-1})	TGW (g)	No. of spikes/ m^2	Seeds/spike	Seed weight (g/spike)
Genotype (G)	.000*	.000*	.000*	.000*	.000*
Year (Y)	.000*	.000*	.000*	.000*	.000*
Density (D)	.000*	.066 ns	.000*	.000*	.000*
Genotype $\times$ Year (G $\times$ Y)	.000*	.000*	.001*	.000*	.000*
Genotype $\times$ Density (G $\times$ D)	.889 ns	.000*	.061 ns	.000*	.001*
Year $\times$ Density (Y $\times$ D)	.000*	.000*	.000*	.011 ns	.000*
Genotype $\times$ Year $\times$ Density (G $\times$ Y $\times$ D)	.998 ns	.000*	.151 ns	.000*	.000*

* = significant; ns = non-significant

Among the two-way interactions, the genotype and year interaction (G $\times$ Y) was statistically significant for all five traits studied ($p \leq 0.05$), demonstrating that genotypes responded differently to the environmental conditions of each growing season - a finding that underlines the importance of multi-year evaluation in variety testing. The genotype and density interaction (G $\times$ D) was significant for TGW, seeds per spike, and seed weight per spike, but non-significant for grain yield ($p = .889$, ns) and number of spikes per m^2 ($p = .061$, ns), indicating that the yield response to sowing density was largely consistent across genotypes. The year and density interaction (Y $\times$ D) was significant for grain yield, TGW, number of spikes per m^2 , and seed weight per spike, but not for seeds per spike ($p = .011$, ns), reflecting the fact that the optimal density response varied with seasonal conditions. The three-way interaction (G $\times$ Y $\times$ D) was statistically significant for TGW, seeds per spike, and seed weight per spike, indicating complex interdependencies between genotype,

year, and sowing density for these components. In contrast, grain yield ($p = .998$, ns) and number of spikes per m^2 ($p = .151$, ns) were not significantly influenced by the three-way interaction, suggesting that the combined effect of all three factors did not produce differential responses beyond those already captured by the other interactions. Overall, the analysis of variance confirms that both genetic and agronomic factors, as well as their interactions with climatic conditions, must be taken into account when optimising the productivity of two-row barley genotypes. Substantial variation in mean grain yield was observed among growing seasons, confirming the dominant effect of the year factor identified in the ANOVA (Table 3). The lowest yields were recorded in the 2020-2021 growing season, with means of 5011 kg/ha^{-1} at D1 (reduced density) and 5225 kg ha^{-1} at D2 (conventional density), while the 2019-2020 season produced intermediate yields of 5387 kg ha^{-1} and 4735 kg ha^{-1} at the lower and higher densities, respectively (Figure 2).

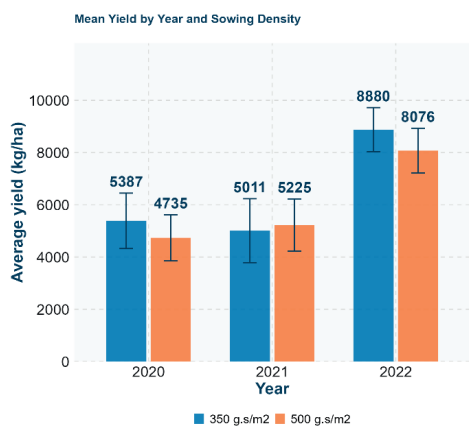


Figure 2. Mean grain yield by growing season and sowing density

The highest yield was recorded in the 2021-2022 season, with mean yields of 8880 kg ha⁻¹ at 350 g.s/m² and 8076 kg/ha⁻¹ at 500 g.s/m², a difference of 804 kg ha⁻¹. This variation between studied years is attributable to the contrasting climatic conditions described earlier, particularly the severe rainfall deficits recorded in 2019-2020 and 2021-2022.

The distribution of grain yield (kg ha⁻¹) across the two sowing densities, averaged over all tested genotypes and growing seasons, is illustrated in Figure 3 as violin plots overlaid with box plots. The diamond symbol represents the mean, and the median is indicated by the horizontal line within the interquartile-range box.

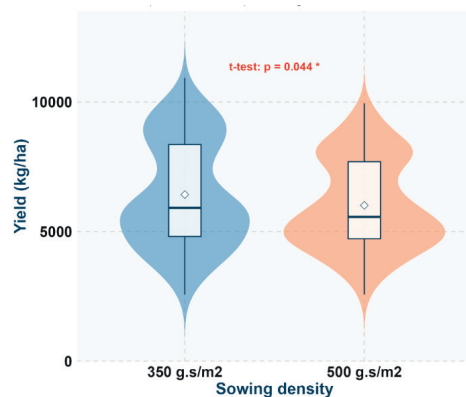


Figure 3. Effect of sowing density on grain yield distribution

An independent-samples t-test revealed a statistically significant difference in mean grain yield between the two sowing densities ($p = 0.044$). The lower-density treatment (350 g.s. m⁻²) registered a mean of 6426 kg/ha⁻¹ compared with 6012 kg/ha⁻¹ (conventional density) as a mean for 3 studied years, suggesting that, on average, the reduced plant competition at 350 g.s/m² was more favourable for overall grain productivity. Both treatments exhibited a wide overall yield range (per replication), from approximately 2568 kg ha⁻¹ in 2020 to 10928 kg ha⁻¹ in 2022, reflecting the strong influence of genotype and year on productivity, as confirmed by the ANOVA (Table 3). The distributions were broadly similar in shape and spread, although a small number of low outliers were recorded at the higher density.

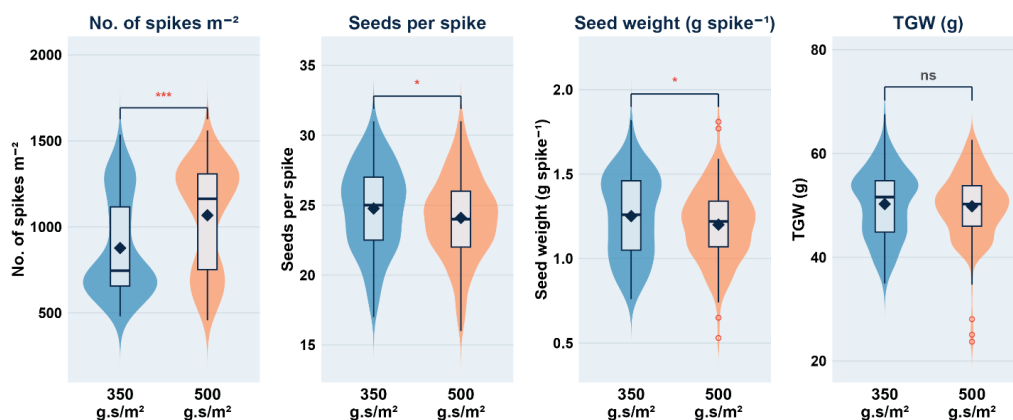


Figure 4. Yield key components under the two sowing densities tested

The number of spikes/m² increased with density, from a mean of 876.8 at D1 to 1067.4 at D2 confirming the expected direct relationship between sowing density and the number of plants. Genotype DH 431-1 recorded the lowest spike density at both sowing rates (754.3 and 910.4 spikes m⁻², respectively), while the highest values were obtained by Andreea (1002.7 spikes m⁻²) at 350 g.s. m⁻² (D1) and by DH 432-6 (1164.3 spikes m⁻²) at 500 g.s. m⁻² (D2).

In contrast, grain weight showed a slight decreasing trend with increasing density, from a mean of 1.25 g at D1 to 1.20 g at D2, reflecting the typical trade-off between spike number and individual grain filling under higher plant competition. DH 384-1 consistently produced the heaviest grains at both densities (1.44 g and 1.38 g), while the lowest values were recorded

by Andreea (1.03 g) at D1 and DH 439-3 (1.07 g) at D2.

The number of grains per spike remained relatively stable across densities, with means of 24.76 and 24.09 at D1 and D2, respectively. The maximum value (26.11 grains/spike) was reached by two different genotypes depending on density: DH 315-10 at D1 and DH 403-12 at D2, while the minimum was recorded by the closely related genotypes DH 425-3 (21.89) and DH 425-4 (22.33).

Overall, a higher sowing density (500 seeds/m² instead of 350) led to more spikes per square meter, but slightly lower yield and grain weight. The non-significant response of TGW to sowing density is consistent with the ANOVA results (Table 3) and suggests that individual grain-filling capacity was largely maintained regardless of plant density.

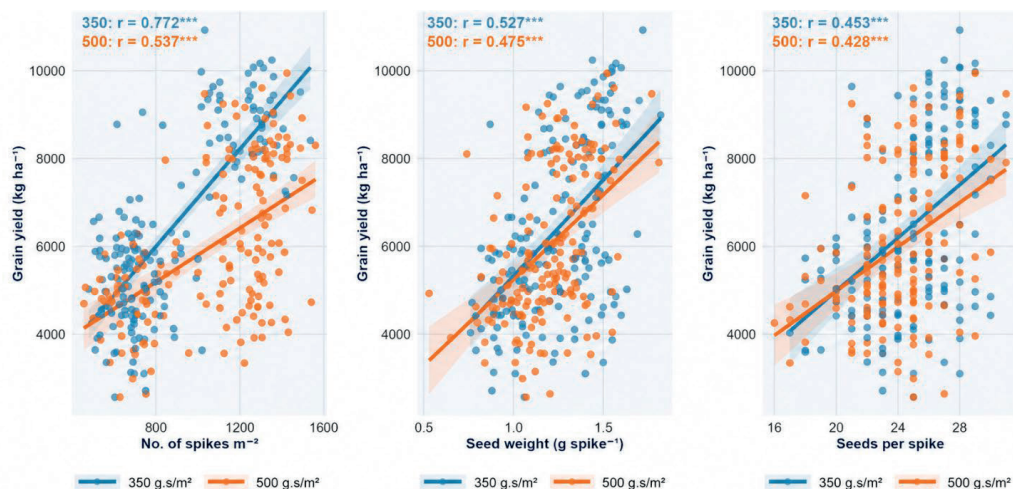


Figure 5. Correlations between yield components and grain yield across sowing densities

Pearson correlation coefficients (*r*) between grain yield (kg/ha⁻¹) and three key yield components - number of spikes/m², seed weight/spike (g/spike⁻¹), and seeds/ spike - are shown in Figure 5, analysed separately for the two sowing densities (350 and 500 g.s/m²). All correlations were statistically highly significant (*p* ≤ 0.001) across both density treatments, confirming that each of the three components contributed meaningfully to grain yield variation under the experimental conditions.

The strongest association with grain yield was found for the number of spikes per m² (left

panel), with correlation coefficients of *r* = 0.772 at 350 g.s./m² and *r* = 0.537 at 500 g.s./m². Both values were the highest recorded among the three components, indicating that spike density was the primary determinant of grain yield in two-row barley under the tested conditions. Notably, the regression slope was markedly steeper at the lower density (350 g.s/m²), suggesting that individual spikes contribute more to yield when plant competition is reduced. Seed weight per spike (central panel) showed moderate positive correlations with grain yield at both densities (*r* = 0.527 at 350 g.s/m²; *r* =

0.475 at 500 g.s. m⁻²). The two regression lines were nearly parallel, with the reduced-density treatment showing a slightly higher slope, indicating that heavier spikes translated more efficiently into yield at the lower density. Seeds per spike (right panel) showed the weakest, though still highly significant, correlations with grain yield among the three components evaluated ($r = 0.453$ at 350 g.s/m²; $r = 0.428$ at 500 g.s/m²). The regression lines for the two densities were nearly coincident, suggesting that the relationship between seeds per spike and yield was largely consistent regardless of plant density. The wide horizontal spread of observations, particularly at higher seed numbers per spike, indicates considerable variability in this trait across genotypes and growing seasons. Overall, the correlation analysis confirms that spike number per unit area was the dominant yield-trigger component in this experiment, followed by seed weight per spike and seeds per spike.

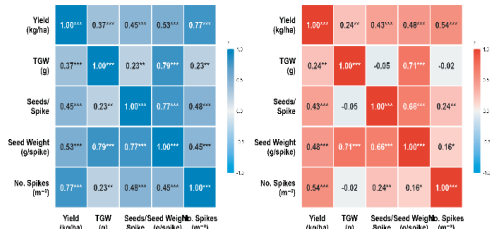


Figure 6. Pearson correlation matrix between grain yield and its key components, by sowing density

At the lower sowing density (blue panel), all correlations between grain yield and the four yield components were positive and statistically significant (Figure 6). The strongest association was observed between yield and number of spikes per m² ($r = 0.77***$), confirming spike density as the primary yield driver at this density level. Grain yield was also strongly correlated with seed weight per spike ($r = 0.53***$) and seeds per spike ($r = 0.45***$), while the correlation with TGW was moderate ($r = 0.37***$). Among the yield components themselves, seed weight per spike showed the strongest inter-component correlations, particularly with seeds per spike ($r = 0.77***$) and number of spikes ($r = 0.45***$), suggesting

that at lower densities, spike-level productivity traits are narrowly co-regulated. At the higher sowing density (D2), the correlation structure changed considerably. While the association between yield and number of spikes per m² remained significant and positive ($r = 0.54***$), several other correlations shifted in sign or lost significance. Most notably, TGW showed negative, though largely non-significant, correlations with yield ($r = -0.05$, ns), seeds per spike ($r = -0.95***$), and number of spikes per m² ($r = -0.02$, ns). The strongly negative correlation between TGW and seeds per spike ($r = -0.95***$) at D2 is particularly noteworthy, indicating a pronounced trade-off between grain size and seed number per spike under conditions of increased intraspecific competition. The mean yield difference (kg) between the two sowing densities shown in Figure 7 highlights that 18 out of 19 two-row barley genotypes evaluated across the three experimental growing seasons had a positive effect on sowing density. Positive values (blue bars) indicate a yield advantage at the lower density (D1) while negative values (orange bars) indicate superior performance at the higher density (D2).

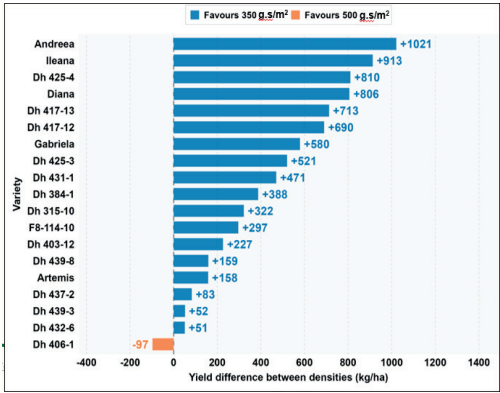


Figure 7. Genotype-specific yield response to sowing density

Yield advantages ranged from 51 kg/ha⁻¹ (DH 432-6) to 1021 kg/ha⁻¹ (Andreaea). The variety Andreaea exhibited the strongest density response, followed by Ileana (913/kg ha⁻¹), DH 425-4 (810 kg/ha⁻¹), and Diana (806 kg/ha⁻¹), all showing yield differences exceeding 800 kg/ha⁻¹ in favour of the lower density.

CONCLUSIONS

Sowing density significantly influenced yield components across all varieties: the lower density of 350 germinating seeds/m² consistently produced higher grain yields than 500 seeds/m², with an average gain of 414 kg/ha over three growing seasons.

Climatic variation caused by year was the dominant factor affecting barley yield, accounting for yield differences of 68% between the least favourable (2020: 5061 kg/ha⁻¹) and most favourable (2022: 8478 kg/ha⁻¹) season, outweighing both variety and sowing density effects.

From 19 evaluated genotypes, we have identified varieties Gabriela, Artemis, and perspective line DH 384-1 that demonstrated the highest and most stable yield performance across both sowing densities and all three experimental years, confirming their adaptability to the pedoclimatic conditions of the study area.

RECOMMENDATIONS

A sowing density of 350 germinating seeds/m² is recommended as the optimal density for winter two-row barley tested genotypes in Southeastern Romania. The classical density of 500 g.s/m² did not improve yield and led to increased competition for water, light, and nutrients between plants.

ACKNOWLEDGEMENTS

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MAIN PESTS ATTACK ON POTATO AND SUGAR BEET CROPS IN THE CONDITIONS OF THE CENTRAL AREA OF MOLDOVA (ROMANIA)

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Abstract

*During 2024-2025, under the conditions of the Central area of Moldova, the purpose of the research carried out was to establish the harmful organisms to sugar beet and potato crops, but also the degree of attack produced. The results obtained over the two years show that in sugar beet, the number of recorded pests varied, with nine insect species and two pathogens identified in 2024, and 12 species and two pathogens in 2025. The soil pest complex has been attacking since the emergence of the plants, with the highest attack rate observed in 2024, when adults of the *Bothynoderes punctiventris* species were present, at 16.5%. In 2025, the attack rate was 7.2% due to the presence of *Cheatoecema* sp. species. In potatoes, the most widespread pests were *Leptinotarsa decemlineata*, with the degree of attack between 0.7% and 22.3% in 2024 and between 10.9% and 100% in 2025, and the pathogen *Phytophthora infestans* produced attacks from 7.9% to 27.6% in 2024 and between 12.5% and 28.5% in 2025.*

Key words: potato, beet, attack, entomofauna, pathogens, climatic conditions.

INTRODUCTION

Sugar beet and potato crops provide, through their primary yield, raw materials for the production of goods used in human nutrition, animal feed, and various industrial sectors (Hermeziu & Adam, 2024; Górska-Warsewicz et al., 2021; Devaux et al., 2021). Both the quality and quantity of sugar beet and potato yields are significantly reduced by the attack of harmful organisms (pests, pathogens, and weeds), which require appropriate control measures to maintain their populations below the economic threshold of damage (ETD). At the same time, these measures must ensure the production of safe food while protecting the environment and human health (Alyokhin, 2007; Adekanmbi et al., 2023).

Chemical interventions using pesticides should be applied only when pest and pathogen pressure exceeds the economic threshold and when alternative control measures prove ineffective (Mohamed, 2023). Climatic conditions represent another major factor influencing crop productivity. In recent decades,

these conditions have negatively affected agricultural systems through uneven precipitation distribution, alternating periods of extreme heat and cool temperatures during the growing season, as well as shifts in seasonal patterns and climatic parameters (Skendzić et al., 2021; Memari et al., 2022).

Along with climatic conditions that reduce production and cultivated areas, there are also changes in agricultural policy, higher impact of pests and disease, variations of imports/exports or shifts in farmer preferences toward other crops (Struik & Wiersema, 1999; Memari et al., 2022). This context highlights the need for innovative approaches to revitalize potato farming in Romania (Cojanu et al, 2025; Paul et al., 2017). Simultaneously, the dynamics of harmful organisms are continuously evolving. In recent decades, the spectrum of pests affecting sugar beet has expanded, including aphid infestations due to the absence of seed treatments with insecticides, as well as the emergence of leafhopper species such as *Cixius wagneri*, *Hyalesthes obsoletus*, and *Pentastiridius leporinus*, which act as vectors of

stolbur phytoplasma and the γ -3 proteobacterium associated with SBR disease (Behrmann et al., 2022; Bressan et al., 2008). These are accompanied by a wide range of soil-borne pathogens (*Macrophomina phaseolina*, *Phoma betae*, *Aphanomyces cochlioides*, *Pythium aphanidermatum*, *Phytophthora drechsleri*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *F. solani*, *F. avenaceum*) responsible for root blackening and rot in sugar beet (Puiu et al., 2023). In potato crops, alongside major pathogens such as *Phytophthora infestans* and *Alternaria* spp., and the most destructive pest, *Leptinotarsa decemlineata*, aphids and other secondary pests have also become increasingly significant (Vernon, 2022).

The present study reports results obtained in 2024-2025 on the inventory of harmful organisms and the assessment of damage levels in sugar beet and potato crops under the environmental conditions of Central Moldova (Romania).

MATERIALS AND METHODS

Location

The research was conducted at the Agricultural Research and Development Station Secuieni - Neamț (Romania), located at 26°5' E longitude and 46°5' N latitude, in a hilly region with altitudes reaching up to 250 m above sea level (a.s.l.). The regional topography consists of interfluvial plains, grasslands, and terraces (Trotuş et al., 2020). The area is characterized by a temperate continental climate (Dfb according to the Köppen-Geiger classification), with short springs, relatively cool summers, and cold winters (Kottek et al., 2006).

Experimental design

Sugar beet and potato trials were established in the experimental field of the Plant Protection Laboratory on a typical cambic chernozem soil, with the following characteristics: pH (H₂O) = 6.29, humus content = 2.3%, nitrogen index = 2.1, available phosphorus (P₂O₅) = 39 ppm, and potassium (K₂O) = 161 ppm.

Monitoring methodology

Pest occurrence, distribution, and population dynamics were monitored periodically using soil sampling with a 25 × 25 cm frame, barber

pitfall traps, yellow sticky traps installed in the field.

Observations were carried out from crop emergence until harvest.

Damage assessments were performed visually on plants (25 plants × 10 replicates). The intensity of attack was scored on a 0-6 scale, where 0 = no attack and 6 = more than 75% of the plant affected. Based on the scores awarded, the degree of attack was calculated for each pest ($GA\% = F\% \cdot I\% / 100$).

Climatic conditions

Climatic data were recorded using a Wireless Vantage Pro 2 Plus weather station (SC Rom Tech SRL, Sibiu, Romania) located near the experimental field.

To characterize the years from a climatic point of view, we used data on the average air temperature (°C) recorded at 2 m height and recorded precipitation Σ (mm).

RESULTS AND DISCUSSIONS

Climatic conditions

Analysis of climatic data over the two experimental years showed that monthly average temperatures exceeded the multiannual average by 0.8°C to 4.1°C (Figure 1).

The spring months are characterized by an increase in temperatures in March and April, followed by a cooling in May, when temperatures dropped by -1.1°C, which made it difficult for plants to emerge and develop. The summer months were warm and very warm from a thermal point of view, which determined faster plant growth and development, with average temperatures recorded as 2.4°C (June) and 3°C (August) above the multiannual average (Figure 1).

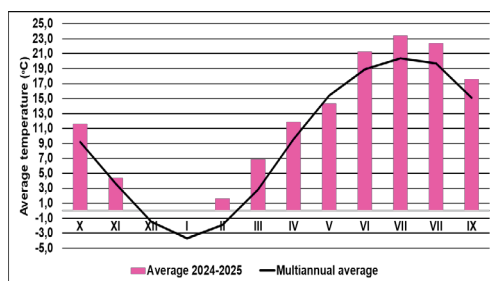


Figure 1. Average monthly temperatures, 2024-2025 average, Secuieni - Neamț

From a pluviometric perspective, both annual and monthly rainfall sum were deficient and unevenly distributed. April was particularly dry (–22 mm compared to the multiannual average), affecting soil preparation, sowing/planting, and crop emergence, and the months of March and May were rainy, recording 10.3 mm (March) and 11.9 mm (May) above the multiannual average (Figure 2).

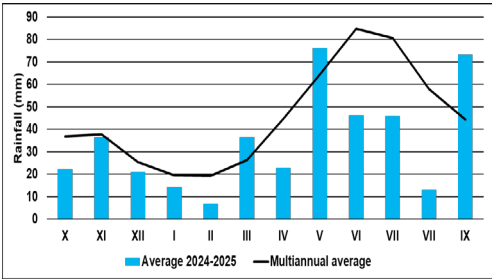


Figure 2. Monthly precipitation, average 2024-2025, Secuieni - Neamț

An increase in the average monthly temperature, respectively the multiannual average in the area of influence, is observed. Also, the uneven rainfall distribution in the area of influence is observed (Isticioaia et al., 2020). In the two years of experimentation, it is observed that spring begins with high temperatures in March and April and cools down in May. The lack of

precipitation leads to uneven emergence of the crop, and extreme temperatures during the summer accelerate the evolution of the crop; the plants reach maturity faster, but the yields were small and of poor quality.

Potato crop

Following the entomofauna inventory in potato crops, three species were identified: *Agriotes* spp., *Leptinotarsa decemlineata* and *Harpalus* spp., which were included in the order Coleoptera. We found that, of the three species, *Leptinotarsa decemlineata* was present from the emergence to the senescence of the plants, totaling 153 specimens. The number of specimens identified increases from the beginning of June (36 specimens), and the density of this pest remains high until harvest (12 specimens) (Table 1). The *Agriotes* species was present at the beginning of the potato growing season, with 6 specimens recorded. *Harpalus* spp. were recorded throughout the growing season, summing up 85 specimens captured. This species does not pose a threat to the future tuber harvest, being spread across the entire surface of the experimental fields; respectively, it is part of the entomofauna structure identified from other crops in the Moldavian area (rapeseed, cereals, corn, sunflower) (Troțuș et al., 2017).

Table 1. Entomofauna harmful to potato crops inventoried in the period 2024 - 2025, Secuieni – Neamț

Species/Order	V			VI			VII			VII	Σ
	I	II	III	I	II	III	I	II	III	I	
Coleoptera											
Agriotes spp	0	0	0	2	4	0	0	0	0	0	6
Leptinotarsa decemlineata	1	3	3	27	22	26	34	31	3	5	153
Harpalus spp.	0	0	2	8	9	15	19	14	12	7	85
Σ = three species, one order	1	3	5	36	34	40	53	45	15	12	243

The most widespread and abundant species in both years of experimentation was *Leptinotarsa decemlineata*. As a series of results indicate (Cingel et al., 2016; Hermezi and Adam, 2024), the attack produced by larvae and adults is difficult to control, with a wide range of insecticides being applied that aimed to limit their spread and attack. Under the conditions in Secuieni, it was found that in 2024, the number

of adults/plant varied from 0.3 adults/plant to 2.6 adults/plant, and that of larvae was between 3 and 8 larvae/plant (Figure 3a). In contrast, in the following year, the most widespread stage was the larval stage, with up to 33 larvae/bush recorded, and the density of the egg and adult stages was lower compared to the previous year (Figure 3b).

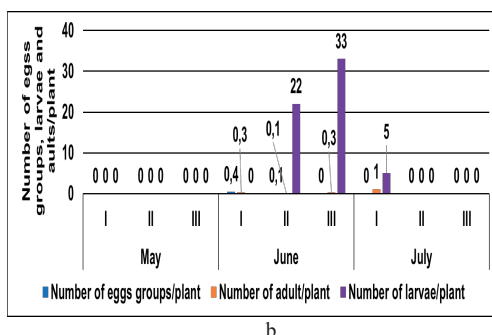
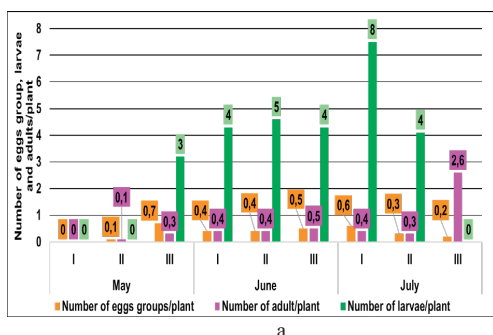


Figure 3. Density of the *Leptinotarsa decemlineata* species in potato crops, Secuieni 2024 (3a - left) - 2025 (3b right)

The high density of larvae led to an attack rate of between 10.9% and 100% in 2025 compared to the previous year, when the attack ranged from 0.7% to 22.3% (Figure 4).

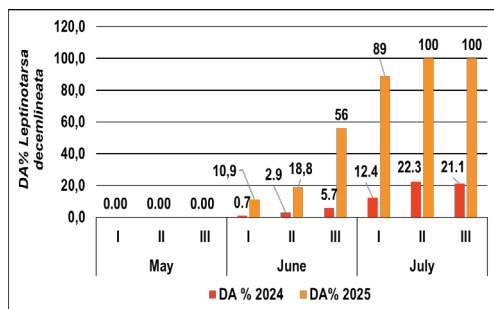


Figure 4. The degree of attack produced by the *Leptinotarsa decemlineata* species on potatoes, Secuieni 2024-2025

Pathogen attack leads to production losses, one of the most important agents being *Phytophthora infestans* (Haas et al., 2009). The attacks recorded in both years of experimentation ranged between 7.9% and 27.6% in 2024 and between 12.5% and 28.4% in 2025 (Figure 5).

Although the attack did not have a high impact on the plant, being recorded at the end of the vegetation period, monitoring the appearance and spread of the pathogen must be carried out carefully, and, when necessary, chemical treatments must be applied.

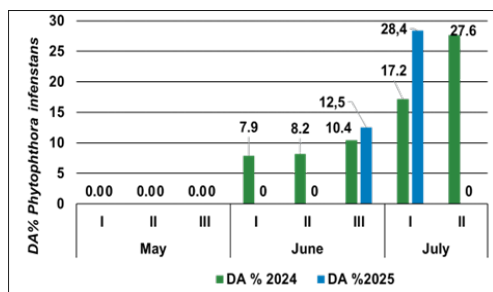


Figure 5. The degree of attack produced by the pathogen *P. infestans* on potatoes, 2024-2025, Secuieni - Neamț

Sugar Beet crop

The structure of the entomofauna in sugar beet crops was different in the two years of experimentation, both in terms of the number of species identified and in terms of their density. In 2024, nine species belonging to three systematic orders were identified, and in 2025, we found that the number of species identified in the beet crop increased to 12 species that were classified in four systematic orders (Coleoptera, Lepidoptera, Homoptera, and Diptera), totaling 760 specimens and 42 colonies (Table 2).

The highest density values were recorded for the species *Chaetocnema* spp. (242 specimens) which had density values ranging between 1 and 62 specimens, totaling 242 specimens/m² (Table 2). In 2025, *Pegomyia betae* and *Lixus junci* were additionally identified in the beet crop, which recorded 36 specimens/m² and 64 specimens/m² (Table 2).

Table 2. Entomofauna harmful to sugar beet crops inventoried during the period 2024-2025, Secuieni - Neamț

Species/Order	IV	V			VI			VII			VIII			IX			Σ
	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	
Coleoptera																	
<i>Agriotes</i> spp*	2	2	0	0	0	4	4	4	5	5	3	0	0	0	0	0	29
<i>Tanymecus</i> spp*.	3	3	3	2	4	1	1	0	0	0	0	0	0	0	0	0	17
<i>Opatrum sabulosum</i> L.*	4	13	10	4	7	2	0	0	0	1	0	0	0	0	0	0	41
<i>Gonocephalum pusillum</i> L.*	0	1	2	1	3	1	1	0	0	0	0	0	0	0	0	0	9
<i>Bothynoderes punctiventris</i> L.*	3	10	13	6	10	3	3	1	1	2	0	0	0	0	0	0	52
<i>Chaetocnema</i> spp*	3	3	4	2	1	5	4	8	5	6	22	19	45	62	33	20	242
<i>Psylliodes chrysocephala</i> L.**	0	0	2	2	3	8	10	7	6	3	10	5	12	5	13	7	93
<i>Lixus junci</i> Boh.**	0	0	0	0	0	1	7	8	12	10	4	5	2	7	3	5	64
<i>Harpalus</i> spp*	0	8	13	12	21	5	8	13	12	10	12	13	12	8	10	5	162
Lepidoptera																	
<i>Autographa gamma</i> L.*	0	0	2	3	1	1	1	1	0	0	0	0	0	6	0	0	15
Homoptera																	
<i>Aphis fabae</i> Scopoli*	0	0	2	2	5	5	8	3	3	4	0	0	0	0	5	5	42
Diptera																	
<i>Pegomyia betae</i> Curtis	0	0	0	0	6	3	3	5	4	5	0	0	0	0	5	5	36
** = four orders; 12 species	15	40	49	32	56	34	42	47	45	42	51	42	71	88	64	42	760 sp + 42 col

*Species present in 2024 and 2025

**Specie present in 2025

During the growing season of beet, the attack produced by five harmful organisms was identified in 2024, and seven in 2025. The attack produced by harmful organisms was recorded throughout the growing season. From emergence to the development of the leaf rosette, the main pests were adults of the species *B. punctiventris* and *Chaetocnema* spp., respectively aphids, as well as, in the second part

of the growing season, the pathogens *Phoma betae* and *Cercospora beticola*.

The highest attack was produced by the species *Aphis fabae* of 28.5% (BBCH 31), followed by *B. punctiventris* L., which recorded an attack rate of 21.6% (BBCH 10-12), and the attack rate produced by *Chaetocnema* spp. varied between 2.2% and 5.7% (Figure 6).

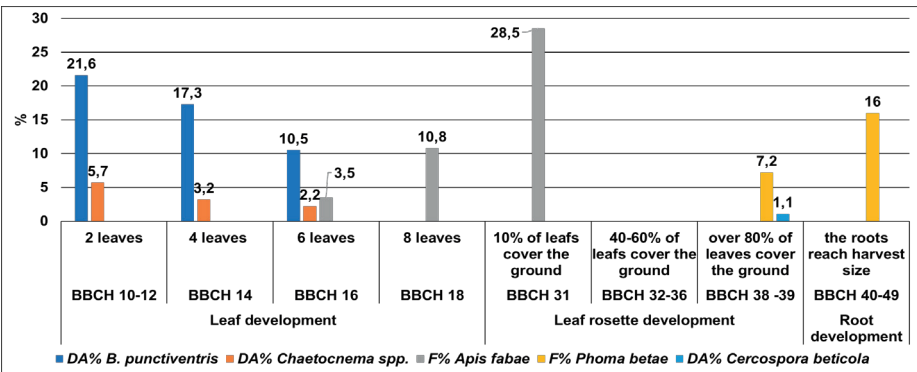


Figure 6. Dynamics of pest attack on sugar beet, 2024, Secuieni - Neamț

The dynamics of the attack produced by harmful organisms on sugar beet in 2025 show that five insect species and two pathogens were responsible for the recorded attacks. From

emergence to the development of the eight leaves, the attack varied between 1% and 9% in the species *B. punctiventris* L., the degree of attack produced by *Chaetocnema* spp. was

between 5.3% and 9.4%, the frequency of plants attacked by mining larvae of the species *Pegomia betae* was 11.4% - 18.8%, and that produced by *A. fabae* was 18.8%. During the leaf rosette development period, beet plants were affected by aphids (*Aphis fabae*), which recorded attack frequencies ranging from 18.7% to 20.6%, flea beetles (*Chaetocnema* spp.), which had an attack rate of 6.8-7.1%, the attack frequency produced by leafminer larvae ranged

from 5% to 12.3%, and adults and larvae of *Lixus junci* had an attack rate of 78.8%. The pathogens *Phoma betae* and *Cercospora beticola* were recorded in beet crops, the frequency of attacked plants was 7.7% and increased to 16.7% towards the end of the vegetation period (BBCH 40-49), and the attack rate of *Cercospora* was 3%, increasing to 5.1% at BBCH 40-49 (Figure 7).

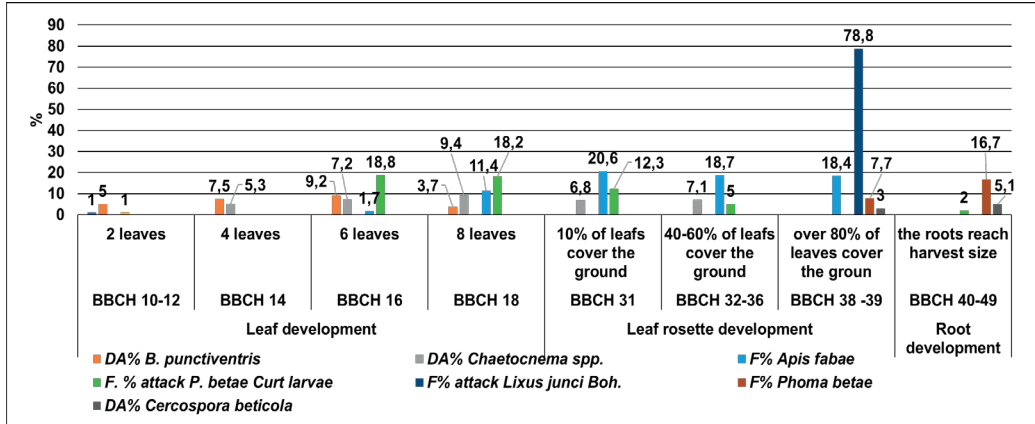


Figure 7. Dynamics of pest attack on sugar beet, 2025, Secuieni - Neamț

CONCLUSIONS

In sugar beet, the number of recorded pests varied, with five insect species and two pathogens identified in 2024, and 12 species and two pathogens in 2025.

The entomofauna harmful to the beet crop during the growing season was made up of 12 species, belonging to the orders Coleoptera, Lepidoptera, Homoptera, and Diptera.

The soil pest complex produced attacks from the emergence of plants, the highest being the attack produced by adults of the *Bothynoderes punctiventris* species in 2024, the DA was 16.5%, and in 2025, by the *Cheatoconema* sp. species, the DA was 7.2%.

In potatoes, the most widespread pests were *Leptinotarsa decemlineata* and the pathogen *Phytophthora infestans*.

The *Leptinotarsa decemlineata* species was present from emergence to plant maturity, the degree of attack produced ranged from 0.7% to 22.3% in 2024 and between 10.9% and 100% in 2025.

Phytophthora infestans produced attacks from 7.9% to 27.6% in 2024 and between 12.5% and 28.5% in 2025.

ACKNOWLEDGEMENTS

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YIELD, QUALITY AND PHYSIOLOGICAL TRAITS OF MONOECIOUS HEMP (*Cannabis sativa* L.) AS INFLUENCED BY SOWING TIME AND FERTILIZATION

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Abstract

Hemp is gaining increasing interest due to its high yield potential and multiple uses. This study was conducted under field conditions at ARDS Secuieni to assess the influence of sowing time and fertilization on yield performance, physiological traits, and biochemical quality of an early monoecious hemp variety, using a bifactorial split-plot experimental design. The E1 × F1 combination enhanced vegetative biomass accumulation, resulting in the highest inflorescence biomass (5300 kg·ha⁻¹ fresh and 1600 kg·ha⁻¹ dry) and stem yield (4380 kg·ha⁻¹), whereas seed production reached its maximum under the E1 × F2 combination (3293 kg·ha⁻¹). The second sowing time (E2) was associated with the highest photosynthetic rate (14.5 μmol CO₂·m⁻²·s⁻¹) and the greatest amount of carbon stored in the soil (68.3 t·ha⁻¹), while F1 fertilization recorded the highest photosynthetic rate (15.5 μmol CO₂·m⁻²·s⁻¹). Moreover, the interaction between the second sowing time and F1 fertilization maximized vitamin C content (67.3 mg·100 g⁻¹) and soluble solids (12.3 °Brix). Overall, the results highlight the importance of appropriate sowing time and fertilization in improving productivity and quality in monoecious hemp.

Key words: inflorescence biomass, stem yield, seed yield, photosynthetic rate, biochemical traits.

INTRODUCTION

Cannabis sativa L., commonly known as industrial hemp, is one of the oldest cultivated plants in human history originating from Central Asia, with documented uses spanning thousands of years across diverse civilizations (Rupasinghe et al., 2020). In recent decades, however, hemp has experienced a remarkable renaissance in agricultural research and commercial cultivation, driven by growing recognition of its extraordinary versatility and its potential to contribute to more sustainable agricultural systems (Struik et al., 2000). The plant is capable of producing a wide range of valuable outputs, including fiber, seeds, oil, and bioactive compounds, making it one of the most multi-purpose crops available to modern

agriculture (Naeem et al., 2023).

From an agronomic perspective, hemp is particularly valued for its rapid growth rate, high biomass production capacity, and relatively low input requirements compared to other industrial crops (Matvejeva et al., 2026). The deep root system of hemp contributes to improving soil structure, while its vigorous growth and dense crop stand effectively suppress weed development, thereby reducing the need for herbicide applications. Hemp is a particularly beneficial crop for soil organic carbon (SOC) accumulation, as its extensive root biomass penetrates deeper soil layers, contributing to long-term carbon sequestration. Furthermore, hemp has been recognized for its capacity to accumulate and sequester carbon both in aboveground biomass and soil organic matter

(Chimento and Amaducci, 2015; Adesina et al., 2020). Soil organic carbon stock is an increasingly important indicator for evaluating the environmental sustainability of cropping systems, as it reflects the capacity of agricultural soils to store carbon and maintain long-term fertility (Nwaogu et al., 2026).

Based on the distribution of floral sexes, industrial hemp can be classified into dioecious and monoecious forms. Monoecious cultivars are particularly valued in breeding programs and commercial production due to their more uniform maturation and greater compatibility with mechanized harvesting (Habán et al., 2022; Ona et al., 2025).

The quality of agricultural production is influenced by the interaction of multiple factors, including genotype, environmental conditions, and crop management practices (Agapie and Sala, 2022).

Sowing time is a critical determinant of crop performance, as it directly influences the photoperiodic and thermal conditions experienced during key developmental stages, including vegetative growth, flowering, and seed formation. Inappropriate sowing dates may expose crops to unfavorable environmental conditions, resulting in reduced yields, altered biochemical composition, and decreased physiological efficiency. Conversely, appropriately timed sowing synchronizes crop development with favorable climatic conditions, thereby maximizing productivity and quality (Butkevičienė et al., 2021).

Within crop management technologies, fertilization plays a key role in determining both the level and the quality of agricultural production (Agapie and Sala, 2023), as nutrient availability, particularly nitrogen, is considered one of the main factors limiting crop productivity worldwide (Agapie et al., 2023). In the case of hemp, fertilization is also an important factor influencing crop yield and quality. Nitrogen, in particular, plays a central role in stimulating vegetative growth and biomass accumulation, while organic and humic-based fertilizers have been increasingly investigated for their capacity to improve soil biological activity, enhance nutrient use efficiency, and support root development (Teliban et al., 2020, 2022). The growing interest in sustainable agriculture has stimulated

research into the use of biostimulants and organic fertilizers as alternatives or complements to conventional synthetic inputs, with promising results reported for various crops, including hemp (Da Cunha Leme Filho et al., 2024).

Given this background, the present study was conducted under field conditions at the Agricultural Research and Development Station Secuieni, Romania, to evaluate the effects of two sowing times and three fertilization treatments on yield performance, biochemical quality traits, photosynthetic activity, and soil carbon storage in the monoecious hemp variety Secuieni-Jubileu. The study aimed to identify suitable combinations of sowing time and fertilization in order to optimize both the quantitative and qualitative performance of the crop and to contribute to the development of agronomic recommendations for monoecious hemp production under temperate continental conditions, particularly in the context of current climate change.

MATERIALS AND METHODS

Experimental Location and Design

The research was carried out in 2025 at the experimental field of the Agricultural Research and Development Station (ARDS) Secuieni, Neamț County. The study was organized as a bifactorial experiment, arranged in a split-plot design with three replications.

The experimental factors considered in the study were: A = sowing time [a_1 – sowing time I (first decade of May); a_2 – sowing time II (second decade of May)] and B = fertilization treatment (b_1 – non-fertilized control; b_2 – fertilized with PromoStart®; b_3 – fertilized with Terrenova®). The experimental plot had an area of 40 m² and was sown with a mechanical seeder at a row spacing of 70 cm. At harvest maturity, the crop density was 7-8 plants·m⁻².

The soil of the experimental field was characterized by a high content of nitrogen, phosphorus, and potassium, as well as adequate levels of magnesium, sulfur, iron, copper, and zinc, providing favorable nutritional conditions for hemp crop development. The moderate humus and organic carbon content contributed to maintaining soil structure and good water retention capacity. The soil reaction was slightly

acidic (pH = 6.72), with no salinity issues recorded.

Biological Material

The biological material used in the study was the monoecious hemp variety Secuieni-Jubileu, developed and homologated in 2012 at the Agricultural Research and Development Station Secueni.

Fertilizers used

PromoStart® is a controlled-release nitrogen-based fertilizer used for foliar application, which stimulates vegetative growth and supplies available nitrogen to plants. Its composition includes total nitrogen (30.0%), urea nitrogen (5.0%), and formaldehyde nitrogen (25.0%).

Terrenova® is an organic fertilizer based on humic and fulvic acids, intended to improve soil fertility and stimulate root system development. It contains total humic extract (18.2%), humic acids (9.1%), fulvic acids (9.1%), potassium (11.4%), free amino acids (5.7%), phosphorus (5.7%), and total nitrogen (0.91%).

Fertilizers were applied in three stages, at the beginning, middle, and end of the flowering phase, at rates of 8 L·ha⁻¹ for PromoStart® and 10 L·ha⁻¹ for Terrenova®.

The meteorological conditions during the experimental period, namely air temperature and precipitation, are presented in Figures 1 and 2.



Figure 1. Multi-year and monthly average temperature (°C)

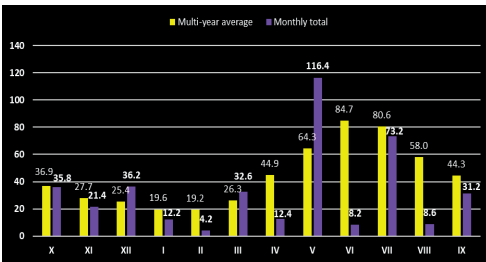


Figure 2. Multi-year and monthly total precipitations (mm)

Measurements and Analyses

The measurements aimed to evaluate seed and biomass production (stems, inflorescences - fresh yield and inflorescences - dry yield). Biochemical and physiological traits were also assessed, including vitamin C content, total soluble solids (TSS), photosynthetic rate, and soil organic carbon stock.

Ascorbic acid content (vitamin C) was determined reflectometrically using the Reflectoquant™ system (RQflex® 20, Merck KGaA), following extraction in a 1% oxalic acid stabilizing solution.

Total soluble solids (TSS) were determined using a Zeiss refractometer, with results expressed in °Brix according to OECD standards (2018).

Leaf gas exchange parameters were measured using the portable photosynthesis system LCpro T® (ADC BioScientific Ltd., UK). The photosynthetic rate was expressed in μmol CO₂·m⁻²·s⁻¹.

Soil organic carbon (SOC) content (%) was determined according to SR ISO 10694:1998 using a Vario MICRO Cube elemental analyzer. Soil organic carbon stock (SOCS) was calculated according to the formula proposed by Pearson and Buraka (Pearson et al., 2005; Buraka et al., 2022):

$$SOCS = BD \times D \times \%C$$

where: SOCS (t·ha⁻¹) represents the soil organic carbon stock, BD (g·cm⁻³) is the soil bulk density, D (cm) is the soil depth, and %C represents the soil organic carbon concentration expressed as percentage.

Statistical Analysis

The experimental data were subjected to analysis of variance (ANOVA) for bifactorial experiments. Differences between means were evaluated using Tukey's test at a significance level of $p < 0.05$.

To highlight the relationships among the analyzed variables and to reduce the dimensionality of the dataset, Principal Component Analysis (PCA) was applied. Correlations among the parameters studied were determined using the Pearson correlation coefficient in Origin ProLab 2026 free trial.

Table 1. Influence of sowing time and fertilization on biomass and seed yield in hemp crop

Variant	Fresh inflorescence yield (kg·ha ⁻¹)	Dry inflorescence yield (kg·ha ⁻¹)	Stems yield (kg·ha ⁻¹)	Seed yield (kg·ha ⁻¹)
Sowing time				
E1	4867 ± 108	1467 ± 26	4193 ± 123	3152 ± 17
E2	4650 ± 36	1383 ± 23	3961 ± 59	2772 ± 37
<i>Significance</i>	<i>ns</i>	<i>ns</i>	*	*
Fertilization				
NF	4150 ± 33 b	1275 ± 40 b	3943 ± 53	2782 ± 49 b
F1	5225 ± 230 a	1575 ± 4 a	4318 ± 142	2948 ± 41 b
F2	4900 ± 104 a	1425 ± 49 ab	4188 ± 64	3157 ± 39 a
<i>Significance</i>	*	*	<i>ns</i>	*

E1 - sowing time I, E2 - sowing time II, NF - non-fertilized control, F1 - fertilized with PromoStart®, F2 - fertilized with Terrenova®;

Values are presented as mean ± standard error (SE). Within each column, ns - no statistically significant difference, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSIONS

The influence of sowing time and fertilization treatment on biomass and seed yield of hemp is presented in Table 1.

Regarding sowing time, the E1 variant recorded significantly higher stems and seed yields compared to E2, with increases of approximately 6.0% and 13.7%, respectively. Fresh and dry inflorescence yields did not show statistically significant differences between the two sowing epochs.

A management strategy aimed at reducing inputs while maintaining adequate yield levels can be achieved by optimizing the sowing time, with the optimal choice depending on the specific production objectives, such as higher seed or biomass yields (Cosentino et al., 2012). Regarding fertilization, both F1 and F2 significantly outperformed the non-fertilized control (NF) for fresh inflorescence yield (+26.0%, +18.1%, respectively) and dry inflorescence yield (+23.5%, +11.8%, respectively). For seed yield, only F2 produced a statistically significant increase over NF (+13.5%). Stems yield was not significantly affected by fertilization treatment.

Nitrogen from fertilizers should be applied in appropriate amounts, as both excess and deficiency can negatively affect crops and the environment. Nitrogen fertilization influences hemp seed and biomass yields to a greater extent than phosphorus and potassium fertilization, particularly under field conditions (Deng et al., 2019; Bruce et al., 2022; Kaur et al., 2023).

The interaction between sowing time and fertilization significantly influenced fresh and dry inflorescence yield and seed yield, while stems yield was not significantly affected (Table 2). E1 × F1 recorded the highest fresh (5300 kg·ha⁻¹, +34.2%) and dry inflorescence yield (1600 kg·ha⁻¹, +33.3%) compared to the lowest-performing E2 × NF combination. Seed yield peaked under E1 × F2 (3293 kg·ha⁻¹), exceeding E2 × NF by 29.6% and E2 × F1 by 19.4%. The E2 × NF combination consistently recorded the lowest values across all yield components, confirming that delayed sowing without fertilization is the least favorable for hemp productivity.

The combination of early May sowing and appropriate fertilization can enhance both yield and crop quality, as also highlighted in the study conducted by Caplan (Caplan et al., 2017).

Table 2. Influence of the interaction between sowing time and fertilization on biomass and seed yield in hemp crop

Variant	Fresh inflorescence yield (kg·ha ⁻¹)	Dry inflorescence yield (kg·ha ⁻¹)	Stems yield (kg·ha ⁻¹)	Seed yield (kg·ha ⁻¹)
E1 x NF	4350 ± 247 ab	1350 ± 119 ab	3997 ± 62	3023 ± 37 abc
E1 x F1	5300 ± 212 a	1600 ± 14 a	4380 ± 231	3140 ± 56 ab
E1 x F2	4950 ± 136 ab	1450 ± 121 ab	4203 ± 93	3293 ± 62 a
E2 x NF	3950 ± 309 b	1200 ± 68 b	3890 ± 130	2540 ± 67 d
E2 x F1	5150 ± 428 ab	1550 ± 19 ab	4257 ± 99	2757 ± 30 cd
E2 x F2	4850 ± 185 ab	1400 ± 55 ab	4173 ± 43	3020 ± 76 bc
<i>Significance</i>	*	*	<i>ns</i>	*

E1 - sowing time I, E2 - sowing time II, NF - non-fertilized control, F1 - fertilized with PromoStart®, F2 - fertilized with Terrenova®;

Values are presented as mean ± standard error (SE). Within each column, ns - no statistically significant difference, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p < 0.05$.

Table 3 presents the effect of sowing time and fertilization on vitamin C content and total soluble solids (TSS) in monoecious hemp. Both factors produced statistically significant differences ($p < 0.05$) for both parameters. The superiority of the second sowing epoch (65.61 mg/100 g) compared to the first epoch can be attributed to the increased solar radiation intensity and higher temperatures during the vegetation period.

In plant physiology, ascorbic acid acts as a primary protective agent against photo-oxidative stress. According to Smirnov, vitamin C synthesis is directly stimulated by photon flux, functioning as an essential protective mechanism of the photosynthetic apparatus (chloroplasts) against reactive oxygen species (ROS) (Smirnov, 2018).

Regarding fertilization treatment, it also influenced the vitamin C content of the fresh biomass. The average values ranged from 61.58 mg/100 g (non-fertilized control, NF) to 66.17 mg/100 g (F1), indicating a high antioxidant potential of the fresh biomass. The F1 variant represented the optimal accumulation threshold, while the slight decrease observed in the F2 variant (65.00 mg/100 g) suggests the occurrence of a possible “dilution effect”. As reported by Gallie, Lee and Kader, higher levels of fertilization, particularly those associated with nitrogen supply, may stimulate vegetative growth and water accumulation in plant tissues, which can lead to a relative decrease in the concentration of bioactive compounds when expressed on a fresh weight basis (Gallie, 2013; Lee and Kader, 2000).

Table 3. Influence of sowing time and fertilization on biochemical traits of hemp

Variant	Vitamin C (mg/100 g fresh product)	TSS (°Brix)
Sowing time		
E1	62.89 ± 0.24	10.80 ± 0.20
E2	65.61 ± 0.20	11.83 ± 0.05
<i>Significance</i>	*	*
Fertilization		
NF	61.58 ± 0.22 c	11.55 ± 0.05 ab
F1	66.17 ± 0.22 a	11.77 ± 0.37 a
F2	65.00 ± 0.14 b	10.63 ± 0.07 b
<i>Significance</i>	*	*

E1 - sowing time I, E2 - sowing time II, NF - non-fertilized control, F1 - fertilized with PromoStart®, F2 - fertilized with Terrenova®; Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p < 0.05$.

According to Table 4, the interaction between sowing time and fertilization produced

statistically significant effects ($p < 0.05$) on vitamin C content and total soluble solids (TSS). The E2 × F1 combination recorded the highest values for both parameters, representing an increase of approximately 12.9% in vitamin C content and 13.4% in TSS compared to the E1 × NF combination. The elevated levels of soluble solids in the second sowing epoch suggest enhanced carbon assimilation efficiency, favored by the higher temperatures during the vegetation period.

Table 4. Influence of the interaction between sowing time and fertilization on biochemical traits of hemp

Variant	Vitamin C (mg/100 g fresh product)	TSS (°Brix)
E1 x NF	58.67 ± 0.33 c	10.87 ± 0.07 bc
E1 x F1	65.00 ± 0.29 b	11.20 ± 0.70 abc
E1 x F2	65.00 ± 0.29 b	10.33 ± 0.07 c
E2 x NF	64.50 ± 0.29 b	12.23 ± 0.09 ab
E2 x F1	67.33 ± 0.17 a	12.33 ± 0.07 a
E2 x F2	65.00 ± 0.29 b	10.93 ± 0.07 bc
<i>Significance</i>	*	*

E1 - sowing time I, E2 - sowing time II, NF - non-fertilized control, F1 - fertilized with PromoStart®, F2 - fertilized with Terrenova®; Values are presented as mean ± standard error (SE). Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p < 0.05$.

Furthermore, there is a direct physiological correlation between TSS and vitamin C: hexoses (glucose and galactose), which constitute a significant fraction of the soluble solids, act as direct biochemical precursors in the ascorbic acid metabolic pathway (the L-galactose pathway) (Cruz-Rus et al., 2011; Smirnov, 2000). Thus, the high °Brix values observed in the E2 × F1 variant metabolically support the maximum vitamin C concentration (67.33 mg/100 g), indicating an optimal state of physiological activity. The data presented in Table 4 confirm that hemp biomass exhibits high metabolic plasticity. The combination of meteorological conditions characteristic of the E2 sowing epoch and fertilization with F1 favors the accumulation of nutritional compounds, highlighting the potential of mature hemp biomass as a valuable plant source of antioxidants.

Maluleke and Thobejane investigated the combined effects of different types of chemical and organic fertilizers across various growing environments on the yield and biochemical constituents of *Cannabis sativa* for commercial production purposes. The authors reported that vitamin C content ranged from 26 to 66 mg/100 g dry weight (DW), depending on the fertili-

zation type and growing environment (Maluleke and Thobejane, 2025).

Figure 3 presents the influence of sowing time and fertilization treatment on the photosynthetic rate in monoecious hemp. E2 recorded a higher photosynthetic rate than E1, with an improvement of approximately 21.3%. Among fertilization treatments, F1 (PromoStart®) achieved the highest photosynthetic rate ($15.53 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), outperforming both F2 (Terrenova®) and the non-fertilized control.

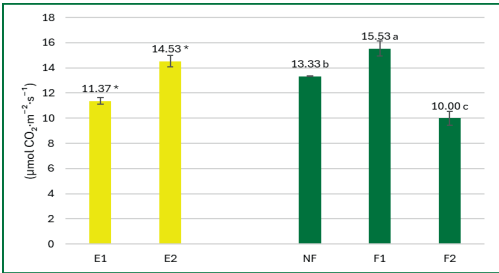


Figure 3. Influence of sowing time and fertilization on the photosynthetic rate

Figure 4 presents the interaction effects between sowing time and fertilization on the photosynthetic rate of monoecious hemp, with statistically significant differences recorded among all six treatment combinations ($p < 0.05$). The E2 $\times$ F1 combination achieved the highest photosynthetic rate ($18.30 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, group a), while the E1 $\times$ F2 combination recorded the lowest value ($9.25 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, group d).

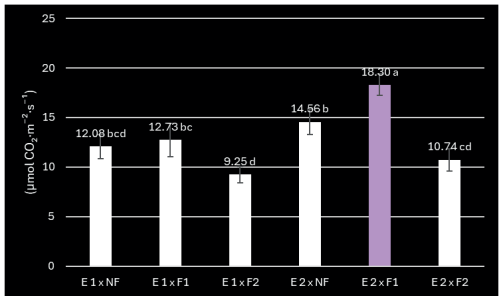


Figure 4. Influence of the interaction between sowing time and fertilization on the photosynthetic rate

Hemp has demonstrated notable photosynthetic capacity, with light-saturated leaf photosynthesis rates recorded at $19 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ under field conditions (Meijer et al., 1995), and more recent studies reporting a

maximum light-saturated net photosynthesis rate (A_{max}) of up to $31.2 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ at 25°C , further highlighting hemp's high resource-use efficiency and bio-economic potential as a crop (Tang et al., 2017).

Figure 5 presents the influence of sowing time and fertilization treatment on soil organic carbon stock (SOCS) in the studied monoecious hemp crop. The soil organic carbon stock (SOCS) values determined at the time of soil sampling were higher in the E2 treatment by approximately 9.4% compared to E1 (68.3 vs. $61.9 \text{ t} \cdot \text{ha}^{-1}$). Regarding fertilization treatments, no statistically significant differences were detected (ns), with the NF variant recording the highest value, exceeding F1 and F2 by approximately 3.7% and 6.5%, respectively.

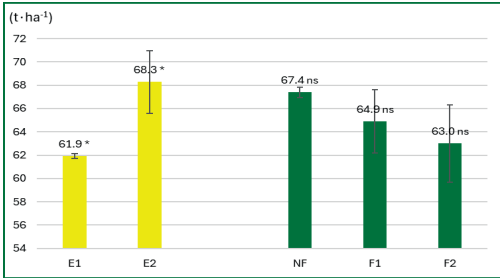


Figure 5. Influence of sowing time and fertilization on soil organic carbon stock (SOCS)

Figure 6 presents the interaction effects between sowing time and fertilization on soil organic carbon stock (SOCS) in the studied monoecious hemp crop, revealing statistically significant differences among treatment combinations ($p < 0.05$).

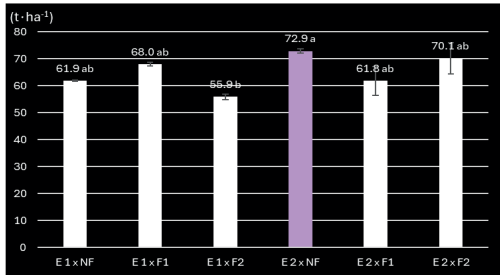


Figure 6. Influence of the interaction between sowing time and fertilization on soil organic carbon stock (SOCS)

The SOCS values determined at the time of soil sampling were highest under the E2 $\times$ NF

treatment (72.9 t·ha⁻¹, group a), exceeding the lowest value observed under E1 × F2 (55.9 t·ha⁻¹, group b) by approximately 23.4%. Furthermore, SOCS values under E2 × NF were higher compared to E2 × F1 and E2 × F2 by approximately 15.2% and 3.8%, respectively. The second sowing date (E2) was associated with higher soil organic carbon stock values, suggesting root - soil interactions and residue dynamics that may favor carbon accumulation in soil under specific phenological conditions. Organic inputs may further modulate soil organic carbon through improvements in soil biological activity and overall soil functioning. Although short-term increases in SOC are promising, longer-term monitoring is necessary to confirm their persistence across seasons and crop rotations. The PCA results revealed that the first two principal components (PC1 and PC2) accounted for 84.59% of the total variance, with PC1 contributing 49.88% and PC2 34.71%. The third component provided an additional 9.66%, bringing the cumulative explained variance to 94.25%. The remaining components contributed marginally to the total variance, indicating that the first two principal components adequately describe the variability in the dataset (Table 5).

Table 5. Eigenvalues and explained variance of principal components

PC	Eigenvalue	Percentage of Variance (%)	Cumulative Percentage of Variance (%)
1	3.99	49.88	49.88
2	2.78	34.71	84.59
3	0.77	9.66	94.25
4	0.45	5.60	99.85
5	0.01	0.15	100.00

PC1 was positively associated with fresh inflorescence yield (0.447), dry inflorescence yield (0.426), stems yield (0.440), and seed yield (0.439), indicating that this component primarily represents overall productivity traits. PC2 showed strong positive loadings for photosynthetic assimilation rate (0.498), TSS (0.466), and vitamin C (0.465), suggesting that this axis is primarily associated with physiological and quality parameters. The PCA biplot depicted in Figure 7 illustrates the relationships between the interaction of sowing time and fertilization with various hemp characteristics. PC1 distinguishes the experimental variants based on seed yield,

revealing that E1 × F2 and E2 × F2 demonstrate superior performance. In contrast, E2 × F1 is linked to improved photosynthesis, total soluble solids (TSS), and soil carbon content. PC2 emphasizes variations in vitamin C and inflorescence yield, with E1 × F1 showcasing higher yields of inflorescence and stems, alongside moderate levels of vitamin C. The experimental variants without fertilization (E1 × NF and E2 × NF) are positioned at the periphery, indicating lower overall performance.

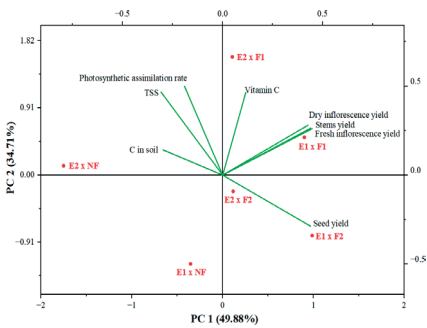


Figure 7. Two-dimensional PCA showing the distribution of analyzed hemp traits as influenced by sowing time and fertilization

The correlation analysis (Figure 8) reinforces the findings from the PCA by elucidating the relationships among various traits. Fresh and dry inflorescence yields, along with stems yield, display a strong positive correlation (0.97–0.99), underscoring that PC1 predominantly reflects overall productivity.

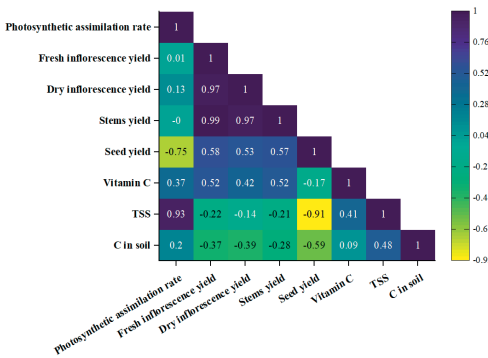


Figure 8. Pearson correlation analysis of hemp traits under the sowing time and fertilization interaction

Seed yield demonstrates a moderate correlation with inflorescence traits (0.53-0.58) but exhibits

strong negative correlations with both the photosynthetic rate (-0.75) and total soluble solids (TSS) (-0.91). This suggests a trade-off between seed production and physiological or quality traits. Similarly, vitamin C is positively correlated with inflorescence yields but negatively correlated with seed yield, aligning with PC2's representation of physiological and quality parameters. Soil carbon content shows moderate correlations with various traits, further emphasizing the distinction between productivity and quality axes in the PCA.

CONCLUSIONS

The results of this study confirm that both sowing time and fertilization treatment significantly influence yield performance, physiological traits, biochemical quality, and soil carbon dynamics in monoecious hemp under temperate continental conditions.

Early sowing proved most effective for maximizing stem and seed yield, as well as inflorescence biomass, by synchronizing crop development with favorable temperature and photoperiod conditions. Later sowing, however, showed advantages in terms of improved photosynthetic efficiency, higher soil organic carbon stock values, and improved biochemical traits.

Among the fertilization treatments, both PromoStart® and Terrenova® consistently outperformed the unfertilized control for the majority of yield and quality parameters. PromoStart® proved most effective in promoting inflorescence and stem biomass, the analyzed biochemical compounds, and photosynthetic rate, while Terrenova®, an organic fertilizer based on humic and fulvic acids, showed particular effectiveness in supporting seed production.

Overall, no single combination of sowing time and fertilization proved universally optimal across all evaluated traits. Future studies should investigate multi-year responses and additional varieties to further optimize agronomic recommendations for this crop.

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WHEAT GRAIN QUALITY DEPENDING ON CULTIVATION TECHNOLOGY AND PREVIOUS CROP

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Abstract

Maintaining wheat quality in the context of climate change is a crucial challenge for ensuring food security and the economic value of agricultural production. In a difficult economic environment, farmers often tend toward cereal-dominated crop rotations, which increases the risk of yield and quality decline, particularly due to the unfavorable effect of preceding crops. The study comparatively evaluates the effect of conventional tillage and no-tillage on the yield and quality of winter wheat grains cultivated in a four-crop rotation, with soybeans, maize and winter rapeseed as preceding crops. The parameters analyzed include yield, crude fiber content, ash content, starch content, total fat, crude fiber and crude protein (both expressed on a dry matter basis), and moisture. The results confirm the clear influence of the preceding crop type and soil tillage system on wheat yield and quality, emphasizing the importance of choosing appropriate technologies to optimize crop performance, promote agricultural sustainability, and increase the competitiveness of the cereal sector.

Key words: crop rotations, tillage, winter wheat.

INTRODUCTION

Due to its high carbohydrate content, wheat (*Triticum aestivum* L.) is frequently perceived as a primary energy source, an aspect that confers it particular importance from this perspective. It also contains significant amounts of essential nutrients, including proteins, fibers, and minor components such as lipids, vitamins, and minerals that can contribute to a healthy diet (NDNS, Bates et al., 2014a, b). These characteristics make it a crucial agricultural crop globally, essential for human nutrition and the agricultural economy (Shewry & Hey, 2015). The quality and quantity of winter wheat production determine its economic value. These parameters make it possible to utilize the grain. Wheat intended for food consumption must have a high baking and milling value, or processing value. Therefore, in wheat production, both high yields and favorable grain quality parameters are important, such as protein content, quantity and quality of gluten, sedimentation value, and specific grain weight. Climate variability is already reshaping how wheat responds to disease pressure, altering both infection timing and pathogen aggressiveness across southern Romania (Paraschivu et al., 2023).

Wheat grain yield and quality are significantly influenced both by the previous crop and by the tillage system, with higher yields and superior quality parameters being obtained when wheat is cultivated after soybean and under conventional tillage (Gawęda and Haliniarz, Agriculture 2021; Slafer & Satorre, 1999). Thus, adapting cultivation technologies and applying appropriate agrotechnical measures play an essential role in increasing productivity and improving crop quality parameters, particularly under the variable climatic conditions of southern Romania (Iancu et al., 2009). Under conditions of good quality soils, characterized by high fertility, favorable structure and texture, and adequate porosity, high yields can be achieved, which can be realized under normal conditions through the application of appropriate technologies (Popescu et al., 2024). The implementation of modern cultivation technologies, including fertilization, crop rotation, and phytosanitary protection practices, affects not only yield but also quality parameters such as protein content, gluten, and hectoliter weight (Rusu et al., 2014). The application of alternative organic products with fungicidal effect in the management of major wheat diseases not only supports sustainable

agricultural practices compared to traditional fungicides, but can also contribute to improving grain quality, positively influencing its parameters (Paraschivu et al., 2024). An important role in improving soil structure and reducing diseases, with a direct impact on wheat production quality, is represented by the choice of the previous crop (García del Moral et al., 2003).

Furthermore, climate change represents a global challenge facing humanity today, due to continuously rising temperatures, which trigger numerous extreme meteorological phenomena such as heat waves, drought, and flooding, affecting crop production. (Soare et al., 2024).

Emphasizing the importance of adopting measures to counteract climatic variability in order to maintain the production and quality potential of crops, it is necessary to consider all crop protection methods starting from the time of planting. (Prioteasa et al., 2025).

Wheat grain quality depends on the interplay between genetics, cultivation technology, the previous crop, and environmental factors, including climate change. Adopting modern cultivation technologies, using alternative organic products, and making appropriate choices regarding the previous crop can improve both yield and grain quality.

Against this backdrop, selecting the tillage system and previous crop in southern Romania is no longer just an agronomic decision it is an economic and climate-adaptation strategy.

Climatic and environmental factors can considerably restrict agricultural performance through the occurrence of extreme water regimes, characterized either by excess or deficit of water during the growing period. Among these, drought represents the primary abiotic stress, and the interaction between atmospheric and pedological drought generates major effects on crop growth and development, manifested in significant yield reductions or even total crop failure (Nițu et al., 2024).

Intensive interventions such as overseeding and fertilization do not guarantee profitability, especially under unfavorable climatic conditions. Future decisions should integrate both economic calculations and sustainability objectives, possibly correlated with agri-

environmental policies or subsidies to support ecosystem restoration (Acatrinei et al., 2024).

Conservation agriculture systems, of which no-tillage represents the most advanced form, have demonstrated significant benefits in the context of climate change, through conserving soil moisture, improving soil structure, and reducing erosion losses (Kassam et al., 2019). At the same time, the choice of previous crop substantially influences the productive potential of wheat, through mechanisms involving the nutrient cycle, water reserve dynamics, and phytopathological pressure (Plaza-Bonilla et al., 2015).

MATERIALS AND METHODS

Experimental Location and Pedoclimatic Conditions

The experiment was conducted over two consecutive years (2022/2023 and 2023/2024) in southern Romania, in the Boian Plain, a subdivision of the Romanian Plain. The experimental site is characterized by cambic chernozem (Haplic Chernozem in WRB classification), a moderately developed chernozem subtype with high natural fertility and favorable structure in the upper horizon. The agrochemical properties determined in the 0-30 cm layer (horizon A) were as follows: humus content 3.7%, total nitrogen (N) 0.19%, available phosphorus (P_2O_5) 37 ppm, and exchangeable potassium (K_2O) 98 ppm. These characteristics provide favorable conditions for cereal cultivation, supporting optimal plant development, drought tolerance, disease resistance, and superior grain quality.

The study area is characterized by a temperate-continental climate with sub-Mediterranean influences, with a mean annual temperature of 11.6°C (average of the last 30 years) and mean annual precipitation of 541.7 mm (Romanian National Meteorological Administration, www.meteoromania.ro). The growing period of the 2022/2023 agricultural year was marked by deficient climatic conditions, with a cumulative rainfall deficit of 64.3 mm compared to the multi annual average and monthly mean temperatures 3.2°C above the climatological norm, which created conditions of moderate to severe water stress for the wheat crop.

Experimental Design and Treatments

The objective of this study was to evaluate the effects of the conventional tillage system versus the no-tillage system on yield and grain quality of autumn wheat. Two high-performing Romanian autumn wheat varieties were selected Glosa and Boema created at the National Agricultural Research and Development Institute Fundulea and recognized for their high productivity and superior drought tolerance.

The experiment was established according to a three-factor factorial design: Factor A (Variety): Glosa and Boema, both characterized by early maturity and drought resistance; Factor B (Tillage System): conventional tillage (conventional plowing followed by secondary tillage operations) and no-tillage system (direct seeding without soil tillage); Factor C (Previous Crop): rapeseed (*Brassica napus* L.), soybean (*Glycine max* L.), and maize (*Zea mays* L.).

The research was conducted according to a complete factorial design of $2 \times 2 \times 3$, resulting in 12 distinct experimental variants. Each experimental plot measured 30 m² (4 m $\times$ 7.5 m), with 1 m buffer zones between plots to prevent cross contamination.

The experimental factors were selected based on both scientific and practical considerations. The two varieties, Glosa and Boema, were chosen for their scientific relevance, representing distinct genotypic profiles in terms of drought tolerance and yield potential, as well as for their practical importance, together accounting for approximately 40-45% of the wheat cultivated area in Romania (Glosa 35%, Boema 10%). The tillage systems were selected to reflect the current transition from conventional to conservation agriculture in the context of climate change. The previous crops were chosen from distinct botanical families (Brassicaceae, Fabaceae, Poaceae), given their differentiated effects on soil fertility, edaphic structure, and phytopathological pressure.

Sowing and Seedbed Preparation

Due to the pedological drought conditions observed in the autumn of 2022, sowing was postponed until the third decade of October (October 25, 2022, for the 2022/2023 season and October 23, 2023, for the 2023/2024 season). All experimental variants were sown on the same day for each agricultural season, using a seeding rate of 550 viable seeds/m². This density

was established based on the variety characteristics (high tillering capacity, 600-650 plants/m² at harvest) and local pedoclimatic conditions (drought risk, estimated germination losses of 5-10%).

For conventional tillage, soil preparation included: plowing at 25 cm depth, performed immediately after harvesting the previous crops (June-July for rapeseed, September for soybean and maize); disk harrowing at 12-15 cm depth for breaking clods and leveling.

For the no-tillage variants, wheat was sown using a purpose-built no-tillage drill without any prior soil tillage. After harvesting the previous crops (soybean, rapeseed, maize), the crop residues were uniformly chopped and distributed on the soil surface using a straw chopper mounted on the combine harvesters. Sowing depth was 4-5 cm for both systems, with a row spacing of 12.5 cm, resulting in 32 rows per plot.

Regarding fertilization, this was uniform for all plots. Before sowing, the following were applied: N: 40 kg/ha (urea 46%), P₂O₅: 60 kg/ha (applied as simple superphosphate), and K₂O: 40 kg/ha (applied as potassium chloride). During the growing season, in March N: 60 kg/ha was applied (ammonium nitrate 34% N) and in April N: 40 kg/ha (as urea 46%). The total nitrogen dose was reduced by 20 kg N/ha in variants following soybean, which fixes an estimated 60-80 kg N/ha of atmospheric nitrogen, with 30-40% remaining available for the subsequent crop. Weed, pest, and disease control followed the principles of integrated pest management.

Harvesting and Data Collection

Yield Determination

Harvesting was performed at complete physiological maturity, when grain moisture was between 16-18%. Mechanized harvesting was carried out over the entire surface of each plot using a small-scale experimental combine harvester. Yield was weighed immediately after harvesting using a precision balance.

Grain Quality Analysis by NIRS Spectrophotometry

For the qualitative characterization of the harvested biological material, near-infrared spectrophotometry (NIRS) was implemented. This instrumental method presents decisive advantages in the context of analyzing cereals and products intended for the feed and food

industry: non-invasiveness, high execution speed, and no requirement for complex sample preparation. The spectrum of investigated parameters included protein content, starch and sugar concentration, digestibility of non-structural carbohydrates (NCGD), crude lipid fraction (ether extract EE), and the *Fah*_{as} indicator. The data obtained provides essential information for two distinct directions of utilization: evaluation of milling-baking aptitudes and establishing the potential for integration into industrial processing flows. Representative 500 g grain samples were collected from each plot.

RESULTS AND DISCUSSIONS

Climatic Aspects

The study area is under the influence of a temperate continental climate, characterized by non-uniform precipitation patterns. Given non-irrigated agriculture, autumn moisture deficit can compromise adherence to the optimal calendar for establishing wheat crops. From a thermal perspective, 2022 ranked among the three warmest years recorded in Romania. October showed notable climatic anomalies: precipitation was limited to 15 mm, representing a decrease of 16.8 mm compared to the climatological norm of 46.0 mm, making it one

of the driest Octobers in the meteorological record. At the same time, the monthly mean temperature of 13.6°C was considerably above the usual values for this period. These conditions had direct consequences for the germination process, which was compromised, negatively influencing the entire ontogeny of the plants and, implicitly, the qualitative characteristics of the grain yield.

In November, above-average temperatures persisted, with a mean temperature of 8.6°C, exceeding the multi-annual average by 3.6°C, placing it among the warmest Novembers on record. The rainfall deficit extended into February, when only 13.2 mm of precipitation was recorded, 14.7 mm below the reference value. The combination of atypically high soil and air temperatures in February led to the premature interruption of winter dormancy, activating vegetative growth processes before the normal physiological time. In contrast to the cold season, the spring period benefited from more favorable conditions, with rainfall within acceptable limits, which allowed the soil water reserve to be maintained at near-optimal parameters. It is important to note that, during the growing period, monthly mean temperature values were consistently above multi-annual averages (Table 1).

Table 1. The meteorological parameters in the experimental period 2022-2023
(Source: Romanian National Meteorological Administration, www.meteoromania.ro)

Specification	Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Average Total
Temperature °C	Monthly Average	13.7	8.6	2.8	4.0	3.6	7.7	10.6	16.4	21.2	25.8	25.7	21.5	13.4
	Multiannual Average Last 30 Years	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	22.8	23.5	18.1	11.6
	Deviation Amount Monthly	+2.0	+3.5	+2.5	+5.3	+2.8	+1.7	-1.4	-1.3	-0.4	+3.0	+2.2	+1.4	+1.8
Precipitation mm	Multiannual Media Last 30 Years	15.0	78.8	33.8	103.4	13.2	20.8	68.8	78.6	44.4	120.0	40.8	35.8	653.4
	Deviation	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	62.1	46.6	43.5	541.7
	Deviation	-16.8	+87.2	-17.7	-4.0	-14.7	-11.8	-21.8	+13.2	-38.1	+33.1	-40.6	-24.5	+111.7

The 2023-2024 agricultural campaign was marked by significant water deficits. In October, the month in which the experiment was established, precipitation was 16.8 mm below the climatological norm, with only 21.4 mm recorded. November brought a substantial improvement, with 124.2 mm of precipitation an

amount that exceeded the multiannual average by 87.2 mm ensuring a satisfactory reconstitution of soil moisture reserves. The winter and early spring period was characterized by insufficient rainfall, with a negative impact on plant growth dynamics. February represented the minimum seasonal

rainfall; with only 11.6 mm a deficit of 14.7 mm compared to the 30-year average of 26.3 mm. May played a crucial role in supplying the crop with water; the pluviometric input of 71.8 mm was 13.2 mm above the norm of 58.6 mm. This amount proved beneficial, occurring during critical phenological stages that require maximum water consumption: heading, flowering, and the processes of grain formation and filling.

From a thermal perspective, the entire growing period was dominated by positive anomalies, with monthly temperatures consistently exceeding reference values. The exception was

May 2024, when the mean temperature of 17.2°C was 0.5°C below the multi-annual average of 17.7°C. The most pronounced positive thermal deviations were recorded in February (+7.2°C) and October (+4.2°C), while in the summer months (June, July, August) the thermal excess remained constant between +3.3°C and +4.4°C (Table 2).

Over the entire growing period (October-September), the cumulative precipitation deficit reached 64.3 mm (477.4 mm vs. the norm of 541.7 mm), while the mean annual temperature of 14.8°C was 3.2°C above the multi-annual average of 11.6°C.

Table 2. The meteorological parameters in the experimental period 2023-2024
(Source: Romanian National Meteorological Administration, www.meteoromania.ro)

Specification	Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Average Total
Temperature °C	Monthly Average	15.9	8.2	3.9	0.9	8.0	8.8	14.9	17.2	26.0	27.2	26.8	20.2	14.8
	Multiannual Average Last 30 Years	11.7	5.1	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	22.8	23.5	18.1	11.6
	Deviation Amount Monthly	+4.2	+3.1	+3.6	+0.4	+7.2	+2.8	+2.9	-0.5	+4.4	+4.4	+3.3	+2.1	+3.2
Precipitation mm	Multiannual Media Last 30 Years	21.4	124.2	21.4	26.8	11.6	22.4	26.0	71.8	31.6	95.2	6.0	19.0	477.4
		46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	62.1	46.6	43.5	541.7
	Deviation	-16.8	+87.2	-17.7	-4.0	-14.7	-11.8	-21.8	+13.2	-38.1	+33.1	-40.6	-24.5	-64.3

Production and Quality

For both varieties and all three previous crops studied, the no-tillage system generated lower yields compared to the conventional system, with absolute differences ranging from -50 kg/ha to -380 kg/ha. This systematic trend suggests that, under the pedoclimatic conditions of 2022/2023, conventional primary tillage favored a more uniform seedbed and superior nutrient mobilization, conferring a net competitive advantage over the conservative variant.

Relative reductions range between -0.9% and -7.8%, a moderate interval. Irrespective of the tillage system, soybean proves to be the most valuable previous crop for wheat in both varieties, generating the maximum absolute yields (5.820 kg/ha for Glosa Conventional; 5.580 kg/ha for Boema Conventional). The beneficial effect of soybean was attributed primarily to biological nitrogen fixation, improved soil structure, and reduced pressure from cereal-specific diseases.

Regarding rapeseed as a previous crop, it occupied an intermediate position, maintaining high yields and offering recognized phytosanitary advantages in rotation with wheat. Notably, for the Glosa variety under no-tillage, the yield after rapeseed (5.330 kg/ha) was higher than that obtained after maize under conventional tillage (4.860 kg/ha), which underscores that the effect of the previous crop can partially compensate for the technological disadvantage of no-tillage.

With maize, the minimum absolute values (4.480 kg/ha for Glosa no-tillage; 4.460 kg/ha for Boema no-tillage) may signal the high risk of contamination with *Fusarium* spp., difficulties in incorporating voluminous crop residues, and imbalances in nitrogen dynamics.

For the Glosa variety, the maximum penalty under no-tillage is -7.8% (after maize), while for Boema it does not exceed -4.5% (also after maize). This behavioral difference suggests that the Boema variety shows superior adaptability to the conditions characteristic of conservation

systems, namely more compact soil, lower soil temperatures at germination, and increased competition with crop residues. This result recommends it preferentially for farms oriented toward conservation agriculture.

The fact that the conventional system surpasses no-tillage in the Boian Plain is easily explained by the nature of the chernozem and the climatic specifics of the area. The cambic chernozem of the Boian Plain, a moderately developed chernozem subtype with high organic matter content and good natural fertility, tends to form a compact surface crust in the absence of mechanical tillage, a phenomenon accentuated by late summer and autumn torrential rains followed by dry periods. In the no-tillage system, this crust limits the infiltration of autumn and spring precipitation water, reducing the hydrological reserve accessible to the crop precisely during the critical phases of tillering and stem elongation. Conventional plowing, through mechanical loosening of the superficial horizon, eliminates this impediment and restores hydraulic conductivity, with a direct positive effect on mineral nutrition through the

intensification of aerobic microbial activity and organic nitrogen mineralization.

Another characteristic specific to the Boian Plain is the increased risk of no-tillage compaction at 25-35 cm depth, which can become impermeable and restrictive for the wheat root system, limiting access to water from lower horizons precisely the strategic water reserve under drought conditions. Yields recorded in 2023, ranging between 4.460 and 5.820 kg/ha, reflect a medium to favorable agricultural year for the Romanian Plain, with sufficient precipitation during the crop's early phenophases, but with moderate water deficit in May-June. Differences of 4-8% in favor of the conventional system are agronomically significant and indicate superior adaptation of the conventional system to local pedoclimatic conditions.

The Glosa after soybean variant, with the smallest difference (-0.9%), suggested that the high nitrogen input from the previous legume crop could partially attenuate the disadvantages of no-tillage, compensating for part of the mineralization deficit (Table 3).

Table 3. Production results obtained for wheat crops in 2023 and 2024

Year	Variety	Previous Crop	Conventional System kg/ha	No-tillage System kg/ha	Relative Difference %
2023	Glosa	Rapeseed	5570	5330	-4.3
		Soybean	5820	5770	-0.9
		Maize	4860	4480	-7.8
	Boema	Rapeseed	5330	5150	-3.4
		Soybean	5580	5340	-4.3
		Maize	4670	4460	-4.5
2024	Glosa	Rapeseed	4900	4840	-1.2
		Soybean	5100	4950	-2.9
		Maize	4550	4480	-1.5
	Boema	Rapeseed	4810	4760	-1.0
		Soybean	4920	4800	-2.4
		Maize	4460	4370	-2.0

The 2023/2024 campaign was marked by severe drought, a recurring phenomenon in this area of southern Romania, with significant rainfall deficits in March-May and high temperatures that accelerated evapotranspiration. The reduction in yields compared to the 2022/2023-year ranges between 600 and 1.160 kg/ha, confirming the devastating impact of water stress on the productive potential of both varieties. Differences between the two technological systems vary from 180-380 kg/ha

in 2023 to only 50-150 kg/ha in 2024. This convergence can be explained by the partial reversal of advantages: under extreme drought conditions, conventional soil, better aerated and with superior porosity, loses moisture more rapidly through direct evaporation from the loosened layer, while no-tillage maintains a more favorable moisture gradient in the superficial horizon due to the mulch of crop residues. The two opposing effects partially compensate each other, bringing the

performance of the systems closer together. This tendency is confirmed by the research of Pittelkow, C.M. et al. (2015), Hemmat, A. & Eskandari, I. (2004), Balwinder-Singh et al. (2016), which demonstrate that the relative benefits of no-tillage increase with intensifying aridification.

From the perspective of climate change, this dynamic has implications: if the frequency and severity of droughts in the Boian Plain continue to increase a well-documented trend for southern Romania the advantage of the conventional system will gradually diminish, and no-tillage will gradually become competitive and possibly superior in the long term. The benefits of no-tillage (He et al., 2011) appear after the progressive accumulation of organic matter over the long term, not in the initial years.

Soybean excels as a previous crop due to biological nitrogen fixation, essential in a region where the cost of mineral nitrogen is high and where its use efficiency decreases under drought conditions. Rapeseed, although excellent from a phytosanitary standpoint, benefits less from the structural advantages of the taproot in the local chernozem, which already exhibits a naturally favorable structure in the upper horizon.

The Glosa variety records higher absolute yields than Boema in all variants, with an average advantage of approximately 250-350 kg/ha under normal conditions and 200-300 kg/ha under drought conditions. This indicates a superior genetic potential of the Glosa variety in the conditions of the Boianu Plain, but also a greater sensitivity to water stress, reflected in the more pronounced yield decline in 2024. The Boema variety presents superior inter-annual productive stability an essential quality for farms in southern Romania where drought is an endemic, not exceptional, risk.

The data obtained in the Boian Plain over the 2023-2024 campaigns demonstrate that, under the specific conditions of the chernozems of southern Oltenia and the highly continental climate, the conventional tillage system ensures yields superior to the no-tillage system in years with relatively normal pluviometry, due to the positive effects of periodic decompaction and accelerated nitrogen mineralization. At the same time, the 2024 data reveal that severe drought

significantly reduces the difference between systems, opening the prospect that conservation agriculture will gradually become more competitive as the region undergoes progressive climatic aridification. This tendency requires continuous monitoring of the experiment for a minimum of 5-7 years and the inclusion of complementary indicators, soil water reserve, compaction indices, and organic carbon balance for a complete basis for long-term agrotechnical decisions. From the perspective of current agricultural practice in the Boian Plain, the rotation with soybean as the previous crop and the Glosa variety in years with medium climatic risk, respectively the Boema variety in years with high drought risk, represents the optimal combination identified by the present study.

Biochemical parameters

Regarding the biochemical parameters, crude protein represents the most important qualitative indicator of bread wheat, directly conditioning gluten content, water absorption capacity, and the technological behavior of flour in the baking process. The synthesis and accumulation of storage proteins in the endosperm, respectively gliadins and glutenins, the main constituents of gluten are determinately influenced by nitrogen availability during the critical phases of grain filling, by the variety genotype, and by climatic conditions during the maturation period (Pechanek et al., 1997; Shewry & Hey, 2015). Protein values from the 2022/2023 campaign ranged from 12.559% to 13.808%, an interval lower than that recorded in the 2024 campaign, reflecting the different climatic context of the two years. The better water supply in 2023 stimulated higher biomass accumulation and more abundant grain filling with starch, diluting the available nitrogen across a greater amount of dry matter and thus reducing the relative protein concentration, a phenomenon known as the protein dilution effect (Triboi & Triboi-Blondel, 2002). The higher yields of 2023 thus came at the expense of grain protein quality, a classical negative relationship between quantitative yield and protein content, with direct practical implications for the fractioned nitrogen fertilization strategy during critical phenophases of wheat cultivation in the conditions of the Boian Plain. None of the variants reaches the premium quality threshold of 14% (Figure 1).

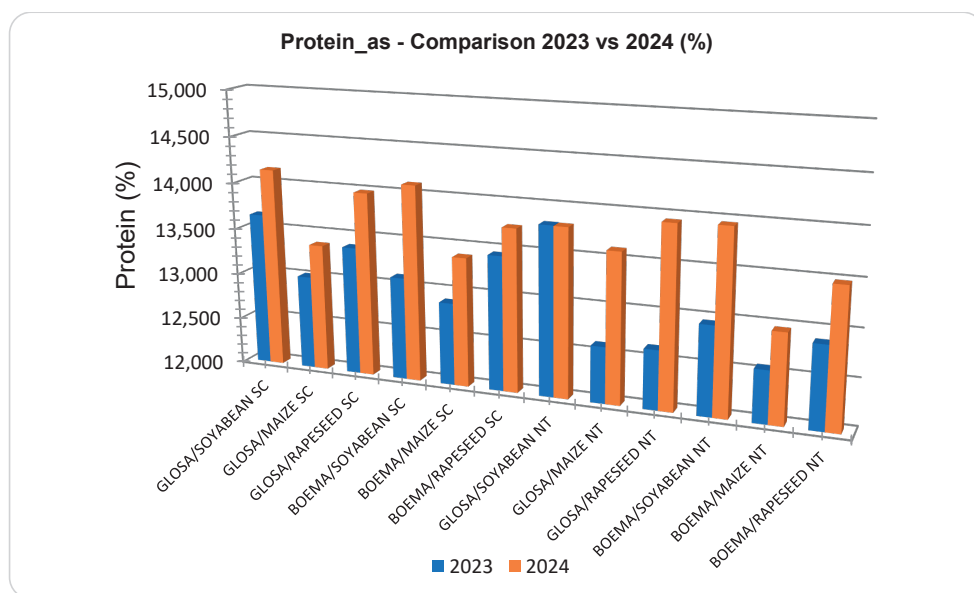


Figure 1. Comparative analysis of protein for the years 2023 vs 2024 (%)

Regarding the previous crop, soybean brings the highest protein values in both systems (12.95-13.81%), followed by rapeseed (12.63-13.44%), then maize (12.55-13.01%). This is logical because soybeans and rapeseed leave better residual nitrogen in the soil. Regarding the two tested varieties, the Glosa variety generally has higher protein than Boema, regardless of the tillage system or previous crop, making it more competitive for the bread wheat market.

Starch values ranged from 58.09% for Glosa Conventional after soybean to 62.33% for Boema No-tillage after maize, confirming the inverse protein-starch relationship, whereby variants with the highest protein content recorded the lowest starch values and vice versa. The Boema No-tillage after maize variant reached the maximum starch value of 62.33%, corresponding also to the lowest protein value, thus confirming the inverse relationship mentioned. This variability is important from the perspective of industrial applications: wheat with high starch and low protein content is preferred for biscuit manufacturing and industrial starch processing, while wheat with high protein content is intended for bread-making and traditional baking. Sugar content varied between 0.003% (Boema Conventional after maize) and 2.251% (Glosa No-tillage after rapeseed), with no negative values recorded.

Previous crops of rapeseed consistently generated the highest sugar values, while maize produced the lowest values regardless of variety or tillage system, suggesting a specific interaction between Brassica residues and soluble carbohydrate dynamics in the grain.

NCGD values ranged between 90.22% and 93.13%, reflecting high and relatively uniform digestibility of non-structural carbohydrates across all variants. The maximum value was recorded in Boema Conventional after soybean (93.13%), indicating particularly favorable grain composition in this variant from a nutritional and feed processing perspective.

Starch values showed little variation between the two experimental years, ranging from 58.09% to 62.33% in 2023 and from 58.29% to 62.43% in 2024, despite yield differences of 600-1.160 kg/ha between the two campaigns. This narrow variation of only 0.10-0.20% between years confirms that the 2024 drought primarily affected protein accumulation and grain size, not the carbohydrate composition of the endosperm (Blumenthal et al., 1994). Regarding grain moisture, values are below the critical threshold of 14%, guaranteeing storage stability. The low value of 9.24% for Boema after soybeans in the conventional system may be associated with faster maturation or more pronounced grain dehydration, possibly related

to early leaf senescence in the context of the high temperatures of summer 2023 in southern Oltenia. Crude fiber (ADF) ranges between 1.82% and 2.66%, and NDF values between 10.04% and 11.75%, showing that the cell wall structure of the grain is a genetically determined and stable parameter, little influenced by annual conditions or the tillage system. This stability confirms that fiber in wheat grain is determined predominantly by genotype and degree of maturity, not by external agronomic factors (Caporaso, 2018). Total fat values expressed as ether extract equivalents (0.834-1.481%) are within the normal range for common wheat. The absence of an effect of the agricultural year on lipid content confirms that grain fats are genetically

stable parameters, with low variability in response to environmental or agronomic factors (Pomeranz, 1988). Fah_as values ranged between 0.710 and 1.726, with the lowest values recorded in the conventional system after soybean for both varieties (0.862 and 0.710), suggesting a specific interaction between the previous legume crop and this indicator under conventional tillage conditions. Ash values ranging between 1.38% and 1.78% are within the normal range for bread wheat. No systematic differences are observed between varieties, technological systems, or previous crops, confirming that grain minerality is a relatively stable parameter under the conditions of the area (Table 4).

Table 4. Variation of wheat quality parameters depending on the sowing system and previous crops for the year 2022-2023

Variety	Previous Crop	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as	Ash_as
Conventional System	Glosa Soybean	13.647	58.088	0.3298	90.225	2.4355	1.1443	0.8619	1.6343
	Boema Maize	13.009	60.22	0.1907	90.403	2.3564	1.4805	1.7058	1.5942
	Rapeseed	13.379	61.064	1.1911	90.568	1.9612	1.3009	1.7255	1.4713
Conventional System	Glosa Soybean	13.103	61.399	0.3231	93.129	2.6558	1.1259	0.7100	1.7845
	Boema Maize	12.886	61.343	0.0031	90.771	2.4636	1.4040	1.6427	1.6190
	Rapeseed	13.444	61.100	2.0351	92.411	1.9404	1.0818	1.5762	1.3801
No-tillage System	Glosa Soybean	13.808	61.364	0.4332	91.619	2.3405	1.0612	1.4021	1.5891
	Boema Maize	12.604	60.364	0.1997	91.456	2.4849	1.3283	1.4201	1.7009
	Rapeseed	12.635	60.171	2.2517	90.821	2.0396	0.8339	1.4251	1.4153
No-tillage System	Glosa Soybean	12.952	60.692	0.9108	90.604	1.8185	1.1819	1.6320	1.4753
	Boema Maize	12.559	62.326	0.1219	91.179	2.2399	1.3870	1.3117	1.5334
	Rapeseed	12.881	61.522	0.6223	92.643	2.6065	1.1374	1.3006	1.5519

According to the international standard SR ISO 7970:2001, Wheat (*Triticum aestivum* L.), adopted also in Romania, as well as according to the commercial requirements established by EC Regulation No. 687/2008 on quality conditions for intervention wheat, the minimum protein content accepted for wheat destined for baking is 11.5% relative to dry matter, and premium-quality wheat for the milling and baking industry requires a minimum protein content of 12.5%, with a value of 14.0% and above constituting the premium quality threshold, with a direct impact on the commercial price and the technological yield of flour. From this perspective, both the Glosa variant with 14.147% and the Boema variant with 14.103% both after soybeans sown in the conventional system reach the premium quality threshold, explicable by the high residual

nitrogen input from soybeans as a legume previous crop. The year 2024 showed generally higher protein content. The average in the conventional sowing system increased from 13.24% to 13.79%, recording an increase of 0.54%. In the no-tillage sowing system, the average varies from 12.90% to 13.62%, with an increase of 0.72%. The no-tillage sowing system recorded the largest gains compared to the year 2022/2023, with the gap between systems narrowing from 0.34% to only 0.17%. Starch values range between 58.29% and 62.43%, indicating normal values of 60-70% in dry matter (Gooding & Davies, 1997). An inverse relationship between protein and starch is observed. Variants with high protein after soybean record the lowest starch values, due to the protein dilution effect, explained by competition between nitrogen and carbon

allocation during the grain-filling phase (Jenner et al., 1991). The Boema after maize no-tillage variant records the highest starch value (62.43%), corresponding also to the lowest protein value, thus confirming the inverse relationship mentioned. This variability is important from the perspective of industrial applications: wheat with high starch and low protein content is preferred for biscuit manufacturing and industrial starch processing, while wheat with high protein content is intended for bread-making and traditional baking. Moisture varies between 9.24% and 12.14%, confirming that samples were harvested and stored appropriately, without risk of microbiological degradation or excessive enzymatic activity. One outlier was the Glosa after rapeseed no-tillage variant (12.14%), slightly higher than the other samples, but within acceptable limits. The low moisture of the

Boema after soybean conventional sample (9.24%) may indicate more advanced field dehydration, possibly due to accelerated senescence during the 2024 drought. ADF values ranging between 1.12% and 2.72% reflect the cellulose and lignin content of the grain, non-digestible fractions. In quality wheat, low ADF is an indicator of a less lignified cellular structure and a higher degree of digestibility. The recorded values are normal for bread wheat (Caporaso, 2018), which presents ADF values of 2.0-3.5% for field wheat. The NDF parameter encompasses cellulose, hemicellulose, and lignin and varies between 10.03% and 11.75%. Values are relatively uniform between variants, without significant systematic differences between the two tillage systems or varieties, suggesting that the technological system does not significantly influence the cell wall structure of the grain.

Table 5. Variation of wheat quality parameters depending on the sowing system and previous crops for the year 2023-2024

Variety	Previous Crops	Protein	Starch (%)	Sugar (%)	NCGD (%)	Fibre	Fat (EE) (%)	Fah_as	Ash_as
Glosa	Soybean	14.147	58.288	0.339	90.425	2.5355	1.1543	0.8719	1.7343
	Maize	13.369	61.22	0.192	90.453	2.3664	1.5805	1.7658	1.6042
Conventional System	Rapeseed	13.979	61.164	1.192	90.668	1.9712	1.3003	1.7355	1.4713
	Boema	14.103	61.499	0.333	93.329	2.6658	1.2259	0.8100	1.7745
Conventional System	Maize	13.386	61.443	0.004	90.871	2.4836	1.5040	1.6627	1.6190
	Rapeseed	13.744	62.106	2.045	92.511	1.9604	1.0918	1.5962	1.3801
Glosa	Soybean	13.804	61.464	0.444	91.719	2.3505	1.0712	1.4121	1.5991
	Maize	13.604	60.564	0.200	91.476	2.4949	1.3383	1.4401	1.7029
No-tillage System	Rapeseed	13.935	60.271	2.271	90.841	2.0496	0.8539	1.4351	1.4173
	Boema	13.952	60.892	0.919	90.704	1.9185	1.2819	1.7420	1.4853
No-tillage System	Maize	12.959	62.426	0.121	91.199	2.2599	1.4870	1.5117	1.5434
	Rapeseed	13.481	61.622	0.632	92.843	2.6165	1.1474	1.2006	1.5619

Fat (EE) values total fat expressed as ether extract equivalents range between 0.85% and 1.58% (Table 5), a normal interval for wheat, which naturally has a low lipid content of 1.5-2.5% (Pomeranz, 1988). Fats in wheat grain are located predominantly in the germ and bran, influencing the oxidative stability of whole-wheat flour. The recorded values are at the lower end of the normal range, which is characteristic of wheat with well-developed endosperm relative to the germ, a positive aspect for white flour. Ash varies between 1.38% and 1.77% (Table 5), reflecting the total mineral content of the grain. For wheat destined for milling, the ash content of whole grain is normally between 1.5-2.0% (Codex Alimentarius, Standard 152-1985). The

recorded values are slightly below the lower limit in some variants, indicating grain with rich endosperm and relatively low bran content, a favorable characteristic from the perspective of white flour extraction yield.

CONCLUSIONS

The comparative study conducted in the Boian Plain over the 2023-2024 agricultural campaigns provides an evaluation of the interaction between the tillage system, previous crop, variety genotype, and climatic conditions in the autumn wheat crop. From the perspective of quantitative production, the conventional tillage system proved superior to the no-tillage system in both campaigns, with

the important caveat that the magnitude of this advantage is strongly conditioned by the rainfall regime.

In the 2023 campaign, with relatively favorable pluviometry, differences ranged from 50 and 380 kg/ha, while the severe drought of 2024 drastically reduced this gap to 50-150 kg/ha, confirming that the relative benefits of conservation agriculture increase with intensifying water stress. This dynamic has major strategic implications in the context of progressive climatic aridification of southern Romania, suggesting that the conventional advantage will gradually erode and no-tillage will progressively become competitive in the long term.

The previous crop was confirmed as a determining agronomic factor, with effects that can partially compensate for technological disadvantages. Soybean consistently generated the highest yields and the highest protein content in both years and both tillage systems, due to biologically fixed nitrogen contribution, positive phytosanitary effect, and soil structural improvement. The Glosa after soybean conventional variant reached the premium quality threshold of 14% crude protein in 2024, with direct implications for the commercial value of production. At the opposite end, maize maintained its status as the most unfavorable previous crop, amplifying phytosanitary risk, degrading soil structure through heavy harvesting equipment traffic, and generating the lowest yields and quality parameters in all studied variants.

From the perspective of biochemical quality, the data confirms the classical negative relationship between quantitative yield and grain protein concentration, explained by the protein dilution effect.

The 2023 campaign, more favorable in terms of yield, generated average protein values of 13.07%, compared to 13.71% in the dry year of 2024. The structural parameters of the grain, namely starch, ADF, NDF, ether extract, and ash, proved stable between years and tillage systems, confirming that these parameters are determined predominantly genetically and are not significantly influenced by the agronomic or climatic factors analyzed.

The differentiated behavior of the two varieties reveals a opposing patterns of productive

potential versus stability. The Glosa variety recorded yields higher by 250–350 kg/ha than Boema under normal climatic conditions but showed greater sensitivity to drought conditions. The Boema variety, in contrast, demonstrated superior inter-annual yield stability, a critical trait in a region where drought represents a persistent risk. This genotypic complementarity underpins the practical recommendation to use the Glosa variety in years with medium climatic risk and the Boema variety in years with high water deficit risk.

Overall, the data obtained argues for an adaptive and differentiated approach to cultivation technology in the Boianu Plain, in which the choice of tillage system, variety, and crop rotation should be aligned with climatic forecasts and the qualitative objectives of the farm. Continuing the experiment for a minimum of 5-7 years, complemented by monitoring soil water reserves, compaction indices, and organic carbon balance, remains a necessary condition for well-founded long-term agrotechnical decisions.

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MULTIVARIATE STATISTICAL ANALYSIS OF PRODUCTIVE TRAITS IN POA PRATENSIS UNDER WESTERN ROMANIAN CONDITIONS

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Abstract

This study evaluated the growth performance and productive potential of smooth meadow-grass genotypes under the pedoclimatic conditions of western Romania. The local populations were analyzed from which 20 genotypes were selected, evaluated based on morphological and productive characters, such as bush diameter, plant height, number of generative shoots, leaf area and green mass per clone. Pearson correlation analysis revealed very strong positive relationships between green mass, bush diameter and number of generative shoots, underlining the importance of these characters in determining biomass production. Principal component analysis (PCA) and cluster analysis revealed the existence of significant phenotypic variability between genotypes and allowed the identification of groups with different levels of productivity. Overall, the results demonstrate that environmental conditions strongly influence vegetative development and the expression of productive characters, highlighting at the same time the potential for selection of valuable genotypes for Poa pratensis breeding programs.

Key words: smooth meadow-grass, biochemical composition, biomass quality, generative shoots.

INTRODUCTION

In Europe, the multifunctional nature of permanent grassland ecosystems is particularly appreciated as a fundamental element of the rural landscape, contributing not only to fodder production, but also to biodiversity conservation, soil protection, hydrological stability and a host of social aspects (Hopkins & Holz, 2006; Peeters, 2009; Conant et al., 2017; Samfira et al., 2022; Butnariu, 2026).

Recent studies indicate that appropriate grassland management can increase carbon sequestration capacity and reduce the impact of climate change on agricultural systems (Frame et al., 1998; Hoskin et al., 1999; White & Hodgson, 1999; Soussana et al., 2019).

The genus *Poa*, belonging to the *Poaceae* family, includes over 500 species distributed in different climatic regions of the globe (Soreng et al., 2017). Among them, *Poa pratensis* L., commonly known as smooth meadow-grass, is one of the most important species from an agronomic and ecological point of view. This species is naturally distributed in temperate

regions of Europe, Asia, and North America and is appreciated for its high adaptability to different environmental conditions (Beard, 2002; Turgeon, 2011; Parker & Roberts, 2016). In forage production systems, smooth meadow-grass is valued for its ability to produce high-nutritional-quality plant biomass. Under favorable soil and climate conditions, dry matter production can range from 4 to 8 t/ha, depending on fertilization, the cropping system, and the meadow's floristic composition (Hopkins & Holz, 2006). In grasslands dominated by perennial grasses, this species frequently contributes to a significant proportion of total forage production.

In addition to biomass production, forage quality is an essential indicator of the agronomic value of grass species. The main parameters used to assess forage quality include crude protein content, organic matter digestibility, and structural fiber content (Van Soest, 1994; Butnariu et al., 2012). In the case of *Poa pratensis*, crude protein content can range from 10 to 18% of the dry matter, depending on

vegetation stage and growing conditions (White et al., 1999).

The digestibility of organic matter is an important indicator of forage nutritional value. Studies on the digestibility of grassland forages show that plant biomass from *Poa pratensis* can have digestibility values between 60 and 70%, depending on the stage of the plant development and management practices (Hopkins & Holz, 2006).

The content of structural fibers, as measured by neutral detergent fiber (NDF) and acid detergent fiber (ADF), directly influences the digestibility and energy value of forages (Orloff & Putnam, 2007; Rayburn et al., 2007).

The leaf/stem ratio is another important indicator of forage quality, since leaves contain higher concentrations of nutrients compared to stems. In this species, the high proportion of leaves improves the nutritional value of the forage and increases palatability for animals (Frame et al., 1998).

In recent years, much research has focused on improving the agronomic performance of *Poa pratensis* by selecting genotypes with high tolerance to abiotic stresses, such as high temperatures or water deficit (Bonos & Huff, 2013; Huang et al., 2021; Chen et al., 2021; Butnariu & Koirala, 2025). In the context of climate change, these characteristics are becoming increasingly important for maintaining the productivity of grassland ecosystems (IPCC, 2021).

The smooth meadow-grass exhibits morphological peculiarities that distinguish it from other *Poa pratensis* varieties, especially its narrower leaves and greater adaptability to variable pedoclimatic conditions. Some studies suggest that this variety may exhibit greater stability in biomass production and better tolerance to climatic stress conditions (Chen et al., 2021).

In Europe, the evaluation of grass species' performance under regional pedoclimatic conditions is essential for optimizing forage production systems. The productivity and nutritional value of plant biomass can vary considerably depending on climatic conditions, soil fertility, and agricultural management practices (Peeters, 2009). In Romania, grassland productivity is an important component of the agricultural system, offering high potential for

forage production, but it depends on the floristic composition and local pedoclimatic conditions (Ciobanu et al., 2012; Samuil et al., 2012). In this context, the evaluation of the productive and qualitative parameters of this variety, cultivated in the pedoclimatic conditions of western Romania, can provide relevant information regarding the agronomic potential of this species and its adaptability to regional conditions.

MATERIALS AND METHODS

The **purpose** of the work was to study, under the specific cultivation conditions of the Banat Plain, the phenotypic externalization of the morpho-productive traits in smooth meadow-grass and to identify productively valuable clones. This objective is important because the species is allelogamous and apomictic (many selected descendants reproduce by seeds that largely preserve the maternal genotype). The improvement process is largely based on selecting valuable clones.

The **biological material** consisted of local genotypes collected from the spontaneous flora of the Banat area, Romania. The genotypes were introduced into the collection field where 20 genotypes were selected and subjected to mass selection to eliminate the unvalued provenances. The identification of the parents required the investigation of the following characters throughout the vegetation period: bush diameter (cm), bush height (cm), number of generative shoots, number of leaves per main shoot, foliar surface (cm²), green mass/clone (g).

The **soil conditions** are typical of a chernozem, weakly gleyed, medium clay-clayey. The pH value indicates a weakly alkaline reaction (7.3-8.4) at 20-100 cm, and a moderately alkaline reaction (8.5-9.0) between 100-130 cm.

Climatic conditions. The observation period exhibited a rainfall regime with values well below the multiannual monthly average in the majority of months. The total amount of precipitation available to plants was 499.2 mm, with a negative deviation of 22.2 mm from the multiannual average. The precipitation deficit, together with excessive temperatures, induced an atmospheric heatwave and exacerbated pedological and atmospheric drought.

Statistical Analyses and Software Used. The relationships between morphological and

productivity characters were evaluated using the Pearson correlation coefficient (r). The Pearson coefficient measures the intensity and direction of the linear relationship between two variables. The statistical significance of the correlations was assessed based on the p-value, using the following significance thresholds: • $p < 0.05$ – significant (*) • $p < 0.01$ – highly significant (**) • $p < 0.001$ – very highly significant (***). The correlation matrix enabled the identification of associations between characters contributing to biomass production and plant architecture. Principal Component Analysis (PCA) was used to evaluate the structure of phenotypic variability between genotypes. Before performing the PCA analysis, all variables were standardized (z-score transformation) to eliminate the effects of differences in units of measurement. PCA reduces the dimensionality of the data set by transforming the original variables into a smaller set of principal components that explain most of the variation in the data. The first two principal components (PC1 and PC2) were used to create a biplot, which visualizes the distribution of genotypes across the analyzed characters. This analysis facilitates the identification of groups of genotypes with similar morphological and productive characteristics. The similarity between genotypes, based on the analyzed morphological characters, was evaluated through hierarchical cluster analysis.

The grouping procedure was performed using Euclidean distance as a measure of similarity and Ward's method as a clustering algorithm. The results of the analysis were graphically represented as a dendrogram, which illustrates the relationships among genotypes and allows the identification of clusters with similar phenotypic characteristics. Cluster analysis is frequently used in plant breeding studies to identify groups of genotypes with similar agronomic performances and valuable potential for breeding programs.

RESULTS AND DISCUSSIONS

To highlight the relationships between the main morphological and productivity characters of the smooth meadow-grass genotypes, a Pearson correlation matrix was performed using the mean values of the following variables: bush diameter, bush height, number of generative shoots, number of leaves on the main shoot, leaf area, and green mass per clone (Table 1). In this table, the first two columns show the variable pairs analyzed for correlations. The obtained correlations highlight the degree of association between these characters and identify those that significantly influence biomass production. Interpretation of correlation coefficients provides important information for selecting valuable genotypes in breeding programs.

Table 1. Expressing the degree of linear association of productive characters in *Poa pratensis* in Pearson's correlation matrix

The pairs of variables analyzed for correlation		Pearson's r	p	Lower 95% CI	Upper 95% CI
Bush diameter cm	Bush height cm	0.325	.162	-0.137	0.671
Bush diameter cm	Generative shoots	0.827***	< .001	0.607	0.929
Bush diameter cm	Leaves per main shoot	-0.215	.364	-0.600	0.252
Bush diameter cm	Leaf area cm ²	0.730***	< .001	0.425	0.886
Bush diameter cm	Green mass g	0.903***	< .001	0.768	0.962
Bush height cm	Generative shoots	0.362	.117	-0.096	0.693
Bush height cm	Leaves per main shoot	0.300	.198	-0.164	0.656
Bush height cm	Leaf area cm ²	0.427	.061	-0.020	0.731
Bush height cm	Green mass g	0.428	.060	-0.018	0.732
Generative shoots	Leaves per main shoot	-0.123	.605	-0.537	0.338
Generative shoots	Leaf area cm ²	0.538*	.014	0.125	0.792
Generative shoots	Green mass g	0.908***	< .001	0.778	0.963
Leaves per main shoot	Leaf area cm ²	-0.092	.699	-0.514	0.365
Leaves per main shoot	Green mass g	-0.010	.968	-0.450	0.435
Leaf area cm ²	Green mass g	0.730***	< .001	0.424	0.886

Significance: * $p < .05$, ** $p < .01$, *** $p < .001$.

The interpretation of the Pearson correlation matrix results highlights strong and very strong correlations among the main productivity characters of the *Poa pratensis* genotypes. These relationships indicate that certain morphological characters are closely related to plants' ability to produce biomass.

The analysis of the relationship between the Green Mass – Generative shoots characters shows a correlation coefficient $r = 0.91$ and a statistical significance level of $p < 0.001$, indicating a very strong, highly statistically significant correlation. This result suggests that the number of generative shoots is one of the main factors influencing green mass production, since a higher shoot density implicitly determines greater biomass accumulation.

Also, the analysis of the relationship between the Green Mass – Bush diameter characters shows a high correlation coefficient ($r = 0.90$), with a significance level $p < 0.001$.

These results indicate that genotypes with larger bush diameters generally produce more green mass. The biological explanation for this relationship is that plants with a more developed bush have a more complex vegetative structure, being able to support a higher biomass production. Similarly, the analysis of the relationship between Bush diameter and Generative shoots revealed a correlation coefficient of $r = 0.83$, with a significance level $p < 0.001$, indicating a very strong correlation between these two characters. This result suggests that genotypes that develop larger bushes also tend to produce a greater number of generative shoots, thereby increasing the plants' productive capacity.

Important interrelationships were also observed between the Leaf area – Green mass characters, with a correlation coefficient of $r = 0.73$ and a significance level of $p < 0.01$, indicating a strong, statistically significant correlation. This relationship can be explained by the fact that a larger leaf area indicates higher photosynthetic capacity, which favors the accumulation of more organic matter and, implicitly, higher biomass production.

On the other hand, the Pearson correlation matrix also highlights weak or insignificant correlations among certain characters. In this sense, the Leaves per main shoot character shows no significant correlations with the other

variables analyzed. This result suggests that the number of leaves per main shoot does not directly influence total biomass production; rather, the main role is attributed to the characters that determine bush architecture and shoot density.

Overall, the results of the correlation analysis indicate that bush diameter, number of generative shoots, and leaf area are the characters that contribute most to green mass production in smooth meadow-grass genotypes. These characters may represent important selection criteria in breeding programs, being relevant indicators of the productive potential of the studied genotypes.

To more clearly highlight the relationships among the analyzed characters, the Pearson correlation matrix was also presented as a heat map (Figure 1). This graphic representation allows for a quick observation of the intensity of the correlations between the variables, by means of a color scale, where the more intense shades indicate strong positive correlations, and the open ones weak or non-existent correlations. The heat map confirms strong correlations among green mass, the number of generative shoots, and bush diameter, as well as between leaf area and green mass, highlighting the importance of these characters in determining the productivity of *Poa pratensis* genotypes. In contrast, the character number of leaves on the main shoot shows low correlations with the other analyzed variables.

To highlight the variability structure among the analyzed characters and identify the main factors contributing to the differentiation of *Poa pratensis* genotypes, Principal Component Analysis (PCA) was performed.

This multivariate statistical method allows the reduction of the dimensionality of the data set by transforming the initial variables into a smaller number of principal components, which together explain most of the total variation. The significance of the PCA model was evaluated using the Chi-square test, which yielded $\chi^2 = 17.447$, $df = 9$, and $p = 0.042$, confirming that the model is statistically significant and adequate for interpreting the relationships among the analyzed variables. The Scree plot highlights the contribution of each component to explaining the total variability of the data. According to the eigenvalue criterion

(eigenvalue > 1) and parallel analysis, the first principal component (RC1) explains most of the variation, which indicates that most of the

analyzed characters are influenced by a common factor associated with plant productivity and vigor.

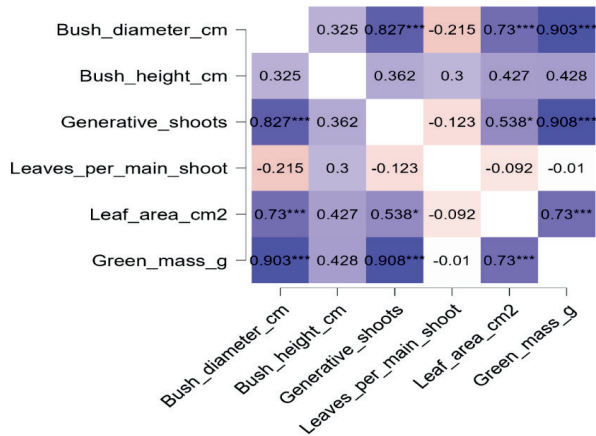


Figure 1. Heat map of the Pearson correlation matrix between morphological and productivity characters of the *Poa pratensis* genotypes

The component loadings show the contribution of each variable to the formation of the principal component RC1. The highest values are recorded for green mass (0.966), bush diameter (0.935), and the number of generative shoots (0.894), indicating that these characters have the greatest influence on the variability between genotypes (Table 2). Also, leaf area (0.819) contributes significantly to this component, suggesting the leaf apparatus plays an important role in biomass accumulation. In contrast, the character number of leaves per main shoot makes a very low contribution, an aspect also highlighted by the high uniqueness value (0.992), which indicates a limited influence on overall variability (Table 3).

The graphic representation of the relationships between the principal component and the analyzed variables, illustrated by a path diagram, confirms that the main characters that determine the variability of genotypes are those associated with productivity and bush size, respectively green mass, bush diameter, and number of generative shoots (Figure 2).

The Scree plot of the eigenvalues from the PCA, together with the results of the parallel analysis, used to determine the optimal number of components to retain in the model (Figure 3). In this representation, the eigenvalues obtained from the experimental data set are compared

with the values generated by a simulated model based on random data.

Table 2. Chi-square test used to check the adequacy of the PCA model

	Value	Df	p
Model	17.447	9	0.042

Table 3. Loadings of variables in the principal component RC1 resulting from PCA analysis

Component Loadings	RC1	Uniqueness
Green mass g	0.966	0.066
Bush diameter cm	0.935	0.125
Generative shoots	0.894	0.201
Leaf area cm ²	0.819	0.329
Bush height cm	0.531	0.718
Leaves per main shoot		0.992

Parallel analysis is a robust method for validating PCA, as it compares the observed structure of the data with a theoretical model in which the variables exhibit no significant correlations. Thus, the principal components are considered relevant when the eigenvalues obtained from the experimental data exceed those generated by the simulated model.

Therefore, the Scree plot analysis confirms that the first principal component explains the dominant factor explaining the variability in the analyzed characters, being mainly associated with productivity characters and bush size. This

observation also supports the results obtained in the analysis of component loadings, where variables such as green mass, bush diameter, and

number of generative shoots contribute the most to the formation of the principal component.

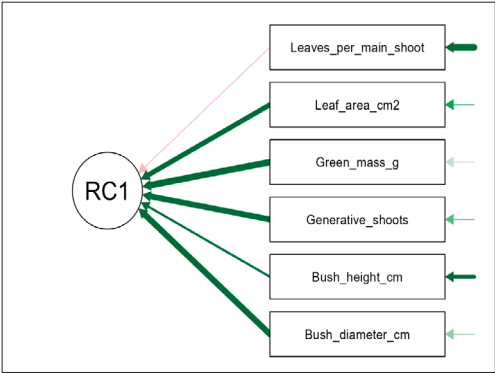


Figure 2. Scree plot of the eigenvalues resulting from principal component analysis (PCA) for the analyzed characters in *Poa pratensis* genotypes

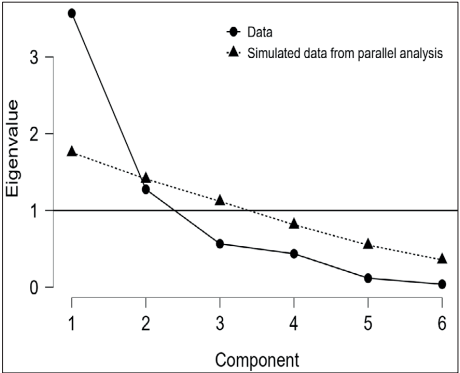


Figure 3. Scree plot of the eigenvalues resulting from principal component analysis (PCA) for the analyzed characters in *Poa pratensis* genotypes

Figure 4 shows the distribution of the 20 *Poa pratensis* genotypes in the plane of the first two principal components (PC1 and PC2), resulting from the PCA analysis. The graphic representation highlights the degree of similarity or differentiation between the genotypes based on the morphological and productivity characters analyzed.

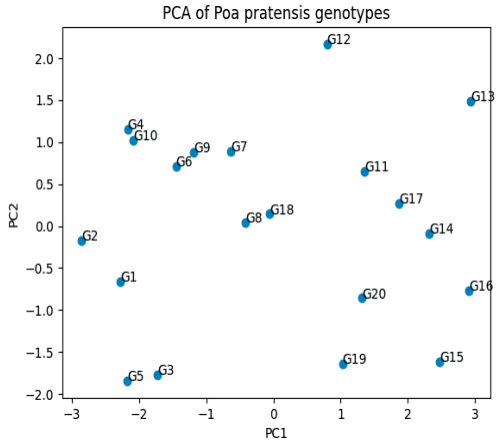


Figure 4. Representation of *Poa pratensis* genotypes in the PC1 and PC2 principal components plot

A clear separation of genotypes is observed along the PC1 axis, which mainly reflects variation in characters associated with productivity, such as green mass, bush diameter, and number of generative shoots. The genotypes

located on the right side of the graph, such as G13, G16, G15, G14 and G17, are characterized by high values of these characters and can be considered genotypes with higher productive potential. In contrast, the genotypes located on the left side of the graph, such as G2, G1, G5 and G3, exhibit lower values for productivity characters, indicating lower productive potential compared to the other genotypes analyzed. The genotypes positioned in the central area of the graph, such as G7, G8, G6, G9 and G18, exhibit intermediate characteristics, suggesting moderate variability in the analyzed characters. It is also noted that certain genotypes, such as G12 and G13, are located at a relatively large distance from the rest of the points on the graph, indicating a high degree of phenotypic differentiation compared to the other genotypes studied. These genotypes may represent valuable sources of genetic variability and are of interest to breeding programs. Overall, the distribution of genotypes on the PCA plot highlights considerable phenotypic variability within the analyzed biological material, offering opportunities to select genotypes with superior performance.

To assess the structure of phenotypic variability and highlight the similarity relationships among the studied genotypes, hierarchical cluster analysis was performed using Euclidean distance and the Ward agglomeration method. The results are presented as a dendrogram,

which illustrates the degree of proximity or differentiation among genotypes based on the morphological and productivity characters analyzed (Figure 5).

The structure of the dendrogram highlights the formation of two main clusters, separated by a relatively high similarity distance, indicating considerable phenotypic diversity within the analyzed biological material.

The first cluster includes genotypes G6, G10, G8, G7, G9, G3, G5, G4, G1 and G2, which are relatively similar and, in general, characterized by moderate values of characters associated with productivity and vegetative development.

The second cluster brings together genotypes G18, G11, G12, G13, G14, G17, G15, G16, G19 and G20, which are clearly differentiated from the first group and are associated with higher values of important morphological characters,

such as bush diameter, number of generative shoots, and green mass. Within this cluster, internal subgroups are also observed, reflecting varying degrees of similarity among genotypes. The clear separation of these two groups confirms the results of the PCA analysis, in which genotypes with higher productive potential were predominantly distributed in the positive area of principal component PC1. Thus, cluster analysis provides a complementary view of phenotypic variability and contributes to identifying genotypes with valuable agronomic characteristics. Overall, the dendrogram results highlight a differentiated genetic structure within the analyzed population, indicating strong potential for these genotypes in *Poa pratensis* breeding programs to obtain varieties with superior productive performance.

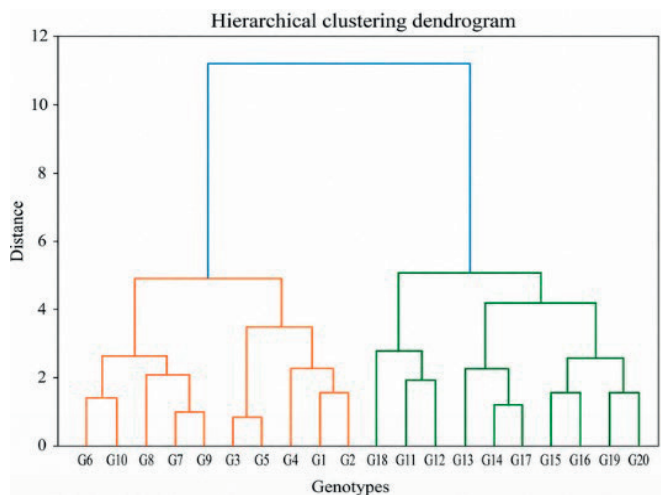


Figure 5. Dendrogram of *Poa pratensis* genotypes obtained by hierarchical cluster analysis based on the morphological and productivity characters analyzed

The statistical analyses performed highlight significant phenotypic variability among the studied *Poa pratensis* genotypes. The Pearson correlation matrix and heat map demonstrated strong correlations among the main productivity characters, especially among green mass, bush diameter, and number of generative shoots, confirming the role of these characters in determining the productive potential of the genotypes.

The principal component analysis highlighted that most of the variability is explained by characters associated with plant vigor and

vegetative development, and the distribution of genotypes on the first principal components plane allowed the identification of genotypes with superior performance.

The results of the cluster analysis, illustrated by a dendrogram, confirm these observations and highlight the formation of two distinct genotype groups, corresponding to different levels of development and productivity. Overall, these multivariate analyses provide valuable information on the structure of phenotypic variability and the relationships between genotypes, contributing to the identification of

genetic material with high potential for *Poa pratensis* breeding programs.

The cluster analysis presented in Figure 5 highlights the formation of two main genotype groups. Group 1 includes genotypes G1-G10 and is characterized by a lower productive potential. Genotypes in this group generally have smaller bushes, fewer generative shoots, and lower biomass production, indicating a moderate level of productive performance. Group 2 includes genotypes G11-G20, which are distinguished by a higher vegetative vigor. These genotypes are characterized by more developed bushes, a larger leaf area, a greater number of generative shoots, and higher green mass production. Within this group, genotypes G13, G14, G15 and G16 stand out for the highest values of productivity traits. Depending on the objectives of breeding programs, this classification of genotypes can be a useful tool for guiding selection, facilitating the identification of genetic material with superior potential for developing new productive varieties. Cluster analysis is frequently used in decision making related to the breeding process, particularly in crop science such as maize, highlighting the practical importance of this method in selecting the most appropriate fertilization response (Agapie & Sala, 2022).

CONCLUSIONS

The results of statistical analyses on smooth meadow-grass genotypes highlight significant phenotypic variability among the studied materials.

Pearson correlation analysis demonstrated very strong correlations among green mass, bush diameter, and the number of generative shoots, indicating that these characters play an essential role in determining the productive potential of the genotypes. In contrast, the character number of leaves per main shoot does not show significant correlations with the other variables analyzed, suggesting a reduced influence on total biomass production.

Principal component analysis showed that the first two principal components explain over 80% of the total variability, confirming that most of the differences between genotypes are determined by a small number of characters,

mainly associated with vegetative vigor and productive capacity.

The results of cluster analysis highlighted the separation of genotypes into two main groups, corresponding to different levels of development and productivity. The first group comprises genotypes characterized by lower values for the analyzed characters, whereas the second group comprises vigorous genotypes with high values for bush diameter, number of generative shoots, and green mass production. Within this superior group, genotypes G13, G14, G15 and G16 stand out for their highest productive potential and are considered valuable genetic materials for breeding programs of the smooth meadow-grass species to obtain new varieties with superior productive performance.

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ASSESSMENT OF HETEROSIS PERFORMANCE IN SUNFLOWER HYBRIDS DERIVED FROM INBRED PARENTAL LINES

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Abstract

*This study investigates the expression of heterosis in sunflower (*Helianthus annuus* L.) hybrids by analyzing their performance in comparison with newly developed parental inbred lines. The experiment was conducted under field conditions in Romania, within the experimental fields of the Agricultural Research Company Fundulea. A total of four sunflower hybrids and their corresponding parental inbred lines were evaluated. Key agronomic traits, including seed yield and selected morphological characteristics, were recorded and subjected to statistical analysis. Heterosis effects were quantified relative to both mid-parent and better-parent values, providing a comprehensive assessment of hybrid vigor. The results indicated consistent positive heterosis across most traits, with several hybrids surpassing their parental lines. Significant differences among genotypes ($p < 0.05$) emphasized the influence of genetic background on heterosis expression. Variability among hybrid combinations further highlighted the importance of selecting genetically diverse and complementary parental lines for breeding programs.*

Key words: sunflower hybrids, heterosis, inbred lines, hybrid vigor, genetic variability.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide, playing a key role in vegetable oil production due to its high oil content and adaptability to diverse environmental conditions (Seiler, 2007; Kaya et al., 2009). The increasing global demand for vegetable oils has intensified the need for developing high-yielding and stable sunflower hybrids (Skoric et al., 2007).

One of the most efficient approaches to improve crop performance is the exploitation of heterosis, also known as hybrid vigor. Heterosis refers to the superior performance of hybrid offspring compared to their parental lines, particularly in terms of yield, growth rate, and stress tolerance (Shull, 1908; Hochholdinger & Hoecker, 2007). The magnitude of heterosis is strongly influenced by the genetic divergence between parental lines and their combining ability (Melchinger, 1999).

The development of inbred lines is a fundamental step in hybrid breeding programs. Through repeated self-fertilization, a high degree of homozygosity is achieved, allowing the fixation of desirable traits and the creation of

genetically stable parental forms (Wright et al., 2013). These inbred lines serve as the genetic basis for hybrid combinations, where their interaction determines the level of heterosis expressed.

In sunflower breeding, fertility restorer (Rf) lines are essential for the production of commercial hybrids, especially when used in combination with cytoplasmic male sterile (CMS) lines (Skoric et al., 2007). The selection of genetically diverse and complementary parental lines is crucial for maximizing heterosis and improving agronomic performance.

Given the importance of hybrid vigor in increasing productivity, it is necessary to evaluate the performance of newly developed inbred lines and their hybrid combinations under field conditions. Such studies provide valuable information regarding the efficiency of breeding methods and the potential of genetic material for future breeding programs.

Therefore, the aim of this study was to assess the expression of heterosis in sunflower hybrids derived from newly developed inbred parental lines. The research focused on the evaluation of key agronomic traits, including yield and oil-related parameters, in order to identify superior

hybrid combinations and to highlight the influence of genetic background on heterosis expression.

MATERIALS AND METHODS

Study location and experimental conditions

The research was conducted in 2025 under field conditions within the Agricultural Research Company Fundulea, located in Fundulea, Călărași County, Romania. The specific pedoclimatic conditions of the area allowed the evaluation of the biological material under conditions representative for sunflower cultivation.

Biological material

The biological material used in the study consisted of commercial sunflower hybrids and inbred lines originating from both commercial sources and a gene bank.

In order to obtain inbred lines through self-fertilization, the commercial hybrids *Suria* and *Andrada* (Ciproma) were used. Self-fertilization was carried out over several generations with the aim of increasing homozygosity and stabilizing genetic traits.

Development of inbred lines

Two breeding approaches were applied to develop inbred lines:

1. Self-fertilization method

This method involved repeated self-pollination of the selected hybrids (*Suria* and *Andrada*) across successive generations to achieve genetic stabilization and a high degree of homozygosity.

2. Hybridization followed by selfing

The second method consisted of crossing fertility restorer (Rf) inbred lines followed by self-fertilization in subsequent generations. The following combinations were performed:

- CONFSCCL-R6 (female parent, emasculated) × RHA-480 (pollen donor);
- RF-3023 (female parent, emasculated) × RHA-478 (pollen donor).

The parental lines used in these crosses were obtained from the Gene Bank of the United States of America and are recognized for their genetic value in sunflower breeding programs.

Controlled pollination and line stabilization

Controlled pollination was performed manually by emasculating the female parental lines

(CONFSCCL-R6 and RF-3023) and applying pollen from the donor lines to obtain F₁ hybrids. The resulting biological material was subsequently subjected to repeated self-fertilization in order to fix traits and obtain stable inbred lines.

Obtained inbred lines

Following the application of the breeding methods, several inbred lines with high genetic potential were developed:

- LC-853 (derived from hybrid *Suria*);
- LC-861 (derived from hybrid *Andrada*);
- LC-623 (from CONFSCCL-R6 × RHA-480);
- LC-635 (from RF-3023 × RHA-478).

Hybridization scheme

The obtained inbred lines were used as male parental forms in hybridization schemes. These were crossed with three cytoplasmic male sterile (CMS) tester lines:

- LC-492-A;
- LC-411-A;
- LC-485-A.

These CMS lines served as female parents for the production of experimental hybrids.

Experimental hybrids

As a result of the crosses, the following hybrids were obtained:

- HS-56/24 = LC-492-A × LC-853;
- HS-76/24 = LC-411-A × LC-861;
- HS-13/24 = LC-492-A × LC-623;
- HS-32/24 = LC-485-A × LC-635.

These hybrids were further evaluated for combining ability and heterosis expression.

Traits evaluated and statistical analysis

A set of key agronomic traits was evaluated in order to assess the performance of the parental lines and their corresponding hybrids. The analyzed traits included plant height (cm), stem diameter (cm), number of leaves per plant, head diameter (cm), seed weight per head (g), number of seeds per head, thousand kernel weight (TKW, g), hectoliter mass (Mh), seed yield (kg/ha), oil content (%), and oil yield per hectare (kg/ha).

The recorded data were used to evaluate the expression of heterosis in the studied hybrid combinations. Heterosis was estimated by comparing hybrid performance with both parental lines, considering mid-parent values and better-parent values as reference points.

Descriptive statistical analysis was applied to characterize the variability of the studied traits. In addition, an analysis of variance (ANOVA) was performed in order to evaluate the significance of differences between genotypes. The significance of differences was assessed at the level of $p < 0.05$.

The analysis focused on highlighting the magnitude of heterosis for yield-related traits, as well as the contribution of parental lines to hybrid performance. Special attention was given to seed yield and oil yield per hectare, as these represent the most important economic traits in sunflower cultivation.

RESULTS AND DISCUSSIONS

Analysis of hybrid HS-56/24

For hybrid HS-56/24, all evaluated traits showed superior values compared to both parental lines, indicating a strong expression of heterosis, according to Table 1.

The most relevant trait, seed yield, recorded a value of 3914 kg/ha, significantly higher than both parental lines (1579 kg/ha and 351 kg/ha). The analysis of variance (ANOVA) indicated that these differences were statistically significant ($p < 0.05$).

Table 1. Agronomic traits of parental lines and F1 hybrid (HS-56/24)

Trait	LC-492-A (♀)	LC-853 (♂)	F1 (HS-56/24)
Plant height (cm)	107	126	157
Stem diameter (cm)	1.5	1.7	2.5
Number of leaves	18	17	20
Head diameter (cm)	17.5	11	22.9
Seed weight per head (g)	39.98	8.18	105.6
Number of seeds per head	951	780	1528
Thousand kernel weight (g)	42	26.2	65.8
Hectoliter mass (Mh)	40.9	31.5	44.3
Seed yield (kg/ha)	1579	351	3914
Oil content (%)	49.55	37.91	46.04
Oil yield (kg/ha)	792	133	1803

The calculated mid-parent heterosis reached +305.6%, while better-parent heterosis was +147.8%, indicating a very strong heterotic effect for productivity. Similar trends have been

reported in previous studies, where significant positive heterosis for seed yield and yield-related traits was observed in sunflower hybrids (Abdel-Rahem et al., 2021).

The superior performance of this hybrid was also reflected in key yield components such as head diameter, seed number, and seed weight per head, which are known to be strongly influenced by heterosis, as reported in the literature.

Analysis of hybrid HS-76/24

Hybrid HS-76/24 showed strong vegetative development and high productivity, exceeding both parental lines for most traits, according to Table 2.

Seed yield reached 4007 kg/ha, compared to 1657 kg/ha and 376 kg/ha in the parental lines. The analysis of variance (ANOVA) confirmed that the differences between the hybrid and its parental lines were statistically significant ($p < 0.05$).

The calculated mid-parent heterosis was +294.2%, while better-parent heterosis reached +141.7%.

This hybrid also recorded the highest plant height and number of leaves, indicating strong vegetative vigor, which contributed to increased yield potential. These findings are in agreement with previous studies, which highlighted that vegetative traits such as plant height and leaf number are positively correlated with hybrid performance (Kaya et al., 2009).

Table 2. Agronomic traits of parental lines and F1 hybrid (HS-76/24)

Trait	LC-411-A (♀)	LC-861 (♂)	F1 (HS-76/24)
Plant height (cm)	104	112	160
Stem diameter (cm)	2,1	1,7	2.2
Number of leaves	16	17	26
Head diameter (cm)	18	12.5	24.7
Seed weight per head (g)	36.26	8.77	80.8
Number of seeds per head	603	1076	1554
Thousand kernel weight (g)	60.1	20	52
Hectoliter mass (Mh)	34.5	41.4	40.8
Seed yield (kg/ha)	1657	376	4007
Oil content (%)	48.8	44.35	46.04
Oil yield (kg/ha)	808	167	1844.8

Analysis of hybrid HS-13/24

Hybrid HS-13/24 exhibited a balanced performance across all traits, with particularly strong results for reproductive characteristics, according to Table 3.

Seed yield reached 3963 kg/ha, compared to 1599 kg/ha and 742 kg/ha in the parental lines. Statistical analysis (ANOVA) revealed significant differences among genotypes for this trait ($p < 0.05$).

The calculated mid-parent heterosis was +238.6%, while better-parent heterosis was +147.9%.

This hybrid also showed the highest oil yield per hectare (1863 kg/ha), indicating its strong economic potential. Similar observations have been reported in the literature, where moderate heterosis for oil content combined with high seed yield leads to significant increases in oil production per unit area (Ebrahimi et al., 2021).

Table 3. Agronomic traits of parental lines and F1 hybrid (HS-13/24)

Trait	LC-492-A (♀)	LC-623 (♂)	F1 (HS-13/24)
Plant height (cm)	107	110	125
Stem diameter (cm)	1.5	1	2.3
Number of leaves	18	17	24
Head diameter (cm)	17.5	9.5	24.6
Seed weight per head (g)	39.98	18.57	115.7
Number of seeds per head	951	719	1987
Thousand kernel weight (g)	42	25.8	58.2
Hectoliter mass (Mh)	40.9	48.6	44.5
Seed yield (kg/ha)	1599	742	3963
Oil content (%)	49.55	17.62	47
Oil yield (kg/ha)	792	353	1863

Analysis of hybrid HS-32/24

Hybrid HS-32/24 showed the highest overall performance among all studied hybrids, according to Table 4.

Seed yield reached 4203 kg/ha, compared to 2755 kg/ha and 843 kg/ha in the parental lines. The analysis of variance (ANOVA) indicated that these differences were statistically significant ($p < 0.05$).

The calculated mid-parent heterosis was +133.6%, while better-parent heterosis was +52.6%.

Although heterosis percentages were lower compared to other hybrids, the absolute yield

was the highest, indicating superior genetic potential. This supports previous findings that high-performing parental lines may lead to lower heterosis values but higher absolute productivity (Melchinger, 1999).

Table 4. Agronomic traits of parental lines and F1 hybrid (HS-32/24)

Trait	LC-485-A (♀)	LC-635 (♂)	F1 (HS-32/24)
Plant height (cm)	120	125	145
Stem diameter (cm)	1.6	1.5	2.3
Number of leaves	22	18	26
Head diameter (cm)	19.5	10.5	25
Seed weight per head (g)	68.4	21.9	120.6
Number of seeds per head	1108	1004	2057
Thousand kernel weight (g)	61.7	21	59.8
Hectoliter mass (Mh)	44.6	54	44
Seed yield (kg/ha)	2755	843	4203
Oil content (%)	48.89	41.43	44
Oil yield (kg/ha)	1347	349	1850

Comparative analysis of hybrids

A comparative evaluation of the four hybrids highlights important differences in heterosis expression depending on the genetic background of parental lines, according to Figure 1.

The statistical analysis confirmed significant variability among hybrid combinations ($p < 0.05$), highlighting the influence of genetic background on heterosis expression.

Hybrids HS-56/24 and HS-76/24 showed the highest heterosis values, suggesting a strong genetic divergence between parental lines. In contrast, HS-32/24, although having the highest yield, showed lower heterosis percentages, indicating a closer genetic relationship between its parental lines.

Hybrid HS-13/24 demonstrated a balanced performance, combining high yield with the highest oil production, making it particularly valuable from an economic perspective.

These results are consistent with previous research indicating that heterosis expression varies significantly among hybrid combinations depending on the combining ability of parental genotypes (Skoric et al., 2007).

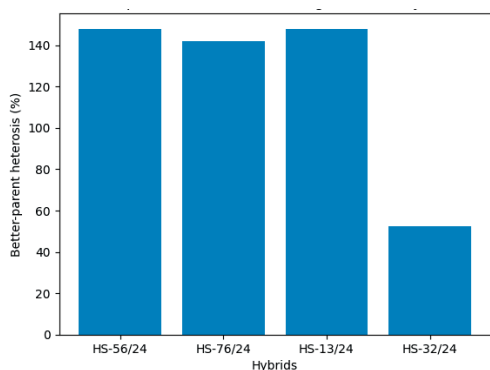


Figure 1. Comparison of heterosis (better-parent %) among sunflower hybrids

Influence of breeding method

The results clearly indicate that the breeding method significantly influenced the expression of heterosis.

Hybrids derived from crosses between genetically distant parental lines showed higher heterosis values, confirming that genetic divergence is a key factor in hybrid performance. Similar conclusions have been reported in sunflower breeding studies, where parental diversity was identified as a major determinant of heterosis expression (Hochholdinger & Hoecker, 2007).

In contrast, hybrids obtained from less divergent parental material showed lower heterosis, even if absolute productivity remained high.

These findings emphasize the importance of selecting appropriate breeding strategies and parental combinations in order to maximize hybrid performance and improve sunflower productivity.

CONCLUSIONS

The present study highlighted the significant role of genetic background and breeding strategy in the expression of heterosis in sunflower hybrids.

The experimental results demonstrated that all analyzed hybrids outperformed their parental lines for the majority of evaluated traits, particularly for seed yield and yield components, confirming the efficiency of hybrid breeding in sunflower.

The highest heterosis values were recorded in hybrids HS-56/24 and HS-76/24, indicating that crosses between genetically divergent parental

lines lead to a strong heterotic response. These findings confirm that genetic distance between parental genotypes is a key factor influencing hybrid performance.

Hybrid HS-32/24, although exhibiting the highest absolute seed yield, showed lower heterosis values, suggesting that high-performing parental lines may reduce the magnitude of heterosis while still ensuring superior productivity. This result emphasizes the importance of distinguishing between heterosis expression and absolute performance. Hybrid HS-13/24 showed a balanced performance, combining high seed yield with the highest oil yield per hectare, highlighting its potential for both agronomic and economic efficiency.

Statistical analysis (ANOVA) confirmed the existence of significant differences among hybrids ($p < 0.05$), validating that the observed variations are genetically determined and not due to random environmental factors.

Overall, the results demonstrate that the choice of breeding method and parental combinations plays a crucial role in maximizing hybrid performance. The use of genetically diverse parental lines is recommended when the objective is to enhance heterosis, while the use of elite parental material may ensure high absolute productivity.

These findings contribute to the optimization of sunflower breeding programs by providing practical insights into the relationship between genetic background, heterosis expression, and yield performance.

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THE USE OF SENSORY VEGETATION INDICES IN POTATO CLONAL SELECTION FOR THE IDENTIFICATION OF RESILIENT GENOTYPES

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Abstract

At NIRDPSB Brasov, in order to evaluate the response to the thermo-hydric stress of 2025, NDVI and SPAD vegetation indices were dynamically evaluated. Their quantification allowed obtaining a predictive image on the growth, development and adaptive capacity of plants, underpinning the efficient selection of the most valuable potato genotypes. Of the 28 genotypes evaluated in relation to the control variety Castrum, genotypes 1979/5, 2011/5 and 2018/1 maintained a high photosynthetic capacity until the end of the season (late maturity), unlike the genotype 2151/10 which, although it preserved a reasonable leaf cover (NDVI 0.89), manifested rapid cellular senescence through drastic pigment degradation (decrease to 38.8 SPAD units), standing out as a potential new early potato variety.

Key words: breeding, NDVI, SPAD, phenotypic selection.

INTRODUCTION

Among various crops, potato (*Solanum tuberosum* L.) stands out as both a staple food and a commercially valuable cash crop, feeding millions worldwide and supporting agro-industries (Biswal et al., 2025b).

Vegetation indices are extensively used to assess and monitor the vegetation and health status of the agricultural crops (Ivanovici et al., 2024). They can provide complementary information on vegetation physiology and ecology (Kulig et al., 2024; Yang et al., 2025), on leaf function, which is essential in capturing light and CO₂ needed for the growth of underground organs (Hermeziu et al., 2025). Good development of plants is indicative of their good nutrition, normal course of their life processes and, thus, optimum yielding and nutrient accumulation (Zarzecka et al., 2021). Adam (2025) showed that the dynamics of NDVI (Normalized Difference Vegetation Index) and SPAD (Soil Plant Analysis Development) indices values reveal substantial potential for employing these non-invasive metrics as robust tools for monitoring vegetation performance. From the agronomic point of view, accurate yield prediction is critical for sustainable potato production, NDVI being the best parameter to

use at the peak vegetative stage of potato (Lin et al., 2023; Biswal et al., 2025a), also based on the SPAD values, as revealed in the works of Dvořák and Král (2019), Trawczyński (2019) and Drăghici et al. (2024). For breeding purpose, these findings contribute to understanding the genetic mechanisms underlying potato tolerance to nitrogen deficiency stress, offering valuable insights for the development of future marker-assisted selection programs aimed at improving nitrogen use efficiency and stress resilience (Iribar et al., 2025). Rapid and non-destructive phenotyping approaches represent a pivotal advancement in optimizing the selection processes in potato breeding programs.

MATERIALS AND METHODS

The evaluated biological material comes from the breeding program of the National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Brasov. The planting of potato genotypes took place in the third decade of April (April 22, 2025), respecting the scheme of establishing the breeding field and the specific cultivation technology. The process of creating new potato varieties is a long-term and highly complex effort, spanning a period of 12 years, from the first generative hybrid population to

comparative orientation and competition cultures, going through rigorous selection stages. In 2025, 28 breeding lines in the Vegetative Descendants stage were selected for phenotypic evaluation, compared to the Castrum control (medium-early potato variety created at NIRDPSB). To assess the response to thermohydric stress, the portable FieldScout CM 1000 NDVI Meter was used. This instrument served as a non-destructive and efficient screening method, facilitating real-time data acquisition. To obtain physiological data complementary to those obtained by NDVI, there was performed real-time monitoring of chlorophyll concentration in potato leaves using a SPAD 502 plus FieldScout Minolta chlorophyll meter. The SPAD index value is a direct indicator of the relative chlorophyll content, which reflects the photosynthetic capacity of the plant, while the NDVI index measures the density and overall health of the canopy, being a good indicator of green biomass. Whereas NDVI decreases as plants age and turn yellow, the SPAD value shows a precise decrease in pigment in individual leaves, thus confirming the state of cellular degradation and stress. The formulas underlying the evaluated indices are $NDVI = \frac{NIR-RED}{NIR+RED}$ and $N\ index = \frac{\text{readings average}}{\text{control average}} \times 100\%$ (for SPAD values), as described by Stefan et al. (2019). Data acquisition was done dynamically on two different calendar dates, June 23 and August 14, 2025. These dates correspond to the end of the vegetative development phase or, depending on the precocity of the genotypes, the beginning of flowering, respectively, the tuber filling stage and maturity, a critical period for the translocation of assimilates, in which the products of photosynthesis are directed from the leaf apparatus to the tubers, for the accumulation of dry matter. From 3 plants of each genotype, 3 measurements were taken from the upper half of the plants and the average was calculated per plant. To identify genetic variability and select superior breeding lines, the average values used as repetitions were processed by analysis of variance (ANOVA) and the Pearson correlation test, the results being complemented by the calculation of the standard deviation in the SPSS statistical program. The correlation analysis targeted the liaison between SPAD, respectively NDVI indices and the studied genotypes. To

enable the statistical procedure in SPSS, the genotype variable was treated as an ordinal variable and coded numerically (1 to 29), following their ranking from Tables 1 and 2. This numerical assignment permitted the evaluation of linear associations between the breeding lines and the physiological indicators. The climatic conditions of the agricultural year 2024-2025 were evaluated using climatic data provided by the local meteorological station Brasov-Ghimbav, belonging to the National Meteorological Administration.

RESULTS AND DISCUSSIONS

The 2024-2025 agricultural year was warmer than the multiannual average (MAT), especially during winter, and was marked by a significant water deficit during both winter and growing periods. This combination of high temperature and rainfall deficit indicated unfavourable agrometeorological conditions for achieving optimal potato crop yields, requiring stress mitigation solutions, such as the use of new climate-resilient genotypes in cultivation. During the growing season (April - September), an average temperature of 16.1°C was recorded, slightly warmer than the MAT (15.6°C), with a positive deviation of 0.5°C and a deficit in precipitation, with an amount of 361.9 mm compared to the MAT of 420.6 mm, resulting in a deficit of 58.7 mm. To measure the potential and resilience of potato breeding lines, we used dynamic measurement of NDVI and SPAD indices, thus gaining insight into the efficiency with which plants transform light energy into organic matter. Under optimal vegetation conditions, plants show maximum absorption of solar radiation for photosynthesis and high infrared reflectance. Measurements carried out in June revealed an average NDVI index value of 0.89 for the genotypes under selection, with a variation between 0.84 and 0.92. Since NDVI values close to +1.0 indicate maximum green leaf density and optimal photosynthetic vigor, these results confirm that the evaluated genetic material presented a good vegetative state at the time of data acquisition. All genotypes under selection manifested a health state and a photosynthetic vigor superior to the Castrum control (0.84) at the date of evaluation. Most genotypes fell within the range 0.89-0.90,

significantly exceeding the NDVI index value of the control. The highest values were achieved by the genotypes 2151/10, 25-1901/6, 1940/4 and 1901/12. Only two genotypes, 2008/5 and 2011/5 (0.86), although slightly higher than the control, were close to the control index value, but in general there were no genotypes clearly inferior in terms of NDVI index. At the August data acquisition, the average NDVI index was 0.88. The NDVI index ranged between 0.81 (genotype 1979/4) and 0.92 (genotype 2011/5). Although the values are still high, they were generally lower compared to June. The control Castrum recorded a value of 0.92, placing itself among the genotypes with the best vigor at this late date. Genotypes 2011/5 (0.92) and 25-1901/6, 1979/5, 1897/2 (0.91) showed the highest vigor, indicating later maturity. Genotypes such as 2008/5 and 1979/4 (both with NDVI index 0.81) recorded significantly lower values, implying more advanced senescence and susceptibility to the thermo-hydric stress factor characteristic of August. Compared to the June assessment, although some genotypes maintained or even slightly increased the value (such as the Castrum control), a large part of the genotypes that had very high values in June (>0.90) recorded a slight decrease or stagnation in August. This is normal, because in mid-August, the potato usually enters the senescence phase, where the leaves start to lose chlorophyll, decreasing the red absorption and implicitly the NDVI value. The genotype 2151/10, which was the leader in June (0.92), decreased to 0.89 in August, while genotypes such as 2011/5 took over the leading position. This dynamic denotes differences in the precocity of maturation and in the resistance of the genotypes to stress. The fact that the Castrum control increased from 0.84 to 0.92 is an unexpected result from the point of view of senescence, given its classification in the semi-early maturity group, but given the vegetation period of 110-120 days (Hermeziu et al., 2015), in June it was in an accelerated growth phase. In summary, the transition from June to August reflects a specific dynamic of the vegetation curve of each genotype, highlighting the genotypes that maintain an active green leaf surface, i.e. photosynthetic vigor for longer, an important character for the continuous accumulation of biomass.

SPAD index measurements, which indicate the relative chlorophyll content and thus the photosynthetic capacity, showed a good general vigor of the genotypes at the end of June 2025. Most genotypes showed SPAD values between 43.2 and 51.7, respectively an active chlorophyll content specific to the intensive growth phase. The highest chlorophyll concentrations were recorded by the genotypes 1979/4 (51.7), 2008/4 (51.6) and 2002/5 (50.3). These genotypes demonstrated a superior photosynthetic efficiency at this date. The Castrum control recorded a SPAD value of 47.9 units. There were genotypes that showed SPAD values below or equal to those of the control. The lowest values were observed at 2151/7 (43.2), 1927/3 (44.0) and 2165/2 (44.5), therefore a slower development compared to the NDVI index, which was high for most. When taking SPAD data in August, a high variability in chlorophyll content was observed comparable to the June records, reflecting differences in the rate of maturation and senescence. SPAD index values ranged between 38.8 (genotype 2151/10) and 54.3 (genotype 2018/1). The range is wider than in June, which led to the highlighting of genotypes that had already entered senescence from those that maintained a high chlorophyll concentration. The highest concentrations were shown by the genotypes 2018/1 (54.3), 1979/5 (53.4) and 2021/2 (52.9). These genotypes behave as later genotypes. The Castrum control recorded a SPAD value of 47.2, a value similar to the 47.9 SPAD units in June. Genotypes with values below 40.0 (2151/10 at 38.8, 2165/6 at 39.8, 2027/1 at 39.2) show advanced senescence at the cellular level.

Statistical interpretation of the results

In Tables 1 and 2 it is observed that the results of the analysis of variance (ANOVA) confirm that there are significant differences between the potato breeding lines studied for both indices (NDVI and SPAD).

Although both observation data provide statistically valid results ($p < 0.05$), the measurements performed in August provide a much greater discrimination power (higher F values), being the most relevant moment for the ranking and selection of genotypes according to vegetative vigor and chlorophyll content.

Table 1. Analysis of variance for SPAD index values

Breeding line	SPAD (23.06.2025)			SPAD (14.08.2025)		
	Value	Difference	Significance	Value	Difference	Significance
1979/4	51.66	3.76	*	52.69	5.51	*
2008/4	51.64	3.74	*	50.90	3.72	*
2002/5	50.32	2.42	*	40.10	-7.08	o
24-1895/4	50.23	2.33	*	47.46	0.28	*
2151/10	50.19	2.29	*	38.76	-8.42	oo
2008/5	49.46	1.56	*	45.27	-1.91	o
2011/4	49.20	1.30	*	50.41	3.23	*
2018/1	49.02	1.12	*	54.27	7.09	*
2027/1	48.83	0.93	*	39.16	-8.02	oo
25-1901/6	48.79	0.89	*	48.03	0.85	*
2021/3	48.64	0.74	*	52.62	5.44	*
2021/2	48.30	0.40	*	52.92	5.74	*
2008/3	47.94	0.04	*	48.56	1.38	*
1968/1	47.73	-0.17	o	47.00	-0.18	o
1979/5	47.69	-0.21	o	53.40	6.22	*
2011/5	47.57	-0.33	o	52.00	4.82	*
1940/4	47.41	-0.49	o	46.19	-0.99	o
1901/12	47.29	-0.61	o	44.84	-2.34	o
2008/2	47.26	-0.64	o	47.64	0.46	*
1901/11	46.91	-0.99	o	52.13	4.95	*
2008/1	45.87	-2.02	o	43.67	-3.51	o
2002/4	45.88	-2.02	o	41.38	-5.80	o
1930/3	45.71	-2.19	o	48.46	1.28	*
1897/2	45.13	-2.77	o	42.32	-4.86	o
2165/6	44.69	-3.21	o	39.76	-7.42	o
2165/2	44.54	-3.36	o	44.44	-2.74	o
1927/3	44.09	-3.81	o	41.04	-6.14	o
2151/7	43.23	-4.67	o	47.68	0.50	*
CASTRUM (Ct)	47.90			47.18		
DL 5%		0.28		DL 5%		0.37
DL 1%		5.95		DL 1%		7.84
DL 0,1%		7.69		DL 0,1%		10.12

Table 2. Analysis of variance for NDVI index values

Breeding line	NDVI (23.06.2025)			NDVI (14.08.2025)		
	Value	Difference	Significance	Value	Difference	Significance
1979/4	0.89	0.046	**	0.81	-0.112	ooo
2008/4	0.89	0.046	**	0.87	-0.050	oo
2002/5	0.90	0.063	***	0.90	-0.017	oo
24-1895/4	0.89	0.046	**	0.88	-0.040	oo
2151/10	0.92	0.076	***	0.89	-0.033	oo
2008/5	0.86	0.020	*	0.81	-0.111	ooo
2011/4	0.87	0.029	*	0.90	-0.016	oo
2018/1	0.90	0.062	***	0.85	-0.068	ooo
2027/1	0.90	0.059	***	0.88	-0.036	oo
25-1901/6	0.91	0.068	***	0.91	0.068	***
2021/3	0.89	0.051	**	0.87	-0.052	oo
2021/2	0.87	0.033	*	0.89	-0.032	oo
2008/3	0.88	0.044	**	0.89	-0.027	oo
1968/1	0.89	0.046	**	0.85	-0.069	ooo
1979/5	0.89	0.054	**	0.91	-0.010	oo
2011/5	0.86	0.016	*	0.92	-0.004	oo
1940/4	0.91	0.070	***	0.84	-0.083	ooo
1901/12	0.91	0.071	***	0.89	-0.028	oo
2008/2	0.88	0.043	*	0.87	-0.047	oo
1901/11	0.89	0.053	**	0.87	-0.048	oo
2008/1	0.89	0.054	**	0.85	-0.074	ooo
2002/4	0.90	0.062	***	0.87	-0.054	oo
1930/3	0.89	0.051	**	0.88	-0.037	oo
1897/2	0.88	0.040	*	0.91	-0.011	oo
2165/6	0.89	0.046	**	0.88	-0.042	oo
2165/2	0.90	0.057	**	0.85	-0.070	ooo
1927/3	0.90	0.058	***	0.89	-0.029	oo
2151/7	0.88	0.036	*	0.90	-0.024	oo
CASTRUM (Ct)	0.84			0.92		
DL 5%		0.002		DL 5%		0.002
DL 1%		0.044		DL 1%		0.045
DL 0,1%		0.057		DL 0,1%		0.058

In the Pearson correlation tables, the link between the breeding lines and their physiological performance was evaluated. Thus, there is a significant positive correlation ($r=.166$, $p=.028$) between the genotype and the SPAD index (Table 3), but the Pearson coefficient value of .166 is considered a weak correlation. That is, although the genetic influence exists and is certain, there are other factors that contribute to the total variation of the SPAD index.

Table 3. Correlations between genotype and SPAD index

Correlations			
SPAD	Pearson Correlation	1	.166*
	Sig. (2-tailed)		.028
	N	174	174
Genotype	Pearson Correlation	.166*	1
	Sig. (2-tailed)	.028	
	N	174	174

*. Correlation is significant at the 0.05 level (2-tailed).

There is a significant negative correlation ($r=-.224$, $p=.003$) of weak to medium intensity between genotype and NDVI index (Table 4), but since the correlation is significant at the 0.01 threshold, the link is stronger than in the case of SPAD. Thus, the NDVI index proves to be a more suitable selection tool than the SPAD index to differentiate potato breeding lines.

Table 4. Correlations between genotype and NDVI index

Correlations			
Genotype	Pearson Correlation	1	-.224**
	Sig. (2-tailed)		.003
	N	174	174
NDVI	Pearson Correlation	-.224**	1
	Sig. (2-tailed)	.003	
	N	174	174

**.. Correlation is significant at the 0.01 level (2-tailed).

The standard deviation, an indicator of the variability of measurements within the same genotype, detected the homogeneous breeding lines with high stability from the heterogeneous lines with high variability. Thus, according to the SPAD index (Figure 1), genotypes with short error bars (e.g. 1968/1, 2165/2) are stable and have a uniform manifestation, while genotypes such as 1979/4, 1979/5, 2011/4 (in June) or

2018/1 (in August) present wide error bars, which denotes a greater sensitivity to environmental conditions.

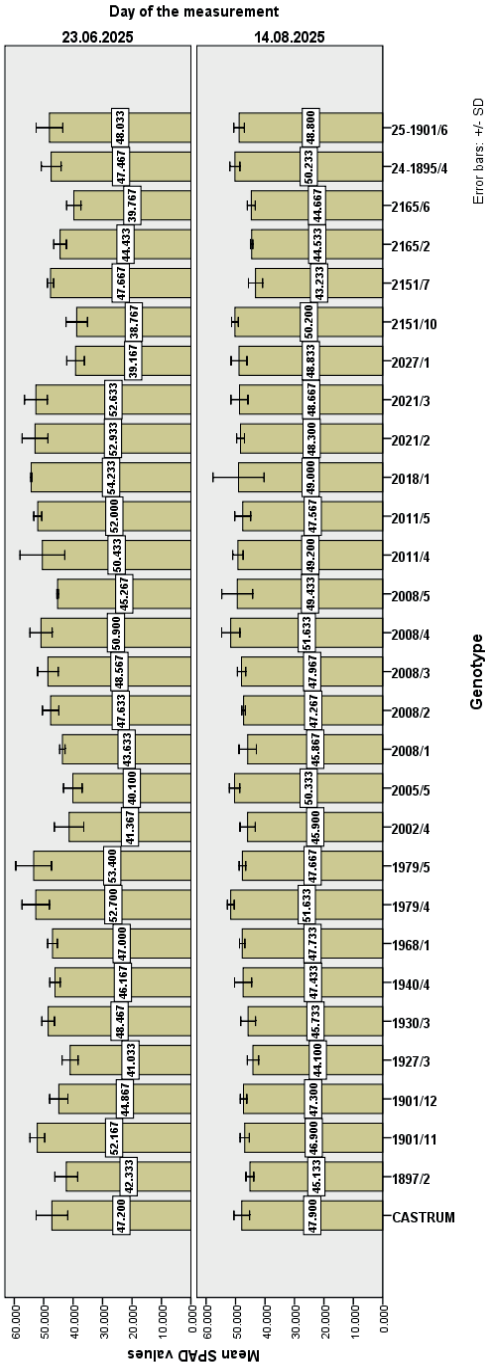


Figure 1. Standard deviation of the SPAD index



Figure 2. Standard deviation of the NDVI index

From the analysis of the stability and uniformity of the NDVI index measurements (Figure 2),

phenotypic canopy stability is observed, given by the small error bars in most genotypes, especially in August, but genotypes such as 1940/4 are also distinguished that had a non-uniform response to environmental conditions, observed by the increase in the standard deviation.

CONCLUSIONS

Modelling and identifying new potato genotypes with climate resilience is the most versatile way to respond to climate change.

The analysis of vegetation indices contributed to the identification of differences in the aging process of potato genotypes, for selection.

The data highlighted genotypes with discrepancies between biomass (NDVI) and cellular health (SPAD), indicators of early senescence processes.

As a global dynamic from June to August 2025, the August data acquisition statistically proved to be more beneficial for discrimination between genotypes.

Thus, the genotype 2151/10 stood out through rapid senescence despite still reasonable leaf cover (NDVI 0.89) becoming one of the genotypes with the lowest chlorophyll concentration (38.8 SPAD units), respectively a potential new early potato variety.

Genotypes such as 2011/5 and 1979/5 maintained or reached values higher than the control in both NDVI and SPAD in August. This indicates a well-maintained photosynthetic capacity throughout the season and a late maturity.

For the selection of future new potato varieties, it is necessary to monitor the efficient conversion into tubers.

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RESEARCH ON THE INFLUENCE OF SOWING TIME ON THE HEIGHT OF CORN GROWN IN THE CARACAL AREA

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Abstract

Six Corteva hybrids placed in three FAO groups (300-350, 350-450, 450-500): P 8834, P9398, P9889, P9944, P0450, P0710, were sown on four different dates, two weeks apart, on the chernozem of Caracal in 2024 and 2025. The aim of the paper was to establish the influence of the sowing date on the height as well as to carry out the distribution of yields according to the height of the plants but also to the earliness groups. The distribution of the height according to the FAO groups was also established. The height was influenced by the sowing period, the ascending order of the height values being period I, period II, period IV and period III. The distribution of yield by height showed that the most productive hybrids were placed in the 190-200 cm and over 200 cm height groups. Regardless of the sowing date, among the tested hybrids, the P0710 hybrid from the FAO 450 group was the most productive - 7087 kg/ha.

Key words: corn, sowing season, chernozem, height, yield.

INTRODUCTION

Corn (*Zea mays*) is one of the most important and versatile cereals cultivated worldwide, ranking second among the most cultivated plants in the world. Originally from Central America, it has become a central pillar of modern agriculture due to its high yield capacity and remarkable adaptability to diverse climatic conditions (Guzzon et al., 2021; Tanumihardjo et al., 2020).

In the context of a growing global population, advances in genomics and biotechnology are key to ensuring food security, providing the tools needed to develop highly productive and resilient corn hybrids (De Souza and Bonciu, 2022a, 2022b). One such character is plant architecture, largely defined by the height of the stem. This is closely linked to photosynthetic efficiency, with a direct relationship between the growing season in cereal species and the area of the standard leaf through production.

Agrotechnical research indicates that the sowing time exerts a direct influence on the height of corn, acting through the thermal and photoperiod regime in the early stages of vegetation (Domokos et al., 2024; Shibuskhov et al., 2021).

Sowing at the early stage (when the soil temperature reaches 8-10°C) often favors a larger plant height. Lower temperatures in early spring extend the vegetative growth period, allowing the accumulation of a larger biomass before panicle emergence (Walne and Reddy, 2022).

Delaying sowing usually causes a decrease in plant height. The rapid increase in temperatures in May-June accelerates the passage of phenological stages, shortening the time available for internode elongation.

Although plant height is a genetic trait, its phenotypic expression is strongly influenced by environmental factors and technological management, especially the sowing time. The latter dictates how the plant goes through critical stages of development, influencing vegetative vigor and, implicitly, the role of the flag leaf in cereal production, as the main source of assimilates for filling the grain (Rădoi et al., 2022, 2025). Moreover, the quantity and especially the nutritional profile of the harvest are directly influenced by the conditions of the plant's development, with subsequent ramifications on changes in food quality during storage (Bonciu et al., 2022).

Early hybrids tend to be less influenced in terms of height by the sowing date compared to late hybrids, which can significantly reduce their height if sown outside the optimal window (Varma et al., 2014). If late sowing coincides with periods of drought during the straw elongation phase (intense growth), the plant height remains reduced, directly affecting the leaf area (Oner, 2024).

The aim of this work is to evaluate the impact of the sowing time on the height of corn plants, under the specific pedoclimatic conditions of Caracal area, Romania.

MATERIALS AND METHODS

Six hybrids of company Corteva placed in three FAO groups (300-350, 350-450, 450-500): P 8834, P9398, P9889, P9944, P0450, P0710, were sown on 4 different dates, two weeks apart, on the chernozem of Caracal in 2024 and 2025. The first era was placed in mid-March (second decade), the second in late March (third decade), the third in mid-April (second decade) and the last in late April (third decade). The bifactorial experience was carried out in subdivided plots where factor A was the hybrid with 6 graduations and factor B – the sowing period with 4 graduations. Since the corn sown

in the first period in 2025 did not sprout, it was excluded from both years of experimentation and reporting was carried out compared to the second period (control).

The soil on which the experiment was located was the argic chernozem from Caracal, a soil characterized by a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing to 1.24% in the B horizon. It is medium supplied in total nitrogen, with 0.172% in Ap, very well supplied in Ap with mobile phosphorus (168 ppm) and well supplied with mobile potassium (248 ppm).

The technology applied was conventional and consisted of sowing the crop at a distance of 0.7 m between rows, with a density of 64,000 plants/ha. Fertilization was carried out with NPK 20:20:0 fertilizers in a quantity of 250 kg/ha when preparing the seedbed and with an input of 250 kg/ha of ammonium nitrate in vegetation. Herbicide was applied pre-emergent with Dual Gold in a dose of 1.5 l/ha and post-emergent with Elumis in a dose of 2 l/ha.

The climatic conditions for the two years highlighted a surplus of 40.97 mm compared to the multi-year average for March–September 2024 (Table 1) and a deficit of 105 l/m² during the corn growing season in 2025 (Table 2).

Table 1. Climate data for the corn growing season in 2024

Specification		III	IV	V	VI	VII	VIII	IX	Total vegetation period
Temperature °C	Monthly Average	7.7	10.7	15.0	21.4	25.8	25.8	21.1	
	NORMAL	6	12	17.7	21.6	22.8	23.5	18.1	
	Difference ±	+1.7	-1.3	-2.7	-0.2	+3.0	+2.3	+3.0	
Rainfall mm	Monthly Total	20.8	68.8	78.6	44.4	120	40.8	21	394.4
	Multi-year average	34.2	47.8	58.6	69.7	52.9	50.7	39.6	353.5
	Difference±	-13.4	+21.0	+20.0	-25.3	+67.1	-10.1	-18.6	

Table 2. Climate data for the corn growing season in 2025

Specification		III	IV	V	VI	VII	Average/Total vegetation period
Temperature oC	Monthly Average	9.3	12.5	16.0	24.0	23.9	17.1
	NORMAL	6.0	12.0	17.7	21.6	22.8	16.0
	Difference ±	+3.3	+0.5	-1.7	+2.4	+ 1.1	+ 1.1
Rainfall mm	Monthly Total	19.0	22.0	104.6	21.8	0.0	167.4
	Multi-year average	34.2	47.8	58.6	69.7	62.1	272.4
	Difference±	-15.2	-25.8	+46.0	-47.9	- 62.1	- 105.0

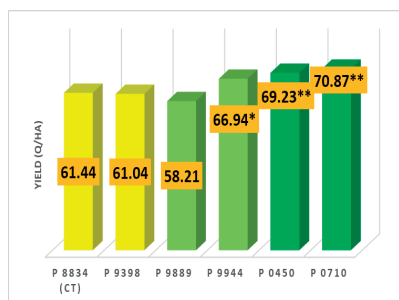
The size, insertion height, yield, and moisture content were determined for all variants and in all repetitions. The latter was used to calculate the yield at STAS moisture content, which is 14% for the corn crop.

The results were interpreted using statistical calculations for two-factor experiments and distributions.

RESULTS AND DISCUSSIONS

The results obtained were interpreted in terms of the singular influence of the studied characters (height, cob insertion height, yield), the influence of the interaction hybrid x sowing time for the same characters and their distributions.

In terms of the yields obtained, the hybrids P9944, P0450, P0710 stood out, the last two from the FAO 400-450 group, the increases being statistically assured in relation to the hybrid P8834 - an early hybrid (FAO 300-350) (Figure 1).

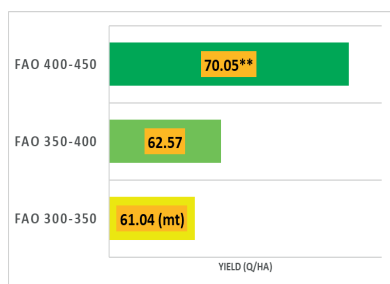


DL 5%=4.64 q/ha; DL 1%=6.60 q/ha; DL 0.1%=9,56 q/ha

Figure 1. Yield of tested corn hybrids, regardless of sowing time – Caracal 2024-2025

The minimum average yield of the experiment was recorded by the hybrid P8834 sown at the end of April (50.84 q/ha) and the maximum average, by the hybrid P0710 sown at the end of March (75.65 q/ha), practically a month in advance.

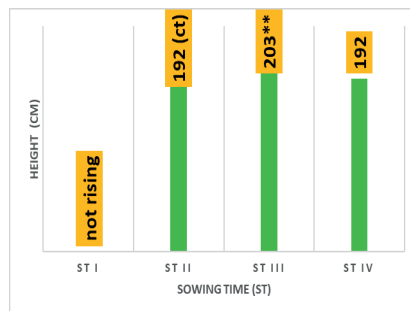
The yield increase obtained by hybrids classified as late under Caracal conditions was distinctly significant compared to the yield of hybrids in the FAO 300-350 group (Figure 2).



DL 5%=4,64 q/ha; DL 1%=6,60 q/ha; DL 0,1%=9,56 q/ha

Figure 2. Yield of tested corn hybrids, depending on classification in FAO groups – Caracal 2024+2025

The plants sown in the first period in 2025 did not sprout. As a result, the reporting was made on the average values obtained in the second period. In terms of height, regardless of the hybrid tested, the height in the third period was distinctly significantly higher than the one recorded in corn sown in mid-April (Figure 3).

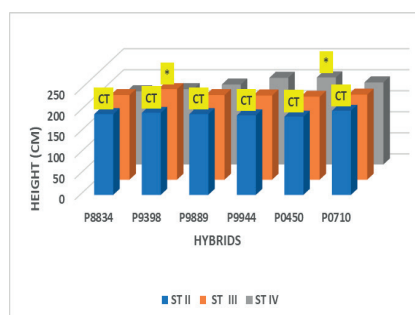


DL 5%=8 cm; DL 1%=11 cm; DL 0.1%=15 cm

Figure 3. Height of tested corn hybrids, depending on sowing period – Caracal 2024-2025

The interaction between the hybrid and the sowing period highlighted the fact that it influenced height to a small extent. Significant increases in height were recorded for the P9398 hybrid in period III and for the late P0450 hybrid sown in period IV (Figure 4).

Some studies show that although the dominant factor for height is the genotype (hybrid), its interaction with the sowing period can produce significant variations, depending on the climatic conditions of the year (Branković-Radojčić et al., 2017; Rajičić et al., 2025).



DL 5%=20 cm; DL 1%=27 cm; DL 0.1%=36 cm

Figure 4. Hybrid x sowing time interaction through the perspective of height – Caracal 2024-2025

Other results suggest that early corn hybrids are not the best choice for Southeast Europe in the

following years, as they are more sensitive to heat waves than late-maturing ones (Buhiniček et al., 2021).

The sowing time also influenced the cob insertion height. As in the case of height, there was a distinct increase of 6 cm in period III compared to period II (Figure 5).

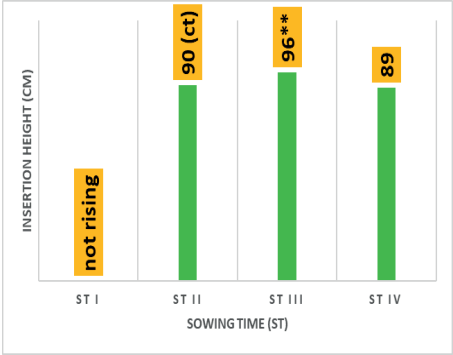


Figure 5. Insertion height of tested corn hybrids, depending on sowing period – Caracal 2024+2025.

The interaction between hybrid and sowing time had a minor influence on the trait studied. Only hybrid P9889 showed a significant increase in insertion height when sown in mid-April compared to sowing period II (end of March) (Figure 6).

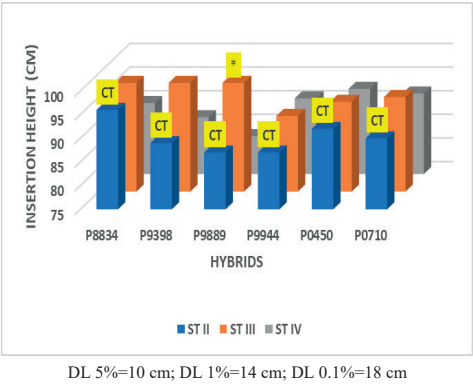


Figure 6. Hybrid x sowing time interaction through the perspective of corn insertion height – Caracal 2024+2025

Yield was also influenced by the sowing period, with the increase in yield in period III being very significant compared to the yield of the variant sown two weeks earlier. At the end

of April, the yield obtained was practically equal to that in period II (sown one month earlier) (Figure 7).

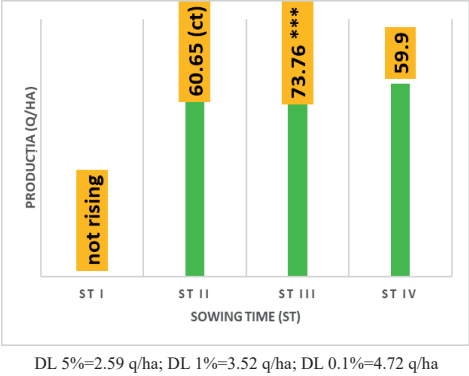


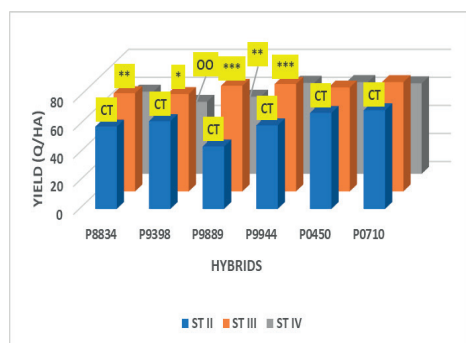
Figure 7 Yield of tested corn hybrids, depending on sowing period– Caracal 2024-2025

Some studies show that continuously adapting the sowing period and maturity of hybrids can counteract negative climate effects, even allowing for increased yields. In both France and China, studies indicate a favorable trend toward replacing early or medium varieties with late ones as temperatures rise (Butler et al., 2013; Caubel et al., 2018; Liu et al., 2013; Moore et al., 2014).

On the other hand, according to the study by Maryam and colleagues (2011), it was found that sowing at the optimal time favors higher cob weight compared to late sowing. Dekhane and Dumbre (2017) reached the same conclusion following experiments conducted on sweet corn.

The interaction between hybrid and sowing time greatly influenced yield. In the third sowing period, four of the hybrids showed statistically significant increases (those in the FAO 300-350 and 350-400 groups). Both hybrids belonging to the FAO 350-400 group recorded the highest increases, which were very significant (Figure 8).

The height distribution showed that the largest share of its values fell in the category over 201 cm (40.8%) but the highest average yield was recorded in the group 191-200 cm – 68.44 q/ha. A higher plant height allows for stronger growth and nutrition capable of developing larger cobs that are the basis for obtaining a higher yield (Figure 9).



DL 5%=6.34 q/ha; DL 1%=8.63 q/ha; DL 0.1%=11.56 q/ha

Figure 8. Hybrid x sowing time interaction through the perspective of yield – Caracal 2024-2025

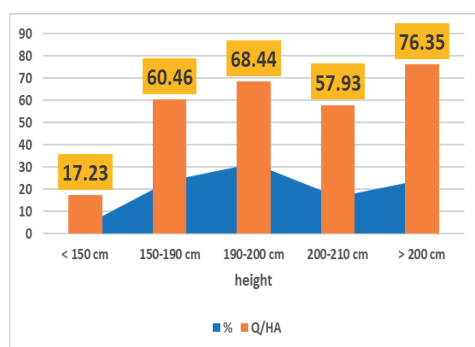


Figure 9. Height distribution of tested hybrids and related yields – Caracal 2024-2025

As the results in the literature show, the correlation between plant height, growth rate and grain yield in corn shows significant variations depending on the time of monitoring and environmental conditions (Anderson et al., 2019; Arslan et al., 2022; Sweet et al., 2025).

CONCLUSIONS

Sowing season influenced the height, insertion height and yield of the hybrids tested on the chernozem of Caracal.

The interaction between hybrid and sowing time highlighted the fact that it influenced the height, but only to a small extent.

The most productive hybrids were those in the FAO 400-450 group sown in mid-April.

The distribution of yield (yield) according to height showed that the most productive hybrids were placed in the 190-200 cm and over 200 cm height groups.

The distribution of yield according to the earliness groups showed that the hybrids placed in the FAO 350-450 and FAO 450-500 groups were more productive compared to the hybrids in the FAO 300-350 group.

The minimum yield of the experiment was recorded by the P8834 hybrid sown at the end of April (50.84 q/ha) and the maximum by the P0710 hybrid sown at the end of March (75.65 q/ha), practically a month in advance.

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TECHNOLOGICAL AND BIOCHEMICAL PARAMETERS OF *Triticum durum* INFLUENCED BY NPK FERTILIZATION

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Abstract

The aim of the study was to track the change in the values of some technological and biochemical parameters under the influence of prolonged application of synthetic nitrogen, phosphorus and potassium. The experiment was conducted in the experimental field of the Field Crops Institute-Chirpan, Bulgaria. The study showed data from 2020/2024. The results of the study showed that meteorological conditions strongly influenced the biochemical traits and to a small extent on the technological parameters. Nitrogen fertilization had a major impact on the technological and biochemical traits of durum wheat compared to phosphorus and potassium fertilizers. High nitrogen rates strongly influenced biochemical traits, while moderate fertilization was more effective for technological parameters. Average for the test period, fertilization with N₈₀P₁₂₀ increased thousand kernel weight (47.1 g) to the greatest extent. Combined fertilization with N₄₀P₄₀ had the strongest effect on test weight (78.5 kg/ha). Grain vitreousness and protein content were highest under the influence of N₁₆₀ (75.6% and 15.2%). Gluten content was maximized by fertilization with N₁₆₀P₄₀ (32.3%).

Key words: biochemical traits, nitrogen, phosphorus, potassium, technological traits, *triticum durum*.

INTRODUCTION

Globally, durum wheat ranks second in cultivation after bread wheat. It is one of the few cereals intended exclusively for human consumption (Samson et al., 2023). In many regions around the world, it is of paramount importance for feeding the population. In the Western world, durum wheat is used to make pasta, while in the East it is used to make couscous, bulgur and freekeh. Due to the higher yields per unit area, durum wheat was inferior in production to bread wheat. Of the total wheat production areas in the world, durum wheat accounts for only 8% (Mariam et al., 2024). Despite the advantages, nitrogen fertilizers has a high financial cost, and its synthesis, transport, and application in the field demand a considerable amount of energy derived from fossil fuels (Dragov, Taneva and Bozhanova, 2021).

The three most commonly required and widely used nutrients in agriculture are nitrogen (N), phosphorus (P), and potassium (K) (Dhillon et al., 2019). Farmers invariably resort to the use of traditional nitrogen, phosphorus, and potassium fertilizers (NPK fertilizers) worldwide to achieve high crop yields

(Amoakwah et al., 2024). Conventional fertilizer use efficiency have not improved significantly over the years, with N, P and K efficiencies hardly exceeding 30-35%, 18-20%, and 35-40%, respectively (Kumar et al., 2023). The N, P and K fertilizer application rates need to be optimized to reduce the nitrate residue in the soil while maintaining a high wheat yield (He et al., 2024). Phosphorus is one of the most widely occurring nutrients for development and growth of wheat (Boukhalfa-Deraoui et al., 2020). Phosphorus plays many key functions in plant life especially in the storage and transfer of energy, photosynthesis, respiration, cell division, and enlargement (Shafi et al., 2020). Without adequate amount application of P, a plant cannot reach its maximum yield potential (Desta, 2022). However, in recent decades, the excessive application of phosphate fertilizer in the soil in pursuit of higher crop yields has raised some environmental problems (Liu et al., 2024). For this reason, improvement in phosphorus management is of high priority (Kominko, Gorazda and Wzorek, 2019). Potassium promotes diseases resistance, regulates water balance, strengthens stalks, build proteins, regulates respiration, stretches water, and acts as a disease retarder, but it is

ineffective without nutrients like nitrogen and phosphorus (Kumar et al., 2024).

The most important traits for the milling industry are the vitreous body of the grain and the protein and gluten content of the grain. Basically, the protein content in the grain primarily depends on the nitrogen content in the soil (Lalevic et al., 2022). Nitrogen is material basis for the synthesis of wheat protein (Noor et al., 2023). Nitrogen fertilizers have a significant effect on the protein content of wheat grain and as a consequence, on wheat prices (Klikocka and Szczepaniak, 2023). White endosperm is typically found in starchy kernels with low protein content (Lachutta and Jankowski, 2024). N fertilization improves grain filling (by reducing empty spaces and decreasing the content of α -amylase), delays ripening, and prolongs grain dormancy (Dargie, Wogi and Kidanu, 2020). It is a key component of enzymes and structural proteins, nucleic acid, pigments and a wide range of secondary metabolites (Ivanova-Kovacheva et al., 2024). Variation in quantity and quality of the gluten-forming proteins has been shown to greatly affect wheat end-use quality (Rathan et al., 2020). Therefore, intensive production technologies based on the extensive use of fertilizers (Sulek et al., 2023). Despite the advantages, nitrogen fertilizers have a high financial cost, and its synthesis, transport, and application in the field demand a considerable amount of energy derived from fossil fuels (Monteiro et al., 2024). Nitrogen fertilization has become one of the main criticisms of modern agriculture in society (Neuweiler et al., 2021).

For the evolution of grain yield, thousand kernel weight (TKW) is an important parameter, being used in research as a measurement indicator (Temneanu and Marghitas, 2019). It is strongly influenced by environmental conditions and food supplies (Mohsin et al., 2014). On the other hand, the key features of grains that strongly influence yield are the good kernel size, high test weight (TW) and high percentage of hard vitreous kernels (Sissons, 2022). For these reasons, increasing TKW and TW are main targets in wheat breeding, in addition to total yield (Desiderio et al., 2019).

As the population grows, farmers are expected to increase their final output, while at the same time requiring higher quality of produce. To

achieve this increase, farmers have been applying unnecessarily large amounts of synthetic fertilizers for years. The forecasts currently do not look optimistic but at the same time, a reduction of fertilizer use by at least 20% is expected by 2030 (Orsini et al., 2023).

This study aimed to track the change in the values of some technological and biochemical parameters under the influence of prolonged application of synthetic nitrogen, phosphorus and potassium.

MATERIALS AND METHODS

The experiment was conducted in the experimental field of the Field Crops Institute-Chirpan, Bulgaria (42°11'58"N, 25°19'27"E). The experiment represented a sample of a multi-year fertilizer experiment that began in 1966. This study showed data from 2020/2024. Sowing of durum wheat was carried out in November with the *Saya* variety.

Four rates of nitrogen and phosphorus fertilizer were tested – 40, 80, 120 and 160 kg/ha and one rate of potassium fertilizer – 80 kg/ha. N, P and K fertilizers were applied individually in the appropriate doses and in combinations: N40P40, N80P40, N120P40, N160P40, N40P80, N80P80, N120P80, N160P80, N40P120, N80P120, N120P120, N160P120, N40P160, N80P160, N120P160, N160P160, N₁₂₀P₁₂₀K₈₀, respectively. A variant without fertilization was adopted as the control. P (triple superphosphate), K (potassium sulfate) and 1/3 of N (ammonium nitrate) were incorporated before sowing, and 2/3 of N in the spring – in the tillering phase.

For a period of 5 years, the 1000-grain weight (TKW), test weight (TW), grain vitreous (GV), protein content (PC) and gluten content (GC) in the grain were monitored.

To establish reliable differences between the variants (at a probability level of $P \leq 0.01$), the data were subjected to analysis of variance (ANOVA) for each year separately and the average value of the years of the study.

Table 1 showed the sum of temperature and precipitation over the vegetative period of durum wheat and multi-year period. The observed growing seasons were significantly higher than the multi-year period in terms of temperatures. The period 2023/24 had the

largest sum of temperature – 2815.3°C, with the difference with the multi-year period being 805.6°C. The lowest accumulated sum of

temperature was 2021/22 - 2226.0°C. For the Chirpan region, the wheat requirement is around 1865°C.

Table 1. Sum of temperature (°C) and precipitation (mm) over the vegetative period of durum wheat and multi-year period (1928/2013)

Years/mounts	XI	XII	I	II	III	IV	V	VI	sum
Sum of temperature, °C									
2019/20	335.5	110.9	44.6	154.0	256.9	314.0	515.9	614.9	2346.7
2020/21	197.4	181.1	99.3	125.7	161.7	309.4	524.4	616.4	2215.4
2021/22	237.2	120.4	57.1	117.6	130.4	367.3	537.0	659.0	2226.0
2022/23	304.1	165.5	169.5	184.3	262.4	332.8	474.6	627.9	2521.1
2023/24	278.7	120.3	80.0	303.4	306.0	463.8	496.0	767.1	2815.3
1928/2013	215.9	61.1	-6.2	49.4	188.9	357.9	511.5	630.7	2009.7
Precipitation, mm									
2019/20	82.4	21.6	1.5	55.5	67.4	62.2	50.3	62.6	403.5
2020/21	7.4	70.4	108.6	25.8	39.1	84.0	34.9	42.8	413.0
2021/22	14.2	108.8	21.4	40.1	22.4	36.0	29.4	80.5	352.8
2022/23	46.9	44.4	120.0	0.2	34.4	68.2	54.8	69.5	438.4
2023/24	45.2	75.1	44.8	5.2	31.4	51.4	59.7	24.8	337.6
1928/2013	47.3	54.0	44.3	37.7	37.0	45.2	64.1	65.4	395.0

In this respect, the closest was the period 2021/22. The higher temperatures had provided the plants with good conditions for entering the winter period with well-tillering plants. In the ripening phase, temperature sums above 500-600°C help accumulated a greater amount of dry matter in the grain. Precipitation for the study period was close to that of the multi-year period, but unevenly distributed. The exception was 2023/24, where precipitation was significantly lower (337.6 mm). During this period, the wheat had passed the ripening phase with extremely insufficient soil moisture (24.8 mm). However, the 5 observed years met the soil moisture requirement during the growing season for about 280-380 mm.

RESULTS AND DISCUSSIONS

Table 2 showed TKW for each of test years and average for the test period. TKW varied between 41.7-50.4 g in different years and 44.7-47.1 g on average for the period. Higher values were observed in 2021, where the highest TKW (50.4 g) was recorded from fertilization with N₈₀P₁₂₀. However, the difference with N₈₀P₈₀ was only 0.2 g. In 2020, N₁₆₀P₁₂₀ had the strongest effect (48.9 g), 2022 - N₁₂₀P₁₂₀ (47.1 g), 2023 - N₈₀P₁₆₀ (48.6 g) и 2024 -K₈₀ (49.5 g). The application of N and P alone showed a moderate increase. The implementation of K in 2024 was impressive. This year, the K₈₀ variant was the only one with a confirmed impact by ANOVA. NPK

fertilization did not have a strong impact in two of the years studied (2020 and 2021) and on average for the study. Moderate fertilization with NP fertilizers showed the best results. Mussarat et al. (2021) reported that increasing the rate of independently applied nitrogen and phosphorus fertilizers leads to a decrease in values. The authors reported that N₄₀ had a TKW of 37.36 g, while N₁₆₀ had a TKW of 33.12 g, and P₆₀ had a TKW of 38.16 g and P₉₀ had a TKW of 36.37 g. In a study by Chetan et al. (2024), it was reported that NP fertilization significantly increased TKW by increasing N rate – N₃₆P₉₂ – 32.89 g; N₁₀₈P₉₂ – 33.98 g (ns) and N₁₄₁P₉₂ – 34.63 g (*) in spring wheat. A study by Obaid et al. (2019) reported the positive influence of P on TKW in bread wheat. Increasing the norm to P₉₀ shows the highest value, and with the next increase (P₁₀₅) TKW decreases.

Table 3 showed TW for each of test years and average for the test period. TW varied between 65.4-81.7 kg/hl in different years and 75.6-78.6 kg/hl on average for the period. Only in 2020 did mineral fertilization have a significant effect and all variants have a confirmed effect. In 2024, all values were lower than the unfertilized control. A similar trend was observed by Allende-Montalban et al. (2024). The authors reported the highest TW from the unfertilized control (80.6 kg/hl). Under the influence of N fertilizer, the values decreased – N₂₇ – 79.3 kg/hl and N₅₄ – 78.7 kg/hl, respectively.

Table 2. Thousand kernel weight (g) for each of test years and average for the test period

Fertilization	2020	2021	2022	2023	2024	Average
N ₀ P ₀ K ₀	43.0	45.7	41.7	44.2	48.7	44.7
N ₄₀	44.6***	47.7***	42.6*	46.4***	46.1 ^{ns}	45.5 ^{ns}
N ₈₀	46.3***	46.0 ^{ns}	42.6*	45.9***	47.2 ^{ns}	45.6 ^{ns}
N ₁₂₀	45.3***	46.4 ^{ns}	43.7***	44.1 ^{ns}	44.9 ^{ns}	44.9 ^{ns}
N ₁₆₀	46.8***	46.6 ^{ns}	43.4***	45.0**	43.4 ^{ns}	45.0 ^{ns}
P ₄₀	43.0 ^{ns}	44.6 ^{ns}	41.8 ^{ns}	46.5***	48.9 ^{ns}	45.0 ^{ns}
P ₈₀	43.6 ^{ns}	45.2 ^{ns}	42.1 ^{ns}	45.2**	47.5 ^{ns}	44.7 ^{ns}
P ₁₂₀	43.9*	48.8***	42.4 ^{ns}	43.7 ^{ns}	47.1 ^{ns}	45.2 ^{ns}
P ₁₆₀	48.7***	46.0 ^{ns}	42.2 ^{ns}	44.3 ^{ns}	48.8 ^{ns}	46.0 ^{ns}
N ₄₀ P ₄₀	46.7***	47.6***	43.0**	46.9***	45.7 ^{ns}	46.0 ^{ns}
N ₈₀ P ₄₀	45.6***	48.4***	43.1**	45.8***	45.7 ^{ns}	45.7 ^{ns}
N ₁₂₀ P ₄₀	46.3***	45.4 ^{ns}	44.1***	43.6 ^{ns}	46.1 ^{ns}	45.1 ^{ns}
N ₁₆₀ P ₄₀	44.2**	46.0 ^{ns}	44.7***	44.1 ^{ns}	44.9 ^{ns}	44.8 ^{ns}
N ₄₀ P ₈₀	47.8***	48.5***	43.0**	47.6***	47.0 ^{ns}	46.8*
N ₈₀ P ₈₀	47.5***	50.2***	43.6***	46.3***	45.4 ^{ns}	46.6 ^{ns}
N ₁₂₀ P ₈₀	44.7***	46.6*	45.2***	45.1**	44.0 ^{ns}	45.1 ^{ns}
N ₁₆₀ P ₈₀	44.4***	45.2 ^{ns}	45.0***	44.4 ^{ns}	44.7 ^{ns}	44.7 ^{ns}
N ₄₀ P ₁₂₀	45.9***	48.8***	44.4***	45.5***	45.9 ^{ns}	46.1 ^{ns}
N ₈₀ P ₁₂₀	44.6***	50.4***	46.3***	46.9***	45.3 ^{ns}	46.7*
N ₁₂₀ P ₁₂₀	44.2***	47.1***	47.1***	44.1 ^{ns}	45.2 ^{ns}	45.5 ^{ns}
N ₁₆₀ P ₁₂₀	48.9***	46.1 ^{ns}	46.5***	43.8 ^{ns}	43.5 ^{ns}	45.8 ^{ns}
N ₄₀ P ₁₆₀	48.4***	49.3***	44.4***	45.9***	46.8 ^{ns}	47.0*
N ₈₀ P ₁₆₀	48.4***	47.8***	45.9***	48.6***	45.0 ^{ns}	47.1*
N ₁₂₀ P ₁₆₀	44.4***	47.5***	45.3***	45.9***	46.3 ^{ns}	45.9 ^{ns}
N ₁₆₀ P ₁₆₀	45.0***	46.6*	45.4***	43.5 ^{ns}	46.4 ^{ns}	45.4 ^{ns}
K ₈₀	44.1**	44.4 ^{ns}	42.5*	45.7***	49.5*	45.2 ^{ns}
N ₁₂₀ P ₁₂₀ K ₈₀	48.3***	47.2***	46.8***	44.2 ^{ns}	44.5 ^{ns}	46.2 ^{ns}
LSD	5%	0.8	0.8	0.6	0.8	2.0
	1%	1.1	1.1	1.1	1.1	2.7
	0,1%	1.4	1.4	1.5	1.4	3.4

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

Table 3. Test weight (kg/hl) for each of test years and average for the test period

Fertilization	2020	2021	2022	2023	2024	Average
N ₀ P ₀ K ₀	74.8	76.0	79.1	78.8	81.7	78.1
N ₄₀	75.5***	75.9 ^{ns}	80.0***	78.8 ^{ns}	80.0 ^{ns}	78.0 ^{ns}
N ₈₀	77.3***	76.7***	77.9 ^{ns}	80.0***	79.5 ^{ns}	78.3 ^{ns}
N ₁₂₀	77.6***	75.4 ^{ns}	78.0 ^{ns}	78.3 ^{ns}	78.5 ^{ns}	77.6 ^{ns}
N ₁₆₀	77.3***	74.7 ^{ns}	76.7 ^{ns}	76.5 ^{ns}	78.3 ^{ns}	76.7 ^{ns}
P ₄₀	77.4***	76.4***	79.8**	78.5 ^{ns}	80.9 ^{ns}	78.6 ^{ns}
P ₈₀	76.6***	75.4 ^{ns}	79.5 ^{ns}	78.7 ^{ns}	80.4 ^{ns}	78.2 ^{ns}
P ₁₂₀	76.9***	75.1 ^{ns}	79.1 ^{ns}	78.5 ^{ns}	80.2 ^{ns}	78.0 ^{ns}
P ₁₆₀	76.7***	75.8 ^{ns}	79.6*	78.8 ^{ns}	81.0 ^{ns}	78.4 ^{ns}
N ₄₀ P ₄₀	78.8***	75.6 ^{ns}	79.2 ^{ns}	78.5 ^{ns}	80.2 ^{ns}	78.5 ^{ns}
N ₈₀ P ₄₀	77.2***	75.6 ^{ns}	78.5 ^{ns}	78.8 ^{ns}	79.8 ^{ns}	78.0 ^{ns}
N ₁₂₀ P ₄₀	76.1***	75.3 ^{ns}	79.2 ^{ns}	78.0 ^{ns}	78.9 ^{ns}	77.5 ^{ns}
N ₁₆₀ P ₄₀	76.1***	74.5 ^{ns}	77.7 ^{ns}	77.0 ^{ns}	78.6 ^{ns}	76.8 ^{ns}
N ₄₀ P ₈₀	78.0***	75.6 ^{ns}	78.9 ^{ns}	78.8 ^{ns}	79.9 ^{ns}	78.2 ^{ns}
N ₈₀ P ₈₀	77.5***	76.0 ^{ns}	78.7 ^{ns}	78.8 ^{ns}	78.3 ^{ns}	77.9 ^{ns}
N ₁₂₀ P ₈₀	76.7***	74.7 ^{ns}	78.8 ^{ns}	78.3 ^{ns}	78.1 ^{ns}	77.3 ^{ns}
N ₁₆₀ P ₈₀	75.9***	73.9 ^{ns}	78.2 ^{ns}	76.0 ^{ns}	78.5 ^{ns}	76.5 ^{ns}
N ₄₀ P ₁₂₀	78.1***	75.8 ^{ns}	79.1 ^{ns}	78.8 ^{ns}	79.1 ^{ns}	78.2 ^{ns}
N ₈₀ P ₁₂₀	76.4***	75.9 ^{ns}	79.0 ^{ns}	78.8 ^{ns}	79.3 ^{ns}	77.9 ^{ns}
N ₁₂₀ P ₁₂₀	77.0***	75.7 ^{ns}	78.7 ^{ns}	78.3 ^{ns}	79.5 ^{ns}	77.8 ^{ns}
N ₁₆₀ P ₁₂₀	76.0***	75.0 ^{ns}	78.3 ^{ns}	77.0 ^{ns}	77.2 ^{ns}	76.7 ^{ns}

Fertilization	2020	2021	2022	2023	2024	Average
N ₄₀ P ₁₆₀	78.1***	75.3 ^{ns}	79.4 ^{ns}	79.0 ^{ns}	79.3 ^{ns}	78.2 ^{ns}
N ₈₀ P ₁₆₀	77.5***	75.9 ^{ns}	79.6*	79.0 ^{ns}	79.6 ^{ns}	78.3 ^{ns}
N ₁₂₀ P ₁₆₀	76.7***	75.1 ^{ns}	78.2 ^{ns}	78.5 ^{ns}	79.3 ^{ns}	77.6 ^{ns}
N ₁₆₀ P ₁₆₀	76.4***	75.3 ^{ns}	78.5 ^{ns}	77.5 ^{ns}	78.8 ^{ns}	77.3 ^{ns}
K ₈₀	77.1***	75.9 ^{ns}	65.4 ^{ns}	79.3***	80.5 ^{ns}	75.6 ^{ns}
N ₁₂₀ P ₁₂₀ K ₈₀	78.0***	75.6 ^{ns}	77.9 ^{ns}	78.3 ^{ns}	79.3 ^{ns}	77.8 ^{ns}
LSD	5%	0.3	0.11	0.5	0.3	1.7
	1%	0.4	0.14	0.6	0.4	2.3
	0.1%	0.5	0.19	0.8	0.5	3.0

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

Over the years of the study, a trend towards a stronger influence of low N-P rates in the range of 40-80 kg/ha was observed. In three of the years studied (2021, 2022, 2023), nitrogen application alone showed the best results, N₈₀ – 76.7 kg/hl, N₄₀ – 80.0 kg/hl и N₈₀ – 81.7 kg/hl, respectively. The results in this study confirmed the results of De Santis et al. (2024), who published data from two locations on the impact of N fertilization on TW. The authors reported that in both locations the rate of 80 kg N ha⁻¹ had the strongest effect compared to N₉₀ and the unfertilized control, but the differences were small. Eshetu and Tafes (2025) observed the response of TW to increasing N rates from 0 to 115 and reported little difference between the variants, while Chadda et al. (2025) reported the highest values from the unfertilized control (80.7 kg/hl).

A large variation in GV was observed over the years of the study (Table 4). Which showed that GV was determined by environmental growth conditions (Lullien-Pellerin, 2020). The data ranged between 18.2-96.2%. 2021 had the lowest values, which can be explained by the lower sum of temperatures compared to other years. 2024 had values typical for Bulgarian durum wheat varieties. The highest sum of temperatures and low rainfall during grain ripening this year resulted in vitreousness above 80% of all variants, reaching 96.2% of N₁₆₀P₈₀. Overall, all variants were influenced by the higher fertilization rate 160 kg N ha⁻¹. This trend was also maintained in the average values. A similar pattern was reported by Bozek et al. (2022). In a study by Bogole et al. (2017), a gradual increase in GV was observed with increasing N rate, with the highest rate (N₁₇₅) having the highest value– 89.9%. Compared to N, the action of P alone had no effect on the values. This result was in contradiction with the claim of Panayotova et al. (2017) that increasing

P by 80 and 160 kg ha⁻¹ led to an increase in durum wheat vitreousness by 5.4% and 13.6%, respectively.

Regarding protein content, the values over the years varied between 9.0-16.4% (Table 5). The P and K application had little or no effect. Although PC is a varietal characteristic, nitrogen fertilization had a confirmed influence. The rate of 160 kg/ha had the strongest effect on the values, both alone and with NP and NPK fertilization. A number of authors have reported the positive impact of nitrogen (Ayadi et al., 2022; Pop et al., 2024). For example, Pop et al. (2024) reported that the unfertilized control was 10.63%, and the highest PC was the highest rate - N₁₂₀ - 15.40%. The results of phosphorus fertilization also matched. The authors reported a weakly demonstrated increase with P₈₀ fertilization (13.68%). Other authors, such as Chen et al. (2022), reported minimal increases under the influence of N fertilizer. The authors reported a PC of 16.3% when fertilizing with N₆₅ and an increase of only 0.5% (16.8%) when fertilizing with N₁₃₅.

Over the years of the study, GC varied widely – 15.0-36.2% (Table 6). Gluten, as the main protein component, was mainly affected by nitrogen fertilization and less by P and K, which confirmed the results of Jastrebska et al. (2024) that phosphorus had no significant effect on GC. Increasing the N rate led to an increase in the values, whether in combination or alone. The dose of N₁₆₀ affected GC the most. During the years of the study, the variant with the highest value was the N₁₆₀P₄₀ (2020) with a GC of 36.2%, and on average for the tested period – 32.3% of the same variant. A similar trend was reported by Kizilgeci et al. (2021) and Liu et al. (2021).

Conversely, Abera et al. (2020) reported that GC increases to the norm from N₁₂₂, with values decreasing as it increases to N₁₈₃.

Table 4. Grain vitreous (%) for each of test years and average for the test period

Fertilization	2020	2021	2022	2023	2024	Average
N ₀ P ₀ K ₀	58.8	18.2	50.2	39.2	85.8	50.4
N ₄₀	61.2	28.6	67.2	38.8	91.6	57.5 ^{ns}
N ₈₀	68.0	43.4	77.0	52.6	94.4	67.1 ^{**}
N ₁₂₀	74.8	52.6	77.8	65.2	93.2	72.7 ^{***}
N ₁₆₀	66.8	70.2	80.2	66.0	95.0	75.6^{***}
P ₄₀	52.0	20.8	51.0	42.4	84.6	50.2 ^{ns}
P ₈₀	69.0	25.0	47.2	35.6	81.8	51.7 ^{ns}
P ₁₂₀	56.2	29.0	46.8	35.0	83.2	50.0 ^{ns}
P ₁₆₀	53.8	23.8	45.6	40.2	86.8	50.0 ^{ns}
N ₄₀ P ₄₀	54.2	35.6	69.8	34.0	93.2	57.4 ^{ns}
N ₈₀ P ₄₀	70.2	50.8	74.2	52.8	93.0	68.2 ^{***}
N ₁₂₀ P ₄₀	73.2	57.6	75.8	59.8	90.2	63.3 ^{**}
N ₁₆₀ P ₄₀	80.2	57.2	77.6	70.0	92.4	75.5^{***}
N ₄₀ P ₈₀	54.0	26.2	60.6	38.2	88.8	53.6 ^{ns}
N ₈₀ P ₈₀	65.8	40.6	72.8	47.0	94.2	64.1 ^{**}
N ₁₂₀ P ₈₀	70.8	52.8	77.0	62.0	93.8	71.3 ^{***}
N ₁₆₀ P ₈₀	68.2	56.8	80.6	62.8	96.2	72.9^{***}
N ₄₀ P ₁₂₀	57.0	29.0	64.0	37.6	91.6	55.8 ^{ns}
N ₈₀ P ₁₂₀	60.2	30.8	69.6	43.6	91.4	59.1 ^{ns}
N ₁₂₀ P ₁₂₀	73.4	49.0	72.6	62.8	94.4	70.4 ^{***}
N ₁₆₀ P ₁₂₀	78.0	56.0	76.4	68.2	92.4	74.2 ^{***}
N ₄₀ P ₁₆₀	58.2	31.2	59.2	38.4	92.8	56.0 ^{ns}
N ₈₀ P ₁₆₀	61.0	39.8	71.0	46.8	93.8	62.5 [*]
N ₁₂₀ P ₁₆₀	73.2	44.4	76.2	63.4	94.6	70.4 ^{***}
N ₁₆₀ P ₁₆₀	74.2	52.6	78.8	65.0	94.0	72.9 ^{***}
K ₈₀	55.0	34.0	48.0	38.8	82.8	51.7 ^{ns}
N ₁₂₀ P ₁₂₀ K ₈₀	76.8	54.2	81.6	60.6	93.0	73.2 ^{***}
LSD	5%					9.2
	1%					12.2
	0,1%					15.8

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

Table 5. Protein content (%) for each of test years and average for the test period

Fertilization	2020	2021	2022	2023	2024	Average
N ₀ P ₀ K ₀	10.9	10.4	11.6	9.7 ^{ns}	11.6	10.8
N ₄₀	13.9 ^{***}	11.0 ^{***}	12.8 ^{***}	9.5 ^{ns}	13.1 ^{***}	12.1 ^{**}
N ₈₀	13.7 ^{***}	12.8 ^{***}	15.0 ^{***}	11.7 ^{***}	15.3 ^{***}	13.7 ^{***}
N ₁₂₀	15.1 ^{***}	13.2 ^{***}	16.0 ^{***}	12.9 ^{***}	16.2 ^{***}	14.7 ^{***}
N ₁₆₀	14.0 ^{***}	14.7^{***}	16.3 ^{***}	14.7^{***}	16.3^{***}	15.2^{***}
P ₄₀	11.6 ^{***}	9.9 ^{ns}	11.5 ^{ns}	10.0 [*]	11.6 ^{ns}	10.9 ^{ns}
P ₈₀	11.7 ^{***}	10.5 ^{ns}	10.9 ^{ns}	9.7 ^{ns}	10.6 ^{ns}	10.7 ^{ns}
P ₁₂₀	11.5 ^{**}	11.1 ^{***}	11.1 ^{ns}	9.0 ^{ns}	12.6 ^{***}	11.1 ^{ns}
P ₁₆₀	11.2 ^{ns}	10.4 ^{ns}	11.1 ^{ns}	9.4 ^{ns}	11.3 ^{ns}	10.7 ^{ns}
N ₄₀ P ₄₀	11.1 ^{ns}	11.4 ^{***}	13.1 ^{***}	8.9 ^{ns}	13.6 ^{***}	11.6 [*]
N ₈₀ P ₄₀	13.0 ^{***}	12.5 ^{***}	14.6 ^{***}	10.6 ^{***}	14.9 ^{***}	13.1 ^{***}
N ₁₂₀ P ₄₀	14.8 ^{***}	13.6 ^{***}	16.0 ^{***}	13.0 ^{***}	15.1 ^{***}	14.5 ^{***}
N ₁₆₀ P ₄₀	14.2 ^{***}	13.9 ^{***}	16.4^{***}	13.9 ^{***}	15.1 ^{***}	14.7 ^{***}
N ₄₀ P ₈₀	11.1 ^{ns}	10.3 ^{ns}	12.5 ^{***}	9.2 ^{ns}	13.3 ^{***}	11.3 ^{ns}
N ₈₀ P ₈₀	12.7 ^{***}	12.3 ^{***}	14.4 ^{***}	10.3 ^{***}	14.9 ^{***}	12.9 ^{***}
N ₁₂₀ P ₈₀	14.7 ^{***}	12.8 ^{***}	15.3 ^{***}	12.5 ^{***}	15.7 ^{***}	14.2 ^{***}

Fertilization	2020	2021	2022	2023	2024	Average
N ₁₆₀ P ₈₀	15.9***	13.7***	16.0***	13.4***	15.6***	14.9***
N ₄₀ P ₁₂₀	11.3*	10.6*	12.9***	9.8 ^{ns}	13.9***	11.7*
N ₈₀ P ₁₂₀	12.1***	10.5 ^{ns}	13.8***	10.4***	14.8***	12.3***
N ₁₂₀ P ₁₂₀	14.9***	12.4***	14.7***	12.5***	15.1***	13.9***
N ₁₆₀ P ₁₂₀	16.0***	13.3***	16.3***	13.3***	15.9***	15.0***
N ₄₀ P ₁₆₀	11.4**	10.9***	12.6***	9.5 ^{ns}	13.5***	11.6*
N ₈₀ P ₁₆₀	12.2***	10.7**	13.8***	10.5***	14.6***	12.3***
N ₁₂₀ P ₁₆₀	15.0***	11.8***	15.2***	12.1***	14.7***	13.8***
N ₁₆₀ P ₁₆₀	15.3***	13.0***	15.5***	13.2***	14.9***	14.4***
K ₈₀	11.2 ^{ns}	10.6*	11.1 ^{ns}	9.7 ^{ns}	11.3 ^{ns}	10.8 ^{ns}
N ₁₂₀ P ₁₂₀ K ₈₀	14.9***	12.9***	14.9***	11.9***	15.4***	14.0***
LSD	5%	0.4	0.2	0.3	0.1	0.8
	1%	0.5	0.3	0.3	0.2	1.1
	0,1%	0.7	0.4	0.4	0.5	1.4

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

Table 6. Gluten content (%) for each of test years and average for the test period

Fertilization	2020	2021	2022	2023	2024	Average
N ₀ P ₀ K ₀	22.4	19.6	22.3	17.2	22.1	20.7
N ₄₀	23.3 ^{ns}	21.5***	25.6***	16.5 ^{ns}	26.9***	22.8 ^{ns}
N ₈₀	29.6***	26.6***	31.3***	22.0***	30.3***	28.0***
N ₁₂₀	34.2***	27.8***	34.5***	26.2***	32.6***	31.1***
N ₁₆₀	30.3***	30.0***	35.8***	31.1***	32.5***	31.9***
P ₄₀	23.2 ^{ns}	20.2 ^{ns}	20.8 ^{ns}	17.9*	22.9***	21.0 ^{ns}
P ₈₀	30.1***	21.4***	20.2 ^{ns}	16.6 ^{ns}	20.0 ^{ns}	21.8 ^{ns}
P ₁₂₀	22.1 ^{ns}	22.5***	20.7 ^{ns}	15.0 ^{ns}	24.3***	20.9 ^{ns}
P ₁₆₀	21.7 ^{ns}	22.2***	20.7 ^{ns}	16.2 ^{ns}	21.7 ^{ns}	20.5 ^{ns}
N ₄₀ P ₄₀	21.4 ^{ns}	22.8***	26.6***	14.4 ^{ns}	26.2***	22.3 ^{ns}
N ₈₀ P ₄₀	27.1***	20.8***	31.0***	19.4***	30.1***	26.6***
N ₁₂₀ P ₄₀	33.4***	21.1***	34.0***	25.8***	30.6***	30.4***
N ₁₆₀ P ₄₀	36.2***	21.7***	35.8***	29.1***	30.5***	32.3***
N ₄₀ P ₈₀	21.2 ^{ns}	25.4***	25.0***	15.3 ^{ns}	26.5***	21.8 ^{ns}
N ₈₀ P ₈₀	26.1***	25.1***	29.9***	18.4***	30.6***	26.0***
N ₁₂₀ P ₈₀	32.7***	21.3***	32.5***	24.3***	31.3***	29.5***
N ₁₆₀ P ₈₀	31.5***	21.6***	34.7***	27.5***	31.6***	30.9***
N ₄₀ P ₁₂₀	21.8 ^{ns}	28.2***	25.7***	17.1 ^{ns}	27.4***	22.6 ^{ns}
N ₈₀ P ₁₂₀	22.4 ^{ns}	26.9***	28.2***	18.8***	29.6***	24.1*
N ₁₂₀ P ₁₂₀	33.1***	25.3***	31.3***	24.9***	30.2***	29.0***
N ₁₆₀ P ₁₂₀	36.2***	23.4***	35.2***	27.7***	31.7***	31.8***
N ₄₀ P ₁₆₀	20.5 ^{ns}	29.8***	24.2***	15.3 ^{ns}	26.4***	21.6 ^{ns}
N ₈₀ P ₁₆₀	24.6***	29.2***	28.6***	18.7***	29.8***	24.7***
N ₁₂₀ P ₁₆₀	33.9***	28.0***	32.2***	24.3***	29.9***	28.4***
N ₁₆₀ P ₁₆₀	34.7***	26.3***	33.1***	27.3***	30.5***	30.4***
K ₈₀	21.0 ^{ns}	21.3***	20.8 ^{ns}	17.1 ^{ns}	22.2 ^{ns}	20.5 ^{ns}
N ₁₂₀ P ₁₂₀ K ₈₀	33.1***	26.5***	31.5***	23.2***	30.4***	28.9***
LSD	5%	1.2	0.7	0.6	0.3	2.7
	1%	1.6	0.9	0.8	0.4	3.6
	0,1%	2.1	1.2	1.0	0.6	4.6

ns: no significant; *, **, *** significant at P=5%, P=1% and P=0.1%

CONCLUSIONS

The results of the study showed that meteorological conditions strongly influenced the biochemical indicators (protein and gluten content in the grain and vitreousness of the grain) and to a small extent on the technological parameters – thousand kernel weight and test weight. Nitrogen fertilization had a major impact on the technological and biochemical traits of durum wheat compared to phosphorus and potassium fertilizers. High nitrogen rates strongly influenced biochemical traits, while moderate fertilization was more effective for technological parameters. Fertilization with N₈₀P₁₂₀ increased thousand kernel weight (47.1 g) to the greatest extent. Combined fertilization N₄₀P₄₀ had the strongest effect on test weight (78.5 kg/hl). Grain vitreousness and protein content were highest under the influence of N₁₆₀ (75.6% and 15.2%, respective). Gluten content was maximized by fertilization with N₁₆₀P₄₀ (32.3%).

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BIOMASS PRODUCTIVITY OF THREE CORN HYBRIDS AFTER OPTIMIZING THE NUTRIENT AND IRRIGATION REGIME

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Abstract

A field experiment was conducted with three maize hybrids: DKC4416, LG 31.390 and Premeo under irrigated and non-irrigated conditions. The experiment was carried out during the period 2022-2024 at the Academic Technological Complex (ATC) of Trakia University, Stara Zagora, Bulgaria. The productivity of the green mass of the three maize hybrids was determined. After foliar fertilization with a complex of fertilizers Aminosol, Lebosol B, Lebosol Zn and Nutriplant 36, an increase of 22.8% (for LG 31.390), 24.4% (for DKC 4416) and 31.8% (for Premeo) was determined, on average for the three-year study period. The parameters of the additional yield of green mass have been calculated. Optimization of nutrients and irrigation regime contributes to increasing the values of additional yield by 276.8% (Premeo) to 353.5% (DKC 4416). An increase was also reported after applying the organic fertilizer Kinsidro Grow and the nitrogen stimulator N-Lock of 338.3% in DKC 4416, 318.6% in LG 31.390 and 276.8% in Premeo. The efficiency coefficient of irrigation water use has been established. The highest values were recorded for variant 3 with the highest coefficient calculated for DKC 4416 (40.0).

Key words: corn, irrigation, fertilization, yield, efficiency.

INTRODUCTION

Maize is one of the most widely grown and widely cultivated cereals in the world. According to data on world grain production in 2022/2023, maize has become the third most important cereal crop in the world after wheat and rice (Shahbandeh, 2024). The crop is ecologically plastic, but its responsiveness to optimizing available moisture has been proven. (Ivanova et al., 2021; Sharifnasab et al., 2023; Stoyanova A. et al. 2024b). Ecological plasticity and a short growing season are prerequisites for high productivity and versatile use (Popova et al., 2008; Stoyanova A. et al. 2024a). Nyirenda et al. (2021) add that for newly created hybrids, their higher yield and constant adaptability to changing environmental conditions are of high importance.

The impact of climate change trends can be predicted after studying the influence of these climate trends on crop yields. By developing statistical and mathematical models, the relationship between productivity and climate change can be found. Global climate change is associated with fluctuations in the amount and distribution of precipitation, which affects the

balance between plant needs for available moisture and moisture supply (Simić et al., 2023). Determining crop water consumption and irrigation water productivity is an important step towards developing more efficient systems for allocating limited water resources (Wang et al., 2022; Rank et al., 2023; Dimitrov, 2023). Research and updating technologies and policies can have a significant impact on more efficient use of water resources (Žarski et al., 2023). Against the backdrop of global warming and drought, Rank et al. (2023) believe that it is necessary to seek and implement water-saving technologies in agriculture, combined with fertilization, mulching and other approaches to reduce the water footprint of crops.

Managing plant nutrition technologies and optimizing the water regime is an important approach that is fundamental to every agricultural crop. Therefore, updating the irrigation and nutrient regime, aiming at a balance between the volume of irrigation water and the number of fertilizers applied, is a key factor in the use of these resources (Camp et al., 2003; Guo et al., 2022; Žarski et al., 2023). Drip irrigation can effectively improve the growth, yield, water use efficiency, leaf area index and

economic benefit of maize in northwest China reported Liu et al. (2023).

Foliar fertilization is an effective way to treat nutrient deficiencies, improve plant nutritional status, and help plants overcome periods of stress. Foliar biostimulants are an important factor in providing the opportunity to stimulate plant metabolism and improve plant defense mechanisms. Combined root and foliar application and fertigation methods significantly improve corn growth and yield (Khalafi et al., 2021; Fan et al., 2023; Stoyanov et al., 2024; Stoyanov, 2025; Stoyanov et al., 2025). Several researchers have reported on the positive effect of applying foliar nutrition to crops, analyzing the increase in biometric parameters in the three corn hybrids (Bagrintseva et al., 2023; Kovalenko et al., 2023). According to studies by Weisany et al. (2021) foliar application of Zn-Ch and Zn-NCh significantly reduces the adverse effects of drought stress. Other authors also support the thesis that micronutrient fertilization is an effective strategy for reducing the harmful effects of water stress in plants (Solanki, 2021; Umar et al., 2022).

Improving nutrient use efficiency and crop yield through improved nutrient and water management practices is an important approach to resource optimization (Noulas et al., 2023; Qin, 2024). Improving strategies for more effective and efficient use of irrigation water in agriculture is important for alleviating water scarcity and related environmental problems. According to Li et al. (2020) spatiotemporal review of agricultural water use provides guidance for improving irrigation water utilization ratio in China.

The aim of this study is to analyze the influence of irrigation and corn nutrition on the green mass productivity of three corn hybrids, as well as to establish the efficiency of irrigation and the productivity of irrigation water.

MATERIALS AND METHODS

To achieve the set goal, a field trial was conducted with the following corn hybrids: DKC4416 is a Bayer hybrid, from the FieldShield hybrids group; LG 31.390 is a hybrid from Limagrain, a representative of Hydraneo technology, and Premeo is a hybrid from Syngenta, from Artesian technology.

The field experiment was carried out at the Academic Technological Complex (ATC) of Trakia University, Stara Zagora, Bulgaria during the period 2022-2024. The study was conducted on a soil type typical of meadow-cinnamon soil in a fertilizer experiment under irrigated and non-irrigated conditions. The experiment was conducted using the fractional plot method, in 4 replications, with a crop plot size of 15 m².

After sowing, before the seed germinates, soil fertilization was carried out with N₁₄ (Ammonium nitrate). Nutrition during the growing season of the studied corn hybrids was carried out with organic multicomponent and single-component fertilizers. The influence of the foliar fertilizers Aminosol (AS), Lebosol B and Lebosol Zn, Nutriplant 36 (N36), Kinsidro Grow (KG) and N-Lock were tested. The field trial options include: 1. Control - fertilization with N₁₄ and no irrigation; 2. Fertilization with N₁₄ + irrigation; 3. Fertilization with N₁₄ + foliar feeding (Aminosol, Lebozol B and Lebosol Zn, Nutriplant 36) + with irrigation; 4. Fertilizing with N₁₄ + foliar feeding (Kinsidro Grow and N-Lock) + with irrigation. In case of lime. 3 feeding with Aminosol, Lebozol B and Lebosol Zn is applied in the 4-6 leaf phase, and with Nutriplant 36 it is treated in the 8-10 leaf phenophase. In lime. 4 the biostimulator Kinsidro Grow is applied in the phenophase 2-8th leaf, and the nitrogen stimulator N-Lock in the phenophase 3-6th leaf.

Irrigation was carried out with a drip irrigation system. In the first year, 4 irrigations were carried out, with an irrigation rate of 30 mm, and in the second and third, 6 and 5 irrigations were carried out, respectively, to maintain 75-80% FC (Field Capacity) in the soil layer 0- 0.80 m.

The irrigation water efficiency coefficient was calculated. The productivity of irrigation water in the studied hybrids was established. Irrigation water productivity is defined as the ratio between yield and irrigation rate.

Statistical analysis was performed with ANOVA.

RESULTS AND DISCUSSIONS

Climatic Conditions

The region in which the experimental field is located belongs to the European-continental climate zone. The years of the field study, in

terms of average daily temperatures, are characterized by significant differences from the measured temperatures compared to multi-year periods (Figure 1).

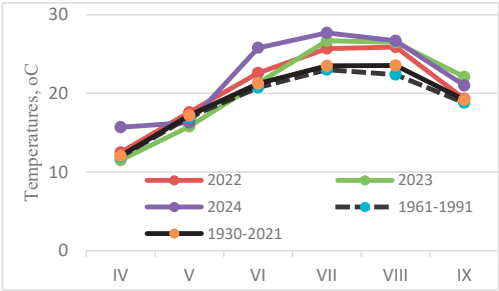


Figure 1. Dynamics of average daily temperatures for the period 2022-2024

The graph shows that the values during both applied multi-year periods are characterized by lower values. In the analysis, we apply two periods - one covers a multi-year period from 1930 to 2021, and the other period 1961-1991 is considered a representative climate norm by the National Institute of Meteorology and Hydrology (NIMH) - Bulgaria. The dynamics of the average daily temperature values by month for the corn growing period shows a permanent increase in temperatures compared to previous periods. On average for the study period, the average daily temperatures were higher by 12.1% (June), 16.1% (July) and 17.9% (August) compared to the average daily temperature for the period 1961-1991.

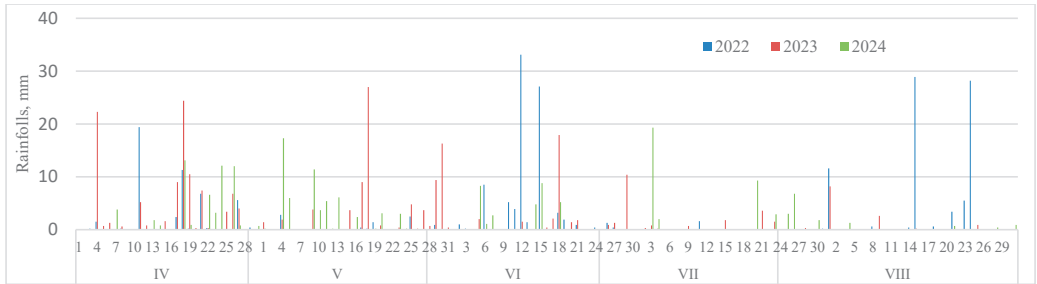


Figure 2. Amount and distribution of rainfall during the growing season of maize 2022-2024, the Stara Zagora region

In terms of precipitation, with a precipitation rate of 302.3 mm (1961-1991), 15.7% less precipitation was recorded in the first year, and 8.91% in the second year.

Table 1. Amount of precipitation for the growing season period

Years	IV	V	VI	VII	VIII	IX
2022	48.3	8.3	88.5	1.8	79.2	28.8
2023	98.6	83.6	39.7	9.2	11.9	32.5
2024	55.8	59.4	30.9	45.1	3.3	98.5
1961-1991	58.5	65.9	50.1	49.0	31.4	47.3
1930-2021	45.7	60.5	66.9	48.2	41.2	34.7

In 2024, the amount is close to the climate norm, only 3.1% less than the same for the period 1961-1991. The period of the field study is characterized by an uneven distribution of precipitation. In the first and second years, precipitation of 1.8 mm (2022) and 9.2 mm (2023) was recorded in July (Figure 2). The

uneven distribution is characteristic, which provides insufficient moisture for crop development. For normal growth and development of corn, moisture characteristics are optimized through irrigation.

Productivity of corn hybrids

The yields of the studied corn hybrids vary by year and treatment options under the influence of different moisture and nutrient availability. Experimental results for green mass yield show an increase in values after application of irrigation and foliar fertilization. Under conditions of natural moisture supply and without fertilization during the growing season, yields ranged from 18100.0 (DKC 4416) to 20166.7 kg/ha (Premeo), on average for the study period (Table 2).

In the variants without foliar fertilization, but under irrigation conditions, during the vegetation period, yields of 37133.0 kg/ha (LG 31.390), 39866.7 kg/ha (Premeo) and 43466.7 kg/ha (DKC 4416) were obtained in the first

experimental year. After foliar fertilization with the organic and mineral products Aminosol + Lebosol B + Lebosol Zn, Nutriplant 36, an increase in yields was found in the hybrids by 21.1% to 50.9%, respectively in Premeo and LG 31.390. In the second year, an increase in the productivity of the hybrids was observed after

the application of organic fertilizers and nitrogen stimulators Kinsidro Grow, N-Lock. The increase was 33.3% (Premeo), 38.6% (DKC 4416) and 43.7% (LG 31.390). The hybrid LG 31.390 stands out as the most responsive to foliar fertilization and to both fertilization technologies during the growing season.

Table 2. Green mass yield (kg ha⁻¹), for the three hybrids, for the period 2022-2024

Corn Hybrids	Variants	2022	2023	2024	2022-2024
DKC 4416	1. N ₁₄ , non-irrigation	14000.0	21800.0	18500.0	18100.0
	2. N ₁₄ with irrigation	43466.7	72648.9	55648.9	57254.8
	3. AS, B, Zn, N36	57267.0	100545.7	88455.4	82089.4
	4. N-Lock, KG	49133.3	100675.7	88172.9	79327.3
LG 31.390	1. N ₁₄ , non-irrigation	16400.0	22300.0	18700.0	19133.3
	2. N ₁₄ with irrigation	37133.0	70978.4	53974.2	54028.5
	3. AS, B, Zn, N36	56033.0	101137.5	85312.0	80827.5
	4. N-Lock, KG	53633.0	101976.5	84682.3	80097.3
Premeo	1. N ₁₄ , no- irrigation	17200.0	24100.0	19200.0	20166.7
	2. N ₁₄ with irrigation	39866.7	74774.2	60954.3	58531.7
	3. AS, B, Zn, N36	48273.3	102320.9	86826.0	79140.1
	4. N-Lock, KG	43533.3	99668.3	84785.5	75995.7
LSD. P <0.05		14620.9	4617.2	5088.7	7634.7
LSD. P <0.01		11850.53	7656.6	8438.5	10414.0
LSD. P <0.001		45342.4	14318.9	15781.1	14089.4
VC, %		0.393	0.449	0.468	0.440

After applying the first technology (variant 3), the increase in 2024 was 42.4% for Premeo, 58.15 for LG 31.390 and 59.0% for DKC 4416. Treatment with the products Kinsidro Grow, N-Lock (variant 4) contributed to an increase in yields by 39.1% for Premeo, 56.9% for LG 31.390 and 58.4% for DKC 4416. In the last year of the field study, a positive trend towards an increase in biomass yield parameters after treatment with foliar fertilization products was again established. Higher results were reported for variant 3, in which feeding during the growing season contributes to an increase in productivity of 42.5 (Premeo) to 58.9% (DKC 4416). The rates of increase in biomass yield after application of the foliar fertilizers Kinsidro Grow and N-Lock range from 39.1% (Premeo) to 58.5% (DKC 4416). The hybrid DKC 4416 proved to be extremely responsive to foliar fertilization in the last year.

The analysis of the results shows an increase in biomass productivity in the three corn hybrids studied. Yields when optimizing moisture in the soil horizon lead to an increase in the indicator. The most responsive, on average for the period, was the hybrid DKC 4416, which recorded an

increase of 216.3% compared to the non-irrigated variant. In the hybrids LG 31.390 and Premeo, an increase of 182.3% and 190.2% was recorded. After optimizing the nutritional and irrigation regime, a trend towards an increase in the productivity of the hybrids was reported after

The highest yield was found in DKC 4416 (82089.4 kg/ha), followed by LG 31.390 and Premeo. The application of the organic and mineral products Aminosol + Lebosol B + Lebosol Zn, Nutriplant 36 contributes to increasing productivity by 292.4% in Premeo, by 322.4% in LG 31.390 and by 353.5% in DKC 4416 compared to the non-irrigated and non-fertilized variant. The results after applying the organic fertilizer Kinsidro Grow and the nitrogen stimulator N-Lock are also high. An increase of 338.3% was recorded for DKC 4416, 318.6% for LG 31.390 and 276.8% for Premeo.

Irrigation water productivity

During the period of the field study, a different number of irrigations were carried out in different years. In 2022, 4 irrigations were carried out to maintain moisture in the soil

horizon. In 2023, 6 irrigations were implemented and 5 irrigations in 2024. The amount of the implemented irrigation rate varied over the years of the field study from 1200 mm to 1800 mm.

Table 3 presents the parameters of the additional green mass yield, which is formed under the influence of irrigation. Productivity values increased after irrigation in all three corn hybrids. The increase in green mass yield under the influence of the optimized irrigation regime is in the range of 34895.2 (LG 31.390) to 39154.8 kg/ha (DKC 4416). There is a trend towards an increase in additional yield after optimizing the irrigation and nutrient regime. The analysis of the results shows that after feeding with the organic fertilizers Aminosol, boron, zinc and N36, productivity is higher. The influence of complex fertilization with the products Kinsidro Grow and the nitrogen stimulator N-Lock is very well manifested in LG 31.390 and gives results similar to fertilization with the complex of fertilizers in variant 3. The

difference between the yield of green mass is only 730.2 kg/ha. More responsive to fertilization with the organic fertilizer Aminosol and the single-component boron and zinc and Nutriplant 36 are the hybrids DKC 4416 and Premeo.

Irrigation water productivity is an important factor in modern conditions, when water resources are limited and of high value. In the present study, the productivity of water was calculated for different fertilization and irrigation options (Table 3). Irrigation water productivity is defined as the ratio between yield and irrigation rate. Irrigation water productivity ranges from 33.8 to 54.9 kg/mm, on average over the study period. In the first year, it was found that the productivity of irrigation water had the lowest values, with an average for all variants of 43.4 kg/mm. The highest values were found in the variants fed with the complex of foliar fertilizers, the organic fertilizer Aminosol and the single-component boron and zinc and Nutriplant 36, in all three hybrids.

Table 3. Additional green mass yield, irrigation water productivity and effect of 100 m³ of irrigation water for the period 2022-2024

Corn Hybrids	Variant	Additional yield (AY), kg/ha				Irrigation water productivity (IWP), kg/mm				Effect of 100 m ³ of irrigation water, kg/ha			
		2022	2023	2024	2022-2024	2022	2023	2024	2022-2024	2022	2023	2024	2022-2024
DKC 4416	2	29466.7	50848.9	37148.9	39154.83	36.2	40.4	37.1	35.8	3622.2	4036.1	3709.9	3578.4
	3	43267	78745.7	69955.4	63989.37	47.7	55.9	59.0	51.3	4772.3	5585.9	5897.0	5130.6
	4	35133.3	78875.7	69672.9	61227.3	40.9	55.9	58.8	49.6	4094.4	5593.1	5878.2	4958.0
LG 31.390	2	20733	48678.4	35274.2	34895.2	30.9	39.4	36.0	33.8	3094.4	3943.2	3598.3	3376.8
	3	39633	78837.5	66612	61694.17	46.7	56.2	56.9	50.5	4669.4	5618.8	5687.5	5051.7
	4	37233	79676.5	65982.3	60963.93	44.7	56.7	56.5	50.1	4469.4	5665.4	5645.5	5006.1
Premeo	2	22666.7	50674.2	41754.3	38365.07	33.2	41.5	40.6	36.6	3322.2	4154.1	4063.6	3658.2
	3	31073.3	78220.9	67626	58973.40	40.2	56.8	57.9	49.5	4022.8	5684.5	5788.4	4946.3
	4	26333.3	75568.3	65585.5	55829.03	36.3	55.4	56.5	47.5	3627.8	5537.1	5652.4	4749.7

In the second year of the field experiment, the values ranged from 40.4 to 57.8 kg/mm, with an average for the year of 51.1 kg/mm. Again, the trend for higher values is observed for lime. 3. In 2024, the highest productivity results (60.0 kg/mm) of irrigation water were recorded for Premeo, variant 3. On average for all variants, the analyses show 51.6 kg/mm is the water productivity. Summarizing the results obtained, an average productivity of 46.1 kg/mm was established for the three hybrids over the three-

year study period. Establishing the productivity parameters of irrigation water is important in the construction of hydro-reclamation systems, Wang et al. (2022). The effect of 100 m³ of irrigation water is another important indicator by which the role of irrigation is determined. It is defined as the ratio between the additional yield and the irrigation rate. The conducted data analysis shows that the effect is highest in 2024. The average across variants was 5160.9 kg/ha. In the second year, 5113.6 kg/ha were

established, and in the first year 4335.8 kg/ha. In terms of fertilization options, a trend towards increasing the effect after foliar fertilizer application was observed in the three hybrids. After applying the second technology with foliar fertilization, the data show that this leads to a

higher effect than 100 m³ of irrigation water. After applying the organo-mineral fertilizer Kinsidro Grow and the nitrogen stimulator N-Lock, the effect is again high compared to lime. 2, in which no feeding was carried out during the vegetation period.

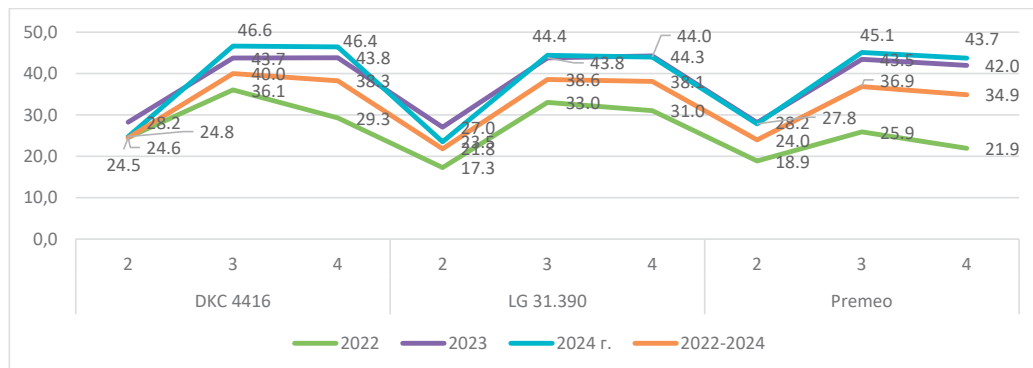


Figure 3. The irrigation efficiency coefficient for three corn hybrids for the period 2022-2024

Figure 3 presents the results of the calculated irrigation water efficiency coefficient (IWEC). The irrigation efficiency coefficient is defined as the ratio between the additional green mass yield and the amount of irrigation rate. The diagram shows that in the second and third years, more efficient use of irrigation water was registered, which generated higher additional yield, with indicator values, on average for the year, of 37.4 and 37.2, respectively. In the first year, lower indicator values were established, ranging from 17.3 to 36.3, with an average of 27.9 across variants. The IWEC values vary by variant, with a trend for the highest values in the three hybrids for variant 3 and all three hybrids.

CONCLUSIONS

The parameters of green mass productivity growth in three corn hybrids have been established. After foliar fertilization with the complex of fertilizers Aminosol, Lebosol B, Lebosol Zn and Nutriplant 36, an increase of 22.8% (for LG 31.390), 24.4% (for DKC 4416) and 31.8% (for Premeo) was found, on average for the three-year study period.

The parameters of the additional yield of green mass were calculated. Optimizing the nutrient and irrigation regime contributes to increasing the additional yield values by 276.8% (Premeo) to 353.5% (DKC 4416).

The efficiency coefficient of irrigation water use has been established. The highest values were registered for variant 3, with the highest coefficient calculated for DKC 4416 (40.0).

ACKNOWLEDGEMENTS

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THE USE OF URBAN SEWAGE SLUDGE IN AGRICULTURE – A SOURCE OF ORGANIC MATTER AND NUTRIENTS OR A SANITARY AND ENVIRONMENTAL RISK?

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Abstract

Urban sewage sludge generated by municipal wastewater treatment plants represents a resource for agricultural use due to its high content of organic matter and plant nutrients, particularly nitrogen and phosphorous. When it is applied as an organic soil amendment, sewage sludge can restore soil fertility, improve soil structure, stimulate biological activity and reduce dependence on mineral fertilizers. However, sewage sludge may also contain hazardous substances, including heavy metals, organic contaminants, micro plastics and pharmaceutical residues. Moreover, it can act as a vector for pathogenic microorganisms, such as bacteria, viruses and helminth eggs, posing risk to soil, water resources and public health. Consequently, agricultural reuse of sewage sludge is regulated by strict legislative frameworks defining permissible pollutant concentrations and requiring stabilization and hygienization before application. Sludge application must be carefully managed through appropriate dosing, crop selection, avoidance of fresh-consumed crops and regular monitoring of sludge and soil to ensure environmental and human health protection.

Key words: urban sewage sludge, sustainable agriculture, organic matter, nutrients, environmental risk.

INTRODUCTION

The increasing pressure on soil resources and the growing demand for mineral fertilizers have brought the use of sewage sludge (“urban sludge”) back into focus as a resource for agriculture. Sludge originating from urban wastewater treatment plants contains significant amounts of stabilized organic matter, nitrogen, phosphorus, and trace elements, which makes it a potential organic amendment and fertilizer with a role in increasing humus content, improving soil structure, and stimulating biological activity. Recent meta-analyses show significant increases in soil organic matter, nitrogen and phosphorus content, and certain micronutrients (Zn, Cu) following sludge application at controlled rates (Paganini et al., 2024; Li et al., 2024).

At the same time, sewage sludge is a concentrate of pollutants resulting from the mixture of domestic and industrial wastewater: heavy metals (Cd, Pb, Hg, Ni, Cr), persistent organic compounds, microplastics, pharmaceutical residues, endocrine disruptors, PFAS (“forever chemicals”), and other emerging contaminants (Council Directive 86/278/EEC, 1986; Mara et

al., 1989; Yakamercan et al., 2021; Frišták et al., 2024; Balkrishna et al., 2025; Chenu, 2025). In addition, sludge may contain a significant microbiological load: pathogenic bacteria, enteric viruses, protozoa, helminth eggs and larvae, which can survive for long periods in the environment and be transmitted to humans and animals through direct contact, aerosols, or the consumption of contaminated plant products (FAO, 1995; Jiménez et al., 2010; Amoah et al., 2018).

In the European Union, Directive 86/278/EEC on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture, establishes limit values for heavy metals in soil and sludge, as well as treatment requirements for pathogen reduction, in order to encourage the proper use of sludge and prevent harmful effects on soil, vegetation, animals, and human health (Council Directive 86/278/EEC, 1986; Baltic Water Hub, 2022). Some Member States have introduced additional regulations (codes of good practice, local protocols) that define the frequency of sludge and soil analyses and impose restrictions on crops intended for direct human consumption (Scottish Executive, 2002; US EPA, 2019).

On the other hand, there are also critical or restrictive positions. Some countries have completely banned the use of sludge in agriculture, opting instead for incineration and phosphorus recovery from ash, citing the risks associated with emerging contaminants and uncertainties regarding long-term effects on soil and the food chain (FAO, 1995; De Brouwere et al., 2006; Chenu, 2025).

Other analyses draw attention to documented cases of odors, discomfort, and possible illness in areas where insufficiently treated sludge was applied (Reilly, 2001; Yakameran et al., 2021). Against this background, this paper aims to synthesize recent bibliographic data regarding:

- the composition of urban sludge in terms of organic matter and nutrients;
- the content of pollutants and emerging contaminants;
- the load of parasite eggs, viruses, and other pathogens;
- the effects on agricultural yields and on product quality;
- the requirements and frequency of control analyses;
- to propose an evaluation and monitoring methodology for the controlled use of sludge in agriculture.

MATERIALS AND METHODS

The methodology is conceptual in nature and integrates recommendations from directives, international guidelines, and experimental studies (Council Directive 86/278/EEC, 1986; Scottish Executive, 2002; Navarro et al., 2010). A combined design is proposed, including sludge characterization studies, soil monitoring, and a factorial field experiment.

Sludge characterization. For each wastewater treatment plant, a quarterly sampling program of dewatered/stabilized sludge is carried out. The minimum recommended analyses include: dry matter, organic matter (organic C), total N, total P, total K, pH, and electrical conductivity; heavy metals (Cd, Cu, Ni, Pb, Zn, Hg, Cr) according to Directive 86/278/EEC; selected emerging contaminants (for example, representative pharmaceuticals, PFAS, microplastics, where analytical capacity exists) (Council Directive 86/278/EEC, 1986; Mara et al., 1989; US EPA, 2019; Frišták et al., 2024; Chenu, 2025). For the

microbiological component, bacterial indicators (*E. coli*, *Salmonella* spp.), viable helminth eggs, and, where justified, enteric viruses (adenovirus, norovirus) are determined (FAO, 1995; Jiménez et al., 2010; Gholipour et al., 2022).

Soil characterization and experimental design. Before application, agrochemical and pedological mapping of the experimental field is performed: texture, pH, organic C, total N, available P, available K, cation exchange capacity, and background heavy metals (FAO, 1995). A randomized experiment is proposed with at least four treatments: control (no sludge), recommended mineral fertilizers, moderate sludge dose (equivalent to 50-60% of the recommended N dose), and high sludge dose (equivalent to 100% of the N dose), with four replications (FAO, 1995; Navarro et al., 2010; Singh et al., 2010; Kildare County Council, 2023; Marin et al., 2023).

Monitoring. Each year, the following are monitored: (i) soil fertility parameters (organic C, N, P, K, micronutrients); (ii) accumulation of heavy metals and possible emerging contaminants in soil; (iii) microbiological soil load (bacterial indicators, helminth eggs); and (iv) crop yields and heavy metal content in edible plant organs (grains, leaves, roots) (FAO, 1995; Navarro et al., 2010; Singh et al., 2010; Zhai et al., 2018; Trinomics, 2020; Kildare County Council, 2023). The frequency of analyses is adjusted according to the results, in line with the regulatory framework and good practice guidelines (Council Directive 86/278/EEC, 1986; Scottish Executive, 2002; Navarro et al., 2010; US EPA, 2019).

RESULTS AND DISCUSSIONS

Characteristics of urban sludge as an alternative source of fertilization in agriculture

Organic matter and nutrient content. Urban sludge originating from municipal wastewater treatment plants is rich in stabilized organic matter and nutrients, especially nitrogen and phosphorus, and to a lesser extent potassium and micronutrients (Fe, Mn, Zn, Cu) (FAO, 1995; Kildare County Council, 2023; Marin et al., 2023; Paganini et al., 2024). The meta-analysis conducted by Paganini et al. (2024) shows that sludge application at adapted rates significantly

increases soil organic matter content, improves structure and porosity, and stimulates microbial activity, with favorable effects on medium-term fertility (Paganini et al., 2024).

Field studies indicate positive crop responses (maize, wheat, barley, legumes) to sludge application, as a result of the supply of mineralizable N and P (FAO, 1995; Navarro et al., 2010; Singh et al., 2010; Zhai et al., 2018; Kildare County Council, 2023; Marin et al., 2023). In an experiment conducted in Belgium, De Brouwere et al. showed that wheat and barley yields increased up to threefold in sludge-fertilized treatments compared with the control, at rates that did not exceed the heavy metal thresholds in soil (De Brouwere et al., 2006). In Turkey, sludge application in a wheat-cabbage-tomato rotation system led to significant yield increases for all species, associated with improved nutritional status and some physical soil properties (Trinomics, 2020).

Experiments with composted sludge show that, in addition to nutrient supply, composting contributes to the partial reduction of pathogens and to the stabilization of organic matter, resulting in yield increases of over 100% compared with the control under certain conditions (Li et al., 2024). At the same time, an increase in microbial carbon and enzymatic activity is observed, indicating an intensification of nutrient cycling (Mara et al., 1989; Kildare County Council, 2023; Li et al., 2024).

Pollutants and emerging contaminants content. The main cause for concern regarding sludge use in agriculture is the accumulation of inorganic pollutants and persistent organic compounds. The heavy metal content of sludge varies depending on the city's industrial pretreatment. Studies from different countries show typical concentrations for Zn, Cu, Cr, Pb, Ni, Mo, As, Cd, Se, and Hg, in descending order of frequency and average levels: Zn > Cu > Cr > Pb > Ni > ... > Hg (Council Directive 86/278/EEC, 1986; FAO, 1995; Yakameran et al., 2021).

Directive 86/278/EEC sets limits for heavy metals in sludge and soil, but recent assessments suggest that these limits, established in the 1980s, may be insufficient for the long-term protection of European soils (Council Directive 86/278/EEC, 1986; Baltic Water Hub, 2022).

Proposals to tighten the limits for Cd, Pb, Hg, and other metals are based on repeated sludge application scenarios and on the risk of bioaccumulation in the food chain (Council Directive 86/278/EEC, 1986; US EPA, 2019).

In recent decades, attention has also turned to emerging contaminants: pharmaceutical residues, endocrine disruptors, PFAS, microplastics and nanoplastics, brominated flame retardants, etc., identified in sludge at significant levels (Mara et al., 1989; Frišták et al., 2024; Balkrishna et al., 2025; Chenu, 2025). Recent investigations regarding sludge application on large agricultural areas have highlighted significant accumulations of microplastics in soil and the presence of PFAS and other persistent organic compounds in the sludge matrix, while regulations still focus almost exclusively on heavy metals (Council Directive 86/278/EEC, 1986; US EPA, 2019; Frišták et al., 2024; Chenu, 2025).

At high doses or in the absence of rigorous source selection, sludge application may lead to exceeding heavy metal limits in soil or to significant accumulation in edible plant organs, especially for Cd, Zn, and Cu (De Brouwere et al., 2006; Singh et al., 2010; Trinomics, 2020). In a study on mung bean, higher sludge doses increased yield, but also resulted in greater metal accumulation in seeds, limiting their use for human consumption (Singh et al., 2010). Thus, the fertilizing effect must always be balanced against the risk of chemical contamination of the soil and crop.

Presence of parasite eggs, viruses, and other pathogens. Sewage sludge is recognized as a major reservoir of pathogens: enteric bacteria (*Salmonella* spp., *Shigella* spp., pathogenic *E. coli*), helminth eggs (*Ascaris*, *Trichuris*, *Taenia*), protozoa (*Giardia*, *Entamoeba*), and enteric viruses (adenovirus, norovirus, rotavirus, hepatitis A), which may survive for long periods in sludge and even after soil application (FAO, 1995; Jiménez et al., 2010; Amoah, I.D. et al., 2018). A number of studies have reported high concentrations of viable helminth eggs in dewatered sludge from Africa and other regions, associated with high infection risks for farmers applying sludge and for consumers of vegetables cultivated on insufficiently protected amended soils (Navarro et al., 2010; Amoah et al., 2018; Sabbahi et al., 2022; Lucia et al., 2025).

WHO guidelines for the safe use of wastewater, excreta, and sludge propose strict criteria regarding the concentration of helminth eggs in sludge intended for agriculture, considering helminths the main public health risk category in developing countries (Mara et al., 1989; WHO, 2006; Navarro et al., 2010). In general, a maximum level of 0.25-1 helminth egg/g dry matter is recommended, which is difficult to achieve in the absence of advanced sanitization technologies (thermophilic composting, high-temperature anaerobic digestion, thermal drying, or sludge irradiation) (WHO, 2006; Navarro et al., 2010; Sabbahi et al., 2022).

Another group of biological contaminants is represented by enteric viruses. A recent systematic review shows that adenoviruses and noroviruses are the most frequently identified in urban sludge, and these viruses may persist in sludge even after certain treatment stages and may be transferred to soil, groundwater, or crops (Gholipour et al., 2022). The COVID-19 pandemic drew attention to the presence of SARS-CoV-2 genetic material in sludge, which led to additional recommendations regarding treatment and withholding periods, although the risk of transmission through properly disinfected sludge appears low (ANSES, 2020).

Influence of fertilization with urban sludge on agricultural production. Numerous field studies confirm that sewage sludge, when applied at moderate doses and according to good practices, can consistently increase agricultural yields (FAO, 1995; Navarro et al., 2010; Singh et al., 2010; Trinomics, 2020; Marin et al., 2023; Kildare County Council, 2023). Sludge application increased maize, wheat, and barley yields, correlated with higher N concentrations in reference leaves and improved phosphorus nutrition (Zhai et al., 2018; Kildare County Council, 2023). In vegetable rotation systems, sludge promoted high cabbage and tomato yields while improving soil physical quality (lower bulk density, greater structural stability) (Trinomics, 2020).

At the same time, studies underline the existence of an optimal threshold: at excessively high doses, yield gains tend to plateau or even decrease, while the risk of salinization, phytotoxicity, and heavy metal accumulation in plants increases (De Brouwere et al., 2006; Singh et al., 2010; Trinomics, 2020). For

example, in the case of mung bean, the authors recommend doses below 9 kg m^{-2} , at which a good compromise is achieved between fertilizer valorization and limiting metal accumulation in seeds (Singh et al., 2010).

Interesting results also come from the use of composted or thermochemically treated sludge, which maintains fertilizing benefits while reducing pathogen load and increasing organic matter stability. Long-term experiments with composted sludge indicate yield increases of over 100% compared with the unfertilized control (Mara et al., 1989; IAEA, 2002; Li et al., 2024). The use of sludge transformed into biochar or other stabilized forms represents an emerging direction with the potential to better separate benefits from risks (Mara et al., 1989; Institute for European Environmental Policy, 2014; Frišták et al., 2024; Balkrishna et al., 2025).

Frequency of analyses and monitoring requirements. The European framework and national guidelines emphasize the role of continuous monitoring of sludge and of the soils on which it is applied. Directive 86/278/EEC provides that the frequency of analyses is determined according to the results of the initial analysis, changes in the nature of the treated wastewater, and other relevant factors, with the recommendation that a complete sludge analysis be carried out at least once every 6–12 months (Council Directive 86/278/EEC, 1986; Institute for European Environmental Policy, 2014; US EPA, 2019). National codes of practice go further, recommending sludge analyses for regulated parameters (heavy metals, basic parameters) at least every 6 months, and soil analyses every 2 years, with heavy metals determined every 5 years or more frequently in the case of high application rates (Scottish Executive, 2002; Kildare County Council, 2023).

International organizations (WHO, FAO) recommend that, in situations where sludge is applied to crops consumed fresh (leafy vegetables, root crops), microbiological monitoring should be more frequent (for example, annually or even before each application campaign) and combined with minimum withholding periods between application and harvest (FAO, 1995; Navarro et al., 2010). In addition, where analytical

infrastructure allows, the gradual introduction of indicators for emerging contaminants is encouraged, at least at screening level (for example, PFAS, selected pharmaceutical residues, microplastics) (Mara et al., 1989; Frišták et al., 2024; Chenu, 2025).

Based on these recommendations and the specialized literature, the following minimal monitoring framework can be proposed for the agricultural use of urban sludge:

Sludge: 2–4 analyses/year for basic parameters (moisture, organic matter, N, P, K), at least 1–2 analyses/year for regulated heavy metals; microbiological analyses (bacterial indicators, helminth eggs) at least annually or prior to application on crops consumed fresh.

Soil: agrochemical analyses (pH, organic C, N, P, K) every 2 years; heavy metals every 3–5 years or more frequently in the case of repeated applications; microbiological monitoring at critical points or in pilot projects.

Risk reduction methods and good practices.

To maximize benefits and reduce the risks associated with urban sludge, the literature describes a wide range of technologies and good practices. From a technological point of view, stabilization and sanitization processes are recommended:

mesophilic/thermophilic anaerobic digestion, thermophilic composting, thermal drying, and, in certain contexts, sludge irradiation (IAEA, 2002; Navarro et al., 2010; Institute for European Environmental Policy, 2014; Sabbahi et al., 2022; Balkrishna et al., 2025). Irradiation, for example, can ensure substantial pathogen reduction, allowing sludge use on sensitive crops, although costs and infrastructure requirements limit large-scale application (IAEA, 2002; Sabbahi et al., 2022). From an agronomic point of view, the following are recommended:

- application of sludge outside the harvest season, to allow further pathogen reduction in soil through the action of time, UV radiation, and microbial competition;

- prohibition of direct application on crops consumed fresh (leafy vegetables, aromatic plants) and establishment of appropriate withholding periods (for example, a minimum of 30–36 days between application and harvest for certain vegetables) (Amoah et al., 2018; Lucia et al., 2025);

- adjustment of doses to the actual nutrient requirements and to the heavy metal and other pollutant content of sludge and soil, avoiding exceedance of regulated limits and long-term accumulation;

- alternation of organic matter sources (manure, plant compost) with sludge, in order to reduce the risk of one-directional accumulation of certain elements.

From the perspective of public policy management, recent evaluations of the Sewage Sludge Directive underline the need to update limits and gradually introduce new parameters (PFAS, microplastics, pharmaceutical residues), as well as to move from models based predominantly on heavy metals toward an integrated risk approach (Navarro et al., 2010; Trinomics, 2020; Frišták et al., 2024; Chenu, 2025). At the same time, the importance of source control is emphasized (preventing uncontrolled industrial discharges into sewer networks), as a more efficient solution than simply restricting sludge application in agriculture.

The balance between resource and risk. The analysis of bibliographic data shows that urban sludge has real potential to contribute to the circular economy of nutrients, especially through the supply of N and P and by increasing soil organic matter content (FAO, 1995; Navarro et al., 2010; Institute for European Environmental Policy, 2014; Kildare County Council, 2023; Li et al., 2024; Paganini et al., 2024). At the same time, its agricultural use involves a series of risks related to inorganic pollutants (heavy metals), emerging contaminants, and pathogens, whose management requires adequate treatment technologies, an updated regulatory framework, and a rigorous monitoring system (Council Directive 86/278/EEC, 1986; FAO, 1995; Jiménez et al., 2010; Navarro et al., 2010; US EPA, 2019; Yakameran et al., 2021; Frišták et al., 2024).

If these conditions are met, sludge can be valorized as an organic amendment and source of nutrients, reducing dependence on mineral fertilizers and contributing to carbon sequestration in soil. In their absence, however, there is a risk of transforming sludge from a “resource” into a pollution vector and sanitary hazard, with possible long-term consequences

for agricultural soils, water, and public health (FAO, 1995; Reilly, 2001; Yakamercan et al., 2021; Frišták et al., 2024).

CONCLUSIONS

The use of urban sludge in agriculture represents an attractive technical option in the context of the transition toward a circular economy and rising costs of mineral fertilizers. The scientific literature shows that, under conditions of rigorous control of sludge and soil quality, it can lead to increased agricultural yields, improved soil fertility, and reduced pressure on phosphate and mineral nitrogen resources (FAO, 1995; Navarro et al., 2010; Institute for European Environmental Policy, 2014; Zhai et al., 2018; Kildare County Council, 2023; Paganini et al., 2024).

On the other hand, the presence of heavy metals, persistent and emerging organic contaminants, microplastics, PFAS, and a wide range of pathogens (bacteria, viruses, parasites) means that this practice is not without risks. Some studies and critical reports highlight cases of soil and agricultural product contamination, as well as dissatisfaction among rural communities exposed to the application of insufficiently treated sludge (FAO, 1995; Reilly, 2001; Yakamercan et al., 2021; Frišták et al., 2024).

The balance between benefits and risks depends on:

- the technological level of wastewater treatment plants (effective stabilization and sanitization);
- the strict application of the regulatory framework and, where needed, the updating of limits and the introduction of new parameters;
- the responsible design of application rates and crop rotations;
- the implementation of a coherent monitoring program for sludge, soil, and yields.

The proposed methodology - which combines detailed characterization of sludge and soil, a controlled field experiment, and multiannual monitoring of yields and contaminants provides a practical framework for evaluating risks and benefits under specific local conditions.

In the long term, the integration of advanced recovery technologies (composting, anaerobic digestion, biochar, phosphorus recovery) may help transform sludge from a potentially

problematic waste into an agricultural resource managed responsibly.

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MULTI-ANNUAL ANALYSIS OF THE PERFORMANCE OF MAIZE HYBRIDS CULTIVATED UNDER A MINIMUM TILLAGE SYSTEM IN IRRIGATED CONDITIONS IN DOBROGEA (2022-2025)

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Abstract

The paper presents the results of a field experiment conducted between 2022 and 2025 in the Dobrogea region, under an irrigation system, evaluating the behavior of three maize hybrids (P9911, P9889, and MAS43P) cultivated under a minimum tillage system. The study aimed to assess the influence of the technological itinerary on grain yield, thousand kernel weight (TKW), test weight (TW), plant height, and plant density at harvest. Weed control was achieved through an integrated management system using pre- and post-emergence herbicides (Callisto and Clinic Xpert), in order to reduce weed infestation and preserve soil moisture. The data were statistically analyzed using the ANOVA method, highlighting significant differences among hybrids regarding average yield, which ranged from 7.8 to 9.2 t/ha. Hybrid P9889 showed the highest adaptability and yield stability under irrigated conditions. The combination of minimum tillage, irrigation, and effective weed control contributed to maintaining soil fertility and increasing the energy efficiency of maize cultivation. The results indicate that thermal stress during the May-July period represents the main limiting factor for maize productivity in Dobrogea, even under irrigated conditions, while hybrid-specific adaptability plays a decisive role in ensuring yield stability under increasing climate stress.

Key words: hybrids, irrigation system, maize, minimum tillage, weed control.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most strategic crops globally, ranking third worldwide by cultivated area after wheat and rice, while being of primary importance for the livestock and energy sectors due to its versatility and high economic value (Erenstein et al., 2022). In Europe, Romania consistently ranks among the leading countries in terms of cultivated area and total maize production, with maize playing a key role in national food security and agricultural competitiveness (Botoș et al., 2025; Stroe & Panaitescu, 2026). In recent years, increasing research attention has focused on adapting maize production technologies to the growing frequency and intensity of drought and aridity episodes associated with climate change (Habibur-Rahman et al., 2022).

Climate change has expanded the agricultural areas exposed to severe heat stress, particularly in southern and south-eastern Romania, where summer temperatures frequently reach or

exceed the critical physiological thresholds for row crops (Prăvălie et al., 2014; Popescu et al., 2023). Dobrogea is considered one of the most climatically vulnerable agricultural regions of the country, being characterized by low annual rainfall, high evapotranspiration rates and recurrent heat waves during July and August (Smuleac et al., 2020; Dumitru et al., 2023). Under these conditions, soil-conservation technologies become essential for preserving soil moisture and preventing structural degradation.

Minimum tillage systems have been increasingly adopted in arid and semi-arid regions due to their capacity to reduce evaporation losses, improve water-use efficiency and limit soil disturbance (Stroe & Panaitescu, 2025). The use of conservative tillage practices, often complemented by cover crops, contributes to soil protection and improves water availability during critical growth stages (Filip & Marin, 2018). Nevertheless, even when water losses are

reduced, maize crops remain highly sensitive to extreme temperatures during reproductive stages, particularly during flowering, when elevated thermal thresholds impair fertilisation and reduce kernel number per ear (Yanev, 2023). Dobrogea is frequently affected by extreme temperatures during maize pollination, with numerous days exceeding 32-35°C, a condition that substantially reduces grain yield even under irrigated conditions (Mircea et al., 2023). In the Topraisar area, where both pedological and atmospheric drought are pronounced, irrigation represents an indispensable technological component; however, its efficiency is strongly constrained by the intensity of heat stress affecting key physiological processes (Risnoveanu et al., 2023). Similar responses to heat stress during flowering have been reported in other southern regions of Romania, where high temperatures during pollination resulted in reduced kernel set and lower yields (Simionescu et al., 2016; Zafiu & Mihalache, 2021). Several studies conducted in south-eastern Europe have demonstrated that the performance of maize hybrids under arid conditions is strongly dependent on their adaptability to high temperatures during flowering (Haraga & Ion, 2022). Consequently, hybrid selection should account not only for drought tolerance but also for sensitivity to extreme thermal conditions, which has become increasingly relevant in the context of climate change and the intensification of heat-wave events (Kirchev, 2025). In this framework, the effectiveness of conservative tillage systems is closely linked to the genetic adaptability of the hybrids cultivated under specific pedoclimatic conditions (Dunăreanu & Bonea, 2022; Aedin et al., 2024). Irrigation systems remain a key technological tool for mitigating water stress in spring crops such as maize, which exhibits maximum water demand during grain formation and filling (Filip & Marin, 2018). However, recent studies indicate that irrigation cannot fully compensate for the negative effects of extreme temperatures on pollen viability and fertilisation, making thermal stress during pollination a major determinant of yield variability in south-eastern Romania (Zhang et al., 2021). In this context, minimum tillage systems have expanded considerably in Dobrogea, contributing to improved soil structure, reduced evaporation

and enhanced resilience to drought, although their long-term effectiveness depends on hybrid response to heat stress during flowering (Amuza & Ilie, 2023; Manole et al., 2023; Dumitru et al., 2023).

Recent research has highlighted the importance of optimizing technological components in maize cultivation systems under irrigation and arid conditions. The interaction between agronomic practices, such as fertilization management and hybrid selection, plays a decisive role in improving yield performance and stability in water-limited environments. For instance, optimizing nitrogen application timing has been shown to significantly enhance grain yield in maize-based systems under arid conditions (Hu et al., 2020). At the same time, quantitative models have been developed to assess the combined influence of technological factors on maize productivity under irrigation, emphasizing the complexity of yield formation processes (Vozhehova et al., 2023). In addition to yield-related aspects, increasing attention has been given to the biochemical and nutritional quality of maize cultivars, which is influenced by both genetic background and cultivation technology (Popoviciu et al., 2024). These findings underline the need for integrated approaches that combine technological optimization with hybrid adaptability in order to ensure stable maize production under conditions of increasing climatic stress.

The aim of this study was to evaluate the multi-annual performance of drought-tolerant maize hybrids cultivated under minimum tillage and irrigation in the specific climatic conditions of the Topraisar area, southern Dobrogea. The objectives were to assess yield stability and key productivity components under contrasting climatic conditions and to identify hybrids with superior adaptability to thermal stress, using ANOVA-based statistical approaches to support technological decision-making in semi-arid regions.

MATERIALS AND METHODS

The research was carried out between 2022 and 2025 in south-eastern Romania, in the Topraisar area (Dobrogea), a region characterized by frequent episodes of pedological and atmospheric drought and high sensitivity of

agricultural crops to heat stress (Dumitru et al., 2023; Smuleac et al., 2020). The experimental site is representative of the Dobrogea Plateau, where moderate soil fertility and a permanent climatic water deficit justify the use of conservative tillage systems and irrigation for spring crops (Filip & Marin, 2018; Dunăreanu & Bonea, 2022).

The biological material consisted of three maize hybrids with high tolerance to drought and elevated temperatures (P9911, P9889 and MAS43P), selected based on their recent performance in south-eastern European environments (Haraga & Ion, 2022; Călugăr et al., 2024). The experiment was organized as a randomized complete block design, with hybrid as the single experimental factor, and was conducted under identical technological conditions for all variants, including sowing date, plant density, fertilization scheme and irrigation regime, in order to allow a realistic comparison of genetic performance (Mircea et al., 2023; Stroe & Panaiteanu, 2025).

Each experimental plot covered an area of 1 ha, and three replications were used for each hybrid in each experimental year. This experimental layout allowed both intra-annual comparisons among hybrids and interannual evaluation of climatic effects under uniform management conditions.

The cultivation technology was based on minimum tillage, involving shallow stubble operations and a reduced number of soil interventions, according to recommendations for areas affected by severe aridity (Dumitru et al., 2023; Risnoveanu et al., 2023). In all vegetation cycles, irrigation was applied using sprinkler systems, ensuring an adequate water supply for crop development, although without eliminating the impact of extreme temperatures during the pollination period, which represents the main limiting factor for maize productivity in Dobrogea (Zafiu & Mihalache, 2021; Kirchev, 2025). Weed control was performed through the application of pre- and post-emergence herbicide treatments based on mesotrione and glyphosate, following technological recommendations for maize cultivated under minimum tillage systems (Yanev, 2023).

The assessed parameters included average grain yield, thousand-kernel weight (TKW), test weight (TW), plant height and plant density at

harvest. Experimental data were statistically analysed using analysis of variance (ANOVA), in accordance with methodologies commonly applied in studies addressing the effects of heat stress, genotype and cultivation system on maize production in south-eastern Europe (Prăvălie et al., 2014; Habib-ur-Rahman et al., 2022).

In addition to ANOVA, correlation and regression analyses were applied to evaluate the relationships between grain yield and the main biometric and quality parameters (TKW, TW, plant height and plant density). Pearson correlation coefficients were calculated and tested for statistical significance at $p < 0.05$ and $p < 0.01$. Linear and polynomial regression models were used to assess the dependence of grain yield on thermal conditions during the flowering period and on selected productivity components. Prior to statistical analysis, data distribution and variance homogeneity were verified in accordance with standard biometric requirements for agricultural field experiments. Statistical processing and graphical interpretation were performed using standard statistical software packages commonly employed in agronomic research, following methodological approaches reported in recent maize studies from south-eastern Europe (Haraga & Ion, 2022; Kirchev, 2025).

The aim of this study was to evaluate the performance of the maize hybrids P9911, P9889 and MAS43P under the pedoclimatic conditions of the Topraisar area and to identify technological solutions applicable to agriculture in south-eastern Romania under conditions of intensifying heat stress (Popescu, 2023). The main objectives were the comparative analysis of key productivity elements, the assessment of the impact of high temperatures on yield components, and the identification of hybrids with superior yield stability under irrigation and minimum tillage conditions.

RESULTS AND DISCUSSIONS

Maize was grown each year after winter wheat, a dominant crop rotation system in south-eastern Dobrogea, considered technologically suitable for the land-use structure of the region. At the preceding crop, swine manure was applied as organic fertilization, contributing to improved soil structure and nutrient availability under the

moderate fertility conditions typical of the Dobrogea Plateau. Basal fertilization of maize included annual applications of nitrogen (200 kg/ha as ammonium nitrate), phosphorus (100 kg/ha as superphosphate) and potassium (80 kg/ha as potassium sulfate), supplemented by a top-dressing of urea (80 kg/ha, 46% N) at the 6-8 leaf stage. The combined use of organic amendments and moderate mineral fertilization is recommended for arid regions and has been reported as an efficient strategy for sustaining crop productivity under limited natural nutrient supply (Filip & Marin, 2018; Dumitru et al., 2023; Stroe & Panaitescu, 2025).

Maize was sown annually between 12 and 17 April at a uniform density of 68,000 plants/ha, ensuring a consistent basis for genetic comparison among hybrids. Weed control followed an integrated management approach, combining mechanical cultivations with pre- and post-emergence herbicide applications based on mesotrione (0.4 L/ha) and glyphosate (3 L/ha), reducing both weed competition and soil moisture losses. Pest control targeted mainly *Ostrinia nubilalis* and *Helicoverpa armigera*, while fungicide treatments were applied in years with favourable conditions for foliar diseases, particularly *Cercospora zeae-maydis*. Similar technological approaches have been widely reported for maize cultivation in south-eastern Romania, where organic fertilization, integrated weed management and irrigation are essential components of conservative tillage systems (Simionescu et al., 2016; Haraga & Ion, 2022; Manole et al., 2023; Yanev, 2023; Kirchev, 2025).

Irrigation was applied through sprinkler systems during all vegetation cycles, ensuring adequate water supply during critical growth stages. However, even under regular irrigation, high temperatures during pollination remained the main limiting factor for grain production, negatively affecting fertilisation and kernel set. The uniform application of the cultivation technology across the four experimental years allowed a clear assessment of hybrid performance against the background of pronounced interannual climatic variability and increasing thermal stress typical of the Dobrogea region (Zafiu & Mihalache, 2021; Mircea et al., 2023). The climatic data recorded in the Topraisar area during 2020-2025 indicate a pronounced

positive deviation of monthly mean temperatures relative to the long-term climatic normal (1980-2019), particularly during the agricultural season (Figure 1). Across all experimental years, the April-September period showed temperature increases of approximately 2.0-4.0°C above the multiannual average, highlighting the intensification of thermal stress characteristic of arid regions. The highest positive deviations were consistently recorded in June, July and August, in agreement with recent studies reporting a marked increase in summer temperatures in south-eastern Europe (Prăvălie et al., 2014; Smuleac et al., 2020; Haraga & Ion, 2022; Mircea et al., 2023). In July, mean temperatures ranged between 26.7 and 28.5°C, substantially exceeding the multiannual average of 22.6°C and approaching or surpassing the physiological thresholds considered limiting for maize pollination and fertilisation (Yanev, 2023). Elevated temperatures persisted in August, with mean values 3-4°C above the climatic normal, indicating prolonged thermal stress during the reproductive phase. Under these conditions, even in irrigated systems, temperature emerged as the primary limiting factor for yield formation in the analysed experimental years, confirming observations previously reported for south-eastern Romania (Dumitru et al., 2023; Zafiu & Mihalache, 2021).

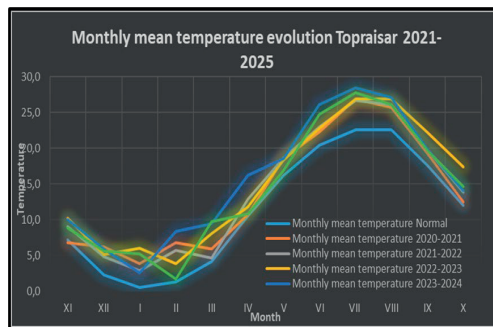


Figure 1. Monthly mean temperature evolution (Topraisar, 2020-2025)

The analysis of absolute maximum and minimum temperatures recorded during March–September confirms the occurrence of pronounced thermal extremes with direct effects on crop establishment and reproductive development in the Topraisar area. In several

years, April registered minimum temperatures below the biological tolerance threshold of maize, reaching values as low as -2.6°C and -11.1°C , which delayed emergence and reduced early seedling vigour. Similar early-season cold events have been reported in Dobrogea and are considered disruptive for uniform crop establishment under conservation tillage systems (Dumitru et al., 2023).

During the reproductive period, absolute maximum temperatures in July-August frequently exceeded $36-39^{\circ}\text{C}$, confirming the presence of severe heat stress during pollination. Such values surpass the thresholds considered critical for pollen viability and fertilisation and are associated with reduced kernel set even under irrigated conditions (Yanev, 2023). The occurrence of temperatures above 35°C as early as June indicates an advancement of heat stress towards the beginning of summer, potentially limiting biomass accumulation during stem elongation, while low minimum temperatures recorded in May ($0.8-3.4^{\circ}\text{C}$) reveal a high thermal amplitude during early vegetative stages.

Overall, the combination of extreme minimum temperatures in early spring and extreme maximum temperatures during July-August demonstrates that maize phenological success in the Topraisar area is jointly constrained by early-season and reproductive heat stress. Consequently, even under irrigation, thermal stress remains the main limiting factor of crop performance in the analysed agricultural years,

in agreement with previous findings from south-eastern Romania (Haraga & Ion, 2022; Mircea et al., 2023) (Figure 2).

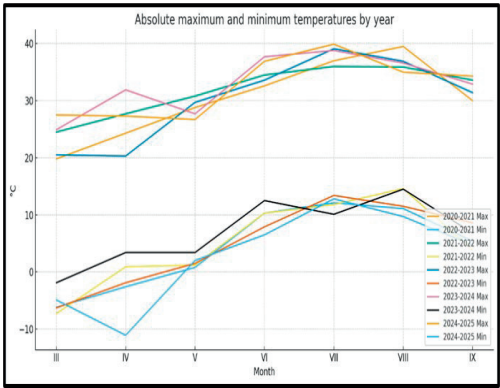


Figure 2. Absolute temperature extremes in Topraisar during the vegetation season (2020-2025)

The analysis of precipitation distribution during the 2020-2025 interval reveals pronounced interannual variability, with large positive and negative deviations from the multiannual climatic norm of 436 mm (Table 1). The agricultural year 2020-2021 was the wettest of the analysed period, recording 629 mm (approximately 44% above the norm), with precipitation surplus concentrated mainly in winter and early spring (January-April), which favoured soil water reserve accumulation at the beginning of the vegetation period, an uncommon situation for southern Dobrogea (Dumitru et al., 2023).

Table 1. Analysis of precipitation distribution during the 2020–2025

Month/year	Precipitation (mm)					
	Normal	2020-2021	2021-2022	2022-2023	2023-2024	2024-2025
XI	40.4	20.6	40.8	39.5	85.0	56.6
XII	34.0	72.0	69.6	20.3	28.0	92.8
I	31.0	89.0	14.8	45.2	14.4	17.4
II	33.0	30.4	27.2	10.8	1.2	5.4
III	21.7	59.7	19.2	23.6	22.0	4.6
IV	33.5	51.8	30.7	99.5	27.6	23.4
V	50.2	66.0	19.8	11.4	26.2	52.4
VI	53.2	100.4	45.0	28.4	11.2	0.2
VII	35.6	33.5	41.6	27.8	7.4	20.8
VIII	31.6	6.8	5.4	3.6	17.0	21.0
IX	41.6	20.8	64.2	0.2	86.2	47.4
X	30.2	78.4	5.4	7.8	79.6	95.0
Total per year	436.0	629.4	383.7	318.1	405.8	437

In contrast, the years 2021-2022 and 2022-2023 were characterised by marked pluviometric deficits, with total precipitation values 12-27% below the multiannual average, reflecting a typical semiarid climatic regime. The year 2022-2023 was particularly severe, with extremely low spring precipitation (March-May), totalling only 36 mm compared to the normal 105 mm, leading to the early onset of water stress during crop establishment.

The 2023-2024 agricultural year also exhibited a dry character, despite total precipitation close to the norm, due to its uneven seasonal distribution, with higher values in autumn and pronounced deficits during the critical May-July period. Similarly, although total precipitation in 2024-2025 (437 mm) was close to the multiannual average, rainfall scarcity during May-July directly affected soil moisture availability during panicle emergence and pollination. These patterns confirm that, under the climatic conditions of south-eastern Romania, total annual precipitation is not a reliable indicator of climatic risk, as crop performance is primarily determined by rainfall distribution during critical phenophases (Zafiu & Mihalache, 2021; Mircea et al., 2023).

Beyond its impact on yield formation, precipitation distribution during the vegetation period also plays an important role in determining grain quality parameters. Adequate water availability during grain filling contributes to proper kernel development, influencing both grain consistency and the accumulation of bioactive compounds. Variations in moisture conditions during this stage may lead to changes in kernel structure and nutritional composition, as reported in studies evaluating maize quality traits under different environmental conditions (Popoviciu et al., 2024). Therefore, irregular precipitation patterns not only affect yield levels but may also influence the qualitative attributes of maize production under semiarid conditions.

Overall, only the 2020-2021 season can be considered favourable in terms of rainfall, while the remaining years were characterised by moderate to severe hydric deficits during the vegetation period, a situation typical of highly arid regions such as Dobrogea (Smuleac et al., 2020; Popescu et al., 2023).

During the vegetation period (March-September), precipitation amounts were below the multiannual norm (267.4 mm) in most of the analysed years, confirming the semiarid character of the Topraisar region (Figure 3). Only the 2020-2021 season recorded precipitation above the normal level (339 mm), whereas the remaining years exhibited marked hydric deficits, with values ranging between 169.8 and 225.9 mm, corresponding to reductions of 15-36% relative to the climatic average.

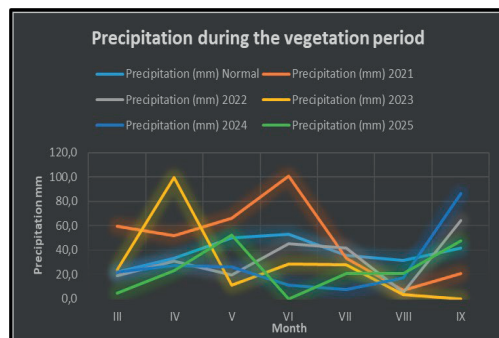


Figure 3. Precipitation during the vegetation period (March-September) in Topraisar (2020-2025)

The hydric deficit was most pronounced during the critical months for yield formation, June and July, when precipitation dropped in some seasons to as little as 0.2-11 mm, far below the physiological water requirements of maize during peak demand. Consequently, except for the first experimental year, crop development relied almost entirely on irrigation inputs.

These results confirm that total annual precipitation is not a reliable indicator of hydric stress, as maize productivity in Dobrogea is primarily determined by rainfall distribution during critical phenological stages rather than by cumulative annual amounts (Smuleac et al., 2020).

Plant density at harvest showed marked interannual variation, reflecting the influence of early-season climatic conditions, particularly spring drought and low minimum temperatures occurring between emergence and the 6-8 leaf stage. Years characterised by higher precipitation during March-May and the absence of severe early frosts favoured uniform emergence and higher final plant densities, whereas reduced rainfall and low minimum

temperatures led to delayed emergence and substantial stand losses.

Against this variable climatic background, clear and consistent differences among hybrids were observed. In the favourable year 2020-2021, average harvest density reached approximately 61,800 plants/ha, with hybrid P9889 recording the highest values ($\approx 62,500$ plants/ha), followed by MAS43P ($\approx 58,700$ plants/ha) and P9911 ($\approx 56,200$ plants/ha). In less favourable years, such as 2021-2022 and especially 2022-2023, when spring precipitation was reduced and minimum temperatures dropped below -1.9°C , average densities declined to approximately 52,600-54,200 plants/ha. Even under these stress conditions, P9889 maintained a clear advantage, exceeding the other hybrids by 3,000-8,000 plants/ha.

In 2023-2024, improved precipitation during June partially compensated early-season stress and resulted in higher final densities ($\approx 58,500$ plants/ha), while in 2024-2025, despite extremely low minimum temperatures in April (-11.1°C), irrigation and partial rainfall recovery during summer enabled stand recovery. In both cases, P9889 consistently recorded the highest plant density at harvest ($\approx 60,800$ - $61,000$ plants/ha), followed by MAS43P and P9911.

These results indicate that, although early-season climatic stress, particularly drought, strongly influences plant establishment, hybrid response plays a decisive role in determining final stand density. The ability of P9889 to maintain higher and more stable plant populations under contrasting hydro-thermal conditions highlights its superior tolerance to early water and thermal stress. This establishment stability constitutes a key physiological advantage, directly contributing to the observed differences in yield performance under irrigated conditions in Dobrogea (Figure 4).

Plant height was strongly influenced by hydro-thermal conditions during May-July, the period of maximum vegetative growth, but consistent differences among hybrids were observed across all experimental years. In seasons characterised by near-normal precipitation and moderate summer temperatures (e.g. 2020-2021 and 2024-2025), average plant height ranged between 200 and 210 cm, with hybrid P9889 consistently exhibiting the highest values (≈ 215 - 218 cm).

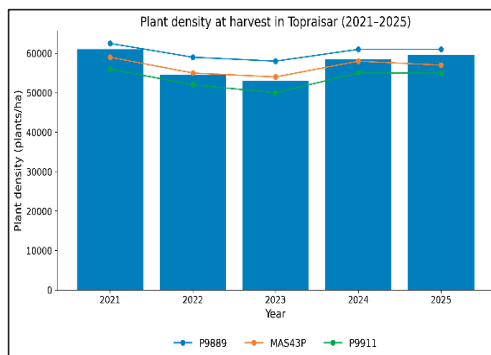


Figure 4. Plant density at harvest in Topraisar (2021-2025)

In contrast, years affected by spring-summer drought and elevated temperatures (2021-2022 and 2022-2023) showed reduced vegetative growth, with average heights declining to approximately 185-195 cm. Even under these stress conditions, P9889 maintained a clear advantage, exceeding P9911 and MAS43P by 8-15 cm, indicating enhanced tolerance to thermal and hydric stress during the peak growth period. Despite strong interannual climatic variability, the genetic ranking of hybrids for plant height remained stable (P9889 > P9911 > MAS43P), highlighting the dominant role of hybrid adaptability in determining vegetative vigour. Across the four experimental years, P9889 consistently recorded the greatest plant height (≈ 205 - 220 cm), confirming its high genetic potential and superior adaptability to the pedoclimatic conditions of Dobrogea.

Ear insertion height followed a similar pattern, being jointly influenced by hydro-thermal conditions during June-July and hybrid genetic characteristics. Under favourable moisture conditions, ear insertion ranged between 102 and 111 cm, while under drought and high-temperature stress it declined to 93-100 cm. In all years, hybrid P9889 maintained the highest ear insertion values (≈ 100 - 111 cm), followed by P9911 and MAS43P, indicating a stable morphological response even under pronounced thermal stress.

The combined stability of plant height and ear insertion observed for P9889 reflects a superior capacity to sustain vegetative growth under contrasting climatic conditions, a trait of particular importance for irrigated maize production systems in south-eastern Dobrogea.

These differences, clearly illustrated by the multiannual means in Figure 5, support the interpretation that morphological vigour

contributes to the higher yield stability of this hybrid under conditions of intensifying heat stress.

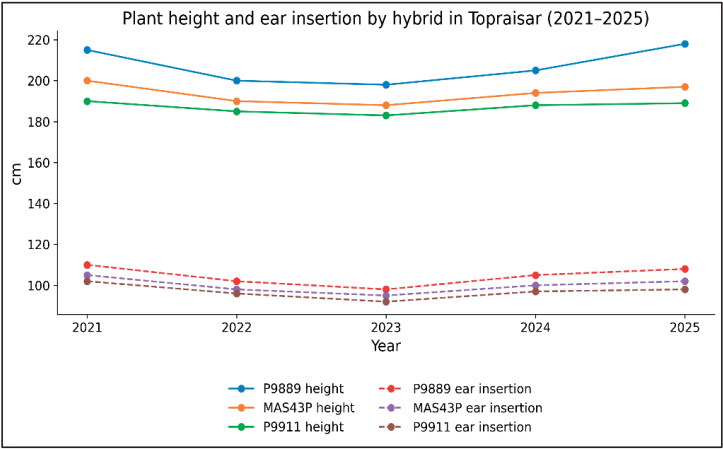


Figure 5. Plant height and ear insertion height of maize hybrids under contrasting climatic conditions in Topraisar (2021-2025)

The determinations regarding kernel weight per ear (without cob) showed clear differences among hybrids, with P9889 recording the highest multiannual mean value (161.29 g), ranging between 172.62 g in 2021 – the year with above-average spring precipitation – and 152.71 g in 2023, strongly affected by heat stress during grain filling. MAS 43P recorded intermediate values, with a mean of 141.14 g, ranging between 162.22 g (2021) and 129.13 g (2023), whereas P9911 exhibited the lowest values (mean 126.67 g), decreasing to 114.42 g in 2023 under severe water deficit conditions. The two-factor ANOVA (hybrid × year), followed by LSD test at $p<0.05$, confirmed significant differences among hybrids as well as a significant hybrid × year interaction, indicating combined influences of genetic potential and yearly climatic variability in the specific conditions of Dobrogea (Filip & Marin, 2019). To obtain a robust estimation of year effect, the statistical analysis was conducted within a mixed model framework, in which hybrid was considered as a fixed effect and year as a random effect, allowing inter-annual climatic variability to be properly integrated in the experimental model. The results regarding total ear mass (kernels + cob) further confirmed the superiority of hybrid P9889, which recorded a mean value of 198.2 g,

followed by MAS 43P (163.14 g) and P9911 (167.56 g). Significant differences ($p<0.05$) were detected within the mixed model ANOVA, confirming physiological differences related to ear development and biomass accumulation, as previously reported for drought-tolerant maize hybrids under arid environments (Haraga & Ion, 2022). For both parameters (kernel weight per ear and total ear mass), hybrid P9889 achieved the highest values, confirming its superior genetic performance and adaptability under irrigation and heat stress conditions specific to Dobrogea (Figure 6).

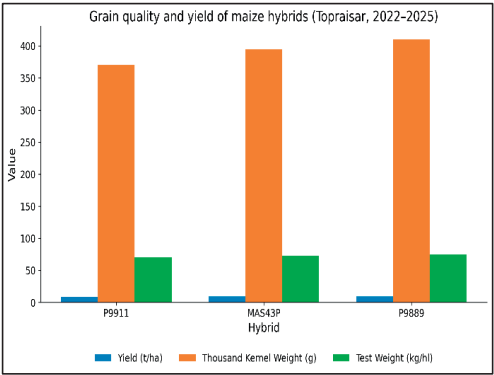


Figure 6. Grain quality parameters and yield for the evaluated maize hybrids (Topraisar, 2022-2025)

The consistently higher ear biomass observed for P9889 indicates a superior capacity to sustain assimilate accumulation during grain filling under heat stress, which represents a key physiological mechanism underlying its higher yield stability in irrigated systems of southern Dobrogea.

Pearson correlation analysis based on multi-annual averages revealed a significant negative relationship between mean temperatures during May–July and grain yield ($r = -0.73$; $p < 0.05$), with a determination coefficient of $R^2 \approx 0.54$, indicating that more than half of the yield variation can be explained by the intensity of thermal stress occurring during the reproductive phase (Figure 7A). This result is consistent with previous findings showing that elevated temperatures during flowering reduce pollen viability and kernel set, leading to substantial yield penalties even under irrigated conditions (Yanev, 2023).

In contrast, cumulative precipitation during April–June showed only a weak positive correlation with grain yield ($r \approx 0.27$; $R^2 \approx 0.07$), suggesting a secondary role of natural rainfall under the experimental conditions (Figure 7B). This limited relationship can be attributed to the use of irrigation, which partially compensated for early-season water deficits. Under rainfed conditions, a stronger precipitation–yield relationship would be expected, as reported for semiarid maize systems (Zafiu & Mihalache, 2021).

Two-way ANOVA (hybrid $\times$ year), followed by LSD/Tukey post-hoc tests at $p < 0.05$, confirmed significant effects of both hybrid and year for all productive traits, highlighting the strong influence of interannual climatic variability. The mixed-model approach, with hybrid treated as a fixed effect and year as a random effect, further emphasized the dominant role of environmental factors under the arid conditions of southern Dobrogea, while allowing reliable discrimination among hybrid responses.

Hybrid $\times$ year interactions demonstrated that maize performance is highly dependent on the intensity of thermal stress during reproductive stages. In this context, hybrid P9889 consistently exhibited the highest interannual yield stability, accompanied by superior thousand-kernel weight and test weight values, reflecting enhanced genetic adaptability and

water-use efficiency. These results are in agreement with previous studies conducted in semiarid regions of south-eastern Europe (Filip & Marin, 2018) and represent a key contribution of the present multi-year, field-based evaluation under minimum tillage and irrigation.

Overall, maize productivity under the analysed conditions was primarily controlled by thermal stress during May–July and only secondarily by early-season water availability. Although irrigation mitigated rainfall deficits, it could not offset the negative effects of extreme temperatures during flowering. Consequently, the adoption of thermotolerant hybrids such as P9889 is essential for maintaining yield performance under the intensifying climatic stress characteristic of Dobrogea.

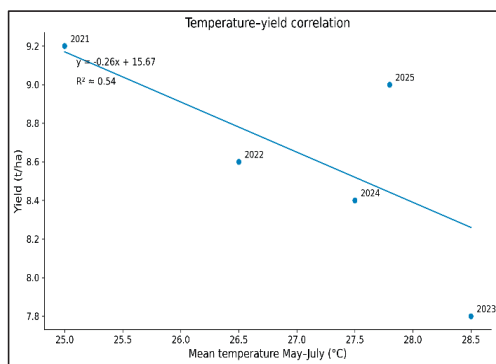


Figure 7A. Temperature-yield correlation for irrigated maize (Topraisar, 2022-2025)

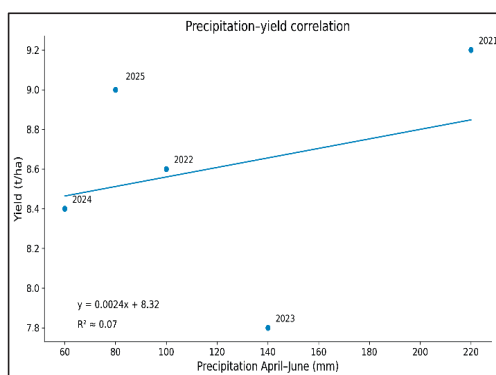


Figure 7B. Precipitation-yield correlation for irrigated maize (Topraisar, 2022-2025)

The results confirm that maize yield under the pedoclimatic conditions of Dobrogea is primarily driven by the thermal regime during

May–July, particularly during flowering and grain filling, in line with findings from other arid regions of south-eastern Europe. Hybrid P9889 showed superior yield stability, supporting previous evidence that genetic traits can mitigate thermal stress effects. Thousand-kernel weight and test weight values were within the range reported for semiarid-adapted hybrids, although lower than those observed in central Romania, where thermal stress during pollination is less severe. Overall, minimum tillage combined with irrigation proved suitable for maize production, although its effectiveness remains constrained under extreme temperature conditions.

The originality of this study lies in its multi-year comparative assessment of thermotolerant maize hybrids under minimum tillage and irrigation in the Topraisar area, one of Romania's most climatically vulnerable regions. The findings demonstrate that yield variability is primarily controlled by May–July temperatures, with hybrid performance shaped by both genetic potential and interannual climatic variability, as confirmed by mixed models and two-factor ANOVA. The superior adaptability of hybrid P9889 supports its inclusion in maize production systems of southern Dobrogea.

A key limitation is the single-location experimental design, which may reflect site-specific pedoclimatic effects, while irrigation partially buffered natural precipitation variability. Future research should expand to multi-location trials, include non-irrigated or controlled water-stress conditions, higher plant densities, a broader hybrid pool, and alternative tillage systems. Integrating predictive thermal-stress models would further improve the assessment of hybrid adaptability under climate change.

CONCLUSIONS

The multi-year study (2022–2025) conducted under the pedoclimatic conditions of the Topraisar area demonstrated that thermal stress during May–July represents the main limiting factor for maize yield, even under irrigated conditions. Interannual climatic variability strongly influenced crop performance, confirming that yield formation in southern Dobrogea is primarily controlled by temperature during the reproductive period rather than by cumulative water availability.

The results highlight that hybrid response to heat stress plays a decisive role in determining yield stability under semi-arid conditions. Differences among hybrids were consistently observed for yield components and biometric traits, indicating that genetic adaptability can partially buffer the negative effects of extreme temperatures. In this context, hybrid P9889 exhibited superior performance across years, reflecting an enhanced capacity to maintain plant density, vegetative growth and ear development under contrasting hydro-thermal conditions.

From a methodological perspective, the combined use of multi-year field experimentation and mixed-model statistical analysis proved essential for separating genetic effects from environmental variability. The significant hybrid $\times$ year interactions underline the importance of long-term evaluations when assessing hybrid adaptability under climate stress, particularly in regions characterized by high interannual climatic fluctuations.

Although minimum tillage combined with irrigation can sustain high yield levels, its effectiveness remains constrained by extreme temperatures during flowering and grain filling. Irrigation mitigates water deficit but cannot fully compensate for the physiological limitations induced by thermal stress, emphasizing the need to integrate technological solutions with appropriate hybrid selection.

For farms located in southern Dobrogea, the adoption of thermotolerant hybrids such as P9889 is recommended to improve yield stability under increasingly frequent heat-wave conditions. Moderate plant densities, together with sprinkler irrigation and soil-conservation technologies, represent suitable management strategies for reducing yield risks in years characterised by pronounced thermal stress.

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IMPACT OF NATIVE SOIL MICROBIOTA ON NODULATION AND SEED YIELD IN WHITE LUPIN (*Lupinus albus* L.) CULTIVARS

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Abstract

During the 2025 growing season, a field experiment was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest to evaluate the effect of native soil microbiota on biological nitrogen fixation and seed yield in four white lupin (*Lupinus albus* L.) cultivars. The results showed that, even without artificial inoculation, plants developed an average of 10.59 nodules per root. The cultivars Medi and Mariuca exhibited the highest nodulation (11.33 and 11.15 nodules, respectively) and achieved the highest seed yields: 1,440 kg ha⁻¹ and 1,363 kg ha⁻¹. These values represent an increase of 13.11% and 7.06%, respectively, over the trial mean (1,273 kg ha⁻¹). A strong positive correlation ($r = 0.962$; $R^2 = 0.926$) between nodule count and seed yield suggests that nodulation serves as a key indicator of nitrogen fixation potential and overall productivity. These findings indicate that native soil microbiota significantly support lupin development, with important implications for cultivar selection and sustainable crop management focused on reducing synthetic nitrogen inputs.

Key words: *Lupinus albus* L., native soil microbiota, nodulation, biological nitrogen fixation, seed yield, correlation.

INTRODUCTION

White lupin (*Lupinus albus* L.) is native to the Mediterranean basin and the coastal regions of the Black Sea, where it has been cultivated for millennia for its high-protein seeds and significant ecological benefits (Gladstones et al., 1998; Hondelmann, 1984). Although it historically played a vital role in traditional agriculture across Southern Europe and Western Asia, it remains globally underrecognized compared to other major legumes (Kurlovich, 2002). Taxonomically, *L. albus* belongs to the *Fabaceae* family. It is an annual, dicotyledonous species, typically reaching heights of 0.5 to 1.5 meters. While primarily self-pollinating, low levels of cross-pollination can occur depending on environmental factors and pollinator activity (Wolko et al., 2011). Cytogenetic studies identify white lupin as a diploid species with a chromosome number of $2n = 50$ (Naganowska et al., 2003). Its seeds possess a remarkable nutritional profile, comparable to soybean in protein content, essential amino acids, and dietary fiber (Lucas et al., 2015), making it a valuable resource for both human consumption and livestock feed.

Beyond its nutritional value, *L. albus* is distinguished by its highly efficient symbiosis with soil bacteria, specifically *Bradyrhizobium* strains, which enables biological nitrogen fixation (BNF). This process enhances soil fertility and reduces the ecological footprint of agriculture by decreasing dependency on synthetic nitrogen fertilizers (Sulas et al., 2016; Vance, 2001). As the global agricultural sector faces energy crises and an urgent need for sustainable protein, white lupin has emerged as a crop of considerable scientific interest. In rainfed Mediterranean environments, it can exhibit a remarkable nitrogen-fixing capacity, with peak values exceeding 300 kg N ha⁻¹ (Sulas et al., 2016), underscoring its potential under favorable conditions.

The efficiency of biological nitrogen fixation in white lupin is regulated by complex molecular interactions between the host plant and symbiotic bacteria, leading to the formation of specialized "lupinoid" nodules. Unlike peas or beans, which typically associate with *Rhizobium leguminosarum*, *Lupinus* species are primarily nodulated by slow-growing *Bradyrhizobium* bacteria, particularly *Bradyrhizobium lupini* (Clúa et al., 2018). Within these nodules,

bacteria differentiate into bacteroids that convert atmospheric nitrogen into a bioavailable form in exchange for plant carbohydrates. This symbiosis allows the plant to meet its nitrogen requirements independently, supporting vigorous growth and high seed yields even in nitrogen-deficient soils (Huyghe, 1997). Recent research indicates that the success of this symbiosis is influenced by intricate interactions between host genotypes and native soil microbiota (Tounsi-Hammami et al., 2021). The diversity of indigenous microbial populations allows different cultivars to adapt to specific pedoclimatic conditions, directly impacting nitrogenase activity and productivity. Despite this potential, white lupin remains a neglected crop in many regions, including Romania, where it is considered a minor species. To promote wider adoption, it is essential to understand how native soil microbiota facilitate effective nodulation without artificial inoculation. This study aims to evaluate the impact of native soil microbiota on nodulation patterns and seed yield in four white lupin (*Lupinus albus* L.) cultivars, providing a scientific basis for integrating this species into sustainable crop rotation systems in temperate climates.

MATERIALS AND METHODS

Experimental Site and Setup

The field trial was conducted at the experimental site of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, during the 2025 growing season. The experiment was carried out on reddish preluvosol soil, characterized by a slightly alkaline pH of 7.79, moderate humus content of 2.95%, moderate total nitrogen level of 0.166%, high mobile phosphorus ($P_{AL} = 92$ mg/kg), and very high mobile potassium ($K_{AL} = 329$ mg/kg). Soil analysis was performed in 2023 at the National Institute of Research and Development for Pedology, Agrochemistry, and Environmental Protection in Bucharest (Szilagyi & Gidea, 2025). The trial was arranged in a Randomized Complete Block Design (RCBD) with three replications. Each experimental plot covered an area of 12 m² and comprised six rows, each 6 m in length. Sowing was performed

on April 2, 2025, at a depth of 3-4 cm, maintaining an inter-row spacing of 0.4 m.

Cultural Practices

Conventional tillage was applied. To evaluate the natural symbiotic potential of the soil, no nitrogen fertilization or artificial inoculation with *Bradyrhizobium* sp (*Lupinus*) was used. During the growing season, weed control was maintained through two manual hoeings. Additionally, two irrigation events of 30 mm each were performed: the first immediately following seedling emergence (BBCH 09-10) and the second prior to the flowering stage (BBCH 59-61). Phenological stages were identified according to the extended BBCH scale (Meier, 2001). All other agrotechnical measures were performed according to local standard practices for white lupin (*Lupinus albus*) cultivation. Manual harvesting was conducted between July 10 and 13, 2025, when the plants ranged between harvest maturity and overripeness (BBCH 89-92).

Plant Material

The biological material consisted of four white lupin (*Lupinus albus* L.) cultivars with distinct phenological, morphological, and chemical profiles (Table 1). Mihai, Mariuca, and Ruxandra varieties were developed at the Iasi University of Life Science (IULS), while 'Medi' originated from the Agricultural Research and Development Station (ARDS) Livada. According to the Official Catalogue of Varieties of Agricultural Plant Species in Romania, the genotypes were classified by seed alkaloid content: 'sweet' (Mariuca, lacking bitter substances) and 'bitter' (Mihai, Ruxandra, and Medi, which contain bitter substances in the seed). The selection included both determinate (Dt) and indeterminate (Ndt) growth habits.

Table 1. Characteristics of the white lupin cultivars used in the study

Cultivar	Origin	Maturity	Growth habit*	Alkaloid Type
Mihai	IULS	Mid-early	Ndt	Bitter
Mariuca	IULS	Mid-early	Dt	Sweet
Ruxandra	IULS	Mid-late	Dt	Bitter
Medi	ARDS Livada	Mid-early	Ndt	Bitter

*Dt – determinate upright, Ndt – indeterminate upright

Weather Conditions

In 2025, according to data from the National Meteorological Administration (ANM) and Meteoblue, Bucharest recorded a positive deviation in the average annual temperature compared to the 1991-2020 multi-annual average, ranging between +0.8°C and +1.2°C. This temperature increase had a detrimental impact on agricultural crops, being particularly severe from June to August, when maximum values exceeding 35°C induced significant heat and water stress, negatively affecting yields. The precipitation regime in 2025 was characterized by rainfall slightly below the climatological average, with an uneven distribution cumulating approximately 520-540 mm/year (Figure 1). The spring months and early summer were marked by prolonged periods of drought, followed by short episodes of torrential rain, often accompanied by convective phenomena such as hail and frequent electrical discharges. This alternation between water deficit and pluvial excess further amplified the physiological stress on the agricultural crops.

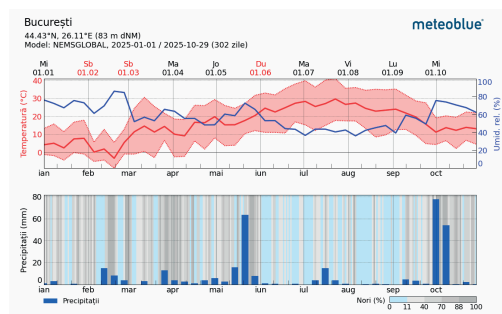


Figure 1. Climate conditions (average temperature and precipitation) in Bucharest - January-October 2025 (Source: <https://www.meteoblue>)

Data Collection

Nodulation: to quantify the number of nodules, a sample of six plants per replicate was randomly collected for each lupin cultivar during the flowering phenophase, which is recognized as the stage of maximum symbiotic nitrogen-fixing activity (Zahran, 1999).

These authors emphasize that nitrogen fixation efficiency is driven by the metabolic sink strength of the flowers and developing pods, reaching its absolute maximum at full bloom.

Seed yield: the total seed yield from each plot was bulked and weighed, with the results

extrapolated to calculate seed yield in kilograms per hectare (kg ha^{-1}).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Mean values were compared using Fisher's Least Significant Difference (LSD) test at a significance level of $p < 0.05$. Additionally, Pearson's correlation coefficient (r) was calculated to assess the relationship between nodule count and seed yield.

RESULTS AND DISCUSSIONS

Symbiotic Performance (Nodulation)

The assessment of nodule formation in the absence of artificial inoculation serves as a critical indicator of the soil's indigenous rhizobial potential. Our results revealed that the experimental site hosts a viable and functionally active population of native *Bradyrhizobium* strains capable of establishing symbiosis with white lupin (*Lupinus albus* L.). Across all tested cultivars, plants developed a trial mean of 10.59 nodules per root system (Table 2).

A key result of this research is the substantial nodule development observed even without artificial inoculation.

This spontaneous nodulation can be attributed to the established rhizobial presence within the experimental site. Although the preceding crop in 2024 was winter barley (*Hordeum vulgare* L.), the field's extensive history, marked by the frequent cultivation of legumes like beans, chickpeas, lentils, peas, white lupin and blue lupin, has likely maintained a persistent microbial reservoir. The survival of these indigenous populations highlights the environmental persistence of native *Bradyrhizobium*, which continued to colonize the lupin roots effectively without the need for new inoculants. Statistically the cultivars Medi and Mariuca exhibited the highest numerical values, with 11.33 and 11.15 nodules per plant, respectively (Table 2). These figures align with findings by Tounsi-Hammami et al. (2021), who observed that in non-inoculated Mediterranean soils, *Lupinus albus* maintains a baseline nodulation similar to our results. However, as noted by those authors, while baseline nodulation exists, it often falls below the threshold required for maximum biomass

production compared to inoculated treatments. The Analysis of Variance (ANOVA) for nodule count (Table 3) yielded a p-value of 0.6326, indicating that the differences between cultivars were not statistically significant at the (5%) threshold. The calculated LSD (0.05) of 3.20 was not exceeded by any pair of cultivars, suggesting a relatively uniform compatibility across the tested cultivars with the indigenous soil microbiota.

Table 2. Descriptive statistics, variability, and significance indicators for the number of nodules per plant in white lupin cultivars

Cultivar	Mean (nodules/plant)	SD*	Observations
Medi	11.33	3.29	Highest variability
Mihai	9.86	0.11	Most consistent results across replications.
Ruxandra	10.01	0.55	Homogeneous results
Mariuca	11.15	0.64	High and stable performance
Trial Mean	10.59		
LSD ($p < 0.05$) - 3.20; CV (%) -16.03			

*SD – standard deviation

Despite the lack of significance, the Coefficient of Variation (CV = 16.03%) and the Standard Deviation (SD) analysis provide deeper insights into cultivar-specific stability. The higher variability observed in Medi (SD = 3.29) implies that while this cultivar possesses a high symbiotic potential, its interaction with native bacteria is more sensitive to micro-environmental fluctuations or soil heterogeneity. In contrast, Mihai demonstrated exceptional stability (SD = 0.11), though at a lower numerical frequency.

Table 3. ANOVA analysis for nodules number to white lupin

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value ns*
Between cultivars	3	5.1912	1.7304	0.6005	0.6326
Residual Error	8	23.0521	2.8815		
Total	11	28.2433			

*(ns) – not significant

These results suggest that in soils with established native populations, cultivar selection may influence the stability of the symbiotic interaction as much as its magnitude.

Seed Yield Performance

The results revealed highly significant differences among the white lupin cultivars studied, with seed yields ranging from 1,080 to 1,440 kg ha⁻¹ (Table 4). The experiment showed a high level of accuracy, evidenced by the low coefficient of variation (CV = 2.86%) and relatively small standard deviations (less than 40 kg ha⁻¹), indicating a strong consistency and uniformity among the replicates.

The cultivar Medi demonstrated the highest adaptive capacity and production potential, recording a very highly significant positive difference of +167 kg ha⁻¹ (113.11%) over the trial mean of 1,273 kg ha⁻¹, establishing this genotype as a reference for high productivity in the analyzed environment. Similarly, Mariuca showed a distinctly significant yield increase of +90 kg ha⁻¹ (107.06%), confirming its status as a high-performing cultivar (Table 4).

In contrast, Ruxandra yielded 1,209 kg ha⁻¹ (94.97% relative yield). Although it produced 64 kg ha⁻¹ less than the control, this difference did not exceed the critical LSD 5% threshold (68.58 kg ha⁻¹), indicating that its performance was statistically comparable to the trial average. The lowest yield was recorded for Mihai (1,080 kg ha⁻¹), which exhibited a very highly significant deficit, producing 15.17% less than the mean, suggesting limited genetic adaptation to the experimental conditions or lower resource-use efficiency for this specific cultivar.

Table 4. Seed yield performance and statistical significance of white lupin cultivars compared to the trial mean (control)

Cultivar	Mean yield (kg ha ⁻¹)	Relative yield (%)	Difference ± Control (kg ha ⁻¹)	SD	Sig.
Medi	1,440	113.11	+167	±39.05	***
Mihai	1,080	84.83	-193	±30.41	000
Ruxandra	1,209	94.97	-64	±37.32	ns
Mariuca	1,363	107.06	+90	±38.43	**
Trial Mean	1,273	100			

Note: LSD 5% = 68.58 kg ha⁻¹; LSD 1% = 99.76 kg ha⁻¹; LSD 0.1% = 149.88 kg ha⁻¹

*** = $p < 0.001$; ** = $p < 0.01$; ns = not significant; 000 = significantly lower at $p < 0.001$.

Table 5. ANOVA analysis for seed yield to white lupin cultivars

Source of Variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value ***
Between cultivars	3	231,342.0	77,114.0	58.14	<0.0001
Residual Error	8	10,610.7	1,326.3		
Total	11	241,952.7			

***– highly significant at $p < 0.001$

The analysis of variance (ANOVA) confirmed the highly significant effect of the cultivar on seed yield ($p < 0.001$) (Table 5).

These results align with findings by López-Bellido et al. (2000), who noted that white lupin yields are highly dependent on genotype-environment interactions, with modern cultivars showing superior harvest indices even under varying climatic conditions.

Correlation Between Nodulation and Seed Yield

The strong positive correlation ($r = 0.962$) suggests that for these specific cultivars (Medi, Mihai, Ruxandra, Mariuca), nitrogen fixation is the primary limiting factor for productivity (Figure 2), a relationship consistent with the findings of Sulas et al. (2016).

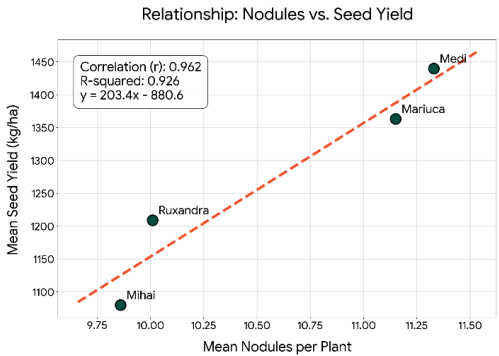


Figure 2. Correlation between nodulation and seed yield in white lupin cultivars

The data indicates that cultivars capable of supporting higher nodule populations, such as Medi (11.33 nodules/plant), are significantly more effective in achieving superior seed yields (1,440 kg ha⁻¹). Medi and Mariuca demonstrated superior symbiotic efficiency. Their ability to maintain higher nodule counts suggests either an enhanced genetic compatibility with

Bradyrhizobium bacteria or a more vigorous root system capable of supporting more infection sites, aligning with the breeding priorities for *Lupinus* species identified by Wolko et al. (2011). Conversely, Mihai was the least productive (1,080 kg ha⁻¹), directly corresponding to its lowest nodule count (9.86). This suggests a potential genetic barrier or a reduced physiological demand for nitrogen that limits its symbiotic potential relative to the top-performing cultivars. With a coefficient of determination R^2 of 0.926, nearly all observed yield variation is dictated by nodulation intensity. This implies that while environmental factors (e.g., soil moisture, temperature) affected the trial uniformly, genotype-specific affinity for local microbiota remained the principal driver of the results, supporting the conclusions of Annicchiarico et al. (2010) regarding the genotypic basis of adaptation as the primary determinant of white lupin productivity.

CONCLUSIONS

This study highlights the crucial role of native soil microbiota, sustained by a long-term persistent microbial reservoir from previous leguminous crops, which provides a solid foundation for white lupin productivity even without artificial inoculation. The strong positive correlation between nodulation and seed yield identifies biological nitrogen fixation as the primary determinant of yield potential in these conditions.

The superior performance of cultivars Medi and Mariuca highlights the importance of genotype-specific affinity for indigenous *Bradyrhizobium* populations. Furthermore, the discrepancy between the high correlation observed and the non-significant variance in nodule quantity (ANOVA) suggests that nodule effectiveness and nitrogen translocation efficiency are more critical to final output than the absolute number of nodules. Therefore, breeding and cultivar selection for sustainable management should prioritize symbiotic functionality as a key indicator of adaptive capacity and high production potential in white lupin.

These findings provide a vital baseline for future research, which should explore whether artificial inoculation can further bridge the yield

gap or enhance the performance of these cultivars.

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EFFECTS OF SOWING DENSITY, TILLAGE SYSTEM (PLOWING VERSUS SCARIFICATION), AND FERTILIZATION LEVEL ON THE PERFORMANCE OF WINTER OILSEED RAPE

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Abstract

The study evaluated the effects of sowing density, tillage system, and fertilization level on the performance of winter oilseed rape (Brassica napus L.). The research was conducted under field conditions at the Agricultural Research and Development Station (SCDA) Lovrin, Romania. A factorial experimental design was used, including two sowing densities, two tillage systems (plowing and scarification), and two fertilization levels, with emphasis on nitrogen input. The observations focused on the production components (thousand kernel weight and seed production). Sowing density significantly influenced plant architecture and yield formation, while the tillage system affected crop establishment and growth dynamics. Plowing generally ensured better early development, whereas scarification represented a suitable alternative depending on soil and climatic conditions. Higher fertilization levels led to increased yield performance, with responses varying according to sowing density and tillage practice. The interaction between experimental factors highlighted the importance of integrated crop management for optimizing winter oilseed rape productivity. The results contribute to improving cultivation strategies adapted to local pedoclimatic conditions in western Romania.

Key words: winter oilseed rape, sowing density, tillage system, nitrogen fertilization, yield.

INTRODUCTION

Winter oilseed rape (*Brassica napus* L.) is one of the most important oil crops worldwide, playing a key role in food, feed, and biofuel production (Suvet et al., 2024). In Europe, and particularly in Romania, rapeseed has become a strategic crop due to its high economic value, favorable place in crop rotations, and contribution to soil structure improvement (Gherasimescu et al., 2023). However, achieving stable and high yields remains challenging, as crop performance is strongly influenced by agronomic management practices and their interaction with local pedoclimatic conditions (Sitnicki et al., 2024).

Among the main agronomic factors affecting winter oilseed rape productivity, sowing density, soil tillage system, and fertilization – especially nitrogen application – are considered decisive (Li et al., 2014; Bouchet et al., 2016). Each of these factors influences plant establishment, canopy structure, nutrient uptake,

and yield components, and their combined effects often determine crop success or failure (Berry et al., 2010). Optimizing these practices is therefore essential to maximize yield while maintaining sustainable input use.

Sowing density plays a crucial role in shaping plant architecture and yield formation in oilseed rape (Bagheri et al., 2011; Lin et al., 2024). Previous studies have shown that low densities favor individual plant development through increased branching and silique number per plant, whereas higher densities enhance yield stability per unit area but may increase competition for resources (Ma et al., 2014; Ren et al., 2017; Kuai et al., 2016). The response of rapeseed to sowing density is also closely linked to nitrogen availability, as sufficient nitrogen supply can partially compensate for higher interplant competition by supporting biomass accumulation and reproductive development (Khan et al., 2017). Liu et al. (2019) demonstrated that inappropriate nitrogen management amplifies yield losses under

suboptimal plant densities, highlighting the importance of adjusting density to fertilization level.

Nitrogen fertilization remains the most influential nutrient factor in rapeseed cultivation due to its strong effect on vegetative growth, photosynthetic capacity, and yield components. Numerous studies have confirmed that increasing nitrogen rates generally leads to higher seed yield, up to an optimal threshold beyond which yield gains diminish and environmental risks increase (Ren et al., 2017; Yahbi et al., 2022). Moreover, recent research emphasizes the need for improving nitrogen use efficiency by adapting nitrogen rates to planting density and crop management systems, rather than relying on uniformly high inputs (Luo et al., 2024; Li et al., 2024). These interactions underline the importance of integrated nitrogen and stand management strategies.

Soil tillage system is another critical factor influencing winter oilseed rape performance, as it affects soil physical properties, root development, water availability, and nutrient dynamics. Conventional plowing has traditionally been favored for rapeseed due to its positive effects on seedbed quality and uniform crop establishment. However, alternative tillage practices, such as scarification or reduced tillage, have gained increasing attention as potential solutions for reducing fuel consumption, labor costs, and soil degradation. Rabiee et al. (2021) reported that different tillage systems interact significantly with nitrogen fertilization in determining yield and agronomic traits of oilseed rape, suggesting that reduced tillage methods can be viable under appropriate fertilization regimes. Similarly, Seepaul et al. (2023) showed that tillage system influences crop response to nitrogen by modifying soil moisture and nutrient availability.

Despite the extensive body of international research on individual effects of sowing density, nitrogen fertilization, and tillage systems, studies assessing their combined impact – particularly under the specific pedoclimatic conditions of Eastern Europe – remain limited (Ahmadi & Bahrani, 2009; Chițu et al., 2024). Romanian agroecosystems are characterized by high interannual climatic variability, which can significantly modify crop responses to

management practices. Consequently, region-specific research is essential to develop practical recommendations adapted to local conditions (Smuleac et al., 2020).

MATERIALS AND METHODS

The field experiment was conducted during the 2024-2025 agricultural season at the Lovrin Agricultural Research and Development Station (SCDA Lovrin), western Romania, on a chernozem soil characteristic of the Banat Plain. The experimental design was trifactorial, including three experimental factors arranged in a completely randomized design, with one observation per experimental unit:

Factor A - Soil tillage and fertilization system (4 levels):

a₁ – Scarification + NPK 10–20–0;

a₂ – Scarification + NPK 15–15–15;

a₃ – Ploughing + NPK 10–20–0;

a₄ – Ploughing + NPK 15–15–15.

For all fertilization variants included in Factor A, a uniform dose of 200 kg ha⁻¹ commercial fertilizer was applied, irrespective of the fertilizer type (NPK 10–20–0 or NPK 15–15–15). Factor B – Hybrid (33 levels): Thirty-three commercial winter oilseed rape (*Brassica napus* L.) hybrids (LID TEBO, CS CALCULATI CL, CS BESSITO, LID INVICTO, LDC 23135, LDC 23140, LID CALIENTO, GENEROSO, ES CAPELLO, DK EXCITED, DK EXUM, DK EXBURY, DK IMMORTAL CL, DK IMPRINT CL, DK EXPOSE, SY GLORIETTA, SY ROBOT CL, SY FLORETTA, PT 312, PT 315, PT 303, PT 314, NS RAS, NS PEK, NS VIR, NS VID, NS DIV, NS DUNAV, NS MARIN, ZLATNA, ANNA, ZORICA, ILIA).

Factor C – Plant density (2 levels):

c₁ – 35 plants m⁻²;

c₂ – 50 plants m⁻².

Sowing was performed in autumn under optimal agrotechnical conditions for the area. Crop management practices (weed, pest, and disease control) were applied uniformly across all experimental variants.

The thousand seed weight (TSW) was determined at harvest maturity, according to standard laboratory procedures. Data were subjected to analysis of variance (ANOVA) to assess the effects of the studied factors on TSW.

Mean comparisons were performed using the Duncan multiple range test, at a 5% significance level. The relative contribution of each experimental factor to TSW variation was also calculated. Statistical analyses were carried out using STATISTICA software.

RESULTS AND DISCUSSIONS

Effect of soil tillage and fertilization system on thousand seed weight (TSW)

The analysis of variance (ANOVA) for thousand seed weight (TSW) revealed a very significant effect ($p < 0.001$) of the soil tillage and fertilization system (Factor A), as shown in Table 1. The mean TSW values varied between 4.86 g and 4.43 g, indicating a clear differentiation among the four technological variants.

Table 1. Effect of soil tillage and fertilization system on thousand seed weight (TSW)

Soil tillage and fertilization system	TSW	Std. Err.	-95.00%	+95.00%
a ₁ – Scarification + NPK 10–20–0	4.86	0.045553	4.769025	4.950975
a ₂ – Scarification + NPK 15–15–15	4.63	0.045033	4.544911	4.724786
a ₃ – Ploughing + NPK 10–20–0	4.79	0.064097	4.663655	4.919678
a ₄ – Ploughing + NPK 15–15–15	4.43	0.045193	4.343834	4.524348

As illustrated in Figure 1, the highest TSW values were recorded under scarification combined with NPK 10–20–0 (a₁: 4.86 g) and ploughing combined with NPK 10–20–0 (a₃: 4.79 g). According to Duncan's multiple range test (Table 2), both treatments were grouped in class A, showing statistically significant superiority compared with the other fertilization systems.

Conversely, the lowest TSW value (4.43 g) was obtained under ploughing combined with NPK 15–15–15 (a₄), which formed a distinct statistical group (class C).

These results indicate that the fertilizer formulation (10–20–0) had a stronger influence on seed weight than the soil tillage system itself, as similar TSW values were obtained under both scarification and ploughing when this fertilizer was applied.

Influence of hybrid on thousand seed weight

Hybrid genotype (Factor B) exerted a highly significant influence on TSW ($p < 0.001$), as indicated in Table 3 and illustrated in Figure 2.

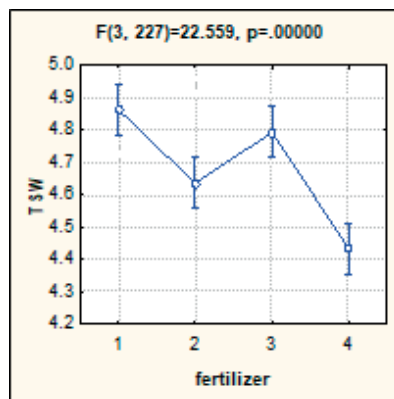


Figure 1. Variation TSW, factor A - test F

Table 2. DUNCAN TEST for factor A DL 5% = 0.11g

Original Date	Sorate Date
Mean 1 = 4.860 A	Mean 1 = 4.860 A
Mean 2 = 4.630 B	Mean 3 = 4.790 A
Mean 3 = 4.790 A	Mean 2 = 4.630 B
Mean 4 = 4.430 C	Mean 4 = 4.430 C

a₁—scarified fat used: 10-20-0; a₂—scarified fat used: 15-15-15
a₃—fat ploughing used: 10-20-0; a₄—fat ploughing used: 15-15-15

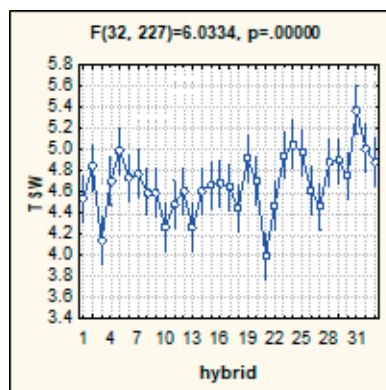


Figure 2. Variation TSW, factor B - test F

Substantial variability was observed among the 33 rapeseed hybrids, with mean TSW values ranging from 4.00 g to 5.37 g.

The highest TSW was recorded for hybrid ANNA (b31), which reached 5.37 g and was classified alone in Duncan class A (Table 4). Other hybrids exceeding 5.00 g included NS PEK (b24) and ZORICA (b32), confirming their superior seed development potential under the tested conditions.

In contrast, the lowest TSW values were obtained for PT 303 (b21: 4.00 g) and CS BESSITO (b3: 4.14 g), which were placed in the lowest statistical classes.

Table 3. Influence of hybrid on thousand seed weight

Hybrid	TSW	Std.Err.	-95.00%	+95.00%
LID TEBO	4.54	0.078848	4.351055	4.723945
CS CALCULATI CL	4.84	0.111515	4.572560	5.099940
CS BESSITO	4.14	0.181610	3.705561	4.564439
LID INVICTO	4.70	0.108447	4.443564	4.956436
LDC 23135	4.99	0.128521	4.689846	5.297654
LDC 23140	4.74	0.108595	4.478214	4.991786
LID CALIENTO	4.78	0.127692	4.476807	5.080693
GENEROSO	4.60	0.093052	4.378717	4.818783
ES CAPELLO	4.59	0.077982	4.403101	4.771899
DK EXCITED	4.28	0.129931	3.967761	4.582239
DK EXUM	4.49	0.079614	4.299243	4.675757
DK EXBURY	4.60	0.109739	4.344259	4.863241
DK IMMORTAL CL	4.26	0.116840	3.984968	4.537532
DK IMPRINT CL	4.61	0.056740	4.474581	4.742919
DK EXPOSE	4.66	0.060723	4.517663	4.804837
SY GLORIETTA	4.68	0.101941	4.436448	4.918552
SY ROBOT CL	4.65	0.124656	4.351486	4.941014
SY FLORETTA	4.45	0.093269	4.226954	4.668046
PT 312	4.91	0.240278	4.343082	5.479418
PT 315	4.70	0.132166	4.387477	5.012523
PT 303	4.00	0.186193	3.554723	4.435277
PT 314	4.46	0.196666	3.997458	4.927542
NS RAS	4.94	0.191915	4.488694	5.396306
NS PEK	5.04	0.054511	4.911102	5.168898
NS VIR	4.97	0.100694	4.726896	5.203104
NS VID	4.61	0.116258	4.333844	4.883656
NS DIV	4.46	0.114532	4.192924	4.734576
NS DUNAV	4.89	0.094130	4.663667	5.108833
NS MARIN	4.89	0.110023	4.631086	5.151414
ZLATNA	4.76	0.085670	4.552424	4.957576
ANNA	5.37	0.222999	4.842691	5.897309
ZORICA	5.01	0.126786	4.706448	5.306052
ILIA	4.88	0.136433	4.553636	5.198864

The wide range of Duncan groupings (A–O) highlights the predominant role of genetic factors in determining thousand seed weight, a finding consistent with previous reports on rapeseed yield components

Effect of plant density on thousand seed weight

Plant density (Factor C) did not significantly affect TSW ($p = 0.933$), as shown in Table 5 and Figure 3. The mean values were 4.682 g for 35 plants m^{-2} and 4.679 g for 50 plants m^{-2} , indicating almost identical seed weights.

Table 4. Table TEST DUNCAN for Factor B [hybrid]
DL 5% = 0.32 g

Original Date		Sorate Date	
Mean 1 = 4.540	IJKLM	Mean 31 = 5.370	A
Mean 2 = 4.840	BCDEFGHI	Mean 24 = 5.040	B
Mean 3 = 4.140	NO	Mean 32 = 5.010	BC
Mean 4 = 4.700	CDEFGHIJK	Mean 5 = 4.990	BCD
Mean 5 = 4.990	BCD	Mean 25 = 4.970	BCDE
Mean 6 = 4.740	BCDEFGHIJK	Mean 23 = 4.940	BCDEF
Mean 7 = 4.780	BCDEFGHIJ	Mean 19 = 4.910	BCDEFG
Mean 8 = 4.600	GHIJK	Mean 28 = 4.890	BCDEFGH
Mean 9 = 4.590	HIJKL	Mean 29 = 4.890	BCDEFGH
Mean 10 = 4.280	LMNO	Mean 33 = 4.880	BCDEFGH
Mean 11 = 4.490	JKLM	Mean 2 = 4.840	BCDEFGHI
Mean 12 = 4.600	GHIJK	Mean 7 = 4.780	BCDEFGHIJ
Mean 13 = 4.260	MNO	Mean 30 = 4.760	BCDEFGHIJ
Mean 14 = 4.610	GHIJK	Mean 6 = 4.740	BCDEFGHIJK
Mean 15 = 4.660	EFGHIJK	Mean 4 = 4.700	CDEFGHIJK
Mean 16 = 4.680	DEFGHIJK	Mean 20 = 4.700	CDEFGHIJK
Mean 17 = 4.650	FGHIJK	Mean 16 = 4.680	DEFGHIJK
Mean 18 = 4.450	KLMN	Mean 15 = 4.660	EFGHIJK
Mean 19 = 4.910	BCDEFG	Mean 17 = 4.650	FGHIJK
Mean 20 = 4.700	CDEFGHIJK	Mean 26 = 4.610	GHIJK
Mean 21 = 4.000	O	Mean 14 = 4.610	GHIJK
Mean 22 = 4.460	KLM	Mean 12 = 4.600	GHIJK
Mean 23 = 4.940	BCDEF	Mean 8 = 4.600	GHIJK
Mean 24 = 5.040	B	Mean 9 = 4.590	HIJKL
Mean 25 = 4.970	BCDE	Mean 1 = 4.540	IJKLM
Mean 26 = 4.610	GHIJK	Mean 11 = 4.490	JKLM
Mean 27 = 4.460	KLM	Mean 27 = 4.460	KLM
Mean 28 = 4.890	BCDEFGH	Mean 22 = 4.460	KLM
Mean 29 = 4.890	BCDEFGH	Mean 18 = 4.450	KLMN
Mean 30 = 4.760	BCDEFGHIJK	Mean 10 = 4.280	LMNO
Mean 31 = 5.370	A	Mean 13 = 4.260	MNO
Mean 32 = 5.010	BC	Mean 3 = 4.140	NO
Mean 33 = 4.880	BCDEFGH	Mean 21 = 4.000	O

Table 5. Effect of plant density on thousand seed weight

Density	TSW	Std.Err.	-95.00%	+95.00%
c1 – 35 pl/mp	4.6818	0.038083	4.606481	4.757155
c2 – 50 pl/mp	4.6785	0.038774	4.601781	4.755189

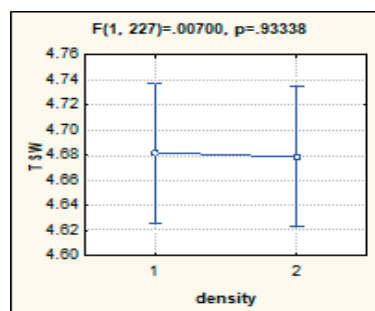


Figure 3. Variation TSW, factor C - test F

These results suggest that, within the tested density range, rapeseed plants compensated effectively through morphological and physiological adjustments, maintaining stable seed weight regardless of stand density.

Contribution of experimental factors to TSW variability

The relative contribution of the studied factors to TSW variability is presented in Figure 4. Hybrid (Factor B) accounted for the largest share (39.6%), followed by the soil tillage and fertilization system (Factor A) with 14%, while plant density contributed only 0.001%.

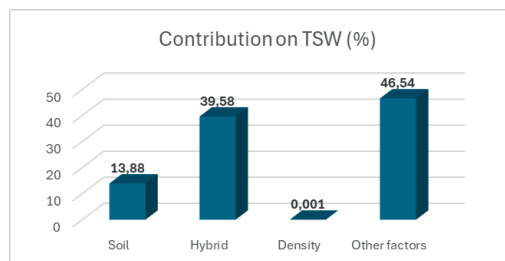


Figure 4. Contribution on TSW of factors A [Soil tillage and fertilization system], B [hybrid], C [density]

The three factors explained approximately 53% of the total TSW variation, indicating that environmental conditions and other unmeasured factors also played an important role in determining seed weight.

Cluster analysis

Cluster analysis further supported the differentiation among treatments and hybrids. The dendrogram for the soil tillage and fertilization system (Figure 5) clearly separated treatments based on fertilizer type, while the dendrogram for hybrids (Figure 6) highlighted distinct genetic groupings associated with TSW performance.

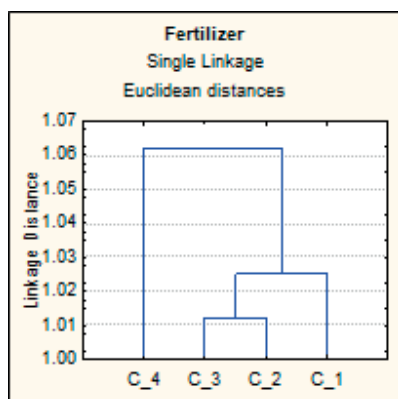


Figure 5. Dendrogram for the soil tillage and fertilization system

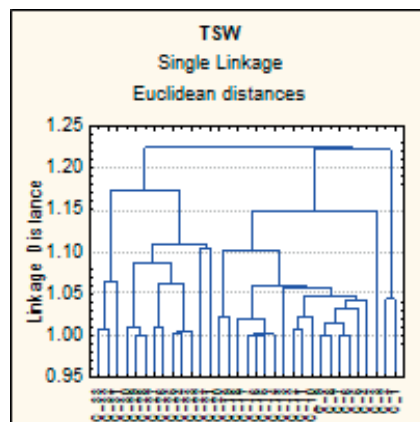


Figure 6. Dendrogram for hybrids

These results confirm the strong genetic control of thousand seed weight and the secondary, yet significant, influence of fertilization strategy.

Interpretation of Yield Results (kg ha^{-1})

The analysis of variance for seed yield (Table 6) showed that the soil tillage and fertilization system (Factor A) and hybrid (Factor B) had a highly significant effect on rapeseed yield ($p < 0.001$), whereas plant density (Factor C) had no statistically significant influence ($p > 0.05$). Therefore, the null hypothesis was rejected for Factors A and B but accepted for Factor C.

Effect of Soil Tillage and Fertilization System

As presented in Table 7, average yield values ranged from $3,221 \text{ kg ha}^{-1}$ under scarification combined with NPK 10–20–0 (a_1) to $4,913 \text{ kg ha}^{-1}$ under ploughing combined with NPK 15–15–15 (a_4).

Duncan's multiple range test classified treatment a_4 in the highest significance group (A), with yields significantly higher than all other treatments. Conversely, treatments a_1 (scarification + NPK 10–20–0) and a_3 (ploughing + NPK 10–20–0) were included in the lowest significance group (C), with yields around $3,200\text{--}3,400 \text{ kg ha}^{-1}$.

These results indicate that the balanced NPK 15–15–15 fertilizer significantly enhanced yield, regardless of the tillage method, while the NPK 10–20–0 formulation was less effective.

Table 6. The analysis of variance for seed yield

Source of variation	SSP [SP]	Weighted Sum of Squares [s ²]	Test F		Significance
			Value	p	
A[Soil tillage and fertilization system]	132812745.0	44270915.01	53.35	0.00000	***
B[Hybrid]	89668904.8	2802153.27	3.38	0.00000	***
C[Density]	2613666.0	2613666.00	3.15	0.07729	ns
Error	188382550.0	829879.07			
Total	413477865.8				

Table 7. Effect of factor A on rapeseed production

Factor A [Soil tillage and fertilization system]	Production kg/ha	Std.Err.	-95.00%	+95.00%
a ₁ – Scarification + NPK 10–20–0	3221	88.4415	3044.203	3397.463
a ₂ – Scarification + NPK 15–15–15	4520	160.1383	4200.560	4840.197
a ₃ – Ploughing + NPK 10–20–0	3449	86.9697	3275.082	3622.463
a ₄ – Ploughing + NPK 15–15–15	4913	156.1832	4600.884	5224.722

Effect of Hybrid

Significant yield differences were recorded among the 33 hybrids (Table 8). The highest average yield was obtained for hybrid PT 315 (b20), with approximately 5,523 kg ha⁻¹, which was significantly superior to all other hybrids. Other high-yielding hybrids included PT 303 (b21) and PT 314 (b22), with yields exceeding 5,200 kg ha⁻¹. In contrast, the lowest yield was recorded for SY ROBOT CL (b17), with approximately 2,821 kg ha⁻¹, significantly lower than the remaining hybrids.

Table 8. Effect of factor B on rapeseed production

Factor B (Hybrid)	Production kg/ha	Std.Err.	-95.00%	+95.00%
LID TEBO	3518	257.8950	2907.925	4127.575
CS CALCULATI CL	4171	363.3661	3311.776	5030.224
CS BESSITO	3713	276.2755	3060.087	4366.663
LID INVICTO	3742	217.7027	3226.965	4256.535
LDC 23135	4133	160.5304	3752.906	4512.094
LDC 23140	4393	169.3057	3992.906	4793.594
LID CALIENTO	3791	168.2138	3393.613	4189.137
GENEROSO	4151	177.0700	3732.296	4569.704
ES CAPELLO	4066	170.9087	3661.365	4469.635
DK EXCITED	3341	97.8788	3109.054	3571.946
DK EXUM	3493	139.7894	3162.201	3823.299
DK EXBURY	3520	206.0956	3032.411	4007.089
DK IMMORTAL CL	3522	191.5164	3069.136	3974.864
DK IMPRINT CL	3672	102.3671	3430.190	3914.310
DK EXPOSE	3915	102.5394	3672.033	4156.967

Factor B (Hybrid)	Production kg/ha	Std.Err.	-95.00%	+95.00%
SY GLORIETTA	2910	92.9942	2690.479	3130.271
SY ROBOT CL	2821	430.2410	1803.517	3838.233
SY FLORETTA	3817	534.2624	2553.295	5079.955
PT 312	4706	532.4656	3447.294	5965.456
PT 315	5523	694.1504	3881.470	7164.280
PT 303	5346	576.4453	3982.549	6708.701
PT 314	5206	512.8761	3993.366	6418.884
NS RAS	4291	539.1326	3016.529	5566.221
NS PEK	4075	476.4807	2948.177	5201.573
NS VIR	4079	525.8430	2835.579	5322.421
NS VID	3906	490.6645	2745.888	5066.362
NS DIV	3969	417.2657	2981.948	4955.302
NS DUNAV	4072	574.9981	2712.346	5431.654
NS MARIN	4319	530.5699	3063.902	5573.098
ZLATNA	4253	464.9040	3153.802	5352.448
ANNA	4165	606.7718	2729.838	5599.412
ZORICA	4500	679.5666	2893.080	6106.920
ILIA	3752	693.0147	2112.906	5390.344

Effect of Plant Density

Mean yields for the two plant densities were 3,926 kg ha⁻¹ at 35 plants m⁻² (c₁) and 4,125 kg ha⁻¹ at 50 plants m⁻² (c₂) (Table 9). However, the differences were not statistically significant (p = 0.077), indicating that both densities provided comparable yield levels under the experimental conditions.

Table 9. Effect of Plant Density

Factor C (Density)	Production kg/ha	Std.Err.	-95.00%	+95.00%
c ₁ – 35 pl/mp	3926	108.8473	3710.871	4141.523
c ₂ – 50 pl/mp	4125	109.1450	3909.282	4341.112

CONCLUSIONS

The results obtained under the pedoclimatic conditions of SCDA Lovrin highlight the differentiated influence of soil tillage and fertilization system, hybrid, and plant density on thousand seed weight (TSW) and seed yield of winter oilseed rape.

Thousand seed weight was significantly influenced by the soil tillage and fertilization system as well as by the hybrid factor, while plant density had no statistically significant effect. The highest TSW values were consistently associated with the fertilization variant based on NPK 10–20–0, regardless of

whether scarification or ploughing was applied. This indicates that phosphorus availability plays a key role in seed filling processes. Hybrid variability accounted for the largest proportion of TSW variation, confirming the strong genetic control over this trait and emphasizing the importance of hybrid selection in achieving superior seed quality.

Seed yield was significantly affected by both the soil tillage and fertilization system and the hybrid, whereas plant density showed no significant effect within the tested range. The highest yields were obtained under ploughing combined with balanced fertilization (NPK 15–15–15), suggesting that higher nitrogen input, together with adequate soil loosening, enhances biomass accumulation and yield formation. Yield differences among hybrids were substantial, reflecting contrasting genetic potential and adaptability to local conditions. The results demonstrate that hybrid choice and fertilization strategy are the main drivers of both yield and seed quality, while moderate variations in plant density exert a limited influence. These findings support the optimization of fertilization regimes and hybrid selection as key components for improving winter oilseed rape performance in western Romania.

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HYBRID PERCOLATION-ULTRASOUND TECHNOLOGY TO IMPROVE EXTRACTION OF BIOACTIVE SUBSTANCES FROM HEMP BUDS FOR AGRICULTURAL APPLICATIONS

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Abstract

The increasing interest in hemp (Cannabis sativa L.) as a source of high-value bioactive compounds has driven the development of efficient and environmentally responsible extraction technologies. Hemp buds contain substantial levels of polyphenols with antioxidant, anti-inflammatory, and antimicrobial properties, making them valuable for functional food, nutraceutical, and cosmetic applications. Conventional extraction methods often require long processing times, high solvent volumes, and provide limited recovery of thermolabile compounds. This study evaluates a hybrid extraction technique that combines percolation with ultrasound-assisted extraction to improve the yield and functionality of polyphenolic fractions from hemp buds. Key parameters - solvent type, percolation rate, ultrasound intensity, and extraction duration - were systematically varied and optimized using response surface methodology. Extraction performance was assessed through total polyphenol content, total flavonoid content, and antioxidant activity, with chromatographic analyses used to quantify major phenolic constituents. The hybrid system outperformed individual techniques, achieving higher polyphenol yields with shorter extraction times and reduced solvent consumption. The resulting extracts showed strong antioxidant capacity associated with their enriched chemical profiles.

Key words: ultrasound-assisted extraction, bioactive substances, polyphenols, percolation, flavonoids.

INTRODUCTION

Hemp buds and inflorescences represent the anatomical structures of the plant with the highest concentrations of bioactive secondary metabolites, including terpenes (α -pinene, myrcene, limonene, β -caryophyllene), polyphenolic compounds, and phytocannabinoids such as cannabidiol (CBD) and cannabidiolic acid (CBDA). These compounds are predominantly localized within glandular trichomes, conferring a broad and complex biological activity profile (Ascrizzi et al., 2024; Yusoff et al., 2021). The extracts obtained from the inflorescences have been shown to be biostimulants, stimulating the germination and development of plants,

increasing biomass, chlorophyll and carotenoid content, and improving the antioxidant activity of the treated crops, which suggests an improved photosynthetic efficiency and stress response (Varga et al., 2025). At the same time, hydrophilic extracts from buds can manifest significant inhibitory effects on fungi due to their high content of polyphenols and antioxidants (Kursa et al., 2024). The essential oils extracted from the inflorescences showed insecticidal and repellent effects, causing high mortality in the larvae of some insect pests and over 80% effectiveness against the *Tetranychus urticae* mite, due to the high concentrations of volatile terpenes (Rossi et al., 2020; Prvulović et al., 2023). At higher concentrations, inflorescence extracts may exhibit allelopathic

activity, suppressing the germination of certain plant species. This suggests their potential applicability in the development of bioherbicide formulations and their use in sustainable soil management practices (Kojić et al., 2025).

Given this diversity of bioactive compounds and their application potential, the process of obtaining extracts becomes an essential element for the optimal use of plant resources. However, traditional extraction methods have several disadvantages: they often use toxic solvents in large quantities, are time-consuming and consume a lot of energy (Cao et al., 2025). In contrast, modern hybrid technologies, such as ultrasonically assisted percolation extraction, offer a much more efficient solution for obtaining these valuable substances. Ultrasound facilitates the rapid release of compounds from plant cell structures, reduces extraction time and increases yield, while requiring lower energy consumption (Nenciu et al., 2023; Tăbărașu et al., 2025).

In the study (Kobus et al., 2022), the extraction of bioactive compounds from hemp leaves and buds was performed by ultrasonically assisted extraction, using an aqueous solution of 60% ethanol as a solvent. The extraction process was carried out at a frequency of 20 kHz and at a controlled temperature of 30°C. The efficiency of the extraction was evaluated by determining the total flavonoid content, the values obtained varying between 3.02 and 9.35 mg QE/g plant material. In another study (Tiago et al., 2022), the extraction of total phenols from hemp was achieved by both ultrasound-assisted extraction and conventional Soxhlet extraction. Ultrasonic extraction was performed at 60°C for 90 minutes and a solid-liquid ratio of 1:10, resulting in a high polyphenol yield of approximately 7.76 mg GAE/g plant material. In contrast, the Soxhlet ethanol extraction, carried out for 90 minutes, generated a significantly lower content of phenolic compounds, of about 0.26 mg GAE/g plant material.

In the study (Lazarević et al., 2025), the extraction of total phenols from the flower buds was performed by conventional extraction and ultrasonically assisted extraction. Conventional extraction was performed at room temperature for 24 hours using a solid-liquid ratio of 1:10. The results showed that 50% ethanol led to the highest concentrations of phenolic compounds,

with the total phenol content varying, depending on the stage of harvest, between approximately 0.4996 and 1.7045 mg GAE/mL extract. For ultrasonically assisted extraction, a 40 kHz ultrasonic bath was used, maintaining the same solid-liquid ratio (1:10) and choosing 50% ethanol as solvent. The varied process parameters were temperature (40-80°C), extraction time (20-60 min) and ultrasonic power density (24-60 W/L). Under these conditions, the total phenol content of the extracts ranged from 1.0552 to 1.7795 mg GAE/mL.

In the study (Chemat et al., 2025), the extraction of phenolic compounds from *Cannabis sativa* L. seeds was achieved by subcritical extraction with microwave-assisted water. The extraction process was carried out at essential parameters such as temperature (100-180°C), extraction time (5-60 min) and solid-liquid ratio (1:5-1:30 g/v). The maximum reported value was approximately 6.57 g GAE/100 g extract.

The synergistic integration of cannabinoids and polyphenols into crop protection strategies represents a novel approach to sustainable agriculture, transforming the secondary metabolite spectrum of the *Cannabis sativa* L. plant into a multipurpose biochemical shield. While cannabinoids exert direct insecticidal and repellent pressure on pests by disrupting neurophysiological processes, the polyphenolic fraction adds an essential layer of protection through its robust antioxidant and antimicrobial properties (Benelli et al., 2018). These phenolic compounds, including flavonoids and phenolic acids, act as inhibitors of insect digestive enzymes and strengthen the structural barriers of treated plants, while simultaneously providing protection against oxidative stress induced by environmental factors (Bass et al., 2014). Unlike synthetic pesticides, this hybrid phytochemical complex exhibits superior biodegradability and minimal toxicity to non-target organisms, facilitating the ecological management of agroecosystems (Lupi et al., 2013). Utilizing these bioactive fractions not only minimizes dependence on conventional chemical inputs but also valorizes industrial biomass in the spirit of a circular economy, transforming extraction by-products into renewable solutions for restoring soil health and optimizing horticultural productivity (Benelli et al., 2017).

From the analyzed studies, it appears that conventional extraction methods are associated with longer process times and lower yields of phenolic compounds caused by the increased temperatures affecting certain classes of thermosensitive polyphenols (Tăbărașu et al., 2024; Oprescu et al., 2023).

The use of hemp extracts is a promising solution for organic farming, due to the high content of bioactive compounds with antifungal and insecticidal effect. These properties allow crops to be protected from pathogens and pests, reducing the need for the use of synthetic chemical pesticides (Ona et al., 2022; Selçuk et al., 2022). In the study (Kursa et al., 2024), hemp extracts, rich in polyphenols, flavonoids and cannabinoids, demonstrated pronounced antifungal activity against phytopathogenic fungi such as *Botrytis cinerea*, *Alternaria alternata* and *Colletotrichum coccodes*, supporting their potential as natural alternatives to conventional chemical fungicides.

The aim of the present research was to assess the potential of a hybrid ultrasound-percolation technology for extracting polyphenols from hemp buds, for an improved recovery of polyphenolic compounds, total cannabinoids, nitrogen, flavonoids, and minerals. The integration of acoustic cavitation with continuous solvent displacement provides a superior extraction kinetic, significantly enhancing process efficiency while maintaining a low-cost, scalable operational profile.

MATERIALS AND METHODS

The hybrid extraction equipment consists of a Timatic DUO percolator (Tecnolab SRL, Spello, Italy) (Figure 1) and a UP400St ultrasonic generator (Hielscher Ultrasonics GmbH, Teltow, Germany). The percolator includes an extraction chamber, two hydropneumatic cylinders, a pump, solenoid valves, an expansion vessel, a pneumatic distributor, a compressor, a control panel, a pressure sensor and a tank for collecting the extract. For the realization of the hybrid extraction, the extraction chamber of the percolator was technically adapted by integrating the ultrasonic probe of the ultrasonic generator UP400St (Figure 2).



Figure 1. Timatic DUO percolator: 1 - extraction chamber; 2 - control panel; 3 - extract collection vessel; 4 - expansion vessel; 5 – compressor

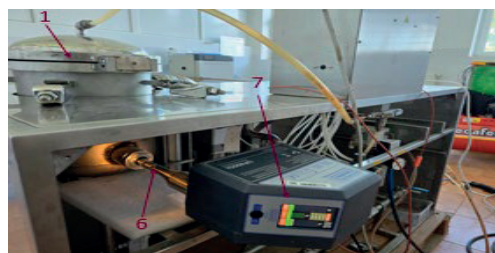


Figure 2. UP400St Ultrasonic Generator: 1 - chamber extractive; 6 - sonotrode; 7 - panel ultrasonic generator control

The hybrid extraction process begins by supplying the system with compressed air generated by the compressor, distributed through the pneumatic distributor to the solenoid valves and hydropneumatic cylinders. The solvent is pumped out of the expansion vessel with the help of the pump and introduced into the extraction chamber, where the plant material is located. Here the pressure extraction process takes place, monitored by the pressure sensor, and assisted by ultrasound generated by the ultrasonic device. After the extraction is completed, the extract is discharged through the automatically controlled valves and collected into the extract tank. The total polyphenols were extracted in a water/methanol mixture (1:1), followed by centrifugation and analysis by the Folin-Ciocalteu method. A permeable bag was used to immobilize the plants in the extraction chamber (Figure 3).

The hybrid ultrasound-assisted percolation (UAP) extraction process optimizes the recovery of bioactive compounds by integrating acoustic cavitation forces into a continuous solvent flow.

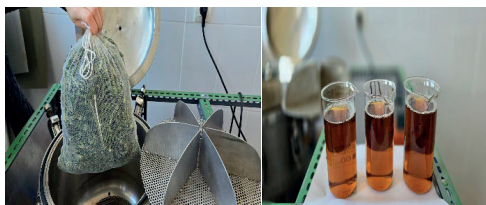


Figure 3. Biomass sample introduction into the extraction chamber and the obtained extracts

Simultaneously, percolation (pressure increase to 7 bar, maintaining it for 5 minutes, followed by a rapid decreasing in pressure to 0.15 bar and maintaining it for 2 minutes) maintains a constant concentration gradient, transporting the extracted metabolites out of the plant matrix and preventing solvent saturation. This synergy drastically reduces processing time and solvent consumption while preserving the thermal integrity of sensitive molecules through controlled power output.

To evaluate the biochemical profile, extracts were obtained from the buds and inflorescences of the Marideea hemp variety, a monoecious industrial cultivar developed at SCDA Secuieni, utilizing an optimized ultrasound-assisted percolation framework. This variety constitutes an ideal feedstock for industrial-scale extraction, providing chemical uniformity and high biomass availability, while being characterized by its adaptability to Eastern European pedoclimatic conditions and a THC profile strictly compliant with current European regulations. Its significant concentration of secondary metabolites, particularly polyphenols and cannabinoids within the floral by-products, makes it an ideal candidate for intensification through hybrid ultrasound-percolation modalities.

The total phenolic content was quantified using the Folin-Ciocalteu spectrophotometric assay and expressed as Gallic Acid Equivalents (GAE). Antioxidant capacity was evaluated through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, measuring the percentage of radical inhibition. For the nutritional profile, total nitrogen was determined via the Kjeldahl method, involving sulfuric acid digestion followed by distillation and titration. Total cannabinoids (CBD and CBDA), were analyzed using High-Performance Liquid Chromatography (HPLC-UV) to ensure the separation of neutral and

acidic forms without thermal degradation. vitamin C levels were assessed through redox titration, while the structural and lipid components, specifically crude fiber and crude fat, were quantified using enzymatic-gravimetric digestion and Soxhlet extraction with non-polar solvents, respectively. Soluble solids were monitored using refractometry and expressed in degrees Brix.

RESULTS AND DISCUSSIONS

By utilizing ultrasound-assisted percolation, the research evaluates the technical capacity to mobilize bioactive compounds into a highly bioavailable liquid format. The objective of this study was not the analysis of the hemp specie (which would have required a multi-year analysis), but prioritized the efficiency of the extraction method considering the plant genetic limitations.

Table 1. Evaluation of bioactive compounds from Marideea (Secuieni) cultivar biomass and ultrasound-assisted percolates extraction method

Compound Group	Values in Plant	Ultrasound-Percolation Extraction	Role as Fertilizer / Biostimulator
Total Cannabinoids (CBD/CBDA)	0.73%	0.61%	Antifungal effect and pest repellent.
Total Polyphenols	34.71 mg GAE/g	29 mg/g	UV protection and resistance to pathogens.
Organic Nitrogen (N)	3.68%	3.15%	Primary source for protein synthesis in new crops.
Amino Acids (glutamic, aspartic)	155 mg/g	105 mg/g	Natural chelation of micronutrients and cold resistance.
Phenolic Acids & Flavonoids	34.71 mg GAE/g	31.8 mg/g	Strong antioxidant; protects plants from thermal stress.
Chelated Minerals (K, Mg, Ca)	K: 1.8%	K: 1.1%	Essential for photosynthesis and cell wall rigidity.
	Mg: 0.4%	Mg: 0.25%	
	Ca: 1.2%	Ca: 0.9%	
Phytohormones (Cytokinins, Auxins)	9 µg/g	6 µg/g	Stimulates cell division and root development.
Vitamin C	65 mg/100 g	52 mg/100g	Enzyme cofactor; helps detoxify plants under stress.
Sugars (Brix)	14.06 °Bx	12.9 °Bx	Energy source for soil microorganisms.

The integration of ultrasonic cavitation effectively bypasses the plant's significant fiber

matrix to release a high-performance concentration of bioactive compounds. The high recovery rate ensures that the resulting liquid serves as a potent nitrogen source for protein synthesis, while the vitamin C acts as a critical enzyme cofactor to help plants detoxify under environmental stress. Furthermore, the extraction process successfully captures amino acids like glutamic and aspartic acid at concentrations which are essential for the natural chelation of micronutrients and increasing a crop's resistance to cold temperatures. Beyond basic nutrition, the Marideea variety extract stands out due to its massive antioxidant capacity, evidenced by the 34.71 mg GAE/g of total polyphenols measured in the sample. The ultrasound-assisted percolation efficiently transfers these into the extract at levels between 31.0 and 33.0 mg/g, providing a "biological shield" that offers UV protection and increased resistance against pathogenic agents. This is complemented by the

CONCLUSIONS

Experimental data showed that the hybrid ultrasound-assisted percolation (UAP) technology is suitable for the extraction of target bioactive substances from the Marideea hemp variety. Analytical assessments of the resulting extracts indicate a superior recovery rate for both cannabinoids and polyphenols, surpassing the yields typically achieved through conventional maceration or simple percolation. This increased efficiency is attributed to the mechanical effects of acoustic cavitation, which ensures the total disruption of glandular trichomes and parenchymal tissues, thereby maximizing the mass transfer of secondary metabolites into the solvent. Furthermore, the continuous flow nature of the system maintained a high kinetic gradient, preventing the degradative thermal accumulation often associated with prolonged sonication. The stability of the phytochemical profile confirms that the UAP parameters were optimally calibrated to balance extraction power with molecular integrity. Given that monoecious hemp varieties exhibit lower cannabinoid concentrations compared to high-resin dioecious cultivars, the present research focused primarily on optimizing extraction

0.61% total cannabinoids successfully extracted from the initial 0.73% found in the plant, which contributes an antifungal effect and acts as a natural repellent for various pests.

The mineral profile is equally impressive, with the process yielding 1.6% Potassium, 0.35% Magnesium, and 0.9% Calcium in chelated forms that are vital for maintaining photosynthesis and structural cell wall rigidity in treated plants. When these minerals are combined with the extracted phytohormones (up to 12 µg/g) and a sugar content of 13.5 °Bx, the resulting biostimulant not only triggers root development and cell division but also provides an immediate energy source for beneficial soil microorganisms. Ultimately, the high fiber content of 25.91% and ash content of 8.30% in the base plant material are effectively bypassed by the ultrasonic method to create a concentrated, bioactive fertilizer that far exceeds the performance of standard organic inputs.

efficiency rather than on the inherent genetic limitations of the plant material.

ACKNOWLEDGEMENTS

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INFLUENCE OF BIOFERTILIZERS TO ORGANIC WINTER PEA CROP IN THE CONDITIONS OF THE COBADIN PLATEAU, ROMANIA

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Abstract

Organic peas (Pisum sativum L.) play an important role in agroecology by naturally fixing nitrogen, improving soil health and organic matter content through crop rotation, and reducing the need for synthetic nitrogen fertilizers and pesticides. Agroecological practices like cultivating organic peas increase the resilience of farming systems to climate change. By boosting soil fertility, reducing chemical inputs, and supporting a healthier ecosystem, the peas contribute to a more sustainable and stable food production system. From an agronomic point of view, peas are of great importance, because the crop is fully mechanized, it is harvested early, being a very good previous crop for many crops, especially autumn crops as wheat; it improves soil fertility, enriching it with nitrogen. This paper presents the results obtained on an organic winter pea crop to which 7 variants of biofertilisers were applied, in three repetitions. The results demonstrated the importance of applying these products in ensuring a balanced nutrition of the crop for achieving an optimal plant density and mass grains under the conditions of the Cobadin plateau in southeastern Romania.

Key words: agroecology, biofertilisers, organic winter pea, yield, soil fertility.

INTRODUCTION

The continuous growth of the global population is generating an ever-increasing demand for food, making it necessary to adopt agricultural practices capable of ensuring long-term productivity without compromising natural resources (Marjanović et al., 2025). In this context, agroecological practices aim to reduce or eliminate the use of controversial inputs, such as synthetic fertilizers and pesticides, and replace them with sustainable alternatives that enhance ecological interactions within agroecosystems. These practices contribute not only to increasing crop yields but also to improving the quality of agricultural production (Szczepanek et al., 2024). At the same time, modern agriculture is expected to meet both economic and environmental objectives by ensuring stable yields, promoting the efficient use of resources, adapting to climate change, and conserving biodiversity (Tamburini et al.,

2020). However, a continuous decline in biodiversity associated with agricultural systems and a degradation of essential ecosystem services, such as soil formation, nutrient cycling, and biological regulation, are being observed worldwide. These effects are primarily driven by land-use change, agricultural intensification, and the simplification of cropping systems (IPBES, 2019; Díaz et al., 2019; Beillouin et al., 2019; FAO, 2019). Increasing crop diversity by introducing a greater number of species and incorporating legumes, such as peas (*Pisum sativum* L.), into crop rotations represents an important strategy for promoting sustainable agriculture (Molla et al., 2025). These practices contribute to improving soil fertility and structure, enhancing biological nitrogen fixation, reducing the pressure of diseases, pests, and weeds, and increasing the stability and productivity of agricultural systems. In addition, pea plays a key role in improving the sustainability and productivity of cropping

systems owing to its unique ability to fix atmospheric nitrogen (N_2), thereby enriching soil nitrogen availability and benefiting subsequent crops (Molla et al., 2025). Furthermore, the root system of pea contributes to improved soil water retention and helps reduce nutrient losses through leaching. This is particularly relevant for winter pea, which can make more efficient use of autumn and winter precipitation, thereby contributing to more stable yields over time (Toader et al., 2025). Cultivation of winter pea in either autumn or spring planting systems is possible and could be decided depending on the immediate needs of farmers (Barbieru, 2022). The cultivation of peas presents a promising opportunity for promoting sustainable agriculture and ensuring food security in the face of contemporary challenges (Eana et al., 2025). This paper presents the results obtained regarding the effects of selected biofertilizers on an organically grown winter pea crop cultivated on the Cobadin Plateau in southeastern Romania.

MATERIALS AND METHODS

The field experiment was established in the autumn of 2025 in the Adamclisi area, Constanța County, Romania, in a certified organic farm. The experimental site is characterized by a chernozem soil with a slightly alkaline pH (8.2) and an organic matter content of 2.4%. The climatic conditions showed considerable variation in both air temperature and rainfall to the long-term averages (Figure 1 and Figure 2). Monthly average air temperatures ranged from -2.4°C in January to 22.5°C in July. Negative temperature deviations predominated during late winter and early spring, with the largest anomaly recorded in February (-6.5°C), followed by April (-2.9°C) and March (-2.6°C). Conversely, warmer-than-average conditions occurred in January ($+1.9^{\circ}\text{C}$), May ($+0.8^{\circ}\text{C}$), June ($+2.3^{\circ}\text{C}$), and October ($+0.9^{\circ}\text{C}$). Monthly rainfall varied from 31.1 mm in February to 72.3 mm in May. Overall, the climatic conditions were characterized by below-average temperatures and above-average rainfall in February, March, and April, followed by relatively warm and less rainfall conditions during late spring and early summer.

The winter pea experiment followed a protocol with eight experimental treatments, including

seven fertilization products approved for organic farming and one unfertilized control.

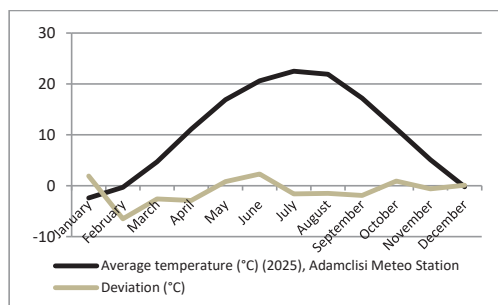


Figure 1. Average temperature, Adamclisi Meteo Station, 2025

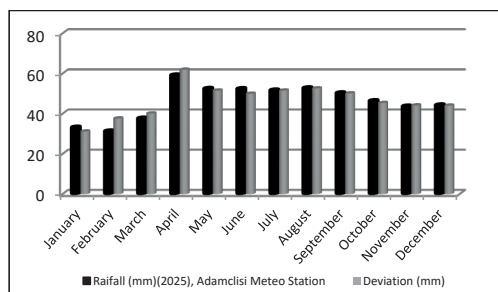


Figure 2. Rainfall (mm), Adamclisi Meteo Station, 2025

The trial was established as a single-factor experiment using a randomized complete block design with three replications. Each experimental plot covered an area of 12 m². The treatments were as follows: V1 - unfertilized control; V2 - Dolomite of Băița (minimum 92% calcium carbonate) applied at 1 t/ha soil during seedbed preparation (this product can be incorporated into the soil during seedbed preparation, applied at sowing, or used as a top-dressing during crop growth); V3 - Bio-Ceres NPK (pelletized organic poultry manure enriched with NPK 4-3-3 + 1% MgO) applied at 250 kg/ha and incorporated into the soil during seedbed preparation; V4 - granulated vermicompost produced by earthworms, applied at 25 kg/ha during seedbed preparation before sowing (incorporated at sowing depth); V5 - BlackJak liquid biostimulant containing a minimum of 28% organic matter, including humic, ulmic, and fulvic acids, applied before sowing at 4 L/ha; V6 - liquid Biohumus applied at 2 L/ha in combination with Etamin, an organic NPK biostimulant enriched with

micronutrients and L-amino acids, applied at 1 L/ha at crop emergence; V7 - Effective Microorganisms (EM), consisting of 500 mL EM-1 and 500 mL molasses, applied once before sowing and once at crop emergence; and V8 - Aminotop N biostimulant containing 30% amino acids, 2% humic substances, and 1.2% seaweed extract (*Ascophyllum nodosum*), applied at 1 L/ha in 300 L water/ha at crop emergence.

Winter wheat was the previous crop, and the winter pea cultivar used in the experiment was Frosen. This cultivar is characterized by high lodging resistance, frost tolerance down to -18°C , and high yield potential. Sowing density was of 90-100 germinating seeds/ m^2 , corresponding to a seeding rate of 200 kg/ha. Sowing was carried out on 14th November 2025. The crop management practices included shallow disc harrowing immediately after harvesting the preceding crop during the second half of July. Seedbed preparation was performed using a Tiger cultivator (Gruber) at a depth of 15-18 cm before sowing. Disease and pest control consisted of a single application of the insecticide-fungicide Bioprotekt at a rate of 0.5 L/ha during the second half of April, when the presence of the major pests and diseases was detected in the crop. The treatment targeted anthracnose (*Ascochyta pisi*), pea moth (*Cydia nigricana*), and pea weevil (*Bruchus pisorum*), which are among the most common and economically important biotic constraints affecting pea production. Throughout the growing season, numerous observations and measurements were performed. This paper present the results obtained at harvest, including plant height, number of nodes/stem, leaflets/ stem, number of pods/plant, and plant density at harvest (plants/ m^2). The experimental data were analyzed by calculating treatment means of variants and replications.



Figure 3. Organic autumn pea experiment (2025, Adamclisi, Experimental Field)

RESULTS AND DISCUSSIONS

Height of pea plants. The tallest plants were recorded in V7 (Effective Microorganisms), with a mean height of 83.53 cm, representing an increase of 6.00 cm (7.74%) compared to the control variant (77.53 cm). This was followed by V2 (Dolomite of Baita), which reached 82.27 cm, an increase of 4.74 cm (6.11%) over the control. Intermediate values were observed for V4 (Granulated vermicompost) and V5 (Blackjak biostimulant), both producing an average plant height of 80.90 cm, corresponding to an increase of 3.37 cm (4.35%) relative to the control. The Aminotop N (V8) treatment resulted in a modest increase to 78.27 cm, which is 0.74 cm (0.95%) higher than the control. In contrast, Bio-Ceres NPK (V3) showed a plant height (77.43 cm) similar to the control, while Biohumus + Etamin (V6) produced the shortest plants (76.87 cm), 0.66 cm (0.85%) lower than the control. Overall, the treatments based on Effective Microorganisms (EM) and Dolomite of Baita had the most pronounced positive effect on plant height, whereas Bio-Ceres NPK and Biohumus Etamin did not improve plant height under the conditions of this experiment (Figure 4).

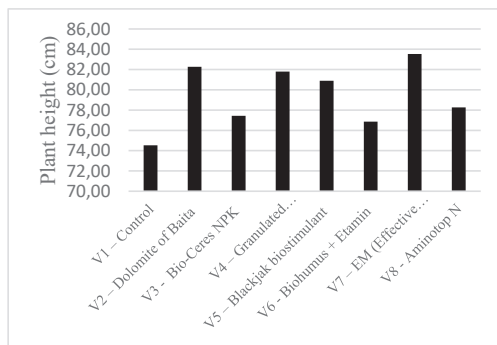


Figure 4. Plants height at harvesting (cm)

Number of nodes/stem. The average number of nodes per stem ranged from 7.63 nodes/stem (V3 - Bio-Ceres NPK) to 12.57 nodes/plant (V5 - Blackjak biostimulant). Compared to the control (10.87 nodes/stem), the highest values were recorded for V5 - Blackjak biostimulant (12.57 nodes/stem) and V7 - Effective Microorganisms (12.30 nodes/stem), whereas V3 - Bio-Ceres NPK showed the lowest value (Figure 5).

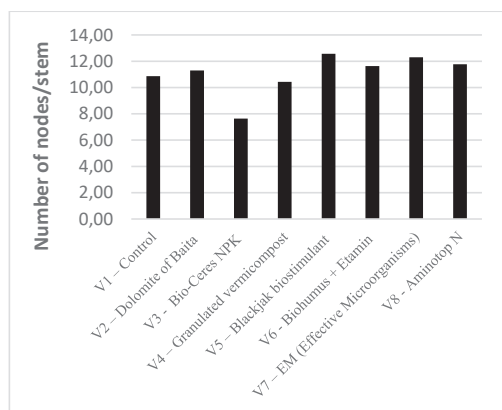


Figure 5. Number of nodes/stem

Number of folioles/plant. Number of folioles per plant ranged from 15.27 folioles/plant in V3 - Bio-Ceres NPK to 25.13 folioles/plant in V5 - Blackjak biostimulant. Compared with the control (21.73 folioles/plant), the higher values were recorded for V5 - Blackjak biostimulant (25.13 folioles/plant), V7 - Effective Microorganisms (24.60 folioles/plants), V8 - Aminotop N (23.53 folioles/plant), V6 - Biohumus + Etamin (23.27 folioles/plant), and V2 - Dolomite of Baita (22.60 folioles/plant). The lowest value was observed in V3 - Bio-Ceres NPK (15.27 folioles/plant). These results indicate that most treatments increased the number of leaves per plant compared to the control, whereas Bio-Ceres NPK reduced this parameter under the experimental conditions (Figure 6).

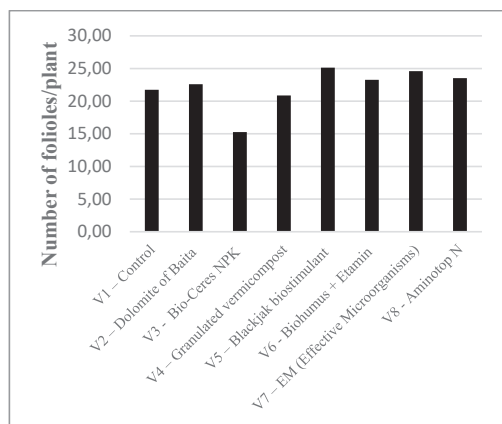


Figure 6. Number of folioles/plant

Number of pods/plant. As shown in Figure 7, the average number of pods per plant ranged from 7.33 in V7 - Effective Microorganisms to 10.43 in V5 - Blackjak biostimulant. Compared to the control variant (8.40 pods/plant), V5 - Blackjak biostimulant recorded the highest average value, while V7 - Effective Microorganisms showed the lowest value. The remaining treatments produced values close to the control, varying between 7.93 and 8.53 pods/plant. The treatments showed only moderate variation in this parameter, with Blackjak biostimulant producing the highest average response under the experimental conditions (Figure 7).

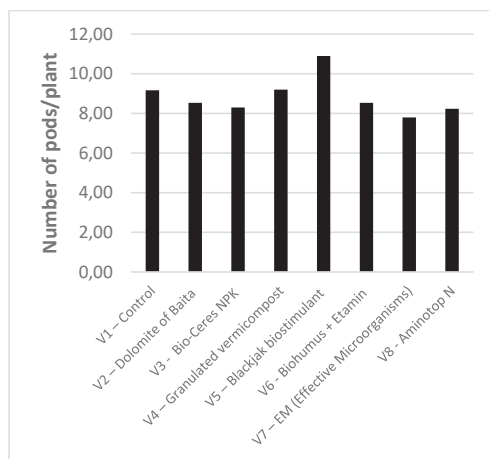


Figure 7. Number of pods/plant

Plant density at harvesting (plants/m²). The highest average plant density was obtained with Biohumus + Etamin (V6), followed by Blackjak biostimulant (V5) and Effective Microorganisms (V7). Harvestable plant density ranged from 55.33 plants/m² in the untreated control variant (V1) to 81.33 plants/m² in V6 - Biohumus + Etamin. Compared to the control, all treatments increased the harvestable plant density, with the highest values recorded for V6 - Biohumus + Etamin (81.33 plants/m), V5 - Blackjak liquid biostimulant (80.33 plants/m²), and V7 - Effective Microorganisms (79.33 plants/m²). These results suggest that the application of biostimulants and biofertilizers improved plant establishment and survival until harvest under the experimental conditions (Figure 8).

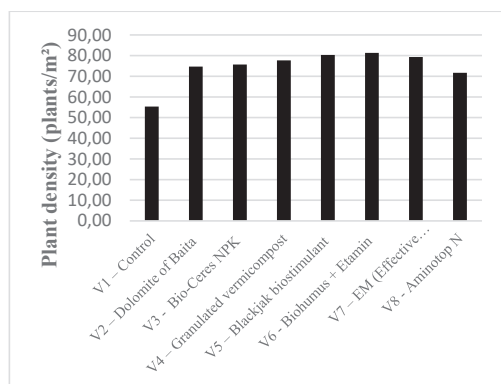


Figure 8. Plant density at harvesting (plants/m²)

Grain mass/plant. Grain mass per plant ranged from 3.25 g in V3 (Bio-Ceres NPK) to 3.80 g in V6 (Biohumus + Etamin). Compared to the control (3.70 g), the highest values were recorded for V6 (Biohumus + Etamin) and V7 (Effective Microorganisms), whereas the lowest value was observed in V3 (Bio-Ceres NPK). Overall, most treatments produced grain mass values comparable to or slightly higher than the control, except for Bio-Ceres NPK and Aminotop N (Figure 9).

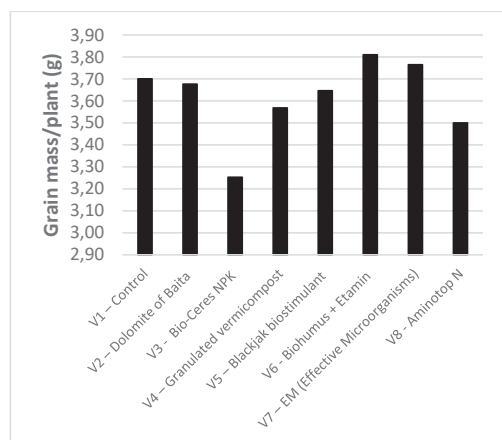


Figure 9. Grain mass/plant (g)

CONCLUSIONS

The application of the tested biostimulants and biofertilizers influenced the vegetative growth of pea plants and grain mass/plant. Although numerical differences were observed among treatments, not all measured parameters were significantly affected.

Plant height was not significantly influenced by the applied treatments indicating that none of the products substantially altered stem elongation under the experimental conditions.

Similarly, the number of pods per plant did not differ significantly among treatments, suggesting that pod formation was relatively stable regardless of the applied treatment.

In contrast, the number of nodes per stem was significantly affected by the treatments. The Blackjak liquid biostimulant (V5) produced the highest mean number of nodes, whereas Bio-Ceres NPK (V3) recorded the lowest value, indicating that these products differed in their influence on vegetative development.

The number of folioles per plant was generally higher in plants treated with Blackjak liquid biostimulant, Biohumus + Etamin, and Effective Microorganisms, while Bio-Ceres NPK consistently resulted in lower values. Harvestable plant density was also improved by all treatments compared with the untreated control, with the highest plant density recorded for Biohumus + Etamin (V6), followed by Blackjak liquid biostimulant (V5) and Effective Microorganisms (V7).

Overall, the treatments based on Blackjak liquid biostimulant, Biohumus + Etamin, and Effective Microorganisms showed the most consistent positive effects across the evaluated parameters. Conversely, Bio-Ceres NPK generally resulted in lower values for several vegetative traits, indicating a less favorable response under the conditions of this experiment.

ACKNOWLEDGEMENTS

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STUDY ON THE EFFECT OF BIOLOGICAL FOLIAR AND SOIL FERTILIZERS AND CONVENTIONAL FERTILIZERS ON THE YIELD OF COMMON WINTER WHEAT

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Abstract

*The aim of the present study is to investigate the effects of biological soil improvers - liquid organic fertilizers obtained by different methods - through nanotechnology and through hydrolysis of plant meals - and to determine the optimal doses of these preparations for maximizing grain yields from new genotypes of common winter wheat (*Triticum aestivum*) developed by the method of combinatorial breeding. The experimental plot is established in the biological experimental field of the Institute of Plant Genetic Resources in Sadovo (Bulgaria), with three replications of seven fertilization treatments, over two consecutive growing seasons: 2023/2024 and 2024/2025. In the older hybrid wheat selections, grain yields are more strongly influenced by the type and quantity of applied fertilizers, as well as by agrometeorological conditions. The highest and most stable yields are obtained from the newest breeding lines. The effect of the fertilizer containing phytohormones depends primarily on agrometeorological conditions; when sufficient moisture is available, the active ingredients of the fertilizer are effectively absorbed, and increasing the dose leads to higher grain production. The effect of the nanofertilizer liquid humus on yields is weaker, limited at a level of 1000 ml/da, and the results of its use depend on the sink capacity of the genotype.*

Key words: *Triticum aestivum*, nanofertilizer, grain yield.

INTRODUCTION

Contemporary research shows that both biological fertilizers including organo-mineral and microbiological products and conventional mineral fertilizers have a positive effect on the yield of winter wheat, though with some differences in their effects depending on the growing conditions. Conventional fertilizers ensure the direct input of essential macro-nutrients such as nitrogen, phosphorus, and potassium, which are critically important for plant growth and productivity. Biological preparations containing effective microorganisms and humic acids improve soil structure, enhance microbiological activity, and increase nutrient uptake, which can result in greater plant resilience and more efficient nutrient utilization (Sydiakina & Dvoretzkyi, 2020; Dehodyuk & Muliarchuk, 2023; Ashurbekova et al., 2024). Nanofertilizers are a promising innovation for increasing grain yields, but they require precise application and integration into an overall fertilization strategy to achieve optimal results.

Studies have shown high values of plant cell viability when treated with nanoparticles, which indicates an absence of toxicity at appropriate concentrations. Nanofertilizers containing iron, molybdenum, and silicon dioxide improve physiological and biochemical plant parameters by enhancing growth and viability, and they support higher activity of soil enzymes, leading to increased yields and improved quality of wheat grain products (Gavrish et al., 2019; Ahmadian et al., 2021; Elahi et al., 2025).

Comparative field-trial data indicate that conventional farming systems provide higher grain yields compared to organic systems. Organic cultivation is a strategic choice based on key advantages for environmental protection and sustainable development. In organic agriculture, yields are lower but stable, and long-term increases in soil organic matter, improved soil structure, and enhanced biodiversity are observed, contributing to sustainable production of healthy food and maintaining agrosystem fertility under adverse conditions (Novokhatsky et al., 2019; Le

Campion et al., 2020; Zastavnyy, 2020; Vinyukov, 2023). Currently, no widely published systematic reviews or meta-analyses exist comparing the effectiveness of nanofertilizers and biofertilizers containing phytohormones in cereal crops, as this is a relatively new and rapidly developing field.

The aim of the present study is to investigate the effects of biological soil improvers - liquid organic fertilizers obtained by different methods, namely through nanotechnology and through hydrolysis of plant meals - and to determine the optimal doses of these preparations for maximizing grain yields from new *Triticum aestivum* genotypes developed through combinatorial breeding. The results contribute to the development of adaptive agroecosystems and the search for synergistic effects in integrated agrotechnologies.

MATERIALS AND METHODS

The biological materials used in the experiment include the wheat varieties Sadovo 1, Pobeda, and Avenyu, as well as the breeding lines Magizhi, MH 258/3355 and RU 251/268. The experimental plot was established in the biological experimental field of the Institute of Plant Genetic Resources in Sadovo (Bulgaria), with three replications of seven fertilization treatments, over two consecutive growing seasons: 2023/2024 and 2024/2025. The crop rotation involved a black fallow, and the soil type was Chromic Vertisol.

To compare the results, two controls were used: an untreated control in the biological field and a control grown under conventional technology (outside the biological field, in a stationary fertilizer experiment). In the conventional system, ammonium nitrate (NH_4NO_3) was applied at an equivalent of N_{12} , along with the herbicides Derby (florasulam 150 g/kg, aminopyralid 300 g/kg) and Axial (pinoxaden 50 g/l), and the insecticide Oasis (esfenvalerate 50 g/l).

The biological cultivation system included pre-sowing spraying with 300 ml/da *Bacillus megaterium* and two treatments with the fungicide Sulfeco (nano-sulfur) in March and April at a dose of 50 ml/da. The study examined the effects of the soil improvers Humeko (liquid humus obtained through nanotechnology) and

Lumbreko (liquid organic fertilizer obtained through hydrolysis of plant meals), which were applied by spraying in March at the following doses: Humeko: 500 ml/da, 1000 ml/da, 1500 ml/da; Lumbreko: 100 ml/da, 200 ml/da, 300 ml/da, and 400 ml/da. The data were subjected to variation and dispersion (ANOVA) analysis.

RESULTS AND DISCUSSIONS

The sowing period begins with drought in October, which is more pronounced in the second year of the experiment and delays both sowing and seed germination and emergence. High temperatures and late emergence lead to weaker root development and hardening, but during the winter months there are no conditions for winterkill. Stands with normal density, uniformity, and viable plants are formed. Vernalization conditions are present for two months during both growing seasons. Nutrient supply during the light stage and the organogenesis of the inflorescences is ensured, despite the uneven distribution of rainfall across phenophases. Plants with genotype-specific habitus are formed.

During the stem-elongation stage in the first growing year, drought is observed, whereas in the second year temperatures and soil moisture are favorable for accelerated biomass accumulation and for ensuring floret fertility. During the heading stage, which is critical in terms of water demand, temperatures and soil moisture favor rapid and uniform emergence and full development of the inflorescences. Pollination and grain formation occur under favorable assimilation conditions.

The processes of grain filling, milk ripeness, and waxing maturity are accelerated by the high temperatures ($+4^\circ\text{C}$ and $+3^\circ\text{C}$ above the long-term averages in the two consecutive years). In the first harvest year (2024), the phase is further shortened by water deficit, while in the second year good water supply to the plants is maintained due to soil moisture reserves. Conditions during the waxing maturity and full ripeness stages are favorable for the accumulation of reserve nutrients in the grain. Meteorological conditions in 2024/2025 are comparable to the long-term norms for the region, whereas the experimental year 2023/2024 is dry and hot (Table 1).

Table 1. Agrometeorological conditions

Month	1991-2020 r	2023/ 2024 r	Deviation	2024/2025 r	Deviation
Mean monthly temperature (T avg, °C)					
X	13.3	16.2	2.9	13.9	0.6
XI	7.4	9.0	1.6	5.5	-1.9
XII	2.2	4.7	2.5	5.5	3.3
I	0.9	3.8	2.9	3.0	2.1
II	3.1	8.9	5.8	2.1	-1.0
III	7.6	10.1	2.5	10.5	2.9
IV	13.0	16.0	3.6	13.0	0.0
V	17.9	20.7	2.9	17.6	-0.3
VI	22.0	26.0	4.0	25.0	3.0
Σ	87.3	115.7	28.4	96.3	9.0
Monthly precipitation sum (ΣR, mm)					
X	49.7	3.3	-46.4	0.0	-49.7
XI	40.0	62.7	22.7	22.1	-17.9
XII	49.7	58.6	8.9	134.0	84.3
I	45.9	52.2	6.3	27.0	-18.9
II	41.4	2.4	-39.0	26.6	-14.8
III	47.8	43.5	-4.3	60.3	12.5
IV	46.0	20.0	-26.0	64.0	18.0
V	53.1	78.4	25.3	68.9	15.8
VI	58.0	9.4	-48.6	11.0	-47.0
Σ	431.3	330.7	-100.6	413.5	-17.8

Variation analyses show that in the older varieties bred in the 1970s - Sadovo 1 (standard bread-making wheat, group B) and Pobeda (standard strong wheat, group A for 25 years) - the average grain yields are more strongly influenced by the type and amount of applied fertilizers and by agrometeorological conditions compared to the newly developed genotypes. In the varieties Avenyu and Magizhi (bred in 2011/2012), variation due to growing conditions is weaker, and consistently higher yields than the standard are obtained from the newest breeding lines included in the study (Table 2).

Table 2. Variation in grain yield (kg/da) by genotype

Genotype	Mean (M)	VC (%)	p-value
Sadovo 1	579	21	st
Pobeda	532	21	-
Avenyu	570	11	-
Magizhi	655	12	-
MH 258/3355	689	8	5%
RU 251/268	782	9	0.1%

Notes: VC = coefficient of variation. "st" indicates the standard (reference) variety. p-values reflect the significance level of differences from the standard.

Statistically significant differences in grain yield compared to the unfertilized control were obtained for the fertilization treatments with Lumbreko and for the conventional treatment with ammonium nitrate. In the Humeko variants,

the variation in grain yield increases due to agrometeorological conditions, and greater differences in genotype response are observed (Table 3).

Table 3. Variation in grain yield (kg/da) under different fertilization treatments

Fertilizer	Mean (M)	VC (%)	p-value
N 0	542	13	St.
N 12	722	10	1%
L 100	641	9	5%
L 200	674	12	5%
L 300	667	15	5%
L 400	697	12	5%
Hu 500	591	18	—
Hu 1000	612	28	—
Hu 1500	591	27	—

Notes: "St." indicates the standard (unfertilized control). L = Lumbreko; Hu = Humeko. N12 = ammonium nitrate at N12 equivalent.

The diagram (Figure 1) illustrates the individual response of each genotype to the applied soil improver.

Both fertilizers are 100% organic, but the uniform response and unidirectional effect of Lumbreko across all genotypes are likely due to the presence of auxins and gibberellins in its composition. Comparative studies indicate that both types of fertilizers (nanofertilizers and biofertilizers containing phytohormones) show potential to increase productivity, with varying degrees of effectiveness depending on specific conditions and varieties; levels of statistical significance vary with dosage and crop characteristics (Dhage & Biradar, 2020; Madlala et al., 2024; Kekeli et al., 2025).

The line RU 251/268 is the most strongly influenced by Humeko, and only in this genotype was a statistically significant difference in yield observed at all treatment levels (Table 4), showing a linear dependence on increasing fertilizer dose. In the remaining genotypes, a yield peak was recorded at a dose of 1000 ml/da, followed by a decline. The nanofertilizer Humeko contains Gram-positive bacteria, polysaccharides, and a wide range of microelements, whose effectiveness decreases with increasing dosage. According to available publications, the effect of nanofertilizers on wheat yield is stronger in cultivars that possess the following distinguishing characteristics: higher photosynthetic activity and biochemical mechanisms for nutrient uptake, greater sensitivity to controlled (precision) nutrient

delivery provided by nanofertilizers, rapid growth, early maturity, and improved technological quality (Garg et al., 2023; Ahmad et al., 2024). The line RU 251/268, due to its inherited high productive tillering capacity, large and

heavy grain, and more intensive growth, is able to utilize the higher levels of nutrient inputs. For the other genotypes, a more balanced nutrient supply using the tested nanofertilizer is recommended.

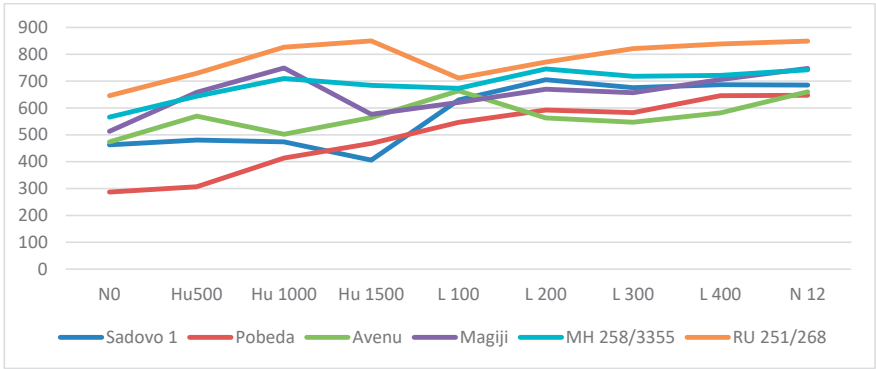


Figure 1. Response of the genotypes to fertilizer type and application rate

Table 4. Statistical significance of differences in grain yield, kg/da

Fertilizer	N 0	Hu500	Hu 1000	Hu 1500	L 100	L 200	L 300	L 400	N 12
Sadovo 1	463 st.	481 n.s.	474 n.s.	406 n.s.	630 n.s.	705 +	676 +	687 +	685 +
Pobeda	288 n.s.	307 n.s.	414 n.s.	468 n.s.	588 n.s.	547 n.s.	592 n.s.	583 n.s.	646 n.s.
Avenue	474 n.s.	570 n.s.	502 n.s.	564 n.s.	664 +	563 n.s.	547 n.s.	582 n.s.	660 +
Magizhi	513 n.s.	658 n.s.	749 +	576 n.s.	621 n.s.	670 +	657 +	706 +	747 ++
MH 258/3355	566 n.s.	644 n.s.	710 +	684 n.s.	673 +	745 ++	718 ++	722 ++	742 ++
RU 251/268	646 n.s.	729 +	826 ++	850 ++	711 +	771 ++	821 +++	839 +++	849 +++

Notes: n.s. = not significant; +, ++, +++ indicate levels of significance. “st.” = control standard.

Statistically significant difference in grain yield was observed between the two growing seasons (Table 5), while the variation attributable to genotype and fertilization was of intermediate magnitude.

Table 5. Variation in yields by genotype and fertilization

Year	Mean (M)	VC (%)	p-value
2024	550	18	-
2025	720	26	5%

The tests of between-group effects confirm the key role of agrometeorological conditions in modifying the influence of soil improvers on the productivity of wheat genotypes (Table 6). The individual effects of the studied factors are statistically significant across all variants of the experiment, but the combined effects of the

independent variables differ. The effect of Humeko is weaker, self-limiting at a level of 1000 ml/da, and the results of its application vary depending on the sink capacity of the genotype. The effect of Lumbreko depends primarily on the environmental conditions during the year; when sufficient moisture is available, the active ingredients of the fertilizer are effectively absorbed and lead to increased grain yield. As reported by other authors, nanofertilizers mainly enhance the delivery and efficiency of mineral nutrients, whereas biofertilizers containing phytohormones modulate growth and adaptation processes, creating a more complex effect on plants and providing more effective biological support for the development and productivity of cereal crops (Semykin et al., 2016; Yousefzadeh et al., 2021).

Table 6. Effect of factors on grain yield, kg/da

Tests of Between-Subjects Effects				
Source	Humeko		Lumbreko	
	Sig.	Effect	Sig.	Effect
genotype	0	22.3	0	9.7
dose	0	7.2	0	7.9
year	0	6.0	0	46.7
genotype *dose	0.051	8.4	0.772	3.0
genotype *year	0.628	0.7	0.033	1.9
dose *year	0.711	0.7	0	6.0
genotype *dose *year	0	24.0	0.917	2.4
Error	30.6		22.3	
Total	100		100	
$\alpha=0.05$	R Squared = 0.694		R Squared = 0.777	
	(Adj R Sq = 0.543)		(Adj R Sq = 0.666)	

CONCLUSIONS

In the older hybrid wheat breedings, grain yield is more strongly impacted by the type and quantity of applied fertilizers, as well as by agrometeorological conditions. The highest and most stable yields are obtained from the newest breeding lines MH 258/3355 and RU 251/268.

The effect of the phytohormone-based soil improver Lumbreko depends primarily on agrometeorological conditions, and when sufficient soil moisture is available, the active components of the fertilizer are efficiently absorbed. Increasing the dose leads to higher grain production, with yields at 400 ml/da being comparable to those under conventional fertilization.

The effect of the nanofertilizer Humeko on yield is weaker, limited at the 1000 ml/da level, and the results of its application depend on the sink capacity of the genotype. Only in the line RU 251/268 was a statistically significant difference in yield detected at all treatment levels and a linear response to increasing fertilizer dosage.

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PHYSIOLOGICAL AND AGRONOMIC RESPONSE OF MAIZE TO FOLIAR APPLICATION OF UTRISHA N: EVIDENCE FROM REAL FARM FIELD TRIALS IN WESTERN ROMANIA

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Abstract

The present study evaluated the effectiveness of the biological product Utrisha™ N (Methylobacterium symbioticum) on maize cultivated under real commercial farming conditions, without major changes to standard crop management practices. The experiment was conducted in 2024 at the Neudorf experimental field (Arad County, Western Romania) using a paired strip design consisting of four independent strip pairs (Utrisha N vs. untreated control), each covering 1 ha. Statistical analysis was performed using a paired t-test (n = 4). Application of Utrisha N resulted in a significant mean grain yield increase of +461 kg ha⁻¹ (+7.0%, p < 0.001). This yield response was associated with a significant increase in chlorophyll index (+3.05 AT Leaf units, p = 0.007), indicating enhanced photosynthetic activity. The physiological benefit confirmed by chlorophyll measurements suggests a measurable and biologically relevant mechanism, supporting the scientific credibility of the treatment. Grain moisture at harvest was slightly higher in the treated variant (+0.45 percentage points, p = 0.016), indicating a marginal delay in physiological maturity. Overall, the results confirm the effectiveness of Methylobacterium symbioticum in improving maize yield performance and physiological status under the pedoclimatic conditions of Western Romania.

Key words: biofertilizer, chlorophyll index, *Methylobacterium symbioticum*, Utrisha N.

INTRODUCTION

The increasing global demand for agricultural products, together with pressures generated by climate change, soil degradation, and the need to reduce chemical inputs, necessitates the identification of sustainable solutions for intensifying agricultural production. Maize (*Zea mays* L.), one of the most important cereal crops worldwide, is highly dependent on nitrogen supply, and improving nitrogen management efficiency represents a major priority for modern agriculture (Sheoran et al., 2021). In this context, biological nitrogen fixation and the use of microbial biostimulants have attracted growing scientific and practical interest. Biological nitrogen fixation (BNF) is a natural process through which diazotrophic microorganisms reduce atmospheric nitrogen into forms assimilable by plants. Although this mechanism is well established in legumes, its extension to cereal crops was long considered limited (Guo et al., 2023). However, recent research has

demonstrated that bacteria associated with cereal roots can be genetically or agronomically manipulated to express nitrogenase activity under controlled conditions (Ryu et al., 2020; Bloch et al., 2020). These findings have opened new perspectives for integrating BNF into conventionally fertilized agricultural systems (Wen et al., 2022). In parallel, the development of agricultural biostimulants has expanded significantly. Biostimulants are defined as substances or microorganisms which, when applied to plants or soil, stimulate natural processes to enhance nutrient use efficiency, stress tolerance, and crop quality (Calvo et al., 2014; Shahwar et al., 2023). Recent meta-analyses indicate that the application of biostimulants can lead, on average, to significant yield increases, although crop responses depend on species, pedoclimatic conditions, and the type of product used (Li et al., 2022). Of particular interest are bacteria belonging to the genus *Methylobacterium*, which are recognized for their ability to colonize leaf surfaces and the

rhizosphere, solubilize phosphates, and interact with plant metabolism (Agafonova et al., 2013; Chibeba et al., 2025). Certain strains, such as *Methylobacterium symbioticum*, have been developed as foliar inoculants capable of supplying biologically fixed nitrogen to maize under field conditions (Corteva Agriscience, 2022). However, the results obtained are variable: while some studies report increases in productivity and nitrogen recovery (Santos & Bundt, 2025), others indicate limited effects or responses dependent on nitrogen fertilization rates (Rodrigues et al., 2024; Quemada et al., 2026). The interaction between nitrogen nutrition and plant metabolism is closely linked to the role of free amino acids, which function both as structural molecules and as metabolic signals during seed development and vegetative growth (Amir et al., 2018; Ryu et al., 2020). By influencing nitrogen availability, biostimulants and nitrogen-fixing microorganisms can alter these metabolic fluxes, with direct implications for yield and crop quality. The adoption of microorganism-based technologies is also influenced by agronomic and digital factors. Monitoring crop nitrogen status using optical sensors provides valuable tools for optimizing the timing and rates of biological input applications (Gabriel et al., 2019). At the same time, the integration of digital twin models enables the simulation of agricultural yields and the assessment of the impact of new technologies under variable environmental conditions (Chitu et al., 2024).

The effectiveness of microbial biostimulants is further conditioned by the dynamics of crop-associated microbiota. Recent studies show that inoculants can modify the structure of soil and rhizosphere bacterial communities throughout the growing season, indirectly influencing nutrient uptake and plant health (Kruščić et al., 2025). These effects must be evaluated in the context of climate change and water resource availability, factors that significantly affect agricultural productivity in many regions (Șmuleac et al., 2020).

At the European level, the use of nitrogen-fixing microbial products is increasing; however, their adoption remains heterogeneous, being influenced by agricultural policies, farmer perceptions, and associated economic risks (Visser & Babiker, 2021; Sitnicki et al., 2024).

In this context, the development of next-generation biostimulants, supported by genomic sequencing strategies and microbial engineering, represents a promising direction for sustainable agriculture (Garg et al., 2024). Overall, the current literature highlights the considerable potential of biological nitrogen fixation and microbial biostimulants in maize cropping systems. Nevertheless, the variability of observed responses underscores the need for integrated approaches that combine plant genetics, nutrient management, digital technologies, and adaptation to local pedoclimatic conditions.

MATERIALS AND METHODS

The experiment was carried out in 2024 in Neudorf village, Arad County (Western Romania), under real commercial farming conditions. Maize was sown on 15 April 2024 and harvested on 20 September 2024.

A paired strip design was used, consisting of eight parallel strips of 1 ha each (total area: 8 ha), arranged alternately as Utrisha N treated / untreated control. This layout generated four independent experimental pairs ($n = 4$), which were used as the statistical unit for inferential analysis. The study was conducted on a medium-fertility brown argic soil with a loam-clay texture, neutral reaction, and good water-holding capacity. Particle size distribution indicated approximately 42% sand, 32% silt, and 26% clay. Soil pH ranged from 6.8 to 7.0, with low calcium carbonate content ($\sim 0.9\%$).

Organic matter content was moderate ($\sim 2.0\%$), and total nitrogen was approximately 0.14%, indicating moderate natural fertility. The cation exchange capacity was around 17 me 100 g⁻¹ soil, with base saturation close to 80%. Available phosphorus showed medium to good levels, while available potassium was well supplied. Field capacity was approximately 28%, with plant-available water estimated at 15–16%. Micronutrients (Fe, Mn, Zn, Cu) were present at adequate levels.

The biological product Utrisha™ N (*Methylobacterium symbioticum*) was applied foliarly on 20 May 2024, at the V6 growth stage, at a rate of 333 g ha⁻¹ diluted in 200 L water ha⁻¹. The control received the standard crop technology without biostimulant application.

Measurements: grain yield (kg ha⁻¹): determined at harvest for each strip; grain moisture (%): measured at harvest; chlorophyll index (AT Leaf): measured on the 4th and 5th leaves of 10 randomly selected plants per strip; strip means were used for analysis. All inferential analyses were performed using a paired t-test (n = 4). Results are presented as mean ± SEM with 95% confidence intervals. Statistical significance was set at p < 0.05.

RESULTS AND DISCUSSIONS

Grain production and crop response to the application of Utrisha N

The results obtained in the experiment carried out in 2024 in Neudorf, Arad County, highlight a positive and constant agronomic response of the corn crop to the application of the biological product Utrisha N. The analysis of the yields (Table 1) carried out in the four experimental pairs shows that, in all cases, the treated strips exceeded the control variants, confirming the efficiency of the treatment under real farm conditions. The average values of grain production, presented in Table 1 and Figure 1, indicate a level of 7067 kg/ha for the Utrisha N variant, compared to 6606 kg/ha for the control variant.

Table 1. Influence of the application of Utrisha N on maize grain production (Neudorf, 2024)

Variation	Yield (kg/ha)
Utrisha N	7067 ± 115 a
Control	6606 ± 102 b

The values represent the average of four experimental bands ± the standard deviation. The values followed by different letters (a, b) differ statistically significantly at p < 0.05.

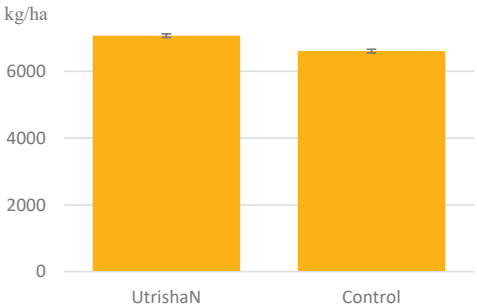


Figure 1. Yield (kg/ha) - paired t-test (n = 4 pairs)
UtrishaN: 7067 ± 63 SEM;
Control: 6606 ± 55 SEM
Difference: +461 kg/ha (95%CI [428.2, 494.3]),
 $t(3)=44.44, p<0.001$
Relative Change: +7.0% (95% CI [6.6, 7.4])

The average production increase was +461 kg/ha, which corresponds to an increase of about 7%. This result is particularly relevant from a practical point of view, as it was obtained without changes in the basic culture technology, but only by introducing a biological product applied foliar in the V6 phenophase.

An important aspect to emphasize is the consistency of the response. The production gains were not the result of a single high-performance belt, but manifested uniformly in all four experimental pairs. This uniformity indicates a stable effect of the product, independent of small local variations of soil or microclimate, and suggests a good adaptation of the bacterium *Methylobacterium symbioticum* to the pedoclimatic conditions in western Romania.

From an agronomic perspective, a production increase of 400-500 kg/ha is significant, especially in the current context, characterized by the increase in input costs and the pressure to reduce the use of chemical fertilizers. Even in years with average production potential, such an increase can make the difference between profitability and the break-even point of the crop.

Grain moisture at harvest and technological implications

In addition to the increase in production, the data obtained indicate a slight increase in the moisture of the grains at harvest in the variant treated with Utrisha N. The average values were 14.64% for Utrisha N, compared to 14.19% for the control. The difference, of about 0.4-0.5 percentage points, is small but constant and was statistically significant.

Table 2. Influence of the application of Utrisha N on corn kernel moisture (Neudorf, 2024)

Variation	Moisture at harvest (%)
Utrisha N	14.64 ± 0.24 a
Control	14.19 ± 0.12 b

The values represent the average of four experimental bands ± the standard deviation. The values followed by different letters (a, b) differ statistically significantly at p < 0.05.

This moderate increase in humidity can be interpreted as a minor delay in physiological ripening, associated with a longer period of metabolic activity of the plants. In the case of biostimulants with a physiological effect, this phenomenon is common and reflects a prolongation of the grain filling phase, in which the plant continues to translocate assimilated to cobs.

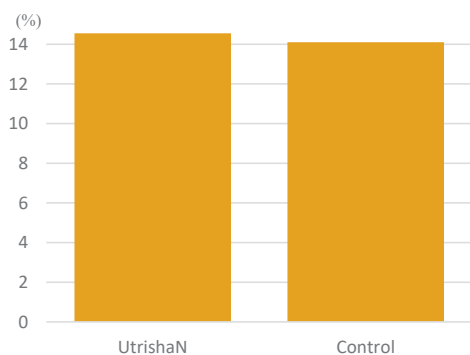


Figure 2. Humidity (%) - paired t-test
Difference: +0.45 pp (95%CI [0.2, 0.7]), $t(3)=4.93$, $p=0.016$

From a practical point of view, the moisture level of the grains remains within normal limits for harvesting and does not imply significant additional costs for drying. Moreover, this slight delay in maturation can be considered beneficial, as it is associated with a greater accumulation of dry matter in the grains, which explains the production increases obtained.

Chlorophyll index as an indicator of plant physiological status

The determinations of the chlorophyll index (AT Leaf) provided essential information on the physiological state of the plants and the mechanism of action of the product Utrisha N. The values obtained on leaf 4 and leaf 5 were constantly higher in the treated variants compared to the control, the differences being statistically significant (Table 3 and Figure 3).

Table 3. Effect of applying Utrisha N on chlorophyll index (AT Leaf) in maize (Neudorf, 2024)

Variation	Analyzed leaf	Chlorophyllin Index (AT Leaf)
Utrisha N	Leaf 4	44.9 ± 2.3 a
Control	Leaf 4	41.9 ± 2.1 b
Utrisha N	Leaf 5	45.8 ± 2.0 a
Control	Leaf 5	42.5 ± 2.2 b

The values represent the average of the determinations made on 10 plants/strip, in four treated strips and four control strips. The values followed by different letters (a, b) differ statistically significantly at $p < 0.05$.

For leaf 4, the chlorophyll index reached an average value of 44.9 AT Leaf in the treated variant, compared to 41.9 AT Leaf in the control. Similar differences were observed on leaf 5, where the mean values were 45.8 AT Leaf at Utrisha N and 42.5 AT Leaf at control. These

differences, of about 3 units of AT Leaf, exceed the natural variability expressed by the standard deviation and indicate a clear treatment effect.

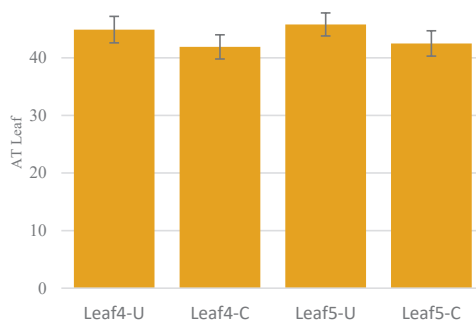


Figure 3. Chlorophyll Index (AT Leaf) - band averages; paired t-test

Leaf 4: $\Delta = +2.77$ (CI95% [0.3, 5.3]), $t(3)=3.50$, $p=0.039$
 Leaf 5: $\Delta = +3.32$ (CI95% [2.2, 4.5]), $t(3)=9.36$, $p=0.003$
 Average leaf 4&5: $\Delta = +3.05$ (CI95% [1.6, 4.5]), $t(3)=6.68$, $p=0.007$

The increase in chlorophyll index reflects an improvement in plant nitrogen nutrition, given the central role of nitrogen in the structure of the chlorophyll molecule. In the case of Utrisha N, the bacteria *Methylobacterium symbioticum* colonize the tissues of the plant and fix atmospheric nitrogen, making it available to the plant in a gradual way, correlated with physiological requirements.

This continuous supply of nitrogen helps keep leaves green for a longer period, delaying senescence and supporting photosynthesis in critical phases for production formation. The high values of the chlorophyll index observed on leaf 5, a fully developed and intensely photosynthetically active leaf, confirm this mechanism.

The relationship between photosynthesis and production accumulation

The correlation of chlorophyll index data with grain yield data shows a direct relationship between plant physiological status and final crop yield. In the variants treated with Utrisha N, higher levels of chlorophyll index were associated with consistent production increases, suggesting that improved photosynthesis is the main mechanism by which the product exerts its effect.

Intensified photosynthesis leads to a higher production of assimilates, which are

subsequently translocated to cobs. In the grain filling phase, this increased availability of assimilates allows the accumulation of a larger amount of dry matter, which is reflected in the increase in the mass of the grains and, implicitly, in the total production.

In addition, maintaining high photosynthetic activity for a longer period can contribute to a more efficient use of water and nutrient resources, especially under moderate stress. Thus, the application of Utrisha N can play an important role in stabilizing productions, not just increasing them.

The results obtained in this experiment have a high practical relevance for farmers, as they demonstrate the efficiency of a biological product in a commercial production system, on a hectare scale. Unlike testing in microplots, where conditions are strictly controlled, the present experiment reflects the reality on the farm, with all its variables.

The application of Utrisha N at a dose of 333 g/ha, in phenophase V6, proved to be an effective solution for increasing maize production and improving the physiological state of plants. The benefits observed are not the result of a direct supply of mineral nitrogen, but of an optimization of the plant's metabolism, which makes the product compatible with modern strategies to reduce chemical inputs.

In the current context, marked by rising prices for nitrogen fertilisers and pressure to adopt more sustainable agricultural practices, the use of nitrogen-fixing bacteria is a promising direction. The results obtained in Neudorf confirm that such solutions can generate concrete economic benefits without compromising the quality of production.

CONCLUSIONS

The application of the biological product Utrisha N resulted in a significant increase in grain production compared to the control variant. The mean of the experimental pairs was 7067 ± 63 SEM kg/ha in the treated version, respectively 6606 ± 55 SEM kg/ha in the control, resulting in an average increase of $+461$ kg/ha (95%CI [428, 494]), $p < 0.001$.

The values of the grain moisture at harvest were slightly higher in the treated version ($+0.45$ percentage points, 95%CI [0.2, 0.7], $p = 0.016$),

indicating a slightly delayed maturation, but without negative impact on recovery.

The determinations carried out on leaf 4 and leaf 5 showed a significant increase in the chlorophyll index in the treated version. The mean on the band indicates an increase of approximately $+3.05$ AT Leaf units (95%CI [1.6, 4.5], $p=0.007$), confirming the intensification of photosynthetic processes, which indicates a real and measurable agronomic mechanism.

The results obtained attest to the potential of Utrisha N to improve the efficiency of nitrogen use and optimize crop performance in real farm conditions. Overall, the experimental data show that the application of the biological product Utrisha N led to a significant improvement in the physiological status of maize plants, reflected by higher chlorophyll index values, and to a stable and reproducible production increase. The correlation of the physiological parameters with the productive ones supports the hypothesis that the main effect of the product is a physiological one, manifested by the intensification of photosynthesis and the prolongation of the period of activity of the foliar apparatus.

These results confirm the usefulness of Utrisha N as an effective tool in modern maize cultivation technologies and provide farmers with a practical solution for increasing productivity in real farm conditions.

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ARTHROPOD FAUNA BIODIVERSITY IN ALFALFA AGROECOSYSTEMS UNDER HYDRIC STRESS CONDITIONS: A PRELIMINARY STUDY

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Abstract

Alfalfa (Medicago sativa L.) provides a long-term agricultural habitat in which arthropod assemblages can develop and persist, making it a suitable system for examining faunal responses to environmental constraints. In recent climatic contexts, hydric stress has emerged as a key factor shaping arthropod distribution and community structure within agroecosystems. This study was conducted during the 2024 growing season in an experimental alfalfa field located in Ghilad, western Romania, at a stage corresponding to the third year of crop establishment and under conditions of reduced water availability. Arthropod fauna was surveyed from April to September through monthly sampling events using sweep netting and direct visual observations, followed by laboratory-based taxonomic identification. The arthropod fauna recorded under hydric stress conditions comprised eight major taxonomic orders, with Coleoptera and Hemiptera accounting for the highest numerical dominance. Community composition was strongly skewed toward phytophagous species, particularly Phytodecta formicata and Aphis craccivora, which together represented a substantial proportion of the total abundance. Statistical analyses revealed significant differences among orders, families, and species, indicating a pronounced functional imbalance within the assemblage. Overall, the observed taxonomic and functional patterns highlight the influence of hydric stress on the distribution and relative dominance of arthropod groups associated with alfalfa agroecosystems.

Key words: arthropod fauna, alfalfa agroecosystem, hydric stress, community structure, taxonomic diversity.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial forage legume belonging to the subfamily *Papilionoideae* and plays a key role in livestock production systems due to its high nutritional value and widespread use in animal feeding (Daud et al., 2025; Varol et al., 2024). Cultivated for more than 9,000 years, alfalfa is among the oldest plants used for forage production and is currently widely distributed across numerous agricultural regions worldwide (Michaud et al., 1988; Bellague et al., 2018; Li and Huang, 2008; Singer et al., 2018). Its remarkable ecological adaptability and strong regenerative capacity have facilitated its extensive integration into agricultural systems, with alfalfa frequently referred to in the scientific literature as the “queen” or “king” of forage crops (Peng et al., 2025; Zhang et al., 2025). In the context of ongoing climate change, the frequency of extreme climatic events has increased considerably, being characterized by rising mean temperatures, reduced precipitation,

and the occurrence of prolonged drought episodes (Tyshchenko et al., 2020). Under such conditions, abiotic stress, particularly water deficit, is considered a major limiting factor for agricultural productivity (Li et al., 2021; Zhang et al., 2025). Although alfalfa exhibits relatively high tolerance to water deficit compared with other cultivated legumes, its productivity remains strongly influenced by environmental conditions, while hydric stress may substantially reduce both biomass yield and forage quality (Daud et al., 2025; Huang et al., 2018; Capstaff and Miller, 2018; Bellague et al., 2018; Liu et al., 2021).

The functioning of agricultural ecosystems largely depends on the biodiversity of organisms associated with crop systems. Insects, mites, and spiders perform essential ecological functions, contributing to processes such as pollination, biological control of pests, and the recycling of organic matter (Schowalter, 2016; Losey & Vaughan, 2006). The structure of these biological communities can reflect the ecological status of agroecosystems and provide

valuable insights for the development of sustainable management strategies (Gurr et al., 2003). Species distribution is influenced by specific ecological traits that determine how organisms respond to environmental variability (Gaston, 2003; Holt, 2003).

In perennial forage crops such as alfalfa, these ecological interactions are particularly pronounced. Alfalfa stands support diverse arthropod communities, including phytophagous species, entomophagous organisms, and pollinators (Razzaq & Al-Saffar, 2016; Flanders and Radcliffe, 2013). More than 1,000 species have been reported in association with this crop, of which approximately 100-150 may cause varying degrees of damage, while the majority fulfil important ecological roles within the agricultural ecosystem (Flanders and Radcliffe, 2013). Previous studies have revealed the complexity of these assemblages, with alfalfa ecosystems supporting hundreds of morphospecies belonging to multiple taxonomic orders and families. For example, Aljoboory et al. (2022) reported 205 arthropod species distributed among 92 families and 10 orders, including representatives of the order Araneae, underscoring the considerable biodiversity associated with alfalfa agroecosystems. This diversity is often dominated by phytophagous species, yet entomophagous organisms and pollinators play an important role in maintaining ecological balance (Bengouga et al., 2026). In Eastern Europe, studies conducted in alfalfa fields have also revealed a considerable diversity of coleopteran species associated with this agricultural habitat (Munteanu et al., 2014). Despite these findings, the biodiversity of organisms associated with forage crops remains insufficiently investigated, particularly within agroecosystems in Romania (Lisenchi - Murariu & Moglan, 2006; Vîrteiu et al., 2012; Pustai et al., 2015; Otavă et al., 2019). At the same time, agricultural intensification and biodiversity loss may reduce the capacity of agroecosystems to maintain ecological balance, whereas perennial crops such as alfalfa may provide favorable habitats for the conservation of biological diversity and the maintenance of associated ecosystem services (Tscharnkte et al., 2005; Kremen & Miles, 2012).

In this context, the present study aimed to evaluate the species diversity and community

structure of arthropods associated with alfalfa agroecosystems under hydric stress conditions in an experimental field located in Ghilad (Timiș County, Romania) during the 2024 growing season.

MATERIALS AND METHODS

Study area and experimental design

The study was conducted in 2024 in an experimental alfalfa field located in Ghilad (Timiș County, western Romania), in the Timiș Plain, part of the Western Romanian Plain. The area is characterized by predominantly flat terrain at an altitude of approximately 85–95 m above sea level, providing suitable conditions for field sampling. Climatic conditions during the study period were characterized by above-average temperatures and a pronounced precipitation deficit, resulting in the development of both atmospheric and pedological drought in western Romania, including the study site in Ghilad (Timiș County). These conditions generated hydric stress in agricultural ecosystems, affecting the growth and productivity of crops such as alfalfa (*Medicago sativa* L.), and provided an appropriate ecological context for investigating the response of arthropod biodiversity to hydric stress conditions.

The experimental field covered approximately 0.5 ha and was cultivated exclusively with alfalfa (*Medicago sativa* L.). At the time of the study, the crop was in its third year of growth. The experiment was arranged in three replications, and a representative sampling area of 100 m² was selected within each replication. Each plot measured 25 m × 4 m, with 16 rows spaced 25 cm apart. No chemical treatments were applied during the monitoring period.

Arthropod sampling

Arthropod sampling was conducted monthly between April and September 2024, resulting in six sampling events. Sweep netting was used as the main sampling method to collect arthropods active on vegetation. For each plot, 50 double sweeps were performed during each sampling (figure 1). The collected specimens were placed in paper bags, immobilized, and transported to the laboratory for identification. Direct visual observations were also performed during field

visits in order to document ecological interactions such as feeding, predation, or flight activity.



Figure 1. Experimental field

Identification of specimens

All specimens were sorted and identified in the Plant Health Diagnosis and Expertise Laboratory of the Faculty of Agriculture using stereomicroscopes and taxonomic keys (Pălăgeşiu et al, 2000; Grozea, 2015). Individuals were classified according to their taxonomic affiliation (order, family, genus, or species). Specimens that could not be identified to species level were assigned to higher taxonomic categories and included in the analyses.

Statistical analysis

Arthropod community structure was characterized using relative abundance (AR%), calculated for each taxon in relation to the total number of collected individuals. Community diversity was evaluated using the Shannon diversity index (H'), Simpson dominance index (D), and Pielou's evenness index (J).

To explore the influence of climatic conditions on arthropod communities, relationships between arthropod abundance and environmental variables (temperature and precipitation) were evaluated using Spearman's rank correlation coefficient.

Climatic data and hydric stress assessment

Meteorological data used in this study were obtained from the historical records available on the Freemeteo platform (https://freemeteo.ro/...), using the nearest available location to the experimental field in Ghilad (Timiș County, western Romania). Monthly mean air temperature ($^{\circ}\text{C}$) and monthly total precipitation (mm) were considered for the

monitoring period between April and September 2024. Official ANM reports on pedological drought in western Romania during 2024 were also used to support the assessment of hydric stress conditions. Hydric stress was defined as the combined effect of below-normal precipitation and elevated temperatures during the vegetation period, associated with reduced water availability and increased atmospheric evaporative demand.

RESULTS AND DISCUSSIONS

During the sampling period, a total of 1230 arthropod individuals belonging to 30 species were collected from the experimental alfalfa field located in Ghilad (Timiș County, Romania). These taxa were distributed among seven arthropod orders, namely *Araneae*, *Orthoptera*, *Hemiptera*, *Hymenoptera*, *Coleoptera*, *Lepidoptera* and *Diptera* (Table 1). The presence of these taxonomic groups reflects a relatively diverse arthropod assemblage associated with the alfalfa crop, including phytophagous insects, predators, parasitoids and pollinators. Among the recorded orders, *Coleoptera* and *Hemiptera* clearly dominated both in terms of species richness and abundance. This pattern is typical for herbaceous agroecosystems, where phytophagous insects exploit the abundant plant biomass provided by the crop canopy, while predators and parasitoids contribute to the ecological complexity of the system. The analysis of relative abundance revealed a pronounced dominance structure within the arthropod community associated with the alfalfa crop. The most abundant species was *Phytodecta fornicata*, representing 37.40% of the total number of individuals collected. Other abundant taxa included *Aphis craccivora* (17.07%), *Adelphocoris lineolatus* (8.62%) and *Sitona lineatus* (6.91%). Together, these four species accounted for approximately 70% of the total individuals recorded during the sampling period. Such a community structure, characterized by the dominance of a limited number of highly abundant species, is commonly observed in herbaceous agroecosystems where specialized herbivores exploit the available host plant resources.

The trophic structure of the arthropod assemblage reflected the coexistence of several

functional groups associated with the alfalfa crop. Herbivorous taxa such as *Phytodecta fornicata*, *Aphis craccivora*, *Adelphocoris lineolatus* and *Sitona lineatus* represented the largest proportion of the collected individuals. Overall, phytophagous species accounted for more than 80% of the total number of individuals, highlighting the strong dependence

of the arthropod assemblage on the plant resources provided by the alfalfa crop. In addition to herbivores, the community also included predatory species, such as *Coccinella septempunctata* and *Adalia bipunctata*, which are well-known biological control agents of aphid populations.

Table 1. Arthropod species recorded in the alfalfa field and their relative abundance during the 2024 sampling period

ORDER	FAMILY	SUBFAMILY	Genus	Species	RA(%)
ARANEAE	LYCOSIDAE	PARDOSINAE	<i>Pardosa</i>	<i>P. agrestis</i>	0.81
	THOMISIDAE	THOMISINAE	<i>Xysticus</i>	-	0.33
ORTHOPTERA	TETTIGONIIDAE	TETTIGONINAE	<i>Tettigonia</i>	<i>T. viridissima</i>	2.93
HEMIPTERA	MIRIDAE	MIRINAE	<i>Adelphocoris</i>	<i>A. lineolatus</i> – a+l	8.62
		DERAEOCORINAE	<i>Deraeocoris</i>	<i>D. trifasciatus</i>	0.49
	NABIDAE	NABINAE	<i>Nabis</i>	<i>N. ferus</i> – l	0.65
	APHIDIDAE	APHIDINAE	<i>Aphis</i>	<i>A. craccivora</i>	17.07
	APHROPHORIDAE	APHROPHORINAE	<i>Philaenus</i>	<i>P. spumarius</i> – a+l	3.66
HYMENOPTERA	EURYTOMIDAE	-	-	-	1.79
	PTEROMALIDAE	-	-	-	1.06
	CHALCIDIDAE	-	-	-	0.81
	ICHNEUMONIDAE	-	-	-	0.57
COLEOPTERA	CHANTARIDAE	CHANTARINAE	<i>Cantharis</i>	<i>C. rustica</i>	0.89
	BYRRHIDAE	BYRRHIDAE	<i>Byrrhus</i>	<i>B. pilula</i>	1.14
	MORDELLIDAE	MORDELLINAE	<i>Mordellistena</i>	<i>M. parvula</i>	0.24
	COCCINELLIDAE	COCCINELLINAE	<i>Coccinella</i>	<i>C. septempunctata</i>	4.96
			<i>Adalia</i>	<i>A. bipunctata</i>	1.07
	CHRYSEMELIDAE	CHRYSEMELINAE	<i>Phytodecta</i>	<i>P. fornicata</i>	37.40
		CRIOCERINAE	<i>Oulema</i>	<i>O. melanopus</i>	0.08
		CEUTORYNCHINAE	<i>Ceutorhynchus</i>	<i>C. pallidactylus</i>	0.08
				<i>S. lineatus</i>	6.91
				<i>S. sulcifrons</i>	0.33
	CURCULIONIDAE	ENTIMINAE	<i>Sitona</i>	<i>S. humeralis</i>	1.63
				<i>S. hispidulus</i>	0.08
				<i>H. variabilis</i>	0.98
		TYCHIINAE	<i>Tychius</i>	<i>T. flavus</i>	0.24
	APIONIDAE	APIONINAE	<i>Eutrichapion</i>	<i>A. ervi</i>	2.28
			<i>Protapion</i>	<i>A. tenue</i>	1.54
LEPIDOPTERA	PLUTELLIDAE	PLUTELLINAE	<i>Plutella</i>	<i>P. xylostella</i> – a	0.89
DIPTERA	SYRPHIDAE	SYRPHINAE	<i>Episyrphus</i>	<i>E. balteatus</i>	0.49

*a - adult; l- larvae; RA(%) - relative abundance

Predatory taxa represented approximately 11% of the total arthropod assemblage, while parasitoids belonging to families such as Ichneumonidae, Chalcididae and Pteromalidae accounted for a smaller fraction of the community. Furthermore, pollinating insects such as *Episyrphus balteatus* were also recorded, highlighting the functional diversity of the arthropod community associated with the alfalfa field. The distribution of species and individuals among the main trophic groups is summarized in Table 2.

Table 2. Trophic structure of the arthropod community recorded in the alfalfa agroecosystem (Ghilad, 2024)

Trophic group	Number of species	Number of individuals	RA (%)
Phytophagous	14	1012	82.28
Predators	9	137	11.14
Parasitoids	4	52	4.23
Pollinators	1	6	0.49
Other / omnivorous	2	23	1.86
Total	30	1230	100

The diversity of the arthropod assemblage was further evaluated using several ecological diversity indices. The calculated values of Shannon, Simpson and Pielou indices are presented in Table 3.

Table 3. Diversity indices of the arthropod community recorded in the alfalfa agroecosystem (Ghilad, 2024)

Index / Parameter	Value
Number of individuals (N)	1230
Number of species (S)	30
Shannon diversity index (H')	2.27
Simpson dominance index (D)	0.18
Simpson diversity index (1-D)	0.81
Pielou's evenness index (J')	0.67

The Shannon diversity index ($H' = 2.27$) indicated a moderate level of species diversity within the arthropod community. Similarly, the Simpson index ($1-D = 0.81$) suggested a relatively high probability that two randomly selected individuals belong to different species. However, the evenness value ($J' = 0.67$) revealed a moderately uneven distribution of individuals among species, reflecting the strong dominance of a few highly abundant phytophagous taxa, particularly *Phytodecta fornicata*, *Aphis craccivora* and *Adelphocoris lineolatus*. This pattern is consistent with the dominance structure previously observed in the arthropod community associated with the alfalfa crop. The rank–abundance curve (Figure 2) illustrates the dominance structure of the arthropod assemblage recorded in the alfalfa agroecosystem. The distribution shows a steep decline in relative abundance from the most dominant species to a large number of rare taxa. *Phytodecta fornicata* clearly dominated the community, largely exceeding the abundance of all other recorded species, while most of the remaining taxa occurred at relatively low abundances. Beyond the dominance structure of the assemblage, the temporal distribution of arthropod abundance revealed pronounced seasonal variation throughout the sampling period.

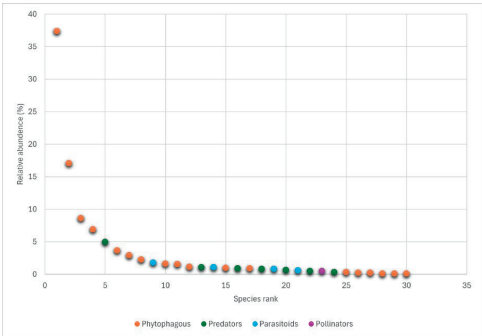


Figure 2. Rank-abundance curve of arthropod species recorded in the alfalfa agroecosystem located in Ghilad (Timiș County, Romania) during the 2024 sampling period. Species are ranked according to their relative abundance (RA%). Colors indicate the trophic affiliation of species: phytophagous (orange), predators (green), parasitoids (blue), pollinators (purple)

The seasonal distribution of arthropod abundance is illustrated in Figure 3. The lowest abundance values were recorded at the beginning of the sampling period, while a marked increase occurred during late spring and early summer, reaching a peak in June and July. After this period, abundance values gradually declined toward the end of the growing season.

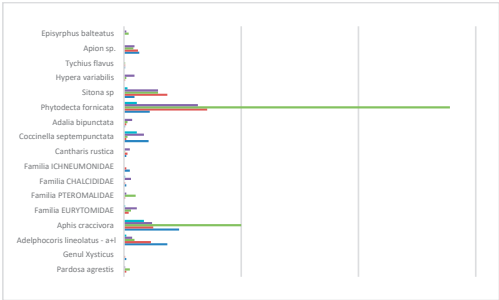


Figure 3. Seasonal structure of arthropod abundance recorded in the alfalfa agroecosystem during the 2024 sampling period

A more detailed view of the species-level dynamics underlying this seasonal trend is provided in the heatmap of dominant species (Figure 3).

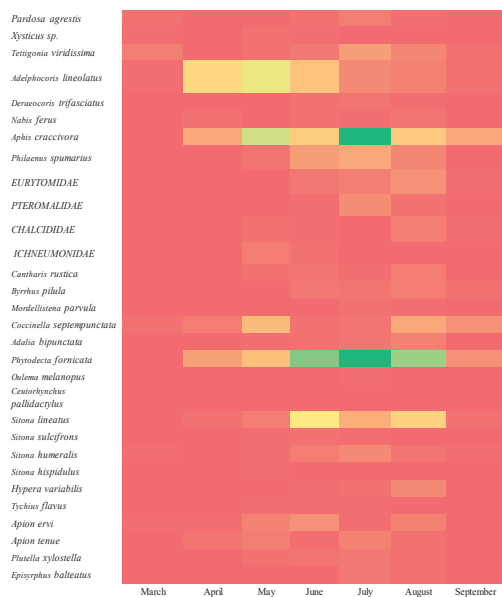


Figure 4. Seasonal distribution of dominant arthropod species recorded in the alfalfa agroecosystem located in Ghilad (Timiș County, Romania) during the 2024 sampling period. Species are arranged according to total abundance (dominance gradient), while columns represent sampling months (April-September). Color intensity reflects abundance values, with warmer colors indicating higher abundance

The heatmap of dominant species (Figure 4) highlights the taxa responsible for the seasonal patterns observed in community abundance. The distribution clearly indicates the predominance of phytophagous species, particularly *Phytodecta fornicata* and *Aphis craccivora*, which exhibited pronounced abundance peaks during June and July.

To further explore the influence of climatic factors on these seasonal patterns, correlations between trophic groups and climatic variables were evaluated using Spearman's rank correlation analysis (Table 5).

Table 5. Spearman correlation coefficients between climatic variables (temperature and precipitation) and arthropod trophic groups

Trophic group	ρ Temperature	p-value	ρ Precipitation	p-value
Phytophagous	0.771	0.072	-0.314	0.544
Predators	0.543	0.266	-0.200	0.704
Parasitoids	0.886	0.019	-0.371	0.468
Pollinators	0.845	0.034	-0.304	0.558

Spearman correlation analysis performed at the trophic-group level indicated that temperature was the main climatic factor influencing arthropod dynamics in the alfalfa agroecosystem under hydric stress conditions. Parasitoids showed the strongest positive and statistically significant correlation with temperature ($\rho = 0.886$, $p = 0.019$), followed by pollinators ($\rho = 0.845$, $p = 0.034$). The phytophagous group, which numerically dominated the assemblage, also exhibited a strong positive association with temperature ($\rho = 0.771$), although the relationship was only marginally significant ($p = 0.072$). Predators displayed a weaker positive correlation with temperature ($\rho = 0.543$, $p = 0.266$), suggesting that their population dynamics were less directly controlled by thermal conditions and possibly mediated by prey availability.

In contrast, precipitation showed generally negative relationships with all trophic groups, although these correlations were not statistically significant. The strongest negative association was observed for parasitoids ($\rho = -0.371$, $p = 0.468$) and phytophagous arthropods ($\rho = -0.314$, $p = 0.544$). These patterns suggest that reduced rainfall and increased temperature during the summer months may have created hydric stress conditions within the alfalfa agroecosystem, favoring the activity and development of several arthropod groups, particularly phytophagous species. Such climatic conditions may alter the functional balance of the arthropod community by promoting herbivore population growth while exerting weaker or indirect effects on higher trophic levels.

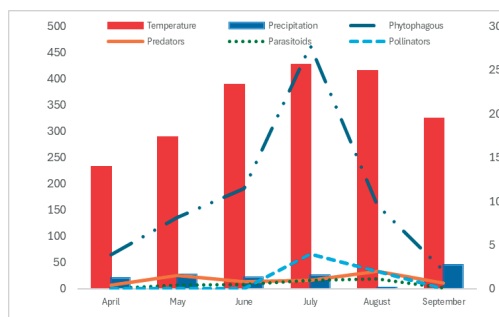


Figure 5. Seasonal distribution of arthropod abundances recorded in the alfalfa agroecosystem during the 2024 growing season

These patterns are further illustrated in Figure 5, which depicts the seasonal distribution of arthropod abundances in relation to temperature and precipitation during the sampling period. The figure reveals a clear expansion of abundance distributions during the mid-summer months, with a pronounced peak in July, corresponding to the period of highest temperatures recorded in the agroecosystem. This seasonal increase supports the positive relationships observed between temperature and several trophic groups, particularly parasitoids and pollinators.

During the same period, precipitation remained relatively low, with a marked reduction in August, indicating the development of hydric stress conditions within the alfalfa crop. Under such circumstances, the arthropod community appears strongly dominated by phytophagous taxa, which contributed most to the broader abundance distributions observed in the figure. In contrast, higher trophic levels, such as predators and parasitoids, were present at comparatively lower abundance levels, reflecting the typical trophic structure of drought-affected agroecosystems.

Overall, both the statistical analyses and the graphical patterns indicate that temperature represents the primary climatic factor structuring arthropod dynamics, while reduced precipitation likely contributed to the hydric stress conditions observed in the alfalfa agroecosystem, potentially influencing the trophic structure of the arthropod community.

The results of the present study indicated the presence and seasonal abundance of 30 arthropod taxa in alfalfa fields located in Ghilad (Timiș County). The occurrence of several taxonomic orders and functional groups is consistent with previous studies reported in Romania (Lisenchi–Murariu et al., 2006 a, b; Dima et al., 2010; Fiera et al., 2013; Pustai et al., 2015), which have shown that perennial forage crops such as alfalfa provide stable habitats for numerous arthropod taxa.

The pronounced dominance of phytophagous species observed in the present study (*Phytodecta*, *Aphis*, *Adelphocoris*, *Sitona*) is typical of herbaceous agroecosystems and reflects the bottom-up influence exerted by plant resources on phytophagous populations (Razzaq

& Al-Saffar, 2016; Kaplin et al., 2020). Similar dominance patterns, characterized by a few highly abundant species and numerous taxa with low abundance, have frequently been reported in alfalfa ecosystems (Shebl et al., 2009). We found that the complex of predators, parasitoids, and pollinators was dominated by *Coccinellidae*, closely associated with parasitoids *Hymenoptera*, which is consistent with other studies (Soroka et al., 2020; Meseguer et al., 2021; Ríos Martínez et al., 2024).

Seasonal dynamics and climatic conditions also played an important role in structuring arthropod abundance. Our data suggests considerable variation in seasonal abundance trends of phytophagous taxa and natural enemies across the sampling months. The highest abundance values were recorded during the warmest months of the growing season, highlighting the positive effects of air temperature on the arthropod community, particularly on phytophagous taxa. These effects may be explained by the dual role of temperature: on the one hand, through the direct stimulation of insect development, reproductive capacity, and survival rates (Nikolova, 2018), and on the other hand, through the indirect influence on the availability of food resources (Lessard et al., 2011).

In addition to air temperature, precipitation also plays an important role in influencing insect species richness and abundance (Zhu et al., 2014). In this context, reduced precipitation during summer contributed to the development of hydric stress conditions in the alfalfa crop. Our results indicate that the lack of precipitation was associated with lower abundance of natural enemies and an increase in phytophagous taxa. These relationships may primarily be explained by unfavorable habitat conditions caused by moisture deficit, which can influence plant physiology and the nutritional quality of resources available to insects (Bao et al., 2026). Consequently, climatic variability, particularly increasing temperatures combined with reduced precipitation, influenced both the seasonal dynamics and the trophic structure of arthropod communities associated with alfalfa agroecosystems.

CONCLUSIONS

The present study highlights the biodiversity and trophic organization of arthropod fauna associated with an alfalfa agroecosystem exposed to hydric stress. The community was strongly dominated by phytophagous species, whereas predators, parasitoids, and pollinators occurred at comparatively lower abundances. Arthropod dynamics showed marked seasonal variation, with peak abundance during the warmest months of the growing season. Our results suggest that the combination of increasing temperature and reduced precipitation reshaped both seasonal dynamics and trophic balance within the arthropod assemblage.

These findings underline the importance of arthropod monitoring during dry years, when environmental stress may favor herbivore proliferation and increase the risk of pest outbreaks. Under such conditions, regular surveillance and integrated management strategies are essential for maintaining ecological balance and ensuring the sustainable management of alfalfa agroecosystems.

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APPLICATION OF IMAZAMOX FOR WEED CONTROL IN SORGHUM (*Sorghum bicolor* (L.) Moench)

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Abstract

In 2023 and 2024, a field trial with sorghum, hybrid Sentinel IG in the experimental field of the Agricultural University - Plovdiv, Bulgaria, was conducted. The experience variants include: 1. Untreated control; 2. Pulsar 40 - 1.20 L ha⁻¹ (BBCH 14-16); 3. Pulsar 40 + Tonivit in rates of 1.20 L ha⁻¹ + 1.00 L ha⁻¹ (BBCH 14-16); 4. Pulsar 40 - 1.20 L ha⁻¹ (BBCH 14-16) + Apetayzer - 0.50 L ha⁻¹ (BBCH 17-19); 5. Pulsar 40 + Tonivit in rates of 1.20 L ha⁻¹ + 1.00 L ha⁻¹ (BBCH 14-16) + Apetayzer - 0.50 L ha⁻¹ (BBCH 17-19); 6. Pulsar 40 - 4.80 L ha⁻¹ (BBCH 14-16). The application of Pulsar 40 (4.80 L ha⁻¹) provides one hundred percent control against *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Sinapis arvensis* L., and *Setaria viridis* L. On the 7th day after application of Pulsar 40 (4.80 L ha⁻¹) it causes phytotoxicity with a score of 3 - slight chlorosis on the leaves, and by the 21st day the crop recovers. The highest yield was recorded after application of Pulsar 40 + Tonivit + Apetayzer (variant 5).

Key words: sorghum, herbicides, weeds, efficacy, yield.

INTRODUCTION

Ensuring sustainable resources for the food sector remains key challenges facing modern society. Crops play a crucial role as a primary source of raw materials. As a result, scientific interest has increased, along with the number of studies focusing on the development and implementation of innovative technological solutions for efficient and sustainable agricultural production (Ahmad et al., 2025; Dulaimy et al., 2025; Mitkov 2025; Thuleau et al., 2025; Tourneur et al., 2025; Panayotov et al., 2024; Panchev & Shopova, 2024; Shevchenko et al., 2024; Kolentsova et al., 2023; Shopova, 2023; Fernández-Aparicio et al., 2022; Ivanov et al., 2021; Kalinova et al., 2021; Tonev et al., 2021; Komitov et al., 2020; Mitkov, 2020; Dimtrova et al., 2019; Ivanov et al., 2019; Goranovska et al., 2017; Shopova & Cholakov, 2015; Uzunova et al., 2015; Dimitrova et al., 2014; López-Bellido et al., 2014; Mitkov et al., 2014; Shopova & Cholakov, 2014; Kalinova et al., 2012; Tonev et al., 2010; Mitkov et al., 2009).

Climate change observed in recent years has further limited the range of crops suitable for cultivation. The trend toward rising temperatures, combined with reduced and unevenly distributed precipitation, highlights

the need to cultivate crops that are better adapted to changing environmental conditions. One of the potential crops capable of thriving under such conditions is sorghum (*Sorghum bicolor* L. Moench) (Fontanet-Manzanegue et al., 2025; Abreha et al., 2022). For example, Hadebe et al. (2017) report that sorghum is well-suited for cultivation in the dry and semi-arid agroecological zones of Sub-Saharan Africa. This suitability is largely due to its root system, which consists of numerous root hairs that facilitate water uptake from the soil, and the waxy layer on its leaves, which reduces water loss (El Bassam, 2010).

Among cereal crops worldwide, sorghum ranks fifth in importance after wheat, rice, maize, and oats (Khalil, 2008; Pacheco, 1998). The high nutritional value of its grains - rich in proteins and dietary fiber, and gluten-free-supports their use as a substitute for wheat flour in baking and confectionery products (Impa et al., 2019; Xiong et al., 2019; McCann et al., 2015). Additionally, sorghum can serve as a raw material for bioethanol production (Mathur et al., 2017; Makuh et al., 2013), and its ability to produce large amounts of biomass makes it valuable as animal feed (Ghani et al., 2015).

Weeds are one of the main factors limiting the growth and development of *Sorghum bicolor* L. Moench (Geier et al., 2009). They compete with

sweet sorghum for essential resources such as water, light, and nutrients (Grabovskiy et al., 2018). In addition, weeds may serve as intermediate hosts for various diseases and pests and may also interfere with the harvesting of the crop (Ottman & Olsen, 2009; Capinera, 2005; Zimdahl, 1999). According to Dille et al. (2020), competition from weeds can reduce sorghum yield by more than 60%. Bagavathiannan et al. (2018) report that grassy weeds are more difficult to control in sorghum compared with broadleaf weeds. Magani (2008) found that weed infestation in sorghum fields resulted in a significant yield reduction of 40-60%, while Stahlman and Wicks (2000) reported yield losses of 30-50%, along with a deterioration in crop quality. More detailed studies have shown that infestation of sorghum with *Parthenium hysterophorus* can reduce yield by up to 81% in Pakistan (Bajwa et al., 2020) and up to 97% in Ethiopia (Tamado et al., 2002).

Effective weed management requires accurate identification of the weed species. In Ludhiana, sorghum fields are heavily weed-infested, with species such as *Trianthema portulacastrum*, *Digera arvensis*, *Commelina benghalensis*, *Dactyloctenium aegyptium*, *Eleusine indica*, *Cyperus rotundus*, *Eclipta alba*, and *Digitaria sanguinalis* predominating (Kaur et al., 2024). In the Kafr El-Sheikh Governorate, Egypt, the most common weeds are *Corchorus olitorius*, *Xanthium strumarium*, *Amaranthus album*, *Portulaca oleracea*, *Ammania aegyptiaca*, *Echinochloa colona*, and *Dinebra retroflexa* (Khaffagy et al., 2014). Sweet sorghum fields in the Kyiv region, Ukraine, were reported to be infested with *Chenopodium album* L., *Setaria viridis* L., *Setaria glauca* L., *Amaranthus retroflexus* L., *Echinochloa crus-galli* L., *Convolvulus arvensis* L., *Capsella bursa-pastoris* L., *Polygonum convolvulus* L., *Cirsium arvense* L., and *Elytrigia repens* L. (Grabovskiy et al., 2018). In Italy, perennial weeds such as *Sorghum halepense* and *Convolvulus* spp. occur at high densities in sorghum fields (Rapparini, 2000).

Various methods can be employed for weed control. Traditional hand weeding is effective but labor- and time-intensive, making it less economically viable under conditions of high labor costs (Vijayakumar et al., 2014). Mechanized weed control offers a more efficient

alternative, requiring less labor and time (Chivinge, 1990). In modern agriculture, chemical control through herbicides is the primary approach. Soil-applied pyroxasulfone (0.1275 kg a.i./ha) has been recommended for controlling broadleaf and grassy weeds in sorghum, with only minimal early-stage phytotoxicity that is overcome in later growth stages (Kaur et al., 2024).

Bajwa et al. (2023) report that pendimethalin followed by bromoxynil effectively controls *Parthenium* weeds. Dimitrova (2005) found that pre-emergence application of isoxaflutole (94 g/ha) followed by 2,4-D + dicamba at the 4-5 leaf stage, combined with a single inter-row cultivation, provides optimal weed control. Post-emergence applications of 2,4-D, sometimes combined with soil-applied atrazine, have also been recommended for controlling broadleaf weeds and sedges (Krishnamurthy et al., 2021; Dan et al., 2010; Kumar et al., 2008; Stahlman & Wicks, 2000).

For environmental reasons, post-emergence herbicides are preferred, even though their action may be shorter than soil-applied herbicides (Takano et al., 2016). Flumioxazin (50 g ha⁻¹) has been identified as a potential herbicide for broadleaf weeds in sweet sorghum, although it may cause a low degree of phytotoxicity (Galon et al., 2016). Although sorghum exhibits mixed weed infestations, grassy weeds remain particularly challenging, and chemical options for their control during vegetative growth are limited (Dille et al., 2020; Bagavathiannan et al., 2018). As a result, sorghum cultivars with enhanced herbicide tolerance, including igrowth™ (resistant to imazamox), Inzen™ (resistant to nicosulfuron), and DoubleTeam™ (resistant to quizalofop), have been developed (Norsworthy et al., 2020).

This study aims to investigate the application of imazamox for weed control in sorghum (*Sorghum bicolor* (L.) Moench).

MATERIALS AND METHODS

In 2023 and 2024, a field trial with sorghum, hybrid Sentinel IG, was conducted in the experimental field of the Agricultural University - Plovdiv, Bulgaria. The experiment was laid out using the long-plot method with four

replications, with a plot size of 50 m² (Dimova & Marinkov, 1999). The experimental treatments included: 1. Untreated control - without tillage or herbicides; 2. Pulsar 40 - 1.20 L ha⁻¹ (BBCH 14-16); 3. Pulsar 40 + Tonivit at rates of 1.20 L ha⁻¹ + 1.00 L ha⁻¹ (BBCH 14-16); 4. Pulsar 40 - 1.20 L ha⁻¹ (BBCH 14-16) + Apetayzer - 0.50 L ha⁻¹ (BBCH 17-19); 5. Pulsar 40 + Tonivit at rates of 1.20 L ha⁻¹ + 1.00 L ha⁻¹ (BBCH 14-16) + Apetayzer - 0.50 L ha⁻¹ (BBCH 17-19); 6. Pulsar 40 - 4.80 L ha⁻¹ (BBCH 14-16). The application of the products was carried out using a SOLO backpack electric sprayer (model 417), with a working solution volume of 200 L ha⁻¹.

Pulsar® 40 is a post-emergence systemic herbicide containing 40 g L⁻¹ imazamox. The active ingredient belongs to the imidazolinone group of herbicides and inhibits the enzyme acetolactate synthase, which is essential for the synthesis of branched-chain amino acids in plants. It is effective against both annual and perennial grassy and broadleaf weeds (BASF, 2022).

The biostimulant Tonivit contains an extract of the brown algae *Ascophyllum nodosum* and provides 13% water-soluble phosphorus pentoxide (P₂O₅) and 5% potassium oxide (K₂O). It also includes biologically active compounds such as amino acids, vitamins, and oligosaccharides, which enhance physiological processes and nutrient uptake in plants (UPL, 2023a).

Apetayzer is a biostimulant based on *Ascophyllum nodosum* filtrate, supplemented with water-soluble manganese and zinc. It incorporates a proprietary technology designed to activate nutrient-related physiological processes in plants (UPL, 2023b).

The preceding crop was wheat. Following its harvest in autumn, deep plowing to a depth of 30-35 cm and two diskings were performed. Pre-sowing fertilization was carried out with a combined NPK fertilizer (15:15:15) at 200 kg ha⁻¹. At the 7-8 leaf stage of *S. bicolor* L. Moench, top-dressing was applied using 200 kg ha⁻¹ NH₄NO₃. Sowing of the Sentinel IG hybrid was performed on 12 May 2023 (first year) and 8 May 2024 (second year), at a seeding rate of 6,500 plants ha⁻¹. The crop was grown under rainfed conditions.

During both years, the experimental fields were naturally infested with *Amaranthus retroflexus* L., *Chenopodium album* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Sinapis arvensis* L., *Setaria viridis* L., *Anthemis arvensis* L., and *Sorghum halepense* (L.) Pers. developed from rhizomes. The biological efficacy of Pulsar® 40 and the biostimulants was assessed at 7, 14, and 28 days after treatment (DAT) using the EWRS visual assessment scale. The selectivity of imazamox, applied alone or in combination with biostimulants, was evaluated at 7, 14, 21, and 28 DAT according to the EWRS scale (score 1 - no crop injury, score 9 - complete crop destruction). In addition to biological efficacy and selectivity, grain yield (t ha⁻¹) was recorded. Statistical analysis was performed using Duncan's multiple range test with SPSS 19 software (Duncan, 1955), and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The meteorological conditions during the study period were variable between the two experimental years. The first year, 2023, was characterized by higher mean monthly temperatures and lower overall precipitation compared to the long-term averages (Tables 1 and 2). In May and June, precipitation exceeded the multi-year mean by 31.3 mm/m² and 47 mm/m², respectively, creating favorable soil moisture conditions for seed germination and normal crop development. However, during July, August, and September, total rainfall was only 57 mm/m², which was 104 mm/m² lower than the multi-year average. Mean monthly temperatures from May until the end of the crop's growing season were above the norm, with a cumulative temperature sum 13.7 °C higher than the long-term average. July, August, and September were particularly hot, with maximum temperatures exceeding 38 °C in July. In contrast, meteorological conditions in the second experimental year, 2024, were less favorable for rainfed sorghum cultivation. Although May rainfall provided adequate soil moisture for germination, it exceeded the long-term average by 51 mm/m². From June to September, a prolonged drought occurred, restricting normal crop growth. Total

precipitation during this period was 141.8 mm/m² lower than the multi-year average (Table 1). Concurrently, mean monthly temperatures throughout the growing season were substantially above the norm, with a cumulative temperature sum from May to September 16.4

°C higher than the long-term average (Table 2). Similar to 2023, temperatures in July exceeded 38 °C. The adverse meteorological conditions during 2024 had a negative impact on grain yield.

Table 1. Precipitation amount during the study period, mm/m²

Month	2023	Average for the period 1916-1970	Deviation	2024	Average for the period 1916-1970	Deviation
May	63.3	32	+31.3	83	32	+51
June	83	36	+47	2	36	-34
July	26.5	42	-15.5	24.2	42	-17.8
August	10.5	54	-43.5	12	54	-42
September	20	65	-45	17	65	-48
Total	202.8	229	-26.2	138.2	229	-90.8

Table 2. Average monthly temperature for the study period, °C

Month	2023	Average for the period 1916-1970	Deviation	2024	Average for the period 1916-1970	Deviation
May	18.9	17.2	+1.7	16.9	17.2	-0.3
June	22.8	20.9	+1.9	26.3	20.9	+5.4
July	26.1	23.2	+2.9	27.6	23.2	+4.4
August	26.4	22.7	+3.7	26.8	22.7	+4.1
September	21.8	18.3	+3.5	21.1	18.3	+2.8
Total	116	102.3	+13.7	118.7	102.3	+16.4

The results indicate that the biological efficacy of the tested products increased progressively from 7 to 28 DAT. On the seventh DAT, control of *Amaranthus retroflexus* L. – the most

abundant weed species in the experiment – was 90% across all herbicide-treated plots, reaching 100% control by 28 DAT (Table 3).

Table 3. Efficacy (%) of the studied products against *Amaranthus retroflexus* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	85	90	100	95	100	100	90	95	100
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	95	100	100	85	90	100	90	95	100
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	90	100	100	90	90	100	90	95	100
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	95	100	100	85	90	100	90	95	100
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	95	100	100	85	90	100	90	95	100

Imazamox applied alone or in combination with biostimulants exhibited lower efficacy against *Chenopodium album* L. compared to *A. retroflexus* L. On the first assessment date, all plots treated with Pulsar 40 at 1.20 L ha⁻¹, either alone or in combination with Tonivit and Apetayzer, showed 65% control. Quadrupling the registered dose of Pulsar 40 significantly increased efficacy to 85%. By 14 DAT, efficacy reached 70% in variants 2, 3, 4 and 5 and 90% in the high-dose treatment. At 28 DAT, the highest control (95%) was achieved with Pulsar

40 at 4.80 L ha⁻¹, while variants 2, 3, 4 and 5 showed satisfactory control at 75% (Table 4). For the late-spring weed *Xanthium strumarium* L., the highest efficacy on the seventh DAT was recorded in the high-dose Pulsar 40 variant (4.80 L ha⁻¹) - 85%, while other treatments achieved 75%. By 14 DAT, efficacy increased, reaching 90% in the high-dose treatment, and by 28 DAT, complete control (100%) was observed. Variants 2, 3, 4 and 5 also showed very good control, reaching 95% efficacy (Table 5).

Table 4. Efficacy (%) of the studied products against *Chenopodium album* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	70	75	80	60	65	70	65	70	75
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	70	75	80	60	65	70	65	70	75
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	65	65	70	65	75	80	65	70	75
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	70	75	80	60	65	70	65	70	75
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	90	95	100	80	85	90	85	90	95

Table 5. Efficacy (%) of the studied products against *Xanthium strumarium* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	70	80	90	80	90	100	75	85	95
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	75	80	95	75	90	95	75	85	95
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	70	85	90	80	85	100	75	85	95
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	70	80	95	80	90	95	75	85	95
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	90	90	100	80	90	100	85	90	100

Table 6. Efficacy (%) of the studied products against *Solanum nigrum* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	80	80	95	80	90	95	80	85	95
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	75	80	90	85	90	100	80	85	95
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	85	90	100	75	80	90	80	85	95
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	80	85	90	80	85	100	80	85	95
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	80	85	100	90	95	100	85	90	100

The results regarding the control of *Solanum nigrum* L. were similar to those observed for *Xa. strumarium* L. On the first assessment date, the highest efficacy - 85% was achieved with Pulsar 40 at 4.80 L ha⁻¹ (variant 6). In the remaining treatments with Pulsar 40, applied either alone or in combination with biostimulants, control was 80%. On the 14 DAT, efficacy in the treated plots ranged from 85 to 90%, while by the 28 DAT it reached 100% for variant 6 and 95% for variants 2, 3, 4, and 5 (Table 6).

The tested herbicides and biostimulants showed limited control of *Portulaca oleracea* L. On the 7 DAT, in all treatments containing Pulsar 40 at

1.20 L ha⁻¹, efficacy against the weed was 25%. In the treatment with Pulsar 40 at 4.80 L ha⁻¹, control was slightly higher (40%) but still unsatisfactory. On the 14 DAT, only a minimal increase in control was observed. In variants 2, 3, 4, and 5, efficacy was merely 30%, whereas in variant 6 it reached 50%. By the 28 DAT, even after some improvement in control of *P. oleracea* L., efficacy in variants 2, 3, 4, and 5 remained unsatisfactory (35%). Control of this weed remained limited (55%) even after applying a fourfold higher than the registered dose of Pulsar 40 at 4.80 L ha⁻¹ (Table 7).

Table 7. Efficacy (%) of the studied products against *Portulaca oleracea* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	20	25	30	30	35	40	25	30	35
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	20	30	30	30	30	40	25	30	35
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	25	25	35	25	35	35	25	30	35
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	25	35	40	25	25	30	25	30	35
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	35	45	50	45	55	60	40	50	55

Table 8. Efficacy (%) of the studied products against *Sorghum halepense* (L.) Pers. developed from rhizomes, day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	15	20	25	25	30	35	20	25	30
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	20	25	25	20	25	35	20	25	30
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	15	20	25	25	30	35	20	25	30
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	15	20	25	25	30	35	20	25	30
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	70	75	80	80	85	90	75	80	85

Table 9. Efficacy (%) of the studied products against *Sinapis arvensis* L., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	75	80	90	75	90	100	75	85	95
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	70	80	90	80	90	100	75	85	95
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	70	85	90	80	85	100	75	85	95
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	75	85	95	75	85	95	75	85	95
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	85	90	100	95	100	100	90	95	100

Similar to the control of *P. oleracea* L., the application of Pulsar 40 at the registered dose of 1.20 L ha⁻¹, either alone or in combination with Tonivit and Apetayzer, failed to effectively suppress *Sorghum halepense* (L.) Pers. developed from rhizomes. The highest efficacy in these treatments was recorded on the 28 DAT after treatment, reaching only 30%. Application of Pulsar 40 at 4.80 L ha⁻¹ provided substantially better control, achieving 85% efficacy against the weed by the 28 DAT (Table 8).

Sinapis arvensis L. was identified as one of the weed species most sensitive to the herbicides used in the study. By the 7 DAT, application of

Pulsar 40 at 4.80 L ha⁻¹ achieved 90% efficacy, which increased to 100% by the 28 DAT. In variants with lower imazamox rates, control of *S. arvensis* L. was 75% on the 7 DAT, reaching 95% by the 28 DAT (Table 9).

Under the conditions of the experiment, control of *Setaria viridis* (L.) P. Beauv. ranged from very good to excellent, depending on the applied imazamox rate. On the 7 DAT, average efficacy across variants 2, 3, 4, and 5 was 75%, while variant 6 reached 85%. By the 28 DAT, 100% control was achieved with Pulsar 40 at 4.80 L ha⁻¹. In the remaining variants involving imazamox, efficacy reached 90% (Table 10).

Table 10. Efficacy (%) of the studied products against *Setaria viridis* (L.) P.Beauv., day after treatment (DAT)

Variants	2023			2024			Average		
	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
1. Untreated control	0	0	0	0	0	0	0	0	0
2. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16	80	85	95	70	75	85	75	80	90
3. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16	75	80	85	75	80	95	75	80	90
4. Pulsar 40 (1.20 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	70	75	85	80	85	95	75	80	90
5. Pulsar 40 (1.20 L ha ⁻¹) + Tonivit (1.00 L ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 L ha ⁻¹) BBCH 17-19	75	80	85	75	80	95	75	80	90
6. Pulsar 40 (4.80 L ha ⁻¹) BBCH 14-16	80	85	100	90	95	100	85	90	100

Regarding the selectivity of the tested products towards *sorghum*, hybrid Sentinel IG, the following key results were obtained. Under the experimental conditions, the application of Pulsar 40 at 4.80 L ha⁻¹ caused phytotoxic effects on the sorghum hybrid. On the 7 DAT, a phytotoxicity score of 3 on the EWRS scale was recorded, manifested as slight leaf chlorosis. By the second assessment at 14 DAT, the crop began to recover, but phytotoxicity was still observed with a score of 2. On the 21 DAT, the crop overcame the herbicide stress, reaching a score of 1 on the EWRS scale. In the other treatments with Pulsar 40 at 1.20 L ha⁻¹ (variants 2, 3, 4, and 5), no visual signs of phytotoxicity were observed, with a consistent score of 1 on the EWRS scale.

In addition to the biological efficacy and selectivity of the tested products, the grain yield of sorghum, hybrid Sentinel IG, was also determined during the experiment. After statistical analysis of the data, the lowest yield was observed in the Untreated control (0.65 t ha⁻¹), where weeds were not controlled. It is noteworthy that yields across all variants in 2024 were lower compared to 2023 (Table 11). This decrease is attributable to the less favorable climatic conditions in 2024, despite the drought tolerance of sorghum (Tables 2 and 3).

The highest grain yield was obtained following the combined application of Pulsar 40 (1.20 L ha⁻¹) + Tonivit (1.00 L ha⁻¹) at the 4-6 leaf stage and Apetayzer (0.50 L ha⁻¹) at the 7-9 leaf stage (variant 5). In this variant, yield reached 2.23 t ha⁻¹ in 2023 and 1.75 t ha⁻¹ in 2024. Similar average yields were observed in variants 3, 4, and 2, with 1.96 t ha⁻¹, 1.92 t ha⁻¹, and 1.90 t ha⁻¹, respectively. It is important to note that no statistically significant differences were found among these four variants across the two

experimental years (Table 11). The yield increase resulting from effective weed control through herbicide application has also been confirmed by Solaimalai & Muthusankaranarayanan (2000).

Table 11. Sorghum grain seed yield (t ha⁻¹)

Variants	2023	2024	Average
1. Untreated control	0.71 c	0.59 c	0.65
2. Pulsar 40 (1.20 l ha ⁻¹) BBCH 14-16	2.13 a	1.67 a	1.90
3. Pulsar 40 (1.20 l ha ⁻¹) + Tonivit (1.00 l ha ⁻¹) BBCH 14-16	2.19 a	1.72 a	1.96
4. Pulsar 40 (1.20 l ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 l ha ⁻¹) BBCH 17-19	2.15 a	1.69 a	1.92
5. Pulsar 40 (1.20 l ha ⁻¹) + Tonivit (1.00 l ha ⁻¹) BBCH 14-16 + Apetayzer (0.50 l ha ⁻¹) BBCH 17-19	2.23 a	1.75 a	1.99
6. Pulsar 40 (4.80 l ha ⁻¹) BBCH 14-16	1.95 b	1.53 b	1.74

Variants with different letters are with proved difference according to Duncan's multiple range test ($p < 0.05$).

Although Pulsar 40 at 4.80 L ha⁻¹ provided the highest weed control, the grain yield obtained with this treatment over the two years was significantly lower than that recorded for treatments with Pulsar 40 at 1.20 L ha⁻¹, either applied alone or in combination with Tonivit and Apetayzer. This reduction is likely attributable to phytotoxic effects on the crop caused by the fourfold increase in the imazamox dose, compounded by adverse meteorological conditions.

CONCLUSIONS

The application of Pulsar 40 at a rate of 4.80 L ha⁻¹ (BBCH 14-16) provides one hundred percent control against *Amaranthus retroflexus* L., *Xanthium strumarium* L., *Solanum nigrum* L., *Sinapis arvensis* L., and *Setaria viridis* L. The highest efficacy against *Chenopodium*

album L. - 95%, *Sorghum halepense* (L.) Pers. developed from rhizomes - 85%, and *Portulaca oleracea* L. - 55% was also recorded with Pulsar 40 at a rate of 4.80 L ha⁻¹.

Under the experimental conditions, Pulsar 40 at a rate of 4.80 L ha⁻¹ caused phytotoxicity on sorghum, hybrid Sentinel IG, at 7 DAT, with a score of 3 on the EWRS scale, expressed as slight leaf chlorosis. By 21 DAT, the crop had overcome the herbicide stress, reaching a score of 1 on the EWRS scale.

The highest yield was recorded following the combined application of Pulsar 40 (1.20 L ha⁻¹) + Tonivit (1.00 L ha⁻¹) + Apetayzer (0.50 L ha⁻¹) - 1.99 t ha⁻¹, followed by Pulsar 40 (1.20 L ha⁻¹) + Tonivit (1.00 L ha⁻¹) - 1.96 t ha⁻¹, Pulsar 40 (1.20 L ha⁻¹) + Apetayzer (0.50 L ha⁻¹) - 1.92 t ha⁻¹, and Pulsar 40 (1.20 L ha⁻¹) - 1.90 t ha⁻¹. No statistically significant differences were observed among these treatments.

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RESEARCH ON THE MANAGEMENT OF BACTERIAL BLIGHT AND THE COMMON RED MITE IN AN IRRIGATED SOYBEAN CROP

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Abstract

The aim of this work is to highlight an effective treatment scheme for combating bacterial blight of soybean (*Pseudomonas savastanoi* pv. *glycinea*) and common red spider mite (*Tetranychus urticae*) under conditions of natural infection and irrigated cultivation. Observations were carried out in 2023, in the experimental field arranged within I.I. Popescu Iulian, in the city of Ianca, Brăila county. The biological material used was the ES Palador variety. Bacterial blight attack was assessed visually, and mites were captured by shaking the leaves onto a white sheet of paper. The frequency of soybean plants attacked by bacterial blight varied between 4.0 and 82.33% in the experimental variants. The common red spider mite attack reduced soybean production by 16.85%. The highest production, of 4895 kg/ha, was obtained in the variants with two treatments against the two biotic factors. Following the research, it can be concluded that among the limiting biotic factors in soybean culture are both the bacterium *Pseudomonas savastanoi* pv. *glycinea* and the mite *Tetranychus urticae*.

Key words: soybean, bacterial blight, common red mite, management.

INTRODUCTION

Soybean -*Glycine max* (L.) Merr. is an important agricultural crop due to its multiple uses: human food (oil, tofu, soybean hamburger or soy milk, due to the quality of proteins, along with high concentrations of isoflavones, phytosterols and minerals), feed (soybean meal fed to farm animals, especially poultry and pigs) and industrial (biodiesel), which is why it occupies close to 6% of the world's arable land (Goldsmith, 2008; Rotundo et al., 2024).

Soybean consumption is beneficial for human health because it contributes to lowering cholesterol levels (Jenkins et al., 2010) and blood pressure, which is reflected in a lower incidence of heart attack and stroke (Yan et al., 2017).

Cultivation is successful in areas with average temperatures of 23-28°C; temperatures below 20°C and above 40°C significantly inhibit growth (Kado, 2010).

Although it has the ability to fix atmospheric nitrogen due to its symbiosis with the bacterium *Bradyrhizobium japonicum* (Kirchner) Jordan, for best yields, it is recommended to inoculate the seeds with bacteria before sowing.

The area cultivated with soybeans in Romania in 2023 was 142,000 ha, and in 2024 145,000 ha. The total soybean production in 2023 was 303,000 tons, which corresponds to an average production of 2,133.8 kg/ha, and in 2024 295,000 tons, the average production being 2,034.5 kg/ha (https://insse.ro/cms/sites/default/files/com_presa/com_pdf/prod_veg_r24.pdf). The area cultivated with soybeans in Brăila County, in the 2023-2024 agricultural year, was 19,276 ha (<https://probr.ro/din-cauza-secetei-pedologice-instalate-in-anul-agricol-2023-2024-in-judetul-braila-a-fost-calamitata-o-suprafata-totala-de-113-125-ha/>).

Climate change will cause soybeans to be affected by certain pathogens and pests in the growth process, which will be reflected in the qualitative and quantitative decrease in production.

Pseudomonas savastanoi pv. *glycinea* (Coerper) Gardan, Bollet, Abu-Ghorrah, Grimont & Grimont is a common pathogen in soybeans grown in Romania and can be found in most soybean fields every year.

Severe infections with *Pseudomonas savastanoi* pv. *glycinea* can cause yield losses of up to 40% (Williams and Nyvall, 1980).

The bacteria enter the plant through natural openings and wounds.

The pathogen overwinters in crop residue and can be seed transmitted.

The common red spider mite-*Tetranychus urticae* Koch, is an important pest of soybean crops in hot and dry areas.

Due to the high number of generations per year, 6-10 (Manolache et al., 1978 cited by Roșca et al., 2011), it can cause plant damage of 10-50% (Biliavska et al., 2025).

Spider mite infestation is recognized by the webbing present on the surface of the leaves and by the discoloration of the leaves.

The stability of global food systems increasingly depends on the resilience of cultivated plants to environmental stress. Yet the productivity of these plants is persistently threatened by a diverse spectrum of pathogens and pests whose development and dissemination are tightly regulated by climatic conditions in different areas worldwide (Cotuna et al., 2021; Cotuna et al., 2022; Paraschivu et al., 2021; Paraschivu et al., 2023; Păunescu et al., 2022; Velea et al. 2021).

MATERIALS AND METHODS

The objective of this research was to estimate the degree of attack of bacterial blight and red spider mite in soybeans grown under irrigation conditions in order to obtain the necessary information for making decisions regarding the application of phytosanitary treatments.

Ratings were based on visual inspection, a conventional method of detecting disease and mite attack based on symptoms induced by them (Zală et al., 2012; Zală et al., 2014).

As shown in Figure 1, the soybean variety of ES Palador cultivated in 2023 were planted and cultivated in the experimental field of II Popescu Iulian, in location Ianca (45°8'6"N 27°28'29"E), Brăila county. ES Pallador is a semi-late soybean variety, with indeterminate growth, adaptable to various soil and climatic conditions, suitable for all growing areas in Romania. It has a protein content of 41.3% and 20.3% oil. Recommended density on irrigated soils: 450-500,000 b.g/ha (<https://lidea-seeds.ro/products/soia-1-1>).

The preceding plant was corn.



Figure 1. Image from the experimental soybean field, ES Palador variety (source: C. R. Zală)

Applied agrotechnics:

- sowing 492000 b.g./ha, 70 cm row spacing + administered N:P:K type fertilizers - 20:20:0, on 20.04.2023;

- post-emergence herbicide against weeds Annual and perennial monocotyledonous and dicotyledonous weeds - 1 l/ha;

- a mechanical harrow;

- application of biostimulator Amino Start - 5l/ha + Wuxal® Boron SiCure - 1 l/ha, before flowering + insecticide Mospilan 20 SG - 0.2 kg/ha;

- application of fungicide Propulse® 250 SE - 1 l/ha + insecticide Coragen - 0.1 l/ha + Wuxal® Boron SiCure - 1 l/ha.

Abiotic factors (especially temperature and relative humidity) exert an important influence on the emergence and evolution of pathogens and pests (Bălașu et al., 2015; Gîdea et al., 2007; Lipianu et al., 2023; Zală et al., 2023).

Temperature and the presence of water on the leaves, from rainfall or sprinkler irrigation (Figure 2) were favorable for the occurrence of bacterial blight.

Also, high temperature values and low amounts of precipitation were favorable for the development of the red mite (Figure 3).

Four irrigations were applied by sprinkling: after sowing, after herbicide application, after mechanical weeding (Figure 4), and at the beginning of flowering, each 50 mm.

The attack value, in the case of bacterial blight, is represented by frequency (F%), intensity (I%) and attack degree (AD%). Frequency represents the percentage of plant attacked out of 100 soybean plants examined.

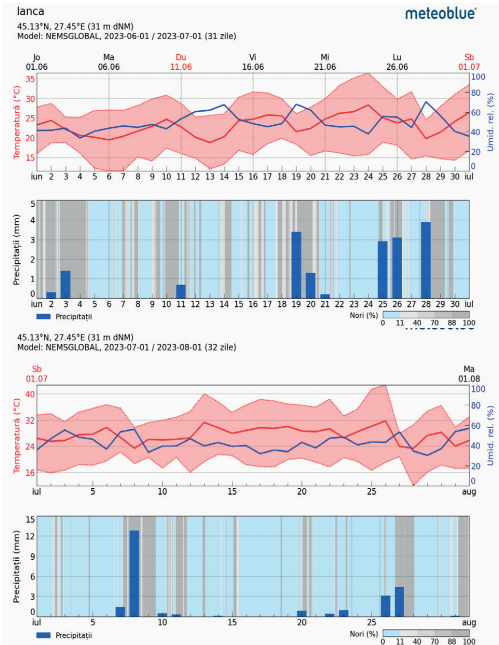


Figure 2. Temperature (°C), relative air humidity (%) and precipitation recorded in July and August 2023 in the Ianca area, Brăila (source:https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/ianca_romania_675840)

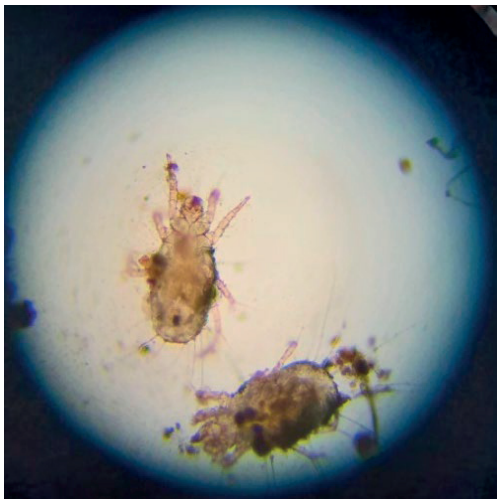


Figure 3. Two specimens of the common red mite through the lens of an optical microscope (source: R. Istrate)

Intensity indicates the degree to which the soybean plant is attacked. The attack degree was calculated using the formula:

$$AD\ (%) = \frac{F\ (%) \times I\ (%) }{100}$$



Figure 4. Irrigation of soybean crop after mechanical weeding (source: C. R. Zală)

Experimental variants:
V1 - control;
V2 - two treatments applied with Triumph 40 WG fungicide (40% metallic copper in the form of Cu(OH)₂ - 2.5 kg/ha against bacterial blight. The first treatment was applied in the R3 phenophase - beginning of pod formation (Fehr et al., 1971): a 5 mm long pod at one of the 4 upper nodes. The second treatment was applied in the R4 phenophase - fully developed pod: a 2 cm long pod at one of the 4 upper nodes.
V3 - two treatments with Nissorun 10 WP (hexitiazox 10%) acaricide - 0.8 kg/ha. The first treatment was applied in the R1-beginning of flowering phenophase: the first flower opened on any of the nodes of the main stem, 8 weeks after crop emergence. The second treatment was applied in the R5-beginning of grain filling phenophase: 3 mm long seed in a pod at one of the 4 upper nodes.
V4 - two treatments with fungicide and acaricide in the above mentioned phenophases.
The scoring of bacterial blight attack was performed 10 days after the application of the second treatment.
Research on the effect of plant protection products used provides data on the possibility of introducing them into crop management schemes (Chiriac and Cristea, 2021).

RESULTS AND DISCUSSIONS

Symptoms of bacterial blight

Bacterial blight occurred on leaves in the middle and upper canopy of soybean plants, because

young leaves are most susceptible. Infections started as small, angular, hydrous, yellow-green spots that turned yellow and then brown. The spots are surrounded by chlorotic halos, as a result of the production of the phytotoxin coronatin. The spots may coalesce and cover large areas of the leaves (Figure 5). The affected tissue may fall off, giving the leaves a perforated appearance. The infected leaves often remain on the plant.

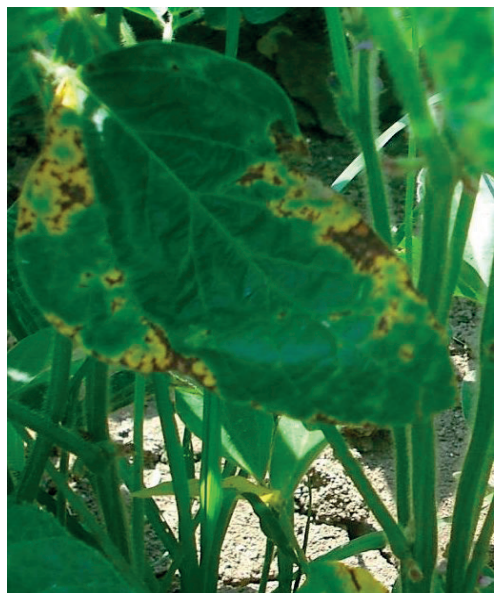


Figure 5. Symptoms of bacterial blight on a soybean leaf (source: C. R. Zală)

Scoring the bacterial blight attack

As can be seen from the data in Table 1, the frequency of bacterial blight attack varied from 4.0% in V2 to 82.33% in V1. The frequency value in V1 is higher than that in V3, because we can assume that in the control variant there were greater chances for infection with *Pseudomonas savastanoi* pv. *glycinea*, including through the holes resulting from the stinging of the leaves by mites. The intensity values of bacterial blight attack ranged from 2.7 to 23.1%. The lowest values were recorded in the variants in which the fungicide-bactericide Triumph 40 WG was applied. Regarding the degree of attack, it was 0.1% in V2, 0.16% in V4, 16.95% in V3 and 19.1% in V1.

Table 1. Scoring the frequency, intensity and calculating the degree of attack of soybean bacterial blight

Variant	Frequency (%)	Intensity (%)	Degree of attack (%)
V1	82.33	23.1	19.01
V2	4.0	2.7	0.1
V3	75.0	22.6	16.95
V4	5.66	2.9	0.16

The productions obtained

The average soybean production in the control variant was 3744.7 kg/ha (Table 2). The average soybean production in variant V2 was 4070.02 kg/ha, 323.32 kg/ha higher than in V1, which represents an increase of 8.7%. The average soybean production in variant V3 was 4569.95 kg/ha, 824.25 kg/ha higher than in V1, which represents an increase of 22.0%. The average soybean production in variant V4 was 4895.0 kg/ha, 1150.3 kg/ha higher than in V1, which represents an increase of 30.7%. If we refer to variants V4, it appears that production in V2, where two treatments were applied against soybean bacterial blight, is reduced by 16.85% as a result of the common red mite attack, and in V3, where two treatments were applied against the common red mite, production was reduced by 6.65% as a result of the bacterial blight attack. From the determinations carried out on 100 soybean leaves at 4 points in the control variant, a colonization of 18.5 specimens/leaf was observed.

Table 2. The influence of biotic factors on soybean production

Variant	Production (kg/ha)	Difference	
		(kg/ha)	(%)
V1	3744.7	-	100
V2	4070.02	325.32	108.7
V3	4569.95	824.25	122.0
V4	4895	1150.3	130.7

CONCLUSIONS

Among the main limiting factors of soybean cultivation, those of a biotic nature are found to a very large extent, in this case *Pseudomonas savastanoi* pv. *glycinea* and *Tetranychus urticae*. Abiotic factors, especially temperature and humidity, favored the emergence, growth, and development of soybean bacterial blight and common red mite during the 2023 growing season.

The biological material was represented by the ES Palador variety and the research was carried out in the experimental field in Ianca, Brăila County.

The typical symptoms of bacterial blight attack were manifested only on the leaves, namely those located on the upper floor of the infected soybean plants.

Copper-based fungicides are plant protection products effective in combating bacterial burn provided they are applied at the first symptoms appear.

Applying two treatments with the fungicide-bactericide Triumph 40 WG, at the appropriate time - the appearance of the first symptoms, contributed to reducing the degree of attack of soybean bacterial blight from 19.01% to only 0.1%.

The application of two treatments each against soybean bacterial blight and common red mite ensures, under irrigation conditions, a production increase of approx. 1150 kg/ha, which represents an increase of approx. 30.0% in production compared to the variants without these treatments.

Bacterial blight of soybean caused a 6.65% reduction in production in the present experiment.

The attack of the common red mite caused a 16.85% reduction in production in the experimental variants.

The application of sprinkler irrigation caused the mites to retreat to the lower areas of the infested plants.

The application of phytosanitary treatments is an extremely important link in the integrated fight to reduce the attack of bacterial blight and the common red mite, along with the other technological measures that must be respected.

The highest average production, of 4895 kg/ha, was recorded in the variants in which two treatments were applied against bacterial blight and common red mite with the fungicide-bactericide Triumph 40 WG and the acaricide Nissorun 10 WP.

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EVALUATION OF FORAGE PRODUCTION AND QUALITY OF TIMOTHY (*Phleum pratense* L.) IN THE PEDOCLIMATIC CONDITIONS OF BRASOV

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Abstract

The species *Phleum pratense* (timothy) presents high variability, especially due to its wide distribution and ecological adaptability. It tolerates cold temperate climates and soils with a neutral to slightly acidic reaction. This paper presents the results regarding the evaluation of the production capacity (t/ha DM) and forage quality of 5 Romanian genotypes (Tirom, Carpatica, Rarău, Syn 1.5 and Syn 1.14) and 2 foreign genotypes (Bobr and Liskra) of the species *Phleum pratense*, studied in comparative crops during 2022-2024, within the Breeding and Genetic Resources Laboratory of the Research and Development Institute for Grassland Brasov. The Romanian varieties respond effectively to the current requirements: high production, good quality of forage, suitability to various type of use, competitiveness and good resistance to disease and pests. The varieties Bobr and Liskra proved to be earlier and more productive in the first years of cropping, but they showed a lower perennality. Regarding the quality, there were differences depending on the genotype and the phenophase of harvesting.

Key words: forage quality, forage production, genotype, *Phleum pratense* L.

INTRODUCTION

Phleum pratense (commonly known as timothy grass) is a perennial, tuft-forming species belonging to the family Poaceae and the genus *Phleum* (Moreno et al., 2023; Pang et al., 2026). *Phleum pratense* grass is native to Europe, temperate Asia and North Africa, and has become naturalized in many cool or temperate regions (Lacefield et al., 1980; Bélanger et al., 2001; Havstad et al., 2013). This is an important forage grass that is used for pasture, hay and silage, which is a widely cultivated in cold, humid regions of the world, including northeastern and northwestern US and Canada, and the Nordic countries in Europe (Bernes et al., 2008; Bélanger et al., 2001; Nissinen et al., 2010).

Timothy is a leafy and tall-growing grass with high biomass production, as well as high nutritive value and digestibility compared to many other forage grasses (Helgadóttir et al., 2014). The forage quality of *Phleum pratense* is regulated by the developmental stage of the plant and is usually highest during the early growth of the crop when most of the tillers are in the vegetative stage (Bélanger et al., 2001; Gustavsson et al., 2004). It is highly palatable for cattle and horses, having a high nutritive

value and digestibility (Becker et al., 2020; Radkowski et al., 2020).

According to Bertrand et al. (2008), the higher dry matter digestibility of *Phleum pratense* species grown under cool temperatures was caused by an increase in cell wall digestibility, in part due to the delay in reaching a given stage of development under cooler temperature. The nutritive value of timothy decreases with time, phenological development and increasing forage yield, and harvesting conditions could reduce its nutritive value, particularly with extended wilting periods (Belanger et al., 2001; Ould-Ahmed et al., 2014). To enhance both dry matter yield and nutritional quality, it is essential to distinguish genotypes that exhibit a reduced trade-off between these two traits.

There are many research studies confirming an inverse relationship between dry matter yield and nutritive value (Bélanger and McQueen, 1996; Bélanger and McQueen, 1998; Casler and Vogel, 1999).

The aim of this research was to evaluate the dry matter production and forage quality of 5 Romanian genotypes of *Phleum pratense* (Tirom, Carpatica, Rarău, Syn 1.5 and Syn 1.14) and 2 foreign genotypes (Bobr from Poland, and Lischka from Germany) which have been

studied in comparative crops during 3 years (2022-2024) and 3 harvesting periods.

MATERIALS AND METHODS

The investigation was based on field trials carried out at Research and Development Institute for Grasslands, Braşov, Romania in the period 2022-2024. The study consisted of determining the dry matter production and forage quality obtained from timothy grass (*Phleum pratense* L.), the experiment being located starting with 2021.

The experimental plot (area of 210 m²) consisted of sowing the 7 varieties of *Phleum pratense*, randomized in three replications. Each block extended over an area of 70 m². Each variety was sown in three replications on an area of 10 m² (2 m width and 5 m length), and the rows were sown at a distance of 12.5 cm. The experiment was conducted by applying a seeding rate of 2000 seeds/m² (10 kg/ha). Each replication was fertilized with an amount of 52.5 kg of active ingredient per hectare of NPK, twice a year.

The soil was a preluvosol soil types with the following characteristics: humus content of 3.02%, available phosphorus 35.8 ppm, and available potassium 131 ppm, with a pH of 6.2. The climatic conditions during the study (2022-2024) indicate a warmer period compared to the multiannual average (Table 1), and generally they allowed for optimal plant growth and development. Regarding the thermal regime, all study years were warmer than the 59-year multiannual average of 7.72°C. In 2022, the annual average temperature exceeded the baseline by 1.41°C. The warming trend continued in 2023 with a deviation of +2.40°C, reaching a peak in 2024. In 2024, the annual average temperature exceeded the multiannual average by 3.73°C, while during the vegetation period (April-September), the thermal deviation reached 3.45°C above the reference value.

In terms of precipitation, the study period was characterized by a general water deficit compared to the multiannual average of 753.2 mm. The year 2023 was particularly dry, recording a total annual deficit of 182.8 mm. During the vegetation period of the same year, a negative deviation of 121.3 mm was observed, significantly affecting the growing conditions. In 2024, although the annual precipitation

(730.6 mm) was closer to the multiannual average with a minor deficit of 22.6 mm, the vegetation period remained dry, recording 43.2 mm less rainfall than the historical average.

Table1. Climatic conditions from Braşov stationary 2022-2024

Years	Annual average I-XII	Dev.*	Vegetatio n period IV-IX	Dev.
Temperature (°C)				
2022	9.13	+1.41	15.92	+1.68
2023	10.12	+2.40	15.98	+1.75
2024	11.45	+3.73	17.68	+3.45
Average 59 years	7,72	0	14.23	0
Precipitation (mm)				
2022	574.2	-179.0	376.6	-152.5
2023	570.4	-182.8	407.8	-121.3
2024	730.6	-22.6	485.9	-43.2
Average 59 years	753.2	0	529.1	0

*Dev.- Deviation

During the three-year study, comparative observations were conducted on *Phleum pratense* to evaluate dry matter production and nutritional indicators, including crude protein (CP), crude fiber (CF), dry matter digestibility (DMD), and organic matter digestibility (OMD). For the analysis, samples were dried at 60 °C and ground using a Grindomix GM 200 mill. The chemical parameters were determined by Near Infrared Spectroscopy (NIRS) using a Perten DA 7200 analyzer. Forage quality was determined by a range of characteristics with nutrient content influenced by factors such as grass species or cultivars, growth stage, and soil conditions (Guţu et al., 2025). All the data was statistically analysed using analysis of variance (ANOVA). The relationships between the observed traits were assessed on the basis of the Pearson’s correlation coefficients, using CORRELATION procedure in the STATISTICA software.

RESULTS AND DISCUSSIONS

The 7 genotypes studied were evaluated from a productive and qualitative point of view. Regarding dry matter production, we present the results from the 3 years studied. The analysis of the variety and year interaction (Table 2) highlights variations in production determined

mainly by annual environmental conditions. In 2022, the production had high values for all varieties, ranging between 9.70 t/ha (Syn 1.5) and 11.03 t/ha (Syn 1.14). Dry matter yield is about 2-9 t/ha depending on the stage of maturity and other factors (Grant and Burgess, 1978; Bélanger et al., 2001; Brégard et al., 2001).

Table 2. Dry matter production (average), obtained in the 3 years studied

Variety x year*		Mean DM t/ha	Std. Err. *	-95.0 %	+95.00 %
Tirom	1	10.90	0.7505	7.6706	14.129
	2	10.83	0.2403	9.7991	11.867
	3	5.50	0.4041	3.7611	7.2389
Carpatica	1	10.90	0.6245	8.2129	13.587
	2	10.73	0.7965	7.3061	14.160
	3	6.37	0.8987	2.4995	10.233
Rarău	1	10.90	0.1527	10.242	11.557
	2	10.90	0.5567	8.5043	13.295
	3	6.57	0.5811	4.0660	9.0673
Syn1.14	1	11.03	0.7859	7.6515	14.415
	2	9.50	0.5567	7.1043	11.895
	3	6.63	0.5364	4.3251	8.9414
Syn 1.5	1	9.70	0.2886	8.4579	10.942
	2	10.77	1.0806	6.1170	15.416
	3	6.13	0.6489	3.3412	8.9254
Bobr	1	10.87	0.405	9.1218	12.611
	2	10.70	0.3785	9.0710	12.328
	3	6.03	0.417	4.2362	7.8304
Lischka	1	10.77	0.5696	8.3158	13.217
	2	10.67	0.7796	7.3123	14.021
	3	6.27	0.6984	3.2616	9.2716
DL 5% = 1.77 t/ha, DL 1% = 2.36% t/ha, DL 0.1% = 3.09 t/ha.					

*Std.Err. – Standard error; Year: 1 - 2022, 2 - 2023, 3 - 2024.

In 2023, the production level was comparable, with values between 9.50 and 10.90 t/ha. In contrast, in 2024, there was a sharp decrease in production for all varieties, the values being between 5.50 and 6.63 t/ha. The differences between 2022 and 2024, respectively between 2023 and 2024, significantly exceed the DL threshold of 0.1% (3.09 t/ha) for all analysed varieties, which indicates a very significant influence of the climatic conditions specific to 2024 on the production level. The decrease of production in 2024 was relatively uniform across all genotypes, the amplitude of the reduction being between approximately 4.3 and 5.4 t/ha. Comparison of varieties within the same year shows that the differences between the average values do not exceed the DL 5%

threshold (1.77 t/ha). Thus, in no experimental year were statistically significant differences recorded between varieties, which indicates a relatively close productive potential of them under the experimental conditions studied.

The variation in production was predominantly determined by the year factor, while the differences between varieties and the interaction between variety and year did not have a statistically significant influence.

The results highlight the relative stability of the tested varieties under the conditions of the preluvosol at the experimental site. Although this soil type has a good water-holding capacity, it could not fully compensate for the severe precipitation deficits recorded in 2022 and 2023, confirming the crop's high sensitivity to extreme climatic fluctuations. The specific climatic conditions of each study year significantly influenced production, with thermal and hydric stress being the primary drivers of yield variability.

The lack of a significant interaction between the varieties and the specific climatic conditions of each study year indicates that the tested varieties maintain stable performance across different environmental contexts (Figure 1).

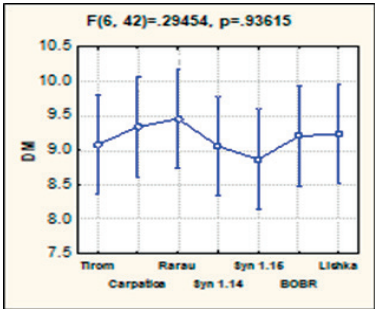


Figure 1. DM variation t/ha, F test

Therefore, the variation in production is predominantly determined by annual environmental conditions, and the productive potential of the analyzed varieties is relatively close under the experimental conditions studied. There are no statistically significant differences between the 7 varieties, according to the p value (0.93615) > 0.05, which indicates that the differences are insignificant. The total highest value of DM t/ha was recorded in the first and the second year, approximately 11 t/ha (Figure 2). The highest value of DM t/ha (9.5 t/ha) was

recorded for the Rarău variety, and the lowest yield, approximately 8.9 t/ha, was obtained for the Syn 1.5 variety (Figure 3).

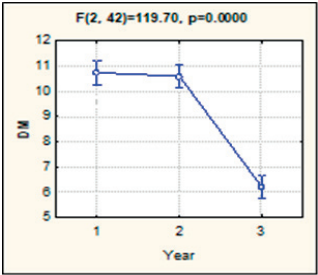


Figure 2. DM variation t/ha, in 2022, 2023 and 2024

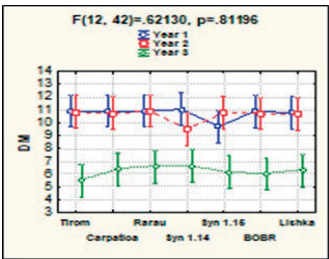


Figure 3. DM variation t/ha, in 2022, 2023 and 2024 for the 7 varieties of *Phleum pratense* L.

The difference in dry matter production between the 3 years factor grades is very significant, according to the value of p ($0.0000 < 0.001$), which show that the differences are very significant. The highest dry matter production was recorded at Syn 1.14, in 2022, approximately 11.03 t/ha, respectively 10.6 t/ha, and the lowest in 2024, approximately 5.5 t/ha, the Tirom variety (Figure 3).

Numerous studies have evaluated the forage quality of *Phleum pratense*, highlighting considerable variability in their biochemical composition depending on growth conditions, cultivar, and harvest stage (Guțu et al., 2025). The crude protein (CP) content is a fundamental indicator of forage quality in perennial grasses, reflecting the plant's capacity for nitrogen uptake and metabolism. In general, perennial grasses exhibit a wide range of CP levels, typically declining as the plant matures. For *Phleum pratense* L. (timothy), literature indicates that CP content is highly sensitive to environmental factors and management, with values often ranging from 12% to 16% in the early stages of growth in Romanian conditions

(Moga and Schitea, 2005). Globally, research shows that while timothy is highly palatable, its protein accumulation is strongly conditioned by the interaction between genotype and thermal or hydric stress (Bélanger et al., 2001; Wang et al., 2014). The analysis of CP content in our study (Table 3) highlights significant differences driven by the specific climatic factors of each experimental period rather than the mere chronological passage of years.

Table 3. Crude protein (%) (average), obtained in the 3 years studied

Variety	Year	Mean CP %	Std. Err. *	-95.00 %	+95.00 %
Tirom	1	16.42	0.4261	14.588	18.255
	2	13.07	0.4073	11.314	14.819
	3	12.82	0.7084	9.7683	15.865
Carpatica	1	16.81	0.4415	14.911	18.710
	2	13.66	0.2328	12.653	14.657
	3	12.68	0.1452	12.058	13.308
Rarău	1	16.98	0.2436	15.929	18.026
	2	12.73	0.2834	11.513	13.953
	3	12.75	0.2500	11.674	13.825
Syn 1.14	1	17.22	0.5690	14.773	19.670
	2	13.52	0.2128	12.606	14.438
	3	13.57	0.5019	11.406	15.726
Syn 1.5	1	17.64	0.7902	14.244	21.044
	2	14.26	0.3087	12.927	15.583
	3	14.00	0.6557	11.178	16.821
Bobr	1	16.26	0.6375	13.512	18.998
	2	12.80	0.1924	11.971	13.628
	3	12.52	0.1740	11.767	13.265
Lischka	1	16.54	0.5938	13.989	19.099
	2	12.92	0.1365	12.334	13.509
	3	11.12	0.4419	9.21	13.018
DL 5%=1,27 %, DL 1%= 1,69 %, DL 0.1%= 2,21 %					

*Std.Err. – Standard error; Year: 1 - 2022, 2 - 2023, 3 - 2024.

In the first experimental period (2022), characterized by a moderate thermal deviation (+1.68°C during vegetation) and a manageable water deficit (Table 1), the CP content reached its peak across all varieties. Values varied between 16.26% (Bobr) and 17.64% (Syn 1.5). The maximum difference of 1.38% exceeded the DL 5% threshold (1.27%), indicating that under favorable conditions, the genetic potential of the Syn 1.5 variety for nitrogen accumulation is significantly superior. These high levels are consistent with the findings of Wang et al. (2014), who documented an upper limit of 17.4% CP for timothy under optimal management. During 2023, a general decline in

CP content was observed (12.73% to 14.26%), primarily due to the severe water deficit recorded during the vegetation period (-121.3 mm, as shown in Table 1). This drought stress limits the mobility of nitrogen in the soil and its subsequent mineralization, a mechanism that directly impacts protein synthesis. Despite this environmental pressure, Syn 1.5 maintained its qualitative lead (14.26%), standing out significantly (DL 5%) from the lower values recorded by the Rarău variety (12.73%). These results align with Tomić et al. (2007), who noted that abiotic stress can significantly compress the qualitative range of timothy forage.

In the 2024 period, the impact of extreme thermal stress became the dominant factor. With a thermal deviation of +3.45°C above the multiannual average during the vegetation season (Table 1), CP content showed the most pronounced differentiation between genotypes. Values varied between 11.12% (Lischka) and 14.00% (Syn 1.5). The very significant difference of 2.88% (exceeding DL 0.1%) reveals a clear contrast in metabolic stability. The Lischka variety showed a sharp qualitative decline, likely due to heat-induced accelerated leaf senescence, whereas the Romanian synthetic lines Syn 1.5 and Syn 1.14 demonstrated a robust resilience, maintaining relatively high CP levels. Our results confirm that under the specific conditions of Brasov, the Romanian genotypes remain in the upper half of this qualitative spectrum, even when faced with significant thermal pressure. Comparing the experimental periods, there is a clear reduction in the crude protein content from 2022 to 2023 and 2024, with the differences exceeding in most cases the DL 0.1% threshold. This highlights a very significant influence of the specific environmental drivers - primarily the thermal acceleration and the precipitation deficit - on the forage quality. Consequently, the environmental context of 2022, characterized by a more balanced thermal regime, was the most favorable for crude protein accumulation. In contrast, the conditions of 2024, marked by the highest thermal deviation (+3.45°C during vegetation, Table 1), proved to be the most restrictive for maintaining high protein levels across all studied varieties.

Comparing the years, there is a clear reduction in the crude protein content from 2022 to 2023

and 2024, with the differences exceeding in most cases the DL 0.1%, which highlights a very significant influence of the annual conditions on the quality of the feed. Thus, the year 2022 was the most favorable for the accumulation of crude protein, and the year 2024 the least favorable. The variation in crude protein content highlights the specific response of the studied varieties to the restrictive environmental factors defined in the experimental methodology. Rather than a mere annual fluctuation, the decline observed in the latter periods is a direct consequence of the thermal stress (+3.45°C deviation) and hydric deficit (-121.3 mm) (Table 1), which limited nitrogen mineralization and uptake. From a scientific perspective, the superiority of the Syn 1.5 variety is not accidental; it reflects its genetic plasticity and ability to maintain metabolic stability under heat stress. While varieties like Lischka showed a higher sensitivity to the complex of factors characterizing the 2024 season, the synthetic lines demonstrated a more robust biological characteristic in translocating nitrogen to the aerial biomass. This differentiated behavior, supported by specialized literature (Wang et al., 2014; Tomić et al., 2007), confirms that under controlled experimental conditions, the genetic factor remains essential in mitigating the negative impact of abiotic stress. To better visualize and synthesize the complex interaction between the studied genotypes and the experimental years, a Two-Way Joining cluster analysis (Heatmap) was performed (Figure 4).

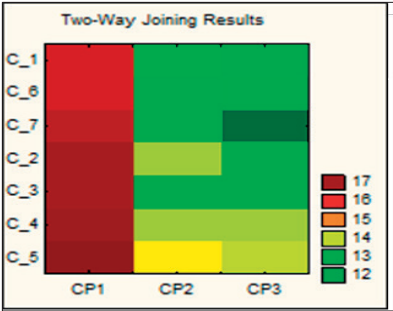


Figure 4. Two-way joining cluster analysis (heatmap) of crude protein (CP) content in the studied *Phleum pratense* varieties across the three experimental periods (Periods: CP1 - 2022, CP2 - 2023, CP3 - 2024; Varietal codes: C_1 - Tirom, C_2 - Carpatica, C_3 - Rarău, C_4 - Syn 1.14, C_5 - Syn 1.5, C_6 - BOBR, C_7 - Lischka)

It is important to know the amount of crude fiber in *Phleum pratense* hay because it can have a negative effect on the relative food value and energy concentration (Țiței et al., 2022). In terms on the crude fiber content %, the results obtained highlight a pronounced influence of annual conditions on the recorded values, as well as differences between varieties within the same year (Table 4).

In 2022, the crude fiber contents were the lowest, ranging between 27.18% (Syn 1.14) and 28.69% (Lischka). The maximum difference between varieties was 1.51%, the lower value, DL 5% (1.69%), which indicates the absence of statistically significant differences between varieties this year.

In 2023, there was an obvious increase in the crude fiber content for all varieties, the range being between 31.26% (Carpatica) and 33.27% (Rarău). The maximum difference was 2.01%, exceeding the DL 5% (1.69%), but being below the DL 1% (2.26%), which indicates significant differences at the 5% level. The Rarău, Lischka and Bobr varieties presented the highest values this year.

Table 4. Crude fiber (%) (average), obtained in the 3 years studied

Variety	Year	Mean CF %	Std. Err. *	-95.00 %	+95.00 %
Tirom	1	27.54	0.098	27.119	27.969
	2	32.41	0.313	31.062	33.759
	3	30.95	1.625	23.954	37.945
Carpatica	1	27.56	0.392	25.865	29.245
	2	31.26	0.481	29.183	33.327
	3	30.65	0.208	29.754	31.545
Rarău	1	27.90	0.157	27.222	28.577
	2	33.27	0.409	31.504	35.029
	3	31.02	0.437	29.135	32.897
Syn 1.14	1	27.18	0.581	24.673	29.681
	2	32.22	1.081	27.568	36.875
	3	29.63	0.142	29.020	30.246
Syn 1.5	1	27.54	0.529	25.267	29.821
	2	31.63	0.101	31.195	32.07
	3	30.15	0.680	27.221	33.078
Bobr	1	28.31	0.298	27.027	29.594
	2	33.08	0.473	31.042	35.113
	3	31.72	0.066	31.429	32.003
Lischka	1	28.69	0.728	25.555	31.822
	2	33.16	0.502	30.994	35.316
	3	32.68	0.693	29.701	35.665
DL 5%=1,69%, DL 1%= 2,26%, DL 0.1% = 2,96%					

*Std.Err. – Standard error; Year: 1 - 2022, 2 - 2023, 3 - 2024.

In 2024, the values remained at a higher level compared to 2022, ranging between 29.63% (Syn 1.14) and 32.68% (Lischka). The maximum difference between the varieties was 3.05%, a value exceeding the DL 0.1% (2.96%), indicating very statistically significant differences. The Lischka variety stood out with the highest value, while Syn 1.14 recorded the lowest level.

Comparing the years with each other, a clear increase in the values is observed from 2022 to 2023, the differences exceeding the DL threshold of 0.1% in most cases, which demonstrates a very significant influence of the annual climatic variations. In 2024, the values decreased slightly compared to 2023, but they were higher than those registered in 2022.

Crude fiber (%) was significantly influenced by the annual conditions, with 2023 being the most favorable. The differences between the varieties were insignificant in 2022, significant at the level of 5% in 2023 and very significant in 2024, which suggests a more pronounced differentiation of the behavior of the genotypes under more variable environmental conditions. Figure 5, illustrates the two-way joining cluster analysis (heatmap), which simultaneously groups both the *Phleum pratense* varieties and the experimental periods based on crude fiber (CF) content.

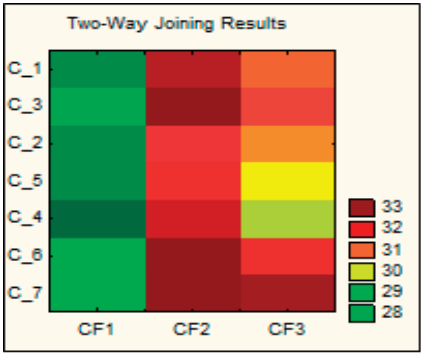


Figure 5. Two-way joining cluster analysis (heatmap) of crude fiber (CF) content in the studied *Phleum pratense* varieties across the three experimental periods. (Periods: CF1 - 2022, CF2 - 2023, CF3 - 2024; Varietal codes: C_1 - Tirom, C_2 - Carpatica, C_3 - Rarău, C_4 - Syn 1.14, C_5 - Syn 1.5, C_6 - BOBR, C_7 - Lischka)

The initial experimental period (2022) is uniformly defined by green hues, confirming that the baseline environmental conditions of

that year systematically restricted crude fiber biosynthesis to its lowest levels (28-29%) across the entire germplasm collection. The second experimental period (2023) marks a radical physiological shift, appearing as a homogenous dark red cluster where synergistic annual factors stimulated maximum fiber deposition, driving values to their absolute peaks (32-33%) in nearly all investigated varieties. The final experimental period (2024) acts as a heterogeneous transitional zone depicted in orange and yellow; unlike the previous years, this phase induced a diversified, variety-

dependent response. The analysis of all cellular constituents shows moderate variability for crude protein (CP) (14.2%) and dry matter (DM) (25.3%), while crude fiber (CF), dry matter digestibility (DMD) and organic matter digestibility (OMD) showed greater stability (7-7.6%).

The overall mean values were: CP = 14.30%, CF = 30.41%, DMD = 63.28%, OMD = 59.36%, DMD = 9.17%. These indices confirm the greater variability of protein and soluble dry matter compared to fiber and DMD (Table 5).

Table 5. Statistical index of cellular constituents

Statistical index of cellular constituents	Mean	Minimum	Maximum	Variance	Std.Dev.	Standard	Cv [%]
CP	14.30	10.6500	18.9667	4.1195	2.0297	0.2557	14.2
CF	30.41	26.0667	34.1500	5.0380	2.2446	0.2828	7.4
DMD	63.28	55.2500	73.5333	23.3489	4.8321	0.6088	7.6
OMD	59.36	51.6000	68.7667	19.2498	4.3875	0.5528	7.4
DM	9.17	4.7000	12.9000	5.4090	2.3257	0.2930	25.3

The correlation between CP and CF is strongly negative ($r = -0.86$), suggesting that an increase in CP is associated with a decrease in CF. In contrast, CP shows very strong positive correlations with DMD ($r = 0.93$) and OMD ($r = 0.90$), indicating a direct relationship between these variables. The correlation between CP and DM is moderately positive ($r = 0.47$) (Figure 6).

The CF shows strong negative correlations with DMD ($r = -0.85$) and OMD ($r = -0.84$), but has a weak correlation with DM ($r = -0.17$), suggesting that the influence of CF on DM is insignificant.

DMD and OMD show an extremely strong correlation ($r = 0.99$), indicating a possible redundancy or collinearity between these two variables. Regarding DM, it is moderately correlated with CP ($r = 0.47$), DMD ($r = 0.44$) and OMD ($r = 0.40$), suggesting a moderate influence of these variables on DM.

CONCLUSIONS

The research highlights that, in the context of current climate changes in the Braşov area, the qualitative stability of the *Phleum pratense* crop is determined more by the genetic resilience of the genotype than by the gross productive potential. The Romanian synthetic lines (Syn 1.5 and Syn 1.14) have a superior capacity for nitrogen translocation to the aerial biomass even under extreme thermal stress conditions (+3.45°C above the average), in contrast to foreign varieties which exhibit accelerated leaf senescence.

Although annual climatic variations (hydric and thermal stress) unified the dry matter production

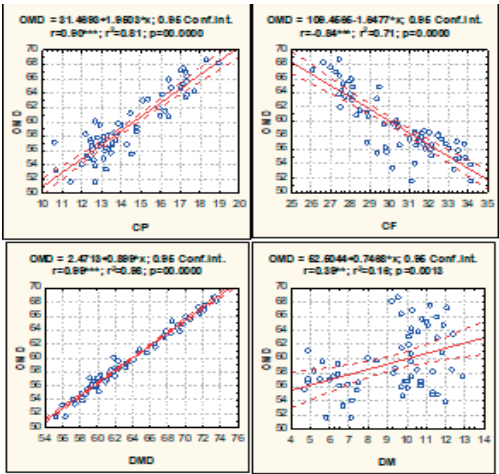


Figure 6. Linear regression plots between OMD and quality parameters

- OMD vs. CP: $y = 31.4693 + 1.9503x$, $r = 0.90***$, $p < 0.0001$;
 - OMD vs. CF: $y = 109.4585 - 1.6477x$, $r = -0.84***$, $p < 0.0001$;
 - OMD vs. DMD: $y = 2.4713 + 0.889x$, $r = 0.99***$, $p < 0.0001$;
 - OMD vs. DM: $y = 52.5044 + 0.7486x$, $r = 0.39**$, $p = 0.0013$.
- Dashed red lines represent the 95% confidence intervals.

across all genotypes, the research reveals that metabolic differentiation between varieties becomes critical under abiotic pressure, directly influencing the nutritional value (crude protein and fiber).

The paper provides new arguments for using protein stability and the limitation of cellulose biosynthesis during drought periods as primary indicators in the selection of genetic material intended for grasslands.

Our results confirm that, on preluvosol soil types, using Romanian synthetic genotypes represents an efficient adaptation strategy for farmers, guaranteeing high-quality forage even in years with severe precipitation deficits, partially offsetting the inevitable quantitative losses.

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MISCELLANEOUS

STUDY OF PHOSPHORUS AVAILABILITY IN PHOSPHO-COMPOSTS MADE FROM POULTRY MANURE AND PHOSPHATE WASTE

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Abstract

To evaluate phosphorus availability in phospho-composts produced from poultry manure (PM) and phosphate waste (Phosphate Sludge – PS and Screening Residue – SR), six composts (A1: 75% PM + 25% PS; A2: 80% PM + 20% PS; A3: 89% PM + 11% PS; B1: 75% PM + 25% SR; B2: 80% PM + 20% SR; B3: 89% PM + 11% SR) were prepared. The results showed that the initial levels of available phosphorus were low, ranging from 0.101 to 0.114% P₂O₅, indicating that microorganisms were not yet active at the beginning of the composting process. By the end of the process, the concentration of available phosphorus increased significantly, reaching 0.395 to 1.195% P₂O₅, representing an increase of 290 to 1071%. Overall, the composts enriched with phosphate sludge (series A) showed higher levels of available phosphorus than those containing screening residues (series B). These phospho-composts therefore represent promising organo-mineral amendments suitable for direct application in soil fertilization.

Key words: phosphorus availability, phosphate waste, poultry manure, compost.

INTRODUCTION

Considered one of the essential nutrients for plant growth (Ochwoh et al., 2005; Wang et al., 2012), phosphorus (P) plays a central role in biochemical processes such as photosynthesis, cellular respiration, and energy transfer (Hinsinger, 2001). However, the availability of this element in agricultural soils is very low in West African countries due to its strong binding to iron and aluminium oxides found in ferralitic soils (Nziguheba et al., 2016). In sub-Saharan Africa, phosphorus deficiency in arable soils remains one of the major constraints limiting the productivity of food crops (Vanlauwe et al., 2015). Limited phosphorus availability, resulting in nutrient deficiencies, frequently compels farmers to rely on phosphate-based fertilizers.

However, previous studies have demonstrated that excessive application of chemical fertilizers can contaminate groundwater, increase soil acidity, degrade soil physical structure, and

reduce soil organic matter (Konaté et al., 2020; Afanou et al., 2025a). Other studies have shown significant accumulations of heavy metals in crops after the application of chemical fertilizers to the soil (Afanou et al., 2025b). Given the limited phosphorus availability in agricultural soils, along with the environmental and food security concerns associated with the use of mineral fertilizers, advancing the adoption of organic and biological fertilizers, especially compost, is imperative. Numerous studies have shown that composting enhances phosphorus availability in composts (Ablede et al., 2019; Bustamante et al., 2016; Wei et al., 2015; Christel et al., 2014) and substantially reduces the formation and release of greenhouse gases emissions into the atmosphere (Sema et al., 2021a).

Compost, the final product of the composting process, improves soil organic matter content (Afanou et al., 2025a). It is also important to note that these amendments, through their organic matter content, contribute to the

immobilization of heavy metals within the soil (Wang et al., 2012). Togo is one of the West African countries suffering from a lack of assimilable phosphorus in its agricultural soils. However, the country possesses a large phosphorus deposit, the exploitation of which generates two main waste products (phosphate sludge and screenings) that create major environmental problems due to their discharge into seawater or their accumulation at the washing site (Afanou et al., 2025c). These wastes are frequently poorly managed, despite containing approximately 14-20% P_2O_5 , which could be utilised locally in agricultural production (Koriko et al., 2021). Additionally, livestock waste, particularly poultry manure produced in large quantities during modern poultry farming (laying and broiler hens), is often stored on-site or applied directly to crops without prior treatment, thereby contributing to

various environmental problems (Lekefack, 2015). Therefore, this study aims to investigate the availability of phosphorus in composts made from poultry manure and this phosphate-rich waste. This research aligns with the promotion of organic farming and the development of the Circular Economy (CE).

MATERIALS AND METHODS

The raw materials and their origin

The poultry manure used in the composting process came from the Regional Centre of Excellence on Avian Sciences (CERSA) at the University of Lomé, while the phosphate waste was obtained from the Société Nouvelle des Phosphates du Togo, a phosphate washing plant located approximately 40 km southeast of Lomé (Figure 1).

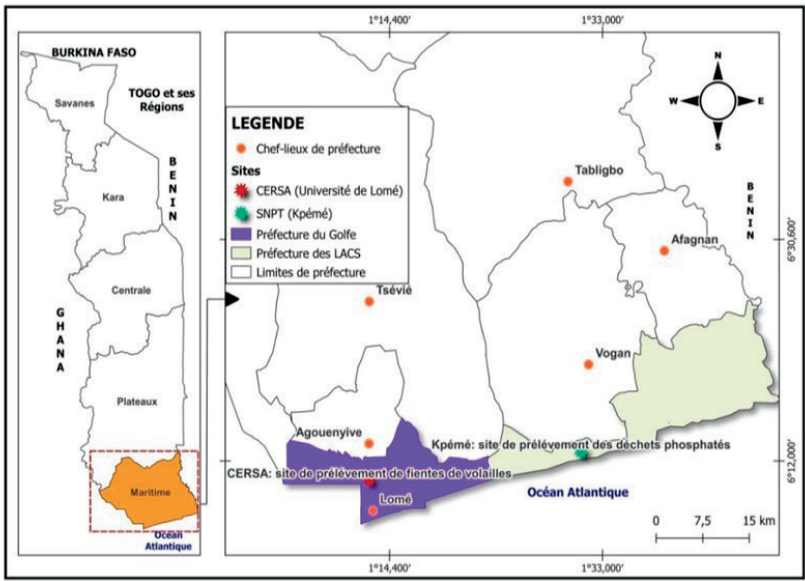


Figure 1. Map of Togo showing the origin of raw materials used for composting

Composting trial for poultry manure and phosphate waste

The composting experiment was carried out from May to July 2025 in plastic containers (pots) at the Laboratory of Applied Sciences for Agriculture and the Environment of the University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest, Romania. Prior to composting, several physicochemical parameters of the raw materials (poultry manure

and phosphate waste) were analysed, including pH, total phosphorus, assimilable phosphorus, and carbon and nitrogen contents (only for poultry manure). In total, two series (A and B) of three composts each were produced. Series A composts were prepared using poultry manure (PM) and phosphate sludge (PS), while series B composts were produced using poultry manure and screen refuse (SC). The composition of each compost, based on the proportion of the various

wastes used, is provided in Table 1. Photo 1 presents: the weighing of the waste (a), mixing of the waste in a bin (b), placement of the mixtures introduced into the composting pots (c) and the phospho-composts during their

evolution (d). Throughout the composting process, pH was regularly monitored at two-week intervals, as this parameter plays a critical role in determining phosphorus availability during compost maturation.

Table 1. Substrate composition of each compost

Series	Composts	PM		PS		SC	
		m (g)	P (%)	m (g)	P (%)	m (g)	P (%)
A	A ₁	500	75	166.67	25	0	0
	A ₂	500	80	125	20	0	0
	A ₃	500	89	62.5	11	0	0
B	B ₁	500	75	0	0	166.67	25
	B ₂	500	80	0	0	125	20
	B ₃	500	89	0	0	62.5	11



Photo 1. Stages preceding and during the composting process: weighing (a) and mixing of waste (b); filling of the composting boxes (c); phospho-composts during their evolution (d)

Physico-chemical analysis methods during composting

The pH was determined using a potentiometric method, following the methodology of the Research Institute for Pedology and Agrochemistry (ICPA) of Bucharest (1983), Chapter 3, PT 126, which is specifically applied to residual products. Total organic carbon (TOC) content was quantified using the Walkley and Black (1934) method. Total nitrogen was determined by the Kjeldahl method in accordance with ICPA (1983), Chapter 8 (2), PT 129. Total phosphorus (P_{tot}) was determined following the ICPA methodology (1983), Chapter 9 (2.1 and 3), PT 132. Assimilable phosphorus (P_{ass}) was assessed through ammonium acetate–lactate extraction according to the Romanian STAS method 7184/19-82; PTL 19; ed.3, rev. 0.

Statistical analysis of data

To assess the normality of the data and the significance of the difference for the results on assimilable phosphorus between the beginning and end of the composting process, statistical analyses (Shapiro Wilk test, t-test and Wilcoxon test) were performed using R software, version 4.5.0.

RESULTS AND DISCUSSIONS

Some physicochemical characteristics of poultry manure and phosphate waste

The pH, organic carbon (%TOC), total nitrogen (%N), total phosphorus (P_{tot} expressed as %P₂O₅) and assimilable phosphorus (P_{ass} expressed as %P₂O₅) of the raw materials used in the composting process are presented in Table 2. The pH of these materials is slightly alkaline,

ranging from 7.53 to 7.77. This alkalinity can be attributed, on the one hand to the high nitrogen content of poultry manure (3.09%), and on the other hand, to the abundance of carbonate (CO_3^{2-}), sodium (Na_2O), calcium (CaO) and magnesium (Mg) in the natural phosphates from which the phosphate waste was generated (Tchangbedji et al., 2003). The high carbon (33.09%) and nitrogen (3.09%) contents of these

poultry manure are normal and are expected to be beneficial during the composting process. The phosphate waste exhibits high total phosphorus concentrations, ranging from 10 to 13% of P_2O_5 , which can be attributed to its apatite content. However, its assimilable phosphorus (Pass) levels are very low, indicating the need for processing to enhance phosphorus availability before this material can be used as fertilizer in agriculture.

Table 2. Some characteristics of the materials used in the composting process

Raw material	pH	%TOC	%N	Ptot (% P_2O_5)	Pass (% P_2O_5)
Poultry manure	7.59	33.39	3.09	4.56	0.1
Phosphate sludge	7.77	-	-	10.68	0.09
Screening residue	7.53	-	-	13.54	0.1

The evolution of pH during the composting process

The pH is one of the important parameters during the composting process (Hellal et al., 2012). The evolution of the pH during the composting process is shown in Figure 2. At the initiation of composting, the pH of the different composts ranged between 7.33 and 7.73, reflecting the comparable pH values of the raw materials (poultry manure and phosphate waste) used. During the first fifteen (15) days of composting, the pH increased considerably, reaching values between 7.86 and 8.64 in both compost series. This rise can be attributed to the release of ammonia or amines resulting from the degradation of nitrogenous organic matter, as well as to the breakdown of organic acids accompanied by CO_2 release (Pan et al., 2011;

Kim et al., 2018; Azis et al., 2023). In series A composts, the highest pH value (8.39) was recorded in compost A3 (compost from 89%PM and 20%PS), whereas in series B, the maximum pH value (8.64) was observed in compost B3 (compost at 89%PM and 20%PS). The high pH values occurring around the 15th day of composting are likely attributable to the high proportions of poultry manure, which is rich in nitrogen. Similar increases in pH have also been reported in several composting studies (Gao et al., 2010; Mengistu et al., 2017). The decline in pH after the 15th day of composting, reaching values around 7, can be attributed to the volatilization of ammoniacal nitrogen (N-NH_4^+) and the release of hydronium ions (H_3O^+) through bacterial nitrification (Huang et al., 2004; Wang et al., 2016; Onwosi et al., 2017).

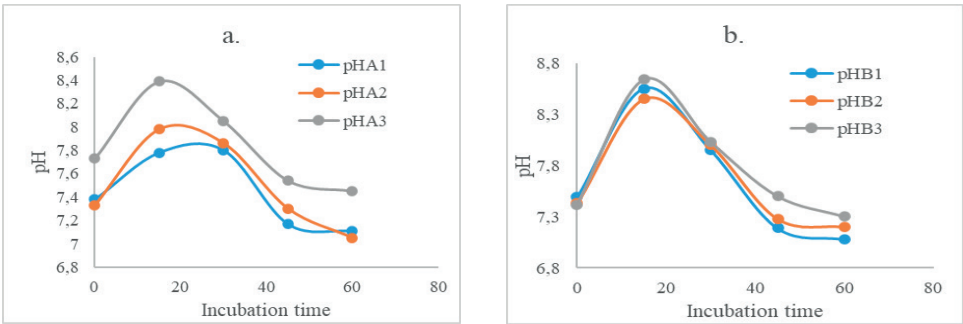


Figure 2. Evolution of compost pH (a. - series A; b. - series B) during composting

Total phosphorus in the different composts

The total phosphorus contents of the phospho-composts are presented in Figure 3. Analysis of

the data shows that the composts in series A contain lower levels of total phosphorus (ranging from 5.27 to 6.60% of P_2O_5) compared

with those in series B, whose values range from 7.90 to 12.55% P_2O_5 . The reduced P_{tot} levels in series A composts are likely attributable to the

use of phosphate sludge, which contains less total phosphorus than the screen refuse used in the series B composts.

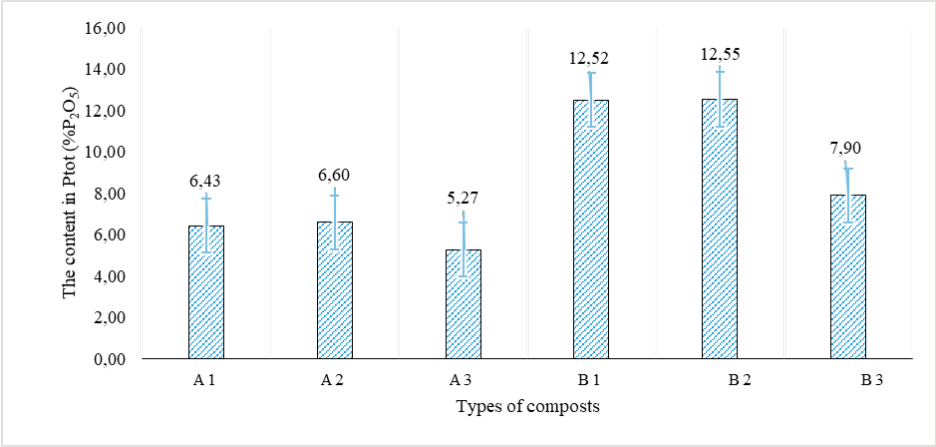


Figure 3: Total phosphorus content in the different phospho-composts

Study of assimilable phosphorus in different composts

Composts that contain high levels of total phosphorus but very low contents of assimilable phosphorus provide limited benefit as plant phosphorus sources, since most of the element remains immobilized in unavailable forms. The Pass contents of the 6 composts are presented in Figure 4. At the initiation of composting, these values were low, ranging from 0.102 to 0.111% P_2O_5 , indicating that phosphatase-producing decomposer microorganisms were not yet fully active. After two months of composting, Pass increased substantially, reaching values between 0.394 and 1.195% P_2O_5 , an increase between 290 and 1071% compared to the initial levels. Similar observations have been made by other authors (Amarasinghe, 2022; Debicka et al., 2023; Kutu et al., 2018; Lee, 2016). We also observed that, throughout the composting process, phosphorus tends to be more available in the series A composts (Pass between 0.74 and 1.195% P_2O_5) than in the series B (Pass between 0.394 and 0.973% P_2O_5). The nature of phosphate waste largely determines this difference. Specifically, phosphate sludge promoted greater phosphorus

availability in the composts compared to the screening refusals.

The results of the statistical tests assessing the significance of the differences in assimilable phosphorus between the beginning and end of composting are presented in Table 3. The Shapiro-Wilk test ($W = 0.896$; $p = 0.351$) indicates that the differences between initial and final values are normally distributed ($p > 0.05$). This normality justified the use of a parametric test, namely the paired t -test ($t = 6.82$; $p = 0.001$), which revealed a highly significant difference between the initial and final means of assimilable phosphorus (P_{ass}), indicating a clear increase in this parameter by the end of the composting process. The non-parametric Wilcoxon test ($W = 0$; $p = 0.031$) further confirmed the t -test results, reinforcing the conclusion that assimilable phosphorus increased significantly from the start to the end of the process. Thus, the statistical analyses consistently demonstrate that the composting process promotes a significant increase in assimilable phosphorus, likely associated with the progressive mineralization of organic matter and the consequent release of nutrients within the compost matrix.

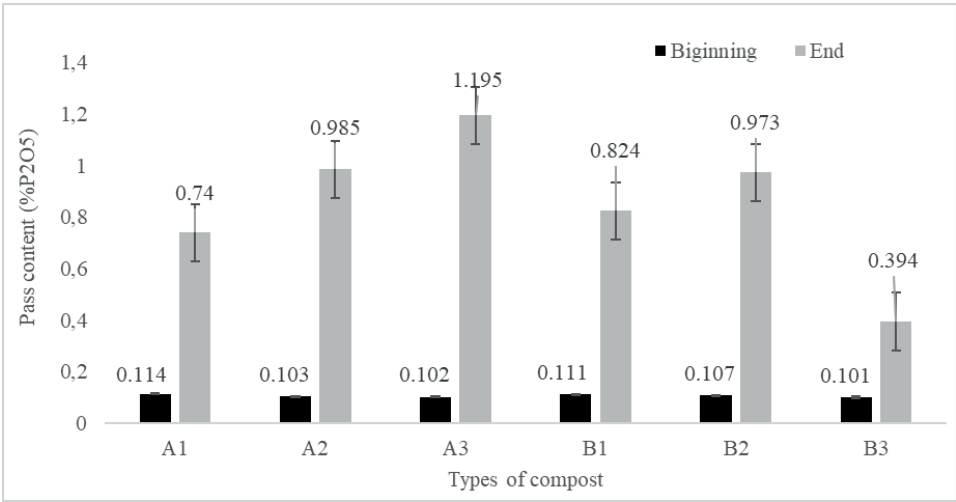


Figure 4: Assimilable phosphorus content of different phospho-composts at the beginning and at the end of composting

Table 3: Results of statistical tests applied to the assimilable phosphorus values of composts

Statistical test	Objectives of the test	Statistic	p-value	Threshold ($\alpha=0.05$)	Interpretation
Shapiro-wilk	Verify the normality of the distribution of differences (End – Beginning)	W = 0.896	0.351	$p > 0.05$	The distribution of differences does not deviate significantly from normality.
Paired t-test (parametric)	Compare the mean available phosphorus at the beginning and at the end of composting	t = 6.82	0.001	$p < 0.05$	Highly significant difference: increase in available phosphorus between the beginning and at the end of composting.
Wilcoxon (non-parametric test)	Confirm the difference without assuming normality (comparison of medians)	W = 0	0.031	$p < 0.05$	This result is also significant, confirming the increase in assimilable phosphorus after composting.

CONCLUSIONS

The study of phosphorus availability in phospho-composts produced from poultry manure and phosphate waste over a 60-day period revealed a pronounced increase in assimilable phosphorus (Pass). Initial Pass levels, ranging from 0.101 to 0.114% P₂O₅, were relatively low at the beginning of composting. During the composting process, and especially around day 60, Pass levels reached values between 0.394 and 1.195% P₂O₅, corresponding to an increase of 290 to 1071% compared to the initial values. In conclusion, the combined management of poultry manure and phosphate waste through a controlled composting process

results in a product enriched in available phosphorus (Pass).

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RESULTS REGARDING THE APPLICATION OF AMENDMENTS IN SOYBEAN CULTIVATION

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Abstract

During the research conducted, the positive influence of calcium on soybean plants was noted. Due to the fact that the experiment was located on an acidic soil (pH around 5.0) we used calcium-based products, namely: agrocalcium (93.6% CaCO₃), doloflor with CaCO₃ and MgO (magnesium oxide) and neutrosol 9 (97.5% CaCO₃). Thus, it was found that total biomass production was on average 6.26 t/ha, pod production was 4.19 t/ha, and grain production was 2.46 t/ha, with significant and highly significant positive differences. Analyzing the thousand grain mass (MTG), small significant positive differences were found. Quality indices of soybeans were also determined and thus the protein content was 38%, oil 25%, fiber 5%, and the moisture content of the beans was 10%. The results obtained show that the Cristina TD soybean variety adapts very well to the conditions in the station.

Key words: amendments, CaCO₃, Cristina TD, soybean.

INTRODUCTION

Due to its high agronomic and economic value, soybean cultivation occupies an important place both in national and global agriculture, making it necessary to expand the cultivated areas in various environments (Bueno et al., 2020). Soybeans are the world's main source of oil (Garima et al., 2020). It is produced across a wide range of latitudes, from the Canadian prairies and the Great Northern Plains of the USA to the tropical areas of the Brazilian Cerrados (Grassini et al., 2021). Long before the advent of ancient writings, soybeans were an important crop in East Asia (Shurtleff & Aayagi, 2013). There is evidence that soybeans were domesticated between 7000 and 6600 BC in China, 5000 and 3000 BC in Japan, and 1000 BC in Korea (Lee et al., 2011).

Calcium is an indispensable macroelement for plants, (Karley & White, 2009) contributing to the optimization of soil characteristics and the proper functioning of physiological processes (Cramer et al., 2009; Dayod et al., 2010). Natural calcium (Ca) reserves come from a wide range of minerals and rocks, the decomposition of which occurs through complex processes. In the case of albic luvisol, there is a dynamic equilibrium between the different forms of

calcium in the soil, an equilibrium that is constantly changing either in the direction of adsorption of Ca ions by clay particles, or in the direction of their consumption by plants and leaching. However, the total amounts of calcium in luvisol are low and insufficient to support proper plant growth. The existing calcium cannot ensure and maintain an adequate degree of base saturation of the soil colloids (Badea et al., 2022). Under these conditions, exchangeable aluminum ions (Al³⁺) predominantly occupy the exchange complex of clay, contributing significantly to the pronounced acidification of the soil. Mobile (soluble) aluminum becomes toxic to most plant species. Calcium deficiency, associated with excess aluminum, causes the appearance of a strongly acidic soil, an unfavorable environment for plant development, which leads to reduced growth and decreased agricultural production (Milivojevic & Stojanovic, 2003). In the cultivated soil, CaCO₃ contributes to improving soil structure, water and air permeability, stimulates the activity of microorganisms and provides an important nutritional supply for plants. Calcium, absorbed in the form of Ca²⁺ ions, is an essential element for the growth and development of fruits (Popescu et al., 2023).

Thus, several calcium carbonate-based formulations were tested, in the absence or presence of magnesium.

MATERIALS AND METHODS

The research was carried out on a stagnant albic luvisol, characterized by acidic pH (around 5), poorly supplied with nitrogen (Nt = 0.130% mg/kg) and phosphorus (P = 33 mg/kg), moderately supplied with potassium (K = 89 mg/kg), clayey structure (clay content below 30%), with a humus of 2.26%.

In the case of the experiment, the biological material used was the soybean variety Cristina TD (00) cultivated in a non-irrigated system. The surface of an experimental variant was 40 m² (5m wide and 8m long) and the method used was that of subdivided plots with 3 repetitions. The research was organized as a bifactorial experiment, with the following gradations of the analyzed factors:

- A factor: -A₁: unamended (0 t/ha);
 - A₂: Powder of the Agrocalcium (2.5 t/ha), 93.6% CaCO₃;
 - A₃: Powder of the Doloflor (2.5 t/ha), 58.87% CaCO₃ and 38.24% MgO;
 - A₄: Granules of the Doloflor (2.5 t/ha), 58.87% CaCO₃ and 38.24% MgO;
 - A₅: Neutrosol 9 (2.5 t/ha), 97.5% CaCO₃;
- B factor: -B₁: unfertilized;
 - B₂: fertilized (N₄₀P₄₀);
 - B₃: fertilized (N₈₀P₈₀).

During the experiment, the following determinations were made on the plants: total biomass, pod biomass, grain biomass, MTG (1000 grain mass), crude protein content (%), fiber content (%) and oil content (%). The determination of grain quality indices was performed using the Infracmatic IM 9500 Plus analyzer. The statistical processing of the data was performed by variance analysis and by studying the correlations between the analyzed characters, the results being graphically represented using the Excel application.

RESULTS AND DISCUSSIONS

Climatic factors (Table 1)

During the experimental period, monthly differences in temperature and, especially, in precipitation were observed.

Table 1. The influence of pedoclimatic conditions on soybean crop productivity

TEMPERATURE °C		May	June	July	August	Sept.
	Dec. I	15,2	22,1	24,7	24,7	22,6
	Dec. II	11,9	21,3	22,9	23,4	18,9
	Dec. II	15,7	24,7	25,8	20,5	14,8
	Monthly average	14,4	22,7	24,5	22,8	18,8
	Multi-year average	16,3	19,5	21,7	21,3	16,9
RAINFALL mm	Deviation	-1,9	+3,2	+2,8	+1,5	+1,9
	Dec. I	54,0	8,2	2,6	2,0	0,0
	Dec. II	43,8	0,0	30,0	9,8	14,0
	Dec. II	80,0	7,4	52,4	14,0	8,8
	Monthly average	177,8	15,6	85,0	25,8	12,8
	Multi-year average	80,5	93,6	81,1	59,7	52,9
	Deviation	+97,3	-78,0	+3,9	-33,9	-30,1

Thus, during the soybean growing season, the thermal regime recorded values higher than the multiannual average by approximately +7.5°C. The analysis of the precipitation regime highlights the fact that, in May, the amount of water exceeded the multiannual average by 97.3 mm, conditions that favored uniform emergence and proper development of plants in the initial phases of vegetation. As a result of the previous rainfall surplus, a satisfactory reserve of water was established in the soil, which partially compensated for the deficit of 78 mm recorded in the following month. In August and September, negative rainfall deviations were recorded compared to the multiannual average, quantified at -33.9 mm and -30.1 mm, respectively.

The total biomass, analyzed according to factor A - the types of amendments - presented production values between 5.96 t/ha (control variant) and 6.63 t/ha (variant with powder of the doloflor), highlighting statistical differences between the variants (Table 2). Regarding pod biomass, the production level varied between 3.82 and 4.37 t/ha, the differences being statistically significantly positive, the best being obtained with the amendments with granules and powder of the doloflor, which recorded increases of 0.55 t/ha and 0.54 t/ha, respectively. In the case of grain production, the granule of the doloflor variant stood out with an input of 0.46 t/ha. Grain biomass was between 2.18 t/ha and 2.64 t/ha. The differences between the variants were significantly positive, and the thousand grain weight (MTG) analysis revealed values between 138.00 and 143.33 g. Of these, the variant treated with powder of the

agrocalcium showed a significant positive increase of 5.33 g. The analysis of the biomass distribution showed that, on average, the pod biomass amounted to 67.41% while the grain biomass represented 37.07%, these values being considered important for the conditions of the cultivation area.

Table 2. Influence of A factor

A factor	Total biomass, t/ha	%	Pod biomass, t/ha	%	Grain biomass, t/ha	%	MTG, gr
Check plot	5,96	100,00	3,82	64,09	2,18	36,57	138,00
Powder of the Agrocalcium	6,04	100,00	4,32	71,52	2,29	37,91	143,33
Powder of the Doloflor	6,63	100,00	4,36	65,76	2,44	36,80	140,11
Granules of the Doloflor	6,46	100,00	4,37	67,64	2,36	36,53	140,78
Neutrosol 9	6,23	100,00	4,24	68,05	2,64	37,56	138,00
LSD 5%	0,219		1,130		0,080		4,755
LSD 1%	0,318		0,189		1,116		6,917
LSD 0,1%	0,478		0,284		1,174		10,394

Table 3. Influence of B factor

B factor	Total biomass, t/ha	%	Pod biomass, t/ha	%	Grain biomass, t/ha	%	MTG, gr
Check plot	5,66	100,00	3,83	67,66	1,89	33,39	131,27
N ₄₀ P ₄₀	6,66	100,00	4,44	66,66	2,42	36,33	142,00
N ₈₀ P ₈₀	6,76	100,00	4,40	65,08	2,77	36,53	146,87
LSD 5%	0,253		0,135		0,108		7,362
LSD 1%	0,345		0,184		0,148		10,040
LSD 0,1%	0,466		0,249		0,200		13,587

B factor included the application of chemical fertilizers based on nitrogen and phosphorus. Under these conditions, the total biomass varied from 5.66 t/ha in the control variant to 6.76 t/ha in the N₈₀P₈₀ variant (Table 3). The pod biomass recorded values between 3.83 t/ha and 4.44 t/ha. Regarding grain production, in the 2 chemically fertilized variants, yields of over 2 t/ha were obtained, with significantly positive differences, more pronounced in the case of the variant fertilized with N₈₀P₈₀. The thousand grain mass (MTG) recorded values between 131.27 - 146.87 g, the differences being very significantly positive.

The analysis of the interaction between the type of amendment and chemical fertilization revealed a total production ranging from 3.93 t/ha in the control variant to 7.45 t/ha in the variant treated with powder of the doloflor in association with N₄₀P₄₀, the differences being very significantly positive (Table 4). The level of pod production ranged between 2.38 and 4.81

t/ha, recording significant positive differences. Regarding grain production, the values ranged between 1.48 t/ha and 3.11 t/ha. The variants: N₈₀P₈₀, Doloflor powder + N₄₀P₄₀, Doloflor powder + N₈₀P₈₀, Doloflor granules + N₈₀P₈₀ with very significant positive differences compared to the control were noted. From the analysis of the mass of one thousand grains (MTG), the values ranged between 126.00 g and 151.00 g, with small significant positive differences. In the experiment, the total biomass of the plants was, on average, 6.26 t/ha, of which the biomass of pods amounted to 4.19 t/ha, and the biomass of grains 2.46 t/ha. The results obtained highlight the good adaptability of the Cristina TD soybean variety to the conditions in the area.

Table 4. The interaction between the two factors

A factor	B factor	Total biomass, t/ha	%	Pod biomass, t/ha	%	Grain biomass, t/ha	%	MTG, gr
Check plot	Martor	3,93	100,00	2,38	58,01	1,48	37,65	126,00
	N ₄₀ P ₄₀	6,54	100,00	3,91	59,78	2,56	39,14	137,00
	N ₈₀ P ₈₀	7,43	100,00	4,78	64,33	3,11	41,85	140,00
Powder of the Agrocalcium	0	5,65	100,00	4,06	71,85	2,31	40,88	141,67
	N ₄₀ P ₄₀	5,98	100,00	4,56	76,25	2,21	36,95	141,67
	N ₈₀ P ₈₀	6,48	100,00	4,35	67,12	2,54	39,19	146,67
Powder of the Doloflor	0	5,55	100,00	3,89	70,09	2,23	40,18	133,00
	N ₄₀ P ₄₀	7,45	100,00	4,81	64,56	2,81	37,71	147,33
	N ₈₀ P ₈₀	6,88	100,00	4,38	63,66	2,88	41,86	151,00
Granules of the Doloflor	0	5,38	100,00	4,19	77,88	2,18	40,52	128,33
	N ₄₀ P ₄₀	7,44	100,00	4,70	63,17	2,65	35,61	145,67
	N ₈₀ P ₈₀	6,55	100,00	4,21	64,27	2,75	41,98	148,33
Neutrosol 9	0	6,32	100,00	4,20	66,45	2,52	39,87	127,33
	N ₄₀ P ₄₀	5,90	100,00	4,24	71,86	2,24	37,96	138,33
	N ₈₀ P ₈₀	6,47	100,00	4,26	65,84	2,47	38,17	148,33
Average		6,26		4,19		2,46		140,04
LSD 5%		0,455		0,254		0,184		12,075
LSD 1%		0,635		0,355		0,256		16,731
LSD 0,1%		0,895		0,503		0,359		23,269

The following figures provide a visual representation of images from the soybean field experiment, illustrating the distinct morphological differences between the control group and the plants treated with amendaments (Figures 1-5).



Figure 1. Control variant without treatment



Figure 2. Influence of powder of the Agrocalcium treatment on plant development



Figure 3. Influence of powder of the Doloflor treatment on plant development



Figure 4. Influence of granules of the Doloflor treatment on plant development



Figure 5. Influence of Neutrosol 9 treatment on plant development

Table 5. Statistical indices obtained in the experiment

Statistical indices	Determined characteristics			
	Protein	Oil	Fiber	Moisture
Mediate, $\bar{x}$	37,85	24,74	5,33	10,4
Variant, s^2	4,00	1,42	0,01	0,41
The standard deviation, s	2,00	1,19	0,10	0,64
<i>LSD</i> 5%	1,677	1,252	0,195	1,065
<i>LSD</i> 1%	2,389	1,732	0,274	1,485
<i>LSD</i> 0,1%	3,476	2,401	0,390	2,087
Coefficient of variation, CV %	5,28	4,82	1,94	6,18

The statistical processing of the obtained data was carried out according to the following formula $1/n-1 * [(\sum x^2) - \sum x^2/n]$ (Table 5). The analysis of the results highlights that the average value of the protein content was 37.85 with a coefficient of 5.28%, the oil content reached 24.74 presenting a coefficient of variation of 4.82%, the average fiber content of the stock was 5.33% with a coefficient of 5.33%. The determined levels of the active principles (protein and oil) indicate a good to very good quality of the beans of the Cristina TD soybean variety.

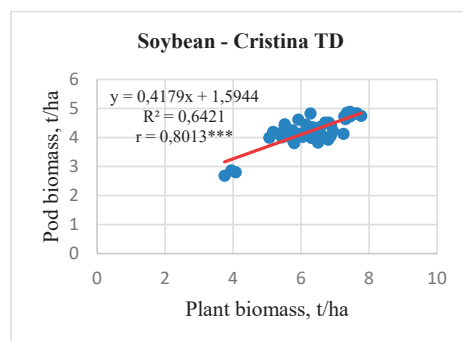


Figure 6. Correlation between plant biomass and pod biomass

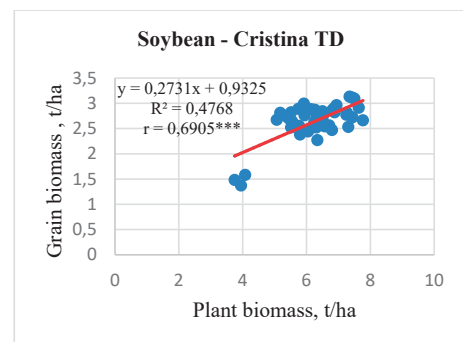


Figure 7. Correlation between plant biomass and grain biomass

The correlation analysis was performed using the mean values for each replicate. The results show a highly significant positive correlation between plant biomass and pod biomass, suggesting that the accumulation of a total biomass of 6.26 t/ha favors pod formation and development, confirming the direct relationship between vegetative growth and reproductive production (Figure 6). Comparably, the correlation between plant biomass and formed grain biomass shows the same significance (Figure 7).

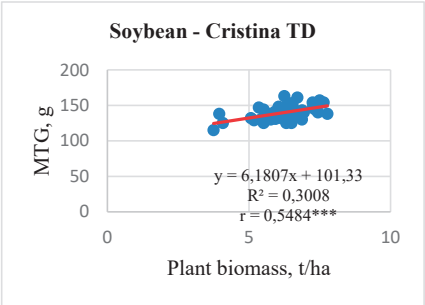


Figure 8. Correlation between plant biomass and MTG

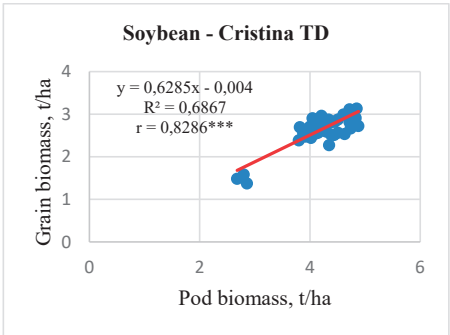


Figure 9. Correlation between pod biomass and grain biomass

The correlation between plant biomass and thousand grain mass (MTG) is positive, but less pronounced, which is explained by the relative stability of the grain weight trait in soybean (Figure 8). This is determined by the genetic peculiarities of the plant, representing an intrinsic characteristic expressed during the progeny formation stage. Correlational analysis indicates a positive correlation between pod weight and grain weight, with a much higher tendency to increase (Figure 9).

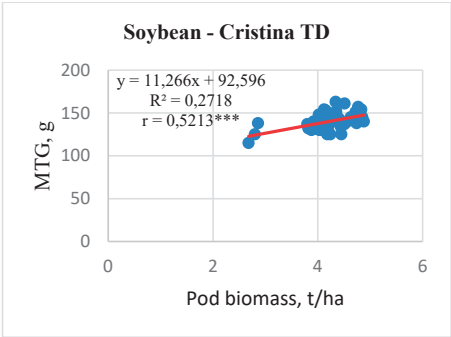


Figure 10. Correlation between pod biomass and MTG

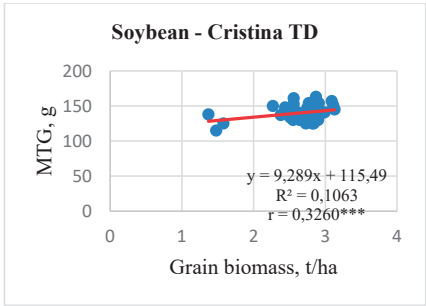


Figure 11. Correlation between grain biomass and MTG

In both correlations, the very positive statistical significance emphasizes that the thousand grain mass (MTG) is a relatively stable character, with reduced changes under the experimental conditions (Figures 10 and 11).

Table 6. Correlations between the main determined characters

	Plant biomass, gr	Pod biomass, gr	Grain biomass, gr	MTG, gr
Plant biomass, gr	1	0,8013***	0,6905***	0,5484***
Pod biomass, gr		1	0,8286***	0,5213***
Grain biomass, gr			1	0,3260***
MTG, gr				1
LSD5% = 0,19 LSD1% = 0,25 LSD0,1% = 0,32				

The relationships between the main determined characters are highly significantly positive, which demonstrates that the Cristina TD soybean variety presented high values in the experiment. The experimental data highlight the variety's ability to adapt favorably to the pedoclimatic conditions specific to the area.

CONCLUSIONS

Against the backdrop of the expansion of soybean cultivation in Romania, the need to promote the latest genetic creations, capable of ensuring high productive potential and efficient adaptation to local pedoclimatic conditions, is highlighted. However, the presence of acidic soils within the station requires the application of measures to correct the soil reaction, in order to create favorable conditions for the development of the plant root system. Based on the data obtained, the Cristina TD variety demonstrated that, in the variants treated with different types of calcareous amendments, the soil reaction was improved, and the plants responded favorably to the culture environment in which the acidity was corrected.

Biomass increased from 3.93 t/ha in the control variant to 7.44 t/ha in the variant with doloflor granules + chemical fertilizers, which indicates a favorable effect of the applied treatment. The analysis of biomass distribution highlighted a weight of 58-77% at the pod level and 37-42% at the grain level, simultaneously with relatively high values of the mass of one thousand grains (MTG), under the culture conditions achieved (126-151g).

There is a very significant positive correlation between plant biomass and pod biomass, demonstrating that achieving a total biomass of 6.26 t/ha is associated with a significant increase in pod biomass.

Total biomass correlates positively, but weakly, with thousand grain mass (MTG), confirming the stability of this trait in soybean.

At the same time, the quality of the grains presented favorable characteristics, reflected by a protein content of 38%, oil content of 25% and 5% fiber.

In terms of application, the experimental data confirm the ability of soybean for grains to adapt

favorably to the pedoclimatic conditions specific to the area.

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SUITABILITY OF POTATO VARIETIES FOR FRENCH FRY PROCESSING UNDER CLIMATIC STRESS

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Abstract

To evaluate the influence of climatic factors on the technological quality of potato tubers during the 2024 and 2025 agricultural years, six potato varieties - Asinaria, Azaria, Castrum, Cezarina, Cosiana, and Darilena - were studied for their suitability for French fry processing. Tubers were analyzed for technological quality parameters, including specific gravity, determined using a PW-2050 digital weigher; dry matter content, assessed by the oven-drying method; and reducing sugar content, measured semi-quantitatively using Quantofix test strips. Frying suitability was evaluated by calculating the frying index, determining the percentage of French fries with brown ends, and assessing the number of colour classes assigned to the fried samples based on comparison with the Munsell USDA Frozen French Fry Color Standard. The frying index ranged from 0.3 to 4.5 among the six varieties, while specific gravity values varied between 1.072 and 1.094.

Key words: potato varieties, processing, climatic stress.

INTRODUCTION

Potato (*Solanum tuberosum* L.), widely cultivated for both fresh consumption and industrial processing, is one of the most important food crops worldwide. French fries and chips are the most important products obtained by frying. The quality of the raw material intended for processing is a very important factor in ensuring the obtaining of products with appropriate technological properties (Singh and Kaur, 2016).

The suitability for frying processing is determined by the technological parameters of potato tubers: specific gravity, dry matter, starch and reducing sugars content. The high dry matter and starch content allows the obtaining of products with low oil absorption, and the low concentrations of reducing sugars lead to the obtaining of light and uniform colour of fried products (Islam et al., 2022; Stark et al., 2003). From a processing perspective, tubers with higher dry matter content and lower concentrations of reducing sugars are preferred for French fries production, as they ensure better frying performance and a lighter color of the fried product. Therefore, the climatic conditions

recorded during the growing season may partially explain possible variations in the technological parameters of the potato varieties, including dry matter content, reducing sugars, and frying quality indices (Hamouz et al., 2005). The period of heat stress with high temperatures during tuber development leads to an increase in the content of reducing sugars, a decrease in the content of dry matter, which leads to colour defects during roasting (Busse et al., 2018).

The suitability for frying processing is influenced by the genetic characteristics of the potato varieties. Technological characteristics that can influence the quality of fried products, dry matter content, starch content, reducing sugars are influenced by genotype (Feltran et al., 2004). Therefore, the selection of varieties suitable for processing is an important objective in breeding programs (Bethke et al., 2019; Seid and Tessema, 2024). Some varieties show high stability of technological characteristics, which makes them more suitable for industrial processing, especially for French fries production.

Genotype × environment interactions shape potato quality traits, making their targeted selection essential for breeding cultivars with

multiple desirable traits under variable conditions (Zhou et al., 2025).

Studies on the influence of climatic factors on tuber quality parameters are important for selecting varieties suitable for the processing industry. Especially for the production of French fries, it is necessary to identify varieties with high dry matter content, low levels of reducing sugars and high stability of these characteristics under different climatic conditions (Islam et al., 2022).

The present study aims to analyze the influence of climatic conditions during the growing season and the variety on the suitability of potato tubers for processing as French fries, by evaluating relevant technological parameters, such as dry matter content, specific gravity, reducing sugar content and color of fried products.

MATERIALS AND METHODS

In order to study the influence of climatic factors on the technological quality of potato tubers in the agricultural years 2024 and 2025, six potato varieties were studied regarding their suitability for processing as French fry. Thus, a bifactorial experiment was undertaken: factor A - crop year with 2 levels: 2024 and 2025 (with 2025 considered the control) and factor B - variety with 6 levels: Asinaria, Azaria, Castrum, Cezarina, Cosiana, Darilena, and the control was represented by the average of the six varieties for each parameter studied. The technological quality parameters of potato tubers were determined before processing: specific gravity, dry matter content, reducing sugars.

After frying, observations were made on the technological parameters of the obtained fried potato strips: frying index, percentage of brown ends, colour class number. Specific gravity was determined by weighing the tuber samples in air and water, using the PW-2050 Weigher digital scale. This device operates using a load cell that converts the applied force (weight) into a proportional electrical signal, which is electronically processed and digitally displayed (Băraescu and Ștefan, 2025). The dry matter content of the potato tubers was determined using the oven drying method. Reducing carbohydrates can influence the colour, taste and

texture of the processed products. The reducing sugar content of potato tubers was determined using QUANTOFIX® test strips, which were read with a QUANTOFIX® Relax digital reader device; this is a semi-quantitative method based on colour reactions.

To determine the suitability of potato samples for French fries, 20 potato sticks, with a section of 1 x 1 cm, taken from the central area of 20 potato tubers, are heat-treated by frying in a standardized manner (4 minutes at 175°C). Each fried potato stick is classified into the predominant colour category (000, 00, 0, 1, 2, 3, 4), compared to a reference scale (USDA Munsell Color Chart for French Fried Potatoes), then the frying index and the number of fries with a browned end are calculated. The suitability of potatoes for frying is designated by:

- The frying index (I_p) which represents the colour of the fries: the lower it is, the lighter the fries are in colour (light yellow); the higher it is, the darker the French fries are (brown). The index value shows the level of suitability of the potato sample for frying (I_p) as follows: $I_p \leq 2.5$ = excellent; I_p between 2.5 and 3.0 = good; I_p between 3.0 and 3.5 = medium; I_p between 3.5 and 4.0 = poor; $I_p > 4.0$ = very poor.
- The number of French fries with a browned ends, with a brown or black colour at one (or both) ends, between 0.5 and 1.5 cm.
- The number of colour classes reflects the homogeneity of the French fries colour. The method used to determine the colour of French fries classifies each of the 20 French fries in the sample into a colour class, ranging from very light (class "000") to very dark (class "4"). The higher the number of classes, the more heterogeneous the French fries colour.

RESULTS AND DISCUSSIONS

The climatic conditions during the growing season shows that the year 2025 was characterized by lower average temperatures during the growing season (15.9°C) compared to 2024 (17.8°C), but with a higher total amount of precipitation (372 mm versus 306.8 mm) (Figure 1).

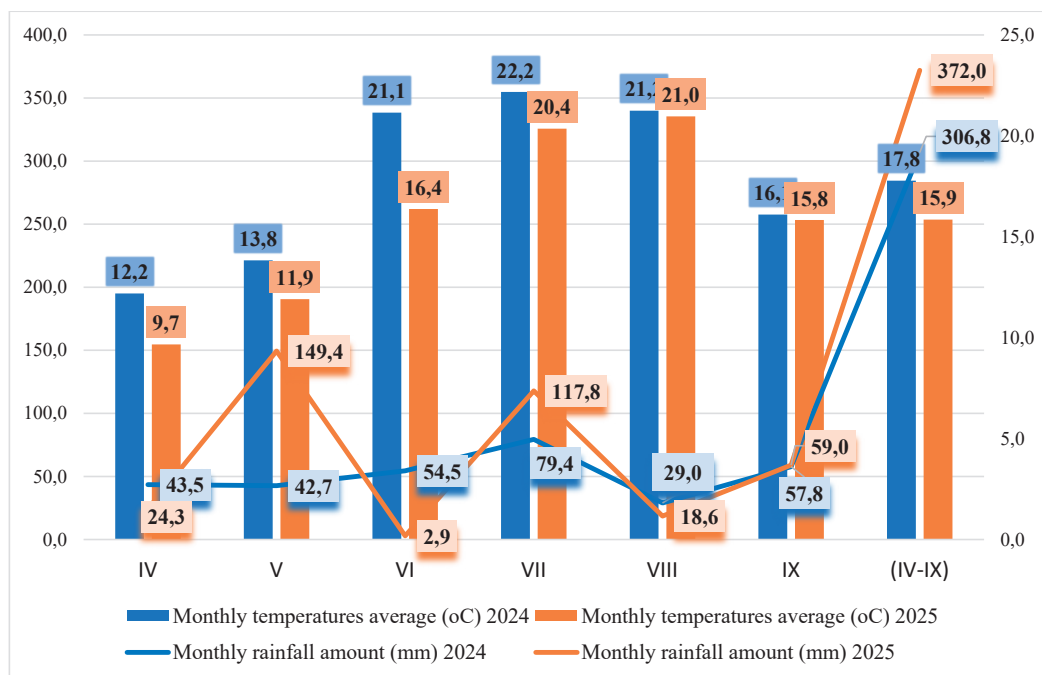


Figure 1. Monthly temperatures average and rainfall amount during the potato growing season (April-September, 2024-2025, Braşov)

During the tuber bulking period (June-August), temperatures in 2024 ranged between 21.1 and 22.2°C, above the optimal range for starch accumulation in potato tubers, potentially affecting dry matter content. In contrast, the cooler conditions recorded in 2025, particularly in June (16.4°C), may have a positive influence on dry matter accumulation.

Stress conditions caused by high temperature or water availability may stimulate the conversion of starch into simple sugars, increasing the concentration of reducing sugars in tubers.

Analyzing the influence of the climatic conditions of the crop year on the technological parameters of fresh potatoes and French fry potatoes obtained by processing them, it was found that in 2024, compared to 2025, climatic conditions had a negative influence on the dry matter content of potato tubers (%), determining a significant difference (-2.02%). For the other parameters taken into study, no statistically significant differences were recorded (Table 1). Following the analysis of the influence of the variety on the technological parameters taken into consideration, according to Table 2, the specific gravity values were between 1.0782

(Cezarina variety) and 1.0877 (Azaria variety). For this parameter there were no statistically significant differences compared to the average of all varieties, considered as a control.

Regarding the dry matter content, the Azaria variety recorded a significantly positive difference of 1.18 (%), compared to the average of the varieties. The Cezarina variety had the lowest accumulation of dry matter in tubers (20.07%).

While the Cezarina variety, with the highest value of reducing sugars content (0.40 mg/g), presented a distinctly significant difference (0.1172 mg/g), the Azaria variety recorded the lowest value of reducing sugars (0.19 mg/g) which led, compared to the average values of all varieties, to a significantly negative difference (-0.0936 mg/g).

The Azaria variety recorded the lowest value of the frying index (1.15), determining the obtaining of a distinctly significant negative difference (1.51), and the Cezarina variety achieved the highest value (4.22) and a very significantly positive difference (1.56). Also, the Castrum variety showed influence on frying, obtaining a significantly positive difference

(1.01). Regarding brown ends, the Cezarina variety surpassed the control with a very significant positive difference (60.14%), while Azaria and Cosiana recorded significant negative differences (24.03%) and distinctly significant (24.86%).

The color class number had values between 2.3 (Azaria) and 4.5 (Cosiana). The Azaria variety

stands out with a significant negative difference (1.3), compared to the control. The low value of this parameter indicates the uniformity of the colour during roasting of this variety, a positive characteristic for processing. For the other varieties, no statistically significant differences were obtained.

Table 1. The influence of culture year on technological quality parameters

Year (a)	Specific gravity	Diff. /Sign.	Dry matter (%)	Diff. (%) /Sign.	Reducing sugars (mg/g)	Diff. (mg/g) /Sign.	Frying index	Diff. /Sign.	Brown ends (%)	Diff. (%) /Sign.	Color class number	Diff. /Sign.
2024 (a ₁)	1.0798	-0.0068 ns	20.09	-2.02 o	0.32	0.06 ns	2.76	0.19 ns	31.39	-0.28 ns	3.4	-0.4 ns
2025 (a ₂) (Ct)	1.0867	-	22.12	-	0.25	-	2.56	-	31.67	-	3.9	-
DL 5% = 0.0068; DL 5% = 1.25; DL 5% = 0.07; DL 5% = 0.69; DL 5% = 8.61; DL 5% = 0.9; DL 1% = 0.0158; DL 1% = 2.89; DL 1% = 0.17; DL 1% = 1.60; DL 1% = 19.89; DL 1% = 2.0; DL 0.1% = 0.0502; DL 0.1% = 9.19; DL 0.1% = 0.55; DL 0.1% = 5.10; DL 0.1% = 63.30; DL 0.1% = 6.3.												

Table 2. The influence of variety on technological quality parameters

Variety (b)	Specific gravity	Diff. /Sign.	Dry matter (%)	Diff. (%) /Sign.	Reducing sugars (mg/g)	Diff. (mg/g) /Sign.	Frying index	Diff. /Sign.	Brown ends (%)	Diff. (%) /Sign.	Color class number	Diff. /Sign.
Asinaria (b ₁)	1.0813	-0.0019 ns	20.66	-0.45 ns	0.24	-0.0478 ns	2.48	-0.18 ns	20.83	-10.69 ns	3.2	-0.5 ns
Azaria (b ₂)	1.0877	0.0044 ns	22.29	1.18 *	0.19	-0.0936 o	1.15	-1.51 oo	7.50	-24.03 o	2.3	-1.3 o
Castrum (b ₃)	1.0833	0.0001 ns	20.99	-0.12 ns	0.34	0.0557 ns	3.67	1.01 *	46.67	15.14 ns	4.0	0.3 ns
Cezarina (b ₄)	1.0782	-0.0051 ns	20.07	-1.03 ns	0.40	0.1172 **	4.22	1.56 ***	91.67	60.14 ***	3.8	0.2 ns
Cosiana (b ₅)	1.0858	0.0026 ns	21.51	0.40 ns	0.28	-0.0090 ns	1.92	-0.74 ns	6.67	-24.86 oo	4.5	0.8 ns
Darilena (b ₆)	1.0832	-0.0001 ns	21.12	-0.02 ns	0.26	-0.0224 ns	2.52	-0.14 ns	15.83	-15.69 ns	4.2	0.5 ns
Mean (Ct)	1.0833	-	21.11	-	0.28	-	2.66	-	31.53	-	3.7	-
DL 5% = 0.0053; DL 5% = 1.01; DL 5% = 0.07; DL 5% = 0.84; DL 5% = 18.16; DL 5% = 1.1; DL 1% = 0.0071; DL 1% = 1.38; DL 1% = 0.10; DL 1% = 1.14; DL 1% = 24.67; DL 1% = 1.4; DL 0.1% = 0.0097; DL 0.1% = 1.87; DL 0.1% = 0.14; DL 0.1% = 1.54; DL 0.1% = 33.45; DL 0.1% = 2.0.												

The comparison of the two crop years shows an increase in specific gravity in 2025 compared to 2024, but the positive difference of 0.0068 is statistically insignificant. The largest differences between years were recorded for the varieties Cezarina (0.0097) and Cosiana (0.0090), these differences being significantly negative (Table 3).

From the analysis of the influence of the variety on the dry matter content, the Azaria variety stood out with a statistically significant positive

difference (1.54%) compared to the control in 2024, indicating a superior capacity of this variety to accumulate dry matter. In 2025, for the 6 varieties analyzed, there were no significant differences compared to the control (Table 4).

The comparison of the two crop years highlights a significant increase in dry matter content in 2025 (22.12%) compared to 2024 (20.09%), the average difference between 2024 and 2025 being significantly negative (2.02%).

Table 3. The combined influence of culture year and variety on specific weight

Year(a)/Variety (b)	Specific gravity		Specific gravity		Diff./Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff./Sign.	2025 (a ₂) (Ct)	Diff./Sign.	
Asinaria (b ₁)	1.0793	-0.0005 ns	1.0833	-0.0033 ns	-0.0040 ns
Azaria (b ₂)	1.0853	0.0055 ns	1.0900	0.0033 ns	-0.0047 ns
Castrum (b ₃)	1.0793	-0.0005 ns	1.0873	0.0007 ns	-0.0080 ns
Cezarina (b ₄)	1.0733	-0.0065 ns	1.0830	-0.0037 ns	-0.0097 o
Cosiana (b ₅)	1.0813	0.0015 ns	1.0903	0.0037 ns	-0.0090 o
Darilena(b ₆)	1.0803	0.0005 ns	1.0860	-0.0007 ns	-0.0057 ns
Mean (Ct)	1.0798	-	1.0867	-	0.0068 ns

DL 5% = 0.0074;

DL 1% = 0.0101;

DL 0.1% = 0.0137.

DL 5% = 0.0087;

DL 1% = 0.0145;

DL 0.1% = 0.0317.

The Castrum variety obtained the largest negative difference of 2.79%, being a distinctly significant difference. Also, the varieties

Cezarina, Cosiana and Asinaria obtained significant negative differences between the two years of cultivation (2.44%; 2.48% and 1.68%).

Table 4. The combined influence of culture year and variety on dry matter (%)

Year(a)/Variety (b)	Dry matter (%)		Dry matter (%)		Diff. (%)/Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff./Sign.	2025 (a ₂) (Ct)	Diff./Sign.	
Asinaria (b ₁)	19.81	-0.28 ns	21.50	-0.62 ns	-1.68 o
Azaria (b ₂)	21.63	1.54 *	22.95	0.83 ns	-1.32 ns
Castrum (b ₃)	19.59	-0.50 ns	22.38	0.26 ns	-2.79 oo
Cezarina (b ₄)	18.85	-1.24 ns	21.29	-0.83 ns	-2.44 o
Cosiana (b ₅)	20.27	0.17 ns	22.75	0.63 ns	-2.48 o
Darilena(b ₆)	20.41	0.32 ns	21.83	-0.28 ns	-1.42 ns
Mean (Ct)	20.09	-	22.12	-	-2.02 o

DL 5% = 1.43;

DL 1% = 1.95;

DL 0.1% = 2.64.

DL 5% = 1.63;

DL 1% = 2.70;

DL 0.1% = 5.78.

From the analysis of the influence of the crop year and the variety on the content of reducing sugars in potato tubers (Table 5) it appears that in 2024, the Cezarina variety had the highest value of reducing sugars (0.4332 mg/g) and a significant positive difference (0.1155 mg/g) compared to the control.

In 2025, the Castrum and Cezarina varieties recorded statistically significant positive differences (0.1144 and 0.1188 mg/g),

compared to the control, while Azaria presented a distinctly significant negative difference (0.1440 mg/g), having a reduced content of reducing sugars.

In 2025 compared to 2024, at the variety level, Azaria presented a significant positive difference, with a reduction in the content of reducing sugars of 0.1636 mg/g. The other varieties showed statistically insignificant differences between the study years.

Table 5. The combined influence of culture year and variety on reducing sugars (mg/g)

Year(a)/Variety (b)	Reducing sugars (mg/g)		Reducing sugars (mg/g)		Diff. (mg/g)/Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff./Sign.	2025 (a ₂) (Ct)	Diff./Sign.	
Asinaria (b ₁)	0.2915	-0.0262 ns	0.1855	-0.0694 ns	0.1060 ns
Azaria (b ₂)	0.2745	-0.0432 ns	0.1110	-0.1440 oo	0.1636 *
Castrum (b ₃)	0.3147	-0.0030 ns	0.3693	0.1144 *	-0.0546 ns
Cezarina (b ₄)	0.4332	0.1155 *	0.3738	0.1188 *	0.0595 ns
Cosiana (b ₅)	0.3320	0.0143 ns	0.2226	-0.0324 ns	0.1094 ns
Darilena(b ₆)	0.2602	-0.0575 ns	0.2677	0.0127 ns	-0.0075 ns
Mean (Ct)	0.3177	-	0.2550	-	0.0627 ns

DL 5% = 0.1049;
DL 1% = 0.1426;
DL 0.1% = 0.1933.

DL 5% = 0.1124;
DL 1% = 0.1771;
DL 0.1% = 0.3486.

Regarding the frying index (Table 6), in 2024, the Cezarina variety recorded the highest value (4.23) and a significant positive difference (1.48).

Table 6. The combined influence of culture year and variety on frying index

Year(a) / Variety(b)	Frying index		Frying index		Diff. /Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff. /Sign.	2025 (a ₂) (Ct)	Diff. /Sign.	
Asinaria (b ₁)	2.80	0.04 ns	2.17	-0.39 ns	0.63 ns
Azaria (b ₂)	1.97	-0.79 ns	0.33	-2.23 ooo	1.63 *
Castrum (b ₃)	3.13	0.38 ns	4.20	1.64 **	-1.07 ns
Cezarina (b ₄)	4.23	1.48 *	4.20	1.64 **	0.03 ns
Cosiana (b ₅)	2.07	-0.69 ns	1.77	-0.79 ns	0.30 ns
Darilena(b ₆)	2.33	-0.42 ns	2.70	0.14 ns	-0.37 ns
Mean (Ct)	2.76	-	2.56	-	0.19 ns

DL 5% = 1.18;
DL 1% = 1.61;
DL 0.1% = 2.18.

DL 5% = 1.21;
DL 1% = 1.84;
DL 0.1% = 3.34.

In 2025, the Castrum and Cezarina varieties presented equal values (4.20) of the frying index and a distinctly significant positive difference (1.64). The very high values of the frying index for the two varieties, in the two years of study, indicate that these varieties are not suitable for processing as French fries. The Azaria variety,

with a significantly positive difference between crop years, with a reduction in the frying index of 1.63 in 2025, ranks first in terms of suitability for processing as French fries. The Cosiana variety, with frying index values of 2.07 in 2024 and 1.77 in 2025, is also suitable for this type of processing.

From the analysis of the influence of the crop year and the variety on the percentage of brown ends observed in French fries sticks (Table 7.), in 2024 the Cezarina variety recorded a statistically very significant increase compared to the control (65.28%), in the brown ends, indicating a very low suitability for processing as French fries. In contrast, the Darilena variety showed a significant negative difference (26.39%), and the other varieties did not show statistically significant differences compared to the control. Also, in 2025 the Cezarina variety showed a very significant difference in the percentage of brown heads (55.00%), compared to the control, and the Azaria and Cosiana varieties recorded significant negative differences (31.67 and 26.67%) compared to the control, indicating their suitability for processing as French fries.

In 2024 compared to 2025, the Asinaria variety recorded a significant increase in the percentage of brown ends (25.00%), and the Castrum variety showed a significant reduction (33.33%) of this parameter in 2024 compared to 2025. The other varieties did not show significant differences between crop years.

Table 7. The combined influence of culture year and variety on brown ends (%)

Year (a) Variety (b)	Brown ends (%)		Brown ends (%)		Diff. (%) /Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff. /Sign.	2025 (a ₂) (Ct)	Diff. /Sign.	
Asinaria (b ₁)	33.33	1.94 ns	8.33	-23.33 ns	25.00 *
Azaria (b ₂)	15.00	-16.39 ns	0.00	-31.67 o	15.00 ns
Castrum (b ₃)	30.00	-1.39 ns	63.33	31.67 *	-33.33 o
Cezarina (b ₄)	96.67	65.28 ***	86.67	55.00 ***	10.00 ns
Cosiana (b ₅)	8.33	-23.06 ns	5.00	-26.67 o	3.33 ns
Darilena(b ₆)	5.00	-26.39 o	26.67	-5.00 ns	-21.67 ns
Mean (Ct)	31.39	-	31.67	-	-0.28 ns

DL 5% = 25.68; DL 5% = 24.40;
DL 1% = 34.89; DL 1% = 34.58;
DL 0.1% = 47.30. DL 0.1% = 53.22.

Regarding colour class number, the differences between varieties and the control were statistically insignificant, indicating a reduced influence of the variety on this parameter under the conditions of 2024 (Table 8).

Table 8. The combined influence of culture year and variety on color class number

Year (a) /Variety (b)	Color class number		Color class number		Diff. /Sign. (a ₁ -a ₂)
	2024 (a ₁)	Diff. /Sign.	2025 (a ₂) (Ct)	Diff. /Sign.	
Asinaria (b ₁)	2.7	-0.8 ns	3.7	-0.2 ns	-1.0 ns
Azaria (b ₂)	2.7	-0.8 ns	2.0	-1.9 o	0.7 ns
Castrum (b ₃)	3.3	-0.1 ns	4.7	0.8 ns	-1.3 ns
Cezarina (b ₄)	3.3	-0.1 ns	4.3	0.4 ns	-1.0 ns
Cosiana (b ₅)	4.7	1.2 ns	4.3	0.4 ns	0.3 ns
Darilena(b ₆)	4.0	0.6 ns	4.3	0.4 ns	-0.3 ns
Mean (Ct)	3.4	-	3.9		-0.4 ns

DL 5% = 1.5; DL 5% = 1.5;
DL 1% = 2.0; DL 1% = 2.3;
DL 0.1% = 2.8. DL 0.1% = 4.2.

In 2025, the Azaria variety presented a significantly negative influence (1.9), compared to the control.

At the variety level, the differences between crop years were statistically insignificant. The correlation matrix highlights several relationships between potato tuber quality traits and climatic factors during the growing season (April–September) in 2024–2025. Specific gravity showed a strong positive correlation with dry matter content ($r=0.87$), while both parameters were negatively correlated with reducing sugars ($r=-0.63$ and $r=-0.66$). Reducing sugars exhibited the strongest positive correlation with the frying index ($r=0.91$), confirming their major influence on frying quality and color development. Average temperature during the growing season was negatively correlated with specific gravity and dry matter content ($r = -0.74$ and $r = -0.88$) and positively associated with reducing sugars ($r = 0.36$), suggesting that higher temperatures may reduce dry matter accumulation while promoting sugar formation in tubers. In contrast, total precipitation during the same period showed only weak correlations with the analyzed quality traits.

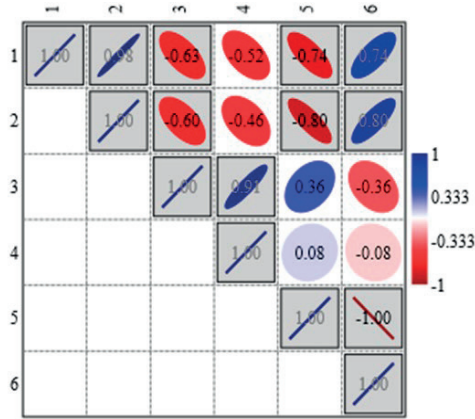


Figure 2. Correlation matrix between potato tuber quality parameters: 1 - Specific gravity, 2 - Dry matter (%), 3 - Reducing sugars, 4 - Frying index, and climatic variables 5 - Temperature, 6 - rainfall (2024-2025)

CONCLUSIONS

The climatic conditions recorded during the potato growing season (April–September) differed between the two studied years (2024, 2025), both in terms of thermal regime and rainfall distribution. Meteorological data revealed that 2024 was characterized by higher temperatures and reduced rainfall during the April–September period, which negatively impacted potato tubers

dry matter accumulation. In contrast, 2025 presented more favourable climatic conditions, for this technological parameter.

The Azaria variety, on average over the two years studied, having the highest dry matter content (22.29%), the lowest reducing sugar content (0.19 mg/g), the lowest frying index (1.15), the very low percentage of brown ends (7.5%) and the lowest number of color classes (2.3), can be considered a variety with high suitability for processing as French fries.

The Cezarina and Castrum varieties are not suitable for processing as French fries because, in the two years of observations, they recorded low dry matter content (20.07% and 20.99%), high reducing sugar content (0.40 mg/g and 0.34 mg/g), high frying index (4.22 and 3.67) and a very high percentage of brown ends (91.67% and 46.67%).

Frying quality is mainly determined by the content of reducing sugars, which is influenced by the genetic characteristics of the potato varieties.

Therefore, the selection of varieties with low levels of reducing sugars and high dry matter content is an essential factor for obtaining high-quality French fries.

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ASSESSMENT OF AVIAN SPECIES DIVERSITY ACROSS THREE PEATLAND ECOSYSTEMS OF N-W ROMANIA AND POSSIBLE INFLUENCE ON THE SURROUNDING AGRICULTURAL FIELDS

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Abstract

*Wetlands and their associated birdlife could impact adjacent agricultural land as they set forth synergistic interactions: wetlands provide habitat and food for birds, which in turn support agriculture through pest control and fertilization. As part of a 3-seasons restoration effort (PeatRO4 2024), we assessed the bird and plant species and described the habitats from three peatland ecosystems with different degrees of human impact, from Cluj County (NW Romania): one in the forest (Negrușul-Finciului), two in the vicinity of agricultural lands (Molhașul Călățele, Dâmbu-Negru). For the active raised bog habitats (Natura 2000 code *7111) the peatland Dâmbu-Negru bore 40% of avian diversity. Most species listed as Least Concern, with 3 on the Near Threatened National Red List and 8 under the Birds Directive Annex I. We found an increased number of insectivorous bird species correlating positively with the presence of typical bog grasses (Eriophorum-Carex associations), shrubs (Vaccinium sp.) and trees (Picea-Pinus associations). Since protected wetlands reduce pesticide dependence and enhance ecosystem resilience, biodiversity, and crop productivity, conserving these habitats is both an ecological imperative and an agricultural strategy.*

Key words: bird diversity, peatland, agricultural impact.

INTRODUCTION

Temperate peatlands cover over 3 million km² globally and store an estimated 455-547 Gt of carbon. They serve as crucial long-term carbon sinks and biodiversity hubs (Harshberger, 1909; Lachance & Lavoie, 2004; Yu et al., 2010; Pasquet et al., 2015). Characterized by acidic conditions that favour specific plant communities and taxa (*Sphagnum* mosses) (Baas Becking & Nicolai, 1934), peatlands have suffered under anthropogenic pressures leading to: altered hydrology, surface drying, and vegetation shifts (shade- and drought-tolerant species are replacing characteristic heliophilous vegetation (Foster & Wright Jr, 1990; Schouwenaars, 1993; Howie & Meerveld, 2011; Pellerin et al., 2009).

Climate change-induced changes to the hydrological regime affect Eastern European wetlands (Kracauer Hartig et al., 1997). These impacts add to drivers of bog forest reshaping, along with land-use changes and agricultural conversion (Minayeva & Sirin, 2012).

Anthropogenic pressures influence ecological interactions, ultimately affecting biodiversity and ecosystem functioning (Evans et al., 2014). Restoration efforts focusing on re-establishing hydrology (ditch blocking; pool creation) promote the recovery of peat-forming vegetation and specific faunal communities (Cuckston, 2017; Hughes et al., 2025; Nicoară et al., 2025). Given that many of these ecosystems have declined across Europe, restoring peatlands is an arduous process that requires long-term efforts, particularly to restore local bird communities, especially peatland-associated species (Alsila et al., 2021; Hughes & Bingham, 2025). Although ecosystem responses vary with microhabitat conditions and management (Mazerolle et al., 2006; Peacock et al., 2013; Beadle et al., 2015), peatlands can become avian diversity hubs. Healthy peatland ecosystems cater to the needs of a variety of species, fostering both trade-offs and synergistic interactions between bird communities and adjacent habitats (Bracken et al., 2008; Calmé et al., 2002; Yapu-Alcazar & Naoki, 2022). In turn,

birds support key ecological functions, providing a wide range of ecosystem services (nutrient cycling, pest control, pollination) (Brewer, 1967; Deng & Yimam, 2020). Moreover, wetlands in general and peatlands in particular help stabilise bird communities, consolidating the ecosystem services they provide and thereby positively impacting agricultural systems. As such, farms established in mosaic landscapes with seminatural and natural wetland ecosystems are less affected by the disservices caused by bird species traditionally considered agricultural pests (Smith et al., 2022). Some flocking species (starlings, crows) are replaced by taxa of higher conservation value (Olimpi et al., 2024), peatland bird species that help reduce crop damage and pre-harvest food safety risks (Smith et al., 2025). Since natural and semi-natural habitat islands can positively influence the farmland matrix through their bird communities (Anderle et al., 2025), protecting such areas is of high importance for preserving regional avian diversity and agricultural welfare. Furthermore, protected area networks, such as Natura2000, highlight the value of habitat-specific species as indicators of biodiversity and ecosystem services change under land-use and climate pressures (Bastian, 2013).

Within this larger framework, our study aims to explore potential outcomes for the agricultural landscape matrix of three peatland areas that network as avian stepping stones between larger conservation areas. To achieve this, we assessed the distribution of bird communities across these peatlands in NW Romania, considering several variables: (i) anthropogenic pressures, (ii) habitat heterogeneity, (iii) representative peatland plant species, (iv) water availability, and (v) land use and proximity to rural/urban areas.

MATERIALS AND METHODS

Study area

The avian and habitat surveys were carried out during the restoration project PEATRO4 (*Restoration of degraded bogs and peatlands in the North West region of Romania*), in three small peatlands with various degrees of

degradation, located in Cluj County, each assigned an identification code: one within Călățele Commune CJ-015 Molhașul Călățele (46°43'43"N, 23°01'15"E) and two within Beliș Commune CJ-020 Dâmbu Negru - Între Drumuri (46°40'52"N, 23°00'51"E) and CJ-022 Negrușul Finciului - Rovina Mare (46°40'42"N, 22°58'20"E) (Figure 1).

Situated at the crossroads of three major avian migration routes across Romania, corridors III and IV – the central-European-Bulgarian and corridor X – the Russian-Adriatic migratory route (Costică, 2011), the surveyed peatlands network as stepping stones between Natura 2000 conservation areas.

Two of the selected sites (Molhașul Călățele and Dâmbu Negru-Între Drumuri) are located outside several designated protected areas, with partially overlapping limits: Parcul Natural Apuseni, Natura 2000 ROSCI0002 Apuseni (Site of Community Interest), Natura 2000 ROSAC0002 Apuseni (Special Area of Conservation) and ROSPA0081 Munții Apuseni-Vlădeasa (Avifaunistic Special Protected Area). Negrușul Finciului-Rovina Mare (CJ-022) is the only peatland located entirely within protected areas, specifically inside the Apuseni Mountains Natural Park Complex (Table 1).

Table 1. Geographic details of the studied peatlands and distance to nearest protected areas

Peatland code	Area (ha)	Altitude (m a.s.l.)	Distance to the border of the protected area		Position relative to the protected area
			ROSPA 0081 Munții Apuseni-Vlădeasa	ROSAC 0002 Apuseni	
CJ-015	3.6	912	3800	3800	exterior
CJ-020	2.32	1122	236	73	exterior
CJ-022	2.08	1089	39	39	interior

Sites evaluation and avian surveys

Data on seasonal variations for avian communities and plant dynamics were obtained through habitat assessment and avian surveys. Both were conducted simultaneously during several outings and covered the spring-to-autumn phenological aspects of 2023, with an additional phase in April 2024 to confirm for late spring migration.

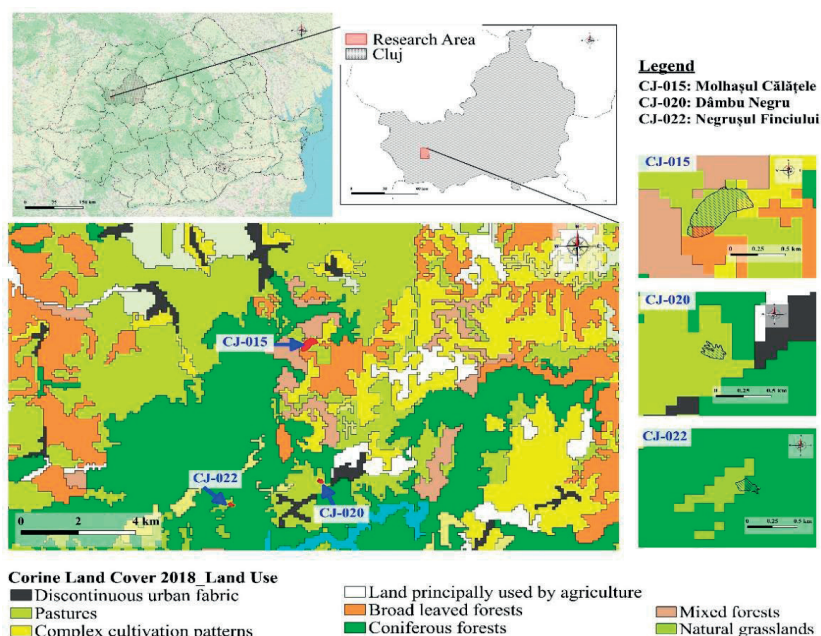


Figure 1. Location and land use for the targeted peatlands, Cluj County, Romania



Figure 2. Drone view from above Dâmbu Negru peatland

For the bird species census, we combined point counts and line transects (Bibby et al., 2000; Sutherland et al., 2004). For each peatland, we established at least five observation points and connected them with transects to ensure a comprehensive species inventory across the entire area. Avian species presence would be recorded 10 minutes/point. For transects, surveys were done every 500 m, with birds detected within 50 m on either side of the transect line recorded to minimise the likelihood of double-counting individuals in adjacent habitats. Counts were done with 10x50 Nikon binoculars, and acoustic recognition and camera

traps were used to confirm the presence of more cryptic species. Photographic documentation completed the ornithological assessment.

For habitat assessment, an initial drone survey was conducted over each site to obtain an overview of site configuration and vegetation structure (Figure 2). The aerial images helped to identify microtopographic features and guided the placement of transects.

Vegetation surveys were then conducted, and based on floristic data, plant communities were classified using the EUNIS habitat classification system (level 3) (EEA, 2012); when this was not possible, the relevant Natura 2000 type was used (Doniță et al., 2005). For each identified community, cover values of the vegetation layers were estimated. Plant species nomenclature follows POWO (2025).

Data analysis

Avian species richness and phenology patterns were processed and illustrated in Excel. To illustrate associations between bird species and key habitat variables, a focused CCA was used as an exploratory tool to visualise potential relationships under chi-square distance, within the constraints of the current model, following Gunnarson et al. (2006). The environmental variables measured on continuous scales were

subjected to z-standardisation (standardised to a mean of 0 and unit variance), prior to analysis to prevent differences in measurement units from influencing ordination results. Binary categorical variables were left untransformed. Species data (presence – ‘absence’/namely species not detected during survey effort) were not standardised, as CCA is based on chi-square distances and weighted averaging. (Legendre et al., 2011). For a more stable p-value estimation in accordance with our sample size, the statistical significance of the CCA model was assessed using a Monte Carlo permutation test with 4,999 unrestricted permutations. CCA analysis was run in PAST (Hammer et al., 2001). Bird guild dots were subsequently overlaid with detailed versions in Canva for illustrative purposes.

RESULTS AND DISCUSSIONS

Habitat assessment

The three study sites display a heterogeneous mosaic of habitats (Figure 3), including open *Sphagnum* bogs, *Carex* fens, shrub communities, conifer woodlands, and heath formations. These correspond to several EUNIS and Natura 2000 habitat types, such as D1.1 Raised bogs, 7120 Degraded raised bogs or 91D0* Bog woodland (Table 2).

Molhașul Călățele (CJ-15), described as an authentic active raised bog (Pop, 1947; 1960), was deeply affected by peat extraction carried out at the site between 1951 and 1989 and, more recently, by waste dumping related to the construction of the access road. Thus, the original ecosystem was disturbed, leading to surface drying and the loss or change of characteristic algal (Momeu et al., 2003) or bryophyte (Andrea et al., 2025) species, a phenomenon we also observed in vascular plants. Today, most of the site is occupied by *Betula* woodland, with variable coverage, classified as Natura 2000 habitat 7120 – Degraded raised bogs still capable of natural regeneration. The banks of the former exploitation are still visible and are currently undergoing regeneration, during which a characteristic vegetation of oligotrophic peatland has re-established: *Eriophorum vaginatum*, *Sphagnum* sp., *Drosera rotundifolia*

in wetter areas, and *Calluna vulgaris* in the drier portions. In the lower parts of the site, excavation ponds dominated by Cyperaceae species (*Carex rostrata*, *Carex echinata*, among the most common) have formed, along with small patches with *Typha* beds.

Table 2. Main habitat types for the studied peatlands

Site	Habitat		EUNIS/N2000 habitat type	Open water (0/1)
	ID	Plant community		
CJ-015	MC1	<i>Betula</i> swamp woodland	7120 Degraded raised bogs still capable of natural regeneration	0
	MC2	<i>Typha</i> beds	C3.23 - <i>Typha</i> beds	1
	MC3	<i>Carex</i> fen	D5.2 - Beds of large sedges normally without free-standing water	0
	MC4	<i>Eriophorum</i> lawns	D1.1112 - Bog cotton sedge-sphagnum lawns and green hummock bases	0
CJ-020	DN 1	open <i>Sphagnum</i> bog	D1.1 Raised bogs	0
	DN 2	<i>Molinia</i> & <i>Juncus</i> lawns	D2.2 <i>Carex nigra</i> , <i>Carex canescens</i> , <i>Carex echinata</i> fens	0
	DN3	<i>Carex</i> fen	D5.2 - Beds of large sedges normally without free-standing water	0
	DN4	<i>Salix</i> scrub	F9.2 - <i>Salix</i> carr and fen scrub	0
	DN5	<i>Picea</i> woodland	91D0*Bog woodland	0
CJ-022	NF1	<i>Picea</i> woodland	91D0*Bog woodland	0
	NF2	<i>Carex</i> fen	D5.2 - Beds of large sedges normally without free-standing water	0
	NF3	Springs	C2.1 Springs, spring brooks and geysers	1

Furthermore, Molhașul Călățele peatland has recently been proven home to rare bryophyte species such as *Sphagnum tenellum*, and confirms the presence of *Sphagnum papillosum* (Ștefănuț et al., 2026)

At **Dâmbu Negru-Între Drumuri** (CJ-020, due to the terrain, which features both inclines and flat areas, a mosaic of communities has developed. The largest area is covered by *Picea* woodland and *Salix* scrub, alternating with wet grasslands dominated by *Molinia caerulea*. The more oligotrophic zones are characterised by *Sphagnum* hummocks interspersed with *Vaccinium* spp. An outer lag or transition zone develops towards *Carex* fens in the more depressed areas, while the steeper and relatively drier slopes sustain dwarf shrub communities dominated by *Calluna vulgaris*.

The highest of the 3 study sites, **Negrușul Finciului**, is dominated by *Picea* bog woodland, with a lower vegetation layer composed of *Sphagnum* spp., *Vaccinium vitis-idaea* and *V. myrtillus*. The entire site is intersected by several springs, and a smaller area supports a Cyperaceae-dominated community.

Alongside structural heterogeneity, land use framing showcases the same diversity, ranging from 2 peatlands dominated and surrounded by agricultural land management (Dâmbu Negru and Molhașul Călățele) to one with little to no human management, due to its protected status (Negrușul Finciului) (Figure 3).

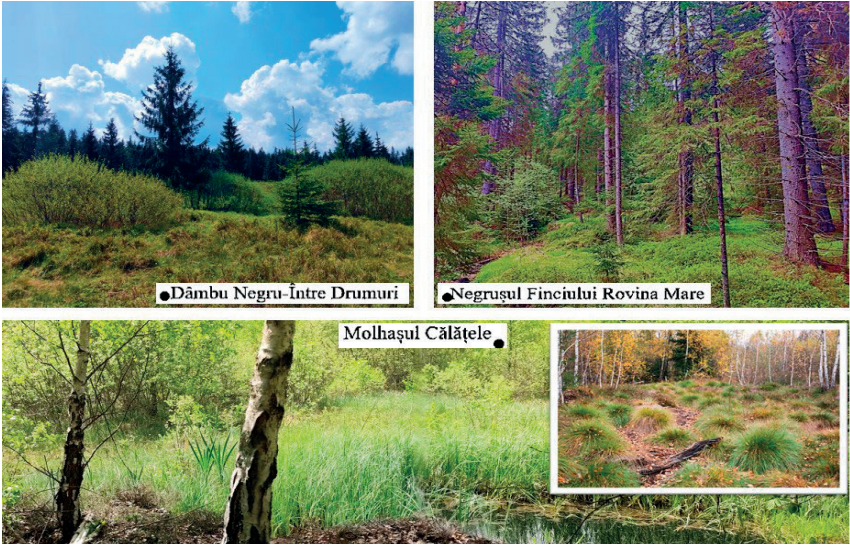


Figure 3. Habitat aspects and plant communities from the three study sites

Avian species richness and distribution

During the field surveys, a total of 39 avian taxa belonging to 17 different families (Table 3) were identified across the three studied peatlands. Out of these, three are considered Nearly Threatened at national level (*Accipiter gentilis*, *Clanga pomarina* and *Anthus trivialis*) and eight fall under the conservation guidelines of the European Birds Directive Annexe I (*Clanga pomarina*, *Falco tinnunculus*, *Dendrocopos leucotos*, *Dryocopus martius*, *Ficedula parva*, *Ficedula albicollis*, *Tetrao urogallus*, *Ciconia nigra*)

Bird communities across all sites differed in both richness and family-level composition, with insectivorous and omnivorous passerines, dominating the assemblages (flycatchers – fam. Muscicapidae at Dâmbu Negru and Molhașul Călățele and warblers – fam. Phylloscopidae for the peatland at Negrușul Finciului). Peatland CJ-020, Dâmbu Negru-Între Drumuri, presented the highest species richness (over 40% of the total avian richness value), also showcasing the most

varied taxonomic diversity at family level. By contrast, CJ-022 Negrușul Finciului counted the lowest value of taxonomic richness (Figure 5). In terms of phenology, spring migrants and summer avian taxa were more abundant in peatlands, attributed to agricultural land use frame (Figure 4).

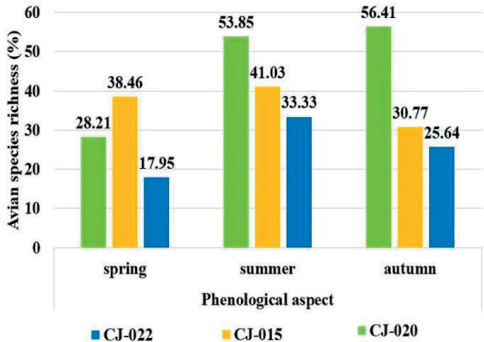


Figure 4. Phenological variability of avian species richness across 3 peatlands, Cluj, Romania

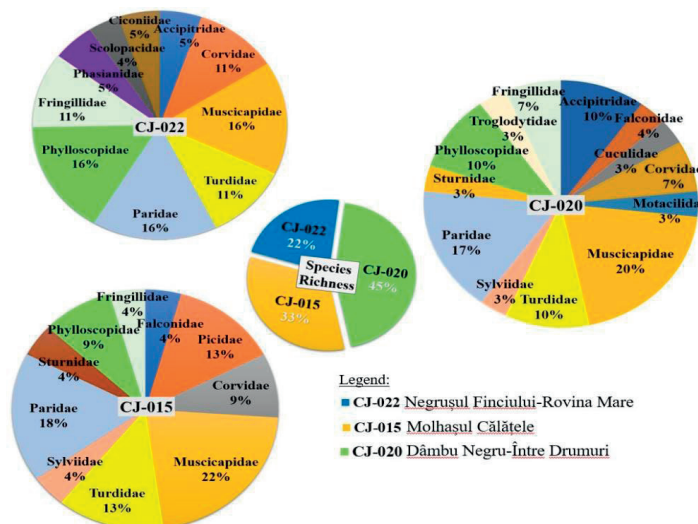


Figure 5. Avian species richness and percentage of taxonomic groups across three peatlands, Cluj, Romania

The CCA ordination (Figure 6) supports and expands on the field survey results. Variables along axis CCA2 have a stronger explanatory weight for avian guild distribution (eigenvalue=0.340). Avian community structure at Negrușul Finciului Rovina Mare (CJ-022) tends to align with the second axis CCA1, therefore it is 30% explained by habitat features (eigenvalue=0.256).

However, guild distribution is ultimately explained by the cumulative constrained variation of the two canonical axes (cumulated eigenvalue=0.595), therefore almost 60% of the matrix for bird species distribution occurs at the intersection of peatland habitat characteristics and anthropogenic variables (ranging from pollution and habitat conversion to agricultural practices).

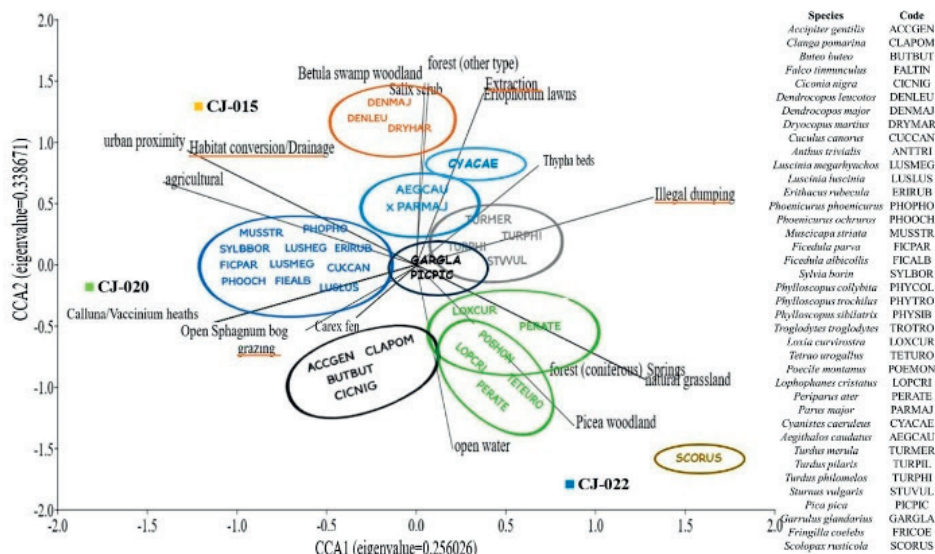


Figure 6. Canonical Correspondence Analysis (CCA triplot; scaling Type 2, amplified. Trace $p = 0.595$, p -value < 0.05) suggesting relationships between avian species composition and habitat variables across three peatland ecosystems, in Cluj, Romania. Bird species with standardised 6-letters alpha code, guild grouped on Eltonian traits – functional ecology grouping based on foraging attributes.

Wetland conservation value and agricultural implications.

The bird species richness and diversity in agricultural areas tend to be determined by the type of habitats in the surrounding area (Yaneva et al., 2020). Since species diversity in restored peatland areas tends to increase with heterogeneity in environmental conditions (Convention on Wetlands, 2021), avian species diversity across the 3 sites is an expression of the different land use categories for each particular site. Shrub coverage, humidity and higher soil moisture favour certain edaphic insect groups (Manu et al., 2020; Niculescu & Mitrea, 2018), offering foraging opportunities for numerous insectivorous birds.

At Dâmbu-Negru and Molhașul Călățele, peatland habitat heterogeneity is influenced by both habitat mosaic features and land use classification, supporting a higher variety of bird species. However, the **Molhașul Călățele** area is marked by habitat conversion, through drainage (CCA1 eigenvalue=0.62217) and overspread presence of birch tree *Betula* sp. This can lead to temperature rises and increased numbers of crop pests (Ma et al., 2025), while there is moderate bird richness and no significant diversity increase for pest control avian taxa (CCA2 eigenvalue for thrushes – 0.17312 and even lower for tits (eigenvalue=0.0207734) or specialised insectivorous species (CCA2 eigenvalue=0.0147887). Likewise, for the same peatland, considerable illegal dumping of construction and excavation waste (CCA2 eigenvalue=0.880171) favours the presence of avian taxa considered traditionally agricultural pests (generalist omnivores like starlings *Sturnus vulgaris* and corvids). Habitat restoration would moderate climate micro-regulation (Convention on Wetlands, 2025) and uphold the presence and diversity of agriculturally friendly bird taxa (Figure 7 – B), which in turn can boost productivity through pest control. (Díaz-Sieffer et al., 2021; Smith et al., 2022). This is particularly relevant for the area since land use classes at CJ-015 map broad leaf forests and pastures and are tangent to rural agricultural areas (the urban fabric of Călățele commune and adjacent complex cultivation patterns – Corine Land Cover, 2018).

CJ-020 Dâmbu-Negru is marginally under the pressure of local livestock management (CLC land use class – pastures and eigenvalue of EIONET A09 pressure grazing = 0.336191). However, peatland habitat heterogeneity supports the highest avian species diversity of all sample areas, noticeably diverse forest insectivores (flycatchers, three warbler families, bush birds such as wrens *Troglodytes troglodytes*, nightingales, thrushes – Figure 3). Diversity of tit species mirrors the habitat matrix and foraging opportunities of the area and can have implications for local pest caterpillar suppression in orchards, consequently increasing fruit yield. (Molls & Viser, 2002). In the case of raptors, with the exception of *Clanga pomarina* recorded at Negrușul Finciului during passage, raptor species presence was also dominant at Dâmbu-Negru.

This can be partially explained by cumulative eigenvalues for variables such as coniferous forest (*Picea* woodland habitat type), open water sources and *Carex* fen – *Sphagnum* sp. – *Vaccinium/Calluna* heath mosaic habitat (resulting value for CCA1+CCA2 = 0.671131). Raptor presence, like the ones identified (goshawks, buzzards and kestrels), was proven to reduce costs in agriculture by pest control in farmland mosaics (Machar et al., 2017)

Lastly, **Negrușul Finciului** peatland displays the characteristic features of a raised bog with coniferous forest, more isolated due to land use and protection status, and less diverse in terms of habitat matrix. As such, avian communities recorded at CJ-022 were more specific to certain habitat traits – wet woodland bird species like the woodcock *Scolopax rusticola*, game species of conservation importance falling under Annexe I, Birds' Directive, like the Western Capercaillie *Tetrao urogallus* (Figure 7) or coniferous patches associated species such as crossbills *Loxia curvirostra*. Last but not least, the area also recorded several particular species with the potential to offer agricultural regulating services during migration or dispersal (Smith et al., 2022). As such, willow and crested tits – *Poecile montanus*, *Lophophanes cristatus*, may contribute to caterpillar and other insect population control, while big waders like the black stork *Ciconia nigra* may help keep invertebrate numbers in check (Korányi, 2025).

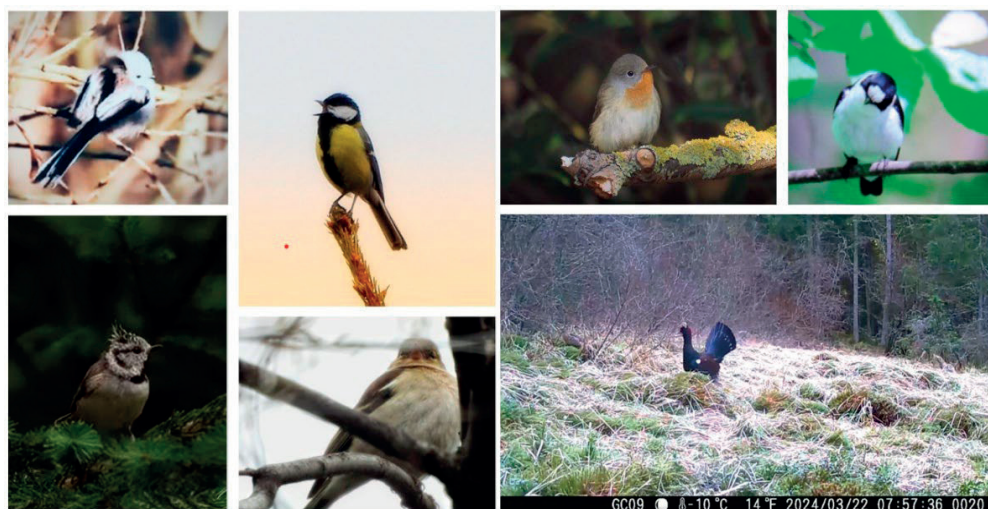


Figure 7. Selection of bird species from the three peatlands in Cluj, Romania relevant to conservation (right corner, down): *Tetrao urogallus* on camera trap at CJ-022; agriculture: insectivore bird taxa (upper row): *Aegitalos caudatus* – CJ-015, *Parus major* – all three areas, *Ficedula parva* – CJ-015/020 (Annexe 1, Birds Directive), *Ficedula albicollis* CJ-020 (Annexe 1 species); (lower row): *Fringilla coelebs* – all three areas & *Lophophanes cristatus*– CJ-022

Table 3. Avian species identified in three peatlands in NW Romania and their conservation status

Avian species			Peatland			Conservation status		
						RL IUCN	B D A I	RL RO
Family	Scientific name	Common name	(CJ-020)	(CJ-015)	(CJ-022)			
Accipitridae	<i>Accipiter gentilis</i>	Northern Goshawk	x			LC		NT
	<i>Clanga pomarina</i>	Lesser Spotted Eagle	x		x	LC	x	NT
	<i>Buteo buteo</i>	Common Buzzard				LC		LC
Falconidae	<i>Falco tinnunculus</i>	Common Kestrel	x	x		LC	x	LC
Picidae	<i>Dendrocopos leucotos</i>	White-backed Woodpecker		x		LC	x	LC
	<i>Dryocopus martius</i>	Black Woodpecker		x		LC	x	LC
	<i>Dendrocopos major</i>	Great Spotted Woodpecker		x		LC	x	LC
Cuculidae	<i>Cuculus canorus</i>	Common Cuckoo	x			LC		LC
Corvidae	<i>Pica pica</i>	Eurasian Magpie	x	x	x	LC		LC
	<i>Garrulus glandarius</i>	Eurasian Jay	x	x	x	LC		LC
Motacilidae	<i>Anthus trivialis</i>	Tree Pipit	x			LC		NT
Muscicapidae	<i>Luscinia megarhynchos</i>	Common Nightingale	x		x	LC		LC
	<i>Luscinia luscinia</i>	Thrush Nightingale		x		LC		LC
	<i>Erithacus rubecula</i>	European Robin	x	x	x	LC		LC
	<i>Phoenicurus phoenicurus</i>	Common Redstart	x	x		LC		LC
	<i>Phoenicurus ochruros</i>	Black Redstart		x	x	LC		LC
	<i>Muscicapa striata</i>	Spotted Flycatcher	x			LC		LC
	<i>Ficedula parva</i>	Red-breasted Flycatcher	x	x		LC	x	LC
Turdidae	<i>Ficedula albicollis</i>	Collared Flycatcher	x			LC	x	LC
	<i>Turdus merula</i>	Common Blackbird	x	x	x	LC		LC
	<i>Turdus pilaris</i>	Fieldfare	x	x	x	LC		LC
Sylviidae	<i>Turdus philomelos</i>	Song Thrush	x	x		LC		LC
	<i>Sylvia borin</i>	Garden Warbler	x	x		LC		LC
Paridae	<i>Parus major</i>	Great Tit	x	x	x	LC		LC
	<i>Periparus ater</i>	Coal Tit	x		x	LC		LC
	<i>Cyanistes caeruleus</i>	Eurasian Blue Tit	x	x	x	LC		LC
	<i>Poecile montanus</i>	Willow Tit	x	x		LC		LC
	<i>Lophophanes cristatus</i>	Crested Tit	x			LC		LC

Avian species			Peatland			Conservation status		
Family	Scientific name	Common name	(CJ-020)	(CJ-015)	(CJ-022)	RL IUCN	B D A I	RL RO
	<i>Aegialos caudatus</i>	Long-tailed Tit		x		LC		LC
Sturnidae	<i>Sturnus vulgaris</i>	Common Starling	x	x		LC		LC
Phylloscopidae	<i>Phylloscopus collybita</i>	Common Chiffchaff	x	x	x	LC		LC
	<i>Phylloscopus trochilus</i>	Willow Warbler	x	x	x	LC		LC
	<i>Phylloscopus sibilatrix</i>	Wood Warbler	x		x	LC		LC
Troglodytidae	<i>Troglodytes troglodytes</i>	Eurasian Wren	x			LC		LC
Fringillidae	<i>Loxia curvirostra</i>	Red Crossbill	x		x	LC		LC
	<i>Fringilla coelebs</i>	Common Chaffinch	x	x	x	LC		LC
Phasianidae	<i>Tetrao urogallus</i>	Western Capercaillie			x	LC	x	LC
Scolopacidae	<i>Scolopax rusticola</i>	Eurasian Woodcock			x	LC		LC
Ciconiidae	<i>Ciconia nigra</i>	Black Stork			x	LC	x	LC

Legend: RL IUCN – The International Union for Conservation of Nature Red List of Threatened Species (LC – Least Concern), BDAI –European Birds’ Directive Annex I, RORL Romanian Red species list (NT – Near Threatened) x – mark for species presence. Species protection status sources: Atlas al speciilor de păsări de interes comunitar din România, (SOR, 2022), Romanian Red List for Bird Species (1991, Monitorul Oficial, online - <https://www.sor.ro/wp-content/uploads/1991/01/Lista-Rosie-Monitorul-Oficial-Partea-I-nr.-798Bis.pdf>)

CONCLUSIONS

Temperate peatlands construct into vulnerable ecosystems requiring monitoring and integrated restoration approaches in order to maintain their ecosystem services that go beyond carbon trapping.

Two of the three sites sampled for this study displayed higher heterogeneity of the habitat matrix and bird species composition due to differences in land use classification and variance in the shape and amplitude of anthropogenic pressures.

The presence of Annexe I avian taxa and Near Threatened bird species confirms the conservation value of small-sized peatlands as stepping stones, particularly between larger Natura 2000 protected areas.

Furthermore, our pilot assessment supports the idea that sustainable agriculture stems from nature-based solutions.

This can lead to mutually beneficial cooperative associations with avian guilds from peatland areas embedded in the farmland matrix.

Understanding bird communities and their dynamics in these habitats becomes vital to further conservation policies.

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***Onobrychis arenaria* (Kit.) DC. - PROSPECTS FOR ITS USE AS A MELLIFEROUS PLANT**

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Abstract

The present study was based on the investigation of the biological characteristics of growth and development of Onobrychis arenaria (Kit.) DC. under the climatic conditions of the Republic of Moldova, with emphasis on its potential use as a melliferous plant. The research was conducted on the experimental plots of the “Alexandru Ciubotaru” National Botanical Garden (Institute). O. arenaria belongs to the family Fabaceae and is a perennial, long-day, heliophilous, polycarpic species. The onset of growth occurs early. The period from the start of the growing season to flowering is approximately 67 days, while seed maturation takes 103-110 days. The flowers are arranged in elongated racemes, comprising 20-100 flowers per inflorescence. Flowering is staggered and prolonged, lasting approximately 20-30 days, depending on plant age and prevailing climatic conditions. Onobrychis arenaria exhibits high melliferous potential, providing nectar and pollen resources for a wide spectrum of insects over an extended period.

Key words: *Onobrychis arenaria* (Kit.) DC., development, insects.

INTRODUCTION

The main food source for insects is the nectar and pollen produced by plants. The melliferous base varies depending on the geographical area and the pedoclimatic conditions characteristic of the region. The quantity of nectar produced by flowers depends on several factors, such as soil, climate, agrotechnical practices etc. (Gluhov, 1973). For example, high temperatures can reduce nectar secretion, heavy rainfall can dilute or wash it away, but moderate rainfall tends to increase nectar production. Since ancient times, honey has been valued not only as food but also for its medicinal properties and energizing effects. Today, it is recognized as a healthy alternative, rich in bioactive compounds that benefit overall health (Ionc, 2024). In the Republic of Moldova, 95% of the beekeeping sector is privately owned. Large producers, which are few in number, typically manage around 400 beehives each. The average local honey production is 24 kg per beehive annually (Programul national de dezvoltare a apiculturii în Republica Moldova pentru 2020-2026). Forecasting the flowering of melliferous crops is based on the flowering period of each botanical species, taking into account the

accumulation of the effective temperature sum during the period from the start of the growing season to the peak flowering of each crop. This heat sum is determined based on an extensive study (Sidarenko, 2015). The same melliferous flower does not always and everywhere produce the same amount of nectar, meaning that the same species may be a highly production honey plant if grown in one area and not productive at all melliferous in another. Studies have shown that to produce 500 grams of honey, bees must make at least 25,000 to 30,000 flights. This immense effort of honeybees is not immediately visible in the field but becomes evident later, in the barns of farmers (Sidarenko, et al, 2010).

O. arenaria is known for its high nectar productivity, flowering earlier than many melliferous crops. It is highly attractive to bees, and its honey has therapeutic properties. Some researchers documented its melliferous productivity, noting that each flower produces about 0.07 mg of nectar and 0.075 mg of pollen. Each inflorescence yields 5.2 mg of pollen, and one plant provides around 262 mg. Under favourable conditions, 15-20 bees can work per square meter planted with sand sainfoin (Atoeva & Olimova, 2024).

The honey productivity of sand sainfoin (*O. arenaria*) can reach up to 225 kg/ha (Pancov, 2009). Other sources indicate that in the second year of growth, honey productivity is around 60 kg/ha, while in the third year, it can increase to as much as 300 kg/ha (Atoeva & Olimova, 2024). Sand sainfoin is valued for its exceptional melliferous qualities, especially in herbaceous pasture mixtures. Pastures provide low- to medium-intensity forage, with peak development in late summer, after the flowering of linden trees and sunflowers, when other forage sources are scarce. (<https://www.scribd.com/doc/77287247/BAZA-MELIFER%C4%82>).

Onobrychis arenaria (Kit.) DC. – sand sainfoin or sandy esparcet – belongs to the genus *Onobrychis* Mill. (family Fabaceae Lindl.) In the Flora Europaea lists, 80 plant species of this genus are described (Kazuharova & Bendassat, 2019), most of them being recognized as forage for grazing animals and honey plants (Mossadegh, 1990; Manning, 2001). In SW Asia, the Mediterranean region and in temperate Europe and Asia, this genus comprises about 170 species (Zohary, 1987). In Armenia, there are records of sainfoin dating back to 1000 BC, and the cultivation of the plant in Armenia and Georgia is described in the 5th century. In Western Europe, it has been known for about 600 years (Şain, 1948; Carasciuc, 1951; Medvedev & Smetannicova, 1981). *O. arenaria* is a tall plant, reaching 30-50 (80) cm in height. The stem is erect, branched, pubescent or nearly glabrous. The stipules are brown, ovate, and acute. The leaves are petiolate, with 5-12 pairs of elliptical leaflets, 10-30 mm long and 2-5 mm wide. The racemes have peduncles 3-4 times longer than the leaves. The calyx is glabrous or pubescent, the corolla is violet-pink, and the pods are sparsely pubescent, 4-6 mm long, with short spines on the surfaces and ridge (Flora Basarabiei, 2020).

MATERIALS AND METHODS

The focus of the study was the plants of the species *Onobrychis arenaria* (Kit.) DC. from the collection of Forage, Honey and Energy Plants at the Laboratory of Plant Resources within the “Alexandru Ciubotaru” National

Botanical Garden (Institute) (NBGI). NBGI is located in the southeastern part of Chişinău, where over 24 types of soil are found, including fertile chernozems. The groundwater is located at depths of 1.5-7.0 meters. The research was carried out during the 2023-2025 growing seasons. The basis of the research was the study of the biological characteristics of growth and development of the plants under the climatic conditions of the Republic of Moldova, conducted according to the methodological guidelines used in NBGI (Novosiolov, 1997). The phenological study was conducted throughout the entire growing season of the plants, from the start of vegetative growth to seed maturation (Beideman, 1974). The phenological data were correlated with the meteorological conditions.

The summer of 2025 was characterized by high temperatures and a significant precipitation deficit. During the season, the total precipitation in the central part of the country ranged from 16 to 60 mm, with a notable lack of rainfall in June and August. In comparison to the summer of 2024, the average air temperature in 2025 was 1.5-2.5°C lower. A similar thermal regime was observed in 2023. The period of research, based on climatic conditions, was marked by high temperatures and a precipitation deficit (Figure 1).

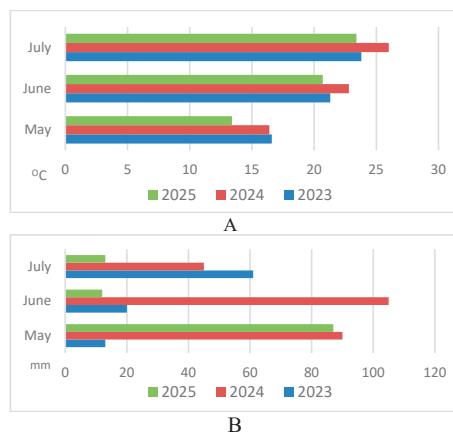


Figure 1. Climatic conditions:
A - air temperature; B - precipitation

The maximum air temperature during the 2024 season reached +39.5°C to +40.9°C, such extreme values being only recorded in 2007 and 2012. A significant precipitation deficit

was noted across almost the entire country in May, July and August. (<https://www.meteo.md/>). The entomological study was conducted during the growing season of the plants, particularly during the generative phase. The list of beneficial insects was updated. The entomological material was studied from spring to autumn, starting from the budding phase of the plants until the end of flowering. Species identification and systematic classification were carried out according to the identification guides: Kryzhanovsky, 1965, Plavilshchikov, 1994.

RESULTS AND DISCUSSIONS

To improve and develop the melliferous base as a branch of beekeeping, it is essential to integrate knowledge about plant morphology, physiology, ecology, phytogeography, phytocenology, breeding and phytotechnology, dendrology, phytophenology, and entomology (Iordache, Roșca, & Cismaru, 2007). Leguminous forages are an important category of plants for sustainable agriculture and beekeeping, being species with ecological plasticity, high nutritional value and vitality, which also influence soil fertilization. Sand sainfoin is part of this group of plants, widespread in steppe and forest-steppe areas. It is a perennial plant with a strong taproot. The aerial part forms a bushy growth with ascending shoots, topped with inflorescences. *O. arenaria* plants, under the climatic conditions of the Republic of Moldova, on the experimental plots of the “Alexandru Ciubotaru” National Botanical Garden (Institute), go through the entire ontogenetic cycle annually, flowering and producing viable seeds starting from the first year of life. It is a long-day, light-loving, perennial, polycarpic species, and under favourable conditions, it can flower and produce seeds twice within one growing season. It belongs to the group of leguminous plants with medium development pace and vitality, with a lifespan of 5-10 years, and is considered early in terms of precocity. Phenological research was conducted on the *O. arenaria* plants growing in the collection, as well as on plants in their first year of life (Figure 2A). Seed incorporation into the soil was performed in early April, when recorded

air temperatures ranged from +13.0°C to +15.5°C.



Figure 2. *O. arenaria* in the collection of NBGI:
A - leaf development vegetative stage,
B - budding-flowering generative stage

The germination capacity of the seeds under laboratory conditions was $68.2 \pm 9.03\%$. In the first year of growth, 21 days passed from seed incorporation into the soil to the onset of vegetation, and approximately 75-80 days passed until peak flowering. Flowering (Figures 2B, 3B) is staggered and prolonged, lasting about 20-30 days, and occurs between May and July. Within a single inflorescence, both buds and flowers develop simultaneously, and in some specimens, fruits begin to form at the base of the inflorescence (Figure 3A).



Figure 3. *O. arenaria* inflorescences: A - at different development stages (number of inflorescences on a shoot); B - *Apis mellifera* visiting flowers

The flowers are arranged in elongated racemose inflorescences, each containing 20-100 individual flowers. These inflorescences exhibit acropetal flowering, with the flowers opening sequentially from the base to the apex of the raceme. The length of the inflorescences

ranges from 5 to 9 cm, and when including the floral peduncle, the total length extends to 13-18 cm. Each inflorescence remains in bloom for 8-12 days. The period from the start of vegetative growth to seed maturity is approximately 103-110 days. Beginning in the second year of growth, the plants initiate early vegetative development, typically during mid- to late March, marked by the formation of a leaf rosette. Following this, the plants experience rapid growth, producing green biomass and accumulating essential nutrients. Under optimal conditions, they grow vigorously and exhibit resilience to low spring temperatures. When exposed to similar climatic conditions, the duration of both the vegetative and reproductive phases is nearly identical. By late July, during the fruit development and seed ripening phases, new shoots may emerge.

These shoots are smaller in size, and some may progress into the reproductive phase by the end of the growing season, producing smaller inflorescences. At the end of the growing season, following seed maturation and the decline in air temperatures, the aboveground parts of the plants enter dormancy. The leaves reach the senescence stage and fall, while the stems become desiccated and turn brown. The plants' frost resistance and tolerance to winter drought are primarily attributed to the basal buds and the root system. The timing and duration of the phenological phases (Table 1) can vary depending on plant age and environmental factors, and the temperate climate conditions of the Republic of Moldova support plant growth and the production of viable seeds.

Table 1. Phenological spectrum of *O. arenaria* Kit. (DC.) plants

Month			March			April			May			June			July		
Year	Sowing	Emergence SVG	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
2023	-	17.03			+	+	+	v	v	v	v	v	v	v			
2024	-	15.03		+	+	+	+	v	v	v	v	v	v	v	v		
2025	02.04	23.04						+	+	+	+	v	v	v	v	v	v

Note: SVG – start of vegetative growth; I, II, III – 10-day period of the month,

The flower structure of sand sainfoin complicates self-pollination, as it is an entomophilous species. Seed production is influenced by the abundance of insect visitors to the flowers (Kempf et al., 2015). Pollination in sand sainfoin is mediated by various insect species, with the honeybee (*Apis mellifera*) accounting for approximately 80% of pollination events (Pancov, 2011). Entomophily is essential for the long-term survival of the species, reflecting the close ecological relationship between entomophilous plants and their pollinators (Mantoptin et al., 2001). Previous and current research has shown a high abundance of diverse pollinating insect species during the flowering period of sand sainfoin. These insects are classified within the trophic chain as primarily phytophagous species that feed on pollen, nectar and other plant fluids, as well as zoophagous species. In experimental plots at the NGBI, 15 insect species were identified on *O. arenaria* plants (*A. mellifera*; *Bombus terrestris*, *B. lapidarius*, *B. hortorum*, *Lasioglossum* sp., *Formica rufa*, *F. nigra*,

Cantharis fusca, *Coccinella septempunctata*, *Agapanthia violacea*, *Oxythyrea funesta*, *Dolycoris baccarum*, *Pyrrhocoris apterus*, *Aphis fabae*, *Polyommatus* sp., classified in four orders (Hymenoptera, Coleoptera, Hemiptera, Lepidoptera), and eleven families (Aphididae, Apidae, Cantharidae, Coccinellidae, Cerambycidae, Pantatomidae, Pyrrhocoridae, Scarabaeidae, Lycaenidae, Formicidae, Halictidae (Cîrlig et al., 2021). Hymenoptera represent 50% of the identified entomofaunal population and play a crucial role in the plant world, as both honey-producing and pollinating insects. Bees gather nectar and pollen from flowers, which they then convert into food sources – honey and bee bread – providing the essential nutrients necessary for their metabolic processes (Burenin & Kotova, 1977). The researcher Gluhov, 1937 highlights sand sainfoin as a crop of significant value for beekeeping, noting that its flowers provide a substantial amount of nectar to bees, irrespective of climatic conditions. A healthy bee colony can produce over 4 kg of honey per

day. The honey is aromatic, has a delicate taste, is transparent and does not crystallize quickly. The insect species identified are active throughout the day during the entire flowering period of *O. arenaria*. Since flowering is staggered, insect activity extends over a longer period. Some researchers suggest that for optimal seed yield in sainfoin, pollination by honeybees should be managed (Ivanov, Peresadin & Šilo, 2017). One key factor for this is placing hives closer to the sainfoin fields, increasing visitation by domestic bees and, consequently, boosting both seed and honey production. It is recommended to have 3-4 bee colonies per hectare, with hives spaced 650-700 meters apart (Starodub, 2015). The research highlights the importance of *O. arenaria* plants in both beekeeping and agriculture. These plants can be utilized to establish monoculture plantations for honey production and can also enhance the floral diversity of natural or artificial pastures, providing both forage and melliferous resources.

CONCLUSIONS

Under the climatic conditions of the Republic of Moldova, *Onobrychis arenaria* (Kit.) DC. can be considered a multipurpose species with high melliferous potential, characterized by a long flowering period allowing its integration into the 'honey conveyor'. The studies conducted at the "Alexandru Ciubotaru" National Botanical Garden (Institute) of MSU confirmed that sand sainfoin is a perennial, long-day, heliophilic, polycarpic species. In its first year of life, the time from seed incorporation into the soil to the start of vegetative growth is approximately 21 days, with peak flowering occurring about 75-80 days later. Flowering is staggered and prolonged, lasting 20-30 days between May and July, with each individual inflorescence blooming for 8-12 days. The period from the start of vegetative growth to full seed maturity spans 103-110 days. During the flowering phase, the plants provide nectar and pollen to a variety of insects over an extended period. The presence of melliferous and pollinating species, such as *Apis mellifera* and *Bombus* spp., underscores the melliferous

potential of *O. arenaria* plants and highlights the availability of nectar and pollen, which serve as essential food sources for these insects.

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THE ENERGY POTENTIAL OF CROP RESIDUE BIOMASS

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Abstract

Crop residues represent an abundant and underutilized source of renewable energy, offering a sustainable alternative to fossil fuels. This study assessed the physicochemical properties and bioenergy potential of post-harvest residues from *Helianthus annuus*, *Zea mays*, *Sorghum* sp., *Fagopyrum esculentum*, *Silybum marianum* and *Rumex patientia* × *Rumex tianschanicus*, to evaluate their suitability for biogas/biomethane, bioethanol, and solid biofuel production. The residues contained 32-89 g/kg crude protein, 69-112 g/kg acid detergent lignin, 348-507 g/kg cellulose, and 208-292 g/kg hemicellulose. Biochemical biomethane potential ranged from 213 to 287 L/kg of dry organic matter, while theoretical ethanol yields reached 404-562 L/t. Net calorific values were 15.33-16.97 MJ/kg, demonstrating substantial energy content. These results highlight the versatility of crop residues as a renewable energy feedstock and support their strategic utilization for sustainable bioenergy production in the Republic of Moldova.

Key words: biochemical composition, biochemical methane potential, crop residues, quality indices, solid biofuel, theoretical ethanol yield.

INTRODUCTION

Agriculture is a cornerstone of the economy of the Republic of Moldova, providing food and raw materials for multiple industries. According to the National Bureau of Statistics, nearly half of the country's revenue derives from the export of agricultural and food products, with cereals and oilseeds accounting for approximately 40% of total exports.

Maize (*Zea mays*, *Poaceae*) has long been a traditional crop in Moldova. However, the total area planted with maize declined from 464,000 hectares in 2024 to approximately 300,000 hectares in 2025, largely due to adverse climatic conditions, including drought and heat waves, and rising costs of fertilizers and phytosanitary products. In response, sorghum (*Sorghum bicolor*, *Poaceae*) has gained renewed attention as a drought-tolerant alternative capable of thriving on saline-affected soils.

Sunflower (*Helianthus annuus*, *Asteraceae*) has become a leading technical crop in the Republic of Moldova, with cultivated areas reaching 414,000-418,500 hectares in 2024-2025 and annual production exceeding 800,000 tons. In addition to its economic value, sunflower is an important honey plant (Ion et al., 2018).

Similarly, milk thistle (*Silybum marianum*, *Asteraceae*) offers high-value applications in agriculture and pharmaceuticals. Its seeds, rich in silymarin, are in demand for liver-protective supplements and teas, and the plant also supports honey production with yields up to 200 kg/ha (Marceddu et al., 2022; Ion et al., 2018). Common buckwheat (*Fagopyrum esculentum*, *Polygonaceae*) has regained popularity as a nutritious, gluten-free crop with high-quality proteins, minerals, and bioactive compounds such as rutin, making it suitable for diabetic and celiac diets. It is an important honey plant. Also appreciable forage crops (Farooq et al., 2016; Heuzé et al., 2019; Țîței, 2023). *Rumex* species, including *Rumex patientia* L. and the hybrid *Rumex tianschanicus* × *Rumex patientia* (syn. *Rumex kioviensis*, *Polygonaceae*), are cultivated in Moldova, Ukraine, and Europe, China for greens, fodder, and high-biomass applications. These robust perennials exhibit rapid growth, early vegetation, and regrowth after seed and stem harvest, making them valuable for both food and bioenergy production (Țîței & Teleuță, 2015; Rolínek, 2018; Rakhmetov et al., 2024). Crop residues represent an abundant and underutilized source of feedstock for circular

economy and production energy, offering a sustainable alternative to fossil fuels.

Currently, crop residues from maize, sorghum, buckwheat, and sunflower are partially used for animal bedding and feed. Given the stagnation of the livestock sector, a significant proportion of these residues could be diverted toward renewable energy applications, providing a sustainable and secure source of biofuels, as renewable energy.

The main objective of this research was to evaluate the quality indices of crop residues remaining after seed harvesting from *Heliathus annuus*, *Zea mays*, *Sorghum* sp., *Fagopyrum esculentum*, *Silybum marianum*, *Rumex patientia* × *Rumex tianschanicus* as feedstock for the production of biogas/biomethane, bioethanol, and solid biofuels as renewable energy.

MATERIALS AND METHODS

Post-harvest crop residues from *Helianthus annuus*, *Zea mays*, *Sorghum* sp., *Fagopyrum esculentum*, *Silybum marianum* and *Rumex patientia* × *Rumex tianschanicus* were collected from experimental plots at the National Botanical Garden of Chişinău (NBGI). For each species, 100 kg of biomass was sampled. The biomass was initially chopped using a mechanical chopper and subsequently ground in a beater mill equipped with a 6 mm mesh. For analytical purposes, samples were oven-dried at 85°C, further ground to a particle size below 1 mm and thoroughly homogenized.

The chemical composition was assessed through the following indices: crude protein (CP), crude fiber (CF), crude ash (CA), acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL), measured using near-infrared spectroscopy (NIRS) on a PERTEN DA 7200 analyzer at the Research and Development Institute for Grassland, Braşov, Romania. Hemicellulose (HC) and cellulose (Cel) contents were calculated following standard procedures. The biochemical methane potential (BMP) was estimated according to Dandikas et al. (2015). The theoretical ethanol potential (TEP) was calculated based on the conversion of cellulose and hemicellulose into hexose and pentose sugars, following Goff et al. (2010).

The elemental composition (carbon (C), hydrogen (H), nitrogen (N), and sulphur (S))

was determined via dry combustion using a Vario Macro CHNS analyzer. The ash content was measured at 550°C in a muffle furnace (HT40AL) according to SM EN ISO 18122, and calorific values (higher and lower heating values) were determined using an automatic calorimeter following SM EN ISO 18125.

This comprehensive analysis allowed for the characterization of crop residues as potential feedstock for biogas/biomethane, bioethanol, and solid biofuel production.

RESULTS AND DISCUSSIONS

The estimation of crop residue availability for bioenergy production requires a comprehensive understanding of both dry matter content and biochemical composition. These parameters are of critical importance for the collection, storage and formulation of homogeneous substrates for biofuel production, as they directly influence processing efficiency, energy yield and feedstock handling characteristics.

At the time of harvest, the investigated crops exhibited relatively similar moisture contents in their stems. Specifically, the dry matter content varied across species as follows: *Fagopyrum esculentum*, 400 g/kg; *Rumex patientia* × *Rumex tianschanicus*, 794 g/kg; *Silybum marianum*, 624 g/kg; *Sorghum* sp., 485 g/kg; *Helianthus annuus*, 723 g/kg; and *Zea mays*, 684 g/kg.

The detailed biochemical composition of the studied crop residues, as summarized in Table 1, revealed wide interspecific variation. Crude protein (CP) ranged from 30 to 89 g/kg, ash from 34 to 132 g/kg, crude fiber (CF) from 408 to 560 g/kg, acid detergent fiber (ADF) from 407 to 501 g/kg, neutral detergent fiber (NDF) from 417 to 614 g/kg, acid detergent lignin (ADL) from 69 to 112 g/kg, cellulose (Cel) from 348 to 507 g/kg and hemicellulose (HC) from 208 to 292 g/kg of dry matter.

Among the species, *Fagopyrum esculentum* residues were distinguished by their relatively high crude protein content and comparatively low levels of structural cell wall components. In contrast, the crude protein concentrations in residues of *Silybum marianum* and *Sorghum* sp. were comparable to those of *Zea mays* stover and significantly higher than those observed in *Rumex patientia* × *Rumex tianschanicus* and *Helianthus annuus*, suggesting their potential as

more balanced feedstock in terms of protein availability.

Ash content was notably elevated in *Silybum marianum*, *Fagopyrum esculentum* and *Sorghum* sp. residues, likely reflecting the prolonged retention of leaves on stems prior to harvest. The distribution of fibrous components and lignin varied considerably among species. The lowest values of ADF, NDF and ADL were observed in *Fagopyrum esculentum*, followed by *Zea mays*, whereas *Silybum marianum* and *Helianthus annuus* exhibited the highest levels, indicating greater recalcitrance to biochemical conversion in these species.

Regarding polysaccharide composition, *Sorghum* straw contained higher cellulose and hemicellulose contents relative to *Zea mays*, *Rumex patientia* × *Rumex tianschanicus* and *Silybum marianum*, but remained lower than that of *Helianthus annuus* stover. This variation underscores the influence of species-specific structural traits on biomass degradability and energy yield potential.

Overall, these results highlight the substantial heterogeneity in dry matter content and biochemical composition among the examined crop residues, emphasizing the importance of species selection and substrate characterization in optimizing bioenergy production.

Table 1. The biochemical composition, biomethane and bioethanol potential of the studied crop residues

Indices	<i>Fagopyrum esculentum</i>	<i>Rumex patientia</i> × <i>Rumex tianschanicus</i>	<i>Silybum marianum</i>	<i>Sorghum</i> sp.	<i>Helianthus annuus</i>	<i>Zea mays</i>
Crude protein, g/kg DM	89	32	53	49	30	52
Crude fibre, g/kg DM	408	520	495	541	560	463
Minerals, g/kg DM	84	53	132	88	34	40
Acid detergent fibre, g/kg DM	417	517	583	583	614	499
Neutral detergent fibre, g/kg DM	625	757	861	875	877	749
Acid detergent lignin, g/kg DM	69	83	112	101	107	82
Cellulose, g/kg DM	348	434	471	482	507	417
Hemicellulose, g/kg DM	208	240	278	292	263	250
Biomethane potential, L/kg VS	287	255	215	236	213	260
Theoretical ethanol potential, L/t ODM	404	490	544	562	559	484
- from hexose sugars, L/t ODM	261	325	353	362	379	313
- from pentose sugars, L/t ODM	143	165	191	200	180	171

Several studies have characterized the biochemical composition of crop residues from the species investigated in this study, providing useful benchmarks for assessing their potential as bioenergy feedstocks. *Rumex patientia* × *Rumex tianschanicus* biomass was reported by Barbash et al. (2011) to contain 37.8% cellulose, 21.9% pentosans, 21.4% lignin and 3.5% ash.

Sunflower (*Helianthus annuus*) residues have been extensively studied. Mathias et al. (2015) further distinguished sunflower stem fractions, reporting 48% cellulose and 14% lignin in the stem bark versus 31.5% cellulose and 2.5% lignin in the stem pith. Yue et al. (2018) found sunflower stalks contained 37.8% cellulose, 17.1% hemicellulose, and 12.3% lignin. Heuze et al. (2015) reported the following indices for sunflower stover (stalks and heads) 758 g/kg dry matter, 5.7% CP, 1.5% EE, 48.1% CF, 66.9% NDF, 56.6% ADF, 15.7% lignin. Gholami-Yangije et al. (2019) reported sunflower stalks contained 2.26% crude protein (CP), 85.09% neutral detergent fiber (NDF), 72.72% acid

detergent fiber (ADF) and 15.25% acid detergent lignin (ADL). Halysh et al. (2016) found that sunflower stover had 36.2% cellulose, 25.2% hemicellulose, 14.1% lignin and 3.4% ash.

Buckwheat (*Fagopyrum esculentum*) residues were reported by Smuga-Kogut et al. (2019) to contain 42.08% cellulose, 8.38% hemicellulose, and 16.97% lignin, while Heuze et al. (2019b) noted the following indices of buckwheat residues 872g/kg dry matter, 4.6% CP, 1.2% EE, 50.4% CF, 73% NDF, 56% ADF, 10.7% lignin. Acar et al. (2015) reported similar compositions with 4.0% CP, 1.7% ether extract (EE), 49% ADF, 73% NDF, 10.7% lignin and 6.9% ash.

Maize (*Zea mays*) stover exhibits substantial variability in biochemical composition. Heuze et al. (2019a) reported the following indices for maize stover 928 g/kg dry matter, 2.9% CP, 0.9% EE, 40.7% CF, 75% NDF, 49.6% ADF, 7.4% lignin. Pattanaik (2019) reported cellulose content ranging from 31.3 to 49.4%, hemicellulose 21.1-26.2%, lignin 3.1-8.8%, CP

3.6-8.7%, lipids 0.3-1.3%, and ash 4.2–6.3%. Dell’Omo (2025) reported 487 g/kg ADF, 794 g/kg NDF, and 89 g/kg ADL in maize stover, while Khan et al. (2024) reported 41.43% cellulose, 26.10% hemicellulose, and 8.13% lignin in maize stalks. Li et al. (2014) found maize stover with 933.8 g/kg dry matter, 95.16% organic matter, 4.05% CP, 1.31% EE, 71.93% NDF, 41.36% ADF, and 6.16% ADL. Nasir and Kamaruddin (2023) determined that kernel maize stalk dry matter contained 4.09% CP, 0.47% fat, 2.22% ash, 27.23% crude fiber (CF), and 60.99% nitrogen-free extract (NFE), whereas sweet corn stalks contained 10.47% CP, 1.32% fat, 4.56% ash, 27.05% CF, and 51.42% NFE. Wattanaklang et al. (2016) reported maize stover with 5.8% CP, 27.38% CF, 1.90% EE, and 2.08% ash, and Zhou et al. (2019) observed sweet corn stover containing 72.3 g/kg CP, 18.1 g/kg EE, 134.1 g/kg water-soluble carbohydrates (WSC), 667.8 g/kg NDF, 426.3 g/kg ADF and 80.8 g/kg starch. Halysh et al. (2016) reported that maize stover had 41.2% cellulose, 20.3% hemicellulose, 20.7% lignin and 2.8% ash.

Collectively, these studies demonstrate significant interspecific and intraspecific variability in the biochemical composition of crop residues, reflecting differences in plant structure, harvest timing and tissue type. Anaerobic digestion is a microbial process in which organic compounds, including proteins, lipids, cellulose and hemicellulose, are converted by microorganisms such as methanogens and sulfate-reducing bacteria into biogas. Biogas primarily consists of methane, carbon dioxide and hydrogen sulfide, and represents an environmentally friendly and renewable energy source. In addition, the residual digestate is nutrient-rich and can be applied as an organic fertilizer, contributing to a circular, sustainable approach to resource utilization.

The biodegradability of lignocellulosic biomass in anaerobic digestion is strongly influenced by its chemical composition, particularly the structural carbohydrates and lignin content, as well as the carbon-to-nitrogen (C/N) ratio.

In the present study, the C/N ratios of the investigated crop residues ranged from 31 to 97, with the most favorable ratio observed in *Fagopyrum esculentum*, followed by *Zea mays* (Figures 1-2).

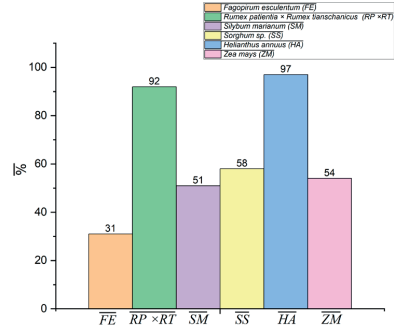


Figure 1. Carbon / Nitrogen Ratio

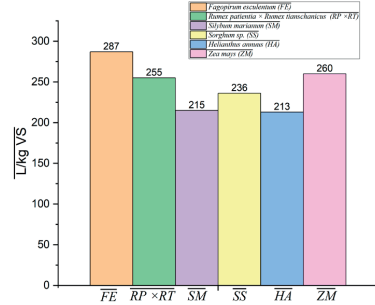


Figure 2. Biochemical Potential, L/kg VS

Differences in hemicellulose and lignin contents among substrates were also critical in determining biomethane yields. The biochemical methane potential (BMP) of the studied residues varied from 213 to 287 L/kg of organic dry matter (ODM). *Fagopyrum esculentum* residues exhibited the highest BMP, while comparable methane potentials were observed in *Zea mays* and *Rumex patientia* × *Rumex tianschanicus*, likely due to their optimal lignin content. In contrast, substrates from *Helianthus annuus*, *Silybum marianum* and *Sorghum* sp. demonstrated lower BMP values, reflecting their higher lignin and structural fiber contents, which reduce biodegradability.

Several studies have reported data on the biomethane production potential of crop residue substrates. Dell’Omo (2025) found that the methane yield and biogas composition of maize stover raw material were 199.6 m³/t VS and 52.0%, respectively, while for pretreated maize stover, the values were 219.8 m³/t VS and 51.1%. Mazurkiewicz et al. (2019) reported that the methane productivity of maize straw substrates ranged from 201 to 207 m³/t. El-Araby (2024) showed that methane production

from the anaerobic digestion of maize stover was 338 L/kg, while wheat straw yielded 290 L/kg. Amon et al. (2007) reported that the specific methane yield of substrates from two sunflower cultivars significantly decreased during the growing season, from 454 to 190 L/kg VS. Mursec et al. (2009) reported that the highest biomethane production was achieved by the sunflower substrate (283 L/kg VS), followed by the sorghum substrate (188 L/kg VS) and maize (187 L/kg VS).

Spyridonidis et al. (2019) reported that the methane yields of sunflower stalk and head residues were 112.2 and 183.0 L/kg TS, respectively, but after alkaline pretreatment, the yields increased to 150.7 and 196.4 L/kg TS. Zhurka et al. (2020) found that the highest methane production, 268.35 L/kg VS, was achieved from pretreated sunflower head residues.

Second-generation liquid biofuels, particularly cellulosic bioethanol, have attracted considerable attention from researchers, industry stakeholders, and policymakers, driven by the depletion of fossil fuel resources and the rapidly rising energy prices. Cellulosic bioethanol is widely regarded as a promising “drop-in” fuel capable of partially or fully substituting gasoline in the transportation sector. Compared with conventional gasoline, ethanol offers several notable advantages when used in internal combustion engines: it possesses a higher octane number, enhancing resistance to engine knock; it has a lower freezing point, which is beneficial in cold climates; and its combustion results in lower net CO₂ emissions, contributing to climate mitigation efforts.

The efficiency of ethanol production is influenced by multiple factors, including the type and chemical composition of the biomass, plant species and variety, growth conditions, and stage of maturity at harvest. Understanding these factors is essential for optimizing substrate selection and maximizing ethanol yield from lignocellulosic residues.

The theoretical ethanol yield of the studied crop residues is presented in Figure 3, illustrating the variability in potential ethanol production among different species.

The estimated theoretical ethanol yield from hexose and pentose sugars varied among the studied crop residues. *Fagopyrum esculentum*

residues exhibited the lowest potential, at 404 L/t, whereas *Sorghum* sp. and *Helianthus annuus* substrates reached the highest values, ranging from 559 to 562 L/t. The substrate from *Silybum marianum* displayed an intermediate yet notably high theoretical bioethanol potential of 544 L/t. In contrast, residues from *Rumex patientia* × *Rumex tianschanicus* and *Zea mays* showed considerably lower ethanol potential, with no significant differences observed between them.

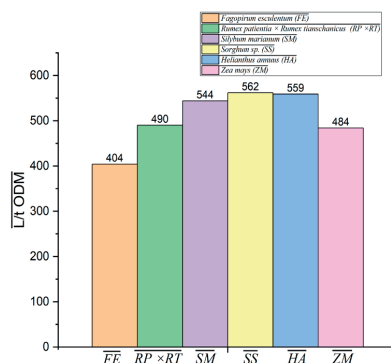


Figure 3. Theoretical Ethanol Potential, L/tonne ODM

The theoretical ethanol potential and chemical composition of various lignocellulosic crop residues have been extensively investigated in the literature, providing critical insights into their suitability as substrates for bioethanol production. Goff et al. (2010) reported that the theoretical ethanol potential of sorghum biomass ranged from 560 to 610 L/t of dry biomass. Kikas et al. (2014) reported that the chemical composition of sunflower plants was characterized by 5.18% hemicellulose, 34.06% cellulose, 7.72% lignin, 9.78% ash, a potential ethanol yield of 49 g/m², hydrolysis efficiency of 33.08%, and fermentation efficiency of 66.36%. In comparison, Jerusalem artichoke harvested in September and October contained 4.50-5.48% hemicellulose, 20.95-25.99% cellulose, 5.05-5.70% lignin, 4.56-5.15% ash, with potential ethanol yield of 38-40 g/m², hydrolysis efficiency of 74.70-77.88%, and fermentation efficiency of 38.14-44.19%.

Mathias et al. (2015) noted that sunflower stem bark contained 48% cellulose and 14% lignin, whereas the stem pith contained 31.5% cellulose and 2.5% lignin. Yue et al. (2018) reported that

sunflower stalks contained 37.8% cellulose, 17.1% hemicellulose, and 12.3% lignin.

According to Pattanaik (2019), maize stover biomass contained 31.3-49.4% cellulose, 21.1-26.2% hemicellulose, 3.1-8.8% lignin, 3.6-8.7% crude protein, 0.3-1.3% ether extract (EE), and 4.2-6.3% ash. In contrast, Jerusalem artichoke stem pith contained 67.7% alpha-cellulose, 4.6% hemicellulose, 7.6% lignin, and 1.3% ash. Gholami-Yangije et al. (2019) reported that sunflower stalks contained 2.26% crude protein, 85.09% neutral detergent fiber (NDF), 72.72% acid detergent fiber (ADF), and 15.25% acid detergent lignin (ADL).

Țiței et al. (2021) revealed that the dry stems of *Helianthus tuberosus* ‘Solar’ contained 276 g/kg cellulose, 176 g/kg hemicellulose, 98.04 g/kg hexose sugars, and 45.4 g/kg pentose sugars, with a theoretical bioethanol yield of 598 L/kg. Overall, these studies highlight the considerable variability in cellulose, hemicellulose, and lignin contents among crop residues, which directly influences their theoretical ethanol potential.

Phytomass, including woody plants and crop residues, has long served as a primary energy source for humans, particularly for heating and cooking. The valorization of energy biomass in the form of densified solid fuels, such as pellets and briquettes, is widely favored due to several advantages: it reduces biomass volume, lowers

transportation costs, facilitates handling, and increases energy density per unit of volume.

Ash content is a critical parameter in determining the quality of solid biofuels. High ash levels can reduce combustion efficiency, accelerate corrosion, promote clinker formation in combustion chambers, and cause wear and physical damage to heating systems. As previously shown in Table 1, the crop residues investigated in this study exhibited considerable variability in ash content. *Helianthus annuus* and *Zea mays* residues were found to have more favorable, lower ash concentrations, whereas very high ash levels were observed in *Silybum marianum*, *Fagopyrum esculentum* and *Sorghum* sp. residues. Consequently, the use of these high-ash residues in densified solid fuel production is recommended in combination with low-ash-content biomass, such as woody materials, to optimize fuel quality.

The utilization of crop residues for densified solid fuel production also requires careful characterization of their elemental composition. During combustion, the oxidation of carbon and hydrogen releases energy, with carbon contributing most significantly to the overall heating value, while increased hydrogen content further enhances the net calorific value. In contrast, high levels of nitrogen (N), sulfur (S) and chlorine (Cl) in biomass can lead to increased emissions of harmful compounds during combustion.

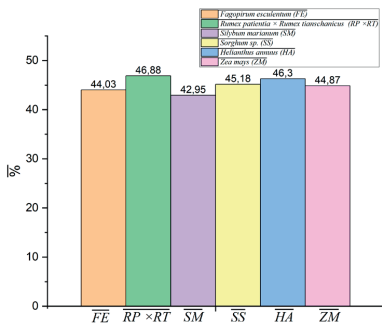


Figure 4. Carbon content, %

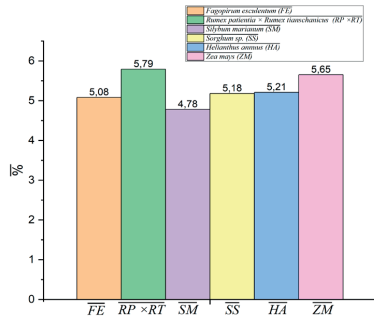


Figure 5. Hydrogen content, %

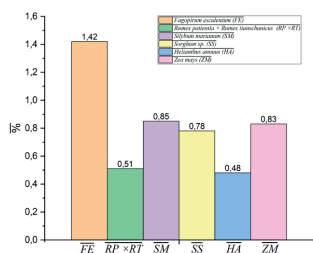


Figure 6. Nitrogen content, %

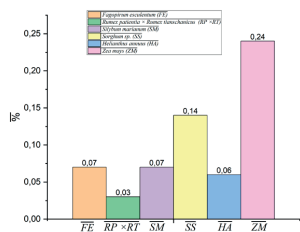


Figure 7. Sulphur content, %

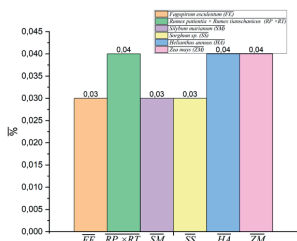


Figure 9. Chlorine content, %

Our results indicate that carbon and hydrogen concentrations vary significantly among the studied crop residues, with relatively higher levels observed in *Rumex patientia* × *Rumex tianschanicus* and *Helianthus annuus*. *Fagopyrum esculentum* residues exhibited particularly high nitrogen content, whereas *Zea mays* residues showed elevated sulphur levels.

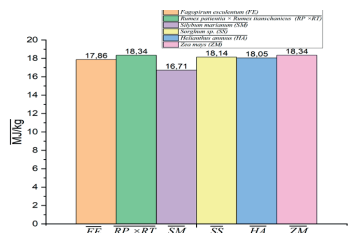


Figure 10. Gross calorific values of the studied residues, MJ/kg

The energy values of studied crop residues are presented in Figures 10-11. It was determined that the gross calorific values (GCV) of the The The elemental composition of the studied crop residues is summarized in Figures 4-11, providing essential information for assessing their suitability for solid biofuel applications residues ranged from 16.71 to 18.34 MJ/kg, while the corresponding lower calorific values (LCV) ranged from 15.33 to 16.97 MJ/kg.

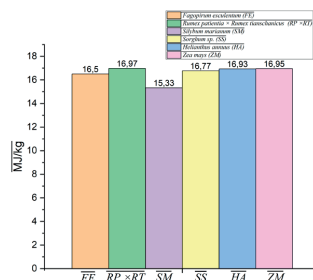


Figure 11. Lower calorific values of the studied residues, MJ/kg

Among the substrates, the net calorific value of *Rumex patientia* × *Rumex tianschanicus* residues was found to be optimal, exceeding that of *Zea mays* and *Helianthus annuus* residues and substantially higher than that of *Sorghum* and *Fagopyrum esculentum*. In contrast, *Silybum marianum* residues exhibited comparatively lower calorific values, likely due to their reduced carbon content coupled with very high ash levels.

Several studies have reported on the quality indices and energy values of crop residues. According to Unal& Alibas (2006) that sunflower stalks had 7.6 % ash and 18.647MJ/kg. Petříková (2011) found that *Rumex* dry matter contained 1.85% ash, 19.17 MJ/kg HHV, 17.89 MJ/kg LHV, 5.5% moisture, 48.62% carbon (C), 0.05% sulphur (S), 0.49% nitrogen (N), and 42.88% oxygen (O). Sulas et al. (2008) reported that the calorific value of whole dry biomass from *Silybum marianum* phytomass was 14.8 GJ/t. Sánchez et al. (2009) found that sunflower biomass contained 8.1% moisture, 74.5% volatile matter, 8.3% ash, 19.8% fixed carbon, 41.21% C, 5.14% H, 49.82% O, 2.54% N and 15.7 MJ/kg calorific value.

Hăbășescu (2011) reported that pellets produced from sunflower stems had 3.78% ash and 14.8 MJ/kg LHV; maize stem pellets contained 5.14% ash and 14.2 MJ/kg LHV; and wheat straw pellets had 6.25% ash and 14.3 MJ/kg LHV. Ledda et al. (2013) analysed milk thistle biomass and found 79.3-87.5% volatile matter, 9.6-16.7% ash, 7.5-10.9% fixed carbon, 37.0-42.9% C, 5.3-5.9% H, 49.8-50.0% O, 0.5-1.2% N, 0.3% S and a calorific value of 16.1-18.1 MJ/kg.

Marian et al. (2013) reported that maize stover contained 4.15-5.32% ash, 17.71-18.16 MJ/kg HHV, and 16.51-16.95 MJ/kg LHV, whereas corn cobs contained 1.25% ash, 14.20 MJ/kg HHV, and 13.62 MJ/kg LHV. Huang (2014) noted that briquettes from sunflower stems had a calorific value of 4,300 kcal/kg (18.0 MJ/kg) and 4.3% ash, wheat straw briquettes had 4,100 kcal/kg (17.1 MJ/kg) and 8.0% ash, compared with wood chips, which contained 4,785 kcal/kg (20.0 MJ/kg) and 1.2% ash.

Heuze et al. (2015) reported that sunflower stover 8.4% ash and 18.6 MJ/kg gross energy; maize stover 7.1 % ash and 18.2 MJ/kg gross energy; buckwheat residues respectively 7.2% ash and 18.7 MJ/kg gross energy. Mathias et al. (2015) found that sunflower stem bark had a heat capacity of 14 MJ/kg, while stem pith had 13 MJ/kg. Muntean et al. (2018) reported that maize stover contained 4.40% ash and 17.8 MJ/kg HHV, while biomass from alnum sorghum contained 3.71% ash and 18.6 MJ/kg HHV. Yue et al. (2018) noted that sunflower biomass contained 79 g/kg ash, 440 g/kg C, 58 g/kg H, 422 g/kg O, 9 g/kg N, and 16.7 MJ/kg LHV. Alatzas et al. (2019) reported that corn agricultural waste had 4.5% ash and 14 MJ/kg LHV. Heuze et al. (2019) found that *Fagopyrum esculentum* straw had an HHV of 18.7 MJ/kg. Rolinec (2018) observed that gross energy values varied from 18 to 19.04 MJ/kg. Kraszkiewicz et al. (2019) determined that buckwheat straw contained 14.49% moisture, 6.28% ash, 44.4% C, 5.56% H, 0.88% N, 0.13% S, 0.12% Cl, and 15.72 MJ/kg combustion heat. Bilyk et al. (2020) reported that pellets from *Fagopyrum esculentum* contained 8.9% moisture, 7.3% ash, 591 kg/m³ bulk density, 1,223 kg/m³ specific density, and 95.3% durability, whereas pellets from *Fagopyrum tataricum* had 9.2% moisture, 6.2% ash, 639

kg/m³ bulk density, 1,237 kg/m³ specific density, and 96.1% durability.

CONCLUSIONS

The investigated crop residues exhibited considerable variability in their biochemical composition, reflecting their suitability for different bioenergy applications. Crude protein ranged from 32 to 89 g/kg, acid detergent lignin from 69 to 112 g/kg, cellulose from 348 to 507 g/kg and hemicellulose from 208 to 292 g/kg, which are key determinants of their conversion potential.

The biochemical methane potential ranged from 213 to 287 L/kg dry organic matter, while the theoretical ethanol potential varied between 404 and 562 L/t organic matter. Net calorific values of the residues were 15.33–16.97 MJ/kg, confirming their suitability as feedstocks for biofuel production.

Specific crop residues show preferential applications: *Fagopyrum esculentum* is particularly promising for biogas production due to its favorable composition. *Silybum marianum*, *Sorghum* sp. and *Helianthus annuus* are well suited for cellulosic bioethanol production. *Rumex patientia* × *Rumex tianschanicus*, *Zea mays*, and *Helianthus annuus* are ideal for the production of densified solid fuels.

These results highlight the versatile utility of crop residues as renewable energy feedstocks and provide guidance for their targeted application in biogas, bioethanol and solid fuel production systems.

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RESEARCH ON THE INFLUENCE OF PLANTING DENSITY ON THE DYNAMICS OF BIOMASS ACCUMULATION IN BAMBOO PLANTATIONS UNDER THE CLIMATIC CONDITIONS SPECIFIC TO THE EMILIA-ROMAGNA REGION, ITALY

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Abstract

Although the cultivation of moso bamboo is currently seen as a challenge, in the near future this crop will certainly be regarded as a sustainable source of biomass, not only because it makes very good use of soils considered unsuitable for other crops, but especially due to the long lifespan characteristic of this species, its accelerated growth rate, the extraordinary capacity of plants for annual regeneration and the amount of biomass produced per unit area. The aim of the research was to determine the dynamics of biomass accumulation in distinct vegetation phases in 8-year-old bamboo plantations under the specific conditions of the temperate humid climate typical of the Emilia-Romagna region, Italy. The research results highlighted that, in an 8-year-old bamboo plantation, after 6 months of growth, the average of fresh and dry aboveground biomass yields were over 796 tons/ha, respectively over 619 tons/ha of dry biomass, thus demonstrating the importance of cultivating moso bamboo as a sustainable alternative energy source.

Key words: moso bamboo, aerial biomass, alternative energy source, sustainability.

INTRODUCTION

Those who produce the raw material are at the top of an industrial pyramid. No chemical treatments are used; bamboo is naturally antifungal and pesticide, and the organic quality of the soil and the purity of the underlying aquifers are maintained (Girard, 2013). This underground biomass enables bamboo to survive and regenerate when the surface biomass is destroyed by fire. Once developed, bamboo is also tolerant to floods and drought (Vitali & Dobrinouiu, 2020).

Several aspects of bamboo biology make it excellent for stabilizing unstable soils and preventing soil erosion, due to its extensive root systems, which can measure up to 100 kilometers per hectare and can live for approximately a century (Lobovikov et al., 2007).

Bamboo is included among phytoremediation plants for its ability to grow in polluted sites, absorb heavy metals present in the soil, and thus perform real remediation work. At present, this

species helps more than 2 billion people meet their basic needs, while also representing a genuine source of growth for environmental resources (Vitali & Dobrinouiu, 2020). It is a species with high ecological plasticity, an extraordinary capacity for regeneration, very productive, versatile, easily accessible, and low-cost; it has great potential to improve the living standards of the population, especially in rural areas of developing countries (Ongugo et al., 2012). Bamboo has a number of characteristics desired in the biofuel market, such as low ash content and alkaline pH. The high calorific value (HHV) of bamboo is superior to most agricultural residues (Boundy et al., 2011). The moisture content of dry bamboo biomass is relatively low (8-23%) (Vitali & Dobrinouiu, 2020), compared to other plants used as a biomass source.

Recently, bamboo has emerged as a new source of biomass for energy production (Astier, 2013). Numerous studies and research have been conducted to assess the use of bamboo as an energy source, identifying bamboo as a viable

alternative for biomass resources to sustainably meet energy demands and generate additional income (Boyle, 2004). Bamboo biomass is used for co-generation to produce electricity or undergoes gasification and pyrolysis processes (Kerlero de Rosbo & De Bussy, 2012). Direct combustion of bamboo biomass can also be applied on an industrial scale, for example, in the form of co-generation, for the production of thermal and electrical energy in thermal power plants or other facilities. Co-generation helps reduce the amount of fossil fuel used in these facilities (Poppens et al., 2013). Bamboo biomass (dry matter) can be used through direct combustion in such a way that it is suitable for domestic use, such as cooking and heating homes in remote rural areas and for the poor. It is also possible to use biomass in co-generation plants to produce electricity. Biomass can undergo various conversion processes that produce so-called charcoal (similar to fossil coal), combustible gases, and biofuels that have characteristics similar to those of fossil fuels (Poppens et al., 2013). In general, biomass is capable of taking on the role of fossil fuels in the future energy sector. The two main advantages of biomass compared to fossil fuels are sustainability and CO₂ emission levels. Bamboo biomass is a renewable source, which means it can be regenerated at a sustainable extraction rate (Vitali & Dobrinou, 2020). Although biomass processing (thermal conversion and biochemical conversion) also releases CO₂, it does not contribute to the increasing concentration of greenhouse gases in the atmosphere, because the CO₂ released through these processes is the same carbon dioxide from the atmosphere fixed through photosynthesis in bamboo plants (Sritong et al., 2012). Currently, the electricity price of power plants using fossil fuels is higher than electricity produced from biomass, so biomass will be more cost-effective than fossil fuels, and the transition will occur naturally.

MATERIALS AND METHODS

The aim of the research was to evaluate the dynamics of aboveground biomass accumulation in moso bamboo plantations of different ages under the specific conditions of the Mediterranean climate characteristic of the Emilia-Romagna Region, Italy.

To achieve the objective outlined in the contract, the following partial objectives were targeted:

- Assessment of the dynamics of above-ground biomass accumulation (fresh substance) in moso bamboo (*Phyllostachys pubescens*) plantations of different ages;
- Assessment of the dynamics of above-ground biomass accumulation (dry substance) in moso bamboo (*Phyllostachys pubescens*) plantations of different ages;
- Assessment of the dynamics of above-ground total biomass yield accumulation in moso bamboo (*Phyllostachys pubescens*) plantations of different ages.

In order to assess the dynamics of biomass accumulation in bamboo plantations of different ages, against the background of using different planting densities, a two-factor experiment was designed according to the split-plot method, in three replications. The experimental factors tested during the research were:

Factor A – planting density, with 2 levels:

a1 – 1200 plants/ha;

a2 – 1080 plants/ha.

Factor B – plantation age, with 3 levels:

b1 – 2 years age;

b2 – 4 years age;

b3 – 8 years age.

By combining the two experimental factors studied, six experimental variants were obtained (Table 1), and the analysis and interpretation of the research results were carried out using the analysis of variance method, according to two-factor experiments, taking the average of the experimental variants tested in the research as the experimental control.

Table 1. Experimental variants tested during the research

Experimental Variants		Factor B		
		b1	b2	b3
Factor A	a1	V1 (a1b1)	V2 (a1b2)	V3 (a1b3)
	a2	V4 (a2b1)	V5 (a2b2)	V6 (a2b3)

The research was conducted in bamboo plantations of different ages (Figures 1 and 2), established in various locations in Italy. Control points (replications) with an area of 1 m² were set up along the diagonal of each plantation, where biometric measurements regarding the number of stems present on plants, the stems height and the stems diameter were carried out.



Figure 1. Collection and labeling of destructive plant samples on biomass components in 2- and 4-year-old bamboo plantations (Source: Personal archive)



Figure 2. Collection and weighing of destructive plant samples on biomass components in 8-year-old bamboo plantations (Source: Personal archive)

After conducting the biometric measurements, destructive plant samples were taken from each predetermined control point to determine the

dynamics of biomass accumulation at each plantation.

The number of control points varied according to the area occupied by each bamboo plantation studied, with 2 control points for areas smaller than 1 ha, 3 control points for areas between 1-3 ha, and 4 or 5 control points for areas larger than 4 ha.

The prelevation of destructive plant samples was carried out in several repetitions, by cutting flush from the ground surface the stems present on the bamboo plants at the initial moment of the research and the new stems formed on the bamboo plants in 1 year of vegetation, given the extraordinary regenerative capacity of the plant, but also the specificity of the species *Phyllostachys pubescens* to differentiate new stems annually from the vegetative buds present in the root system (rhizomes).

The destructive samples were taken separated into the aerial components of the plants, namely stems, branches, and leaves, were labeled and weighed, resulting in the fresh biomass production reported per unit area.

After the determination of fresh biomass, the analysis samples were transferred for drying. The drying of the analysis samples was carried out at a temperature of 95°C for 48 hours for stems and branches, and at a temperature of 60°C for 24 hours for leaves. After drying, these samples were weighed and the results obtained were recorded for the purpose of determining the production of dry biomass achieved per unit area.

Observations and measurements made during the research.

The measurements carried out in the field focused on:

- the density of stems per unit area - by counting differentiated stems per m²;
- the height of stems (m) by measuring the height of the stems from ground level to their top;
- the diameter of stems (cm) by measuring with calipers at the midpoint of the stems.

In the laboratory, the following measurements were made:

- fresh biomass of stems (kg/m²) by weighing with the scale;
- fresh biomass of stem branches (kg/m²) by weighing with the scale;

- fresh biomass of leaves (kg/m²) - by weighing with the scale;
- biomass of stems d.m. (kg/m²) - by weighing with the scale;
- biomass of stem branches d.m. (kg/m²) - by weighing with the scale;
- biomass of leaves d.m.. (kg/m²) - by weighing with the electronic scale;
- total fresh and dry biomass production (kg/m²)
- by summing the biomass of its components, namely stems, branches, and leaves;
- total fresh and dry biomass production (to/ha)
- by relating it to the unit area.

RESULTS AND DISCUSSIONS

Results and discusions regarding the biometric parameters at moso bamboo plants
 Research conducted in 2025 on moso bamboo plantations of different ages and established at

different planting densities in the Emilia-Romagna Region, Italy, revealed, based on biometric determinations, that over 12 months of vegetation, the number of differentiated culms per unit area varied between 9.47 culms/m² and 13.87 culms/m², with the highest number of culms recorded in 2-year-old plantations, under a planting density of 1200 plants/ha (V1-a1b1). Compared to the experiment average (11.18 culms/m²), the number of culms per m² showed highly significant positive differences (***) in the experimental variants where the planting density was 1200 plants/ha (V1, V2, and V3) and highly significant negative differences (ooo) in the experimental variants where the planting density was 1080 plants/ha (V4, V5, and V6), regardless of the plantation age (Table 2).

Table 2. Biometric parameters on moso bamboo plants (Emilia-Romagna, Italy)

Experimental plot	Stems number/m ²	Diff.	Sign.	Stems height (m)	Diff.	Sign.	Stems diameter (cm)	Diff.	Sign.
V1 (a1b1)	13.87	2.69	***	2.43	-3.52	ooo	1.47	-3.73	ooo
V2 (a1b2)	12.03	0.85	***	3.23	-2.72	ooo	2.03	-3.17	ooo
V3 (a1b3)	11.71	0.53	***	11.15	5.20	ooo	10.82	5.62	ooo
V4 (a2b1)	10.13	-1.05	ooo	2.72	-3.23	ooo	1.93	-3.27	ooo
V5 (a2b2)	9.84	-1.34	ooo	3.73	-2.22	ooo	2.47	-2.73	ooo
V6 (a2b3)	9.47	-1.71	ooo	12.41	6.46	***	12.47	7.27	***
Average (Control)	11.18	Ctrl.	Ctrl.	5.95	Ctrl.	Ctrl.	5.20	Ctrl.	Ctrl.
	LSD _{5%} = 0.040; LSD _{1%} = 0.074; LSD _{0.1%} = 0.185			LSD _{5%} = 0.010; LSD _{1%} = 0.018; LSD _{0.1%} = 0.046			LSD _{5%} = 0.009; LSD _{1%} = 0.013; LSD _{0.1%} = 0.019		

The height of the stems after 12 months of growth reached values ranging from 2.43 m (V1) to 12.41 m (V6), with the most intense rate of stem growth and development recorded in plants aged 8 years, where stem heights varied between 11.15 m (V4) and 12.41 m (V6), showing very significantly negative differences (ooo) compared to the experiment average, with the exception of the experimental variant V6, where the differences were very significantly positive (***) (Table 2).
 The diameter measured at the middle of the moso bamboo culms recorded values ranging from 1.47 cm to 12.47 cm, with the largest diameter culms identified in 8-year-old plants, due to the use of a planting density of 1080 plants/ha when establishing the plantation. Differences from the experimental mean were statistically highly significantly negative (ooo), regardless of the experimental factors tested in

the research, with the exception of experimental variant V6, where the difference was highly significantly positive (***), following the provision of a density of 1080 plants/ha at the time of plantation establishment (Table 2).

Results and discusions regarding the dynamics of aboveground fresh biomass accumulation
 By analyzing the dynamics of biomass accumulation in the aerial organs of moso bamboo plants, it is observed that the largest amount of fresh biomass was accumulated in the stems, with values of this biometric parameter ranging from 3.513 kg/m² (35.13 to/ha), the minimum value obtained in 2-year-old plants under a planting density of 1,200 plants/ha (V1), to 60.173 kg/m² (601.73 to/ha) in 8-year-old plants with a planting density of 1,080 plants/ha (V6).

Compared to the experiment average of 23.39 kg/m² (233.9 to/ha), statistically significant differences were recorded, ranging from very significantly negative (ooo) in 2- and 4-year-old

bamboo plants to very significantly positive (***) in 8-year-old plants, regardless of the planting density established at the time of plantation establishment (Tables 3 and 4).

Table 3. Aerial fresh biomass (kg/m²) accumulated in moso bamboo plantations

Experimental plot	Stems fresh biomass (kg/m ²)	Diff.	Sign.	Branches fresh biomass (kg/m ²)	Diff.	Sign.	Leaves fresh biomass (kg/m ²)	Diff.	Sign.
V1 (a1b1)	3.513	-19.88	ooo	2.361	-3.17	ooo	2.138	-3.80	ooo
V2 (a1b2)	6.133	-17.26	ooo	4.241	-1.30	ooo	4.361	-1.58	ooo
V3 (a1b3)	59.670	36.28	***	9.503	3.98	***	10.461	4.52	***
V4 (a2b1)	3.936	-19.45	ooo	2.815	-2.72	ooo	2.638	-3.30	ooo
V5 (a2b2)	6.917	-16.47	ooo	4.513	-1.02	ooo	4.685	-1.26	ooo
V6 (a2b3)	60.173	36.78	***	9.791	4.26	***	11.334	5.39	***
Average (Control)	23.39	Ctrl.	Ctrl.	5.53	Ctrl.	Ctrl.	5.94	Ctrl.	Ctrl.
	LSD _{5%} = 0.008; LSD _{1%} = 0.016; LSD _{0.1%} = 0.040			LSD _{5%} = 0.001; LSD _{1%} = 0.002; LSD _{0.1%} = 0.005			LSD _{5%} = 0.002; LSD _{1%} = 0.004; LSD _{0.1%} = 0.011		

The same dynamic of biomass accumulation is observed when analyzing the fresh biomass accumulated in the branchings of the stems, with recorded variations ranging from 2.361 kg/m² (23.61 to/ha) (V1) to 9.791 kg/m² (97.91 to/ha) (V6), with 8-year-old bamboo plants accumulating over 9.5 kg/m² (95 to/ha) of fresh biomass in the stem branchings, regardless of

plant density. It is observed that in the case of 4-year-old plants, the amount of fresh biomass accumulated in the stem branches exceeded 4 kg/m² under both planting densities (V2 and V4), while for 2-year-old plants, a biomass amount of over 2 kg/m² (23 to/ha) was determined (Tables 3 and 4).

Table 4. Aerial fresh biomass accumulated on the surface unit in moso bamboo plantations

Experimental plot	Stems fresh biomass (to/ha)	Diff.	Sign.	Branches fresh biomass (to/ha)	Diff.	Sign.	Leaves fresh biomass (to/ha)	Diff.	Sign.
V1 (a1b1)	35.13	-198.77	ooo	23.61	-31.76	ooo	21.38	-37.98	ooo
V2 (a1b2)	61.33	-172.57	ooo	42.41	-12.96	ooo	43.61	-15.75	ooo
V3 (a1b3)	596.70	362.80	***	95.03	39.66	***	104.61	45.25	***
V4 (a2b1)	39.36	-194.54	ooo	28.15	-27.22	ooo	26.38	-32.98	ooo
V5 (a2b2)	69.17	-164.73	ooo	45.13	-10.24	ooo	46.85	-12.51	ooo
V6 (a2b3)	601.73	367.83	***	97.91	42.54	***	113.34	53.98	***
Average (Control)	233.90	Ctrl.	Ctrl.	55.37	Ctrl.	Ctrl.	59.36	Ctrl.	Ctrl.
	LSD _{5%} = 0.040; LSD _{1%} = 0.074; LSD _{0.1%} = 0.185			LSD _{5%} = 0.572; LSD _{1%} = 1.068; LSD _{0.1%} = 2.674			LSD _{5%} = 0.009; LSD _{1%} = 0.013; LSD _{0.1%} = 0.019		

The differences compared to the experiment's average were statistically highly significant negative (ooo) for 2-year-old and 4-year-old plants, whereas the differences were statistically highly significant positive (ooo) for 8-year-old bamboo plants (Tables 3 and 4), regardless of the density established at the time of plantation establishment (V3 and V6).

In the leaves of moso bamboo plants, larger amounts of fresh biomass accumulated compared to the amounts of biomass accumulated in the stem branches, with values ranging from 2.138 kg/m² (21.38 to/ha) to

11.334 kg/m² (113.34 to/ha), the highest amounts of biomass being observed in plants aged 8 years (Tables 3 and 4), for which the differences from the average of the trial taken as the experimental control were very significantly positive (***) from a statistical point of view, due to the practice of the two planting densities examined during the study (V3 and V6).

Research results regarding the dynamics of aboveground dry biomass accumulation The aerial organs of moso bamboo plants of different ages contributed differently to the accumulation

of dry biomass per unit area, with the greatest contribution coming from the stems, with over 2.972 kg of dry biomass/m² (29.72 to/ha) in 2-year-old plants, over 5.973 kg of dry biomass/m²

(59.73 to/ha) in 4-year-old plants, and over 49.608 kg of dry biomass/m² (496.08 to/ha) in 8-year-old plants (Table 5).

Table 5. Aerial dry biomass accumulated in moso bamboo plantations (kg/m²)

Experimental plot	Stems dry biomass (kg/m ²)	Diff.	Sign.	Branches dry biomass (kg/m ²)	Diff.	Sign.	Leaves dry biomass (kg/m ²)	Diff.	Sign.
V1 (a1b1)	2.972	-16.72	ooo	1.812	-2.16	ooo	2.019	-2.30	ooo
V2 (a1b2)	5.973	-13.72	ooo	3.669	-0.30	ooo	3.813	-0.51	ooo
V3 (a1b3)	49.608	29.92	***	5.963	1.99	***	6.350	2.03	***
V4 (a2b1)	3.227	-16.46	ooo	2.106	-1.86	ooo	2.394	-1.93	ooo
V5 (a2b2)	6.236	-13.45	ooo	3.851	-0.12	ooo	4.095	-0.23	ooo
V6 (a2b3)	50.111	30.42	***	6.431	2.46	***	7.223	2.90	***
Average (Control)	19.69	Ctrl.	Ctrl.	3.97	Ctrl.	Ctrl.	4.32	Ctrl.	Ctrl.
LSD _{5%} = 0.001; LSD _{1%} = 0.001; LSD _{0.1%} = 0.002				LSD _{5%} = 0.005; LSD _{1%} = 0.011; LSD _{0.1%} = 0.030			LSD _{5%} = 0.003; LSD _{1%} = 0.006; LSD _{0.1%} = 0.014		

The branching of the culms contributed over 1.812 kg of dry biomass/m² (18.12 tons/ha) in 2-year-old bamboo plantations, over 3.669 kg of dry biomass/m² (36.69 tons/ha) in 4-year-old plantations, with the highest amount of dry biomass recorded in the differentiated branches of 8-year-old bamboo plant culms, namely over 5.963 kg of dry biomass/m² (59.63 tons/ha). The contribution of leaves to the production of dry biomass was higher than that of the culm branches, ranging between 2.019 kg of dry biomass/m² (20.19 tons/ha) and 7.223 kg of dry biomass/m² (72.23 tons/ha). By practicing a planting density of 1080 plants/ha, the rate of

biomass accumulation increased significantly throughout the growing season of bamboo plants, regardless of their age. Compared to the average experience in the experimental variants where the plants were 2 and 4 years old, very significant negative differences (ooo) were recorded in terms of dry biomass accumulation in the aerial parts of the bamboo plants, while in the 8-year-old bamboo plantations, very significant positive increases (***) in the rate of dry biomass accumulation were achieved, regardless of the plant density established at the time of plantation establishment (Table 6).

Table 6. Total aerial dry biomass accumulated on the surface unit in moso bamboo plantations (To/Ha)

Experimental plot	Stems dry biomass (to/ha)	Diff.	Sign.	Branches dry biomass (to/ha)	Diff.	Sign.	Leaves dry biomass (to/ha)	Diff.	Sign.
V1 (a1b1)	29.72	-167.16	ooo	18.12	-21.60	ooo	20.19	-22.97	ooo
V2 (a1b2)	59.73	-137.15	ooo	36.69	-3.03	ooo	38.13	-5.03	ooo
V3 (a1b3)	496.08	299.20	***	59.63	19.91	***	63.50	20.34	***
V4 (a2b1)	32.27	-164.61	ooo	21.06	-18.66	ooo	23.94	-19.22	ooo
V5 (a2b2)	62.36	-134.52	ooo	38.51	-1.21	ooo	40.95	-2.21	ooo
V6 (a2b3)	501.11	304.23	***	64.31	24.59	***	72.23	29.07	***
Average (Control)	196.88	Ctrl.	Ctrl.	39.72	Ctrl.	Ctrl.	43.16	Ctrl.	Ctrl.
LSD _{5%} = 0.026; LSD _{1%} = 0.054; LSD _{0.1%} = 0.156				LSD _{5%} = 0.017; LSD _{1%} = 0.036; LSD _{0.1%} = 0.107			LSD _{5%} = 0.099; LSD _{1%} = 0.229; LSD _{0.1%} = 0.730		

Research results regarding total aerial biomass yield

The total production of fresh aerial biomass in the bamboo plantations studied varied over the course of a growing year from 80.12 tons/ha in 2-year-old plantations, with a planting density of 1200 plants/ha (V1), to 812.98 tons/ha in 8-year-

old plantations, with a planting density of 1080 plants/ha (V6). The plants showed an extraordinary capacity for growth and vegetative development, especially during the first six months of the growing season (Table 7). In the case of 4-year-old plantations, total fresh biomass yields of over 147 tons/ha were

recorded, regardless of the planting scheme established at the establishment of the plantations. Compared to the experiment average (348.64 tons/ha), the differences recorded in the 6 experimental variants tested during the research showed a highly significant negative statistical

assurance (ooo) in the case of 2- and 4-year-old Moso bamboo plantations (V1, V2, V4, and V5), while in 8-year-old plantations, fresh biomass production increases of over 447 tons/ha were recorded with highly significant positive statistical assurance (***) (Table 7).

Table 7. Total aerial fresh and dry biomass yield at moso bamboo plantations (to/ha)

Experimental plot	Fresh biomass yield (to/ha)	Difference	Significance degree	Dry biomass yield (to/ha)	Difference	Significance degree
V1 (a1b1)	80.12	-268.52	ooo	68.03	-211.73	ooo
V2 (a1b2)	147.35	-201.29	ooo	134.55	-145.21	ooo
V3 (a1b3)	796.34	447.70	***	619.21	339.45	***
V4 (a2b1)	93.89	-254.75	ooo	77.27	-202.49	ooo
V5 (a2b2)	161.15	-187.49	ooo	141.82	-137.94	ooo
V6 (a2b3)	812.98	464.34	***	637.65	357.89	***
Average (Control)	348.64	Control	Control	279.76	Control	Control
	LSD _{5%} = 0.033; LSD _{1%} = 0.058; LSD _{0.1%} = 0.135			LSD _{5%} = 0.010; LSD _{1%} = 0.018; LSD _{0.1%} = 0.046		

Analyzing the total dry biomass production resulting from the interaction of the two experimental factors tested in the research, it can be observed that the values of this biometric parameter ranged between 68.03 tons/ha and 77.27 tons/ha in 2-year-old Moso bamboo plantations, between 134.55 tons/ha and 141.82 tons/ha in 4-year-old plantations, and between 619.21 tons/ha and 637.65 tons/ha in 8-year-old plantations, where very significant positive increases (***) in dry biomass production of over 339 tons/ha were recorded by ensuring a planting density of 1200 plants/ha and over 357 tons/ha with a planting density of 1080 plants/ha (Table 7).

The other experimental variants studied recorded, compared with the experiment's average taken as control, very significant negative differences (ooo) ranging between 137.94 tons/ha and 211 tons/ha from a statistical point of view (Table 7).

CONCLUSIONS

Based on the observations and measurements carried out between April 2025 and January 2026 in Moso bamboo plantations of different ages located in the Emilia-Romagna Region, Italy, the following conclusions can be drawn: During the research period, due to the pedo-climatic conditions specific to the locations where the studies were conducted, the values of

the biometric parameters recorded quite significant variations, influenced by the two planting schemes taken into study.

Under the condition of ensuring a planting density of 1200 plants/ha, the stem density per unit area recorded average values of 13.87 stems/m², with an average stem height of 2.43 m and an average stem diameter of 1.47 cm in 2-year-old bamboo plantations; 12.03 stems/m² in 4-year-old plantations with an average height of 3.23 m and a diameter of 2.03 cm; and 11.71 stems/m² in 8-year-old plantations, with a height of 11.15 m and a diameter of 10.82 cm.

By increasing the nutrition space, as a result of ensuring a planting density of 1080 plants/ha, the stem density per unit area decreased to 10.13 stems/m² for 2-year-old bamboo plants, with stems reaching a height of 2.72 m and a stem diameter of 1.93 cm, 9.84 stems/m² for 4-year-old plants, with an average stem height of 3.73 m and a diameter of 2.47 cm, and 9.47 stems/m² with an average height of 12.41 m and a stem diameter of 12.47 cm for 8-year-old plants.

The largest contribution to total fresh above-ground biomass production came from the stems, where, by ensuring a planting density of 1080 plants/ha, over 32 tons of fresh biomass/Ha were accumulated for 2-year-old bamboo plants, over 62 tons of fresh biomass/ha for 4-year-old plants, and over 501 tons of fresh biomass/ha for 8-year-old bamboo plants.

The highest yields of fresh aerial biomass after 9 months of vegetation in moso bamboo plants were recorded in the experimental variants where a planting density of 1080 plants/ha was used when establishing the plantations, with values of 93.89 to/ha in 2-year-old plantations, 161.15 to/ha in 4-year-old plantations, reaching maximum values of 812.98 to/ha in 8-year-old plantations.

The total dry biomass yield over 9 months of vegetation in the plants recorded values of 77.27 to/ha in 2-year-old bamboo plantations, 141.82 to/ha in 4-year-old plantations, reaching maximum values of 637.65 to/ha in 8-year-old plantations, in the context of maintaining a density of 1080 plants/ha when establishing the plantations.

Based on the results obtained in the present research, it can be stated that moso bamboo plantations represent a sustainable source for biomass production intended for bioenergy due to the longevity of the plants (80-100 years), their extraordinary annual regeneration capacity, the intensive rate of vegetative growth and development of the plants, and the superior yields achieved per unit area compared to other energy plant species.

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THE QUALITY INDICES OF ENERGY BIOMASS FROM AGRICULTURAL RESIDUES OF *Asteraceae* SPECIES

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Abstract

The objective of this research was to evaluate the quality indices of the dry matter from agricultural residues - stems of *Asteraceae* species: *Artemisia absinthium*, *Cynara cardunculus*, *Silphium integrifolium*, *Silphium perfoliatum*, *Solidago canadensis* collected in the experimental plot of the NBGI Chișinău. It has been found that elemental composition of the collected stems dry matter was 41.36-50.00% carbon, 4.32-6.14% hydrogen, 0.62-1.37% nitrogen, 0.03-0.10% sulphur, 2.18-5.66% ash and gross calorific value varied from 18.2 to 19.0 MJ/kg dry matter. The dry biomass contained 461-545 g/kg cellulose, 180-320 g/kg hemicellulose, 85-112 g/kg acid detergent fibre and the estimated theoretical ethanol yield varied from 522 to 575 L/t organic dry matter and biomethane potential from 167 to 266 L/kg VS. The investigate agricultural residues have optimal concentration of structural carbohydrates and energy value, which make them suitable to be used as a part of a diverse mix for solid biofuel production and feedstock for cellulosic bioethanol as renewable energy.

Key words: agricultural residues, *Asteraceae* species, biomethane potential, quality indices of biomass, theoretical ethanol yield.

INTRODUCTION

Facing climate change, energy security, and sustainable development challenges, biomass has emerged as a crucial alternative to fossil fuels. Phytomass can provide energy and multiple products, with biorefineries offering efficient conversion into bioenergy (solid, liquid, gas) and bioproducts (fibres, starch, oils, chemicals).

The *Asteraceae* (*Compositae*) family is among the largest flowering plant families, comprising approximately 1,911 genera and 32,913 species. Its diversity spans annual and perennial herbaceous plants, shrubs, trees, climbers, succulents, aquatic plants, etc. Economically, it includes food, forage, oilseeds and technical crops, medicinal plants, ornamentals and weedy species. Key genera include *Artemisia* (474 species), *Helianthus* (71 species), *Cynara* (11 species), *Silphium* (17 species), and *Solidago* (138 species), with many species native to North America and some also in South America, Asia and Europe.

In the flora of Moldova, genera *Artemisia* comprises 10 species, while genera *Solidago* (1 species), important medicinal and aromatic

plants used to treat malaria, hepatitis, inflammation, cancer and various infections. Domesticated *Asteraceae* agricultural crops include *Helianthus annuus*, *Helianthus tuberosus*, *Lactuca sativa*, *Cynara cardunculus*, *Cichorium endivia*, *Carthamus tinctorius*. Species such as *Calendula officinalis*, *Echinacea purpurea*, *Inula helenium*, *Matricaria recutita*, *Polymnia sonchifolia*, *Solidago canadensis* have notable pharmacological properties, offering therapeutic potential.

Among the species of the genus *Silphium*, only *Silphium perfoliatum* and *Silphium integrifolium* are of considerable economic significance, as forage, oilseeds and honey crops, also as energy biomass (Rakhmetov, 2018; Reinert et al., 2019; Cumplido et al., 2020; Mueller et al., 2020; Wu et al., 2020; Daraduda, 2024; Titei, 2025; Wöhl et al., 2025).

In our previous studies we present the results regarding the fodder value and energy biomass potential of some *Asteraceae* species (Ivanova et al., 2015; Țîței, 2014, 2019, 2025; Țîței & Roșca, 2021; Cerempei et al., 2023; Guțu et al., 2023).

Harvesting *Asteraceae* plants produces agricultural residues, which require proper collection

and processing to avoid environmental impacts. In recent decades, European energy, agricultural, and environmental policies have promoted Asteraceae as biomass crops. These species are increasingly researched for conversion into solid (pellets, briquettes, chips), liquid (biodiesel) and gaseous (biomethane, syngas) biofuels, providing a renewable energy source from agricultural and industrial residues. This research aimed to assess the biochemical and physical quality indices of dry matter from agricultural residues – stems of five Asteraceae species (*Artemisia absinthium*, *Cynara cardunculus*, *Silphium integrifolium*, *Silphium perfoliatum* and *Solidago canadensis*) – to determine their suitability as feedstock for biofuel production.

MATERIALS AND METHODS

The Asteraceae species *Artemisia absinthium*, *Cynara cardunculus*, *Silphium integrifolium*, *Silphium perfoliatum* and *Solidago canadensis*, grow in the experimental field of the “Alexandru Ciubotaru” National Botanical Garden (Institute) of MSU, Chişinău, were used as research material, while *Helianthus annuus* served as the control. Stem biomass from the investigated Asteraceae species was collected after seed harvest. The material was initially cut into small pieces using a stationary forage chopper, then further reduced in size with a beater mill equipped with a 6 mm mesh sieve. For analytical purposes, the resulting biomass was oven-dried at 85°C, ground to a particle size below 1 mm, and homogenized thoroughly. Elemental composition - total carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) - was determined by dry combustion using a Vario Macro CHNS elemental analyser. The ash content and energy properties, including higher and lower heating values of both raw biomass and derived pellets, were assessed according to standardized methods. The structural components of the plant cell wall were evaluated by determining neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) using near-infrared spectroscopy (NIRS) with a PERTEN DA 7200 analyser at the Research-Development Institute for Grassland in Braşov, Romania. The cellulose content was calculated as the difference between ADF and

ADL, whereas hemicellulose content was obtained as the difference between NDF and ADF. The theoretical ethanol potential (TEP) was estimated based on the conversion of cellulose and hemicellulose into hexose and pentose sugars, following the equations proposed by Goff et al. (2010). The biochemical methane potential (BMP) was calculated according to the methodology described by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

The conversion of biomass into solid biofuels represents an important approach to meeting contemporary energy demands while promoting renewable and sustainable energy sources. Densified biomass in the form of solid biofuels, such as pellets and briquettes, is widely preferred because it reduces volume, lowers transportation costs, facilitates handling and increases the energy content per unit of volume. The application of biomass as a solid fuel for energy production requires a detailed characterization of its elemental composition. It is well established that the energy released during combustion is positively correlated with the carbon and hydrogen content, reflecting the high energy value of these elements. Conversely, higher levels of oxygen and nitrogen reduce the calorific value, thereby diminishing the energy potential of the fuel. Moreover, low nitrogen and sulphur contents are desirable, as elevated concentrations of these elements contribute to environmental pollution. The elemental composition of the dry biomass from the agricultural residues studied – stems of Asteraceae species – is presented in Figures 1-5.

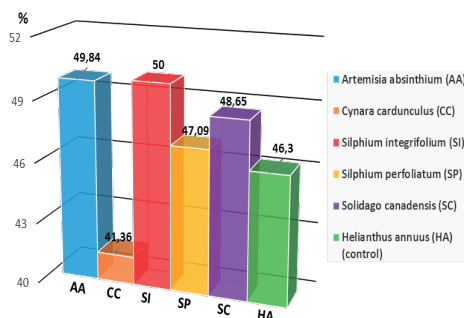


Figure 1. Carbon, %

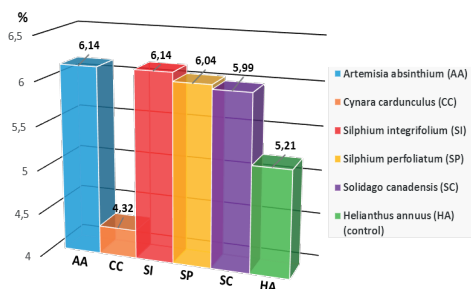


Figure 2. Hydrogen, %

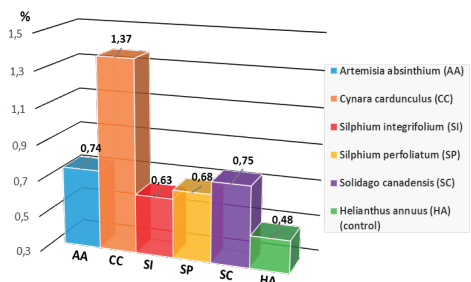


Figure 3. Nitrogen, %

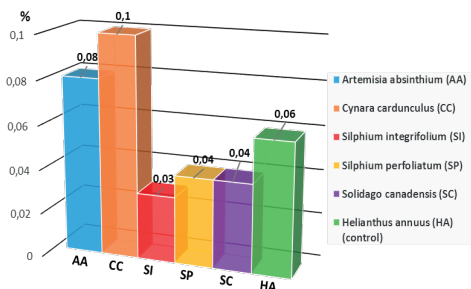


Figure 4. Sulphur, %

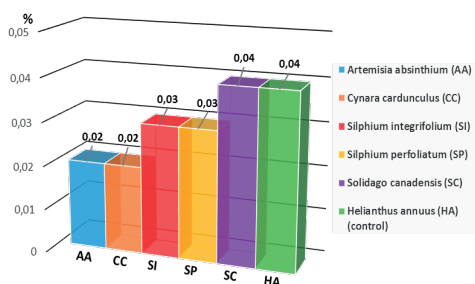


Figure 5. Chlorine, %

The elemental composition of the collected dry biomass ranged from 41.36 to 50.00% carbon, 4.32 to 6.14% hydrogen, 0.62 to 1.37% nitrogen, 0.03 to 0.10% sulphur, and 0.02 to 0.04%

chlorine. Among the studied species, the stems of *Silphium integrifolium* and *Artemisia absinthium* exhibited the highest carbon (50.00%) and hydrogen (6.14%) contents compared with the control, *Helianthus annuus*. In contrast, stem biomass from *Cynara cardunculus* showed lower carbon and hydrogen concentrations. The levels of these elements in *Silphium perfoliatum* and *Solidago canadensis* were higher than in the control, but lower than in *Silphium integrifolium* and *Artemisia absinthium*.

The investigated stem biomass generally contained higher nitrogen concentrations than *Helianthus annuus*, with *Cynara cardunculus* stems exhibiting approximately three times the nitrogen content of the control. Sulphur levels were the highest in *Artemisia absinthium* and *Cynara cardunculus*, while these species also showed lower chlorine concentrations compared with the control and other studied species. Sulfur and chlorine contents in *Silphium integrifolium*, *Silphium perfoliatum*, and *Solidago canadensis* were not substantially different from each other. Ash content is a key parameter for evaluating solid fuel quality, as it represents the non-combustible inorganic residue remaining after combustion. High ash content reduces heating value, increases handling and operational costs, and can lead to issues such as slagging, fouling and corrosion in boilers. Moreover, elevated ash levels can affect environmental compliance by increasing particulate emissions. The ash content of the studied Asteraceae stem residues is presented in Figure 6.

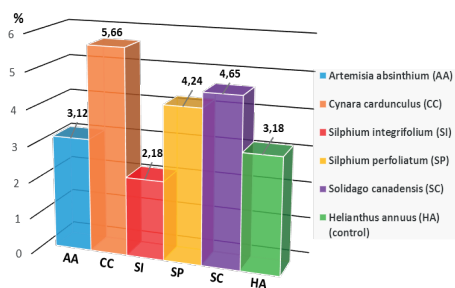


Figure 6. Ash content of biomass, %

Among the species analyzed, *Silphium integrifolium* exhibited the lowest ash content. In contrast, *Cynara cardunculus* stems showed the highest ash concentration at 5.64%, with

Silphium perfoliatum and *Solidago canadensis* also having higher ash levels compared with the *Helianthus annuus* control. This elevated ash content in *Cynara cardunculus*, *Silphium perfoliatum* and *Solidago canadensis* may be attributed to the prolonged retention of leaves on the stems.

Calorific value is the most important property of a fuel, as it determines its energy potential. The energy content of the investigated stem biomass is shown in Figures 7 and 8. The gross calorific value of the stem biomass ranged from 18.20 to 19.00 MJ/kg, while the net calorific value varied between 16.93 and 17.60 MJ/kg. The highest energy values were observed in the biomass of *Artemisia absinthium*, *Silphium integrifolium* and *Solidago canadensis*, followed by *Silphium perfoliatum*, all exceeding the calorific value of the control, *Helianthus annuus* stems. In contrast, the net calorific value of *Cynara cardunculus* stem biomass was comparable to that of the control.

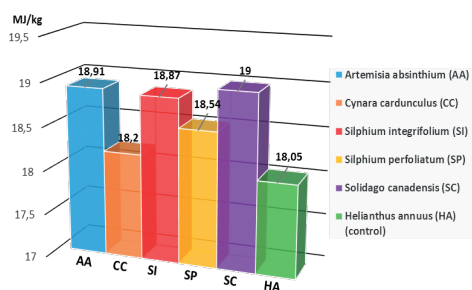


Figure 7. Gross calorific value, MJ/kg

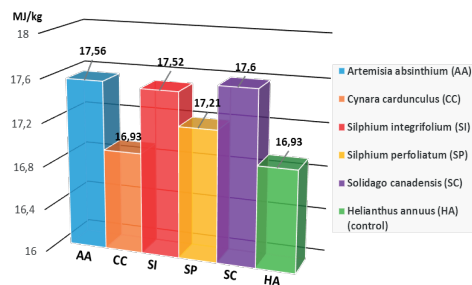


Figure 8. Net calorific value, MJ/kg

According to the literature, Asteraceae species have demonstrated strong potential as feedstock for the production of solid fuels for heat and power generation. Encinar et al. (2002) reported that *Cynara cardunculus* biomass contained

77.3% volatile matter, 14.3% fixed carbon, 8.4% ash, 46.7% carbon, 4.8% hydrogen, 0.7% nitrogen, 0.1% sulphur and a gross calorific value of 18.2 MJ/kg. Dahl & Obernberger (2004) found that cardoon pellets contained 17.4% ash, 1.1% nitrogen, and a gross calorific value of 20.3 MJ/kg. Fernández et al. (2006) reported that *Cynara cardunculus* biomass exhibited a highest heating value of 4083 kcal/kg and a lowest heating value of 3795 kcal/kg. Hua et al. (2007) observed that the energy content of *Solidago canadensis* biomass ranged from 16.7 to 19.2 MJ/kg. Grammelis et al. (2008) analysed *Cynara cardunculus* dry biomass harvested in Central Greece and reported 7.9-8.2% moisture, 6.9-7.2% ash, 70-71.8% volatile matter, 13.4-14.6% fixed carbon, 40.6-43.7% carbon, 5.5-6.0% hydrogen, 0.9-1.8% nitrogen, 0.05-0.1% sulphur and a calorific value ranging from 13.7 to 16.3 MJ/kg. Similarly, Angelini et al. (2009) reported that cardoon biomass contained 10.0% moisture, 73.0% volatile matter, 13.9% ash, 13.1% fixed carbon, 39% carbon, 6.6% hydrogen, 1.5% nitrogen, 0.2% sulphur and a calorific value of 14.9 MJ/kg.

Toscano et al. (2016) documented cardoon pellets with 11.2% moisture, 6.3% ash, high and low heating values of 20,399 and 19,247 J/kg, respectively, along with 0.55% nitrogen, 0.12% chlorine and 0.65% sulphur. Gominhoa et al. (2018) noted that depending on the plant fraction, cardoon biomass calorific value ranged from 18 to 22 MJ/kg dry matter. Hăbășescu (2011) reported that sunflower stem pellets had a net calorific value of 14.8 MJ/kg and 3.78% ash; maize stem pellets contained 14.2 MJ/kg and 5.14% ash, while wheat straw pellets showed 14.3 MJ/kg and 6.25% ash. Stolarski et al. (2014) found that *Silphium perfoliatum* stalks harvested in March-April had 2.97-3.04% ash, 47.40-48.69% carbon, 5.70-5.84% hydrogen, 0.030-0.033% sulphur, 18.70-18.90 MJ/kg high heating value and 13.35-14.95 MJ/kg low heating value. For comparison, *Helianthus tuberosus* stalks contained 3.02-3.12% ash, 46.75-46.98% carbon, 5.67-5.68% hydrogen, 0.032% sulphur, 18.54-18.63 MJ/kg high heating value and 14.04-14.78 MJ/kg low heating value. Meerbeek et al. (2015) reported that *Solidago gigantea* biomass contained 5.44% ash and a high heating value of

19.35 MJ/kg. Ciesielczuk et al. (2016) found that *Solidago canadensis* biomass had 979 g/kg organic matter, 44.8% carbon, 6.46% hydrogen, 0.37% nitrogen, 0.198% sulphur, a high heating value of 18.12 MJ/kg and a calorific value of 16.49 MJ/kg (288.4 GJ/ha). *Artemisia absinthium* biomass contained 979 g/kg organic matter, 44.6% carbon, 6.30% hydrogen, 1.54% nitrogen, 0.252% sulphur, with a high heating value of 17.87 MJ/kg and calorific value of 16.24 MJ/kg (107.2 GJ/ha). Zihare et al. (2018) reported that *Solidago canadensis* biomass had 7.3% moisture, 6.8% ash, 18.24 MJ/kg high heating value and 16.84 MJ/kg low heating value.

Gheorghe & Neacsu (2019) reported high heating values of 14.94 MJ/kg for *Arctium lappa* (3.48% ash, 0.31% nitrogen), 23.02 MJ/kg for *Silybum marianum* (3.35% ash, 0.563% nitrogen) and 16.15 MJ/kg for *Xanthium spinosum* (6.07% ash, 0.306% nitrogen). Bury et al. (2020) found that *Silphium perfoliatum* biomass contained 3.10-4.08% ash, 33.7-34.5% carbon, 1.37-1.62% nitrogen, 0.05-0.09% sulphur and 16.26-16.95 MJ/kg high heating value. Cavalaglio et al. (2020) reported cardoon biomass with 68.1% volatile matter, 9.12% ash, 14.67% fixed carbon, 41.57% carbon and a 16.15 MJ/kg high heating value.

Țiței (2020) found that *Cynara cardunculus* briquettes had a specific density of 918 kg/m³, high heating value of 19.0 MJ/kg and 2.8% ash. Nguyen et al. (2021) observed that *Solidago canadensis* biomass contained 42.46% carbon, 6.14% hydrogen and 1.48% nitrogen; *Cynara cardunculus* biomass had 7.03-12.46% moisture, 61.92-69.83% volatile matter, 5.67-10.27% ash, 10.21-19.25% fixed carbon, 38.57-48.90% carbon, 5.22-5.90% hydrogen, 0.55-0.80% nitrogen, 0.05-0.1% sulphur and 17.73-18.0 MJ/kg calorific value; while *Silybum marianum* biomass contained 79.3-87.5% volatile matter, 9.6-16.7% ash, 7.5-10.9% fixed carbon, 37.0-42.9% carbon, 5.3-5.9% hydrogen, 5.3-5.9% nitrogen and 16.1-18.1 MJ/kg calorific value.

Peni et al. (2022) reported that *Silphium perfoliatum* biomass contained 8.47% ash, 18.51% fixed carbon, 73.02% volatile matter and 17.73 MJ/kg high heating value. Šurić et al.

(2022) found that *Silphium perfoliatum* biomass had 5.86% ash, 15.49% coke, 9.28% fixed carbon, 74.45% volatile matter, 15.46 MJ/kg high heating value, and 14.54 MJ/kg low heating value. In our previous studies (Țiței, 2020, 2023; Țiței & Roșca, 2021), *Silphium perfoliatum* stem biomass contained 45.02% carbon, 5.96% hydrogen, 0.21% nitrogen, 0.03% sulphur, 3.03-3.83% ash, and 17.8-18.3 MJ/kg high heating value. Tóth (2022) reported a high heating value of 15.21-16.79 MJ/kg for *Silphium perfoliatum* biomass. Wiatrowska et al. (2022) found that *Solidago canadensis* dry biomass had 2.39% ash, 19.894 MJ/kg high heating value, and 18.369 MJ/kg low heating value, while *Solidago gigantea* had 4.93% ash, 19.403 MJ/kg high heating value and 17.868 MJ/kg low heating value. Witaszek et al. (2022) reported that *Silphium perfoliatum* lignocellulosic biomass at a moisture content of 7.17% had 15.78 MJ/kg heat of combustion and 14.08 MJ/kg calorific value.

The biochemical composition of the collected dry stem biomass from the studied Asteraceae species is presented in Figures 9-12.

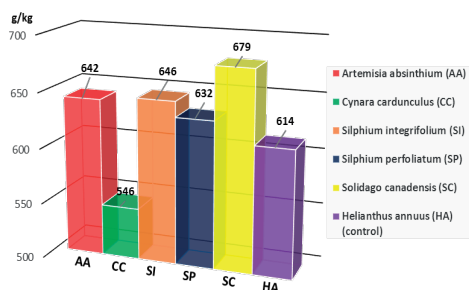


Figure 9. Acid detergent fibre, g/kg

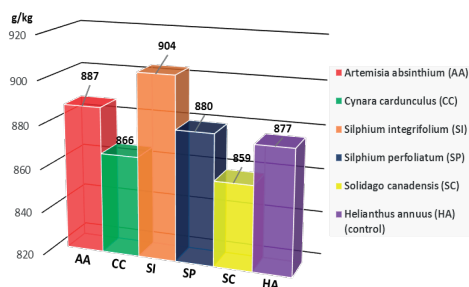


Figure 10. Neutral detergent fibre, g/kg

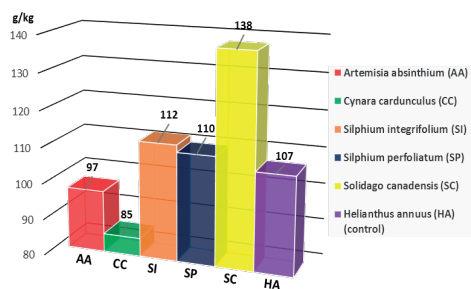


Figure 11. Acid detergent lignin, g/kg

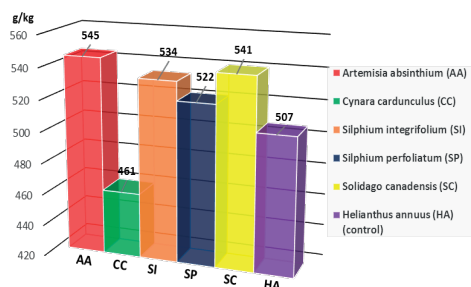


Figure 12. Cellulose, g/kg

The analysed stems contained 461-545 g/kg cellulose, 180-320 g/kg hemicellulose and 85-138 g/kg acid detergent fibre (ADF). For comparison, the control, *Helianthus annuus* stem biomass, contained 507 g/kg cellulose, 263 g/kg hemicellulose and 107 g/kg ADF.

In the context of increasingly stringent environmental regulations and global efforts to reduce greenhouse gas emissions, alternative liquid fuels, including biofuels, are becoming increasingly important. Their development addresses the need to mitigate the environmental impact of transportation while maintaining the accessibility and performance of conventional internal combustion engine systems. Among these fuels, cellulosic bioethanol is regarded as a promising drop-in option that could serve as a viable substitute for gasoline in the transportation sector. Ethanol offers several advantages over gasoline when utilized as a fuel. The theoretical ethanol yield from lignocellulosic substrates of the studied Asteraceae species is presented in Figures 13-15. The estimated yield from hexose sugars ranged from 348 to 405 L/ton, while the yield from pentose sugars was 348-405 L/ton. Compared with the control substrate from *Helianthus annuus*, substrates from *Artemisia*

absinthium, *Silphium integrifolium* and *Solidago canadensis* exhibited higher potential for ethanol production from hexose sugars, whereas *Cynara cardunculus* showed particularly high potential for ethanol production from pentose sugars.

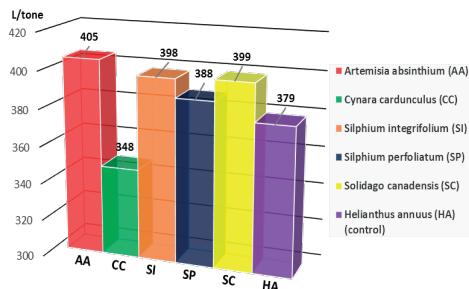


Figure 13. Theoretical ethanol potential from hexose sugars, L/tonne VS

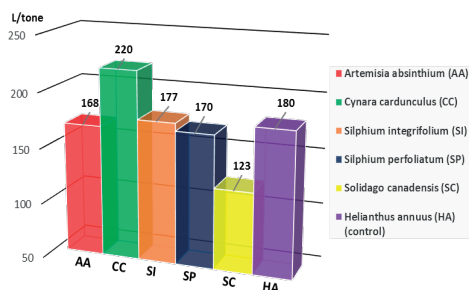


Figure 14. Theoretical ethanol potential from pentose sugars, L/tonne VS

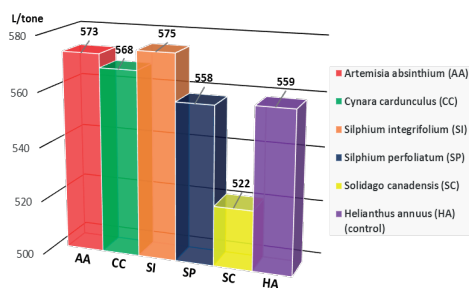


Figure 15. Theoretical ethanol potential from hexose and pentose sugars, L/tonne VS

Several studies have reported on the cell wall composition of Asteraceae species and their corresponding ethanol yields. Coccia et al. (2014) found that *Cynara cardunculus* biomass contained 34.96% cellulose, 16.17% hemicellulose, 4.54% acetyl groups, 11.22%

extractives, and 16.89% lignin. Cotana et al. (2015) reported an ethanol yield from *Cynara cardunculus* biomass of 13.17-13.64 g per 100 g of raw material. Cavalaglio et al. (2020) observed that cardoon biomass contained 37.65% cellulose, 16.70% hemicellulose, 3.96% acetyls, 17.51% lignin and 5.55% extractives. Smuga-Kogut et al. (2020) reported that untreated stems of *Solidago virgaurea* contained 30.4% cellulose, 29.2% hemicellulose, and 19.2% lignin, while other untreated stems contained 38.0-40.6% cellulose, 22.0% hemicellulose and 12.8-13.3% lignin, yielding 2.86 g/L ethanol with up to 81.43% conversion efficiency. Giannoni et al. (2021) noted that prior to organosolv treatment, *Cynara cardunculus* biomass contained 30.52% cellulose, 17.17% hemicellulose, 5.02% acetyls, 14.21% lignin and 7.64% extractives; after treatment, the composition shifted to 87.59% cellulose, 5.06% hemicellulose, and 7.34% lignin, producing 23.42 g of glucose per 100 g raw material (HY of 86.94%). Peni et al. (2022) reported that *Silphium perfoliatum* biomass contained 8.47% ash, 24.47% cellulose, 14.80% hemicellulose and 8.29% lignin. Premjet et al. (2013) analyzed 75 plant species for bioenergy and bioethanol potential, reporting ash content ranging from 2.6 to 17.5%, lignin from 4.6 to 20.4%, cellulose from 18.8 to 56.0%, hemicellulose from 3.3 to 34.2%, and ethanol yields from 239.2 to 584.4 L/t. Ruf and Emmerling (2022) observed that harvest residues (stubbles) from cup plant contained 10.5% hemicellulose, 20.6-31.6% cellulose, and 8.2-11.7% lignin, whereas silage maize contained 16.6-20.0% hemicellulose, 24.2-41.3% cellulose and 10.1-11.2% lignin. In our previous studies (Țiței, 2020, 2023; Țiței & Roșca, 2021), *Silphium perfoliatum* dry stem biomass contained 29.2% hemicellulose, 56.2% cellulose and 11.4% acid detergent lignin (ADL). Tóth (2022) reported lignocellulose quality of *Silphium perfoliatum* as 31.3-48.94% acid detergent fiber (ADF), 34.94-54.69% neutral detergent fiber (NDF), 7.21-12.54% ADL, 24.11-37.30% cellulose, and 2.33-5.75% hemicellulose. Wiatrowska et al. (2022) described *Solidago canadensis* dry biomass as

containing 20.07% pentosans, 38.95% cellulose, 23.78% hemicellulose, 19.17% lignin, 14.42% soluble compounds, and an ethanol yield of 84.2 g/kg biomass; *Solidago gigantea* contained 18.54% pentosans, 38.50% cellulose, 29.59% hemicellulose, 24.79% lignin, 13.27% soluble compounds and an ethanol yield of 50 g/kg biomass.

Biomethanation is a process in which organic material is microbiologically converted under anaerobic conditions into biogas, a mixture primarily composed of methane and carbon dioxide. Phytomass feedstock can be used as a sole substrate for anaerobic digestion or co-digested with one or more additional substrates. The resulting biogas can be directly combusted for cooking and lighting or utilized for electricity generation. The residual digestate and liquid effluent can serve as valuable fertilizers in organic farming systems.

The carbon-to-nitrogen ratio and biomethane potential of the studied agricultural residues stems of Asteraceae species - are presented in Figures 16-18.

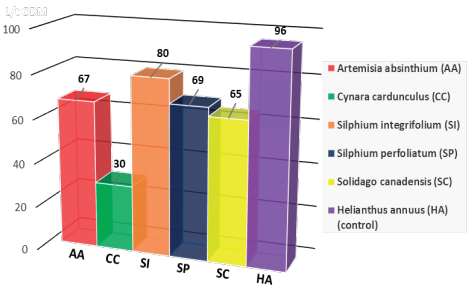


Figure 16. Carbon/nitrogen ratio

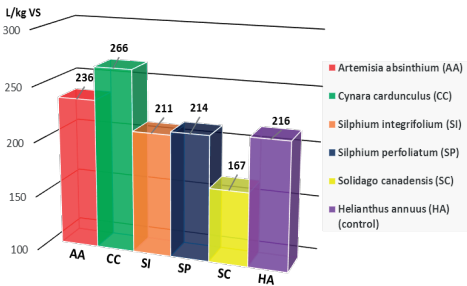


Figure 17. Biomethane potential, L/kg VS

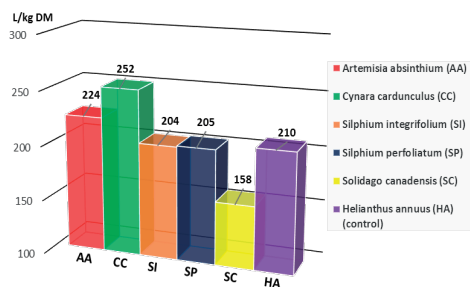


Figure 18. Biomethane potential, L/kg DM

The C/N ratio of the investigated substrates ranged from 30 to 96. The biochemical methane potential of the stems varied between 167 and 266 L/kg VS, corresponding to 158-252 L/kg DM. A notably high biomethane potential was observed in the *Cynara cardunculus* biomass substrate, likely due to its high protein and hemicellulose content combined with relatively low lignin levels. The substrate from *Artemisia absinthium* also exhibited an optimal biomethane potential. The biomethane potential of stem substrates from *Silphium* species did not differ significantly from that of the control substrate, *Helianthus annuus*. In contrast, stem substrates from *Solidago canadensis* displayed lower biomethane potential.

According to the literature, Yao et al. (2016) reported that *Solidago canadensis* biomass contained 90.5% volatile solids, 55.2% carbon, and 1.4% nitrogen, producing a maximum methane yield of 143.7 L/kg. Meerbeek et al. (2015) found that the methane yield of *Solidago gigantea* substrate reached 217.12 L/kg. Havryliuk et al. (2023) reported that the methane yield from *Solidago canadensis* biomass substrate reached 68.2 L/kg of total solids (TS).

CONCLUSIONS

The investigated agricultural residues from Asteraceae species - *Artemisia absinthium*, *Cynara cardunculus*, *Silphium integrifolium*, *Silphium perfoliatum* and *Solidago canadensis* - exhibit high structural carbohydrate content and favourable energy values. These characteristics make them promising candidates for inclusion in mixed solid biofuels, as well as for use as feedstock in the production of cellulosic bioethanol and biomethane. Their utilization not only provides a renewable energy source but

also offers an efficient way to make good use of agricultural residues, contributing to sustainable biomass management.

ACKNOWLEDGEMENTS

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EXPLORATORY STUDIES ON MYCOBIOTA AROUND THE ROOTS OF INVASIVE PLANTS - CASE STUDY *Ailanthus altissima*

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Abstract

It is known that specific and common fungal species can synthesize hydrolytic enzymes (amylases, proteases, cellulases, lipases, pectinases, xylanases) and organic acids, thus causing changes in soil pH and reducing the rate of decomposition processes in the soil. Ailanthus altissima is an aggressive invasive tree in Romania. We aim to analyse if fungal communities from soil are altered by the presence of A. altissima; thus we conducted a field soil sampling in micro-habitats with different Ailanthus individuals' density and age. Mycological analysis assessed the fungal load of the samples and the species with the highest frequency. In the field, we also measured the Ailanthus tree DBH in all stands, recording trees' life stages, and their connection with the mycobiota's species diversity. Results showed mycobiota's biodiversity and its capacity to synthesize metabolites involved in soil decomposition. Analysis of the mycobiota around the roots of adults and root sprouts of A. altissima showed that the composition of the soil fungal community and the fungal activity require a longer invasion time to be affected.

Key words: *Ailanthus altissima*, around the roots, hydrolytic enzymes, soil mycobiota.

INTRODUCTION

Fungi are among the most biologically active and diverse groups of soil microorganisms. They play a crucial role in the decomposition of organic matter and the cycling of nutrients, thus are fundamental to ecosystem processes and plant growth, contributing to improved nutrient uptake and greater soil stability (Berendsen et al., 2012; Singh et al., 2025). Many fungal species - both specific (specialized) and common (ubiquitous) - secrete a variety of extracellular hydrolytic enzymes that break down complex organic molecules into simpler compounds for absorption such as: amylases (hydrolyze starch into simple sugars like glucose and maltose), proteases (degrade proteins into peptides and amino acids), cellulases (break down cellulose, a major structural polysaccharide in plant cell walls, into glucose units), lipases (catalyze the breakdown of fats and lipids into glycerol and fatty acids), pectinases (decompose pectin, a structural polysaccharide found in plant cell walls), and xylanases (degrade xylan, a hemicellulose component of plant biomass) (Dix & Webster,

1995; Gomoiu, 2009; Baldrian, 2014; De Beeck et al., 2021; Zuccarini et al., 2023).

These biochemical products are key to the fungi's ecological role, but they also influence the soil's physicochemical properties, including pH alterations and decomposition rates (Ritz & Young, 2004).

Through these enzymatic activities, fungi facilitate the initial stages of organic matter decomposition, initiating the release of nutrients essential for plant growth and microbial metabolism (Skujiniš & Burns, 1976; Idris, 2026).

In addition to hydrolytic enzymes, many fungal species also produce organic acids such as oxalic, citric, gluconic, and malic acids. These acids: lower the soil pH by releasing protons (H⁺) during metabolic reactions, solubilize metal cations (e.g., Ca²⁺, Mg²⁺, Fe³⁺) and nutrients bound in mineral lattices, making them more bioavailable, aid in the weathering of minerals and chelating of heavy metals, altering soil chemistry. However, the acidification of soil caused by fungal metabolism can also influence microbial community structure and overall soil biochemical balance (Liaud et al., 2014).

Plant invasion can influence litter decomposition, a key process in terrestrial nutrient cycling, through changes in fungal communities associated with both litter type and soil conditioning. Reduced fungal richness in soils conditioned by invasive plants alters decomposition dynamics, leading to differential breakdown of native and invasive litter. By modifying litter traits and reshaping soil fungal communities, invasive plants drive changes in decomposition processes, underscoring the need to consider litter inputs and soil fungi simultaneously when evaluating the impacts of plant invasion on ecosystem functioning (He et al., 2025).

Invasive plants frequently modify soil properties, producing legacy effects that alter soil conditions and, consequently, influence competition among native plant species. Fungal interactions enhance invasive plant success by improving nutrient acquisition, reducing pathogen pressure, and altering soil microbial communities to the disadvantage of native plants (Castro-Díez et al., 2019; Sun et al., 2026).

The development and architecture of plant root systems play a crucial role in shaping the diversity and composition of soil fungal communities, particularly those inhabiting the rhizosphere (the soil zone influenced by roots) (Peng et al., 2025; Martins et al., 2026).

Ailanthus altissima, one of the most widespread invasive non-native species globally, thrives in temperate climates across various habitats, including urban areas, agricultural lands, semi-natural ecosystems, and wetlands (Fotiadis et al., 2011; Lazzaro et al., 2020; Liu et al., 2023; Marzioletti et al., 2025). Its invasion causes drastic changes in community composition, significantly reducing plant richness and diversity (Terzi et al., 2021).

The aggressive growth of *A. altissima* affects the regeneration of native species by altering nutrient cycling and producing toxic compounds (Lawrence et al., 1991; Brooks et al., 2021; Motti et al., 2021). A major advantage for *A. altissima* is its symbiotic relationship with arbuscular mycorrhizal fungi (AMF), which may be driven by resource availability (Badalamenti et al., 2015; 2018).

In contrast, *Ailanthus* negatively impacts seedling growth and ectomycorrhizal (ECM) fungal colonization of *Quercus rubra* (red oak), causing reduced root-to-shoot ratios, possibly

due to stress or inhibited root development induced by *Ailanthus* invasion (Bauman et al., 2013). This allelopathic effect may disrupt the beneficial symbiosis between red oak and ECM fungi.

Although invasive *A. altissima* and *Robinia pseudoacacia* co-occur and share similar growth and reproductive traits, they fulfill distinct ecological functions - *Ailanthus* exhibits allelopathy, while *Robinia* contributes to nitrogen fixation (Call & Nilsen, 2005; Gavrilidis et al., 2023; Miles et al., 2025).

A. altissima produces the allelochemical ailanthone in its roots and bark, which helps it outcompete other plants. Additionally, this compound acts as an anti-herbivore agent, deterring insects from feeding on its leaves (Sasnow, 2014).

Castellano and Gorchov (2012) reported that *Ailanthus altissima* selects for ectomycorrhizal (ECM) fungi that can tolerate allelopathic compounds present in the soil. The observed decrease in ECM root colonization may be a response to reduced carbon allocation to roots by the plant (Bauman et al., 2012).

Removal of *A. altissima* can lead to increased organic matter, soil moisture, and litter accumulation, which may facilitate the succession of ECM fungi and their host plants, thereby promoting long-term ecosystem restoration.

The high content of phenolic compounds in *Ailanthus* leaves, which possess antioxidant and antimicrobial properties, may explain the observed low fungal diversity in invaded soils (Cocîrlea et al., 2024 a, b; Zhao et al., 2005).

From a fungal perspective, some species show potential as biological control agents against *Ailanthus*. The fungus *Verticillium nonalfalfae* is a strong candidate due to its high host specificity, ability to infect new *Ailanthus* individuals through soil or connected roots, and long-term survivability in soil (Miles et al., 2025; Soler & Izquierdo, 2024; Hrabovský & Hladík, 2024). Additionally, Ding et al. (2006) demonstrated that *Fusarium oxysporum* f. sp. *perniciosum* causes wilt in *Ailanthus* in North America, indicating its potential as a control agent.

Small et al. (2010) described the allelopathic effects of *A. altissima* on tree and crop species, showing that these effects reduce crop yields not

only through plant-derived allelopathy but also by altering the biodiversity of soil microorganisms.

Sasnow (2014) emphasized the importance of microbiota - the community of microorganisms associated with plant rhizospheres and roots - in influencing plant health (Poli et al., 2016). This microbial community is shaped by both plant species and soil type. Specific members of the microbiota are either stimulated or suppressed by chemical exudates released into the rhizosphere, the soil region surrounding the roots (Berendsen et al., 2012).

This study aims to characterize the cultivable fungal community (mycobiota) associated with *Ailanthus altissima* and to elucidate the role of these fungi in soil organic matter decomposition through their production of hydrolytic enzymes. Specifically, we intend to compare the hydrolytic enzyme activities of fungal isolates obtained from the rhizosphere and root zones of *A. altissima* individuals at different developmental stages.

MATERIALS AND METHODS

Sample collection - field work

In December 2025, we developed a field trip in *Ailanthus altissima* dense stand outside

Bucharest (Bragadiru area) where the urban constant impact (air pollution, direct human impact, etc.) is lower and *A. altissima* individuals are in different age stages, meaning with different development of above-ground biomass and of the root system (rhizosphere = below-ground biomass) (Figures 1 and 2).

The soil is a mixture of different types of soils discharged here following the construction of the neighboring houses.

For characterizing the tree's age stage, we measured with a tape: the height and DBH (diameter at the breast level) for mature trees and 20 cm above ground level for low height and young stems.

Based on the idea that root system (architecture) development has a strong influence on fungal diversity (Peng et al., 2025; Martins et al., 2026), we collected soil samples from: a hill-like area where the roots of *A. altissima* could not reach the soil (control sample) (noted with number 1), near the trunk of an older trees where the roots are well developed as thickness and length (noted number 2), near the stem of single young shoot less developed (noted number 3, 5, 7 and 8), and groups of suckers originating from the roots: two young shoots (noted with number 4) or three young shoos (noted number 6) (Figure 3).



Figure 1. Location of *Ailanthus altissima* stand in the country and local areas



Figure 2. The above-ground architecture of the *Ailanthus altissima* individuals (left: old growth individual - sample 2, and group of 3 young individuals - sample 6)

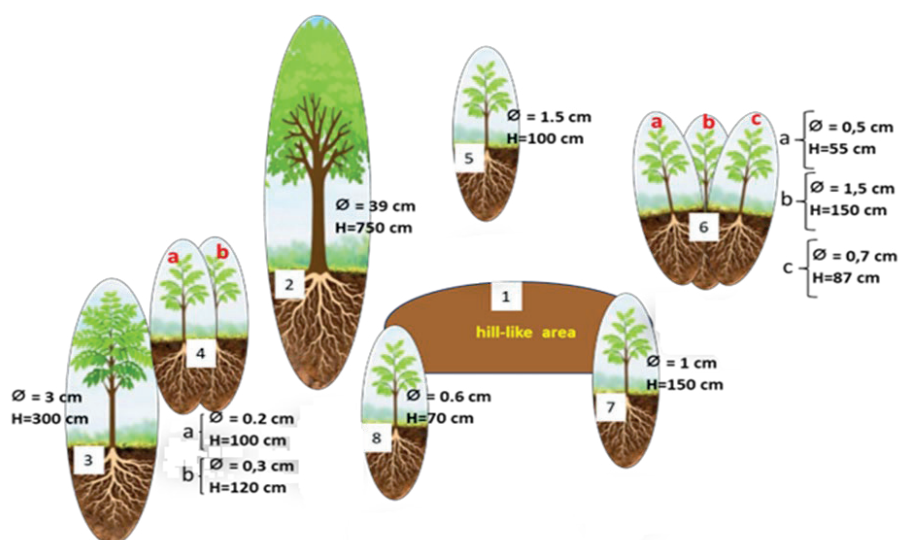


Figure 3. The location of the samples collection at the local area (Legend: H = height of the stem (trunk), Ø = diameter at DBH or 20 cm above ground for young individuals)

Isolation of fungi from the rhizosphere

In order to isolate fungi and estimate their cultivable fraction, soil samples were serially diluted (Sanders, 2012) and plated onto growth media containing yeast extract, glucose, chloramphenicol and agar (YGCA). The fungal load (CFU per g of dry weight) was calculated for the total mycobiota.

Morphological identification of each strain was achieved according to the relevant taxonomic keys (Kiffer & Morelet 1997).

Qualitative evaluation of hydrolase producing fungi

The ability of fungal strains to produce amylase, cellulase, lipase, protease, and xylanase was tested by agar plate-based assays (Ruginescu et al., 2020). The YGCA growth media containing, instead of glucose, one of the substrates of interest (i.e., starch, carboxymethyl cellulose - CMC, Tween 80, xylan and casein) were inoculated in triplicate with diluted soil samples and incubated at 28°C for 14 days.

The production of enzymes was indicated by clear zones around the colonies after flooding the plates with KI solution (amylase), Congo red solution (cellulase and xylanase), or 1N HCl (protease). Lipolytic activity was put in evidence by an opaque halo. The levels of enzyme activities (LEA) were evaluated using the formula LEA = diameter of the hydrolysis zone divided by the diameter of the microbial colonies (in millimeters).

RESULTS AND DISCUSSIONS

Abundance of Cultured Fungi

Soil fungal species decreased significantly when *A. altissima* was present as adult or suckers originate from the roots. The total fungal load for cultivable rhizospheric fungi ranged from $1,3 \times 10^4$ to 60 CFU dwt, with significant differences among the samples. The highest microbial densities were generally obtained on YGCA media (Tabel 1). Even so, the highest number of fungal CFU was found on the control sample represented by hilly-like area. In all the soil samples around the roots of *A. altissima*, the number of CFU was lower, which suggests the possible synthesis of some inhibitory compounds against fungi. The genera with the highest load in the rhizosphere were: *Aspergillus*, *Penicillium*, *Alternaria*, *Trichoderma*, *Chaetomium*, *Botrytis* and *Acremonium*.

Fungal colonies grew on all growth media with specific substrates for hydrolases, but in smaller numbers than on YGCA media. The lowest number of CFU was found in the media with hydrolase substrates for sample 8. Sample 6 contained the lowest number of CFU on the nutrient medium with CMC. Very few UFC were obtained on the nutrient medium with xylan (10) and for the other soil samples they were not identified. The lower number of CFU on the media with hydrolase substrates may be a consequence of the reduction of N total and the release of some inhibitory compounds by the roots of *A. altissima*. Medina-Villar et al. (2016) found that capability of *A. altissima* to decrease soil total N. It could be attributed to changes in the balance between N input and losses from the soil. Also, several properties, the composition of soil microbial communities and microbial activities need longer time since invasion to be altered.

Table 1. Abundance of Cultured Fungi on different growth media)

Sample code	CFU YGCA	CFU on starch nutrient	CFU on casein nutrient	CFU on CMC nutrient	CFU on Tween 80 nutrient	CFU on xylan nutrient
1	140000	13000	12000	1100	110	10
2	12000	100	10000	100	100	0
3	10000	100	12000	1200	100	0
4	13000	100	1400	110	110	0
5	1300	100	100	100	110	0
6	1200	100	100	10	100	0
7	1100	100	100	100	100	0
8	1100	80	75	80	60	0

Soil microbial communities in the field differ between invaded and non-invaded soil due to nitrate concentration and net nitrification rates in *A. altissima* soil. It was proved that the methanolic extracts of the leaves, of the barks and of the fruits have strong antimicrobial activity which can explain reducing the number of microorganisms in the soil where they reach (Caramelo et al., 2021; Zhao et al., 2005).

The fact that the lowest number of UFC was identified on the roots of *A. altissima* with a diameter of 6 cm is evidence that the release of inhibitory compounds begins immediately after the formation of roots. The time required for plant growth allows some fungi to adapt to the new conditions.

Production of Extracellular Hydrolytic Enzymes

The production of hydrolases by fungi was identified in all soil samples collected from MOVILA and from the rhizosphere of *A. altissima* specimens of different ages. Apparently, the percentage of hydrolase producing fungi is higher in the rhizosphere soil of *A. altissima*. The explanation lies in the fact that the number of CFU in the rhizosphere of the *A. altissima* samples was reduced compared to the control sample (hill-like area). Samples 4, 5 and 6 had higher amylase activity than the control, sample 1 comparable and samples 2, 3 and 7 lower than the control. Samples 1-6 had higher protease activity than the control and sample 7 had slightly reduced activity. Samples 1-4 and 6 had higher cellulase activity than the control, sample 7 had slightly reduced activity and in sample 5 this enzymatic activity was

absent. Samples 1, 2, 4 and 6 had higher lipase activity than the control, samples 3 and 5 had activity comparable to the control, and in sample 7 this activity was absent. Xylanase activity was present only in the control and absent in all other samples (Figure 4).

Most likely, the organic compounds present in the soil samples have a heterogeneous distribution as a result of the fact that the analyzed surface comes from the random deposits of waste from the neighboring households. As Fotiadis et al. (2011) mentioned, the spreading of *A. altissima* in urban areas is very intense, mainly in abandoned places (35.29%). The ability of the same species to grow and synthesize several types of hydrolases is the result of the versatility of the metabolism and the presence of enzyme inducers in the soil.

Our results are consistent with those obtained by Motard et al. (2015) revealing that increasing density of *A. altissima* is associated to lower soil microbial activity.

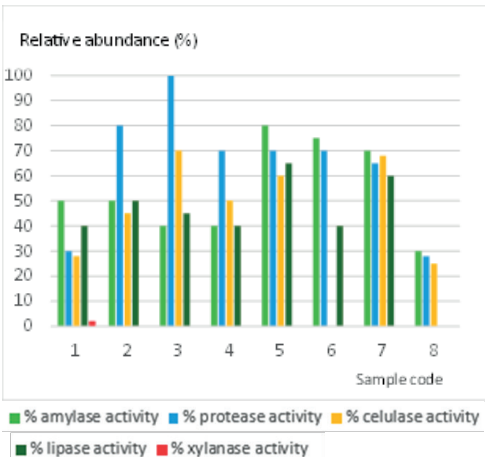


Figure 4. Relative abundances of microbial hydrolase producers recovered from the investigated site

Scavo et al. (2018) revealed that the decay/decomposition of plant materials (root system) releases a large quantity of allelochemicals into the soil with effect on microorganisms. If Hnini et al. (2024) consider that beneficial soil microorganisms, particularly Plant Growth-Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF), is a sustainable solution in general, it could be a new direction of our studies.

Many studies confirm that soil fungal communities and microbial activities do not require long invasion periods to be altered; significant shifts can occur in the short term (under 2-5 years) as invaders immediately modify soil pH, moisture, and nutrient availability (Bauman et al., 2013; Sasnow, 2014; Soler & Izquierdo, 2024; Miles et al., 2025).

CONCLUSIONS

The rhizosphere mycobiota of invasive *A. altissima* trees is significantly shaped by the density of the stands and the age of the individuals. Older stands often show increased soil nutrient pools (carbon and nitrogen) due to sustained leaf litter inputs and biological nitrogen fixation, which in turn alters fungal community structure.

This study provides a characterization of the cultivable mycobiota associated with *Ailanthus altissima*, highlighting the critical role of these fungi in soil organic matter decomposition through hydrolytic enzyme production. Our results demonstrate that fungal enzymatic activity is subject to spatial and temporal variations, influenced by both the root zone and the developmental stage of the host plant. These findings enhance our understanding of how invasive species alter nutrient cycling and soil health.

Future research should focus on integrating these culture - dependent results with metagenomic approaches to capture the full diversity of the non-cultivable fungal community. Additionally, further studies are needed to evaluate the long-term impact of these fungal-mediated processes on native plant re-establishment in post-invasion soils. Experimental trials utilizing the isolated fungal strains could also explore their potential in biotechnological applications or as indicators of soil ecosystem shifts during *A. altissima* expansion.

This research initiated the study of a sophisticated synergy between plant functional attributes and microbial activities in the rhizosphere of *Ailanthus altissima*, identifying the cultivable mycobiota associated with this invasive species as a key driver of soil organic matter decomposition, mediated by specialized hydrolytic enzyme production. We observed

that fungal enzymatic activities exhibit distinct spatial and temporal variations, which align with the broader ecological patterns identified in global meta-analyses regarding fine root biomass (RB) and functional trait dissimilarity. Our findings suggest that the success of *A. altissima* in invaded ecosystems is not only a result of its intrinsic traits - such as growth rate and stress tolerance - but also its ability to modulate belowground processes, i.e. the associated fungal communities facilitate nutrient cycling through enzymatic pathways. As these effects intensify with soil depth and developmental stages, the interplay between root architecture and microbial-mediated decomposition becomes a critical factor in ecosystem carbon sequestration and soil health. The isolated fungal strains and the root trait correlations established here provide a fundamental resource for future research. Integrating these findings with metagenomic approaches will be essential to fully decipher the mechanisms by which invasive - driven functional diversity alters the biogeochemical cycles of native habitats. Understanding spatial and temporal variations in fungal enzymatic activity is crucial for revealing their contribution to nutrient cycling and soil health in invaded ecosystems. The isolation and availability of our fungal cultures from both rhizosphere and roots will serve as valuable resources for further experimental evaluations of fungal-mediated decomposition processes in soils influenced by *A. altissima*.

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AGROECONOMIC POTENTIAL OF SMOOTH BROME (*Bromus inermis*) AND TALL FESCUE (*Festuca arundinacea*) UNDER THE CLIMATIC CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

This study was aimed to evaluate the quality indices of green biomass and hay of local ecotype of smooth brome (*Bromus inermis* Leyss.) and tall fescue (*Festuca arundinacea* Schreb.) cultivated at the “Alexandru Ciubotaru” National Botanical Garden (Institute) of Moldova State University. The biochemical composition of the green biomass varied between grass species: 12.4-13.1% crude protein (CP), 33.6-34.7% crude fibre (CF), 62.9-66.6% neutral detergent fibre (NDF), 36.9-37.8% acid detergent fibre (ADF), 2.8-3.6% acid detergent lignin (ADL), and 11.7-13.5% total soluble sugars (TSS). The forage quality of the green biomass included 594-602 g/kg dry matter digestibility (DMD), 553-563g/kg total digestible nutrients (TDN), relative feed value (RFV) of 87-90, and metabolizable energy (ME) of 9.67-9.76 MJ/kg. The hay had 10.3-12.4% CP, 36.0-36.9% CF, 65.0-69.1% NDF, and 6.2-9.4% TSS, with 528-543g/kg TDN and ME of 9.45-9.60 MJ/kg. The biochemical methane potential of the substrates ranged from 331 to 366 L/kg volatile solids. These findings suggest that smooth brome and tall fescue are both suitable as high-quality forages for livestock and as promising substrates for biomethane production within renewable energy systems.

Key words: biochemical methane potential, *Bromus inermis*, *Festuca arundinacea*, forage value, nutrient content.

INTRODUCTION

The family *Poaceae* is one of the largest plant families, of high economic and ecological value. According to *The Plant List*, the genus *Bromus* family *Poaceae* comprises approximately 170 accepted species, and the genus *Festuca* records 664 accepted species names. Floristic research has identified 11 *Bromus* species and 8 *Festuca* species present in the territory of the Republic of Moldova.

Bromus inermis Leyss. (syn. *Bromopsis inermis* (Leyss.) Holub, *Festuca inermis* (Leyss.) DC., *Forasaccus inermis* (Leyss.) Lunell, *Schedonorus inermis* (Leyss.) P. Beauv., *Zerna inermis* (Leyss.) Lindm.), commonly known as smooth brome, is a creeping, rhizomatous, perennial C₃ grass native to Europe and parts of Asia. It is a widespread species that has been introduced to various regions, including North America. The plant has erect stems, which can grow up to 150 cm in height. Leaf sheaths are either glabrous or shortly hairy, while the leaf blades are flat, 15-40 cm long and 5-10 mm wide. Both surfaces and margins of the leaf blades are scabrid, glabrous, or sparsely ciliate

at the edges. The leaf tips are acuminate, and the ligule measures 1-2 mm. The inflorescence is an open panicle, 5-20 cm long, with 1 to 4 branches per node. The spikelets are purple-brown, typically 15-30 mm long, with 6-12 florets. The rachilla internodes are 2-3 mm long and spinulose. The glumes are lanceolate with membranous margins. The lower glume is 4-7 mm long and 1-veined, while the upper glume is 6-10 mm long and 3-veined. Lemmas are oblong-lanceolate, 8-12 mm long, 5-7-veined, glabrous, scabrid at the base, may be awned (up to 3-4 mm) or awnless. The paleas are shorter than the lemmas and have ciliate keels. Anthers measure 3-4 mm in length. *Bromus inermis* is an open-pollinated, self-incompatible species, with flowering occurring from late May to early July. The caryopses are elliptical, 5-8 mm long, and range in colour from pale yellow to dark brown. The 1000-seed weight is about 2-3 g. The chromosome numbers are $2n = 14, 28$, or 56. Seeds remain viable for 6-10 years. This species is commonly found along roadsides, riverbanks, field edges, meadows, woods, and pastures. It reproduces through seeds, tillers, and rhizomes.

Tall fescue, *Festuca arundinacea* Schreber (syn. *Lolium arundinaceum* (Schreb.) Darbysh.; *Schedonorus arundinaceus* (Schreb.) Dumort.) is a long-lived perennial grass native to Europe, belonging to the C₃ photosynthetic group. It has robust, upright stems that range from 60 to 200 cm in height. The leaves grow in basal tufts, with blades measuring 20-70 cm in length and 3-12 mm in width. Each tuft can produce 10 to 30 flowering stems. The inflorescence is a panicle, which can be open or narrowly branched, ranging from 12 to 35 cm in length. The spikelets typically contain three to nine florets, and the lemmas can be either awnless or short-awned. The fruit is a caryopsis with an adherent pericarp, which is brownish-yellow, glabrous, oblong to ellipsoid, and measures 6-9 mm in length, with a 1000-seed weight of 1.8-2.6 g. Tall fescue develops a deep and extensive root system that can reach up to 150 cm in depth. This root system produces secretions that aid in nutrient mobilization and uptake from the soil. The species is highly adaptable, thriving in both moist and drought conditions, and grows well in soils with a pH between 5.5 and 8.0, including eroded slopes and slightly saline soils. Tall fescue reproduces by seed and also increases vegetatively by producing new tillers from the root crown.

Currently, *Bromus inermis* and *Festuca arundinacea* are being researched and cultivated in various regions around the world. (Tomić et al., 2007; Albayrak & Türk, 2013; Amalevičiūtė-Volungė et al., 2020; Fehlbeg et al., 2020; Dong et al., 2021; Chornolata et al., 2023; Bozhanska et al., 2022; Mackiewicz et al., 2024). Currently, in the Catalogue of Plant Varieties of the Republic of Moldova, no cultivars of these species are officially registered. However, in the Official Catalogue of Varieties of Agricultural Plants of Romania (2024), 10 cultivars of *Festuca arundinacea* and 5 cultivars of *Bromus inermis* are listed. The cultivars of smooth brome and tall fescue developed in Romania show dry matter productivity levels ranging from 12 to 20.3 t/ha (Maruşca et al., 2016; Acatrinei (Dumitru) et al., 2023).

The aim of this study was to assess the quality indices of green mass and hay from smooth brome (*Bromus inermis*) and tall fescue

(*Festuca arundinacea*) as both forage and substrates for biogas production in Moldova.

MATERIALS AND METHODS

The local ecotypes of smooth brome (*Bromus inermis*) and tall fescue (*Festuca arundinacea*), cultivated in monoculture within the experimental sector of the National Botanical Garden (Institute) of Moldova, Chişinău, were selected as experimental material. Plant samples were collected during the first harvest, conducted at the early flowering stage. The harvested biomass was chopped into fragments measuring 1.5-2.0 cm using a laboratory forage chopper. Dry matter (DM) content was determined by oven-drying the samples at 105°C until constant weight was achieved. For chemical analyses, the chopped material was dried in a forced-air oven at 60°C and subsequently ground in a beater mill equipped with a 1 mm mesh sieve.

The principal biochemical parameters assessed included crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), and digestible organic matter (DOM). These parameters were determined by near-infrared spectroscopy (NIRS) using a PERTEN DA 7200 NIR analyser. Hemicellulose (HC), cellulose (Cel), total digestible nutrients (TDN), relative forage quality (RFQ), digestible energy (DE), metabolizable energy (ME), and net energy for lactation (NEL) were calculated in accordance with established standard procedures. The carbon content of the substrates was estimated using the empirical equation proposed by Badger et al. (1979). The biochemical methane potential was calculated based on the equations described by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

During the first year of establishment, no significant differences were observed in the growth and developmental patterns of the studied grass species. By the end of the growing season, stem formation was recorded in some individuals. In subsequent years, vegetative regrowth resumed in March, starting

5-7 days earlier in *Bromus inermis* than in *Festuca arundinacea*. Growth rates accelerated during the second half of April. At the time of harvest (mid-May), the studied local ecotypes of smooth brome and tall fescue attained plant heights ranging from 95 to 109 cm, with smooth brome exhibiting greater mean height. The yield of the local ecotype of *Festuca arundinacea* reached 3.95 kg/m² of fresh biomass and 0.88 kg/m² of dry matter, whereas the local ecotype of *Bromus inermis* produced 4.19 kg/m² of fresh biomass and 1.13 kg/m² of dry matter.

The analysis of the biochemical composition of the harvested biomass (Table 1) indicated that

the dry matter contained 12.4-13.1% CP, 33.6-34.7% CF, 62.9-66.6% NDF, 36.9-37.8% ADF, 2.8-3.6% ADL, 33.0-35.0% Cel, 26.0-28.8% HC, 11.7-13.5% TSS, and 9.4-10.0% ash. The green forage of *Festuca arundinacea* was characterized by a higher crude protein content, whereas *Bromus inermis* forage exhibited lower mineral, cellulose, and hemicellulose concentrations, but higher levels of total soluble sugars and acid detergent lignin. No substantial differences were observed between the two ecotypes with respect to digestibility, relative feed value, or energy value of the harvested biomass.

Table 1. The biochemical composition and the nutritive value of green mass from *Bromus inermis* and *Festuca arundinacea*

Indices	<i>Bromus inermis</i>	<i>Festuca arundinacea</i>
Crude protein, g/kg DM	124	131
Crude fibre, g/kg DM	336	347
Ash, g/kg DM	94	100
Acid detergent fibre, g/kg DM	369	378
Neutral detergent fibre, g/kg DM	629	666
Acid detergent lignin, g/kg DM	36	28
Total soluble sugars, g/kg DM	135	117
Cellulose, g/kg DM	330	350
Hemicellulose, g/kg DM	260	288
Dry matter digestibility, %	60.2	59.4
Total digestible nutrients, g/kg	563	553
Digestible energy, MJ/kg DM	11.89	11.78
Metabolizable energy, MJ/kg DM	9.76	9.67
Net energy for lactation, MJ/kg DM	5.79	5.69
Relative feed value	90	87
Relative forage quality	92	86

The specialized literature reports considerable variation in the chemical composition and nutritive value of whole-plant biomass and forage of *Bromus inermis* and *Festuca arundinacea*. According to Medvedev and Smetannikova (1981), whole plants of *Bromus inermis* contained 60-81% moisture, 2.9-4.4% CP, 5.9-10.5% CF, 0.4% EE, 1.5-2.6% ash, and 7.6-18.3% NFE. For *Festuca arundinacea*, the dry matter of the forage contained 7.5-12.8% CP and 24.4-36.6% CF. May et al. (1998) reported that *Bromus inermis* forage contained 9.9-11.3% CP, 62.9-69.5% NDF, 42.1-43.3% ADF, 4.9-5.5% ADL, 6.3-7.6% ash, and 56.9-70.6% IVDMD. Burlacu et al. (2002) found that green forage of *Bromus inermis* contained 250 g/kg DM, 10.2% CP, 2.8% EE, 33.5% CF, 45.1% NFE, 7.4% ash, 58.2% NDF, 37.8% ADF, 28.3% Cel, and 18.3 MJ/kg gross energy (GE). In comparison, *Festuca arundinacea* forage contained 286 g/kg DM, 16.7% CP, 5.9% EE, 24.7% CF, 42.6% NFE, 10.1% ash,

and 18.8 MJ/kg GE. Flores et al. (2017) reported that tall fescue plants contained 56.5-67.8% NDF, 27.7-34.9% ADF, 28.8-34.0% HC, 25.0-28.1% Cel, and 3.61-10.05% lignin. Tomić et al. (2007) indicated that cultivars of *Festuca arundinacea* contained 10.26-11.80% CP and 26.98-30.46% CF. Zhao et al. (2008) found that crude protein concentration in tall fescue and festulolium forages was 111 g/kg DM, but in smooth brome grass 147 g/kg DM. Utyamishv & Reznichenko (2009) reported that at the heading stage, *Bromus inermis* forage contained 15.16% CP, 2.49% EE, 28.88% CF, 46.27% NFE, 1.12% starch, 1.95% sugars, 7.2% ash, 18.50 MJ/kg GE, and 9.43 MJ/kg metabolizable energy (ME). At the flowering stage, CP decreased to 7.42%, while CF increased to 30.63%, and digestibility declined to 552 g/kg DM, with 18.14 MJ/kg GE and 9.11 MJ/kg ME. Hayes et al. (2010) reported that the herbage of *Bromus stamineus* contained 7.0% CP, 61.3% NDF, 33.3% ADF,

7.0% ash, 58.7% dry matter digestibility (DMD), and 8.5 MJ/kg ME, whereas *Festuca arundinacea* forage contained 9.5-10.8% CP, 58.2-61.8% NDF, 35.9% ADF, 7.0-8.0% ash, 58.6-59.6% DMD, and 8.6 MJ/kg ME. Albayrak et al. (2011) found that *Bromus inermis* forage contained 10.45-11.30% CP, 57.85-59.70% NDF, 41.75-43.50% ADF, 45.16-47.45% TDN, and RFV of 85.57-88.11. Naydenova (2012) reported that green biomass of *Bromus inermis* harvested at the heading-flowering stage contained 16.55-20.29% CP, 27.83-30.04% CF, 33.45-41.62% ADF, 59.44-64.12% NDF, 2.71-5.85% ADL, 22.50-26.00% HC, 30.74-35.77% cellulose, 58.04-70.38% IVDMD, 54.17-68.65% IVOMD, 10.45-11.04 MJ/kg ME, and RFV values ranging from 132 to 172. For *Festuca arundinacea*, the corresponding values were 14.66-17.67% CP, 28.60-30.75% CF, 32.73-37.93% ADF, 52.50-61.84% NDF, 2.58-5.69% ADL, 19.77-23.91% HC, 30.15-34.61% cellulose, 60.69-63.75% IVDMD, 59.92-62.50% IVOMD, 10.00-10.30 MJ/kg ME, and RFV values of 145-159. Albayrak & Türk (2013) reported that smooth brome grass harvested in late May contained 8.5-8.7% CP, 61.1-61.3% NDF, 44.6-46.4% ADF, and an RFV of 80-82. Bulokhov (2014) showed that the dry matter of *Festuca arundinacea* green biomass contained 12.0% CP, 2.7% EE, 32.6% CF, 45.2% NFE, and 9.8% ash, with a nutritive value equivalent to 0.37 feed units per kg fresh mass and 78 g digestible protein per feed unit. McEniry & O'Kiely (2014) reported that in Ireland, *Festuca arundinacea* biomass harvested in mid-May contained 15.2% CP, 8.6% ash, 52.9% NDF, 26.7% ADF, and 16.1% soluble carbohydrates. When harvested in early June, CP decreased to 11.2%, while ash increased slightly to 9.0%, and structural fibre fractions rose to 62.3% NDF and 37.2% ADF; soluble carbohydrates declined to 9.2%. Ünal and Mutlu (2015) observed that smooth brome grass harvested at the flowering (blooming) stage contained 17.82-21.78% CP, 62.00-66.38% NDF, 34.55-37.67% ADF, and RFV of 107.72-113.89. Kshnikatkina et al. (2016) reported that the first-cut green biomass of *Festuca arundinacea* contained 9.25% CP, 6.51% DP, 1.15% EE, 29.57% CF, 52.36% NFE, 8.19% ash, 9.62 MJ/kg ME, 0.75 nutritive unit/kg

DM, and 86.9 g DP per nutritive unit. In the second cut, the respective values were 8.40% CP, 5.90% DP, 1.32% EE, 27.80% CF, 53.91% NFE, 8.42% ash, 9.74 MJ/kg ME, 0.76 nutritive units per kg DM, and 76.8 g DP per nutritive unit. Jenson et al. (2017) found that smooth brome harvested in June contained 13.4% CP and 48.1% NDF, with a neutral detergent fibre digestibility of 73.7%. Pocienė & Kadžiulienė (2016) demonstrated that tall fescue biomass, depending on fertilizer type and application rate, contained 14-20% HC, 34-36% Cel, and 6-9% lignin. Czyż et al. (2017) reported that the forage obtained from *Festuca arundinacea* cultivars at the first cut contained 14.3-15.2% CP, 29.3-30.2% CF, 2.0-2.3% EE, and 5.2-6.9% soluble sugars. Chornolata et al. (2018) reported that the green biomass of *Festuca arundinacea* harvested at the heading stage contained 10.0% CP, 1.88% EE, 5.41% ash, 22.76% CF, 41.51% NDF, 21.23% ADF, 59.95% NFE, 3.39% starch, 16.13% cellulose, 12.30% hemicellulose, and 7.10% lignin. For comparison, *Agropyron intermedium* harvested at the same stage contained 9.76% CP, 2.00% EE, 7.18% ash, 22.40% CF, 45.28% NDF, 25.25% ADF, 54.45% NFE, 1.88% starch, 17.35% cellulose, 14.55% hemicellulose, and 6.75% lignin. Tenikecier & Ateş (2018) reported that *Bromus inermis* harvested at the full flowering stage contained 14.23% CP, 55.11% NDF, 30.19% ADF, 3.88% Ca, and 0.22% P. Türk et al. (2018) found that forage from *Bromus inermis* contained 8.91% CP, 59.55% NDF, 45.11% ADF, 43.11% TDN, and RFV of 83.96. Under different nitrogen fertilization rates, CP ranged from 8.88 to 12.02%, NDF from 51.49 to 58.01%, ADF from 36.38 to 43.34%, TDN from 45.40 to 54.38%, and RFV from 88.40 to 109.38. Bandanova & Butukhanov (2019) reported that awnless brome (*Bromus inermis*) harvested at the heading stage contained 16.3% CP, 2.9% EE, 34.0% CF, 36.8% NFE, 5.3% ash, 1.32% Ca, and 0.37% P. At the flowering stage, CP decreased to 14.8%, CF increased to 37.8%, ash increased to 7.53%, and P decreased to 0.30%, while EE (3.0%), NFE (37.0%), and Ca (1.40%) showed minor variation. Kazarina et al. (2019) reported that local varieties of *Bromus inermis* contained 13.56-16.39% CP, 8.26-13.37% TSS, and 102.6-174 mg/kg

carotene. Pavlova et al. (2019) indicated that the green biomass of *Bromus inermis* grown in monoculture contained 10.6% CP, 1.7% EE, 35.0% CF, 47.5% NFE, 8.5 MJ/kg ME, 0.58 nutritive units per kg, and 63.7 g digestible protein per nutritive unit. Sokolović et al. (2019) reported that the first cut of *Bromus inermis* contained 11.61% CP, 33.23% CF, 3.09% EE, 60.19% NDF, 40.33% ADF, and 7.69% ash. Țiței et al. (2019) found that fresh forage from tall fescue (*Festuca arundinacea*) cultivars contained 114-136 g/kg CP, 74-89 g/kg CA, 582-593 g/kg NDF, 392-396 g/kg ADF, 34-41 g/kg ADL, 322-329 g/kg Cel, and 226-229 g/kg HC. Digestibility and energy values were 60.3-63.8% DDM, 57.2-62.2% OMD, 9.12-9.62 MJ/kg ME, and 5.69-5.86 MJ/kg net energy for lactation (NEI). Amalevičiūtė-Volungė et al. (2020) reported that green biomass of *Festuca arundinacea* contained 15.05% CP, 7.52% ash, 52.19% NDF, 31.3% ADF, 4.02% ADL, 16.4% water-soluble carbohydrates (WSC), 27.3% Cel, 20.9% HC, and 62.5% DDM. Coblenz et al. (2020) found that tall fescue herbage contained 75 g/kg CP, 82 g/kg ash, 107.3 g/kg WSC, 627 g/kg NDF, 356 g/kg ADF, 27.9 g/kg ADL, and 1.40 Mcal/kg NEI. Karbivska et al. (2020) reported that unfertilized *Bromus inermis* forage contained 10.6% CP, 3.3% EE, 29.9% CF, 49.9% NFE, 8.3% ash, 0.41% Ca, 0.34% P, 55% DDM, 0.69 nutritive units per kg, 8.0 MJ/kg ME, and 109 g DP per nutritive unit. Reiné et al. (2020) reported that *Festuca arundinacea* plants contained 455 g/kg DM, with 7.2% CP, 4.4% ash, 2.0% EE, 73.3% NDF, 41.1% ADF, 4.0% ADL, 56.8% DDM, 0.12% P, and 0.35% Ca. Shepelev et al. (2020) reported that the nutrient concentration in green biomass of *Bromus inermis* varieties ranged from 13.39 to 17.07% CP and 29.9 to 33.0% CF. Dong et al. (2021) found that *Festuca arundinacea* forage contained 306 g/kg DM, 7.29% CP, 24.8% ADF, 54.7% NDF, 14.4% WSC, 9.08% ash, and 6.97% EE. Baykalova and Serebrennikov (2022) reported that *Bromus inermis* cultivars harvested at the budding stage contained 17.5–25.5% CP, 24.13-28.4% CF, and 1.7-1.9 MJ/kg ME in fresh biomass. Feoktistova and Leonidov (2022) noted that *Bromus inermis* is characterized by a leaf proportion of 38-50% in the harvested biomass,

with 7.0-12.0% CP and 28.2-34.0% CF. Bozhanska et al. (2022) reported that *Bromus inermis* plants contained 13.26-15.27% CP, 2.17-2.31% EE, 34.30-41.23% CF, 35.51-41.78% NFE, 5.67-6.71% ash, 0.9-2.78% Ca, and 0.06-0.21% P. In comparison, *Festuca arundinacea* plants contained 9.89-15.26% CP, 1.49-2.40% EE, 32.06-37.27% CF, 39.55-48.38% NFE, 6.39-7.26% ash, 0.8-2.31% Ca, and 0.03-0.17% P. Acatrinei (Dumitru) et al. (2023) indicated the crude protein in Romanian cultivars of *Bromus inermis* varied from 12.74 to 14.66%. Becker et al. (2023) reported that *Festuca arundinacea* forage contained 17.6% CP and 6.2 MJ/kg net energy. Chornolata et al. (2023) found that herbage DM of *Bromus inermis* contained 6.71-8.92% CP, whereas *Festuca arundinacea* forage contained 5.21-10.10% CP. Coşman et al. (2023) reported that *Festuca arundinacea* forage contained 6.91-10.59% CP, 2.02-2.80% EE, 31.48-34.89% CF, 37.11-42.27% NFE, 9.80-11.98% ash, 0.23-0.29% Ca, 0.20-0.251% P, and 8.14-8.95 MJ/kg ME. Zaiț et al. (2023) indicated that *Festuca arundinacea* grown in pure stand contained 11.74% CP, 64.53% NDF, and 39.73% ADF, with a relative forage quality (RFQ) of 87.8. Yavuz & Kır (2023) remarked that smooth brome forage had 12.3-12.5 % CP, 53-56% NDF, 39.0-42.6% ADF, 5.3-6.5% ADL. Waliszewska et al. (2024) reported that DM of *Bromus inermis* contained 38.65% cellulose, 14.89% lignin, 68.40% holocellulose, 29.75% hemicellulose, and 8.57% ash. Nazare & Țiței, (2025) found that biochemical composition and nutritional value of green fodder from *Festuca* species was 8.6-12.4% CP, 7.5-8.9% ash, 31.5-42.4% CF, 3.0-5.5% ADL, 30.9-39.1% Cel, 23.5-29.2% HC, 4.0-12.2%TSS, 492-651 g/kg DDM, 443-601 g/kg DOM, 527-646 g/kg TDN, RFQ=70-110, 8.90-10.01 ME, 4.91-6.13 MJ/kg NEI.

Haymaking represents one of the oldest and most widely used methods of forage preservation. However, the dehydration process may alter the chemical composition and nutritive value of forage; therefore, the biochemical characteristics of hay prepared from the studied ecotypes were investigated (Table 2). The biochemical composition of the prepared hay was as follows: 10.3-12.4% CP, 10.6-10.8% ash, 36.0-36.9% CF, 38.4-39.7%

ADF, 65.0-69.1% NDF, 4.2-4.6% ADL, and 6.2-9.4% TSS. The nutritive value ranged between 580 and 590 g/kg digestible dry matter, with RFV values of 76-84, digestible energy of 11.51-11.70 MJ/kg, metabolizable energy of 9.45-9.60 MJ/kg, and net energy for lactation (NEI) of 5.47-5.62 MJ/kg. Compared with the initial green biomass, the haymaking process resulted in increased concentrations of structural cell wall components and minerals, accompanied by reductions in crude protein

and total soluble sugars. These compositional changes led to decreased dry matter digestibility and overall energy value. More pronounced reductions in crude protein and soluble sugars were observed in *Bromus inermis* hay. The hay derived from *Festuca arundinacea* was characterized by adequate crude protein, acid detergent lignin, and hemicellulose contents, but a higher cellulose concentration and slightly lower energy value compared with *Bromus inermis* hay.

Table 2. The biochemical composition and the feed value of the hay from *Bromus inermis* and *Festuca arundinacea*

Indices	<i>Bromus inermis</i>	<i>Festuca arundinacea</i>
Crude protein, g/kg DM	103	124
Crude fiber, g/kg DM	360	369
Ash, g/kg DM	108	106
Acid detergent fiber, g/kg DM	384	397
Neutral detergent fiber, g/kg DM	650	691
Acid detergent lignin, g/kg DM	46	42
Total soluble sugars, g/kg DM	62	94
Cellulose, g/kg DM	338	355
Hemicellulose, g/kg DM	266	294
Dry matter digestibility, %	59.0	58.0
Total digestible nutrients, g/kg	548	523
Digestible energy, MJ/kg DM	11.70	11.51
Metabolizable energy, MJ/kg DM	9.60	9.45
Net energy for lactation, MJ/kg DM	5.62	5.47
Relative feed value	84	78
Relative forage quality	87	80

Published data indicate considerable variability in the chemical composition and nutritive value of hay derived from *Bromus inermis* and *Festuca arundinacea*. For example, Medvedev and Smetannikova (1981) reported that the hay from *Bromus inermis* contained 8.0-12.9% CP, 24.0-29.0% CF, 2.4-2.9% EE, 4.7-8.0% ash, and 37.4-41.4% NFE. Burlacu et al. (2002) found that tall fescue (*Festuca arundinacea*) hay contained 6.9-9.0% ash, 11.5-16.7% CP, 4.7-5.9% EE, 26.9-31.2% CF, 41.5-45.7% NFE, 35.0-39.0% ADF, 4.3-5.2% lignin, and 18.9-19.0 MJ/kg GE. Angima & Kallenbach (2008) reported that, depending on fertilization rate, tall fescue hay contained 6.37-7.99% CP with RFV of 96-104, whereas smooth brome (*Bromus inermis*) hay contained 7.76-8.28% CP and an RFV of 92-94. Phillips et al. (2009) indicated that *Bromus inermis* hay contained 2.10% N, 71.44% NDF, and 37.23% ADF. Utyamishv and Reznichenko (2009) reported that the hay from *Bromus inermis* harvested at the flowering stage contained 7.89% CP, 1.9% EE, 30.0% CF, 44.9% NFE, and 8.6 MJ/kg ME. Ünal and Mutlu (2015) found that smooth brome hay harvested at the flowering stage contained 19.80% CP, 36.10% ADF, 64.18%

NDF, 60.78% DDM, and an RFV of 113.89. Bender et al. (2016) reported that tall fescue hay contained 143 g/kg CP, 107 g/kg ash, 30 g/kg lipids, 644 g/kg NDF, 81 g/kg ADL, and 16 g/kg starch. Kaplan et al. (2017) found that tall fescue hay DM contained 5.56% CP, 64.94% NDF, 41.01% ADF, 1.61% EE, and 12.03% ash, with 48.54% OMD, 7.00 MJ/kg ME, and 3.75 MJ/kg NEI. Jenson et al. (2017) reported that smooth brome harvested in June and conserved as hay contained 13.4% CP, 48.1% NDF, and 73.7% NDFD. Akdeniz et al. (2019) characterized *Festuca arundinacea* hay by 9.86% CP, 9.54% ash, 1.15% EE, 44.85% CF, 64.05% NDF, 47.64% ADF, and an RFV of 75.22. Fehlberg et al. (2020) reported that smooth brome hay contained 9.6% CP, 2.7% EE, 63.3% NDF, 35.6% ADF, and 2.12 Mcal/kg DE for horses. Foster et al. (2021) found that hay from a hybrid brome grass (*Bromus riparius* × *Bromus inermis*) contained 8.3-8.5% CP, 38.9-39.6% ADF, and 563-571 g/kg TDN. Tan and Çoruh (2021) reported that smooth brome hay harvested from plants sown in spring contained 12.36-13.24% CP, 35.15-37.54% ADF, and 40.16-47.12% NDF, whereas summer-sown stands produced hay

containing 12.38-14.01% CP, 33.94-37.66% ADF, and 35.26-47.27% NDF. Yazici and Tan (2021) found that hay from tall fescue cultivars contained 13.47-18.67% CP, 42.48-50.05% NDF, and 20.20-30.97% ADF, with RFV values ranging from 127.1 to 144.4. Coşman et al. (2023) reported the nutrient content of hay from *Festuca arundinacea* as follows: 5.88-12.31% CP, 0.83-2.60% EE, 31.07-37.20% CF, 31.50-44.95% NFE, 8.51-12.31% ash, and 8.90-64.67 mg/kg carotene. Similarly, Țiței (2023) found that prepared tall fescue hays contained 10.84% CP, 2.65% EE, 33.38% CF, 40.54% NFE, 12.59% ash, 2.60 g/kg Ca, 2.30 g/kg P, with 17.44 MJ/kg GE, 8.85 MJ/kg ME, and 4.93 MJ/kg NEL. Nazare & Țiței (2025) reported that tall fescue hay contained 10.0% CP, 39.3% CF, 8.9% ash, 42.7% ADF, 72.0% NDF, 3.5% ADL, 5.5% TSS, 37.2% Cel, and 29.2% HC, with a forage value of 548 g/kg DDM, 9.10 MJ/kg ME, and 5.13 MJ/kg NEL.

The use of grass biomass for anaerobic digestion in biogas reactors has recently gained significant interest in Europe for biomethane production as a renewable energy source. The quality indices of substrates from the studied local grass ecotypes, along with their biomethane potential, are presented in Table 3. It is important to note that the investigated substrates exhibit a favourable carbon-to-nitrogen (C/N) ratio for the activity of methanogenic bacteria (C/N = 23.85-30.25), hemicellulose concentration (260.00-294.00 g/kg), and acid detergent lignin (ADL) content (28.00-46.00 g/kg). Hay substrates were found to have higher ADL content and lower carbon and nitrogen concentrations, which negatively affected their biomethane potential. The biochemical methane potential ranged from 331 to 366 L/kg VS, with the highest biomethane potential observed in *Festuca arundinacea* green mass substrates.

Table 3. The biochemical biomethane production potential of the investigated substrates

Indices	<i>Bromus inermis</i>		<i>Festuca arundinacea</i>	
	green mass	hay	green mass	hay
Crude protein, g/kg DM	124.00	103.00	131.00	124.00
Nitrogen, g/kg DM	19.84	16.48	20.96	19.84
Ash, g/kg DM	94.00	108.00	100.00	106.00
Carbon, g/kg DM	503.33	495.56	500.00	496.66
Ratio carbon/nitrogen	25.37	30.25	23.85	25.03
Acid detergent lignin, g/kg DM	36.00	46.00	28.00	42.00
Hemicellulose, g/kg DM	260.00	266.00	288.00	294.00
Biomethane potential, L/kg VS	351.00	331.00	366.00	343.00

Several researchers have explored the biomethane potential of various grass species and their substrates, with varying results depending on factors such as substrate composition and treatment methods. Mähnert et al. (2002) reported that *Festuca arundacea* fresh mass co-substrates had 139 g/kg dry matter (DM), and gas production ranged from 688 to 836 L/kg VS over 28 days, while co-substrates contained 173 g/kg DM and 89.6% ODM, yielding 818-887 L/kg VS. Ust’ak et al. (2013) reported specific methane yields of 239 L/kg DM for substrates derived from tall fescue, 214 L/kg DM for meadow fescue, 237 L/kg DM for Italian ryegrass, and 222–238 L/kg DM for the festulolium hybrid. Similarly, McEniry and O’Kiely (2014) observed that methane production from *Festuca arundinacea* biomass ranged between 216 and 268 L/kg, depending on the harvest stage. Higher methane yields

were reported by Kandel et al. (2017), who found that first-cut biomass of *Festuca arundinacea* produced between 401 and 428 L/kg, corresponding to a methane productivity of approximately 6871 m³/ha. Țiței et al. (2019) revealed that the biomethane production potential of *Festuca arundinacea* green mass substrates were consistently between 349 and 354 L/kg VS. Żurek & Martyniak (2020) also reported biogas yields of 518.3 L/kg VS with 50.4% methane for smooth brome grass, 611.9 L/kg VS with 54.8% methane for ryegrass, and 485 L/kg VS with 50.23% methane for rescue grass. Amalevičiūtė-Volungė et al. (2021) found that the methane yields of substrate from tall fescue were 191 L/kg VS, perennial ryegrass 205.7 L/kg VS, orchard grass 213.9 L/kg VS, switchgrass 143.4 L/kg VS, and reed canary grass 123.1 L/kg VS. Zhang et al. (2021) reported that methane yields from *Festuca arundinacea*

substrates ranged from 259 to 446 L/kg VS, compared to 220 to 330 L/kg VS for lucerne substrates, and 196-436 L/kg VS for maize substrates. Czubaszek et al. (2024) found that substrates from spring-cut grass verges, predominantly composed of perennial ryegrass, had a dry matter content of 380 g/kg DM, 682.2 g/kg VS, 20.76 g/kg nitrogen, 378.8 g/kg total organic carbon, and a biogas yield of 715.05 L/kg VS with 59.7% methane content.

CONCLUSIONS

The green biomass and hay obtained from the studied local ecotypes of *Bromus inermis* and *Festuca arundinacea* were characterized by substantial nutrient concentrations, confirming their suitability as valuable feed resources for farm animals. In addition, the biochemical composition of the biomass indicates considerable potential for use as feedstock for biomethane production, contributing to renewable energy generation.

Despite these differences, both ecotypes produced forage and hay of adequate nutritive value, suggesting that smooth brome (*Bromus inermis*) may be preferable for higher yields, whereas tall fescue (*Festuca arundinacea*) offers greater protein content, supporting their effective use in livestock feeding systems.

Overall, the obtained results highlight the potential of the studied local ecotypes of *Bromus inermis* and *Festuca arundinacea* as promising genetic resources for breeding programs aimed at developing new locally adapted grass cultivars for sustainable agricultural production.

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GROWTH AND DEVELOPMENT OF THE WEED JOINTED GOATGRASS (*Aegilops cylindrica* Host) EMERGING FROM DIFFERENT DEPTHS

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Abstract

Jointed goatgrass is an overwintering plant from the Poaceae family. It is used as a gene donor for winter wheat in different breeding programs. Nevertheless, in some countries and in Bulgaria as well it has turned into a big issue as a weed in the winter wheat. The plants are resistant to most of the herbicidal substances registered for weed management in wheat. Therefore, different approach for control has to be performed. One of the options is to apply "weed seed burial method" - turning over the arable layer through plowing. A pot experiment for studying the germination depth and development of the jointed goatgrass was performed. The depths of placing the weed's seeds from 0 to 14 cm were studied. The period of emergence from each sowing depth, number and percent of germinated plants, total plant length, aboveground and root length, total plant weight, aboveground and root weight were evaluated. With increasing the depth, a delay of the germination and decreasing of the weed's biomass development was recorded. Plants sown on 12 and 14 cm did not emerge.

Key words: *Aegilops cylindrica*, weed, germination, growth.

INTRODUCTION

Aegilops cylindrica Host, also known as jointed goatgrass, is an annual monocotyledonous plant part of the Poaceae family. The weed is native to southern Europe and Russia, along with winter wheat and other cereals (Donald and Ogg, 1991). The weed species is capable of reducing winter wheat yields from 25 to 50% (Kennedy and Stubbs, 2007).

Due to the weed's relationship to winter wheat, it is very difficult to control. On the one hand, the seeds are similar in shape and size to those of wheat, which makes it difficult to remove by grain cleaning methods. On the other hand, the plants are very similar genetically. This makes chemical weed control extremely difficult (Donald and Ogg, 1991) and creates problems for farmers, who have to bear the losses of diminished yields and decreased grain quality (Kennedy and Stubbs, 2007).

Various studies have been conducted worldwide on methods for control of the Jointed goatgrass:

- Biological control

Donald and Ogg (1991) stated that selective control of *A. cylindrica* seedlings with phytotoxic microorganisms should be investigated. Several studies have evaluated the use of soil microorganisms for selective control

of the weeds. It was found that a great number of naturally occurring bacteria tested for their effect on *A. cylindrica* has inhibited the weed by more than 30% in the field (Kennedy and Stubbs, 2007).

- Mechanical control

According to (Westra, 1989), deep plowing is an effective mean for *A. cylindrica* suppressing. After fallow cultivation, plowing provided 95% weed control.

In a winter wheat-fallow rotation, fall or spring tillage with a chisel plow under a conventional tillage system reduced the infestation with *A. cylindrica* by nearly 50% (Evans et al., 1999).

In a study conducted by Anderson (1993), it was found that incorporating plant residues into the soil reduced the fresh weight of *A. cylindrica* by 70% to 85%.

- Agrotechnical control (crop rotations, variety selection, sowing rates)

Studies have been conducted to document the effects of crop rotation on *A. cylindrica* density in winter wheat. In a three-year crop rotation involving winter wheat, late spring crop, and summer fallow was 10% lower than *A. cylindrica* density found in a two-year crop rotation of winter wheat and fallow (Lyon and Baltensperger, 1995).

Adding sorghum or sunflower to a winter wheat-fallow rotation reduced *A. cylindrica* density more than other cultural practices studied by White et al. (2004).

Anderson (1997) reported that the tall winter wheta variety 'Lamar' provided higher suppression of the weed than the medium tall variety 'TAM 107' (Porter et al., 1987).

Kappler et al. (2002) conducted field trials to investigate the effect of winter wheat sowing density on the competitiveness of *A. cylindrica*. Increased wheat density reduces the reproductive capacity of the weed.

Yenish and Young (2004) investigated the effects of wheat height, sowing rate, and seed size on the competitiveness of *A. cylindrica*. They found that increased wheat sowing rate reduced grain density, total biomass, and ear biomass.

- Chemical control

Geier and Stahlman (1996) tested herbicides for selective control of the weed *A. cylindrica* in winter wheat. The researcher obtained limited success. The weed showed greater sensitivity to sulfosulfuron than winter wheat, but was two to three times more tolerant than *Bromus* spp. Overall, efforts to find herbicides that provide economically significant levels of control of *A. cylindrica* in conventional wheat have been unsuccessful.

Based on the above, the weed seems to be very difficult to control through herbicide applications. However, Newhouse et al. (1992) bred imidazolinone-tolerant winter wheat that allowed the use of *imazamox* for weed control, but Seefeldt et al. (1998) mentioned concerns about the possibility of resistant hybrids between winter wheat and the weed.

Prior to the development of imidazolinone-resistant winter wheat, herbicidal control of *A. cylindrica* in this crop was limited primarily to periods when the plants were harvested. Nonselective herbicides, particularly *glyphosate*, are effective for controlling the weed in fallow fields (Beck et al., 1995; Wiese et al., 1995).

Imazamox-tolerant winter wheat varieties were first introduced to the market in 2001 (Tan et al., 2005) and highly effective and selective control of *A. cylindrica* was achieved. Results from field trials showed that *imazamox* could control the weed up to 97% (Ball et al., 1999).

The aim of the current study was to establish the emergence depth of *A. cylindrica* Host and measure the biomass development of the weed.

MATERIALS AND METHODS

The pot experiment was conducted in 2025. For the purpose of the experiment relatively equal in shape and size seeds of the weed jointed goatgrass were used. The seeds were put in conditions to start germination and were sown after germinated.

For the purposes of the trial 19 litter pots were used. In each pot 10 seeds were sown. The sowing was performed in February.

The soil (Mollic Fluvisols) characteristics were:

- pH 6.3;
- 28.53 mg/kg Total N;
- 17.51 mg/100 g P₂O₅;
- 35.14 mg/100 g K₂O.

The pots were regularly watered depending on the soil moisture.

The trial was designed to evaluate the emergence depth of the weed and the following variants were evaluated:

1. 0 cm (Control);
2. 2 cm;
3. 4 cm;
4. 6 cm;
5. 8 cm;
6. 10 cm;
7. 12 cm;
8. 14 cm.

All variants were in five replications.

The evaluated parameters of the weed:

- Emergence after sowing, days;
- Emergence depth, cm;
- Number of emerged plants and percent of emergence;
- Total plant weight, g;
- Total plant length, cm.

For statistical analyses of collected data, Duncan's multiple range test (1955) of SPSS program was used. Statistical differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The next few figures present the results of the pot experiment conducted to determine the depth of emergence. It should be emphasized that the experiment was conducted in February 2025. So

the weed plant did not have the period of full vernalization, due to which the plants form above-ground and root mass, but did not develop reproductive organs. The need of vernalization of the weed was described by Donald (1984) and Mallory-Smith et al. (2018). The author found that the weed has a quantitative requirement for vernalization in order to flower. This is the reason why there are no results obtained for the total number of tillers, the number of productive tillers, the length of the ears and the mass of the ears. Despite these statements the obtained results showed the great biomass development capacity that the jointed goatgrass possess. According to Donald et al. (1991) the optimal depth for germination and emergence of the weed is 1-3 cm. Therefore, as found by Morrow et al. (1982) the weed did not emerge from below 5 cm in greenhouse and 6 cm in laboratory. After conducting our experiment, it was found that the jointed goatgrass plants that develop from the soil surface at variant 1 (0 cm) sprout the fastest – in 6 days (Figure 1). Plants sprout more slowly from seeds sown at 2 and 4 cm (Variants 2 and 3) – 8 and 10 days after sowing, respectively. Plants placed at 6, 8 and 10 cm sprout for a period of 24 to 28 days.

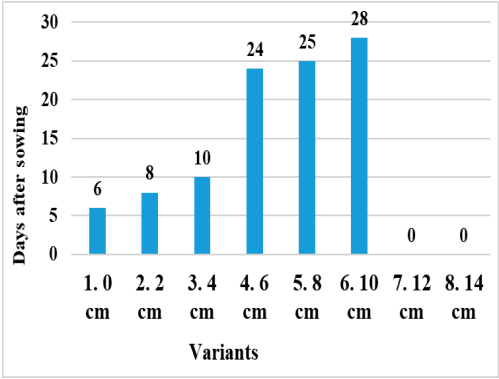


Figure 1. Emergence after sowing, days

Research has shown that the species can germinate from depths of up to 10-15 cm (Donald et al., 1991). In our study it was found that plants sown at 12 and 14 cm do not emerge

and the statement of the author was not confirmed. More information for the weed’s germination could be obtained from the Figures 2, 3.

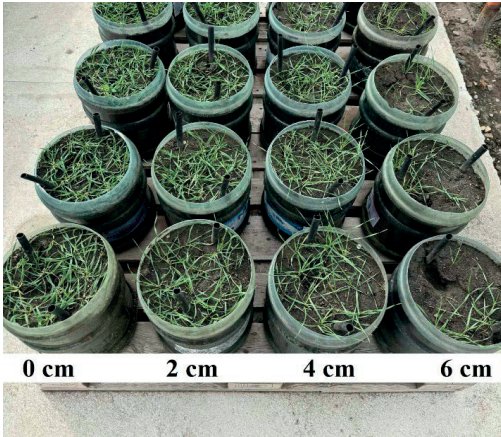


Figure 2. Emergence of the plants at variants from 1 to 4

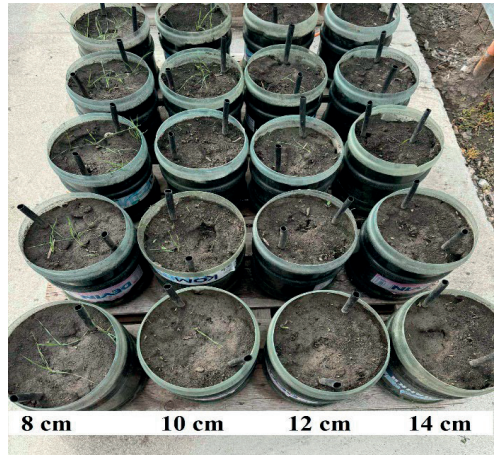


Figure 3. Emergence of the plants at variants from 5 to 8

Figure 4 shows the results related to the number of emerged plants from the different sowing depths. It was found that plants from variants 1, 2 and 3 (sown at 0, 2 and 4 cm) had 100% emergence (all 10 seeds sown). With increasing sowing depth, the percentage of emerged plants decreased from 68% (6.8 sprouted plants) in variant 4 (6 cm) to 18% (1.8 sprouted plants) in variant 6 (10 cm). The percentage of sprouted plants from 12 and 14 depths (Variants 7 and 8) was 0%.

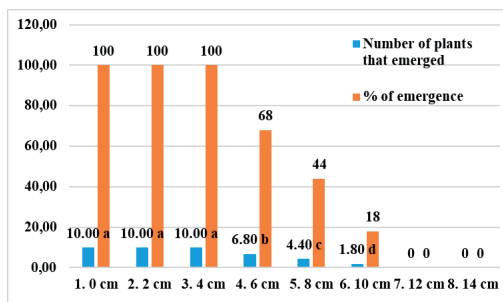


Figure 4. Number of emerged plants and percent of emergence

Numbers with different letters are significantly different according to Duncan's test ($p < 0.05$)

The weight of the formed, developed biomass of the weed is presented in Figure 5. The highest total weight of the biomass (roots + aboveground biomass) was measured in plants from variant 2 - 298.54 g, which emerged from 2 cm of depth, with the weight being relatively evenly distributed between that of the roots and aboveground mass.

The biomass of one plant in variant 1, where the plants develop from the soil surface, had a slightly lower weight - 273.93 g.

With increasing emergence depth, the weight of the plant biomass decreases and reaches 76.22 and 76.50 g in variant 5 and variant 6 respectively.

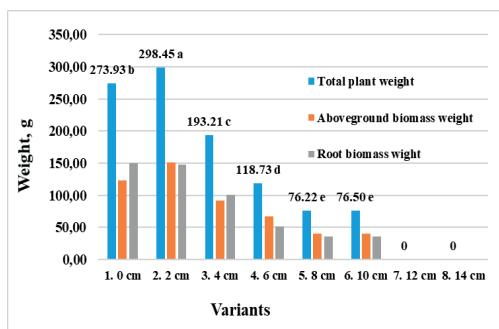


Figure 5. Total plant weight, g

Numbers with different letters are significantly different according to Duncan's test ($p < 0.05$)

The plants that develop from the soil surface were distinguished by the highest length – 76.00 cm. Plants that sprout from 2 and 4 cm in variants 2 and 3 had a length of 74.00 cm. With

increasing emergence depth, the length of the plants decreased – from 76.00 to 59.00 cm. It is worth noting that the plants form longer roots than above-ground mass (Figure 6).

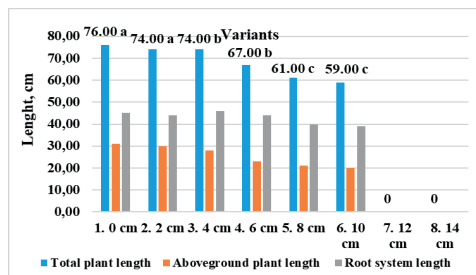


Figure 6. Total plant length, cm

Numbers with different letters are significantly different according to Duncan's test ($p < 0.05$)

The next figures (7-12) give greater idea of the weed jointed goatgrass biomass development.



Variant 1. Germination from 0 cm

Figure 7. Variant 1 (Control). Plants in the emergence stage from 0 cm



Variant 2. Germination from 2 cm

Figure 8. Variant 2. Plants in the emergence stage from 2 cm



Variant 3. Germination from 4 cm

Figure 9. Variant 3. Plants in the emergence stage from 4 cm



Variant 4. Germination from 6 cm

Figure 10. Variant 4. Plants in the emergence stage from 6 cm



Variant 5. Germination from 8 cm

Figure 11. Variant 5. Plants in the emergence stage from 8 cm



Variant 6. Germination from 10 cm

Figure 12. Variant 6. Plants in the emergence stage from 10 cm

CONCLUSIONS

The emergence of the weed was faster – 6-10 days after sowing for the depths of 0, 2 and 4 cm. The plants sown deeper emerge 25 to 27 days after sowing.

The seeds of the weed sown on 12 and 14 cm of depth does not emerge.

There was 100% of emergence of the plants sown from 0 to 4 cm of depth, while the percent of emergence decreased from 68 to 18 for the sowing depths of 6 to 10 cm.

The plants emerging from 2 cm of depth developed the highest total biomass, and were followed from those emerged on the soil surface. The length of the total biomass of the weed was the highest when the plants emerge from the soil surface. The results were followed by the 2 cm emergence depth.

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COMPARATIVE ANALYSIS OF THE STANDS' STRUCTURE OF VIRGIN AND MANAGED BEECH FORESTS FROM THE SEMENIC MOUNTAINS

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Abstract

This paper presents the results of a comparative analysis of the structural characteristics of virgin and managed beech forests in the Semenic Mountains. The research was conducted in eight sample areas, each measuring 2,500 m², with two areas in each forest category (virgin and managed) at two altitudes (1200 m and 1350 m). The structural characteristics were compared using the following parameters: diameter distribution, height distribution, maximum dimensions of living trees (with emphasis on thick trees), Gini index, Shannon index, slenderness coefficient, and regeneration. The density of the stands and their productive characteristics were also studied, using the number of trees, basal area, and volume as attributes. The greatest differences were found in terms of tree diameters, slenderness coefficients, and regeneration, which may lead to the conclusion that silvicultural interventions carried out in managed forests over time have significantly affected their structure.

Key words: beech forest, virgin forest, managed forest, structural diversity.

INTRODUCTION

In Europe, beech is the predominant native species providing multiple ecosystem services, with its range extending from hilly to mountainous regions (Kulla et al., 2023). At the European level, beech occupies approximately 15 million hectares (Brunet et al., 2010), while its share in the total volume of wood is approximately 12% (Forest Europe, 2020). According to the potential vegetation in Europe, beech forests could cover a much larger area than the current one, with an estimated coverage of approximately 92 million hectares (Bohn et al., 2004). Similar to Europe, beech is also the most widespread species in Romania, occupying about 31% of the country's forest area. It has a very wide altitudinal range, forming the upper limit of the forest in the Semenic Mountains (Stănescu et al., 1997).

Intensive exploitation of European forests has led to their homogenization and simplification in terms of composition and structure, leaving only remnants of virgin forest ecosystems in the temperate zone (Kisin et al., 2025). It is estimated that approximately 0.3 million hectares of intact forests (0.4% of the total forest area) are found in strict forest reserves and other protected areas in the European temperate zone (Parviainen, 2005).

Forest management affects their characteristics and natural processes, and numerous studies have shown that managed forests differ from natural ones. The differences between managed and unmanaged forests depend heavily on the intensity of silvicultural interventions, whether we are talking about tending operations or treatment method. Their magnitude and type can have a different effect on biodiversity as well as on forest structure (Baran et al., 2020).

An essential part of modern forestry consists of "copying" the dynamics and natural structure of forests as found in primary forests, without giving up their production function (Stiers et al., 2018), this is all the more important given that, in recent years, due to the tense economic situation, forest districts have been forced to reduce the costs of tending operations and to make full use of the forest's self-regulating processes (Commarmot et al., 2005).

In order to mimic the natural dynamics and structure of managed forests as closely as possible, primary forests must be studied as reference systems and their structural characteristics must be quantified (Stiers et al., 2018).

To quantify these differences, a series of structural attributes are used, such as diameter at 1.3 m, tree height, number of trees/ha, basal area, and stand volume, etc., based on which

conclusions can be drawn about the structure of the entire stand.

Virgin and quasi-virgin forests have the following structural characteristics:

- they have a large biomass volume;
- they contain large, old specimens that can reach the maximum development potential of the species;
- they have significant volumes of dead wood in various stages of decomposition;
- they present a heterogeneous structure, with a mosaic distribution of components (Radu et al., 2004).

In order to achieve such characteristics in managed forests, these characteristics must first be measurable and reproducible (Stiers et al., 2018). Only then we can talk about close to nature forestry, a concept that has been widely accepted and gained popularity in practice, becoming the main or only approach to forest management in an increasing number of countries (Schütz et al., 2016).

The main objective of the research in this article is to compare the structural characteristics of virgin and managed beech forests in the Semenik Mountains in order to determine (I) the impact of past forestry operations on the structural diversity of beech stands, even though such operations have not been carried out in

recent decades (stands in sample areas at 1200 m altitude) and (II) the impact of past and present forestry operations on the structural diversity of beech stands (stands in sample areas at 1350 m altitude).

MATERIALS AND METHODS

The research was conducted in sample areas located in both virgin and managed forests. Data was collected only for living trees in eight circular sample plots, each measuring 2500 m². In order to limit the differences caused by site variability, we chose stands at the same altitude for both forest types, with sample plots located at 1200 m and 1350 m altitude, respectively (two in each forest category at each altitude level).

The sample plots are located in the Semenik Massif (Figure 1), in the Caraș-Severin County, four of them being located within the Izvoarele Nerei Nature Reserve (Forest District Nera, parcels: 113, 123 and 12, 14, 115 in management unit (UP II and III, respectively), with stands aged 180 years (xxx, 2015) and another four in two managed stands nearby, within Văliug Forest District (parcels 39B - management unit (UP XII), and 17A and 21A - management unit (UP II), 100-year-old stands (xxx., 2021).

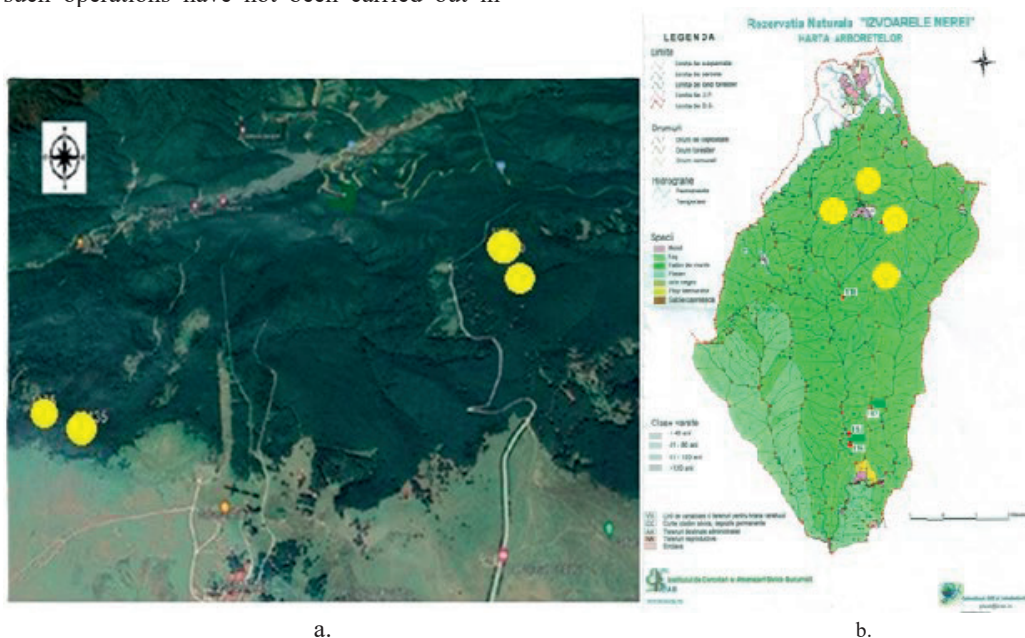


Figure 1. Location of sample plots within the managed forest (a) (<https://earth.google.com/web>) and within the Izvoarele Nerei Nature Reserve (b) (Tomescu et al., 2004)

In order to observe the influence of forestry operations on the structure of the stands, two types of sample areas were selected within the managed forest: those at an altitude of 1200 m were located in the stands where human intervention had been minimal in recent decades, while those at an altitude of 1350 m were chosen in stands where various types of forestry work had been carried out, with varying intensities, some more recently.

Following the analysis of existing forest management plans, it was found that in the forest management units where the sample areas were located at an altitude of 1200 m, only accidental felling was carried out in 2012 (26 m³ – intensity 0.5%), while in the parcels selected for the sample plots located at an altitude of 1350 m, they were carried out: thinnings (8 ha – 41% of the parcel) and principal cuttings (1293 m³ – intensity 11.7%) in 1993, other cuttings (563 m³ – intensity 5.3%), thinning (25 m³ – intensity 0.25%), accidental cuttings (78 m³ – intensity 0.8%) and conversion cuttings to selection system (1316 m³ – intensity 13.2%) in 2002 and thinnings (12 m³ – intensity 0.16%) and accidental cuttings (21 m³ – intensity 0.28%) in 2012.

The diameters at 1.3 m were measured with a forestry caliper, and all trees with a diameter greater than or equal to 6 cm were inventoried. Heights were measured with a Vertex device. The degree of regeneration of the stands was also investigated by inventorying all seedlings taller than 10 cm and with a dbh < 6 cm. In each of the eight sample plots, the regeneration layer was inventoried in 13 sample areas arranged according to the following scheme: one in the center of the 2500 m² sample area, and another 3 sample areas at respectively equal distances (9 m) in the cardinal directions – N, E, S, W. Each regeneration sample plot consists of 3 concentric circles, as follows: RC1 - a 5 m² circle in which all seedlings with a height between 10 and 39 cm were inventoried, RC2 - a 10 m² circle in which seedlings with heights between 40 and 130 cm were inventoried, and RC3 - a 20 m² circle in which all seedlings with a height greater than 130 cm and a dbh < 6 cm were inventoried.

The diversity of diameters was calculated using the Gini index. The Gini coefficient is defined as the area between the line of perfect equality (diagonal) and the Lorenz curve, expressed as

the proportion of the area under the diagonal (Weiner & Solbrig, 1984), and its general formula is: $G = 1 - \sum_{i=1}^k [(ba_{i-1} + ba_i)(ni - ni_{i-1})]$ where ba_i (ba_{i-1}) is the cumulative fraction of the basal area (per cent) of the trees from all diameter classes thinner than or equal to the i th ($i - 1$) diameter class (for $i = 1$, $ba_{i-1} = 0$), ni (ni_{i-1}) is the cumulative fraction of the number of trees (per cent) from all diameter classes thinner than or equal to the i th ($i - 1$) diameter class (for $i = 1$, $ni_{i-1} = 0$) and k represents the number of diameter classes (Duduman, 2011). The values of this coefficient vary between 0 (lack of diversity) and 1 (maximum diversity). In order to highlight the distribution of diameters as accurately as possible, we used a mixed model of Gaussian functions.

Also, using the Shannon index (H), the diversity of diameters and heights was computed, where 0 means no diversity and higher values of the Shannon index indicate a higher degree of diversity. The calculation formula for this index is $H = -\sum_{i=1}^S p_i * \ln p_i$ where $p_i = ni/N$, and p_i is the proportion of species representation, ni is the number of individuals of a species, and N is the total number of individuals in the analyzed perimeter.

The slenderness coefficient (z) was determined, which is expressed by the ratio between tree height h (m) and base diameter d (cm) (Grudnicki, 2004).

The following double logarithmic regression equation was used to calculate the volume of living wood:

$$\log V = a_0 + a_1 * \log(D) + a_2 * \log^2(D) + a_3 * \log(H) + a_4 * \log^2(H),$$

where: D – diameter (cm) at 1.3 m, H – total height (m), a_0 , a_1 , a_2 , a_3 , a_4 – regression coefficients for different species established by Giurgiu and Draghiciu (2004).

RESULTS AND DISCUSSIONS

Density and productivity of the stands

In the virgin forest, the number of living trees per hectare is quite similar at both altitudes. Compared to the managed forest, this number is much lower, with a large number of trees per hectare present in the sample plots of managed forest at an altitude of 1200 m (Table 1). This number can be attributed to the (almost total) lack of forestry interventions, to the age (the

trees are much younger than those in the virgin forest, being in the middle forest development phase, the trees not being at the limit of their physiological longevity), but also to the lack of abiotic disturbances.

In the sample areas of the managed forest at an altitude of 1350 m, the number of trees/ha was closer to that in the virgin forest, with forestry interventions of varying degrees of intensity being carried out in these stands.

For the virgin forests, the results are similar to other virgin beech forests in Central and Eastern Europe in terms of the number of trees per hectare. Therefore, in the Havešova Reserve, the average number of living trees per hectare is 290 (Drössler & von Lüpke 2007), in the Puka Reserve, 325 living trees per hectare were inventoried (Meyer et al., 2003), and in the Rožok Reserve, 270 living trees/ha were inventoried (Kucbel et al. 2012).

Regarding the volume and basal area, the values calculated for the Semenik forest stands are high (Table 1), higher than those in the beech forests in Serbia, where the basal area in managed forests is 27.37 m²ha⁻¹ and the volume is 429.53 m³ha⁻¹ (Matović et al., 2018). In the Havešova Reserve, the basal area is 35 m²ha⁻¹ and the volume was 716 m³ha⁻¹ (Drössler & Lüpke

2007), in the Puka Reserve, the basal area is 45.6 m²ha⁻¹ and the volume was 780.7 m³ha⁻¹ (Meyer et al. 2003), and in the Rožok Reserve, the basal area is 39.5 m² ha⁻¹ and the volume was 744 m³ha⁻¹ (Kucbel et al. 2012).

Table 1. Number of trees, basal area, and volume per hectare by forest category

Forest category	Altitude (m)	Number of trees (Nha ⁻¹)	Basal area (m ² ha ⁻¹)	Volume (m ³ ha ⁻¹)
virgin	1200	294	53,77	962,77
	1350	318	43,12	682,49
managed	1200	1082	52,95	739,90
	1350	516	50,10	631,15

Diameters of the trees

In terms of tree diameters, based on the Shannon index, greater diversity and higher average and maximum values and standard deviations are observed in the virgin forest compared to the managed forest. However, the average tree diameter in the managed forest at 1350 m is close to that in the virgin forest at 1350 m altitude due to the thinning of the forest stands through forestry operations (Table 2).

Table 2. Statistical indicators of tree diameters

Forest category	Altitude (m)	Average (cm)	Standard deviation (cm)	Minimum (cm)	Maximum (cm)	Shannon index (H)
virgin	1200	41.5	24.9	7.6	90.8	2.97
	1350	35.7	21.1	7.1	93.1	2.82
managed	1200	23.7	7.7	7.1	59.4	2.03
	1350	32.6	13.2	6.5	70.1	2.54

The Gini index proved to be the best estimator of tree structure based on tree diameter (Silaghi, 2013).

The average value of the Gini index for the stands in the virgin forest at 1200 m is 0.541, and for the virgin forest at 1350 m it is 0.555.

The Gini index calculated for tree diameters in the managed forest at 1200 m is 0.341, and for

the managed forest at 1350 m it is 0.415 (Figure 2). These values indicate a higher inequality in the distribution of tree diameters in the virgin forest and a greater diversity of size classes.

In the managed forest, the values indicate a more uniform distribution of diameters, trees having similar sizes, which is typical for a managed forest.

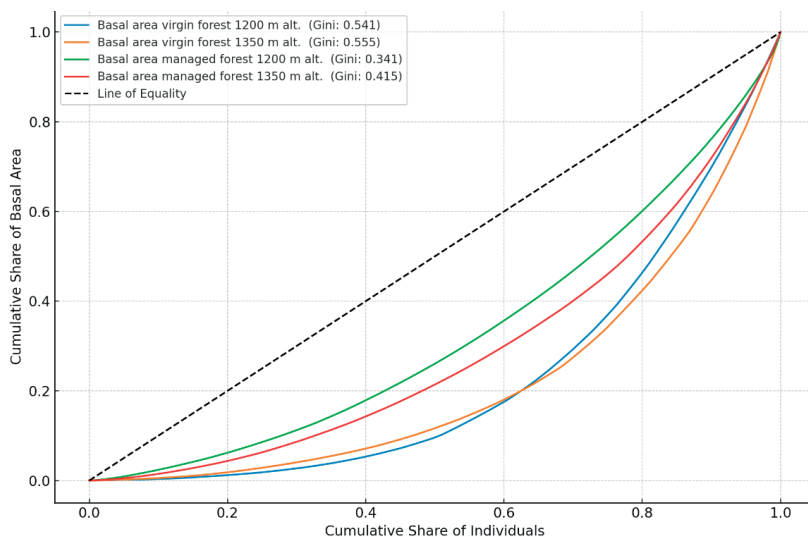


Figure 2. Lorenz curves for tree diameters with Gini Index

In the managed beech forests in Serbia, the Gini index ranged between 0.49 and 0.57, while in virgin forests the values varied between 0.45 and 0.52 (Matović et al., 2018). In managed beech forests in Bohemia, the Gini index values averaged 0.22 (Bilek et al., 2011), below those found in the beech forests of Semenik. In contrast, for virgin forests, the values were higher in Bohemia, ranging between 0.67 and 0.75 (Bilek et al., 2011).

The distribution of trees by diameter categories was modeled as the sum of two normal

distributions using a mixed model of Gaussian functions. In the case of virgin forest, they were observed two major groups of trees, one concentrated around 28 cm (medium-sized, younger trees) and another concentrated around 68 cm (corresponding to older, larger trees) for the forest at an altitude of 1200 m, and a group of trees concentrated around 26 cm (younger trees) and another at 64 cm (corresponding to older, larger trees) for the forest at an altitude of 1350 m (Figure 3).

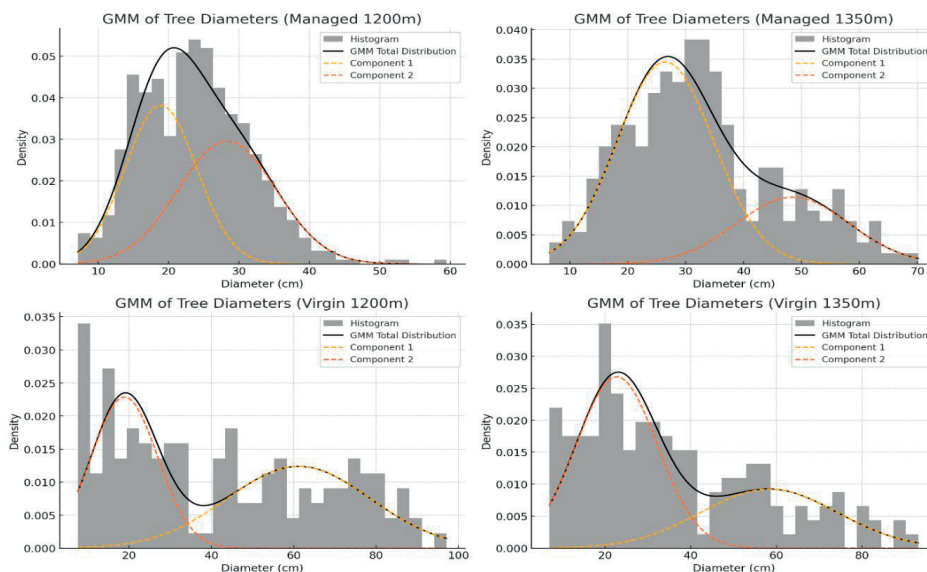


Figure 3. Gaussian Mixture Model Distribution for diameters

Density of thick trees per hectare

We defined the large trees as those with a diameter greater than or equal to 60 cm (Turcu et al., 2015). These are essential components in terms of maintaining biomass accumulation, carbon storage, structural heterogeneity, biodiversity, and forest integrity. They also regulate biotic filtration processes, dominating the structure, dynamics, and functions of many forests in temperate zones (Lutz et al., 2013 and Fichtner et al., 2015). Such trees are also called "habitat trees" because they provide or can provide ecological niches due to their size (Bütler et al., 2013).

Most thick trees are found in the virgin forest at 1200 m, followed by those in the virgin forest at 1350 m altitude. No such trees were found in the managed forest at 1200 m. This fact can be attributed to the lower age but also to the increased density of the stands. In the managed forests at 1350 m, due to the lower consistency of the forest as a result of silvicultural interventions, such trees are found in the diameter category $d \geq 60$ -80 cm (Table 3).

In the old beech forests in Central Europe, inventories have found approximately 30 living trees per hectare with a diameter greater than 70 cm, and the number of living trees with a diameter greater than 80 cm varies between 10 and 17 trees/ha (Nilsson et al., 2003).

Table 3. Distribution of the number of thick trees/ha by diameter categories

Forest category	Altitude (m)	$d \geq 60$ -80 cm	$d \geq 80$ -100 cm
virgin	1200	58	26
	1350	28	16
managed	1200	0	0
	1350	18	0

Heights of the trees

The distribution of trees by height categories within the virgin forest at an altitude of 1200 m is wide and relatively symmetrical, but with a slight tendency towards higher values. Several size classes are present, reflecting a layered structure typical of virgin forests. The forest has an extremely heterogeneous structure: very small and very tall trees coexist.

The absence of forestry interventions has favored the coexistence of young, mature, and old trees. In the case of the virgin forest at an altitude of 1350 m, the distribution suggests a high vertical diversity, but with a lower concentration in a dominant class. The possible influence of more severe climatic conditions (higher altitude) may have led to a lower increase in tree height. Here, too, we find a stable natural structure, with trees of varying sizes and high vertical diversity (Figure 4).

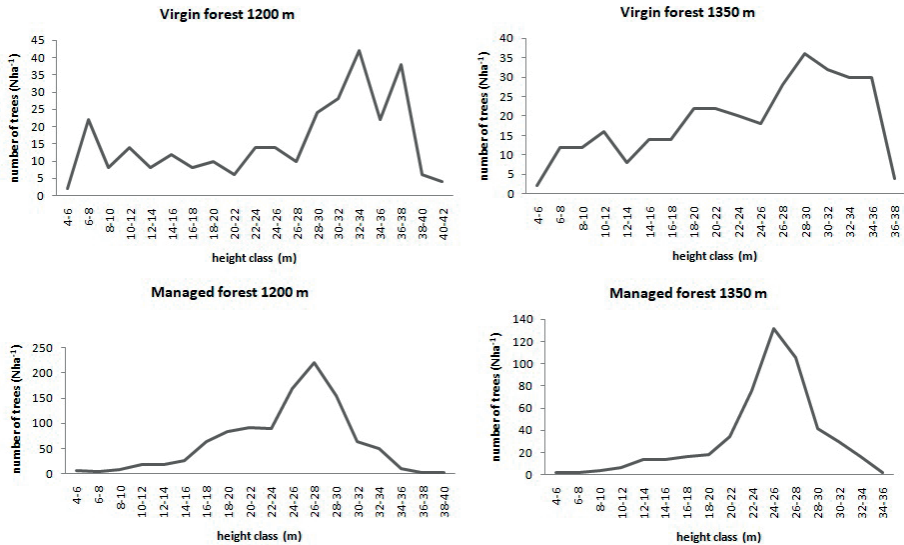


Figure 4. Distribution of the number of inventoried trees/ha, by height categories in the sample plots at 1200 m and 1350 m altitude in virgin forest and managed

Within the managed forest at an altitude of 1200 m, it can be observed that the distribution of trees by height category is positively asymmetrical, with a well-defined peak in the 26-28 m height class. Most trees are of average height, which indicates a dominant cohort, i.e., a forest that is relatively uniform in age, with a homogeneous structure and less vertical structural diversity than in virgin forest. In the managed forest at an altitude of 1350 m, it can be observed that the distribution of trees by height categories is similar to that at 1200 m, positively asymmetric, but with a slight shift

towards lower height values, possibly due to the increased altitude. The forestry interventions applied have led to a fairly uniform structure (Figure 4).

In Table 4, the Shannon index values indicate greater diversity, and we can also observe higher average and maximum values and standard deviations in the virgin forest compared to the managed forest. The exception is the average and maximum values in the managed forest at an altitude of 1200 m, where they are higher compared to the virgin forest at an altitude of 1350 m.

Table 4. Statistical indicators of tree heights

Forest category	Altitude (m)	Average (m)	Standard deviation (m)	Minimum (m)	Maximum (m)	Shannon index (H)
virgin	1200	26.03	10.16	5.2	40.6	2.71
	1350	23.94	8.37	5.1	36.9	2.63
managed	1200	24.45	5.55	4.3	38.6	2.36
	1350	22.35	5.00	3.7	32.8	2.19

The relationship between heights and diameters

The mathematical modeling of these distributions was done using logarithmic equations

(Figure 5). In the case of the virgin forest at an altitude of 1200 m, R² has a value of 0.8602 and 0.8721 for the virgin forest at an altitude of 1350 m.

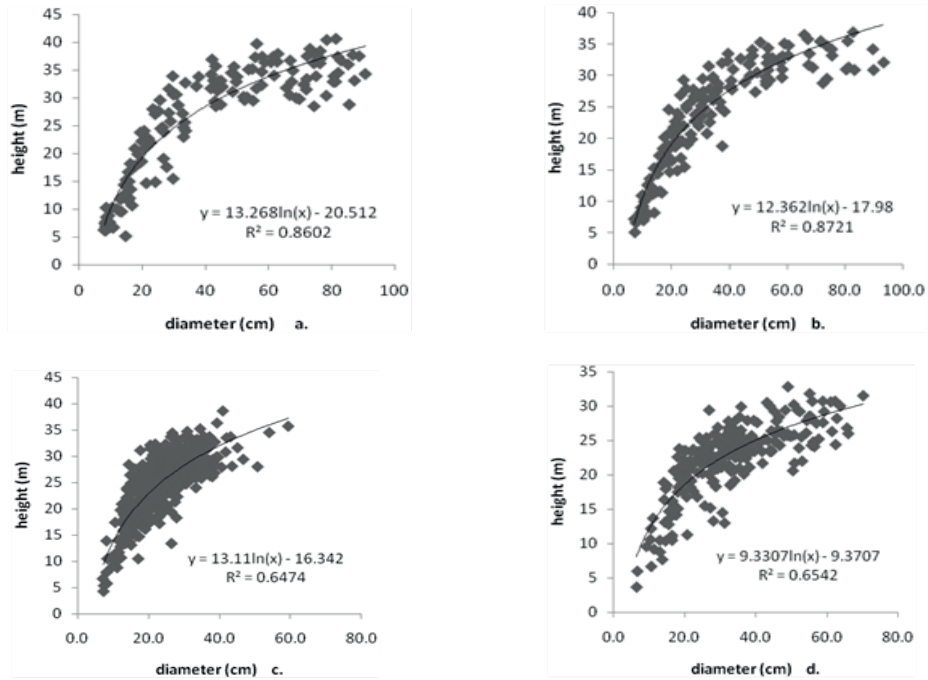


Figure 5. The relationship between heights and diameters in virgin forest (a. - at 1200 m altitude, b. - at 1350 m altitude) and in managed forest (c. - at 1200 m altitude, d. - at 1350 m altitude)

These values indicate a significant correlation between diameter and height, although there is considerable variability, characteristic of a mature ecosystem.

For the managed forest at an altitude of 1200 m, R^2 has a value of 0.6474, and for the forest at 1350 m, the value is 0.6542. A rapid increase in height can be observed at small diameters, which tends to stabilize at larger diameters, which is typical for managed forests (Figure 5)

The slenderness coefficient

This coefficient varies considerably depending on the species, the structure of the stands, their density, age, etc. In dense stands, trees are tall and have small diameters, therefore this coefficient can reach high values (above 1 or even higher).

The density is considered satisfactory when this coefficient has values between 0.6 and 0.9 in pre-logging forests (Florescu and Nicolescu, 1996).

In the virgin forest at an altitude of 1200 m, the minimum and maximum values of the slenderness coefficient were 0.34 and 1.22, respectively, while in the virgin forest at an altitude of 1350 m, the minimum and maximum values of the slenderness coefficient were 0.34 and 1.30, respectively.

For the managed forest, the minimum and maximum values are: 0.39 and 1.34, respectively, for the forest at an altitude of 1350 m, and 0.51 and 1.96, respectively, for the forest at an altitude of 1200 m. Most trees have slenderness coefficients lower than 0.9, with the exception of those in the managed forest at an altitude of 1200 m, where 77% of trees have slenderness coefficients higher than 0.9 (Table 5).

This aspect can be attributed to the thin trees that make up a large proportion of the managed forests. According to Korpel et al., 1991, for beech forests, the slenderness coefficient is considered to have a critical value if it is between 1.8 and 2.2.

Table 5. Distribution of trees by slenderness coefficient categories

Forest category	Altitude (m)	$z < 0.6$ (%)	$z = 0.6-0.9$ (%)	$z > 0.9$ (%)
virgin	1200	32	42	26
	1350	46	39	39
managed	1200	22	1	77
	1350	49	27	24

In the managed beech forests in Serbia, the slenderness coefficient ranged between 0.34 and 2.22, while in virgin forests the values ranged between 0.30 and 1.65 (Matović et al., 2018). In the virgin beech forests of Central Bohemia, the range of this coefficient was higher: from 0.28 to 1.89 (Bilek et al., 2011).

The regeneration

Following the inventory of the seedlings layer, the vast majority of it belongs to beech, the only exceptions being in the sample plots at an altitude of 1200 m in the managed forest, where there are three fir seedlings and two mountain ash seedlings in the 10-39 cm height category and one fir seedling in the 40-129 cm height category. Comparing the regeneration in the

managed forest with the regeneration in the virgin forest, it can be seen that in the managed forest it is practically non-existent. Thus, of the total number of beech seedlings inventoried, 83% are found in the virgin forest in the 10-39 cm height category and only 2% in the managed forest at an altitude of 1350 m, 10% are found in the virgin forest in the 40-129 cm height category and 4% in the virgin forest in the >130 cm height category. In the managed forest at an altitude of 1200 m, regeneration is less represented compared to that in the managed forest at an altitude of 1350 m (these stands had silvicultural interventions in the past - regeneration cuttings, which favors the establishment of seedlings and, implicitly, of a new generation of trees (Table 6).

Table 6. Distribution of beech seedlings per hectare in managed and virgin forests within sample plots at an altitude of 1200 m

Forest category	Altitude (m)	Height category (number of seedlings/ha, %)		
		10-39 cm	40-129 cm	>130 cm
virgin	1200	47769 (67%)	7192 (10%)	2308 (3%)
	1350	11462 (16%)	346 (0%)	538 (1%)
managed	1200	77(0%)	115(0%)	0 (0%)
	1350	1385 (2%)	77 (0%)	0 (0%)

CONCLUSIONS

After the comparative analysis of the structure of the two forest categories (virgin and managed), the following conclusions can be drawn:

1. Past forestry operations in beech stands, followed by their exemption from silvicultural interventions, had the greatest impact on the structural diversity of managed forests. The greatest differences were found between the stands in the managed forest at an altitude of 1200 m and the virgin forests regardless of altitude, but also in comparison with the managed forest at an altitude of 1350 m. These differences were noted both in terms of the size of the largest trees (especially diameters) and in the distribution of diameters and heights, the relationship between these two components, and the values of the slenderness coefficient. These differences are also reflected in the values of the Gini index or the Shannon diversity index (with one exception, this index having the lowest value in the case of tree distribution by height categories for the managed forest at an altitude of 1350 m).

2. Although over time there have been a series of forestry interventions in the managed forest at an altitude of 1350 m, some of them more recent, its structural diversity is higher compared to the managed forest at an altitude of 1200 m and, in some respects, it is close to the structural diversity of the virgin forest at an altitude of 1350 m. These indicators may be a sign of adequate forest management (close to nature), which will enable the long-term conservation of these beech forests and ensure their ecological stability.

3. Even though the managed forest has been affected by human intervention over time, the Gini index and Shannon index show that these beech forests in Semenik still retain a fairly high structural diversity, which is very important,

especially as there is a low diversity of tree species.

4. Monitoring tree diameters in managed forests can provide a good picture of their structural diversity, and maintaining trees in large diameter classes increases the structural diversity of these forests.

5. The results of this study allow for limited conclusions to be drawn, as the stands are of different ages (180 years for the virgin forest and 100 years for the managed forest), and further detailed analysis is needed to provide a better comparison between the two forest categories.

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SCIENTIFIC CONSIDERATIONS REGARDING THE PRESENCE OF CONTINENTAL DUNE HABITATS IN ROMANIA

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Abstract

There are continental dunes habitats of Community interest on the territory of Romania outside the checklist for which two reports were made and submitted to European Commission (2013 and 2019). In the initial checklist for Romania, there were five types of continental dune habitats, present in two biogeographical regions (Black Sea - BLS and Pannonian - PAN) for which seven Natura 2000 sites were designated. In 2025, for the preparation of the third report under Article 17 of the Habitats Directive, it was proposed to extend the list based on information collected from the field and documents referring to dune habitats. During field identification, species characteristic of the plant communities that define the habitats were taken into account. The new list confirmed the presence of all five habitat types, with these being highlighted in nine Natura 2000 sites, including Continental (CON) and Steppic (STE) biogeographical regions. The presence of these habitats in the mentioned biogeographical regions requires the updating and revision of two documents: the standard forms and site management plans, as well as their adoption in appropriate legislative acts.

Key words: checklist, conservation, dunes habitats, Habitats Directive.

INTRODUCTION

At European level, sandy soils are a special category from a geomorphological point of view.

Thus, the soil map has recently been revised (Figure 1) and includes this category (Bertran et al., 2016). Soil data and information is based by a large searchable repository of global and regional datasets and layers available in GIS format and European Atlas of Soil Biodiversity (Jeffery et al., 2010; ISRIC, 2026).

Closely related to soil composition is the establishment of particular types of continental dune vegetation, for the conservation of which habitats have been included in the European Nature directives, respectively defining the Natura 2000 ecological network.

After Romania's accession to the European Union (EU) in 2007, these all these areas were included in the Natura 2000 ecological network as Sites of Community Interest (SCIs).

Special Areas of Conservation (SACs) are sites of community importance for which conservation measures have been established. The Romanian National legal references of SCIs

designation are Ministry Order no. 1.964/2007; Ministry Order no. 2387/2011, and for SAC designation is Government Decision, no. 685/27.05.2022 (G.D., 2022).

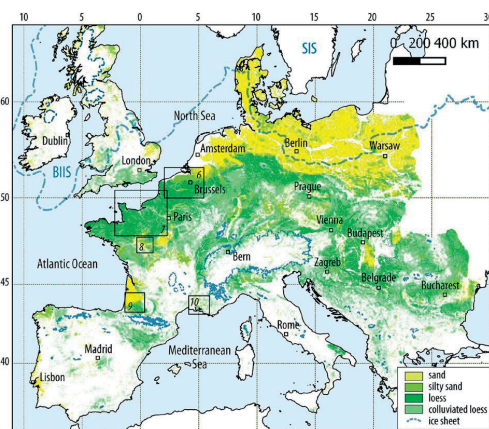


Figure 1. Distribution of European aeolian deposits derived from the LUCAS topsoil database, after Bertran et al., 2016

In Romania the sand-covered lands are present in: Danube Delta Biosphere Reserve, Agigea

Maritime Dune Natural Reserve, Siret and Bârlad valleys, starting from Hanul Conachi and continuing northwest to Tecuci, southern part of Oltenia, in the Ciuperceni – Desa Nature Reserve and Dăbuleni, Western Plain of Romania (Foieni Sand Dunes Natural Reserve and Carei Plain). The sand-covered lands here form the following types of maritime and continental dune habitats:

- 2110 *Embryonic shifting dunes*,
- 2130* *Fixed coastal dunes with herbaceous vegetation ('grey dunes')*,
- 2160 *Dunes with *Hippophae rhamnoides**,
- 2190 *Humid dune slacks*,
- 2340* *Pannonic inland dunes*.

The European Environment Agency (EEA, 2020) checklist included two biogeographical regions: Pannonic (PAN) with only habitat 2340*; and Black Sea (BLS) with 2110, 2130*, 2160 and 2190 habitats.

The presence of sand dune habitats was highlighted in the Danube Delta Biosphere Reserve (ROSCI0065 Delta Dunării), within the perimeter of the sandbanks: Letea Grind, Caraorman Grind, Lupilor Grind and Chituc Grind, which are defined as strictly protected areas. The Lupilor Grind is a lagoonal marine sandy sandbank made up of Holocene age sedimentary formations (Airinei & Pricăjan, 1971; Gâstescu & Şelariu, 1994; Strat, 2002-2003; DDBRA_PM, 2022-2024).

All these areas include coastal dunes (most of the area), marine sandy dunes and interspersed flat surfaces of marine origin with sandy dunes formed by sedimentary rocks (sandy, shell-bearing deposits, of mixed origin: fluvial and marine) with very particularly vegetation types (Sârbu et al., 1995; Popescu et al., 1997; Sârbu et al., 2000; Făgăraş et al., 2008; Făgăraş 2014; Schneider-Binder & Kuhlke, 2015).

The geomorphology of the Black Sea coast has allowed the establishment of specific habitats of sand dunes (Gâstescu et Şelariu, 1994; Gâstescu et Ştiucă, 2006).

The vegetation from the Agiea Maritime Dune Natural Reserve has allowed the inclusion of the habitat of community interest specific to marine coasts. (Boşcaiu, 1975; Popescu & Sanda, 1976-1977; Sârbu et al., 2000; Făgăraş et al., 2008). This area was partially included in the ROSAC0073 Dunele marine de la Agiea (Sea

dunes of Agiea) (M.O. no. 1.964/2007; G.D., 2022).

Since 1937, it was known that sandy soils and sands in Romania are exploited through forestry, agricultural, horticultural and viticultural cultivation (Chiriţă, 1937). Among the sandy formations of Romania, the sands along the Siret and Bârlad valleys, starting from Hanul Conachi and continuing northwest to Tecuci, represented geographical conditions specific to afforestation works. These sands extend in the form of a strip of varying width between 0.5 km and 1 km and 4,250 km long, with an almost north-south orientation, located west and southwest of Hanul Conachi and north of Nămolosa (Chiriţă, 1937).

Afforestation works to stabilize sandy soils have been a constant concern for decades. Currently, the Hanu Conachi Natural Reserve is managed under a forestry regime. This area was partially included in the ROSAC0072 Dunele de nisip de la Hanul Conachi (M.O. no. 1.964/2007; G.D., 2022).

A similar situation was also in the southern part of Oltenia, in the Ciuperceni – Desa Nature Reserve. This area is between Ciuperceni – Desa - Dăbuleni, an area partially included in the ROSAC0039 Ciuperceni Desa site (M.O. no. 1.964/2007; G.D., 2022) where the stabilization of sandy soils has been a permanent concern. In the Oltenia Plain, including Ciuperceni, several alignments of relief rippled dunes have been identified. The main source of sandy material was the alluvium Danube, and the dunes differ from interdunes (Stănilă et al., 2020).

In the last years, fruit and wine varieties have been selected to be adapted to sandy soils (Enache & Croitoru, 2018; Mărcăneanu et al., 2025).

Also in the category of continental sand dunes are areas in the Western Plain of Romania, which border similar areas in Hungary. The continental dunes near the towns of Urziceni and Foeni are located in the Nirului Plain, which is an integral part of the Western Plain (northern sector) of Romania (Raţiu et al., 2000). The Natural Reserve Foieni sand dunes (Dunele de nisip Foieni) was included into the Natura 2000 site ROSCI0020 Câmpia Careiului (Carei Plain) (M.O. no. 1.964/2007; M.O. no. 2.387/2011). The characteristic vegetation was described, including plant communities of Foieni sand

dunes (Karacsonyi, 1995). Recently, the management plan of these protected areas was approved (M.O. 3.221/2023).

Sometimes dramatically associated with the movement of sand dunes in populated areas, desertification actually refers to the land resulting in degradation in arid, semi-arid and dry sub-humid areas from various factors, including climate and human variation activities. For soil biodiversity, desertification is a threat. Habitat fragmentation can be striking as a result of desertification with land degradation continuous vegetation cover to a discontinuous, and finally island, vegetation cover (Jeffery et al., 2010).

In the European Union, all Member States use the *Habitats Directive* (92/43/ECC) on the conservation of natural habitats and of wild fauna and flora as a key instrument for biodiversity conservation (DG Environment, 2017; 2025) and for maintaining and improving the supply of ecosystem services directing their efforts toward designation of protected areas (Maes et al., 2012).

Classification of habitat types was based partly on phytosociology (defined plant communities associations), but this is considered complex and sometimes unclear and has recently been reinterpreted into classification systems based on analysed of accumulated data (Mucina et al., 2016; Rodwell et al., 2018; Chytrý et al., 2020). Thus, assessment of the conservation status of habitat types is based partly on structure (typical/characteristic species), function and future habitat trends (Müller-Kroehling, 2019). In the *Habitats Directive*, monitoring of Annex 1 habitats is required in Article 11, with reporting to the Commission every 6 years of their assessed conservation status, according to the requirements of Article 17, is provided for at national level per each biogeographical or marine region. The EU biogeographical conservation status was assessed by combining the Member State data or by weighting the Member State assessments and colour codes was defined (Table 1).

Table 1. Abbreviations and colour codes for Conservation Status classes (after DG Env., 2017)

Conservation Status	Colour	Abbreviation
Favourable	Green	FV
Unfavourable-inadequate	Amber	U1
Unfavourable-bad	Red	U2
Unknown	Grey	XX

Conservation status is assessed using a standard methodology as being either 'favourable', 'unfavourable-inadequate' or 'unfavourable-bad' (Table 1), based on four parameters as defined in Article 1 of the *Habitats Directive*:

1. range (within the biogeographical region concerned);
2. area covered by habitat type within range;
3. specific structures and functions (including typical species);
4. future prospects (as regards range, area covered and specific structures and functions).

The documents are on the reference portal for reporting under Article 17 of the *Habitats Directive* (EEA, 2020; EEA, 2025a; EEA, 2025b). However, for the designation and management plans of Natura 2000 sites in Romania, the plant communities remain the reference (Mihăilescu et al., 2024).

MATERIALS AND METHODS

The present study justifies the need to expand the checklist for Romania for dune habitats to more biogeographical regions.

The sandy areas were selected based on the documents regarding Romania's accession as well as the scientific references confirming the inclusion of dune habitats in the European classification system. The Annex I of *Habitats Directive* described habitats and sites where whose conservation requires designation of special areas of conservation (SAC, SCIs), so that Romanian habitat types could be integrated into the Natura 2000 network (Doniță et al., 2005; Doniță et al., 2006; Gafta & Mountford, 2008; EUR28, 2013; Chytrý M. et al., 2020).

The *Interpretation Manual of European Union Habitats* (EUR28, 2013) is a scientific reference document, which on 13 April 2007 adopted additional changes for the accession of Bulgaria and Romania.

These types of continental dune habitats are present in the Natura 2000 sites in Romania, as they have been designated since 2007 with Romania's accession to the EU. The Standard Forms have been updated periodically and we present the current situation. In the initial habitat checklist for Romania, there were 5 types of continental dune habitats, present in two bioregions (BLS and PAN) for which 7 occurrences in Natura 2000 sites were

designated. Habitat coverage is taken from the Standard Site Forms according to the official information transmitted by Romania in the European system (BISE, 2025).

The grid is based on the recommendation at the 1st European Workshop on Reference Grids in 2003 and later INSPIRE geographical grid systems 10 x 10 km (ETRS 89 grid cells, LAEA EPSG:3035) (INSPIRE, 2007; DG Environment 2017; 2023). For each country three vector polygon grid shape files, 1, 10 and 100 km, are available. Last updated was in 2016 (EEA, 2016). For the Romanian continental dune habitats, we used 1x1 km for the small area and 10 x 10 km for large area.

At the European level, the last edition of map generated by the EEA was in 2016 and these documents define the biogeographical regions dataset contains the official delineations used in the *Habitats Directive* (92/43/EEC) (Figure 2).



Figure 2. EEA geospatial data catalogue. Biogeographical regions, Europe 2016

Dune habitats were identified in the field based on plant communities as described in *Habitats of Romania – Habitatele din România* (H.R.) (Doniță et al., 2005; Doniță et al., 2006), starting from the description in *Interpretation manual of European Union habitats* (EUR 28, 2013). The edifying plant species and communities present in Romania were taken into account. The description of the habitats according to these follows:

Habitats descriptions

2110 Embryonic shifting dunes

Formations of the coasts of the Atlantic, the North Sea, the Baltic Sea and the Mediterranean, representing the first stages of dune construction, constituted by ripples or raised sand surfaces of the upper beach or by a seaward fringe at the foot of the tall dunes. Along the Black Sea coast (Făgăraș 2014) are present following plant communities according with H.R.: *Elymetum gigantei* Morariu 1957; *Artemisietum Tschernievianae* (arenariae), *Agropyretum juncei* (Br.-Bl. et De L.1936) Tx. 1952; *Secali sylvestri* – *Alysssetum borzeani* (Borza 1931) Morariu 1959, *Aperetum maritimae* Popescu et al. 1980, *Bromo tectori* (Kern 1863) Soó (1925) 1939. Conservation status at Member States of EU level on bioregion: BLS: Poor (U1); CON: Bad (U2) (BISE, 2025).

2130* Fixed coastal dunes with herbaceous vegetation ('grey dunes')

Fixed dunes, stabilised and colonised by more or less closed perennial grasslands and abundant carpets of lichens and mosses, from the Atlantic coasts, the shores of the North Sea and the Baltic. Subtype presents in Romania and Bulgaria is Pal. 16.22B - Pontic fixed dunes - fixed dunes of the coasts of the Black Sea (EUR 28, 2013).

Plant communities according with H.R., 2005-2006: *Ephedro* – *Caricetum colchicae* (Prodan 1939 n.n. Morariu 1959) Sanda & Popescu 1973 (Syn.: Association with *Ephedra distachya* and *Carex ligERICA* (Prodan 1939) Morariu 1959) *Koelerio glaucae* – *Stipetum borysthenicae* Popescu & Sanda 1987; *Scabioso argenteae* – *Caricetum colchicae* (Simon 1960) Krausch 1965, *Scabioso argenteae* – *Artemisietum campestris* Popescu & Sanda 1987; *Holoschoeno* – *Calamagrostetum epigeios* (Syn.: *Calamagrostetum epigeios* Eggler 1933 subas. *arenosum* Popescu & Sanda 1976).

By analysing a large number of plots, errors in classifications were identified, thus recognizing that there are differences in interpretation that can lead to confusion or some cases inconsistent, characterization confused the meaning of the EUNIS habitats. Therefore, the current interpretation of the same habitat type can vary among European countries habitats (Chytrý et al., 2020). The revised EUNIS habitat types and

the number of plots assigned to each of them by the EUNIS-ESy expert system, with the current codes and corresponding codes used in the 2007 version of the EUNIS classification and in the European Red List of Habitats (Chytrý et al., 2020). Based on 967 plots habitat *Black Sea coastal dune grassland (grey dune)* was revised and included as follow: EUNIS code N17; EUNIS2007 code B1.4; Red List code B1.4c. (Chytrý et al., 2020).

The habitat 2130* *Fixed coastal dunes with herbaceous vegetation (grey dunes)*, considered a priority habitat, was included in this category of protection.

Conservation status at Member States of EU level on bioregion: BLS: Unknown (XX), CON: Bad (U2) (BISE, 2025).

2160 Dunes with *Hippophae rhamnoides*

Sea-buckthorn formations of forest colonisation in both dry and humid dune depressions, mostly in Denmark, Germany, the Netherlands, Belgium, Britain and Ireland (EUR 28, 2013). Subtype presents in Romania and Bulgaria: Pal. 16.251 Sea-buckthorn formations of forest colonisation in both dry and humid dune depressions.

Plant communities according with H.R., 2005-2006: *Hippophaë – Salicetum elaeagni* Br.-Bl. et Volk 1940 (Syn.: *Calamagrostio epigei-Hippophaëtum rhamnoides* Popescu et al. 1986 *Hippophaë – Berberidetum* auct. rom. non. Moor, *Viburno – Crataegetum berberidetosum* Mititelu et Barabáš 1970).

Conservation status at Member States of EU level on bioregion: BLS: Good (FV). Presence in bioregion only in Romania (BISE, 2025).

2190 Humid dune slacks

Humid depressions of the dunal systems. Humid dune-slacks are extremely rich and specialised habitats very threatened by the lowering of water tables. Subtype presents in Romania and Bulgaria (EUR 28, 2013):

Pal. 16.34 - Dune-slack grasslands: humid grasslands and rushbeds (see 37.31, 37.4) of dune-slacks, also often with creeping willows (*Salix rosmarinifolia*, *S. arenaria*).

Plant communities according with H.R., 2005-2006: *Saliceto (rosmarinifoliae) – Holoschoenetum vulgaris* Mititelu et al., 1973.

Conservation status at Member States of EU level on bioregion: BLS: Poor (U1) (BISE, 2025).

2340* Pannonic inland dunes

Inland dunes of the Pannonic plain and of neighbouring basis. This habitat represents a mosaic of different plant associations with open sand, dune lichen communities, pioneer swards with many therophytes, open and closed swards. For steppes and meadow-steppes on stabilised sand or sandy soils is not associated with dune complexes as habitat 6260* *Pannonic sand steppes* (EUR 28, 2013).

Plants species composition in Romania: *Cladonia convoluta*, *C. furcata*, *Corynephorus canescens*, *Thymus serpyllum*, *Viola tricolor* ssp. *tricolor*, *Cerastium semidecandrum*, *Spergularia morisonii*, *Alyssum montanum* ssp. *gmelinii*, *Bassia laniflora*, *Cynodon dactylon*.

Plant communities according with HR, 2005-2006: *Festuco vaginatae – Corynephoretum* Soó 1935; *Festucetum beckeri* Popescu & Sanda (1976) 1997 (Syn.: *Festucetum vaginatae* subass. *arenicolum* Popescu & Sanda (1976) 1979); *Mollugietum cervianae* Borza 1963.

Conservation status at Member States of EU level PAN, in our region RO, BG, HU: Bad (U2) (BISE, 2025).

In the checklist for Romania, there were five types of continental dune habitats (Tabel 2), present in two biogeographical regions (BLS and PAN) for which seven Natura 2000 sites were designated.

Table 2. Types of continental dune habitats for Romania distributed on regions

Natura 2000 habitat code	Description	Biogeographical regions
2110	Embryonic shifting dunes	BLS
2130*	Fixed coastal dunes with herbaceous vegetation ('grey dunes')	BLS
2160	Dunes with <i>Hippophae rhamnoides</i>	BLS
2190	Humid dune slacks	BLS
2340*	Pannonic inland dunes	PAN

*priority habitat

RESULTS AND DISCUSSIONS

As we mentioned, for soil biodiversity, desertification is a threat. Analysing the Map of Soil Biodiversity Potential Threats on the European level (Jeffery et al., 2010), the sand dunes in Romania were included partly in the Low category and partly in the Moderate category.

In 2025, for the preparation of the third report under Article 17 of the *Habitats Directive*, it was proposed to extend the list based on information collected from the field (between 2019-2024) and documents referring to continental dune habitats. The distribution map of dune habitats was generated to confirmed the presence of all five habitat types, on biogeographical regions in Romania (Figure 3).

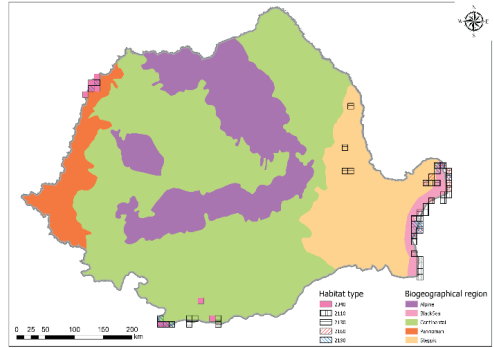


Figure 3. Distribution map of dune habitats in Romania

For this report the field identification took into account the species characteristic of the plant communities that define the habitats. At the European level, the latest edition of the map generated by the EEA in 2016 defines biogeographical regions, but these are not fully correlated with the geomorphology of the terrain.

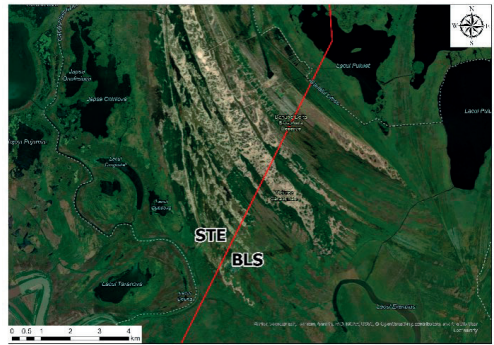


Figure 4. Habitat 2130* identified at Caraorman into the Danube Delta Biosphere Reserve (red line represent the limit between BLS and STE biogeographical regions)

Thus, the dataset used for reporting under Article 17 of the *Habitats Directive* can lead to the separation of plots taken from the field into

two biogeographical regions. This also happens at the level of the Caraorman Grind (Figure 4). Because dunes differ from interdunes in relief and soil, this fact is reflected in the types of vegetation that settle in this area. The habitat 2190 are found in the CON in the site ROSCI0039, also confirmed by the Management Plan of site Ciuperceni – Desa (M.O. 1.248/ 2016). The habitat 2130* present in CON in ROSCI0039 (Figure 5) as well as in the STE at the ROSAC0072, also confirmed by the identified characteristic species.



Figure 5. Habitat 2130* *Fixed coastal dunes with herbaceous vegetation (grey dunes)* identified at ROSCI0039 Ciuperceni – Desa.
Photo: Simona Mihăilescu

The new list confirmed the presence of all 5 habitat types, these being highlighted in 9 occurrences in the Standard Forms of Natura 2000 sites, including Continental - CON and Steppic STE biogeographical regions (Table 3).

Table 3. Types of continental dune habitats distributed on Natura 2000 sites in Romania

Natura 2000 habitat code	Natura 2000 sites		Cover (ha)	Bio-geographic regions
Initial habitat checklist				
2110	ROSCI0065	Delta Dunării	857.23	BLS
2130*	ROSCI0065	Delta Dunării	1355.58	BLS
2130*	ROSAC0073	Dunele marine de la Agigea	6	BLS
2160	ROSCI0065	Delta Duării	125.70	BLS
2190	ROSCI0065	Delta Dunării	645.49	BLS
2190	ROSCI0020	Câmpia Careiului	847.5	PAN
2340*	ROSCI0020	Câmpia Careiului	100	PAN

Proposed expand the habitat checklist after field data processing (2019-2024) to include in 2025 report			
		Cover (ha) estimated	
2190	ROSCI0065 Delta Dunării	129	STE
2190	ROSCI0065 Delta Dunării	516.49	BLS
2130*	ROSAC0072 Dunele de nisip de la Hanul Conachi	34.5	STE
2130*	ROSAC0039 Ciuperceni - Desa	18.98	CON
2160	ROSAC0039 Ciuperceni - Desa	3.75	CON
2190	ROSAC0039 Ciuperceni - Desa	38	CON
2190	ROSCI0020 Câmpia Careiului	469	PAN

*priority habitat

These habitats are also found in the perimeter of the Letea, Caraorman and Lupilor grinds in the R.B.D.D. (ROSCI0065 Delta Dunării) which were reported for the BLS bioregion in 2013 and 2019.

During the period 2019-2024, for the third report (from 2025) field data and information were collected according to the requirements of the reporting guide (DG Environment, 2023) based on Article 17 of the *Habitats Directive*, taking into account the species characteristic of the plant communities that define the habitats when identifying them. Distribution and spread maps updated with recent information were also developed, and for Romania the first short-term trend reporting was carried out.

Between 2025 and beginning 2026, it was necessary to check supplementary some presents of the characteristic species into the habitats (Figure 6, 7, 8, 9) (GBIF, 2023a; 2023b; 2023c).

For the Caraorman sand dunes, we report the partial presence in the BLS bioregion, but also in the STE, of the habitat 2190 *Humid dune slacks*. The habitat was found in this biogeographical region in the last reporting period. The combination is valid due to improved knowledge of the habitat distribution. The priority habitat 2130 * *Fixed coastal dunes with herbaceous vegetation ('grey dunes')* is present in ROSAC0072 Sand dunes at Hanul Conachi, belonging to the STE bioregion, and 2160 *Dunes with Hippophae rhamnoides*, 2190 *Humid dune slacks*, were identified in the CON in the ROSCI0039 Ciuperceni – Desa site, information also confirmed by the management plan for these protected areas. The presence of

habitat 2130 was not included in the Standard Form and in the Management Plan of the ROSAC0072 site, requiring the updating of the two documents.

We mentioned that the priority habitat 2130* *Fixed coastal dunes with herbaceous vegetation ('grey dunes')* is present on Black Sea coast, in Romania and Bulgaria (Valcheva et al., 2021). Taking into account the species characteristic of the plant communities that define the habitats when identifying them.

Plants: *Aira* spp., *Anacamptis pyramidalis*, *Bromus hordeaceus*, *Carex arenaria*, *Cerastium* spp., *Corynephorus canescens*, *Erodium glutinosum*, *E. lebelii*, *Galium verum*, *Gentiana campestris*, *G. cruciata*, *Koeleria* spp., *Milium scabrum*, *Myosotis ramosissima*, *Ononis repens*, *Phleum arenarium*, *Polygala vulgaris* var. *dunensis*, *Silene conica*, *S. otites*, *Trifolium scabrum*, *Tuberaria guttata*, *Viola curtisii*, *V. rupestris* var. *arenaria*.

Conservation status at BLS: Unknown (XX).

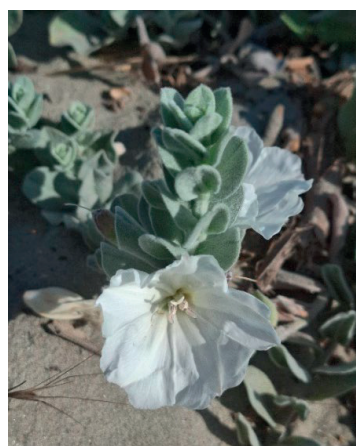


Figure 6. *Convolvulus persicus* L. Identified at Sulina into the Danube Delta Biosphere Reserve. Photo: Simona Mihăilescu

The phytocoenoses with *Convolvulus persicus* L. (Figure 6) were present in some areas of the Danube Delta Biosphere Reserve (Sulina, Sf. Gheorghe, Cardon), in the natural reserve Marine Sand Dunes of Agigea and on Durankulak beach, in Northern Bulgaria into the Natura 2000 site: BG0000154 Ezero Durankulak (Făgăraș & Jianu, 2016; Mitoi et al., 2025). It is typical psammophytes species for the continental dune habitats. The species assessed in the European Red Lists prepared by the IUCN

for the European Commission are also included (EEA, 2020).

On the territory of Romania, there are continental dunes of community interest outside the checklist for which the first two reports were made (2013 and 2019).

In order to update the presence of habitats in the habitat's checklist for Romania, there are scientific arguments to confirm their presence in other biogeographical regions besides BLS and PAN. These are found in CON in the ROSCI0039 Ciuperceni – Desa site (Figure 5), as well as in STE in the ROSAC0072 Sand dunes at Hanul Conachi site.

These habitats have a common geomorphological origin with the Letea Grind and Caraorman Grind in RBDD that were reported for the BLS bioregion (2013 and 2019). During the period 2019-2024, for the 2025 report, field data and information were collected according to the requirements of the reporting guide for art.17 of DH, in identification taking into account the characteristic species of the plant communities that define the habitats. Distribution and range maps updated with recent information were also produced.

Habitat 2130 * *Fixed coastal dunes with herbaceous vegetation (grey dunes)* is present in ROSAC0072 Sand dunes at Hanul Conachi belonging to the STE bioregion, and 2160 *Dunes with Hippophae rhamnoides* and 2190 *Humid intradunal depressions* were identified in CON in the ROSCI0039 Ciuperceni – Desa site, information also confirmed by the management plan for these protected areas (M.O. 1.248/2016).

In the floristic composition of the phytocenoses, in addition to vascular plant species, moss and lichen species were identified in the areas researched during 2019-2024.

These species are particularly for the herbaceous vegetation of coastal dunes: Mosses: *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr (Syn. *Tortula ruraliformis* (Besch.) Ingh.) (Figure 7), and Lichens: *Cladonia* spp.: *Cladonia furcata* subsp. *subrangiformis* auct. non (Sandst.) Abbayes (Figure 8A and 8B) and *Cladonia foliacea* (Huds.) Schrad. (Figure 9). This led to the reporting in 2025 of the habitats mentioned above also in Natura 2000 sites in the CON and STE biogeographical regions.



Figure 7. *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr identified at Hanu Conachi Natural Reserve (voucher Herbarium BUCM). Photo: Simona Mihăilescu



A



B

Figure 8. (A and B). *Cladonia furcata* subsp. *subrangiformis* auct. non (Sandst.) Abbayes identified at Hanu Conachi Natural Reserve (vouchers Herbarium BUCM L4470; BUCM L4411). Photo: Simona Mihăilescu

We confirmed that continental dune habitats and coastal dune meadows present species composition as the main difference between the Atlantic-Baltic and Mediterranean-Black Sea regions (Marcenò et al., 2018; Chytrý et al., 2020).



Figure 9. *Cladonia foliacea* (Huds.) Schrad. identified at Hanu Conachi natural reserve (voucher Herbarium BUCM L4412). Photo: Simona Mihăilescu

Conservation Status

The Conservation Status of habitats was evaluated by Natura 2000 sites and on distribution by biogeographical region (Tabel 4).

Table 4 Conservation status of continental dune habitats for Romania, reported in 2025

Natura 2000 habitat code	Biogeographic regions			
	BLS	CON	STE	PAN
2110	U1			
2130*	U1			
2160	FV			
2190	FV	U1	U1	
2340*				FV

*priority habitat

The conclusions were on overall assessment: range (within the biogeographical region concerned); area covered by habitat type within range; specific structures and functions (including typical species), and future prospects. Relating the Conservation Status, the habitats which are in Unfavourable-inadequate (U1), the necessary conservation measures are:

- Manage the use of natural fertilisers and chemicals in agricultural (plant and animal) production (CA09).
- Other measures related to agricultural practices (CA16).

- Habitat restoration of areas impacted by residential, commercial, industrial and recreational infrastructure, operations and activities (CF02).

CONCLUSIONS

The new list confirmed the presence of all five habitat types of continental dune (2110; 2130*; 2160; 2190; 2340*), with these being highlighted in nine Natura 2000 sites, including Continental (CON) and Steppic (STE) regions. At European level, the EEA, based on the information received from the Member States, should define the biogeographical regions more accurately, namely to make a better correlation with the geomorphology of the terrain. Thus, some confusions can be avoided for the next reporting in 2031.

For the report on 2025, the dataset on biogeographical regions containing their official delimitations leads to the separation of the corresponding areas into two regions. Thus, habitat 2130* becomes present in both the BLS and STE regions, especially in the Danube Delta Biosphere Reserve which includes ROSCI0065. Field data collected between 2019-2024 confirms this fact.

The presence of habitat 2130* was not included in the Standard Form and in the Management Plan of the ROSAC0072 site, this fact requiring the updating of the two documents.

The presence of these habitats in the mentioned biogeographical regions requires the updating and revision of two documents: the Standard Forms Natura 2000 sites and management plans of these, as well as their adoption in appropriate legislative acts for the following Natura 2000 sites: ROSCI0065 Delta Dunării (Danube Delta), ROSAC0039 Ciuperceni – Desa, and ROSAC0072 Dunele de nisip de la Hanul Conachi (Sand dunes at Hanul Conachi).

To maintain habitats in a Favourable conservation status, but especially for habitats in an Unfavourable-inadequate (U1), the necessary conservation measures must be financed and applied with consideration.

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THE IMPACT OF FERTILIZER ON FLORISTIC COMPOSITION AND BIODIVERSITY OF FOREST-STEPPE GRASSLANDS (HNV) IN THE NORTH EASTERN OF ROMANIA

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Abstract

National natural conditions strongly influence the floristic structure of meadows, creating a wide range of meadow types and formations. This diversity of species ensures the functionality, efficiency and resilience of the ecological balance within these natural ecosystems. The objective of this study was to determine the effect of the applied management on the phytodiversity of a *Dichanthium ischaemum* permanent meadow in the Moldavian forest-steppe. The experimental factor was: fertilization with seven treatments: T_1 - unfertilized (control), T_2 - $N_{50}P_{50}$ kg/ha annually, T_3 - $N_{75}P_{75}$ kg/ha annually, T_4 - $N_{100}P_{100}$ kg/ha annually, T_5 -10 t/ha sheep manure annually, T_6 -20 t/ha annually and T_7 -30 t/ha annually sheep manure applied at two years. The applied fertilizers influenced the floristic composition, producing appreciable, quantitative and qualitative changes in the vegetal cover. The dominance and frequency of the species, as well as the variation of the Shannon diversity index, were largely influenced by the amount of mineral NP, the amount of manure, but also by the number of years of the fertilization period.

Key words: forest-steppe grasslands, *Dichanthium ischaemum*, fertilizer treatment, diversity, species frequency, sustainable management.

INTRODUCTION

Grasslands are one of the world's major ecosystems, covering nearly one-third of the Earth's land surface, and should be given greater priority in future landscape planning and management because they play important roles in agricultural landscapes and provide many benefits to society. High Nature Value (HNV) grasslands provide a wide range of services that are important for water management, livestock grazing, climate mitigation, biodiversity, and are closely linked to our agricultural history in many ways (Hopkins & Holz, 2005; Lemaire et al., 2011; Buchmann et al., 2019; Pergola et al., 2020; Petermann et al., 2021). The *Dichanthium ischaemum* (L.) Roberty (sin. *Bothriochloa ischaemum*) grasslands studied in the experiment are NATURA 2000 habitats (Sub-Pannonian Steppe grasslands – 6240*) and are specific to the steppe and forest-steppe areas, which have recently gained increasing importance in terms of climate change and biodiversity conservation. Due to their role as biodiversity hotspots, and the great threats they face, they are priority habitats of the EU Habitats Directive (Poniatowski et al., 2020).

These meadows have a xerophilic character, conferred by the characteristic and dominant species *Dichanthium ischaemum* and are established on poor, shallow, often calcareous or skeletal soils, on sunny slopes, with southern or southwestern exposition and represent a derived type of meadows. *Dichanthium ischaemum* is an oligotrophic, xerophilous species with a wide ecological amplitude (Vintu et al., 2004; Szentes et al., 2025), and due to the increased abundance of the dominant species with low potential, the vegetation has a reduced forage value, which affects sustainable use. The absence of fertilisation, abandonment and traditional land use (extensive grazing) have allowed this type of vegetation to be maintained in the long term (Doniță et al., 2005; Chifu et al., 2014; Szentes et al., 2025). Numerous long-term studies have shown that the application of fertilizers, regardless of their nature, has a significant impact on the floristic composition and biodiversity of grasslands (Vaida et al., 2021; Gaga et al., 2022; Nazare et al., 2023; Ghețe et al., 2026). In contrast, intensive management involving excessive use of mineral and organic fertilizers leads to simplification of phytocenoses and the domination of competitive

grasses and perennial legumes, while a lack of input favors oligotrophic species and reduces forage value.

In this context, the objective of the study was to evaluate how different management scenarios will affect the stability of the plant community dominated by *Dichanthium ischaemum*. For this, in 2018, a multiple treatment experiment was designed to analyze the long-term effects of organic and mineral fertilization on floristic composition and diversity, providing information on community dynamics and its responses under constant management conditions.

MATERIALS AND METHODS

Soil and climatic conditions

The experiment was conducted on a permanent *Dichanthium ischaemum* (L.) Roberty grassland in the Moldavian forest-steppe area on the territory of Andrieșeni village Iași county, between the geographical coordinates 47°02' N latitude and 27°22' - Eastern longitude, on a slightly inclined ground, with SE exposition (Photo 1).

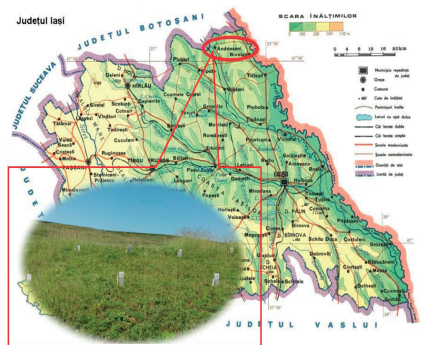


Photo 1. Location of experiences field in Iasi County

The soil type in the experimental field is kastanozem with medium fertility, having a slightly alkaline reaction, with pH values between 7.93 and 8.38 (Gafencu et al., 2023). The studied area has remarkable characteristics in terms of both precipitation and temperatures recorded during the study period and is characterized climatically by significant deviations from normal, with annual temperatures above the multi-year average and an uneven distribution of precipitation, with long periods of water stress, which negatively affects the growth and

regeneration of vegetation on the studied grasslands.

The multi-year average temperature is 9.6°C, and the average temperature during the growing season is 17.3°C. The multiannual average of precipitation is 517.8 mm, and in the area where the research was conducted, from a climatic point of view, the 2023-2024 agricultural year can be characterized as favorable for the exploitation of permanent grasslands in the forest-steppe zone. The total amount of precipitation during the study period (2023-2024) was 658.8 mm, which is higher than the multi-year average, but was unevenly distributed, with short periods of water stress in September-October 2023 and August 2024, as shown in Figure 1. The average annual temperatures were 13.7°C in 2023-2024.

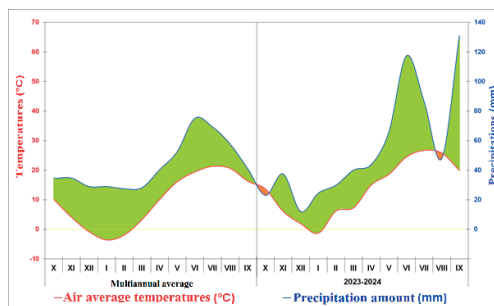


Figure 1. Climadiagram for the agricultural year 2023-2024, where green color show the precipitation that is above the evapotranspiration level, and the red color show the periods of water stress for plants

Experiment set-up

A monofactorial experiment with 7 treatments was organized according to the randomized block method in three replicates.

The research covered three blocks, and the experiment had the following treatments:

- T₁ - unfertilized (control);
- T₂ - N₅₀P₅₀ kg/ha annually (low input);
- T₃ - N₇₅P₇₅ kg/ha annually (medium input);
- T₄ - N₁₀₀P₁₀₀ kg/ha annually (high input);
- T₅ - 10 t/ha sheep manure applied annually;
- T₆ - 20 t/ha sheep manure applied annually;
- T₇ - 30 t/ha sheep manure applied at two years.

Two types of fertilizers were used: an organic one represented by well fermented sheep manure (older than two years) and a mineral one represented by a complex fertilizer with nitrogen and phosphorus (N₂₀P₂₀). The administered

manure from sheep had the following chemical composition: N-0.692%, P₂O₅-0.320% and K₂O-0.811%. Fertilizers were applied manually in early spring before the start of active vegetation growth. The area of each variant was 12 m² (3 x 4 m), and the harvested area was of 8.75 m² (2.5 x 3.5 m).

Measurements and analyses

Harvesting was done at earing-flowering stage of the dominant grasses, and the floristic composition was established using the Braun-Blanquet method (Cristea et al., 2004). For the processing of floristic data, we used the programmes Excel and PC-ORD 6 software. Within the PC-ORD multivariate analysis program (McCune & Grace, 2002), various methods were used to order the floristic relevés, namely: Summary (to determine Specific richness (S) - total number of species per plot; Shannon - Wiener index (H'), which combines species richness and relative abundance; Evenness (E), calculated as the ratio between the observed H' (Shannon-Wiener index) and the maximum possible H' for the number of species ($E = H' / \ln S$); Simpson index (D), which is sensitive to the dominance of abundant species in the community), average frequency and dominance, and multidimensional NMDS Autopilot scaling (Păcurar & Rotar, 2014).

The statistical data was analyzed by SPSS using ANOVA and the Lowest Significant Difference test (LSD 5%).

RESULTS AND DISCUSSIONS

The influence of treatments on the floristic composition

An important factor in determining fertilizer doses is the floristic composition of grasslands. Fertilizer application leads to an increase in the availability of nutrients for plants. Under these conditions, only a few fast-growing plant species can compete for light, eliminating less competitive species, which can result in a decline in species richness. On the other hand, on very poor soils, only slow-growing species are able to compete for nutrients.

The Figure 2, showing the ordering of the floral composition in 2024, that was done using NDMS Autopilot (multidimensional scaling),

confirms that the application of treatments over the years of study has produced significant changes in the floral composition of the vegetation cover. Thus, following the analysis of the ordination graph, a much clearer overlap of the experimental variants is observed, but the fact that the unfertilized phytocenosis (T₁-unfertilized) is at a great distance from the fertilized variants shows us that mineral and organic treatments have caused changes in the floristic composition of the vegetation cover, and the similarity of the phytocenoses is not very high. Depending on the management applied, phytocenoses can be grouped according to their proximity into two groups: the first group includes treatments with NP fertilizers (T₃ and T₄), and the second group includes treatments with sheep manure (T₅, T₆, and T₇). The similarity between the variants within the two groups indicates that there is a high degree of similarity between them in terms of floristic composition. In the case of treatment T₂ – low input (N₅₀P₅₀), the changes recorded in the structure of the vegetation cover were more limited. There are variants in which, following the application of organic fertilizers (T₅, T₆, and T₇), new species appeared, such as *Medicago sativa* L. in variant T₅ (10 t/ha sheep manure applied annually), *Medicago falcata* L. in variant T₆ (20 t/ha sheep manure applied annually), and *Vicia cracca* L. in the variant fertilized with 30 t/ha sheep manure applied at two years (T₇). Regarding the frequency of species in the reference phytocoenosis (type *Dichanthium ischaemum* (L.) Roberty - *Festuca valesiaca* Schleich. ex Gaudin), in addition to the dominant and co-dominant species, there are other species with higher coverage and frequency, such as: *Elymus repens* (L.) Gould., *Achillea millefolium* L., *Potentilla cinerea* Chaix ex Vill., *Thymus glabrescens* Willd and *Astragalus onobrychis* L., but also species with medium coverage and frequency: *Vicia hirsuta* (L.) Gray and *Vicia sativa* L. (Figure 3). The lowest dominance and frequency are found in the species *Poa pratensis* L. ssp. *pratensis*, *Medicago falcata* L., *M. lupulina* L., *Lotus corniculatus* L., *Achillea setacea* Waldst. et Kit., *Koeleria gracilis* Pers., *Eryngium campestre* L., *Lappula squarrosa* (Retz.) Dumort., etc. (Figure 3).

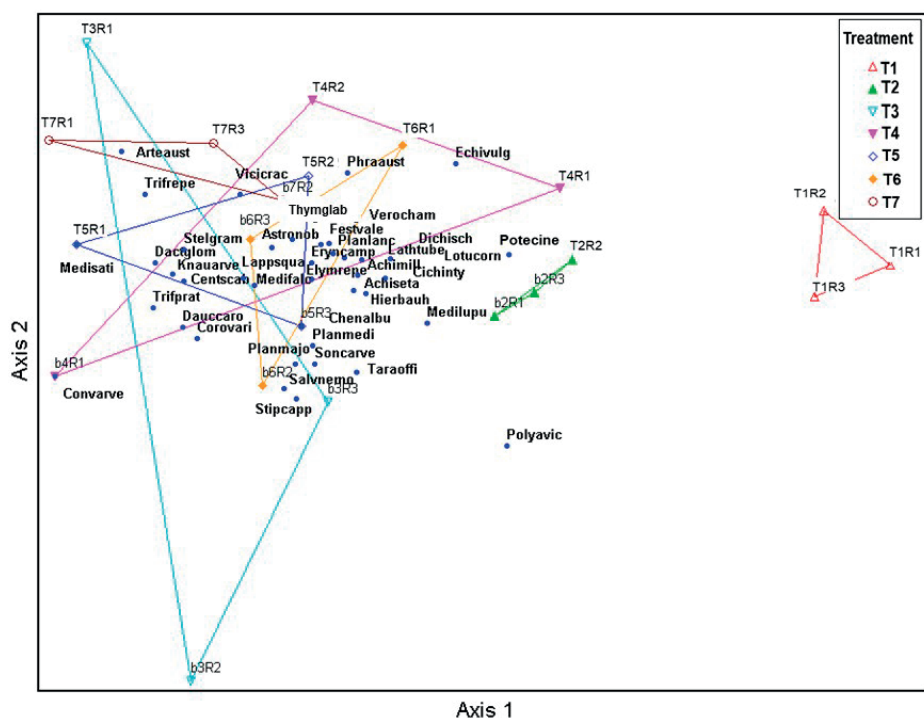


Figure 2. Ordination of floristic composition in 2024, influenced by treatments (T₁ - unfertilized (control); T₂ - N₅₀P₅₀; T₃ - N₇₅P₇₅; T₄ - N₁₀₀P₁₀₀; T₅ - 10 t/ha of sheep manure applied annually, T₆ - 20 t/ha of sheep manure applied annually and T₇ - 30 t/ha of sheep manure applied at 2 years; R₁, R₂, R₃ – replicates)

Legend: Species abbreviations: *Dactylis glomerata* L. = Dactglom, *Dichanthium ischaemum* (L.) Roberty = Dichisch, *Elymus repens* (L.) Gould. = Elymrepe, *Festuca valesiaca* Schleich. ex Gaudin = Festvale, *Poa pratensis* L. ssp. *pratensis* = Poaprat, *Phragmites australis* (Cav.) Trin. ex Steud. = Phraaust, *Stipa cappilata* L. = Stipcapp, *Astragalus onobrychis* L. = Astronob, *Coronilla varia* L. = Corovari, *Lathyrus tuberosus* L. = Lathtube, *Lotus corniculatus* L. = Lotucorn, *Medicago falcata* L. = Medifalc, *Medicago lupulina* L. = Medilupu, *Medicago sativa* L. = Medisati, *Trifolium pratense* L. = Trifprat, *Trifolium repens* L. = Trifrepe, *Vicia cracca* L. = Vicicrac, *Achillea millefolium* L. = Achimile, *Achillea setacea* Waldst. et Kit. = Achiseta, *Artemisia austriaca* Jacq. = Arteaust, *Convolvulus arvensis* L. = Convarve, *Centaurea scabiosa* L. = Centscab, *Cichorium intybus* L. = Cichinty, *Chenopodium album* L. = Chenalbu, *Daucus carota* L. ssp. *carota* = Dauccaro, *Echium vulgare* L. = Echivulg, *Eryngium campestre* L. = Eryncamp, *Hieracium bauhini* Besser = Hierbauh, *Knautia arvensis* L. = Knauarve, *Koeleria gracilis* Pers. = Koelgrac, *Lappula squarrosa* (Retz.) Dumort. = Lappsqua, *Ononis arvensis* L. = Ononarve, *Plantago lanceolata* L. = Planlanc, *Plantago media* L. = Planmedi, *Plantago major* L. = Planmajo, *Potentilla cinerea* Chaix ex Vill. = Potecine, *Salvia nemorosa* L. = Salvnemo, *Sonchus arvensis* L. = Soncarve, *Stellaria graminea* L. = Stelgram, *Taraxacum officinale* Weber. = Tragoffi, *Thlaspi arvense* L. = Thlaarve, *Thymus glabrescens* Willd. = Thymglab, *Vicia cracca* L. = Vicicrac, *Vicia hirsuta* (L.) Gray = Vicihirs, *Vicia sativa* L. = Vicisati, *Veronica chamaedrys* L. = Verocham, nomenclature after Sărbu et al., 2013; Ciocărlan, 2009

Following a comparison between the floristic composition of the control and that of the fertilized variants, changes in the frequency-dominance of certain species were observed (Figure 4). Therefore, some species that had a very low frequency-dominance in the control phytocenosis (T₁-unfertilized), such as the species: *Poa pratensis* L. ssp. *pratensis*, *Achillea setacea* Waldst. et Kit., *Cichorium intybus* L., *Medicago falcata* L., *Lotus corniculatus* L., etc., are found in a higher

percentage with a medium to high frequency (Figure 4). Valuable forage species such as *Dactylis glomerata* L., *Festuca arundinacea* Schreb., and *Medicago lupulina* L., which were absent in the control phytocenosis (T₁), show an increase in frequency-dominance as a result of the management applied (Figure 4). Other species, such as *Thlaspi arvense* L., *Medicago sativa* L., and *Ononis arvensis* L., show low frequency-dominance in most of the variants studied (Figure 4).

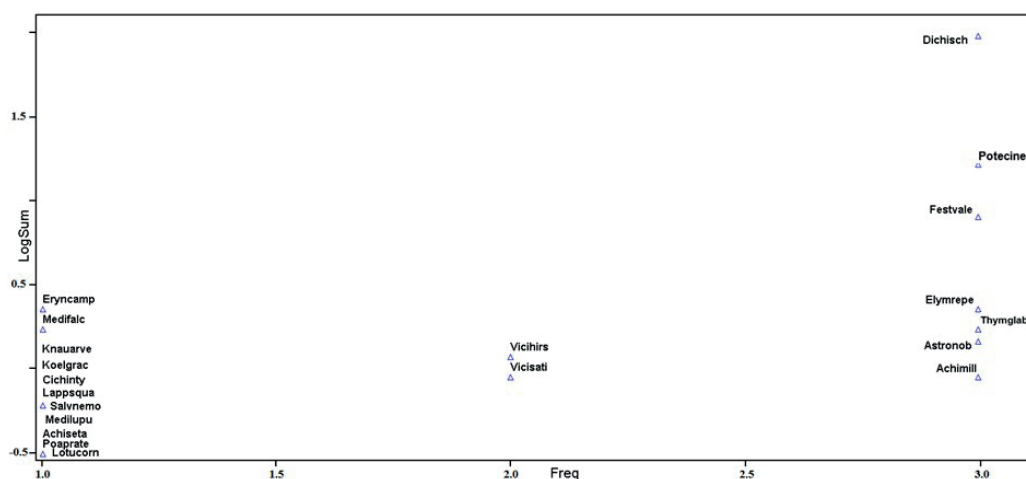


Figure 3. Dominant frequency diagram of witness phytocenosis *Dichanthium ischaemum* (L.) Roberty, in 2024 Legend: Freq= Species frequency, Logsum = Log base 10 of species dominance (for species name see the legend from Figure 2)

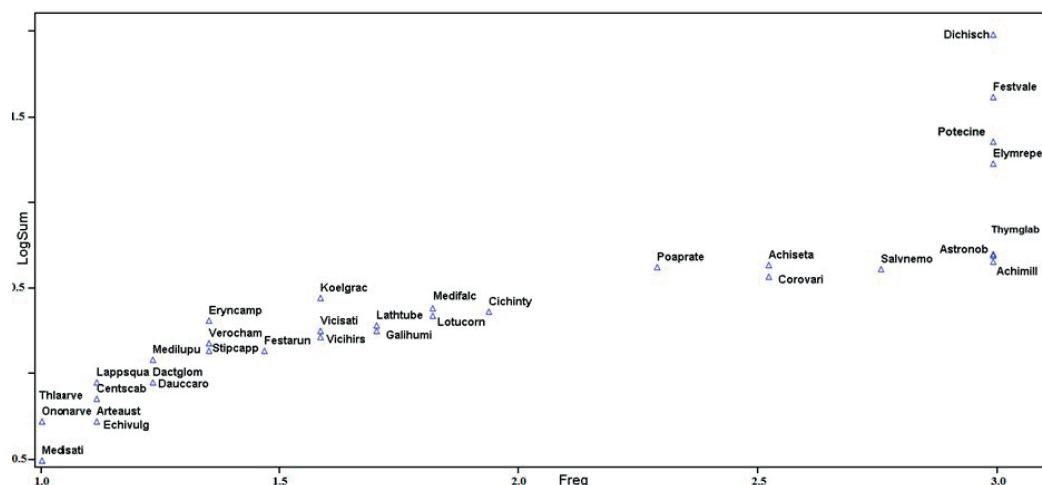


Figure 4. Dominant frequency diagram of organic and mineral fertilized variants phytocenosis *Dichanthium ischaemum* (L.) Roberty, without control variant, in 2024 (Freq= Species frequency, Logsum = Log base 10 of species dominance, for species name see the legend from Figure 2)

The Impact of fertilizer on diversity indices

To evaluate the impact of treatments on diversity indices, four biodiversity indices (S, H', E, D') were calculated, and the results are presented in Table 1. The overall assessment of biodiversity indices highlights a significant effect of mineral and organic treatments on the structure of phytocenoses. Analyzing the phytodiversity of fertilized variants, significant changes can be observed, whether we consider the number of species (S) or the Shannon (H) and Simpson (D') diversity indices. The unfertilized control plots (T₁) showed low

values of species richness and diversity (S = 19; H' = 1.314), characteristic of oligotrophic grasslands with relatively balanced species abundances. The treatments that received reduced mineral and organic inputs (T₂ - N₅₀P₅₀ and T₅; 10 t/ha of sheep manure applied annually) showed higher diversity values compared to the control treatment. Of these, treatment T₅ (10 t/ha of sheep manure applied annually) showed the highest species richness and Shannon diversity (S = 32; H' = 1.806). Moderate mineral and manure inputs (T₃ - N₇₅P₇₅ and T₆ - 20 t/ha of sheep manure applied

annually) resulted in consistently high values of diversity and evenness ($H' \approx 1.950\text{--}1.985$; $E \approx 0.641\text{--}0.625$), indicating a relatively even distribution of species abundance across these treatments (Table 1). This shows a more balanced distribution of species and greater diversity under moderate fertilization conditions. In contrast, at very high fertilizer doses ($T_4 - N_{100}P_{100}$ kg/ha annually and $T_7 - 30$ t/ha sheep manure applied at two years), although the Shannon index remains relatively high ($H' \approx 1.788\text{--}1.806$), diversity tends to be sustained by a few dominant species. Species evenness (E) followed the same trend: low values in the control (0.498) compared to the highest values in the fertilized treatments (0.571-0.625). This indicates that fertilization promotes a more uniform distribution of species abundance up to an entry threshold, after which communities become unbalanced again. The Simpson index (D) reflected the same dynamics (Table 1), with higher values in

fertilized treatments (0.634-0.715) compared to the control (0.562). Our research shows that high biodiversity can be observed in intermediate situations (T_3 and T_6) when competition is low and when a large number of mesotrophic species can coexist with a certain number of oligotrophic and eutrophic species. It also suggests that both the absence of inputs and high fertilization lead to a reduction in diversity (Table 1). These results confirm the results previously obtained by many other authors such as Mrkvička & Veselá, 2002; Rotar et al., 2003; Chytrý et al., 2009; Păcurar & Rotar, 2004; Ciobanu et al., 2013; Samuil et al., 2013; Vintu et al., 2017; Tong et al., 2019; Vaida et al., 2021; Boch ș.a. 2021; Ranta ș.a., 2021; Nazare et al., 2023; Samuil et al., 2025; Canișag et al., 2026; Ghețe et al., 2026).

Table 1. The influence of fertilization treatments on biodiversity indices in grasslands, in 2024

Treatments	Shannon Index (H')	Species Richness (S)	Evenness (E)	Simpson (D)
T_1	1.314 ^c	19 ^c	0.498 ^c	0.562 ^c
T_2	1.547*	21 *	0.571 *	0.634 *
T_3	1.950*	27 *	0.641 *	0.715 *
T_4	1.806*	25 *	0.593 *	0.667 *
T_5	1.959*	32 *	0.609 *	0.697 *
T_6	1.985*	33 *	0.625 *	0.710 *
T_7	1.788*	30 *	0.613 *	0.695 *
LSD 5%	0.31	2	0.036	0.033

Note: T_1 - unfertilized (control); $T_2 - N_{50}P_{50}$; $T_3 - N_{75}P_{75}$; $T_4 - N_{100}P_{100}$; $T_5 - 10$ t/ha of sheep manure applied annually, $T_6 - 20$ t/ha of sheep manure applied annually and $T_7 - 30$ t/ha of sheep manure applied at 2 years; * = $p < 0.05$).

CONCLUSIONS

The current results of our six-year study demonstrate that fertilization is a determining factor in the structure and diversity of oligotrophic grasslands dominated by the species *Dichanthium ischaemum* in the North-East of Romania. The results showed that the application of organic and mineral fertilizers led to a clear difference in the development of plant communities, confirming the hypothesis that management intensity is the main factor influencing the structure and diversity of grasslands in the study area. Therefore, based on the long-term study, we hypothesized that high biodiversity can be maintained in intermediate

situations, when competition is low and when a large number of mesotrophic species can coexist with a certain number of oligotrophic and eutrophic species. Our research shows that in order to preserve the floristic diversity of the studied grassland, the amount of sheep manure should not exceed 10-20 t/ha, and in the case of mineral fertilizer treatments, the dose should not exceed $N_{75}P_{75}$ kg/ha s.a.

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CHOROLOGY, ECOLOGY AND PHYTOSOCIOLOGY OF THE ALPINE SEDGE SPECIES IN NORTHERN OLTENIA, ROMANIA

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Abstract

The alpine sedge (*Carex curvula* All.) is an endemic species, characteristic of alpine meadows. This species of conservation interest has a limited distribution at European level, being found in: the Alps, the Pyrenees, the French Massif Central, the Carpathians and part of the highest mountains in the Balkans. Alpine sedge grow on flat or slightly sloping terrain, on humic-silicate soils, strongly acidophilic, oligotrophic and covered with snow until the beginning of summer. In Oltenia, this species is found in the Parâng, Țarcu, Godeanu, Cernei, Lotru and the Căpățâni Mountains. From a phytocoenotic point of view, this alpine meadow species forms the plant community *Primulo-Caricetum curvulae* Br.-Bl. 1926 em Oberd. 1957, whose composition and floristic physiognomy vary depending on the ecological conditions existing in the different mountain groups in Oltenia. At the same time, this species is part of the floristic composition of other alpine plant communities of *Ericaceae* and other types of secondary alpine meadows, the species presenting a special conservation interest.

Key words: chorology, alpine sedge, phytosociology, distribution, plant communities.

INTRODUCTION

In Europe, *Carex curvula* occurs as the following infrataxa: *C. curvula* ssp. *curvula* and *C. curvula* ssp. *rosae*, especially in the floristic structure of the alpine tundra, but often descends to the subalpine level.

Therefore, the alpine sedge (*Carex curvula* All.) is characteristic species of alpine meadows and has a wide distribution in Europe, especially in the alpine zone of the mountains: Alps, Pyrenees, Massif Central France, Carpathians and on the higher peaks of some mountain ranges in Albania, Bosnia-Herzegovina, Bulgaria and Slovenia. In the Carpathian Mountains the *Carex curvula* ssp. *curvula* infrataxon is found. In Romania the species is found in the Țarcu, Godeanu, Cernei, Retezat, Parâng, Cibin, Căpățâni, Făgăraș, Iezer-Păpusa, Piatra Craiului and Bucegi Mountains, Rodnei Mountains. From Oltenia, alpine sedge meadows were cited from the Țarcu, Godeanu and Cernei Mountains, by Simionkai (1877), Grecescu (1898), Safta (1943), Buia (1943), Popescu P. C. & Bujoreanu G. (1957), Pop, Nemeș, Boșcaiu et al. (1967), Boșcaiu (1942, 1971), Lupșa (1967), Niculescu et al. (1965), Cernelea et al. (1985) from the following locations: Babei Peak, Bloju

Peak, Căleanu Peak, Gugu Peak, Bulzu, Micușă, Cununii Peak, Pietrei Peak, Țarcu Peak, Vulturul Peak, Godeanu Peak, Moraru Peak, Bloju Mountain, Stâna Mare Mountain, Bulzu, between Gugu and Muraru, Micușă, Stâna Mare Mountain, Borăscu Platform, Gârdomanului Plateau, Borăscu Mic Peak, Gârdomanului Peak, Godeanu Peak, Gugu Peak, Mâțului Peak, Moraru Peak, Paltina Peak, Scărișoara Peak.

The studied plant community, built by *Carex curvula*, was also cited from the Parâng Massif, where it occupies the largest area, in the upper basin of the Gilort, meeting on Parâng Peak, Cârja Peak, Gruicul Peak, the Bengai Coast, on Cioara Mountain, Iezeru, Cârja, Mândra, Dengherul, Picleșă, Gruiu, Bălescu, Urdele, Tidvele, Setea Mare, Setea Mică, Mohorul and Păpușă, between 2100-2518 m (Buia, 1943; Buia et al., 1959; Buia et al., 1963; Aur et al., 2001; Resmeriță et al., 1977; Safta, I., 1943; Samiolă, 1960; Sanda et al., 1989; Șerbănescu et al., 1966). Since 2004, important phytocoenoses of *Carex curvula* have been identified and analyzed in the Căpățâni Mountains (Niculescu, 2004). Well-individualized phytocoenoses of *Carex curvula* have been cited from the Lotrului Mountains – Șteflești and Frumoasa Peaks (Drăgulescu, 1995). In order to contribute to the study of the flora and practical vegetation of

Oltenia, as well as to prevent its reduction through irrational exploitation of mountain meadows, we began a study on the chorology, ecology, phytosociology and syndynamics of the plant community built by alpine sedge, a species with unique morpho-anatomical, physiological, ecological and geobotanical characteristics.

MATERIALS AND METHODS

The territory under study is located in the Southern Carpathians in northern Oltenia, in the subalpine and alpine levels, at over 2000 m altitude, where the eco-pedo-climatic conditions allow the development of the *Carex curvula* species.

For the study of the vegetal carpet, we have used methods of phyto-sociologic research characteristic to the Central European phyto-sociologic School, which was based on the principles and methods elaborated by J. Braun-Blanquet (1928) and adapted by A. Borza (1934) to the particularities of our country's vegetation. The plant communities were identified and distinguished according to the characteristic, edifying, dominant and differential species. For the identification of the plant communities and the coenotaxonomic classification, synthesis works of Mucina et al. (2016), Rodwell (2002) were used. Regarding the studies carried out on the vegetation dynamics, they were taken into account according to the methodology described in the works developed by Cristea, Pedrotti and Gafta (1993; 2004). Following the research on the vegetal carpet, we will take into account its analysis and characterization from a structural, physiognomic and syndamic point of view in close correlation with the profound eco-pedo-climatic changes of recent years (Niculescu, 2025).

RESULTS AND DISCUSSIONS

Following studies carried out in the area included in the mountainous area of northern Oltenia, the plant community *Primulo-Caricetum curvulae* Br.-Bl. 1926 em Oberd. 1957 was identified and analyzed, which was classified in the *Juncetea trifidi* Klika et Hada class. *Carx curvula* meadows were identified and analyzed in the Godeanu Mountains on

Godeanu and Gîrdomanul Peaks, in the Parang massif, on Parangul Mare Peak, Parangul Mic Peak, Tidvele, Carja, Urdele, Dengherul, Mandra, Setea Mare, Setea Mică, Mohorul and Păpușa, in the Căpățânii Mountains, in the upper basin of Luncavăț, on Ursu Peak, Căpățâna Peak and Coșana Peak and in the Lotrului Mountains - Șteflești Peak, Cristești Peak, Baluțu Peak, Puru Mountain and Piatra Albă. Following studies conducted in the Parâng Massif, we can mention that *Carex curvula* meadows have the best development and a well-individualized floristic structure with over 30 species, the lowest altitude at which phytocenoses characteristic of these meadows can be found is 2100. In this area, alongside the edifying species, *Carex curvula* and *Primula minima*, some species of *Cyperaceae* and *Juncaceae* are found with high abundance-dominance: *Juncus trifidus*, *Luzula sudetica*, *Luzula spicata*, *Campanula alpina*, *Gentiana verna*, *Hieracium alpinum*, *Gnaphalium supinum*, *Phyteuma nanum*, *Chrysanthemum alpinum*, *Geum montanum*, *Potentilla aurea* ssp. *chrysocraspeda*, *Ligusticum multelina* are cormophyte species that are not absent from the floristic composition of the phytocenosele analyzed, thus being characterized by a high constancy (K) and abundance-dominance (AD m). *Festuca airoides*, *Agrostis rupestris*, *Nardus stricta* and *Avenastrum versicolor* are dominant species within this type of meadow, under certain eco-pedo-climatic conditions the phytocenoses built by these grasses have the capacity to develop excessively, to evolve towards characteristic meadows, replacing *Carex curvula* meadows, especially at lower altitudes, below 2200 m. In the northwestern part of Oltenia, *Carex curvula* meadows occupy quite important areas, they being identified following studies carried out in the period 2014-2024 on Vf. Godeanu, Gîrdomanul. In the Godeanu Mountains, the phytocenoses with *Carex curvula* have a floristic structure in which a small number of species adapted to develop on very acidic oligotrophic soils, on ridges and plateaus or steep slopes exposed to low temperatures are present. During the surveys carried out on the Godeanu Peak and Gîrdomanul, it was found that, along with the edifying species, the following are also found with a high constant: *Festuca airoidis*, *Juncus*

trifidus, *Oreochloa disticha*, *Pedicularis verticillata*, *Phyteuma confusum*, *Avenula versicolor*, *Gnaphalium supinum*, *Veronica bellidioides*, *Potentilla aurea* ssp. *chrysocraspedae*, *Ligusticum mutellina*, *Campanula alpina*, *Cetraria islandica*, *Thamnolia vermicularis*.

In the Lotrului Mountains in Northern Oltenia, in the transition zone to Transylvania, we have carried out complex studies in recent years on the mountain, subalpine and alpine meadows, especially in the Lotrului Valley, the Frumoasa Valley, Mănăileasa and Latorița. Regarding the *Carex curvula* species, we can mention that the most important populations of this species that build well-defined phytocenoses are found in the area of Ștefleşti Peak (2242 m): the species being dominant on these high plateaus. On smaller areas, the species was identified on Cristești Peak (2202 m) but also in the area of Baluțu, Puru and Piatra Albă Peak, at over 2000 m altitude.

In the Căpățânii Mountains, in the upper basin of the Luncavăț, the *Primulo-Caricetum curvulae* plant community is rarely found, occupying small areas on the peaks and ridges exceeding 2000 m altitude. The hornbeam meadows take root on flat or slightly sloping terrain, on humiciferous-iluvial podzol soils, oligotrophic and covered with snow until the beginning of summer. In the Căpățânii Mountains, studies were carried out to re-evaluate and analyze the floristic composition and synphytonomics of the *Carex curvula* phytocenoses on Ursu Peak (2124 m), Coșana Peak (2004 m), Căpățâna Peak (2113 m) and Balota Peak (2094 m), but they were not shown in the phytosociological table, because they occupy much smaller areas.

The vegetation coverage varies primarily with altitude and is in close correlation with the ecopedoclimatic conditions of each phytocenosis, being between being between 70-100%. In the phytocenoses analyzed in the Căpățânii Mountains, a smaller number of species are found in the floristic structure, so besides the two edifying species of the plant community, *Carex curvula* and *Primula minima*, the following species are also found with high constancy: *Juncus trifidus*, *Oreochloa disticha*, *Hieracium alpinum*, *H. villosum*, *Campanula alpina*, *Festuca airoides*, *Agrostis rupestris*,

Luzula spicata, *Carex atrata*, *Cetraria islandica*. Within this plant community, hemicryptophytes predominate as bioforms, and as floristic elements, the largest share is held by circumpolar species. From an ecological point of view, the meadows, following the analyses carried out within the phytocenoses of this plant community, are dominated by mesophilous, hexistothermic and acidophilic species.

Karyologically, the most numerous are polyploid species, the diploidy index has the value of 0.70.

The dendrogram of the *Primulo-Caricetum curvulae* Br.-Bl. 1926 em Oberd. 1957 (Figure 1) plant community highlights the grouping of the surveys into two well-individualized clusters, whose Bray-Curtis quantitative index has very close values, ranging between 0.31 and 0.27, this leading to the idea that, within the association, the floristic composition of the phytocenoses is very homogeneous and stable. The first cluster groups the surveys: 1, 3, 2, 5, 9 and 7, and the second 4, 8, 6 and 10. The separation of the surveys into the two clusters is very obvious, the first cluster groups the surveys in which the abundance-dominance of the *Carex curvula* species has a value of 4, in the second cluster this value being 5. Survey 2 within the first cluster is separated based on the *Primula minima* species, being the only survey in which this has AD=1-2. Also in this cluster, the separation of a subcluster with releves 5, 9 and 7 is observed, this grouping being due to the abundance-dominance value of the *Juncus trifidus* species which has the value 1. Releve 6 within the second cluster is separated by the absence of the species *Oreochloa disticha* and *Avenastrum versicolor*.

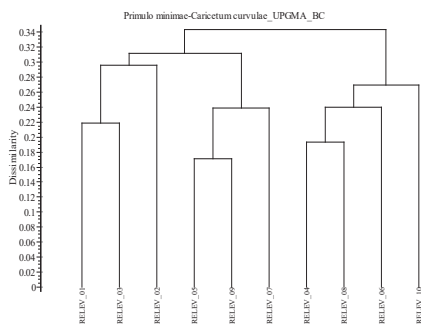


Figure 1. Dendrograma of the *Primula minima*-*Caricetum curvulae* plant community

Table 1. Floristic composition of the *Primula minima*-*Caricetum curvulae* Br.-Bl. 1926 em. Oberd. 1957 plant community

No. of relevée	1	2	3	4	5	6	7	8	9	10	K
Altitude m.o.s. (x 10 m)	212	212	212	223	223	230	232	225	240	221	
Exposure	SV	V	-	-	-	NE	-	E	-	-	
Inclination (in grades)	10	5	-	-	-	10	-	5	-	-	
Canopy (%)	90	90	85	90	95	85	90	90	90	90	
Area (m ²)	10	10	25	25	100	10	25	100	100	25	
Char. ass.											
<i>Carex curvula</i>	4	4	4	4	4	4	4	4	4	4	V
<i>Primula minima</i>	+	1	+	+	+	+	+	+	+	+	V
Caricion et Caricetalia curvulae											
<i>Agrostis rupestris</i>	+	+	-	-	+	+	1	+	+	+	IV
<i>Festuca airoides</i>	+	1	+	+	1	+	1	+	1	+	V
<i>Oreochloa disticha</i>	+	1	+	+	1	-	+	+	+	+	V
<i>Phyteuma confusum</i>	+	+	+	+	+	+	+	-	+	-	IV
<i>Campanula alpina</i> ssp.											
<i>bucegiensis</i>	+	+	+	+	+	+	-	+	+	-	IV
<i>Luzula spicata</i>	+	-	+	+	+	+	+	-	+	-	IV
<i>Carex atrata</i>	1	+	+	+	+	+	+	+	+	+	V
<i>Avenastrum versicolor</i>	+	+	1	1	1	+	1	+	1	+	V
Juncetea trifidii											
<i>Juncus trifidus</i>	1	1	+	+	1	+	1	+	1	+	V
<i>Pulsatilla alba</i>	-	-	-	+	+	+	+	+	+	+	IV
<i>Hieracium alpinum</i>	+	+	+	+	+	-	-	+	+	-	IV
Loiseleurio-Vaccinion											
<i>Vaccinium gaultherioides</i>	+	-	+	-	-	+	-	+	-	+	III
<i>Loiseleuria procumbens</i>	+	-	+	+	+	-	-	+	-	-	III
<i>Cetraria islandica</i>	+	+	+	1	+	1	1	+	1	1	V
<i>Thamnolia vermicularis</i>	+	+	+	1	1	1	+	+	1	1	V
Androsacion et Androsacetalia											
<i>Poa laxa</i>	+	+	+	+	-	-	+	-	-	-	III
<i>Veronica baumgartenii</i>	+	+	-	-	+	-	+	-	-	-	II
Potentillo-Nardion											
<i>Ligusticum mutellina</i>	-	+	-	+	-	+	-	-	+	-	II
<i>Potentilla aurea</i> ssp.											
<i>chrysocraspeda</i>	+	+	+	+	-	+	+	+	+	-	IV
<i>Pedicularis verticillata</i>	+	-	-	+	-	+	+	+	-	-	III
<i>Thymus balcanus</i>	-	-	-	+	-	+	.	+	-	-	II
<i>Geum montanum</i>	+	+	+	-	+	+	+	-	+	+	IV
<i>Poa media</i>	+	+	-	-	-	+	-	-	+	-	II
<i>Poa alpina</i>	+	-	-	+	-	-	-	-	+	+	II
Nadetalia et Nardo-Callunetea											
<i>Nardus stricta</i>	-	+	-	-	+	+	-	+	+	-	III
<i>Festuca nigrescens</i>	+	+	-	.	-	+	+	-	-	+	III
Salicetalia herbaceae s.l.											
<i>Gnaphalium supium</i>	-	+	+	-	+	-	-	+	+	-	III
Variae Syntaxa											
<i>Carex sempervirens</i>	-	-	+	-	+	-	-	-	+	+	II
<i>Deschampsia flexuosa</i>	-	-	+	-	-	-	+	-	+	-	II
<i>Veronica bellidioides</i>	-	-	-	-	-	+	+	+	-	-	II
<i>Rhododendron myrtifolium</i>	+	+	+	-	-	-	-	-	-	-	II
<i>Hieracium villosum</i>	1	+	+	+	1	+	+	+	1	+	V
<i>Polytrichum juniperinum</i>	-	+	-	+	-	-	-	-	-	-	I

Place and data of the relevés: 1, 2, 3 - Ursu Peak, 18.07.2004; 4, 5 - Godeanu Peak, 20.06. 2023; 6, 7 - Setea Mare Peak, 15.06.2024; 8 - Urdele, 10.06.2018; 9 - Parangul Mare Peak, 23.06.2014; 10 - Șteflești Peak, 19.05.2019.

From a syndinamic point of view, these meadows can evolve in close correlation with the existing eco-pedo-climatic conditions towards meadows of *Agrostis rupestris* (when overgrazed), *Festuca airoides* or *Poa alpina* and sometimes they can be replaced by thickets of *Loiseleuria procumbens* or *Vaccinium gaultherioides*.

Importance. Horn meadows have a low economic importance, because in the studied territory they occupy small areas and have a low fodder productivity. However, considering that in the floristic composition there are several species with fodder value, even if the productivity is low, of approximately 2000kg/ha, the nutritional value is good, these meadows being very well consumed by sheep. However, at the end of June and in July, flocks of sheep are frequently found on many of the peaks and ridges on which we have conducted studies, at altitudes exceeding 2000 m.

CONCLUSIONS

Alpine sedge meadows take root on flat or slightly sloping terrain, on humic-silicate soils, strongly acidophilic, oligotrophic and covered with snow until the beginning of summer. In Oltenia this plant community is found in the Parâng Mountains, the Godeanu Mountains, Lotru Mountains and the Căpățâni Mountains. The *Primulo-Caricetum curvulae* plant community occupies larger or smaller areas in the Carpathians of Northern Oltenia, being in close correlation with the current eco-pedo-climatic conditions, meeting on the highest peaks and ridges exceeding 2000 m. From an economic point of view, the described association does not present a particular importance, having a low forage value. Intensive grazing has a negative impact on the biodiversity of the studied area, by decreasing the plant biomass and the number of species with forage value. The large number of sheepfolds as well as the too large number of animals in these sheepfolds can lead to the degradation of meadows with forage value and to the increasing expansion of sedges.

These meadows built by *Carex curvula* have a special role from a pedogenetic point of view but also from a floristic and conservative point of view, so they must be preserved with a favorable conservation status by applying adequate protection measures.

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VEGETATION AND THE PRODUCTIVE POTENTIAL OF PERMANENT GRASSLANDS DOMINATED BY *Festuca rupicola* Heuff. IN BIHOR COUNTY (NORTH-WESTERN ROMANIA)

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Abstract

The present study proposed two main objectives, the first was to investigate *Festuca rupicola* Heuff. grasslands in Bihor County in terms of productivity and biodiversity. The second main objective involves analyzing the floristic composition and comparing the productivity of *Festuca rupicola* Heuff. grasslands in Bihor County with similar phytocoenoses from nine locations elsewhere in Romania. To study the productive potential of *Festuca rupicola* Heuff. grasslands, two plant associations were studied: *Festucetum rupicolae* Burduja et al. 1956 and *Thymo comosi-Festucetum rupicolae* (Csűrös et Gergely 1959) Pop et Hodișan 1985, from seven locations in Bihor County. *Festuca rupicola* Heuff. grasslands are characteristic of the plains and hilly zones of Bihor County, occurring on moderately sloping terrain with generally sunny exposures. Due to less pronounced xerothermic ecological conditions, these grasslands exhibit a complex floristic composition, consisting mainly of xerophilous species, as well as mesoxerophilous ones. The pastoral value and productivity of these grasslands in Bihor County are weak-medium to middle-good, with a yield of 6.07-11.80 t/ha of green mass and a capacity of 0.57-0.98 LU/ha. To obtain a clearer understanding of the pastoral value, green mass production and livestock carrying capacity of these grasslands in Bihor County, a correlation analysis was conducted on *Festuca rupicola* Heuff. dominated grasslands originating from nine distinct regions of Romania.

Key words: grasslands, pastoral value, green mass production, carrying capacity.

INTRODUCTION

The socio-economic development of human communities has, over time, been influenced and shaped by the way natural resources are utilized at the local and/or regional level. In this context, grasslands have been used as important renewable natural resources.

Permanent grasslands natural and/or semi-natural constitute an essential component of natural heritage and, through their use for grazing, represent the most accessible source of forage. At the same time, grasslands are characterized by a pronounced multifunctional nature, fulfilling a wide range of roles, such as: forage production, conservation of plant and animal biodiversity, prevention of soil erosion, water storage, maintenance of groundwater quality, preservation of landscape quality, carbon sequestration (Bernués et al., 2015).

Within agricultural systems, soil and water quality are correlated with productivity levels. Maintaining healthy perennial vegetation contributes significantly to soil formation,

improvement, and protection, and also plays an essential role in preserving water quality.

Pastoral Value (PV) is an integrative synthetic index used in vegetation studies, which combines forage yield, grassland quality, and the degree of palatability for animals, being determined through floristic assessment methods (Marușca, 2019).

This indicator allows for the estimation of the grassland's carrying capacity, in relation to the maximum livestock load corresponding to a specific grazing system. In general, studies and research on grassland vegetation in Romania contain limited or, in some cases, no information regarding forage yield and quality.

Such data on grassland productivity are essential for underpinning optimal decisions related to their improvement, the assessment of grazing capacity, and the establishment of other measures necessary for the rational management of this important form of agricultural land use dedicated to livestock production (Marușca et al., 2020; Pășcuț et al., 2025; Marușca & Pășcuț, 2022).

Furthermore, this evaluation method, characterized by an adequate level of precision, can substitute the direct determination of biomass production through mowing in enclosed experimental plots, a procedure that is difficult to implement and, in certain situations, impracticable under real-world conditions (Pășcuț et al., 2025; Pășcuț et al., 2022; Pășcuț & Marușca, 2020).

The newly developed method for assessing the productive potential of grasslands dominated by *Festuca rupicola* Heuff., was applied in the present study, with the following main objectives:

1. To determine the pastoral value, green biomass production, and livestock carrying capacity of *Festuca rupicola* Heuff. grasslands located in Bihor County;
2. To conduct a comparative analysis of the main pastoral indicators, namely pastoral value, green biomass production, and livestock carrying capacity, characteristic of grasslands dominated by *Festuca rupicola* Heuff. in Bihor County, in relation to ten other distinct regions of Romania.

The method for assessing grassland productivity based on floristic relevés enables the correlation and comparability of vegetation studies, irrespective of the period in which they were conducted, while also facilitating the integration of economic information related to yield and quality.

The estimation of species abundance-dominance is commonly performed using the Braun-Blanquet scale (1964), which is characterized by unequal variation intervals between classes, namely: 4.5% between classes +1, 12.5% between classes 1-2, 20% between classes 2-3, and 25% between classes 3-4 and 4-5 (Pășcuț et al., 2025).

The pronounced discontinuities among these intervals, particularly the 25% gaps between classes 3-4 and 4-5, as well as the 20% gap between classes 2-3, lead to less reliable estimates in the quantitative assessment of biomass (Marușca, 2019; Pășcuț et al., 2025).

In order to reduce these estimation errors, an alternative scale for evaluating the quantitative contribution of species has been developed, based on their constancy (K) (Marușca, 2019; Pășcuț et al., 2025).

Grasslands play a significant role in rural

development and in maintaining ecological balance, as evidenced by their contribution to biodiversity conservation, improvement of soil fertility, regulation of the hydrological regime, and preservation of landscape quality (Wick et al., 2016).

Plant biomass production and the forage quality of plant species constitute key determinants of the performance of grazing animals, as well as of the carrying capacity of grasslands (Novák, 2004; Soder et al., 2007).

The impact of grazing intensity on forage value and floristic diversity varies depending on the type of animal species involved and on specific ecological conditions (Liu et al., 2015).

Some studies indicate that the abandonment of grazing may lead to a decline in floristic diversity (Janišová et al., 2020), while other research highlights an increase in plant species richness (Ford et al., 2012).

Methods for assessing grassland productivity based on the harvesting and weighing of phytomass provide a high degree of accuracy; however, they involve considerable time investment and financial resources (Peratoner & Pötsch, 2019).

In the hilly regions of our country, there are groves or sparse stands of oak, sessile oak, or other tree species on grasslands dominated by *Festuca rupicola* Heuff., which exhibit the characteristics of silvopastoral systems (Marușca 2006; Sicoe et al., 2023; Crainic et al., 2023b).

Pastoral systems in our country, particularly tree-based pastures, operate empirically, according to the needs of local communities that use them for grazing (Crainic et al., 2023a).

MATERIALS AND METHODS

The assessment of the productive potential of grasslands dominated by *Festuca rupicola* Heuff. was conducted in Bihor County, based on seven phytocoenological studies carried out in the following locations:

- 1 - Crișurilor Plain;
- 2 - Ierului Valley;
- 3 - Tășnadului Hills;
- 4 - Middle and lower basin of the Crișul Negru River;
- 5 - Lăzărenilor Hills;
- 6 - Pădurea Craiului Mountains;
- 7 - Codru-Moma Mountains (Figure 1).

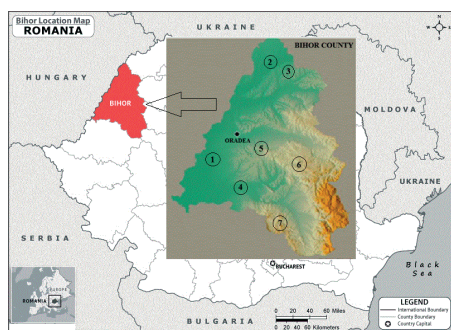


Figure 1. The geographical location of the grasslands dominated by *Festuca rupicola* Heuff. investigated in Bihor County (processed after <https://www.mapsofindia.com>)

The scientific nomenclature of the identified species was adopted in accordance with the works published by Ciocârlan (2009). To assess the degree of floristic similarity among the phytocoenoses of the studied grasslands, the Jaccard index was employed, calculated using the statistical software PAST 5.

The scientific naming of the plant associations was established in compliance with the provisions of the International Code of Phytosociological Nomenclature, 3rd edition (Weber et al., 2000).

The classification of grassland plant associations into higher syntaxonomic units (alliance, order, class) was carried out in accordance with the specialized literature, particularly Mucina (1997) and Chifu (2004-2006). The assessment of species abundance-dominance (AD) in the herbaceous layer of the grasslands was performed using the Braun-Blanquet scale, following the methodology described by Cristea et al. (2004).

After converting the scale of phytosociological ratings in percentage of participation, for each species in the floristic relevé, organized into three major forage groups (*Poaceae*, *Fabaceae*, and other families) the forage quality indicators (F4-F9) and those corresponding to harmful species (F1-F3) are recorded, together with the indicators of useful forage phytomass (M1-M9) and harmful phytomass (M0, associated with categories F1-F3) (Marușca, 2019; Pășcuț et al., 2025).

The conversion of AD values into percentages (Table 1), according to constancy classes (K), was conducted based on the models proposed by Marușca (2019) and Pășcuț et al. (2025).

Table 1. Appreciation of participation (P%) from synthetic floristic relevés, depending on the abundance-dominance scale intervals (AD) and average constancy (K%) for phytocoenoses of permanent grasslands (Marușca, 2019; Pășcuț et al., 2025)

AD Scale Br.-Bl.	AD according to K (%)				
	V (81-100)	IV (61-80)	III (41-60)	II (21-40)	I (<20)
5	87.5*	61.3	43.8	26.3	8.8
4-5	75.0	52.5	37.5	22.5	7.5
3-5	62.5	43.8	31.3	18.8	6.3
2-5	52.5	36.8	26.3	15.8	5.3
1-5	46.3	32.4	23.2	13.9	4.6
+ - 5	44.0	30.8	22.0	13.2	4.4
4	62.5*	43.8	31.3	18.8	6.3
3-4	50.0	35.0	25.0	15.0	5.0
2-4	40.0	28.0	20.0	12.0	4.0
1-4	33.8	23.7	16.9	10.1	3.4
+ - 4	31.5	22.1	15.8	9.5	3.2
3	37.5*	26.3	18.9	11.3	3.8
2-3	27.5	19.3	13.8	8.3	2.8
1-3	21.3	14.9	10.7	6.4	2.1
+ - 3	19.0	13.3	9.5	5.7	1.9
2	17.5*	12.3	8.8	5.3	1.8
1-2	11.3	7.9	5.7	3.4	1.1
+ - 2	9.0	6.3	4.5	2.7	0.9
1	5.0*	3.5	2.5	1.5	0.5
+ - 1	2.8	2.0	1.4	0.8	0.3
+	0.5*	0.4	0.3	0.2	0.1

*Appreciation scale transformation A+D, Braun-Blanquet in percentage, after Cristea et al. (2004).

The forage value indices (F), according to Kovacs (1979), Păcurar & Rotar (2014), and Marușca (2019), are classified as follows: F1 - toxic to animals and humans, F2 - harmful to animal products, F3 - harmful to the grass carpet, F4 - weak fodder (ballast), F5 - mediocre fodder, F6 - forage mediums, F7 - good fodder, F8 - very good fodder, F9 - excellent fodder, X - species of unknown feed value.

The floristic relevés thus developed, which include the percentage participation of species within the vegetation layer, as well as the forage quality indices (F) and the useful phytomass indices (M), enable the determination of pastoral value (PV) based on a specific calculation formula:

$$PV = \sum P(\%) \frac{F}{9}$$

in which: pastoral value (PV), expressed on a scale from 0 to 100, is interpreted as follows: 0-5 (degraded grassland); 5-15 (very poor); 15-25 (poor); 25-40 (mediocre); 40-60 (average); 60-80 (good); 80-100 (very good). Within this calculation, the F index takes values ranging from 4 to 9.

To assess net forage production, an indirect method was used, based on floristic relevés and on the production indices (M) of forage species (F4-F9) within the vegetation layer of grasslands, according to the approach described

by Marușca (2019). The average green biomass production index (IM) of permanent grassland phytocoenoses is determined using a specific formula:

$$IM = \sum P(\%) \frac{M}{100}$$

in which: M - has values between 1-9 only for values of F between 4 - and 9.

After determining the average green biomass index (IM), the corresponding value range is identified according to Table 2 and subsequently multiplied by the conversion coefficient for green biomass production (CMV). Thus, the production is obtained, expressed in tonnes per hectare, along with the corresponding assessment coefficient for this indicator, in accordance with the methodology developed by Marușca (2019) (Table 2).

Table 2. Production indices for feed species and estimating the useful yield per hectare of permanent unfertilized grasslands (Marușca, 2019; Pășcuț et al., 2025)

Average production indices green biomass forage species (IM)	Coefficients of transformation in green biomass production (CMV)	Green biomass production estimate (MV) (t/ha)	Appreciation of production value
0.1 – 0.5	x 1.8	0.18 – 0.90	Very poor
0.6 – 1.0	x 1.9	1.14 – 1.90	
1.1 – 1.5	x 2.0	2.20 – 3.00	Poor
1.6 – 2.0	x 2.1	3.36 – 4.20	
2.1 – 2.5	x 2.2	4.62 – 5.50	Poor-average
2.6 – 3.0	x 2.3	5.98 – 6.90	
3.1 – 3.5	x 2.4	7.44 – 8.40	Average
3.6 – 4.0	x 2.5	9.00 – 10.00	
4.1 – 4.5	x 2.6	10.66 – 11.70	Average - Good
4.6 – 5.0	x 2.7	12.42 – 13.50	
5.1 – 5.5	x 2.8	14.28 – 15.40	Good
5.6 – 6.0	x 2.9	16.24 – 17.40	
6.1 – 6.5	x 3.0	18.30 – 19.50	Good - Very good
6.6 – 7.0	x 3.1	20.46 – 21.70	
7.1 – 7.5	x 3.2	22.72 – 24.00	Very good
7.6 – 8.0	x 3.3	25.08 – 26.40	
8.1 – 8.5	x 3.4	27.54 – 28.90	Excellent
8.6 – 9.0	x 3.5	30.10 – 31.50	

Based on these determinations, the optimal livestock stocking rate, respectively grazing capacity (GC), expressed in livestock units (LU) per hectare, is established using a specific calculation formula:

$$GC(LU/ha) = \frac{MV(kg/ha)}{Nz \times Zp}$$

in which: Nz - the daily requirement of grass for 1 livestock unit (LU), 65 kg (50 kg + 30% (15 kg) seasonal climate fluctuations and unconsumed debris)

Zp = number of grazing days (season)

The grazing season duration in the studied

grasslands of Bihor County averages approximately 180 days, being shorter in the Pădurea Craiului Mountains (175 days) and the Codru-Moma Mountains (165 days).

The indirect estimation of biomass based on floristic relevés offers significant logistical advantages; however, its accuracy is influenced by the degree of observer subjectivity, as visual assessments of vegetation cover may vary among researchers, as well as by the phenological dynamics of vegetation, given that actual biomass changes rapidly depending on the season during which the data are collected.

RESULTS AND DISCUSSIONS

In order to assess the productive potential of permanent grasslands dominated by *Festuca rupicola* Heuff. in Bihor County, two types of grassland associations were analysed, namely *Festucetum rupicolae* Burduja et al. 1956 (Figure 2) and *Thymo comosi-Festucetum rupicolae* (Csűrös et Gergely 1959) Pop and Hodișan 1985, which were phytocoenologically described in seven locations, as follows: Crișurilor Plain (Pop, 1968), Ierului Valley (Czirjak, 2014), Tășnadului Hills (Karacsonyi, 2011), Middle and lower basin of the Crișul Negru River (Gavra, 2014), Lăzărenilor Hills (Herman, 2012), Pădurea Craiului Mountains (Groza, 2008), Codru-Moma Mountains (Pășcuț, 2012).



Figure 2. *Festucetum rupicolae* Burduja et al. 1956, near Cărăsău (Crișul Negru River basin)

These grasslands develop on flat or gently sloping, well-drained surfaces, on a variety of soil types with a reaction ranging from neutral to slightly acidic. Under moderately xerothermic ecological conditions, they exhibit a relatively complex floristic composition, predominantly dominated by xerophilous species, along with mesoxerophilous elements.

From a cenotaxonomic perspective, the two analysed grassland associations are classified as follows:

Festuco-Brometea class (Br.-Bl. et R. Tüxen in Br.-Bl. 1949)

Stipio pulcherrimae-Festucetalia pallentis order (I. Pop 1968)

Thymio comosi-Festucion rupicolae alliance (Pop 1968)

Thymo comosi-Festucetum rupicolae association ((Csűrös et Gergely 1959) Pop et Hodişan 1985)

Festucetalia valesiacae order (Br.-Bl. et R. Tüxen ex Br.-Bl. 1949)

Festucion valesiacae alliance (Klika 1931)

Festucetum rupicolae association (Burduja et al. 1956)

A total of 35 floristic relevés belonging to the grasslands of the association *Festucetum rupicolae* Burduja et al. (1956) were included in the study, originating from six locations in Bihor County: Crişurilor Plain (3 relevés), Ierului Valley (8 relevés), Tăşnadului Hills (3 relevés), Middle and lower basin of the Crişul Negru River (5 relevés), Lăzărenilor Hills (10 relevés), Pădurea Craiului Mountains (6 relevés) (Table 3). These grasslands are characterized by high biodiversity, comprising a total of 183 species, predominantly xeromesophilous. They are distributed across slopes with varied exposures, with inclinations ranging between 0 and 25°, at altitudes between 90 and 995 m.

The physiognomy of these grasslands is determined by *Festuca rupicola* Heuff., with a cover ranging from 46.3% to 87.5% and maximum constancy ($K = V$). Of the total number of species, 23 belong to the family *Poaceae*, among which those with the highest frequency include: *Festuca valesiaca* Schleich. Ex Gaudin, *Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca pseudovina* Hack. ex Wiesb., *Lolium perenne* L., *Koeleria macrantha* (Ledeb.) Schult., *Alopecurus pratensis* L. (Table 3). *Fabaceae* exhibit low cover (0.1-5%), with the most representative species being: *Lotus corniculatus* L., *Trifolium repens* L., *Trifolium pratense* L., *Trifolium campestre* Schreber, *Medicago falcata* L., *Vicia grandiflora* Scop.

Among the species of forage value from other families present in these grasslands, the greatest proportion is held by: *Luzula campestris* (L.) DC., *Achillea collina* (Wirtg.) Heimerl,

Centaurea jacea L., *Daucus carota* L., *Fragaria viridis* Duchesne, *Galium verum* L., *Hieracium pilosella* L., *Leontodon hispidus* L., *Plantago lanceolata* L., *Thymus pulegioides* L., *Potentilla argentea* L., *Prunella laciniata* (L.) L.

In these grasslands, a number of plant species occur that are detrimental to animal products, such as: *Artemisia absinthium* L., *Carduus acanthoides* L., *Carduus nutans* L., *Eryngium campestre* L. and toxic and harmful plants *Euphorbia cyparissias* L., *Hypericum perforatum* L., *Verbascum nigrum* L.

The xeromesophilous phytocoenoses of the grasslands belonging to the association *Thymo comosi-Festucetum rupicolae* occur in the Codru-Moma Mountains on sun-exposed calcareous substrates, on southern, south-eastern, and south-western slopes with inclinations ranging from 5 to 25°. The soils on which they develop are rendzinas, rich in humus and with a neutral reaction. The edificatory and characteristic species are *Festuca rupicola*, with a cover of 40%, and *Thymus comosus*, with a cover of 21.3% and maximum constancy ($K = V$) (Table 3). In the Codru-Moma Mountains region, these grasslands have been identified in seven localities, exhibiting a rich floristic composition totaling 112 species.

The physiognomy of these grasslands is given by *Poaceae* species (45%), with *Fabaceae* species having a reduced presence (3.1%). The useful phytomass of these grasslands is represented by species with medium forage value such as *Festuca rubra* L., *Brachypodium pinnatum* (L.) P. Beauv., *Anthoxanthum odoratum* L., *Lotus corniculatus* L., *Anthyllis vulneraria* L., *Trifolium repens* L., *Galium verum* L., *Fragaria viridis* Duchesne, *Plantago lanceolata* L., *Hieracium pilosella* L.

Among the harmful, toxic, and noxious plants occurring in this type of grassland, we list: *Carduus acanthoides* L., *Carduus nutans* L., *Euphorbia cyparissias* L., *Verbascum phlomoides* L., *Coronilla varia* L.

The dendrogram of *Festuca rupicola* Heuff. grasslands in Bihor County suggests a relatively uniform floristic composition, with the highest similarity occurring between phytocoenoses from the Pădurea Craiului Mountains and the Codru-Moma Mountains (Figure 3).

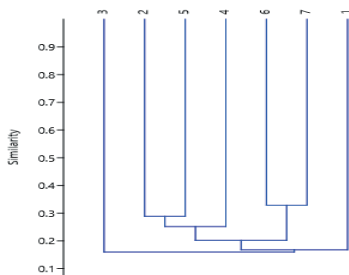


Figure 3. Jaccard similarity index for *Festuca rupicola* grasslands studied from Bihor County (where: 1 - Crișurilor Plain; 2 - Ierului Valley; 3 - Tașnadului Hills; 4 - Middle and lower basin of the Crișul Negru River; 5 - Lăzărenilor Hills; 6 - Pădurea Craiului Mountains; 7 - Codru-Moma Mountains)

The following table synthesizes information on the species composing the floristic structure of *Festuca rupicola* Heuff. grasslands in Bihor County, including study locations, number of relevés, altitude, herbaceous layer cover, exposure, slope, and relevé size. The vertical arrangement of the species presented in the table was based on taxonomic criteria (*Poaceae*, *Fabaceae*, and other botanical families), as well as on their degree of palatability, while also highlighting harmful, toxic, or noxious plants, all of which are listed in alphabetical order. In addition, fodder quality indicators (F) and useful forage phytomass indicators (M) were provided for each species.

Table 3. The floristic composition of *Festuca rupicola* Heuff. grasslands in the Bihor County (where: F - Fodder quality indicators; M - Indicators of useful forage phytomass; K - constancy)

Plant association	Festucetum rupicolae association																		Thymo comosi-Festucetum rupicolae association	Indicators			
Location in Bihor County	1			2			3			4			5			6			7				
	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K	AD according to K (%)	ADm	K		
No. of relevés	3			8			3			5			10			6			7				
Altitude (m)	90-100			105-120			200-250			95-295			150-270			300-660			510-800				
The coverage of grass layer (%)	90.9			80.7			77.2			96.4			89.6			86.9			93.5				
Exposition	-			-			V			V, S, E, NE			S, E, N, V, NE			S, V, E, SV			S, SV, SE				
Slope (degree) (°)	-			-			10			3-6			1-9			4-25			5-25				
Area (m²)	100			100			100			100			100			100			100			F	M
0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Poaceae																							
Agropyron repens	-	-	-	0.2	+	II	-	-	-	0.2	+	II	0.5	+	V	-	-	-	0.2	+	II	6	7
Agrostis capillaris	-	-	-	0.2	+	II	5	I	V	-	-	-	-	-	-	-	-	-	-	-	7	5	
Alopecurus pratensis	5	1	V	2	+1	IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	7	
Anthoxanthum odoratum	0.5	+	V	0.2	+	II	7.9	1-2	II	-	-	-	2.8	+1	V	-	-	-	1.4	+1	III	5	3
Brachypodium pinnatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	+	IV	5	7
Brica media	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	+	III	5	2
Bromus arvensis	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	6	
Bromus inermis	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	8	
Cynodon dactylon	-	-	-	0.2	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	2	
Cynosurus cristatus	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	0.2	+	II	7	4
Dactylis glomerata	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	II	0.2	+	II	0.2	+	II	9	8
Danthonia decumbens	-	-	-	-	-	-	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	5	4	
Festuca arundinacea	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	8	9	
Festuca pratensis	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	9	8	
Festuca pseudovina	9	+2	V	-	-	-	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	5	3	
Festuca rubra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	+1	IV	1.4	+1	III	7	6
Festuca rupicola	50	3-4	V	62.5	3-5	V	50	3-4	V	87.5	5	V	62.5	3-5	V	46.3	1-5	V	40	2-4	V	5	5
Festuca valesiaca	-	-	-	0.2	+	II	0.8	+1	II	0.3	+	III	2	+1	IV	0.1	+	I	0.1	+	I	5	3
Holcus lanatus	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	6	6	
Koeleria macrantha	2.8	+1	V	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	5	3	
Lolium perenne	0.5	+	V	0.1	+	I	-	-	-	0.2	+	II	0.1	+	I	-	-	-	0.1	+	I	9	8
Poa angustifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	7	5
Poa bulbosa	0.4	+	IV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	1	
Poa compressa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	+	IV	0.1	+	I	6	3
Phleum montanum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	6	5
Phleum pratense	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	9	8
Poa pratensis	0.5	+	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	8	6
Fabaceae																							
Anthyllis vulneraria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	+1	II	6	5
Lotus corniculatus	0.1	+	I	0.3	+	III	0.5	+	V	0.3	+	III	2	+1	IV	0.2	+	II	0.5	+	V	8	6
Lotus tenuis	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	3	
Medicago falcata	0.1	+	I	0.3	+1	I	-	-	-	-	-	-	-	-	-	0.3	+	III	-	-	7	6	
Medicago lupulina	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	0.3	+	III	0.2	+	II	8	3
Medicago minima	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	+1	II	-	-	7	1	
Trifolium alpestre	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	-	-	6	3	
Trifolium arvense	-	-	-	-	-	-	0.3	+	III	-	-	-	0.2	+	II	-	-	-	-	-	4	2	
Trifolium campestre	0.3	+	III	0.2	+	II	-	-	-	0.3	+	III	0.3	+	III	-	-	-	0.1	+	I	7	2
Trifolium fragiferum	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	3	

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Trifolium hybridum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	8	6
<i>Trifolium medium</i>	-	-	-	0.1	+	I	-	-	-	0.1	+	I	-	-	-	-	-	-	0.2	+	II	6	4
<i>Trifolium pannonicum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	7	5
<i>Trifolium pratense</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	0.1	+	I	0.2	+	II	0.1	+	I	8	7
<i>Trifolium repens</i>	5	1	V	2	+1	II	-	-	-	-	-	-	-	-	-	-	-	-	0.8	+1	II	8	5
<i>Vicia cracca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	-	-	7	6
<i>Vicia grandiflora</i>	-	-	-	0.4	+	IV	-	-	-	-	-	-	-	-	-	-	0.1	+	-	-	-	7	5
<i>Vicia tetrasperma</i>	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	3
Forage species from other botanical families																							
<i>Achillea collina</i>	0.2	+	II	0.1	+	I	0.4	+	IV	0.4	+	IV	0.4	+	IV	0.2	+	II	0.1	+	I	6	5
<i>Achillea millefolium</i>	-	-	-	0.3	+	III	-	-	-	-	-	-	-	-	-	-	-	-	0.3	+	III	6	4
<i>Achillea nobilis</i>	-	-	-	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	-	-	-	-	6	2
<i>Achillea setacea</i>	0.1	+	I	0.1	+	I	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	-	6	3
<i>Capsella bursa-pastoris</i>	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	3
<i>Carum carvi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Centaurea jacea</i>	0.1	+	I	0.2	+	II	-	-	-	0.2	+	II	0.3	+	III	-	-	-	0.2	+	II	6	3
<i>Cichorium intybus</i>	0.1	+	I	0.1	+	I	0.3	+	III	-	-	-	-	-	-	-	-	-	-	-	-	5	6
<i>Convolvulus arvensis</i>	-	-	-	0.3	+	III	-	-	-	0.1	+	I	0.2	+	II	-	-	-	0.1	+	I	7	6
<i>Daucus carota</i>	-	-	-	0.4	+	IV	0.4	+	IV	-	-	-	0.3	+	III	-	-	-	-	-	-	6	5
<i>Echium vulgare</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	0.2	+	II	4	3
<i>Filipendula vulgaris</i>	0.1	+	I	-	-	-	-	-	-	-	-	-	0.1	+	I	0.1	+	I	0.1	+	I	5	4
<i>Fragaria viridis</i>	0.3	+	III	0.1	+	I	0.2	+	II	-	-	-	0.8	+1	II	-	-	-	2.8	+1	V	4	1
<i>Galium verum</i>	0.2	+	II	0.4	+	IV	-	-	-	0.1	+	I	0.3	+	III	0.1	+	I	2	+1	IV	5	4
<i>Helianthemum nummularium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	+1	IV	0.1	+	I	4	2
<i>Hieracium bauhini</i>	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	4	1
<i>Hieracium pilosella</i>	0.2	+	II	-	-	-	0.2	+	II	-	-	-	0.2	+	II	0.1	+	I	2	+1	IV	4	1
<i>Inula britannica</i>	0.1	+	I	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	4	4
<i>Inula hirta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	4	4
<i>Knautia arvensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	0.2	+	II	4	4
<i>Leontodon autumnalis</i>	0.2	+	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	3
<i>Leontodon hispidus</i>	0.1	+	I	0.2	+	II	0.2	+	II	0.2	+	II	-	-	-	-	-	-	0.3	+	III	5	3
<i>Leucanthemum vulgare</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	0.3	+	III	5	5
<i>Lucula campestris</i>	2.8	+1	V	0.1	+	I	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	-	4	2
<i>Origanum vulgare</i>	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	0.1	+	I	4	4
<i>Pimpinella saxifraga</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	5	3
<i>Plantago lanceolata</i>	0.5	1	V	0.2	+	II	-	-	-	0.1	+	I	0.3	+	III	0.4	+	IV	0.4	+	IV	6	1
<i>Plantago media</i>	-	-	-	-	-	-	0.1	+	I	-	-	-	-	-	-	0.1	+	I	0.1	+	I	6	2
<i>Polygala vulgaris</i>	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	0.1	+	I	0.2	+	II	4	1
<i>Polygonum aviculare</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	-	5	3
<i>Potentilla argentea</i>	0.1	+	I	0.3	+	III	-	-	-	-	-	-	0.4	+	IV	0.2	+	II	0.1	+	I	4	2
<i>Potentilla cinerea</i>	-	-	-	-	-	-	0.1	+	I	-	-	-	-	-	-	15	3-4	II	0.3	+	III	4	1
<i>Potentilla recta</i>	-	-	-	-	-	-	-	-	-	0.1	+	I	0.1	+	I	-	-	-	0.1	+	I	5	2
<i>Potentilla reptans</i>	0.1	+	I	-	-	-	-	-	-	-	-	-	0.2	+	II	0.1	+	I	-	-	-	4	1
<i>Prunella grandiflora</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	4	2
<i>Prunella laciniata</i>	0.1	+	I	-	-	-	-	-	-	0.3	+	III	1.4	+1	III	0.1	+	I	-	-	-	4	2
<i>Prunella vulgaris</i>	0.1	+	I	-	-	-	-	-	-	-	-	-	0.2	+	II	0.1	+	I	0.1	+	I	4	2
<i>Ranunculus polyanthemos</i>	-	-	-	-	-	-	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	-	4	4
<i>Rumex acetosa</i>	0.3	+	III	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	0.2	+	II	4	4
<i>Salvia pratensis</i>	0.5	+	V	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	0.2	+	II	4	4
<i>Sanguisorba minor</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	0.1	+	I	0.3	+	III	0.4	+	IV	6	3
<i>Scabiosa ochroleuca</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	0.4	+	IV	4	4
<i>Taraxacum officinale</i>	0.1	+	I	0.2	+	II	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	3
<i>Thymus glabrescens</i>	0.5	+	V	-	-	-	0.3	+	III	-	-	-	-	-	-	0.1	+	I	-	-	-	4	2
<i>Thymus pulegioides</i>	-	-	-	-	-	-	-	-	-	0.3	+	III	0.4	+	IV	-	-	-	-	-	-	4	2
<i>Tragopogon pratensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	0.2	+	II	5	5
<i>Urtica dioica</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	7
<i>Verbena officinalis</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	0.1	+	I	0.1	+	I	-	-	-	4	4
<i>Veronica chamaedrys</i>	-	-	-	-	-	-	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	-	4	2
Plant species not consumed or with a low degree of consumability (F3), (M0)																							
<i>Acinos arvensis</i>	-	-	-	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	3	0
<i>Agrimonia eupatoria</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	-	3	0
<i>Agrostis genevensis</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	0.2	+	II	-	-	-	0.1	+	I	3	0
<i>Anthemis arvensis</i>	-	-	-	-	-	-	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	3	0
<i>Arabis hirsuta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	0.1	+	I	3	0
<i>Arenaria serpyllifolia</i>	0.3	+	III	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0
<i>Asperula cynanchica</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	0.1	+	I	0.4	+	IV	0.3	+	III	3	0
<i>Bromus commutatus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	3	0
<i>Bromus hordeaceus</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0
<i>Calamagrostis epigeios</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	-	-	-	-	3	0
<i>Campanula glomerata</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	-	-	-	0.1	+	I	3	0
<i>Campanula patula</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	+	II	3	0	
<i>Campanula persicifolia</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	3	0	
<i>Carex caryophylla</i>	2.8	+1	V	-	-	-	-	-	-	0.1	+	I	-	-	-	0.1	+	I	0.1	+	I	3	0
<i>Carex hirta</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	0.3	+	III	-	-	-	-	-	-	3	0
<i>Carex precox</i>	0.5	+	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	0
<i>Carlina acaulis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	+	I	3	0
<i>Carlina biebersteinii</i>	-	-	-	-	-	-	-	-	-	0.3	+	III	0.3	+	III	-	-	-	0.2	+	II	3	0
<i>Carlina vulgaris</i>	-	-	-	0.1	+	I	-	-	-	-	-	-	-	-	-	0.1	+	I	0.1	+	I	3	0
<i>Centaurea micranthos</i>	-	-	-	-	-	-	-	-	-	0.2	+	II	-	-	-	0.1	+	I	-	-	-	3	0
<i>Centaurea scabiosa</i>	-	-																					

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Veronica arvensis</i>	2.8	+1	V																			3	0
<i>Veronica prostrata</i>	0.2	+	II																			3	0
<i>Veronica serpyllifolia</i>	0.1	+	I																			3	0
<i>Veronica spicata</i>										0.2	+	II										3	0
<i>Veronica teucurium</i>																0.1	+	I	0.1	+	I	3	0
<i>Viola arvensis</i>																			0.1	+	I	3	0
<i>Viola tricolor</i>																0.2	+	II	0.1	+	I	3	0
<i>Vinca herbacea</i>													0.2	+	II							3	0
<i>Xeranthemum cylindraceum</i>							2.7	+2	II													3	0
Plants that harm livestock products (F2), (M0)																							
<i>Artemisia absinthium</i>				0.1	+	I							0.2	+	II							2	0
<i>Carduus acanthoides</i>				0.3	+	III							2	+1	IV				0.2	+	II	2	0
<i>Carduus nutans</i>										0.4	+	IV				0.1	+	I	0.3	+	III	2	0
<i>Eryngium campestre</i>	0.1	+	I	0.2	+	II	0.5	+	V	0.3	+	III	0.2	+	II							2	0
<i>Rumex acetosella</i>	0.1	+	I																			2	0
<i>Thlapsi perfoliatum</i>	0.3	+	III																			2	0
Toxic and harmful plants (F1), (M0)																							
<i>Adonis vernalis</i>										0.3	+	III	0.3	+	III							1	0
<i>Colchicum autumnale</i>																0.1	+	I	0.1	+	I	1	0
<i>Coronilla varia</i>													0.1	+	I	0.1	+	I	0.2	+	II	1	0
<i>Euphorbia cyparissias</i>	0.3	+	III	0.3	+	III	0.3	+3	III	0.1	+	I	0.3	+	III	0.1	+	I	0.4	+	IV	1	0
<i>Euphorbia serrulata</i>													0.4	+	IV							1	0
<i>Hypericum perforatum</i>													0.3	+	III	0.1	+	I	0.1	+	I	1	0
<i>Stellaria graminea</i>				0.2	+	II							0.2	+	II				0.1	+	I	1	0
<i>Verbascum blattaria</i>				0.1	+	I													0.1	+	I	1	0
<i>Verbascum nigrum</i>										0.1	+	I	0.1	+	I				0.1	+	I	1	0
<i>Verbascum phlomoides</i>																0.1	+	I	0.1	+	I	1	0
<i>Verbascum phoenicum</i>	0.2	+	II																			1	0
<i>Verbascum speciosum</i>													0.4	+	IV							1	0

Locations in Bihor County taken into study: 1 - Crișurilor Plain; 2 - Ierului Valley; 3 - Tășnadului Hills; 4 - Middle and lower basin of the Crișul Negru River; 5 - Lăzărenilor Hills; 6 - Pădurea Craiului Mountains; 7 - Codru-Moma Mountains.

Following the analysis of the productive potential of grasslands dominated by *Festuca rupicola* Heuff. in Bihor County, it was found that the highest pastoral values are recorded in grasslands belonging to the *Festucetum rupicolae* association in the Crișul Negru Basin (PV=50.67), whereas the lowest values characterize the grasslands classified within the *Thymo comosi-Festucetum rupicolae* association in the Codru-Moma Mountains (PV=32.4) (Table 4).

Table 4. The productivity of *Festuca rupicola* associations in Bihor County

Location in Bihor County	Association	Pastoral value (PV)	Green biomass production (MV) (t/ha)	Livestock units LU/ha	Season duration (days)
Crișurilor Plain	<i>Festucetum rupicolae</i>	47.95 (average)	9.72	0.81	185
Ierului Valley	<i>Festucetum rupicolae</i>	42.48 (average)	9.15	0.76	185
Tășnadului Hills	<i>Festucetum rupicolae</i>	37.82 (mediocre)	7.56	0.63	185
Middle and lower basin of the Crișul Negru River	<i>Festucetum rupicolae</i>	50.67 (average)	11.80	0.98	185
Lăzărenilor Hills	<i>Festucetum rupicolae</i>	44.07 (average)	9.22	0.77	185
Pădurea Craiului Mountains	<i>Festucetum rupicolae</i>	38.29 (mediocre)	6.51	0.57	175
Codru Moma Mountains	<i>Thymo comosi-Festucetum rupicolae</i>	32.4 (mediocre)	6.07	0.57	165
Average of the value	-	41.95 (average)	8.57	0.73	180

Regarding grazing capacity, the highest values are recorded in the grasslands of the Crișul Negru Basin (0.98 LU/ha), while the lowest

values are found in the grasslands of the Pădurea Craiului Mountains (0.57 LU/ha) and the Codru-Moma Mountains (0.57 LU/ha) (Figure 4).

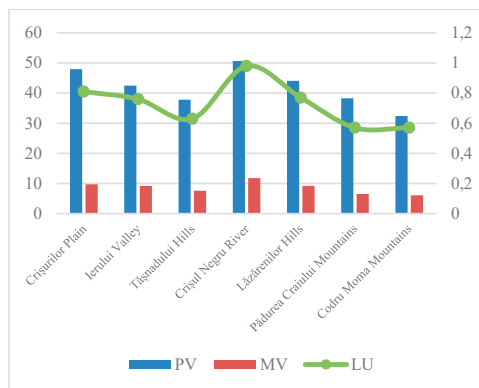


Figure 4. The grazing capacity (LU) of *Festuca rupicola* grasslands in Bihor County, based on pastoral value (PV) and green biomass production (MV).

It can be concluded that the *Festuca rupicola* grasslands in Bihor County exhibit a moderate pastoral value (PV=41.95), associated with an average green biomass production of 8.57 t/ha and a stocking rate of 0.73 LU/ha, for an average grazing period of approximately 180 days.

In order to provide a clearer overview of the productive potential of these grasslands in Bihor County, a comparative analysis was conducted based on similar pastoral studies carried out in nine distinct locations at national level, as

follows: Cașin-Onești Depression (Oituz Basin) (Marușca et al., 2023), Suceava River Basin (Marușca et al., 2022a), Bistrița Vâlcii Basin (Oltenia Region) (Marușca et al., 2025b), Arieș Valley (Apuseni Mountains) (Marușca et al., 2025a), Motru River Basin (Marușca et al., 2024), Orăștie River Basin (Marușca & Vințan, 2022), Timiș River Basin (Marușca & Nicolin), Baraolt Mountains (Marușca et al., 2021), the Mureș Gorge (Marușca et al., 2022b) (Table 5; Figure 5)

The comparative analysis of the data highlights significant variations in pastoral parameters among the studied geographical regions, reflecting differences driven by local ecological conditions, phytocoenotic structure, and the intensity of pastoral use.

Regarding pastoral value (PV), the highest values are recorded in the Suceava River Basin (45.00) and Bihor County (41.95), followed by the Timiș River Basin (39.10) and the Orăștie River Basin (38.80). These regions indicate a superior quality of grasslands, associated with a floristic composition favorable to valuable forage species. At the opposite end of the spectrum, the Mureș Gorge (16.00) and the Bistrița Vâlcii Basin (21.00) exhibit low pastoral values, suggesting a poor quality of the vegetation cover and a high proportion of species with low forage value (Table 5).

Table 5. Comparing the productivity of the *Festuca rupicola* grasslands in Bihor County with some areas of Romania

Geographical region	Pastoral value (PV)	Green biomass production (MV) (t/ha)	Livestock units (LU/ha)	Season duration (days)
Bihor County	41.95	8.57	0.73	180
Casin-Onești Depression (Oituz Basin)	35.40	4.84	0.41	180
Suceava River Basin	45.00	8.16	0.76	165
Basin of Bistrița Vâlcii (Oltenia Area)	21.00	3.20	0.34	145
Arieș Valley (Apuseni Mountains)	31.40	4.94	0.46	165
Basin of Motru River	34.50	4.77	0.46	160
Basin of Orăștie River	38.80	7.75	0.64	185
Basin of Timiș River	39.10	7.43	0.65	175
Baraolt Mountains	38.40	6.07	0.57	165
Mureș Gorge	16.00	2.37	0.22	165

For green biomass production (MV), the same general trends are observed, with maximum values in Bihor County (8.57 t/ha) and the Suceava Basin (8.16 t/ha), as well as comparable values in the Orăștie Basin (7.75 t/ha) and the Timiș Basin (7.43 t/ha). The lowest values are

recorded in the Mureș Gorge (2.37 t/ha) and the Bistrița Vâlcii Basin (3.20 t/ha), indicating a reduced productive capacity of grassland ecosystems in these regions.

Regarding livestock units (LU/ha), the distribution follows a similar pattern, with high values in Suceava Basin (0.76 LU/ha) and Bihor County (0.73 LU/ha), and intermediate values in Timiș Basin (0.65 LU/ha) and Orăștie Basin (0.64 LU/ha). The lowest values are found in the Mureș Gorge (0.22 LU/ha) and the Bistrița Vâlcii Basin (0.34 LU/ha), confirming the productive limitations of these areas (Figure 5).

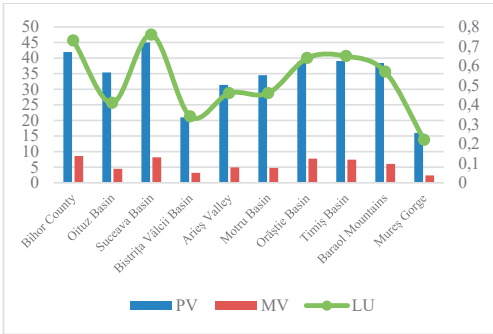


Figure 5. Comparison of the livestock stocking rate associated with *Festuca rupicola* grasslands in Bihor County with other regions of the country, based on pastoral value (PV) and green biomass production (MV).

Grasslands dominated by *Festuca rupicola* Heuff. exhibit significant variations in productivity (biomass yield) from one region to another, differences primarily determined by ecological factors, particularly climatic and edaphic conditions. As a xeromesophilous species, *Festuca rupicola* Heuff. demonstrates a high tolerance to moderate moisture deficit. Consequently, regions characterized by higher annual precipitation levels (500–600 mm) support the accumulation of substantially greater biomass compared with arid areas, where annual precipitation decreases below 400 mm. Maximum productivity levels are recorded on chernozem and humus-rich grey soils, which provide optimal conditions for vegetation development. In contrast, biomass production is significantly reduced on eroded, lithomorphic soils or on rendzinas developed over calcareous substrates. Furthermore, soil texture directly influences water availability: loamy soils, due to their superior water retention capacity, ensure relatively stable yields, whereas sandy or stony

soils intensify drought effects and contribute to reduced plant stature.

CONCLUSIONS

In order to assess the productive potential of grasslands dominated by *Festuca rupicola* Heuff. in Bihor County, two vegetation associations were analyzed, namely *Festucetum rupicolae* Burduja et al. 1956 and *Thymo comosi-Festucetum rupicolae* (Csűrös et Gergely 1959) Pop & Hodişan 1985, identified across seven distinct areas of the county.

These grasslands are distributed from the lowland zone to the lower montane belt, at altitudes ranging between 90 and 800 m, predominantly occurring on slopes with sunny exposures (S, SW, SE), with inclinations varying from 0 to 25°, and developed on a variety of soil types. For the assessment of pastoral value and grazing capacity, a total of 220 species were considered, among which, within the forage-relevant taxa, 25 belong to the *Poaceae* family, 18 to the *Fabaceae* family, and 49 to other botanical families. The *Festuca rupicola* Heuff. grasslands in Bihor County are characterized by a moderate productive potential, reflected by an intermediate pastoral value and grazing capacity, but by a higher green biomass production compared to other analyzed regions.

The internal variability of these parameters, determined by vegetation association type and local ecological conditions, indicates the presence of areas with high productive potential, such as the Crişul Negru Basin, as well as less favorable areas, such as the Codru-Moma and Pădurea Craiului Mountains. At the national level, the grasslands in Bihor County occupy an intermediate position in terms of pastoral value and grazing capacity, while standing out due to their high biomass productivity, which confers them significant importance for pastoral use.

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RESEARCH ON HARMFUL ARTHROPOD SPECIES AND THEIR ANTAGONISTS IN SEVERAL RAPESEED CROPS

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Abstract

*Research on arthropods associated with rapeseed crops was conducted at the Ezăreni Research and Student Training Station of "Ion Ionescu de la Brad" Iași University of Life Sciences during the 2025 growing season. Sampling employed yellow pan traps, Barber-type pitfall traps, sweep nets, and direct plant observations. All collected material was labeled with the sample number, collection method, and experimental variant. In the laboratory, specimens were cleaned of soil, plant debris, and other impurities, then preserved in ~20% ethanol until identification. Taxonomic determination was performed using standard keys, to class, order, family, and species levels. Collected taxa belonged to Arachnida, Insecta, and Crustacea. The most abundant and diverse class was Insecta, with species from Coleoptera, Diptera, Hymenoptera, Heteroptera, Homoptera, Lepidoptera, and others. Coleoptera was the dominant order in both abundance and species richness. Frequently collected beetle species included *Ontophagus ovatus*, *Pseudophonus pubescens*, *Amara familiaris*, *Opatrum sabulosum*, *Coccinella septempunctata*, *Harpalus calceatus*, *Tanymecus palliatus*, *Idiocroma dorsalis*, *Amara aenea*, *Anisodactylus* spp., and *Pterostichus* spp., reflecting the key Coleoptera fauna of rapeseed agroecosystems. This version keeps all essential details while staying concise and clear.*

Key words: yellow pan traps, rapeseed crops, harmful arthropod.

INTRODUCTION

Oilseed crops, particularly rapeseed (*Brassica napus* L.), have had and continue to have a special importance for human society, representing one of the most valuable groups of cultivated plants. Rapeseed is a fundamental source of vegetable oil for human consumption and an important component in animal nutrition, while also providing raw material for various industrial uses, including the production of biodiesel. In recent years, the expansion of rapeseed cultivation has been closely linked to the increasing demand for renewable energy and sustainable agricultural systems (Zheng et al., 2020; Obermeier et al., 2022). In this context, achieving high, stable and superior quality yields in rapeseed cultivation is a constant concern, with major implications for food security and the development of the agri-food industry. Insect pests have a significant impact on rapeseed crops, causing considerable damage by affecting plant metabolism and interfering with essential physiological processes, which ultimately leads to both quantitative and

qualitative yield losses (Williams, 2010; Zhang et al., 2017). Among the most damaging species are those that attack during key phenological stages, such as emergence, flowering and pod formation, when plants are particularly vulnerable (Hausmann, 2021; Thöming et al., 2020). The intensity of pest attacks is strongly influenced by environmental conditions and agricultural practices, which can either favor or limit pest development (Zheng et al., 2020). To reduce these negative effects, various control strategies are applied, including chemical control through the use of pesticides, as well as biological control methods based on the use of natural enemies such as pathogenic fungi, bacteria, viruses and protozoa, alongside parasites and predators of harmful species (Sekulic & Rempel, 2016; Williams, 2010). However, the intensive use of insecticides has raised concerns regarding environmental impact, pollinator decline and the development of pest resistance, which highlights the importance of integrated pest management strategies (Cook & Jędryczka, 2024; Obermeier et al., 2022).

In this context, the present work aims to highlight the diversity of arthropods associated with a rapeseed crop and to emphasize the role of beneficial species in reducing pest pressure, contributing to the development of more sustainable and environmentally friendly agricultural practices (Zheng et al., 2020; Zhang et al., 2017).

MATERIALS AND METHODS

The observations were carried out in several rapeseed (*Brassica napus* L.) crops located within the perimeter of the Ezăreni Teaching and Research Station, belonging to the “Ion Ionescu de la Brad” Iași University of Life Sciences. The research was conducted on a total area of approximately 3 hectares, divided into three experimental plots, each covering 1 hectare. The study focused on the dynamics of harmful arthropod species and the beneficial entomofauna associated with rapeseed crops throughout the entire vegetation period.

The biological material was collected using an entomological net, applying the sweep netting method, with 25 double sweeps performed at each sampling point. Within each plot, 5 observation points were established, arranged diagonally to ensure a good representativeness of the samples. The collections were complemented by direct observations on plants, monitoring the main phenological stages: emergence, rosette development, stem elongation, flowering, and pod formation.

In 2025, 10 successive samplings were carried out at intervals of 10-15 days, during the period March-July, corresponding to the critical development stages of the rapeseed crop (Tălmăciu, 2024). In total, approximately 150 entomological samples were analyzed (3 plots × 5 sampling points × 10 samplings).

The material collected from the field was labeled (date, plot, sampling point), cleaned of plant debris, and preserved in a 40% ethyl alcohol solution (Sipos, 2024). Subsequently, the samples were analyzed in the laboratory, where arthropods were sorted and identified using a binocular magnifying glass (10×–40× magnification), based on specialized entomological identification guides and updated taxonomic keys.

The identification process targeted both phytophagous species of economic importance and the complex of entomophagous arthropods (predators and parasitoids) involved in the natural regulation of pest populations. For each sample, numerical abundance (number of individuals per species) and species frequency were determined.

Statistical data processing included the analysis of variance (ANOVA), used to highlight significant differences between plots, sampling periods, and functional groups of arthropods. Statistical tests were performed at a significance level of $p < 0.05$, and when significant differences were identified, post-hoc tests (Tukey type) were applied for mean comparisons.

Species diversity of the entomofauna was assessed by calculating the Shannon-Wiener index (H'), according to the formula:

$$H' = - \sum_{j=1}^S p_i \ln p_i$$

where:

- p_i represents the proportion of individuals belonging to species i out of the total number of collected individuals, and S is the total number of identified species.

This index allowed the evaluation of both species diversity and the evenness of species distribution within the studied agroecosystem.

The obtained results were used to assess the structure of arthropod communities and their role in maintaining biological balance, contributing to the development of integrated pest management strategies in rapeseed crops (Barascou, 2022; Zheng et al., 2020).

RESULTS AND DISCUSSIONS

Data analysis highlights significant variations in the abundance and structure of the entomofauna during the seven collections carried out in the rapeseed crop in 2025 (Tabel 1). The total number of arthropods collected was 5210 individuals, with an uneven distribution between the harvest periods.

A progressive increase in abundance is observed in the first three collections, from 560 individuals on 15.05 to a maximum of 2261 individuals on 24.06, followed by a sharp decrease in the following period (750

individuals on 07.07 and only 117 on 22.07), and then a slight recovery towards the end of the vegetation period (665 individuals on 21.08). This dynamics is closely correlated with the phenology of the rapeseed crop and with the climatic conditions favorable to the development of arthropods in the early summer. The structure of the collected fauna highlights a clear dominance of phytophagous species during the peak period (June), especially of harmful dipterans and coleopterans. Species such as *Delia brassicae* (789 individuals) and *Dasineura brassicae* (712 individuals) recorded the highest values, being responsible for a large part of the pest pressure on the crop. Also, the presence of the species *Psylliodes attenuata* (63 individuals) and *Phyllotreta nemorum* (23 individuals) indicates specific attacks in the early and middle phases of vegetation.

In parallel, a significant development of useful entomofauna was noted, especially predators from the families Carabidae and Staphylinidae, as well as spiders (Araneae). Dominant species such as *Brachinus crepitans* (633 individuals), *Calodera aethiops* (309 individuals), *Xysticus cristatus* (242 individuals) and *Zelotes electus* (203 individuals) indicate a high potential for biological regulation of pest populations.

Also, the presence of parasitoids, such as *Trissolcus grandis* (72 individuals), *Aphidius*

rhopalosiphi (20 individuals) and *Aphelinus abdominalis* (19 individuals), suggests the existence of well-developed trophic relationships and a natural pest control mechanism. The increase in their number during the peak period of phytophagous populations (June-July) confirms the ecological synchronization between hosts and antagonists. An important aspect is the high share of species from the Carabidae family (e.g. *Amara eurynota* - 187 individuals, *Harpalus distinguendus* - 128 individuals), which significantly contribute to the reduction of biological reserves of pests. At the same time, the high diversity of spiders (*Pardosa agricola*, *Phalangium opilio*) reflects the stability of the agricultural ecosystem and its self-regulation capacity.

The sharp decrease in the total number of individuals in July-August can be explained by the maturation of the crop, the reduction of trophic resources and less favorable climatic conditions, which determine the migration or decrease of arthropod populations.

Overall, the results highlight a complex structure of the entomofauna, characterized by a relative balance between harmful and beneficial species. This aspect suggests a high potential for natural biological control in the studied rapeseed crop, emphasizing the importance of conserving biodiversity within agroecosystems.

Table 1. Structure of useful and harmful insect species collected in rapeseed crops in 2025

No	Name of species	15.05	03.06	24.06	07.07	22.07	02.08	21.08	Total
1.	<i>Actobius cinerascens</i>	6	-	-	-	-	-	-	6
2.	<i>Acylophorus glaberrimus</i>	-	2	-	1	-	-	-	3
3.	<i>Afide</i>	-	-	-	20	5	-	5	30
4.	<i>Agriotes ustulatus</i>	1	-	-	2	-	-	-	3
5.	<i>Aleochara lanuginosa</i>	6	-	27	-	-	-	-	33
6.	<i>Amara aenea</i>	14	16	1	6	4	-	1	42
7.	<i>Amara eurynota</i>	1	5	57	16	8	3	97	187
8.	<i>Amara familiaris</i>	1	5	2	-	-	-	-	8
9.	<i>Amara similata</i>	-	-	-	1	-	-	-	1
10.	<i>Aneclis melanaria</i>	1	-	-	9	-	-	-	10
11.	<i>Anisodactylus binotatus</i>	3	9	26	-	1	-	-	39
12.	<i>Anisodactylus signatus</i>	-	1	-	4	1	-	-	6
13.	<i>Anthicus flavipes</i>	8	2	2	-	-	-	1	13
14.	<i>Aphanogmus abdominalis</i>	-	-	2	10	12	-	2	26
15.	<i>Aphelinus abdominalis</i>	1	-	6	6	1	2	3	19
16.	<i>Aphidius rhopalosiphi</i>	1	-	4	13	2	-	-	20
17.	<i>Athalia rosae</i>	-	-	-	-	3	-	-	3
18.	<i>Atheta trinitata</i>	3	-	-	4	-	-	-	7
19.	<i>Baris chlorizans</i>	10	14	11	8	-	-	-	43
20.	<i>Brachinus crepitans</i>	1	80	176	45	3	9	319	633
21.	<i>Brachyserphus parvulus</i>	2	-	1	-	-	-	-	3

No	Name of species	15.05	03.06	24.06	07.07	22.07	02.08	21.08	Total
22.	<i>Bryocharis analis</i>	-	1	1	-	-	-	-	2
23.	<i>Calodera aethiops</i>	8	10	267	24	-	-	-	309
24.	<i>Cantharis fusca</i>	1	1	1	-	-	-	-	3
25.	<i>Cantharis obscura</i>	5	-	-	-	-	-	-	5
26.	<i>Cantharis pellucida</i>	2	-	-	-	-	-	-	2
27.	<i>Cantharis rustica</i>	1	3	-	-	-	-	-	4
28.	<i>Ceraphron bispinosus</i>	-	-	4	8	-	4	32	48
29.	<i>Ceutorhynchus obstrictus</i>	1	-	7	3	-	-	-	11
30.	<i>Ceutorhynchus pallidactylus</i>	1	1	-	3	-	-	-	5
31.	<i>Chorthippus vagans</i>	-	-	-	-	-	2	-	2
32.	<i>Coccinella septempunctata</i>	-	26	2	-	-	-	-	28
33.	<i>Conosoma pubescens</i>	-	-	4	-	-	-	-	4
34.	<i>Conostigmus rufescens</i>	2	2	3	12	-	1	-	20
35.	<i>Dasineura brassicae</i>	30	140	414	99	25	1	3	712
36.	<i>Delia brassicae</i>	121	123	463	74	1	1	6	789
37.	<i>Dolycoris baccarum</i>	-	1	3	-	-	1	1	6
38.	<i>Epyris niger</i>	-	-	3	1	1	-	5	10
39.	<i>Eurydema ornata</i>	-	-	2	1	1	6	8	18
40.	<i>Eurygaster integriceps</i>	-	1	2	-	1	-	3	7
41.	<i>Forficula auricularia</i>	2	-	-	-	1	-	1	4
42.	<i>Formicomus pedestris</i>	87	9	22	21	4	-	20	163
43.	<i>Geophilus flavus</i>	2	-	-	-	1	-	-	3
44.	<i>Gryllus campestris</i>	3	-	9	14	-	1	7	34
45.	<i>Harpalus aeneus</i>	-	-	6	9	-	-	-	15
46.	<i>Harpalus distinguendus</i>	14	4	79	25	-	1	5	128
47.	<i>Harpalus tardus</i>	2	-	-	-	-	-	-	2
48.	<i>Hister purpurascens</i>	1	-	-	-	-	-	-	1
49.	<i>Hister quadrimaculatus</i>	-	-	2	-	-	-	-	2
50.	<i>Hister stercorarius</i>	1	1	9	-	-	-	2	13
51.	<i>Idiochroma dorsalis</i>	27	208	117	37	2	2	5	398
52.	<i>Lathrobium quadratum</i>	3	-	8	-	-	-	-	11
53.	<i>Locusta migratoria</i>	-	-	-	-	-	-	1	1
54.	<i>Longitarsus lycopi</i>	-	-	2	2	2	-	1	7
55.	<i>Meligethes aeneus</i>	4	1	12	5	-	-	-	22
56.	<i>Metabletus truncatellus</i>	-	-	8	3	-	-	1	12
57.	<i>Micromus variegatus</i>	6	-	-	-	-	-	-	6
58.	<i>Necremnus tidius</i>	-	-	1	3	-	-	-	4
59.	<i>Nephus redtenbacheri</i>	1	-	-	-	-	-	-	1
60.	<i>Ocydromus decorus</i>	-	-	-	1	-	-	-	1
61.	<i>Ocyopus ophthalmicus</i>	1	-	-	-	-	-	-	1
62.	<i>Ommatoiulus sabulosus</i>	1	1	-	-	-	-	-	2
63.	<i>Opatrum sabulosum</i>	38	1	43	14	-	-	-	96
64.	<i>Ophonus azureus</i>	1	2	1	-	-	-	1	5
65.	<i>Oxytelus sculptus</i>	-	-	5	-	-	-	-	5
66.	<i>Paederus riparius</i>	-	-	1	-	-	-	-	1
67.	<i>Parallelomorpha terricola</i>	-	-	-	1	-	-	-	1
68.	<i>Pardosa agricola</i>	30	18	49	24	-	6	14	141
69.	<i>Parophonus maculicornis</i>	1	-	-	-	-	-	-	1
70.	<i>Peirates hybridus</i>	2	1	1	-	-	-	1	5
71.	<i>Phalangium opilio</i>	-	-	10	38	-	-	-	48
72.	<i>Phygadeuon trichops</i>	-	-	2	8	-	-	-	10
73.	<i>Phyllotreta nemorum</i>	15	-	4	2	1	1	-	23
74.	<i>Poecilus dimidiatus</i>	-	4	-	-	-	-	-	4
75.	<i>Pseudophonus griseus</i>	-	-	8	2	-	-	-	10
76.	<i>Pseudophonus pubescens</i>	1	5	7	1	6	1	22	43
77.	<i>Psylliodes attenuata</i>	-	4	9	4	5	19	22	63
78.	<i>Psylliodes chrisocephala</i>	14	-	9	2	1	-	-	26
79.	<i>Pterostichus cupreus</i>	7	4	42	1	-	3	18	75
80.	<i>Pterostichus niger</i>	-	-	-	-	-	-	9	9

No	Name of species	15.05	03.06	24.06	07.07	22.07	02.08	21.08	Total
81.	<i>Quedius lateralis</i>	-	2	40	-	-	-	-	42
82.	<i>Rhagonycha fulva</i>	-	-	-	3	-	-	-	3
83.	<i>Scopaeus laevigatus</i>	-	-	-	-	1	-	-	1
84.	<i>Sipalia circellaris</i>	3	-	32	-	-	-	-	35
85.	<i>Sitona lineatus</i>	1	-	-	-	-	-	-	1
86.	<i>Sminthurus viridis</i>	1	1	-	-	-	-	-	2
87.	<i>Staphylinus caesareus</i>	2	-	1	-	-	-	1	4
88.	<i>Stenomalina gracilis</i>	2	-	-	-	-	-	-	2
89.	<i>Stibeutes curvispina</i>	1	-	-	3	3	-	-	7
90.	<i>Tachyporus hypnorum</i>	7	7	12	2	-	-	-	28
91.	<i>Tachyusa thoracica</i>	-	-	20	-	-	-	-	20
92.	<i>Tanymecus palliates</i>	3	8	1	1	-	-	-	13
93.	<i>Tenuiphantes tenuis</i>	2	-	-	-	-	-	-	2
94.	<i>Trechus quadristriatus</i>	1	1	1	-	-	-	1	4
95.	<i>Trissolcus grandis</i>	2	2	8	43	10	3	4	72
96.	<i>Trybliographa rapae</i>	-	3	2	7	-	-	--	12
97.	<i>Xysticus cristatus</i>	25	33	90	50	4	-	40	242
98.	<i>Zelotes electus</i>	15	27	97	54	7	-	3	203
	Total samples	560	790	1261	750	117	67	665	3958

The analysis of the data obtained from the seven sampling sessions carried out in the rapeseed crop in 2025 highlights significant differences in both the numerical dynamics and the structure of arthropod communities. The total number of individuals (3958) (Tabel 2) showed pronounced variations between sampling dates, with a maximum recorded on June 24 (1261 individuals) and a minimum on August 2 (67 individuals), indicating an uneven distribution influenced by crop phenology and environmental conditions.

The analysis of variance (ANOVA) revealed statistically significant differences between sampling periods ($p < 0.05$), confirming that the timing of sampling had a significant effect on the total abundance of arthropods. Post-hoc tests (Tukey) indicated significant differences between the June 24 sampling and those from the later part of the growing season (July 22 and August 2), reflecting a clear population peak in June followed by a sharp decline.

The faunal structure during the peak period was dominated by phytophagous species, particularly *Delia brassicae* and *Dasineura brassicae*, which contributed substantially to the increase in total abundance. ANOVA applied to functional groups (phytophagous vs. entomophagous) showed statistically significant differences ($p < 0.05$), indicating a significant dominance of pest species during the peak period. However, the consistent and diverse

presence of beneficial entomofauna, including predators (*Brachinus crepitans*, *Amara eurynota*, *Xysticus cristatus*, *Zelotes electus*) and parasitoids (*Trissolcus grandis*, *Aphidius* spp.), suggests the existence of effective natural biological regulation mechanisms.

The Shannon diversity index (H') ranged between approximately 2.10 and 3.25, and statistical analysis revealed significant differences between sampling dates ($p < 0.05$). Higher values recorded during the early sampling periods (May-early June) indicate high diversity and a relatively even distribution of species, whereas the decrease in H' during June–July reflects the dominance of certain phytophagous species. The statistically significant differences in H' values confirm structural changes in the arthropod community throughout the growing season.

Overall, the results demonstrate that the studied agroecosystem exhibits a complex dynamic, with statistically significant variations in both abundance and faunal diversity. Although periods of high pest pressure were observed, the presence of beneficial arthropods and the increase in diversity toward the end of the growing season indicate a tendency toward ecological rebalancing. These findings support the importance of implementing integrated pest management strategies based on the conservation of natural biological control and the reduction of chemical inputs.

Table 2. Structure of the collected species by order, family, and ecological characterization

Order	Family	Name of species	Type of food	No of samples
1	2	3	4	5
Araneae	Lycosidae	<i>Pardosa agricola</i>	Predators	141
	Total samples of family			141
Coleoptera 2	Anthicidae	<i>Anthicus flavipes</i>	Saprofag	13
	Total samples of family			13
	Anthicidae	<i>Formicomus pedestris</i>	Saprofag	163
	Total samples of family			163
	Cantharidae	<i>Cantharis fusca</i>	Predators	3
	Cantharidae	<i>Cantharis obscura</i>	Predators	5
	Cantharidae	<i>Cantharis pellucida</i>	Predators	2
	Cantharidae	<i>Cantharis rustica</i>	Predators	4
	Total samples of family			14
	Carabidae	<i>Amara aenea</i>	Omnivores	42
	Carabidae	<i>Amara eurynota</i>	Omnivores	187
	Carabidae	<i>Amara familiaris</i>	Omnivores	8
	Carabidae	<i>Amara similata</i>	Omnivores	1
	Carabidae	<i>Anisodactylus binotatus</i>	Omnivores	39
	Carabidae	<i>Anisodactylus signatus</i>	Omnivores	6
	Carabidae	<i>Brachinus crepitans</i>	Predators	633
	Carabidae	<i>Harpalus aeneus</i>	Omnivores	15
	Carabidae	<i>Harpalus distinguendus</i>	Omnivores	128
	Carabidae	<i>Harpalus tardus</i>	Omnivores	2
	Carabidae	<i>Metabletus truncatellus</i>	Predators	12
	Carabidae	<i>Ocydromus decorus</i>	Predators	1
	Carabidae	<i>Ophonus azureus</i>	Omnivores	5
	Carabidae	<i>Parallelomorphus terricola</i>	Predators	1
	Carabidae	<i>Poecilus dimidiatus</i>	Predators	4
	Carabidae	<i>Pseudophonus griseus</i>	Omnivores	10
	Carabidae	<i>Pseudophonus pubescens</i>	Omnivores	43
	Carabidae	<i>Pterostichus cupreus</i>	Predators	75
	Carabidae	<i>Parophonus maculicornis</i>	Omnivores	1
	Total samples of family			1213
	Chrysomelidae	<i>Idiochroma dorsalis</i>	Phytophagous	398
	Chrysomelidae	<i>Longitarsus lycopi</i>	Phytophagous	7
	Chrysomelidae	<i>Phyllotreta nemorum</i>	Phytophagous	23
	Chrysomelidae	<i>Psylliodes attenuata</i>	Phytophagous	63
	Chrysomelidae	<i>Psylliodes chrysocephala</i>	Phytophagous	26
	Total samples of family			517
	Coccinellidae	<i>Coccinella 7punctata</i>	Predators	28
	Coccinellidae	<i>Nephus redtenbacheri</i>	Predators	1
	Total samples of family			29
	Curculionidae	<i>Baris chlorizans</i>	Phytophagous	43
	Curculionidae	<i>Ceutorhynchus obstrictus</i>	Phytophagous	11
	Curculionidae	<i>Ceutorhynchus pallidactylus</i>	Phytophagous	5
	Total samples of family			59
	Elateridae	<i>Agriotes ustulatus</i>	Phytophagous	3
	Total samples of family			3
	Histeridae	<i>Hister purpurascens</i>	Predators	1
	Histeridae	<i>Hister quadrimaculatus</i>	Predators	2
	Histeridae	<i>Hister stercorarius</i>	Predators	13
	Total samples of family			16
	Nitidulidae	<i>Meligethes aeneus</i>	Phytophagous	22
	Total samples of family			22
	Staphylinidae	<i>Actobius cinerascens</i>	Predators	6
	Staphylinidae	<i>Acylophorus glaberrimus</i>	Predators	3
	Staphylinidae	<i>Aleochara lanuginosa</i>	Parasitoids	33
	Staphylinidae	<i>Atheta trinotata</i>	Predators	7

	2	3	4	5
	Staphylinidae	<i>Bryocharis analis</i>	Predators	2
	Staphylinidae	<i>Calodera aethiops</i>	Predators	309
	Staphylinidae	<i>Lathrobium quadratum</i>	Predators	11
	Staphylinidae	<i>Oxytelus sculptus</i>	Saprophag	5
	Staphylinidae	<i>Paederus riparius</i>	Predators	1
	Staphylinidae	<i>Conosoma pubescens</i>	Predators	4
	Staphylinidae	<i>Ocypus ophthalmicus</i>	Predators	1
	Total samples of family			382
	Tenebrionidae	<i>Opatrum sabulosum</i>	Phytophagous	96
	Total samples of family			96
Dermaptera	Forficulidae	<i>Forficula auricularia</i>	Omnivores	4
	Total samples of family			4
Diplopoda	Julidae	<i>Ommatoiulus sabulosus</i>	Detritivor	2
	Total samples of family			2
Diptera	Cecidomyiidae	<i>Dasineura brassicae</i>	Phytophagous	712
	Anthomyiidae	<i>Delia brassicae</i>	Phytophagous	789
	Total samples of family			1501
Geophilomorpha	Geophilidae	<i>Geophilus flavus</i>	Predators	3
	Total samples of family			3
Hemiptera	Aphididae	Afide	Phytophagous	30
	Pentatomidae	<i>Dolycoris baccarum</i>	Phytophagous	6
	Pentatomidae	<i>Eurydema ornata</i>	Phytophagous	18
	Reduviidae	<i>Peirates hybridus</i>	Predators	5
	Scutelleridae	<i>Eurygaster integriceps</i>	Phytophagous	7
	Total samples of family			66
Hymenoptera	Aphelinidae	<i>Aphelinus abdominalis</i>	Parasitoids	19
	Total samples of family			19
	Bethylidae	<i>Epyris niger</i>	Parasitoids	10
	Total samples of family			10
	Braconidae	<i>Aphidius rhopalosiphi</i>	Parasitoids	20
	Total samples of family			20
	Ceraphronidae	<i>Aphanogmus abominalis</i>	Parasitoids	26
	Ceraphronidae	<i>Ceraphron bispinosus</i>	Parasitoids	48
	Total samples of family			74
	Eulophidae	<i>Necremnus tidius</i>	Parasitoids	4
	Total samples of family			4
	Ichneumonidae	<i>Aneucelis melanaria</i>	Parasitoids	10
	Ichneumonidae	<i>Phygadeuon trichops</i>	Parasitoids	10
	Total samples of family			20
	Megaspilidae	<i>Conostigmus rufescens</i>	Parasitoids	20
	Total samples of family			20
	Proctotrupidae	<i>Brachyserphus parvulus</i>	Parasitoids	3
	Total samples of family			3
	Tenthredinidae	<i>Athalia rosae</i>	Phytophagous	3
	Total samples of family			3
Neuroptera	Hemerobiidae	<i>Micromus variegatus</i>	Predators	6
	Total samples of family			6
Opiliones	Phalangidae	<i>Phalangium opilio</i>	Omnivores	48
	Total samples of family			48
Orthoptera	Acrididae	<i>Chorthippus vagans</i>	Phytophagous	2
	Acrididae	<i>Locusta migratoria</i>	Phytophagous	1
	Total samples of family			3
	Gryllidae	<i>Gryllus campestris</i>	Omnivores	34
	Total samples of family			34
Total samples	General = 3958	Phytophagous =1737	Predators=170	Omnivoreses = 428
				Parasitoids=125

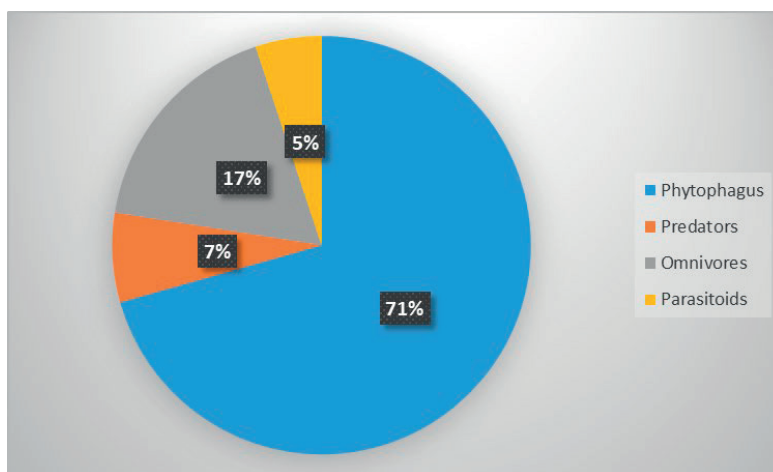


Figure 1. Percentage distribution of species according to ecological characterization

The graph (Figure 1) highlights a clear dominance of phytophagous species (1737 individuals), which indicates a high pest pressure on the rapeseed crop. Beneficial entomofauna groups are represented in smaller proportions, with 170 predators and 125 parasitoids, but they play an essential role in the biological regulation of pest populations.

Omnivorous species (428 individuals) occupy an intermediate position, contributing to the stability of the ecosystem through trophic flexibility. Overall, the distribution shows a relative imbalance in favor of phytophagous species, but also the existence of a potential for natural biological control.

CONCLUSIONS

The dynamics of arthropod populations are strongly influenced by crop phenology and climatic conditions, with a clear peak in abundance in June, followed by a sharp decline in July-August.

There are statistically significant differences between sampling periods ($p < 0.05$), confirming that the timing of sampling has a major impact on the abundance and structure of the entomofauna.

Phytophagous species dominate during the peak period, especially *Delia brassicae* and *Dasineura brassicae*, generating high pest pressure on the rapeseed crop.

Beneficial entomofauna (predators and parasitoids) are well represented and

ecologically synchronized with pests, suggesting the presence of effective natural biological control mechanisms.

Species diversity (Shannon index) varies significantly throughout the season, being higher at the beginning of the growing period and decreasing during the dominance of phytophagous species.

The agroecosystem shows a high potential for self-regulation, despite the temporary imbalance in favor of phytophagous species, supporting the need for integrated pest management strategies based on biodiversity conservation.

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MACROPHYTES AS ECOSYSTEM ENGINEERS: IMPLICATIONS FOR BIODIVERSITY, WATER QUALITY, AND AGRICULTURAL SUSTAINABILITY

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Abstract

Macrophytes are recognized as ecosystem engineers that strongly influence freshwater and wetland ecosystems by shaping habitat structure, regulating nutrient cycling, and supporting key ecosystem services. Through their structural and functional diversity, macrophyte communities sustain high biodiversity, stabilize sediments, and enhance the resilience of aquatic food webs. Macrophytes play a central role in water quality regulation by retaining excess nutrients, reducing turbidity, and supporting biogeochemical processes that mitigate pollution. At the interface between aquatic ecosystems and agricultural landscapes, they contribute to sustainable agriculture by buffering nutrient runoff, improving water availability, and offering potential for nature-based solutions such as phytoremediation and nutrient recovery. Despite these benefits, macrophyte communities are increasingly threatened by eutrophication, habitat alteration, invasive species, and climate change. This paper emphasizes the need to integrate macrophyte conservation and management into water and agricultural policies to enhance biodiversity, maintain water quality, and support long-term agricultural sustainability.

Key words: macrophytes, ecosystem engineering, biodiversity, water quality, agricultural sustainability.

INTRODUCTION

Freshwater ecosystems are increasingly recognized as some of the most vulnerable environments on the planet, undergoing an accelerated process of degradation largely caused by anthropogenic pressures associated with agricultural intensification (Majeed et al., 2025). The expansion of agricultural land has substantially modified riparian vegetation and channel morphology, affecting the structural integrity of aquatic habitats and thus contributing to biodiversity loss (Fares et al., 2020). A determining factor in this decline is the excessive loading of nitrogen (N) and phosphorus (P) from agricultural runoff, which accelerates the eutrophication process and triggers ecological chain effects, such as harmful algal blooms and hypoxia (Yang et al., 2025).

Within these disturbed ecosystems, aquatic macrophytes play a central ecological role that goes beyond the mere presence of vegetation. They function as true ecosystem engineers, with the ability to modify both the physical structure and the chemical conditions of aquatic

environments (Gopal, 2016; Lesiv et al., 2020; Thomaz, 2021). By influencing hydrodynamics, sedimentation processes, and nutrient cycling, macrophytes contribute significantly to the regulation and resilience of freshwater ecosystems (Swe et al., 2021; Poveda, 2022). These ecological engineering effects manifest themselves through complementary mechanisms, both physical and biogeochemical. Structurally, dense macrophyte populations attenuate wave energy and reduce water flow velocity, facilitating the settling of suspended particles and limiting sediment resuspension, an essential process for maintaining water clarity (Yang et al., 2025). At the sediment-water interface, rooted macrophytes further modify environmental conditions through radial oxygen loss (ROL), a mechanism by which oxygen is transported to the rhizosphere and released into otherwise anoxic sediments (Rejmánková, 2011). The resulting aerobic microzones stimulate microbial processes, including nitrification and degradation of organic pollutants (Majeed et al., 2025). In addition, macrophytes act as active regulators of nutrient dynamics, assimilating nitrogen and

phosphorus from both sediments and the water column, thereby reducing the availability of these elements for phytoplankton proliferation (Poveda, 2022; Yang et al., 2025).

Despite these well-documented ecological functions, a significant conceptual and practical gap remains at the intersection of aquatic ecology and agricultural management. Research on macrophytes has largely developed along separate disciplinary lines: ecological studies focus on ecosystem functioning and species interactions, while agricultural perspectives often view aquatic vegetation as a management nuisance or a biomass removal problem (Quilliam et al., 2015). As a result, current management approaches rarely integrate the phytoremediation capacity of macrophytes into nutrient management strategies in agricultural landscapes (Poveda, 2022). Furthermore, limited attention has been paid to developing frameworks that recognize nutrient-enriched macrophyte biomass as a potential resource, capable of recovering nutrients lost from agricultural systems and supporting circular bioeconomy initiatives (Quilliam et al., 2015; Majeed et al., 2025).

To fill this gap, a more integrated perspective is needed, one that connects ecosystem processes with agricultural sustainability. In this context, the present study proposes a conceptual framework that positions macrophytes as key mediators between freshwater ecosystem functioning and agricultural resource management. The central hypothesis is that integrating macrophyte-based processes into hydrological and agricultural management strategies can enhance socio-ecological resilience, simultaneously promoting ecosystem restoration and resource recovery (Quilliam et al., 2015). Accordingly, the objectives of this study are to: (1) examine the role of macrophytes in sustaining biodiversity through habitat structuring; (2) evaluate their contribution to water quality regulation and eutrophication mitigation via nutrient cycling and sediment stabilization; (3) explore their potential applications in sustainable agriculture through nutrient recovery and phytoremediation; and (4) discuss implications for integrated water resources management policies.

MATERIALS AND METHODS

This study is based on a structured literature review, conducted with the aim of synthesizing current knowledge on the ecological and agricultural functions of aquatic macrophytes. Scientific papers were identified in the main academic databases - Web of Science, Scopus and Google Scholar - thus ensuring extensive coverage of peer-reviewed sources.

The selection focused primarily on peer-reviewed journal articles, relevant book chapters, and policy documents published primarily between 2015 and 2025, but some previously relevant studies were also considered. Fundamental ecological studies were included to substantiate the theoretical basis of the concepts related to ecosystem engineering by macrophytes. The search strategy involved combining key terms such as "aquatic macrophytes", "ecosystem engineers", "phytoremediation", "nature-based solutions", and "agricultural sustainability".

The collected literature was screened and subsequently organized through thematic synthesis. Four principal analytical themes were identified: (1) physical and biogeochemical mechanisms through which macrophytes act as ecological engineers; (2) their role in supporting biodiversity and regulating water quality; (3) applications at the agriculture-freshwater interface, particularly nutrient buffering and nutrient recovery processes; and (4) the integration of these ecological functions into environmental governance and policy frameworks.

In addition, information on species-specific functional traits was extracted from the selected literature, in order to assess the practical relevance of different macrophyte taxa for sustainable agricultural systems and nutrient management strategies.

RESULTS AND DISCUSSIONS

MACROPHYTES AS ECOSYSTEM ENGINEERS

Structural Engineering

Aquatic macrophytes function as true ecosystem engineers, physically shaping aquatic habitats and increasing the structural

complexity of these environments. This complexity, often described in terms of fractal dimensions, amplifies habitat heterogeneity and is strongly correlated with higher biodiversity of epiphytic algae and macroinvertebrates (Celewicz-Goldyn & Kuczyńska-Kippen, 2017; Filho et al., 2021; Thomaz, 2021). In addition to providing substrate for numerous organisms, dense macrophyte populations also influence local hydrodynamic conditions, attenuating wave energy and reducing water flow velocity. As a result, suspended particles settle more easily, bank erosion is minimized, and overall turbidity decreases. These processes contribute, at the same time, to changes in sediment distribution and riverbed morphology (Barko & James, 1998; Madsen et al., 2001; Horppila & Nurminen, 2003; Catianis et al., 2022).

Nutrient Cycling and Biogeochemical Regulation

Macrophytes also contribute to the regulation of biogeochemical cycles through interactions between their root systems and the surrounding sediment matrix. One of the key mechanisms involved is radial oxygen loss (ROL), by which rooted species, such as *Phragmites australis*, transport atmospheric oxygen to the rhizosphere, generating localized aerobic microzones within anoxic bulk sediments (Brix, 1994). This localized oxygenation stimulates microbial activity and promotes the coupled nitrification-denitrification pathways, essential for nitrogen removal and the degradation of various pollutants (Brix, 1997; Vymazal, 2013; Kochi et al., 2020; Paul et al., 2022; Ijoma et al., 2024).

In terms of nutrient dynamics, macrophytes have the capacity to store considerable amounts of nitrogen and phosphorus during the active growing season (Preiner et al., 2020). However, this ecological function varies over time. As macrophyte populations enter the senescence phase, they can transform from nutrient reservoirs to potential nutrient sources, releasing previously stored elements into the water column. Consequently, appropriate timing vegetation management becomes an important aspect in preventing internal nutrient loading of aquatic ecosystems (Lu et al., 2017).

Regime Shifts

Submerged macrophytes are a central component in the stabilization of the clear water state of shallow lakes, acting mainly through positive ecological feedback mechanisms. By directly competing with phytoplankton for light and nutrients, and in some cases by releasing allelopathic compounds that inhibit algal growth, they limit the proliferation of phytoplankton. In addition, macrophyte populations reduce sediment resuspension, thereby enhancing water transparency (Scheffer et al., 1993; Hilt & Gross, 2008; Vanderstukken et al., 2011; Bakker et al., 2016).

When macrophyte cover decreases as a result of eutrophication, these stabilization mechanisms can collapse. This phenomenon often leads to a nonlinear transition to a phytoplankton-dominated turbidity state. Once established, this alternative ecological state can become extremely resilient and difficult to reverse without substantial restoration interventions (Jeppesen et al., 2012; Phillips et al., 2016; Zeng et al., 2017; Yang et al., 2025).

BIODIVERSITY AND WATER QUALITY REGULATION

Functional Diversity

Macrophyte assemblages show a clear functional complementarity that is correlated with their growth forms, thus generating heterogeneous “mosaic communities” that enhance ecosystem stability (Wang et al., 2009). Submerged species, such as *Potamogeton* spp. and *Hydrilla* spp., play an essential role in coupling benthic and pelagic processes and contribute to oxygenation of the water column. In contrast, species with floating leaves influence the thermal regime and regulate light penetration in aquatic systems (Rawat & Singh, 2025; Yang et al., 2025). Emergent macrophytes, located at the interface between land and water, contribute to shoreline stabilization and favor sediment oxidation through specialized tissues such as aerenchyma, which also facilitate microbial degradation of pollutants (Rejmánková, 2011).

Higher functional diversity within macrophyte communities has been consistently correlated with increased ecosystem multifunctionality. Such diverse communities support a wider range of invertebrate trophic groups and improve nutrient retention compared to monospecific populations (Thomaz, 2021; Yofukuji et al., 2021; Moi et al., 2021).

Refuge and Trophic Cascades

The structural complexity generated by macrophyte vegetation plays a central role in regulating biodiversity and water quality through trophic cascade mechanisms. Dense populations, especially those formed by macrophytes with complex leaf architectures, such as *Myriophyllum* spp., provide essential diurnal shelter for large zooplankters (e.g., *Daphnia* spp.), protecting them from fish predation (Warfe & Barmuta, 2006). This refuge function allows zooplankton populations to maintain strong grazing pressure on phytoplankton, which contributes to the suppression of algal blooms and supports the persistence of clear water conditions (Burks et al., 2006; Celewicz-Goldyn & Kuczyńska-Kippen, 2017).

Beyond their importance for invertebrate communities, the habitats created by macrophytes also play a key role in fish reproduction. Their structural complexity provides spawning substrates and rearing habitats for phytophilic fish species, such as *Cyprinus carpio* and *Micropterus salmoides*, where the juvenile fish benefit from protection both against predators and against hydrodynamic disturbances (Mandal & Bera, 2025).

Indicator Role

Because macrophytes are sessile organisms whose distribution reflects long-term environmental conditions, they are widely used as biological quality elements in ecological assessments carried out under the European Union Water Framework Directive (Stelzer et al., 2005). The composition of macrophyte communities therefore serves as an integrative indicator of ecological status. For example, the presence of sensitive charophyte species generally indicates good water quality, while the dominance of more tolerant taxa, such as

Potamogeton pectinatus or *Ceratophyllum demersum*, usually signals the onset of eutrophication and ecological degradation (Schneider, 2007; Schneider-Binder, 2018; Costică et al., 2018; Robbe et al., 2024).

To quantify these ecological changes, numerous assessment tools have been developed, including the Reference Index (RI) and the Macrophyte Biological Index for Rivers (MBRI). These metrics assess deviations from reference conditions specific to different types of water bodies (Stelzer et al., 2005; Kozak & Goldyn, 2016).

INTERFACE WITH AGRICULTURAL SUSTAINABILITY

Nutrient Buffering and Nature-Based Solutions

Macrophytes form important vegetated buffer zones, also known as ecotones, at the interface between terrestrial and aquatic ecosystems. Certain emergent species are particularly valued for their functional traits, including high nutrient retention capacity and sediment stabilization (Table 1). For example, *Phragmites australis* and species of the genus *Typha* are highly effective in intercepting agricultural runoff due to their extensive rhizome networks, which attenuate water movement and promote denitrification processes (Kurniawan et al., 2021; Poveda, 2022).

These characteristics make macrophytes essential components of nature-based solutions (NBS) designed to improve water quality in agricultural landscapes. Their application also extends to constructed wetlands (CWs), which have proven highly effective in reducing biochemical oxygen demand (BOD) and removing emerging pollutants from wastewater streams (Agaton, 2025).

Phytoremediation and Irrigation Enhancement

Beyond their passive filtering capacity, macrophytes can actively improve water quality through phytoremediation processes in agricultural reservoirs. Species such as *Lemna minor* and *Ceratophyllum demersum* are particularly effective in nutrient recovery systems due to their rapid uptake rates (Table

1). There is evidence that irrigation with water treated by these macrophytes, which effectively function as a liquid biofertilizer, can significantly increase crop productivity (Poveda, 2022; Patel & Kumar, 2025; Mignoni et al., 2025). In addition, certain macrophyte species act as hyperaccumulators (Table 1), having the ability to remove heavy metals from contaminated waters, thus increasing the safety of water used in agricultural production systems (Ansari et al., 2020; Opitz et al., 2021; Rawat & Singh, 2025).

Socio-Ecological Feedback

Ensuring long-term sustainability requires management strategies capable of generating a positive socio-ecological feedback loop, in which ecosystem functioning and economic

benefits are mutually reinforcing (Figure 1). From this perspective, management should go beyond the traditional approach of simply removing biomass and focus on valorizing aquatic vegetation, thus contributing to closing nutrient cycles. Harvesting macrophyte biomass before senescence sets in is particularly important, as it prevents the re-release of nutrients into the water column and reduces the risk of internal nutrient loading. At the same time, the collected biomass can serve as a valuable resource for the production of compost or biochar, transforming what was previously considered waste into a useful input for sustainable agricultural systems (Quilliam et al., 2015; Gusain & Suthar, 2020; Yatoo et al., 2025).

Table 1. Functional traits of representative macrophyte species relevant for biodiversity, water quality regulation, and agricultural sustainability (based on synthesis of Cook, 1996; Brix, 1997; Cronk & Fennessy, 2001; Cotton et al., 2006; Kadlec & Wallace, 2009; Vymazal, 2013; Ali et al., 2013; Rezanian et al., 2015; Kurniawan et al., 2021; Poveda, 2022)

Species	Growth form	N retention	P retention	Phytoremediation potential	Sediment stabilization	Agricultural application
<i>Phragmites australis</i>	Emergent	High	High	Heavy metals, organic pollutants	Very High	Buffer strips, CWs
<i>Typha latifolia</i>	Emergent	High	Moderate-High	Nutrients, metals	High	Nutrient interception zones
<i>Ceratophyllum demersum</i>	Submerged	Moderate	High	Nutrient pulses	Low-Moderate	Irrigation ponds
<i>Potamogeton pectinatus</i>	Submerged	Moderate	Moderate	Nutrient stabilization	Moderate	Shallow agricultural reservoirs
<i>Myriophyllum spicatum</i>	Submerged	Moderate	High	Nutrient uptake	Moderate	Water quality enhancement
<i>Lemna minor</i>	Free-floating	High	Moderate	Wastewater nutrient removal	Low	Nutrient recovery systems
<i>Elodea canadensis</i>	Submerged	Moderate	Moderate	Nutrient buffering	Moderate	Secondary irrigation canals

POLICY AND MANAGEMENT IMPLICATIONS INTEGRATION INTO WATER AND AGRICULTURAL POLICY

Integration into Water and Agricultural Policy

Contemporary governance frameworks need to evolve to more effectively integrate macrophyte management into large-scale agricultural and water policy strategies. Historically, environmental interventions in vulnerable regions such as the Lower Danube

have focused mainly on damming and drainage aimed at expanding intensive agriculture, often to the detriment of natural capital (Vădineanu et al., 2003). In contrast, recent agricultural policy directions, within the Common Agricultural Policy (CAP), increasingly promote the implementation of nature-based solutions (NBS) as a means of reconciling agricultural productivity with ecosystem conservation (David & Hulea, 2001; Vădineanu et al., 2003; Szoszkiewicz et al., 2025). In this framework, the restoration and reconstruction of wetlands

are encouraged as natural nutrient buffers, capable of reducing agricultural runoff, while contributing to the achievement of the nutrient

reduction objectives set by the European Union Water Framework Directive (WFD) (Vădineanu et al., 2003; Robbe et al., 2024).

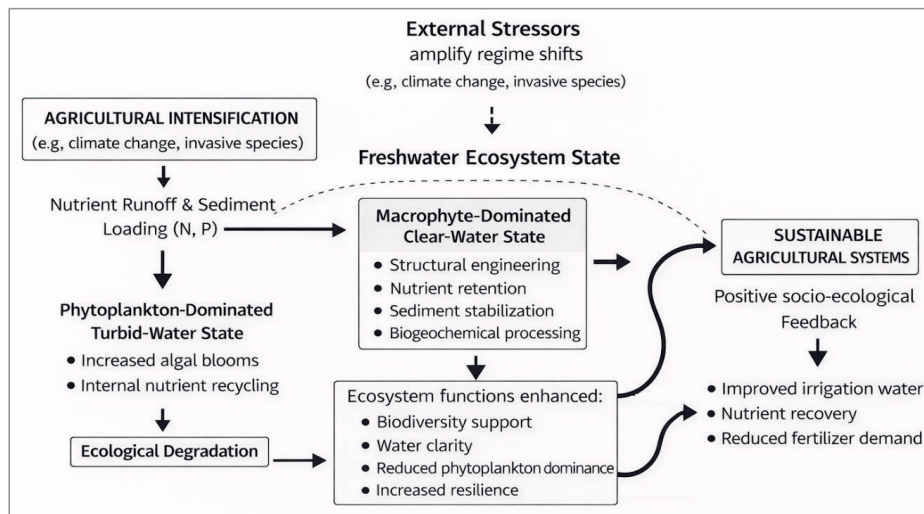


Figure 1. Macrophytes as ecosystem engineers linking agriculture and freshwater resilience (based on Jeppesen et al., 1998; Scheffer et al., 2001)

Conflict and Synergy in Monitoring

Although the WFD mandates the use of macrophytes as biological quality elements for assessing ecological status – using indicators such as the Reference Index (RI) – a gap often remains between ecological monitoring systems and practical agricultural management (Stelzer et al., 2005; Caffrey et al., 2006). Strengthening governance therefore requires identifying practical synergies through which monitoring data can directly inform agricultural decision-making. For example, changes in community composition in favour of tolerant species, such as *Potamogeton pectinatus*, can function as early warning signals for nutrient enrichment associated with agricultural runoff (Schneider-Binder, 2018). Integrating such ecological indicators into land management strategies would allow for a more proactive response to emerging environmental pressures (Schneider, 2007).

Long-term Resilience and Adaptive Management

Achieving sustainable ecosystem resilience requires adaptive management strategies that can address the hysteresis frequently observed in shallow lake systems, where simply reducing

external nutrient inputs may not be sufficient to restore submerged macrophyte communities (Hilt et al., 2018; Troia, 2023). Effective restoration therefore requires more comprehensive approaches that combine multiple interventions, including biomanipulation and water level management, with the aim of restoring structurally diverse “mosaic communities” capable of stabilizing clear water conditions (Kozak & Goldyn, 2016; Wang et al., 2009). In addition, conservation strategies should prioritize the protection of key habitats and rare aquatic plant species, such as *Trapa natans*, in both urban and rural wetlands. Protecting these ecological components is essential for maintaining biodiversity, while balancing the increasing pressures associated with human activities (Sârbu et al., 2006; Strat, 2016; Manea et al., 2016).

CONCLUSIONS

Aquatic macrophytes represent a key socio-ecological component within freshwater systems, connecting water quality regulation, biodiversity conservation, and the sustainability of agricultural landscapes. Through their capacity to engineer habitats, these plants

contribute to sediment stabilization, enhance structural complexity, and support diverse trophic interactions, functioning as one of the main biological mechanisms that maintain clear-water conditions in many freshwater ecosystems.

Despite these ecological functions, the potential of macrophytes remains insufficiently integrated into agricultural management. In many landscapes, they could simultaneously mitigate diffuse nutrient pollution and enable nutrient recovery through the harvesting of aquatic biomass. Reframing aquatic vegetation not as a nuisance but as a valuable biological resource is therefore essential for developing circular bioeconomy strategies, where harvested biomass may be converted into biofertilizers or bioenergy products.

Future management approaches should overcome traditional disciplinary boundaries by encouraging collaboration among ecologists, agronomists, and policymakers. Such interdisciplinary cooperation is necessary to implement integrated catchment-scale strategies that fully recognize and utilize the ecosystem services provided by macrophyte communities.

Ultimately, the conservation and restoration of aquatic macrophytes should be viewed not only as a biodiversity objective but also as a fundamental requirement for maintaining long-term water security and strengthening the resilience of agricultural systems under accelerating global environmental change.

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USE OF GENERATIVE ARTIFICIAL INTELLIGENCE (GenAI)

The authors declare the use of Generative Artificial Intelligence (GenAI) tools in the preparation of this manuscript. GenAI was used exclusively for drafting assistance, language editing, and text refinement. All scientific content, original data, and interpretations presented in this paper were developed and verified solely by the authors.

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COMPARATIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN FENNEL AND CARAWAY SEEDS

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Abstract

Foeniculum vulgare Mill. (fennel) and *Carum carvi* L. (caraway) are aromatic plants widely used in the food, pharmaceutical, cosmetic, and feed industries due to their essential oils rich in biologically active compounds. This study characterized and compared the chemical composition, total phenolics, flavonoid content, and antioxidant capacity of essential oils and extracts from four fennel samples and one caraway sample. Essential oils were obtained by hydrodistillation and analyzed using gas chromatography–mass spectrometry (GC-MS). Fennel oils displayed a largely consistent qualitative profile, with trans-anethole as the predominant constituent (up to 60.5% in cv. Hestia), accompanied by minor compounds such as fenchone, α -pinene, β -pinene, limonene, and estragole, which varied quantitatively among cultivars. Caraway oil showed a distinct profile dominated by carvone and D-limonene, accounting for over 97% of the total volatile fraction. Caraway seeds exhibited the highest flavonoid content, while certain fennel cultivars accumulated more total phenolics, resulting in measurable differences in antioxidant capacity. These findings highlight the influence of species and cultivar on the accumulation of bioactive compounds and suggest that both fennel and caraway seeds have potential as natural feed additives to enhance antioxidant defenses, support metabolic efficiency, and improve dairy animal productivity.

Key words: *Foeniculum vulgare*, *Carum carvi*, bioactive compounds, volatile compounds, feed.

INTRODUCTION

Aromatic and medicinal plants have been used for centuries for their therapeutic properties and as spices to enhance the flavor and aroma of food (Rather et al., 2016). Among them, *Foeniculum vulgare* Mill. (fennel) and *Carum carvi* L. (caraway), members of the Apiaceae family, are extensively cultivated for their essential oils and bioactive compounds (Ahmad et al., 2018; Laribi et al., 2013).

Foeniculum vulgare Mill. is an annual, biennial, or perennial plant widely distributed in Central Europe and the Mediterranean region (Abdesslem et al., 2022). It has long been used as a natural remedy due to its antibacterial, antioxidant, antitumor, anti-inflammatory, galactogenic, and antiviral properties (Rahimi and Ardekani, 2013). These biological effects are attributed to its diverse phytochemical

composition, including flavonoids, hydrocarbons, fatty acids, amino acids, and volatile compounds. Within the volatile fraction of *Foeniculum vulgare* seeds, trans-anethole, estragole (methyl chavicol), fenchone, and α -phellandrene are reported as major constituents, with trans-anethole being the predominant compound, which contributes to the wide use of fennel extracts in the food and pharmaceutical industries (Mokhtari & Ghoreishi, 2019). *Carum carvi* L. is the best-known species of the genus *Carum*, which includes approximately 25–30 species. Caraway is cultivated in Europe, West Asia, Africa, and the United States as a spice and as a source of essential oil with recognized biological and technological value. Its essential oil exhibits diuretic, galactogenic, antibacterial, seed germination inhibitory, and insect-repellent activities (Bouzaid et al., 2024; Simic et al., 2008; Singh et al., 2006). Carvone

and limonene represent approximately 97% of the major components of caraway essential oil, while other compounds such as carveol, camphene, phellandrene, and anethole further contribute to its nutritional and therapeutic value (Sedláková et al., 2003). Essential oils from fennel and caraway are traditionally recognized for their galactogenic potential, mainly attributed to volatile constituents such as trans-anethole, fenchone, carvone, and limonene.

Beyond their volatile fractions, fennel and caraway seeds are important sources of phenolic acids and flavonoids. These secondary metabolites are well known for their antioxidant properties, primarily due to their ability to neutralize reactive oxygen species through hydrogen atom or electron donation. Total phenolic content (TPC) and total flavonoid content (TFC) are widely used indicators of antioxidant potential in plant extracts. Several studies have demonstrated a positive correlation between phenolic concentration and radical scavenging activity in Apiaceae species (Shan et al., 2005; Dai and Mumper, 2010). Therefore, evaluating both essential oil composition and phenolic profiles is essential for understanding the biological activity and functional value of these plants (Figueiredo et al., 2008).

In recent years, aromatic and medicinal plants have gained increasing attention as phytogetic feed additives in dairy nutrition, aiming to enhance productivity and product quality (Iommelli et al., 2025). Mahmoud et al. (2020) reported that supplementation of dairy rations with caraway and fennel seed powder at 0.7% of total dry matter improved animal performance and enhanced the milk fatty acid profile. Similarly, Attari et al. (2018) found that fennel supplementation increased milk yield without altering its composition. Moheghi et al. (2010) also demonstrated that increasing caraway seed powder in cattle diets improved milk fat content. Considering their phytochemical richness and documented biological effects, the aim of this study was to characterize the phytochemical composition, essential oil profile, and antioxidant activity of *Foeniculum vulgare* and *Carum carvi* seeds in order to evaluate their potential application as natural feed additives.

MATERIALS AND METHODS

Plant material

Seeds of *Foeniculum vulgare* Mill. and *Carum carvi* L. were provided by the Plant Genetic Resources Bank of Buzau, Romania. The experimental material included four fennel samples corresponding to different cultivars and one caraway sample (Table 1). The seeds were stored at room temperature in a desiccator prior to characterization.

Table 1. Samples and codification

Sample	Code
<i>Carum carvi</i> L.	C1
<i>F. vulgare</i> Mill. cv. Hestia	F1
<i>F. vulgare</i> Mill. cv. BRGV1 (Napoleon)	F2
<i>F. vulgare</i> Mill. cv. de Florenta	F3
<i>F. vulgare</i> Mill. cv. BRGV2	F4

Determination of dry matter content

Dry matter content was determined by oven-drying 1 g of seed material at 105°C until constant weight was achieved, according to Gül et al. (2023). The dried material was cooled in a desiccator to prevent moisture absorption and then weighed using an analytical balance.

Protein content analysis

Nitrogen content (%) was determined using a CHNS elemental analyzer based on the high-temperature combustion method (Dumas principle), as described by Serrano et al. (2013). Approximately 2 mg of sample was accurately weighed into tin capsules and introduced into the combustion furnace, where complete oxidation occurred at temperatures above 900°C in an oxygen-rich atmosphere. The resulting gases were transported by helium, reduced, and purified before separation by gas chromatography. Quantification was performed using cystine as the analytical standard. Crude protein content was calculated from the measured nitrogen content using the conventional nitrogen-to-protein conversion factor of 6.25, based on the assumption that proteins contain approximately 16% nitrogen ($100/16 = 6.25$) (Mariotti et al., 2008; Posa et al., 2024). Protein content was therefore calculated according to the following equation:

Protein (%) = Nitrogen (%) $\times$ 6.25

Extraction of bioactive compounds

An amount of 1 g of seed sample was mixed with 10 mL of 70% aqueous methanol (v/v) and subjected to conventional extraction. The extraction procedure consisted of macerating and homogenizing the sample in the presence of quartz sand for 24 h in the dark at room temperature. After maceration, the samples were agitated to enhance extraction efficiency and then centrifuged for 5 min at 5000 rpm at 4°C. The supernatant was collected, and the extraction step was repeated twice under the same conditions. The obtained supernatants were pooled, resulting in a final extract volume of 30 mL (Stan et al., 2017).

Determination of total phenolic content

The total phenolic content of the seed extracts was determined using the Folin-Ciocalteu spectrophotometric method, following the protocol described by Ion et al. (2020). An aliquot of 0.5 mL of extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (2 N), previously diluted tenfold with distilled water. A 70% methanol solution was used as the blank. After incubation for 2 minutes at room temperature, 2 mL of sodium carbonate solution (75 g/L) was added. The mixture was then placed in a water bath at 50°C for 15 minutes and subsequently cooled to room temperature in an ice-water bath. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Total phenolic content was quantified using a gallic acid calibration curve ($y = 0.012x + 0.0277$, $R^2 = 0.9984$). The results are expressed as milligrams of gallic acid equivalents per gram of fresh sample (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric assay described by Samatha et al. (2012), with minor modifications. Briefly, 250 μ L of extract was transferred into a test tube, followed by the addition of 150 μ L of aluminum chloride solution (10% w/v). The mixture was incubated at room temperature for 5 minutes. Subsequently, 500 μ L of NaOH solution (5%) was added, and the reaction mixture was thoroughly vortexed. The final volume was

adjusted to 2.5 mL with distilled water. Absorbance was measured at 510 nm using a UV-Vis spectrophotometer. Quantification was performed using a calibration curve prepared with rutin, and the results were expressed as milligrams of rutin equivalents per gram of fresh sample (mg RE/g) (Ahmad et al., 2018).

Essential oils extraction and analysis

Ground seeds (approximately 200 g) were subjected to hydrodistillation for three hours using a Neo-Clevenger apparatus. The obtained essential oils were diluted in hexane and analyzed using an Agilent 6890 gas chromatograph coupled with a 5973 single-quadrupole mass spectrometer equipped with an HP-5MS capillary column (Ion et al., 2020). The compounds were identified based on their retention indices and by comparison of their mass spectra with those from the NIST database.

Determination of antioxidant capacity

Antioxidant activity was evaluated using the DPPH assay with the stable free radical 2,2-diphenyl-1-picrylhydrazyl, following the method described by Bujor et al. (2016). Absorbance was measured at 515 nm using a UV-Vis spectrophotometer. Antioxidant capacity was quantified using a Trolox calibration curve, and the results were expressed as milligrams of Trolox equivalents per gram of fresh sample (mg TE/g).

RESULTS AND DISCUSSIONS

Plant material and seed characteristics

The analyzed fennel (*Foeniculum vulgare* Mill.) and caraway (*Carum carvi* L.) seeds exhibited morphological characteristics typical of umbelliferous fruits, including an elongated shape with longitudinal ridges. Fennel seeds were observed to be curved and cylindrical, with relatively large dimensions ranging from approximately 4 to 10 mm. Their color varied from green to yellowish-brown depending on the cultivar and maturity stage. The seed surface appeared smooth and was marked by five distinct longitudinal ridges. The fennel samples also presented a characteristic sweet and aromatic odor, which is commonly associated with the high trans-anethole content of fennel essential oil (Ahmed et al., 2019).

In comparison, caraway seeds were smaller, measuring approximately 3-6 mm in length. They displayed a dark brown to brownish-gray coloration and a pronounced spicy aroma, which is typically linked to the presence of carvone and limonene in their essential oil (Laribi et al., 2013). As in fennel, five longitudinal ridges were present on the seed surface; however, in caraway these ridges were more sharply defined. The dry matter content of the analyzed samples ranged from 81.50% to 90.33%. Caraway seeds exhibited the highest dry matter content ($90.33 \pm 2.36\%$), indicating a lower moisture level compared to the fennel samples (Figure 1). Among the fennel cultivars, *Foeniculum vulgare* Mill. cv. Hestia (F1) and the BRGV2 cultivar (F4) recorded similar dry matter values ($87.25 \pm 1.06\%$ and $87.00 \pm 0.71\%$, respectively). Consequently, the moisture content of fennel seeds ranged from 13.67% to 18.50%, which is higher than the values reported by Mehra et al. (2021), who observed moisture contents between 5.85% and 8.45%, depending on the variety or genotype.

The high dry matter content observed in *Carum carvi* L. (C1) seeds ($90.33 \pm 2.36\%$) is consistent with previously reported literature data. A comparative study on Apiaceae seeds reported a moisture content of 6.67% for caraway, corresponding to a dry matter level exceeding 90%, which is in agreement with the results obtained in the present study (El-Assri et al., 2023).

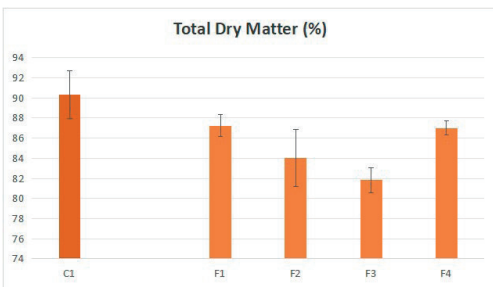


Figure 1. Variation of dry matter content in caraway and fennel seeds

Protein content

Crude protein content ranged from 10.95% to 19.68% (Figure 2), with the highest value recorded in *Foeniculum vulgare* Mill. cv. Hestia (F1), followed by *Carum carvi* L.(C1) ($17.29 \pm 0.90\%$).

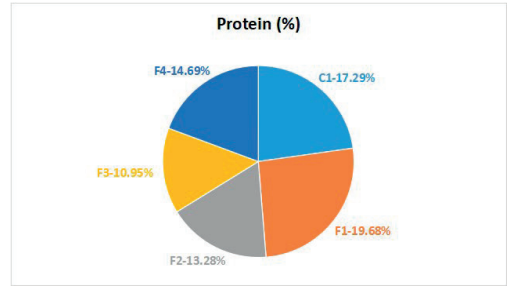


Figure 2. Crude protein content of raw seed

Previous investigations on the chemical composition of *Carum carvi* L. seeds have reported relatively high protein levels (15-20%), which supports the results obtained in the present study (Bouzaid et al., 2024; Filipović et al., 2014). The variability observed among the analyzed samples is consistent with literature data describing fennel seeds as rich in nutritional constituents whose levels may vary depending on cultivar and environmental conditions. For instance, a study evaluating the nutritional composition of seven fennel cultivars from Tunisia reported significant differences in protein content among cultivars, confirming that genetic background strongly influences seed chemical composition, including sugars and proteins (Abdesslem et al., 2022).

Bioactive Compounds

Variation in total phenolic content (TPC) among the analyzed samples is presented in Figure 3. The results revealed noticeable differences between species and cultivars. The highest TPC was recorded in *Foeniculum vulgare* Mill. cv. Florenta (F3) (8.01 ± 0.61 mg GAE/g), followed by the cv. BRGV1 (Napoleon) (F2) with 7.59 ± 0.41 mg GAE/g, indicating a greater accumulation of phenolic compounds in certain fennel cultivars. Intermediate TPC values were observed for *Foeniculum vulgare* Mill. cv. Hestia (F1) (6.70 ± 0.13 mg GAE/g) and the BRGV2 cultivar (F4) (6.47 ± 0.64 mg GAE/g), with relatively low variability recorded for the Hestia cultivar. A comparable TPC value was also recorded for *Carum carvi* L. (C1) (6.34 ± 0.64 mg GAE/g), suggesting a relatively lower accumulation of phenolic compounds under the studied conditions. Similar results were reported by Anwar et al. (2009), who demonstrated that *Foeniculum vulgare* seeds contain considerable amounts of phenolic compounds (627.21-

967.50 mg GAE/100 g), indicating that phenolic content in fennel seeds may vary depending on genetic and environmental factors. In contrast, Cherbal et al. (2023) reported a much higher total phenolic content for fennel seeds purchased from a grocery store (38.2 ± 2 mg GAE/g), which differs markedly from the values obtained in the present study. Such discrepancies may be attributed to differences in extraction methods, solvent type, seed origin, maturity stage, and storage conditions, all of which can significantly influence the phenolic composition of plant materials. In an earlier study, (Bouzaid et al., 2024 found a different level of phenolic compounds in *Carum carvi* L. seeds (13.66 ± 0.06 mg GAE/g), indicating that it fluctuates based on the variety and environmental factors.

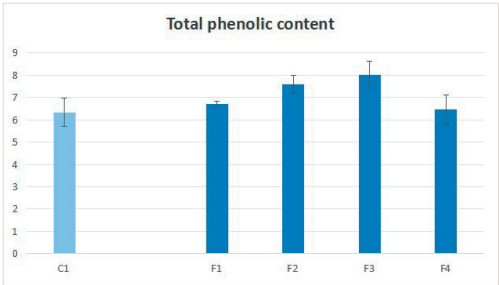


Figure 3. Comparative TPC of fennel cultivars and caraway

Total flavonoid content (TFC) varied among the analyzed samples, with species exerting a stronger influence than cultivar (Figure 4). *Carum carvi* L. (C1) exhibited the highest flavonoid content (7.04 ± 0.56 mg RE/g), indicating a greater accumulation of flavonoid compounds compared with the fennel samples under the studied conditions. In the fennel samples, TFC ranged from 3.99 ± 0.31 to 5.42 ± 0.35 mg RE/g, with the highest value recorded for *Foeniculum vulgare* Mill. cv. Florenta (F3).

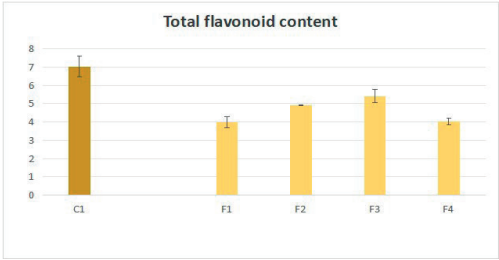


Figure 4. Comparative TFC of fennel and caraway cultivars

These results are consistent with previous reports highlighting caraway seeds as a rich source of flavonoids. For instance, Ulewicz-Magulska et al. (2023) reported a flavonoid content of 5.97 mg RE/g in caraway seeds, while Bamdad et al. (2006) emphasized the substantial phenolic content contributing to their antioxidant potential. In fennel, Ghanem et al. (2012) observed that cultivated varieties contain higher flavonoid levels (1.6%) than wild types (1.2%), confirming that flavonoid accumulation is influenced by genotype and environmental factors. Notably, the values obtained in the present study are substantially higher than those reported by Crescenzi et al. (2023) for hydroalcoholic extracts of various fennel plant parts, which ranged from 0.083 to 1.497 mg RE/g extract, with the highest levels found in leaves.

Antioxidant capacity

The observed antioxidant capacity generally mirrored the pattern of total flavonoid content. Caraway extracts exhibited the highest capacity (97.13 ± 3.14 mg TE/g), indicating strong radical scavenging potential.

As shown in Figure 5, among the fennel samples, *Foeniculum vulgare* Mill. cv. Florenta (F3) and the BRGV1 (Napoleon) cultivar (F2) displayed the highest antioxidant capacity, followed closely by the BRGV2 (F4) cultivar.

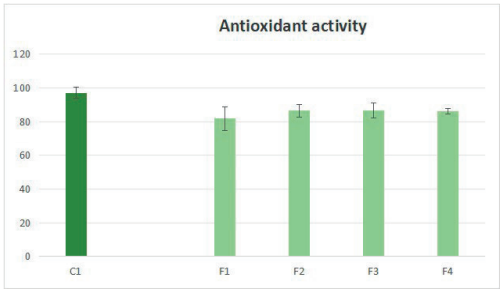


Figure 5. Antioxidant capacity of seed extracts

This correlation between phenolic compounds and antioxidant capacity is consistent with previous reports on fennel and caraway. In methanolic extracts of fennel, total phenolic and flavonoid contents have been shown to significantly contribute to DPPH radical scavenging, indicating that higher phenolic concentrations are generally associated with enhanced antioxidant capacity.

Shan et al. (2005) reported high DPPH radical scavenging activity in caraway, which correlated with elevated phenolic content, highlighting the contribution of these compounds to antioxidant potential.

In this study, antioxidant capacity closely followed the trend of total flavonoid content, suggesting that non-volatile phenolic constituents contribute substantially to radical scavenging capacity, as previously reported (Bamdad et al., 2006). Additionally, variations in phenolic and flavonoid contents, as well as differences in essential oil composition, may explain the observed differences in antioxidant capacity among the samples.

Essential oil composition

GC-MS analysis of fennel essential oils identified 24 constituents, revealing a consistent qualitative profile across all four cultivars, with only minor quantitative differences. Trans-anethole was the major constituent, ranging from 51.41% in the BRGV2 (F4) cultivar to 60.48% in cv. Hestia (F1) (Figure 6), confirming that the analyzed samples belong to the anethole chemotype, the most common for fennel essential oil (Rather et al., 2016). Fenchone was the second major component, varying between 20.73% and 22.42% (Figure 7), reflecting a typical sweet fennel profile. Other significant constituents included α -pinene (5.54-8.00%), D-limonene (4.05-6.80%), and estragole (4.90-5.65%), which contribute to the aromatic profile. Minor volatiles, such as γ -terpinene (1.08-1.67%), β -pinene (0.45-0.54%), camphene (0.86-1.06%), camphor (0.59-0.76%), and linalool derivatives, showed noticeable quantitative variation among cultivars.

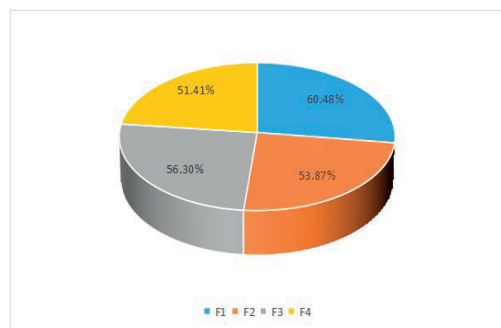


Figure 6. Trans-anethole composition in the fennel essential oil samples

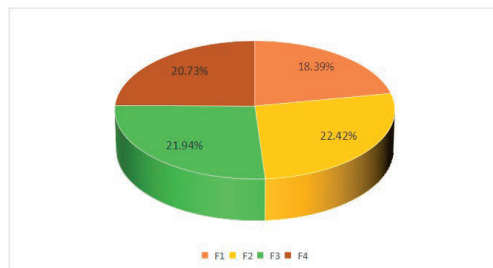


Figure 7. Fenchone in composition in the fennel essential oil samples

Although the qualitative composition was similar across fennel cultivars, the quantitative differences highlight the influence of genetic background and environmental factors. Previous studies have reported variability in fennel essential oil composition depending on geographical origin and genotype. For instance, Egyptian fennel oil was rich in estragole (51.04%), limonene (11.45%), L-fenchone (8.19%), and trans-anethole (3.62%) (Ahmed et al., 2019), whereas Turkish fennel oils contained higher trans-anethole (81.12%) but lower estragole (4.19-5.53%), limonene (2.96-4.60%), and fenchone (1.17-2.65%) (Telci et al., 2009). Similar major compounds were reported in fennel oils from Yugoslavia and Montenegro (Mimica-Dukić et al., 2003; Šunić et al., 2023), while bitter fennel from Turkey exhibited a different profile with estragole (40.49%), fenchone (16.9%), and limonene (17.66%) (Özcan and Chalchat, 2006).

In caraway, essential oil was dominated by carvone (54.25%) and D-limonene (43.37%), together accounting for 97.62% of the total volatile fraction. Minor constituents, including β -pinene, (Z)- β -ocimene, limonene oxide, trans-carveol, perillal, and caryophyllene, were present at <1%, contributing marginally to the overall profile. These results align with previous reports of carvone and limonene as the main components of caraway essential oil (Meshkatsadat et al., 2012; Simić et al., 2008). The composition differs from other *Carum* species, such as *C. nigrum*, where dillapiol and germacrene derivatives dominate (Singh et al., 2006).

A clear interspecific difference was observed: fennel oils are dominated by trans-anethole (phenylpropanoid chemotype), whereas caraway oils exhibit the carvone-limonene

chemotype. These differences are reflected in antioxidant capacity, with caraway showing higher radical scavenging potential, likely linked to its volatile profile. Major volatile constituents such as trans-anethole, fenchone, carvone, and limonene, along with minor compounds like α -pinene, β -pinene, and estragole, contribute to antioxidant effects either individually or synergistically with non-volatile phenolics (Rather et al., 2016; Figueiredo et al., 2008).

The identified bioactive compounds - phenolics, flavonoids, and key volatiles - have practical implications for dairy feed applications. Their antioxidant properties may reduce oxidative stress in animals, which is known to impair metabolism, immune function, and milk production (Celi, 2011). Volatile compounds such as trans-anethole and carvone may enhance appetite, feed intake, and digestive efficiency, supporting nutrient utilization and milk yield (Benchaar et al., 2008). Additionally, essential oil constituents can modulate rumen microbiota, suppressing harmful microbes while promoting beneficial populations, further improving energy availability and nutrient absorption (Patra & Yu, 2012).

CONCLUSIONS

The study demonstrated notable differences between *Foeniculum vulgare* and *Carum carvi* seeds in terms of phytochemical composition, nutritional profile, and antioxidant capacity, highlighting the combined influence of species and cultivar on bioactive compound accumulation. Caraway seeds showed the highest flavonoid content and dry matter, while fennel cultivars exhibited greater variation in total phenolics. Essential oil analysis revealed distinct chemotypes: fennel oils were rich in trans-anethole, whereas caraway oils were dominated by carvone and limonene, compounds known to support digestive health and appetite in livestock.

These findings indicate that both fennel and caraway seeds can serve as natural feed additives to improve antioxidant status, enhance nutrient utilization, and potentially increase milk production in dairy animals. Cultivar selection is crucial for maximizing beneficial traits, such as phenolic and flavonoid content, antioxidant

capacity, and essential oil composition, to optimize animal health and productivity.

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CONTRIBUTIONS TO THE KNOWLEDGE OF THE MORPHO-ANATOMY OF THE CARNIVOROUS PLANTS *Darlingtonia californica* AND *Cephalotus follicularis*

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Abstract

This paper focuses on the morpho-anatomical characteristics of the carnivorous plants Darlingtonia californica and Cephalotus follicularis, which, although not native species, provide valuable information for multiple scientific and practical fields. The analysis includes both the morphological examination of the vegetative organs and the anatomical structure revealed through transverse sections taken from different regions of these organs. Although they originate from completely different families and continents, these plant species have evolved traps that are functionally similar but structurally distinct—both internally and externally—as adaptations to extreme environments. Their study contributes to understanding the relationship between form and function, ecological and physiological adaptations, and the development of conservation strategies. It was also examined the current literature regarding their importance and methods of propagation, particularly since Darlingtonia californica is known for its richness in compounds with potential pharmaceutical and medical applications, while also having a restricted natural habitat. In this context, future research aims to develop conservation methods, cultivation techniques adapted to local climate conditions, and even micropropagation approaches to support the maintenance of existing populations.

Key words: carnivorous plants, *Cephalotus follicularis*, *Darlingtonia californica*, trap structures.

INTRODUCTION

Carnivorous plants live in nutrient-poor environments, which is why they have developed complex food capture mechanisms (leaves modified into traps, special digestive secretions, and even rapid movements to catch prey) that allow them to survive and develop in these habitats.

Darlingtonia californica (cobra lily) lives in damp swamps, cold springs in California and Oregon, USA, while *Cephalotus follicularis* (Australian pitcher plant) lives on the southwest coast of Australia, with sandy soils, humid environments.

Darlingtonia californica has been listed as an endangered plant by the Smithsonian Institution since 1975 (Family Lists of Candidate Endangered and Threatened Plant Species in the Continental United States); it is on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species/ the IUCN Red List or Red Data Book (Schnell et al., 2000).

It was discovered by botanist William D. Brackenridge in 1841 and described by John Torrey in 1853. *D. californica* is a carnivorous plant that is difficult to grow and propagate outside its natural range.

The roots develop in a cooler environment than the rest of the plant.

However, some authors have demonstrated that these plants are able to grow on nutrient media rich in mineral composition, at a temperature of $24 \pm 1^\circ\text{C}$ and do not require low positive temperatures for the root system (Erst et al., 2022).

Morphological and anatomical studies confirm the high level of ecological adaptation of this species to extreme conditions.

It is known that if the geochemical situation meets the requirements of plants, then the influence of the genetic factor is reflected mainly in the elementary chemical composition. At the same time, a genotypic program for the absorption of chemical elements is carried out and qualitative and quantitative regulations for the saturation of tissues with ions are maintained

(Ilyin & Yudanov, 1989; Ilyin & Syso, 2001 cited by Erst et al., 2022).

Brittnacher (2014) published methods for cultivating this plant, including its cumbersome propagation by seeds, and concluded that the best way to propagate is through stolons.

Lyons (2023) has successfully grown *D. californica* by maintaining an optimal root zone temperature (~10°C) using semi-hydroponic floodplain culture, incorporating a chiller that cools the water to the desired temperature as it circulates through the floodplain system. There is a chance that the method can be tested so that it can be successfully grown in more varied climates.

Although initially thought to not produce digestive enzymes, decomposition of captured insects relies on symbiotic bacteria and protozoa; later studies (Koller-Peroutka et al., 2019) have shown that *Darlingtonia* does possess proteases.

Meindl & Mesler (2011) demonstrated that *D. californica* is melitophilous, observing that the bee *Andrena nigrihirta* pollinated its flowers at five sites in northern California, while other regular floral visitors included thrips and several species of spiders.

Miclea (2022) conducted a review aimed at providing further support for the knowledge that secondary metabolites of plants from species belonging to the Sarraceniaceae family are useful in the fight against various diseases and emphasizes the need for further investigations into their biochemistry and the production of pharmaceutically and commercially valuable compounds from their tissues. The Sarraceniaceae family of the order Ericales is a family of carnivorous plants native to the American continent. Currently, the three genera that make up this family are *Darlingtonia*, *Heliophora* and *Sarracenia*.

They are rich in compounds with potential pharmaceutical and medical uses. They belong to several classes such as flavonoids, with flavonol glycosides being the most abundant, monoterpenes, triterpenes, sesquiterpenes, fatty acids, alkaloids and others. Some of them are well characterized in terms of chemical properties and biological activity and have wide commercial applications.

Plants belonging to the Sarraceniaceae family have a limited natural habitat (*Darlingtonia*

californica, *Heliophora* sp.) or are endangered (*S. alabamensis*, *S. jonesii*, *S. oreophila*) due to habitat destruction or excessive collection.

Cephalotus foliularis is the only genus and species in the Cephalotaceae family (Conran, 2004). It is one of the most desirable carnivorous plants in culture, prized by collectors, cultivated and sold worldwide.

Recently, the genome of *C. foliularis* has been sequenced, and analysis of carnivorous vs. non-carnivorous leaves has identified genetic changes associated with carnivory (Fukushima et al., 2017). This shows how the plant has reused stress-related genes for capture, digestion and absorption functions. The anatomy of the trap and the differentiation of the leaves (carnivorous vs. non-carnivorous) is a subject of interest.

This plant is listed as vulnerable by the IUCN (The International Union for Conservation of Nature), due to population decline (over-collection), highlighting the need for a better understanding of its ecology.

The endemic, insectivorous, monotypic family Cephalotaceae of Western Australia has been allied with Nepenthaceae, Sarraceniaceae, Saxifragaceae and Crassulaceae. Recent studies show a close relationship with the latter two families. An examination of the flavonoids in *C. foliularis* showed that the predominant flavonol profile more closely resembles the flavonoid profile of Saxifragaceae than that of Crassulaceae and supports morphological features that suggest it is most closely related to that family (Nicholls et al., 1985).

Pavlovic (2011) performed simultaneous comparative measurements of gas exchange and chlorophyll fluorescence in the two distinct leaf shapes. Stomatal density, an important component of gas exchange, and chlorophyll concentration were also determined. The trap had a lower net photosynthetic rate compared to the non-carnivorous leaf, while the respiration rate was not significantly different. This was in accordance with the lower stomatal density and chlorophyll concentration in the trap, the results obtained being consistent with the hypothesis that changing the leaf shape in carnivorous plants to make it a better trap generally makes it less efficient at photosynthesis.

Concerning the present work, it aims to pursue the morpho-anatomical description of the

carnivorous plants *Darlingtonia californica* and *Cephalotus follicularis* and highlight their importance for subsequently finding the best methods of multiplication.

MATERIALS AND METHODS

For the morphoanatomical study, fresh material belonging to the carnivorous plants *Darlingtonia californica* (Ericales: Sarraceniaceae) and *Cephalotus follicularis* (Oxalidales: Cephalotaceae) purchased online (https://heldros.com/en_US/basket) was used. They were morphologically described and anatomically analyzed by performing numerous cross-sections through the vegetative organ (Andrei, 2003), using the Moti Panthera S binocular microscope model available at the Faculty of Agriculture, Biology program within USAMV Bucharest. Also, for *Darlingtonia californica*, the stomatal index was determined to evaluate the plant's adaptation to the environment. The stomatal index represents the percentage value of the ratio between the number of stomata to the total number of epidermal cells, including stomata, per unit area (Ciulei et al., 1995).

RESULTS AND DISCUSSIONS

I. Morphoanatomy of the carnivorous plant *Darlingtonia californica*

The leaves of *Darlingtonia californica* (Figure 1) are tubular and contain a digestive fluid formed by water (absorbed from the soil and even resulting from the condensation created inside the urn), mucilage (produced by epidermal glands), products of digestion and symbiotic microorganisms that help with decomposition. This fluid prevents insects from getting out, facilitates the digestion of prey and possibly the absorption of nutrients.



Figure 1. *Darlingtonia californica*, general aspect

The upper part (Figure 2), dilated, has the shape of a cobra's head, with translucent areas similar to luminous windows (to confuse prey) and also in this area of the leaf there is a circular opening, preceded by a formation like a bifurcated appendix ("fishtail appendage") through which insects can enter.

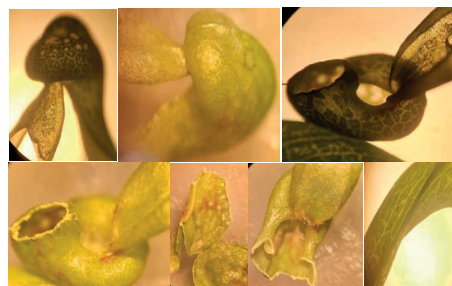


Figure 2. *D. californica*, upper part

The reddish areas observed in the head area are due to anthocyanin pigments in the cells (role in attracting insects), and the nectar glands, which secrete sweet nectar and attract insects, are present in the entrance area (in the external epidermis) but also on the bifurcated appendages.

Along the length of the tube there are downward-facing hairs that prevent insects from exiting the trap (Figure 3). At the bottom of the trap there are symbiotic microorganisms that ensure the decomposition of insects. The plant does not have digestive glands and does not produce digestive enzymes.

For a better understanding of the morphology of these leaves, we fragmented the traps into different areas: "tube base" – i.e. the small-diameter tube area, "under the head" – i.e. the large-diameter tube area, bifurcated appendage – the fin-shaped appendages in the head area and "head" – the dilated part of the tube.

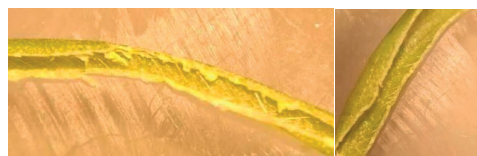


Figure 3. *D. californica*, downward-facing hairs

The cross-sections, both at the "base" and in the "under the head" areas and bifurcated appendix, show two epidermises (external protected by the cuticle and internal), modified mesophyll (with

photosynthetic parenchyma but also weakly pigmented cells), mechanical tissue (collenchyma and sclerenchyma) and conducting tissue (phloem and xylem). In the upper and middle area of the tube, elongated cells transformed into sharp bristles (Figure 4) similar to a saw with teeth facing downwards, for directing the insect (mechanical adaptation), are differentiated in the lower epidermis.

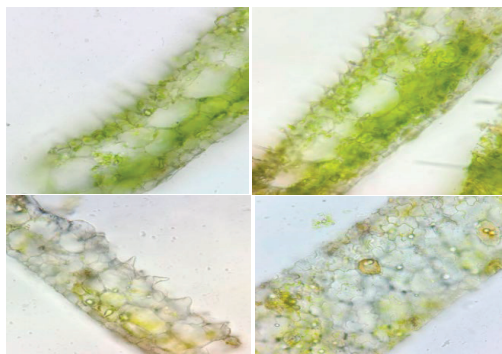


Figure 4. *D. californica*, cross sections: “under the cap” area (top) and “fishtail appendage” area (bottom)

At the base of the urn (Figure 5) the epidermal bristles are small, rounded with a thin external wall, which, possibly, reduce direct contact with cold water.

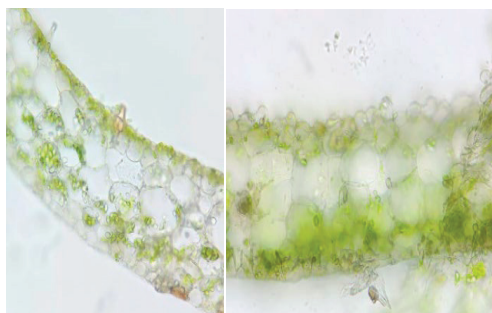


Figure 5. *D. californica*, cross sections: “tube base” area

The “head” area has a specialized internal structure, adapted to both photosynthesis and insect capture. The epidermal cells arranged like a puzzle are transformed, towards the inside of the trap, into short, unicellular, rigid mechanical hairs (Figure 6, arrow), oriented downwards, with a role in retaining the prey. The presence of stomata was observed especially on the external face (Figure 6).

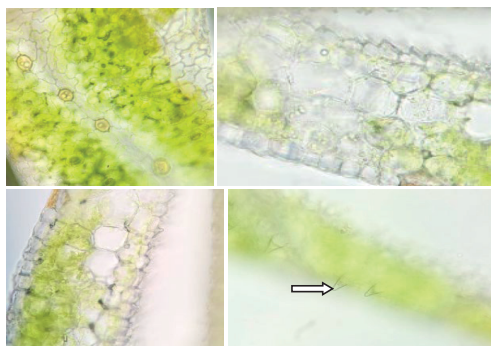


Figure 6. *D. californica*, cross sections: “head” area

The assimilatory tissue is rich in chloroplasts, but in the translucent areas the epidermis is thinner and the cells are poorly pigmented, thus creating the illusion of a window through which insects can exit. Nectar glands are present in the entrance area (in the external epidermis) but also on the bifurcated appendages.

The stomatal index was determined to be moderate (9.37%), indicating that the plant adopts a balanced physiological strategy compatible with its natural environment (cold, humid areas with nutrient-poor soils), a controlled gas exchange.

II. Morphoanatomy of the carnivorous plant *Cephalotus follicularis*

Cephalotus follicularis (Figure 7) is a small herbaceous plant. It has fibrous, thin roots in the soil, later short, thickened underground rhizomes (underground stems) appear, on which the two types of leaves are formed.



Figure 7. *Cephalotus follicularis*, general aspect

Unlike most carnivorous plants that develop a single type of leaf, *Cephalotus* has two very

different types of leaves: non-carnivorous leaves and pitcher-shaped trap leaves.

The non-carnivorous leaves (Figure 8), the first to appear, are simple, hairy, with an entire margin, bright green on the upper surface and dark purple on the dorsal side.



Figure 8. *C. follicularis* - non-carnivorous leaves

In cross-section they present (Figure 9) an internal structure typical of a dorsiventral leaf specialized exclusively for photosynthesis, as follows: upper epidermis formed by a layer of cells, covered by cuticle, without stomata; mesophyll with two rows of assimilatory/palisade tissue arranged towards the upper epidermis formed by elongated cells, rich in chloroplasts; lacunar tissue formed by cells with intercellular spaces; conducting fascicles surrounded by a fascicular sheath, with the wood arranged towards the upper epidermis and the free towards the lower epidermis; lower epidermis formed by a layer of cells and numerous stomata.

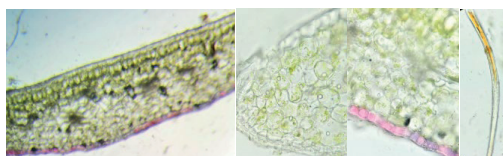


Figure 9. *C. follicularis*, cross-sections through non-carnivorous leaves

Numerous hairs are present on the leaf edge (Figure 10).

The trap leaves (Figure 10) are small, green with red-violet areas (more intense on the upper and inner side) and play a role in capturing and digesting insects.

The traps (small, 2-5 cm) are short, have on the outside three longitudinal, hairy ridges (Figure 11) that guide insects towards the mouth and a fourth, slightly highlighted one.

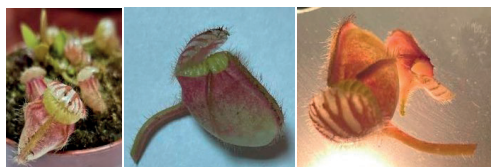


Figure 10. *C. follicularis*, pitfalls

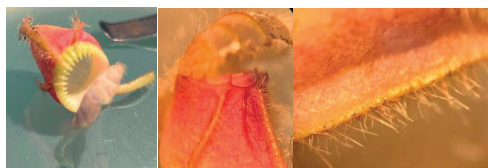


Figure 11. *C. follicularis*, hairy growths

They have a granular, rough, pigmented texture in order to attract insects.

There are three distinct macroscopic areas belonging to the urn of the carnivorous plant: the operculum or lid, the edge of the opening (peristome) and the urn with the waxy area and the digestive area.

Each of these areas has a specific function, with corresponding morpho-physiological characteristics.

The cap or operculum is attached a short distance above the peristome and covers the opening of the urn, preventing rain from filling the urn excessively, but still allowing light to enter. It is hairy (Figure 12, arrow), stiff, with white veins, in places with anthocyanin papillae. It has translucent areas to confuse insects.

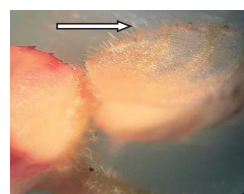


Figure 12. *Cephalotus follicularis*, lid (operculum)

The cap has polygonal epidermal cells, arranged like a puzzle, and downward-oriented bristles to guide prey to the opening of the trap (Figure 13).

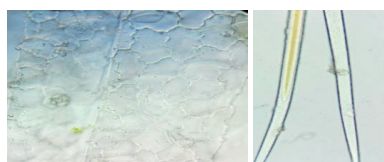


Figure 13. *C. follicularis*, cross-sections through the cap

In cross-section, the cap shows: upper epidermis with large cells, covered with a highly developed cuticle and few stomata, undifferentiated mesophyll clearly in assimilatory (reduced) and lacunar (large-celled) tissue, with few chloroplasts located in the subepidermal cells, lower epidermis formed by a layer of cells with a thin cuticle (Figure 14) and poorly developed and reduced number of conducting fascicles.

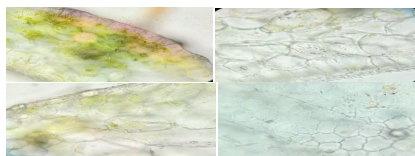


Figure 14. *C. follicularis*, cross section through the lid (operculum)

The peristome (upper edge) has the shape of a glossy, yellowish-white collar, slippery, lobed (large lobes in the shape of teeth arranged radially). The area below the teeth (Figure 15) with a spongy appearance is a nectar secretor that attracts insects, being formed by epidermal nectariferous glands with small, dense secretory cells, protected by a thin cuticle for easy release of nectar.

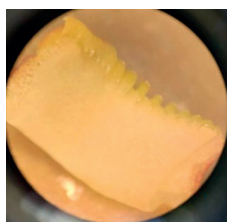


Figure 15. *C. follicularis*, peristome below the teeth

The lobes (Figure 16) curve over the edge of the urn (cause insects to slide inside) and end in a sharp tip facing downwards (prevent insects from exiting). The lateral and frontal lobes are well developed and those arranged posteriorly in the area where the lid covers the urn are small, short, crowded.



Figure 16. *C. follicularis*, the lobes

The urn has two areas: slippery and digestive. The upper part of the interior of the urn is smooth, slippery, formed by specialized cells that have a smooth, waxy cuticle to prevent insects from climbing. The mesophyll is reduced, without chloroplasts.

The lower part is glandular - the digestive zone (Figure 17), has a moist surface and epidermal cells with thin cell walls; here too there are golden-yellow digestive glands, which secrete enzymes and absorb products resulting from digestion.

Subepidermally, there is a homogeneous, compact tissue, formed by weakly pigmented cells with thin walls (Figure 18).

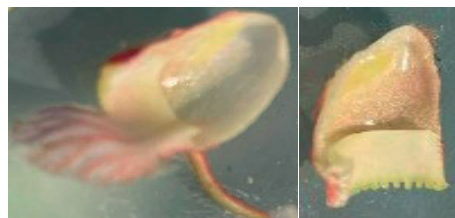


Figure 17. *C. follicularis*, internal surface of the trap

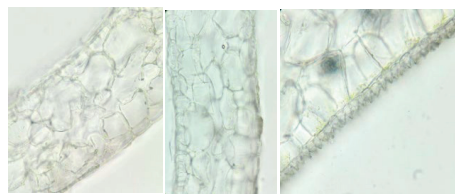


Figure 18. *C. follicularis*, cross section through the urn

CONCLUSIONS

The study of cross-sections made at the level of trap leaves highlighted morpho-anatomical peculiarities that reflect the high degree of specialization of carnivorous plants.

Darlingtonia californica has unique, tubular leaves (hollow tubes), with a widened upper part and a narrowed base, resembling a cobra's head. *Cephalotus follicularis*, unlike *Darlingtonia californica*, has two very different types of leaves: non-carnivorous leaves and carnivorous trap leaves.

The results obtained emphasize the importance of morpho-anatomical studies in understanding the adaptive mechanisms of carnivorous plants and provide useful data for future research on the relationship between anatomical structure,

digestive function and ecological strategies of these species.

The interest in carnivorous plants and substances with chemopreventive and therapeutic potential involves additional research on the chemical composition of the genus *Darlingtonia*, knowing the richness in compounds with potential pharmaceutical and medical uses.

Knowledge of these plants is of great importance, given that the manufacture of products intended for commercialization requires considerable quantities of plant material necessary for the pharmaceutical industry, and in vivo propagation methods or micropropagation can be a lifesaver in this regard.

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PRELIMINARY ASSESSMENT OF THE WATER QUALITY AND LENGTH-WEIGHT RELATIONSHIP IN FISHES OF IALOMIȚA RIVER: A NOTE FROM FIERBINȚI-TÂRG

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Abstract

In 2024 and 2025, several specimens of *Blicca bjoerkna* and *Carassius gibelio* were captured from the Ialomița River, near the locality of Fierbinți-Târg. The length-weight relationship indicated that the white bream and Prussian carp exhibited positive allometric growth. To complete the habitat assessment, two water samples, collected from the fish capture site (S1) and from another section of the same river (S2), were analyzed for physico-chemical parameters and compared with the limits established by national quality standards and recommended values for fish. Considering all measured quality parameters, water from S2 is of lower quality than that from S1. Microbiological analyses revealed the presence of Gram-positive cocci, bacilli Gram-positive, filamentous micromycetes and yeasts. Correlating the biometric data of the fish with the physico-chemical and microbiological parameters of the water could suggest that both *B. bjoerkna*, an autochthonous species, and *C. gibelio*, an invasive species, show positive allometric growth in a less polluted environment, characterized by reduced organic load.

Key words: fish species, Ialomița river, length weight-relationship, water analysis.

INTRODUCTION

The Ialomița River, a major tributary of the Danube in southern Romania, flows through an intensively cultivated plain where agricultural runoff and diffuse pollution strongly influence water quality. Long-term monitoring (2007-2018) of the basin documented variable physicochemical conditions, reflecting the influence of land use and anthropogenic pressures on water quality in the lower basin (Dunea et al., 2020). These conditions are typical for lowland rivers receiving agricultural and urban inputs and can favor eutrophic states that affect aquatic biota.

Fish assemblages in the Ialomița River basin are composed of a mixture of native and introduced species.

Surveys reported 32 fish species in the southern part of the basin, including tolerant taxa such as *Carassius gibelio* and other cyprinids dominant in disturbed habitats. Species such as Prussian carp are known to thrive in slow-flowing,

nutrient-enriched waters and often dominate where habitat degradation and water-quality stressors are present (Davideanu et al., 2010).

Recent investigations of *Carassius gibelio* from urban lakes in Bucharest have reported pollution-associated alterations in antioxidative defense, gut microbiota, and metabolome, supporting the species' high tolerance and adaptive capacity under contaminant exposure (Alistar et al., 2022; Voinea et al., 2023).

Regarded as an invasive species that impacts ecosystems, Prussian carp *Carassius gibelio* (Bloch, 1782) is the most caught species by fishermen in many lowland rivers in Romania (Drăgan et al., 2024), with a common length of 20 cm (Kottelat & Freyhof, 2007). In Ialomița River, *C. gibelio* was reported before (Năstase & Oțel, 2016; Dunea et al., 2020; Drăgan et al., 2024).

On the other hand, White bream *Blicca bjoerkna* (Linnaeus, 1758), a medium-sized native species, with a typical length of 15-20

cm (Mustea, 2023), has not been reported so far in Ialomița River.

Among other water bodies included in some SCIs Natura 2000 from Romania, Năstase & Oțel (2016) investigated Ialomița River in terms of fish fauna and stated that intense pollution has negative impact on its fish community.

However, little data is available, concerning the physico-chemical parameters and microbiological load of Ialomița River, in relation with fish fauna. Therefore, this study aims to explore the data regarding the ichthyofauna of Ialomița River and its environmental quality, considering the need of information upon this topic.

MATERIALS AND METHODS

Study site and fish measurements

The fish specimens (Figure 1a) were caught with a rod and a trap net in November 2024 and February 2025 from the Ialomița River (Figure 1b), near Fierbinți-Târg, at GPS coordinates 44°41'18.8"N, 26° 23'31.4"E (sampling point S1). The town of Fierbinți-Târg is placed in the south east of the Romania, in the historical province of Muntenia, in the western extremity of Ialomița County (<https://romaniadategeografice.net/>).

In 2024 fishing session, only 14 individuals of *Carassius gibelio* (Prussian carp) were captured, while in 2025 were collected: another 14 individuals of *C. gibelio*, *Blicca bjoerkna* (White bream) (N=48), *Gymnocephalus cernua* (Ruffe) (N=3), *Squalius cephalus* (Chub) (N=3), *Rutilus rutilus* (Roach) (N=2), and one individual of *Cyprinus carpio* (carp). Overall, 85 fish individuals belonging to six species were obtained. However, Prussian carp and White bream constitute the main ichthyological material for the biometric assessment, as these species were best represented numerically.

The scientific name and classification of fish species were given according to FishBase database (Kottelat & Freyhof, 2007; <https://www.fishbase.se/>). In order to assess the length-weight relationship (LWR), fish individuals were measured for total length (TL, in cm), standard length (SL, in cm) and

weighed (TW=total body weight, in grams) to the nearest 1.0 mm and 0.1 g, respectively.

The weight-length relationships were computed as $TW=aTL^b$ and $TW=aSL^b$ where intercept a represents the rate of change of weight with length of fish, while slope b gives information regarding the type of growth (Froese, 2006). Also, the relationship between the weight and the length of the fish was calculated through linear regression ($\text{Log } W = \text{Log } a + b \text{ Log } L$) (Stavrescu-Bedivan et al., 2023).

According to Karachle & Stergiou (2012), a positive allometric pattern of growth is indicated when $b > 3$ and a negative allometric or hypoallometric growth type when $b < 3$.

Water sampling and analyses

Water samples were collected in 2025 from the fish capture site (S1 from 44°41'18.8"N, 26° 23'31.4"E) and from another section of the Ialomița river (S2 from 44°41'04.7"N, 26° 20'57.9"E).

Water sampling was designed as a preliminary component of the study, aiming to characterize the habitat conditions associated with fish captures. During the 2025 campaign, two sectors of the river (S1 and S2) were analyzed, selected to allow an indicative comparison between the capture site and a sector with potentially different anthropogenic pressures.

The adopted approach provides an initial contextual assessment, while additional long-term investigations are necessary to support more robust conclusions.

In order to ensure accuracy in the water quality assessment, three samples were collected from each sampling point, whereby each one was analyzed in duplicate. Samples were obtained through manual grab sampling, a method widely used in surface water monitoring studies (Kalkhajeh et al., 2019). Water was collected at a depth of 0.5 m using polyethylene bottles that had been rinsed three times beforehand with the sampled water. All samples were properly labeled and transported to the laboratory in a cooler maintained at 4°C. Prior to analysis, the samples were kept in the laboratory until they reached room temperature.

In Table 1 are presented determined parameters, used methods and instrumentation.

Table 1. Determined parameters, analytical methods and instrumentation.

Parameter	Abbreviation	Unit	Analytical methods	Instrumentation
pH	pH	pH units	Potentiometry	inoLab pH 720, WTW, Germany
Electrical conductivity	EC	$\mu\text{S cm}^{-1}$	Conductometry	SensION7, Hach, USA
Total hardness	TH	mg CaO L^{-1}	Complexometric method	-
Chemical oxygen demand	COD	$\text{mg O}_2 \text{L}^{-1}$	Redox titration	-
Nitrite nitrogen	N-NO_2^-	mg N L^{-1}	Spectrophotometry	MetertechSP830 Plus spectrophotometer, Metertech Inc., Taiwan
Nitrate nitrogen	N-NO_3^-	mg N L^{-1}		
Ammonium nitrogen	N-NH_4^+	mg N L^{-1}		
Phosphate phosphorus	P-PO_4^{3-}	mg P L^{-1}		

Microbiological analysis

From each water sample, a specific volume (0.1 mL/100 μL) was plated, under aseptic conditions, on the surface of the agarized medium in the Petri dish (nutrient agar for the detection of bacteria and Sabouraud Dextrose Agar for the isolation of filamentous fungi and yeasts) by the plating method (Manole, 2025).

To assess the general morphological characteristics, the plates were incubated and the developed colonies were observed macroscopically and microscopically by making preparations, the identification being carried out at a presumptive level, without the use of biochemical or molecular tests

RESULTS AND DISCUSSIONS

I. Biometric analysis of *Blicca bjoerkna* and *Carassius gibelio*

The biometric data for White bream (*Blicca bjoerkna*) sampled in 2025 (N=48, unsexed) were registered as follows: TL (min. 7.5 - max. 15.5 cm, with an average of 11.78 cm); SL (min. 5.8 – max.12.5 cm, 9.01 cm average); TW (min. 3 – max. 40 g, 16.23 g average).

The weight-length relationships for *B. bjoerkna* were calculated as: $\text{Log (TW)}=3.3349 \text{ Log (TL)}-2.4044$ ($r^2=0.9488$, 95% confidence intervals for the intercept and the slope); $\text{TW}=0.0039\text{TL}^{3.3349}$; $\text{Log (TW)}=3.089 \text{ Log (SL)}-1.8075$ ($r^2=0.9687$, 95% confidence intervals for the intercept and the slope); $\text{TW}=0.0156\text{SL}^{3.089}$.

Regarding the biometry of Prussian carp (*Carassius gibelio*) sampled in 2024 (N=14,

unsexed), the following data were obtained: TL (min. 9 – max. 12.20 cm, average 10.65 cm); SL (min. 7.20 – max. 10 cm, average 8.63 cm); TW (min. 14.7 – max. 33.64 g, 23.80 g average); $\text{Log (TW)}=3.164 \text{ Log (TL)}-1.8831$ ($r^2=0.9299$, 95% confidence intervals for the intercept and the slope); $\text{TW}=0.0131\text{TL}^{3.164}$; $\text{Log (TW)}=2.6514 \text{ Log (SL)}-1.1135$ ($r^2=0.9088$, 95% confidence intervals for the intercept and the slope); $\text{TW} = 0.0776\text{SL}^{2.6514}$.

As for *C. gibelio* collected in 2025 (N=14, unsexed), the registered values are: TL (min. 8.80 – max. 14 cm, average 11.81 cm); SL (min. 7.20 – max. 10 cm, average 8.63 cm); TW (min. 10 – max. 48 g, 27.64 g average); $\text{Log (TW)}=3.2622 \text{ Log (TL)}-2.0798$ ($r^2=0.9541$, 95% confidence intervals for the intercept and the slope); $\text{TW}=0.0083\text{TL}^{3.2622}$; $\text{Log (TW)}=3.5161 \text{ Log (SL)}-2.0501$ ($r^2=0.9652$, 95% confidence intervals for the intercept and the slope); $\text{TW} = 0.0089\text{SL}^{3.5161}$.

In LWR (where TL was considered), it can be noticed that the values for slope b were within the expected range of 2.5-3.5 for all analyzed fish individuals. With values of b being higher than 3, the growth type for White bream and Prussian carp sampled in Ialomița River was estimated as positive allometric, which means that fishes grow faster in weight than in length (Karachle & Stergiou, 2012). However, the small samples call for caution in interpreting the results and it is estimated that future catches in various sampling points and seasons will be necessary to track the growth patterns of the fish in the studied ecosystem.

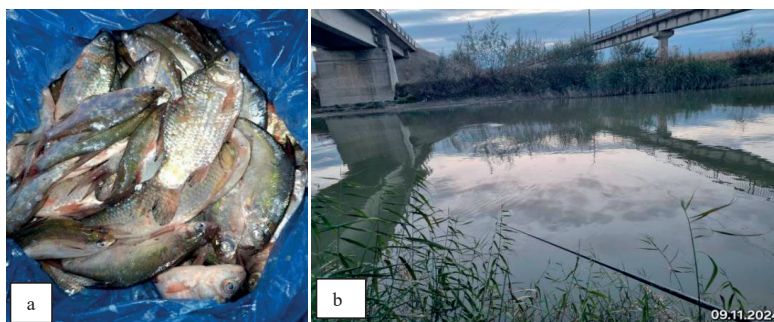


Figure 1. Cyprinid fishes (a) caught in Ialomița River, S1 (b), November 2024

II. Water analysis results and interpretation

Table 2 presents the measured ranges, mean values with standard deviations, and corresponding quality class limits for selected physicochemical parameters in samples S1 and

S2. The classification of each parameter is based on established water quality standards (Order 161/2006), allowing comparison of the samples' water quality status.

Table 2. Ranges, means, standard deviations and the assignment of water quality classes for the samples collected from Ialomița river near Fierbinți-Târg locality

Parameter	Unit	S1			S2		
		Min.-Max.	Mean $\pm$ SD	Limits/Quality classes *	Min.-Max.	Mean $\pm$ SD	Limits/Quality classes *
pH	pH unit	7.52-7.61	7.55 $\pm$ 0.052	6.50-8.50	6.29-6.38	6.33 $\pm$ 0.047	6.50-8.50
EC	$\mu\text{S cm}^{-1}$	908-912	909.67 $\pm$ 2.082	NA	532-535	533.67 $\pm$ 1.528	NA
TH	mg CaO L ⁻¹	17.05-17.50	17.28 $\pm$ 0.224	NA	10.32-10.77	10.47 $\pm$ 0.259	NA
COD	mg O ₂ L ⁻¹	3.92-4.08	4.02 $\pm$ 0.092	I	9.83-9.91	9.86 $\pm$ 0.046	II
N-NO ₂ ⁻	mg N L ⁻¹	0.036-0.038	0.038 $\pm$ 0.001	III	0.010-0.012	0.011 $\pm$ 0.001	II
N-NO ₃ ⁻	mg N L ⁻¹	2.125-2.461	2.322 $\pm$ 0.176	II	0.128-0.163	0.139 $\pm$ 0.020	I
N-NH ₄ ⁺	mg N L ⁻¹	0.140-0.163	0.147 $\pm$ 0.013	I	0.605-0.768	0.682 $\pm$ 0.082	II
P-PO ₄ ³⁻	mg P L ⁻¹	0.025-0.037	0.033 $\pm$ 0.007	I	0.098-0.160	0.127 $\pm$ 0.031	II

Notes: SD = standard deviation; * = according to (Order 161/2006); NA = not available.

a) pH is crucial for aquatic life: high pH increases ammonia toxicity, low pH enhances metal toxicity, and values below 6 promote toxic hydrogen sulfide formation (Sallenave, 2012). Deviations from the optimal pH range also impair fish growth, reproduction, and phytoplankton development (Stone & Thomforde, 2004; Alatorre-Jacome et al., 2011).

The pH values of the analyzed samples ranged between 7.52 and 7.61, with an average value of 7.55 $\pm$ 0.052 for sample S1, and between 6.29 and 6.38, with an average value of 6.33 $\pm$ 0.047 for sample S2 (Table 2). For sample S1, the pH values fall within the range imposed by legislation (Order no. 161/2006), whereas for sample S2 the pH is slightly lower. The optimal pH for fish generally ranges from 6.50 to 9.00, varying by species (Turlybek et al., 2025). Similar research on water quality in relation to the Prussian carp (*Carassius gibelio*) species

indicated pH values ranging between 7.25 and 8.11 (Sandu et al., 2023).

b) Electrical conductivity (EC) reflects dissolved ion concentration; although no official limits exist, surface waters with 60–2000 $\mu\text{S cm}^{-1}$ are generally suitable for most fish (Stone & Thomforde, 2004; Austin et al., 2016). EC values (Table 2) were within the recommended range: 908-912 $\mu\text{S cm}^{-1}$ for S1 (mean 909.67 $\pm$ 2.08) and 532-535 $\mu\text{S cm}^{-1}$ for S2 (mean 533.67 $\pm$ 1.53).

c) Total hardness (TH), reflecting Ca²⁺ and Mg²⁺ essential for aquatic life (Lall & Kaushik), ranged 17.05–17.50 mg CaO L⁻¹ in S1 (mean 17.28 $\pm$ 0.224) and 10.32–10.77 mg CaO L⁻¹ in S2 (mean 10.47 $\pm$ 0.259) (Table 2). Although no regulatory limits exist, 28-84 mg CaO L⁻¹ is considered optimal for aquaculture (Stone & Thomforde, 2004).

d) Chemical oxygen demand (COD) measures the oxygen needed to oxidize reducible

substances in water (Lv et al., 2024). Sample S1 falls in water quality class I, while S2 is class II, with a COD more than twice that of S1 (Table 2).

e) Nutrient levels

Phosphorus and nitrogen are essential for algae and aquatic plant growth but can cause eutrophication at high concentrations by lowering dissolved oxygen (Akinawo, 2023). Phosphate levels of $0.08\text{--}0.10\text{ mg L}^{-1}$ may favour algal blooms (Akhter et al., 2021). Nitrogen, mainly as ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-), primarily comes from agriculture (Vasile Scăteanu & Madjar, 2025) and its oxidation consumes oxygen, exerting additional stress on fish communities. While phosphorus and nitrates contribute to eutrophication, nitrites are directly toxic, converting hemoglobin to methemoglobin, reducing oxygen transport, and causing brown discoloration (Jensen, 2003; Kroupova et al., 2005). Tolerance varies: largemouth and cool-water fish are sensitive even to low levels

(Durborow et al., 1997), highlighting the need for continuous monitoring.

Nitrite levels classified S1 as water quality class III and S2 as class II, with S1's mean over three times higher than S2 (Table 2, Figure 2), though both remain below harmful thresholds for fish ($0\text{--}0.2\text{ mg L}^{-1}$ optimal; $>0.5\text{ mg L}^{-1}$ harmful; $>1.6\text{ mg L}^{-1}$ lethal) (Akhter et al., 2021). Nitrate ranged from $2.322 \pm 0.176\text{ mg N L}^{-1}$ (S1, class II) to $0.139 \pm 0.020\text{ mg N L}^{-1}$ (S2, class I), while ammonium remained low (S1, class I; S2, class II). Phosphate averaged $0.033 \pm 0.007\text{ mg P L}^{-1}$ in S1 (class I) and $0.127 \pm 0.031\text{ mg P L}^{-1}$ in S2 (class II). According to a study (Jeppesen et al., 2000) increased phosphorus concentrations in water lead to higher densities of cyprinid fish, accompanied by a decrease in their average body weight.

Overall, most parameters fell in classes I–II per Romanian legislation (Order no. 161/2006) and the EU Water Framework Directive (Directive 2000/60/EC), except S1, which was class III due to elevated nitrite (Table 2, Figure 2).

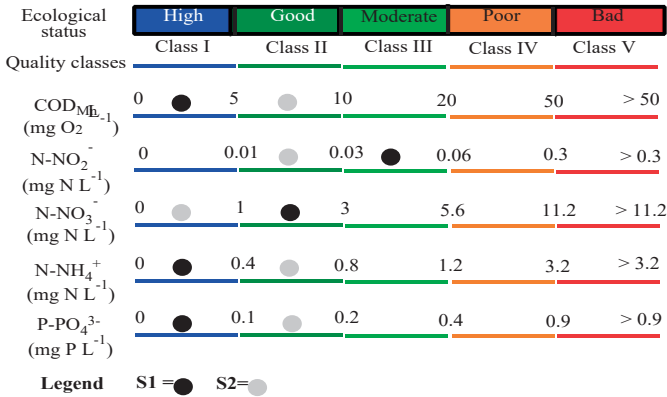


Figure 2. Ecological status and quality classes according to quality parameters' levels for the classification of surface water quality

III. Principal component analysis (PCA) and nutrient correlations

a) PCA analysis

The PCA biplot (Figure 3) reveals a clear separation between fish capture sites S1 and S2, primarily along PC1, which explains 98% of the total variance. This indicates that the major differences between the two sites are driven by the physicochemical characteristics of the water. Site S1 is positioned on the negative side of PC1 and is closely associated with pH, EC, TH,

and nitrogen compounds ($\text{NO}_2^- + \text{NO}_3^-$). This suggests that fish captured at S1 are linked to waters with higher mineral content and hardness, reflecting more stable or less nutrient-enriched conditions.

In contrast, site S2 is located on the positive side of PC1 and shows a strong association with chemical oxygen demand (COD), ammonium (N-NH_4^+), and phosphate (P-PO_4^3). This indicates that fish capture at S2 occurs in waters with elevated organic matter

and nutrient levels, which may be indicative of anthropogenic inputs or eutrophic conditions. PC2 accounts for only 1.5% of the variance, suggesting that differences along this axis are minimal and that PC1 is the dominant factor structuring the separation between sites.

Overall, the PCA graphic demonstrates that fish capture sites S1 and S2 are characterized by contrasting water quality conditions, which likely influence fish distribution and abundance at the two locations.

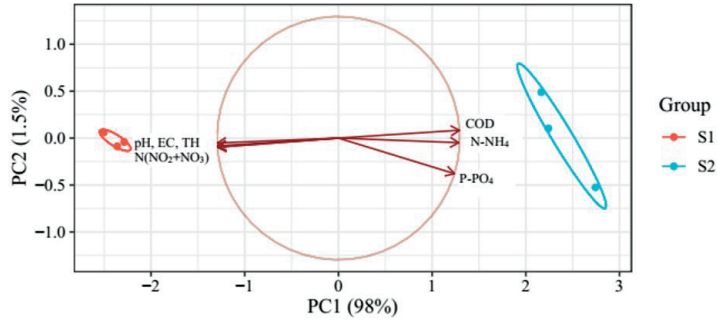


Figure 3. PCA biplot showing the separation of sampling sites based on water quality variables. PC1 explains 98% of the total variance and distinguishes S1 (associated with pH, EC, TH, and $\text{NO}_2^- + \text{NO}_3^-$) from S2 (associated with COD, N-NH_4^+ , and P-PO_4^{3-}), while PC2 explains 1.5% of the variance

b) Correlations between TH, EC and pH

A strong positive relationship between TH and both EC and pH has been evidenced (Figure 4). EC increases linearly with TH ($r = 0.9981$, $p < 0.001$), indicating that higher water hardness is associated with increased ionic content. Similarly, pH also rises with increasing TH ($r = 0.9963$, $p < 0.001$), reflecting a shift toward more alkaline conditions as hardness increases. These strong correlations suggest that mineral enrichment of the water significantly influences both conductivity and pH. The strong positive correlations between TH, EC, and pH suggest that mineral content plays a key role in shaping the water chemistry at the study sites.

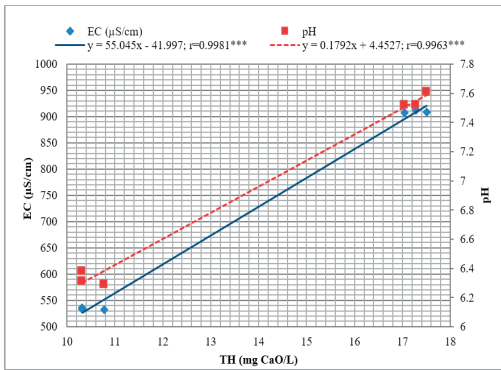


Figure 4. Correlation between TH and EC and pH

As TH increases, the rise in EC indicates a greater concentration of dissolved ions, reflecting enhanced mineralization. Concurrently, the increase in pH with TH suggests a shift toward more alkaline conditions, which could influence the availability of nutrients and the overall aquatic ecosystem health. These relationships highlight how variations in water hardness can impact multiple physicochemical parameters, potentially affecting fish habitats and water quality.

c) Correlations between COD and nutrients

COD was significantly correlated with nutrient concentrations in the water samples. Nitrate (N-NO_3^-) and nitrite (N-NO_2^-) showed strong negative correlations with COD ($r = -0.9959$ and -0.9979 , respectively; $p < 0.001$), indicating that their concentrations decreased as COD increased. In contrast, ammonium (N-NH_4^+) exhibited a strong positive correlation with COD ($r = 0.9850$, $p < 0.001$), while phosphate (P-PO_4^{3-}) showed a moderate positive correlation ($r = 0.9350$, $p < 0.01$). These results suggest that higher organic matter levels, as indicated by COD, are associated with increases in ammonium and phosphate, and decreases in nitrate and nitrite concentrations. The graph (Figure 5) shows how COD, which measures the amount of organic matter

in water (Zhang et al., 2026), relates to different forms of nitrogen and phosphate in the aquatic environment.

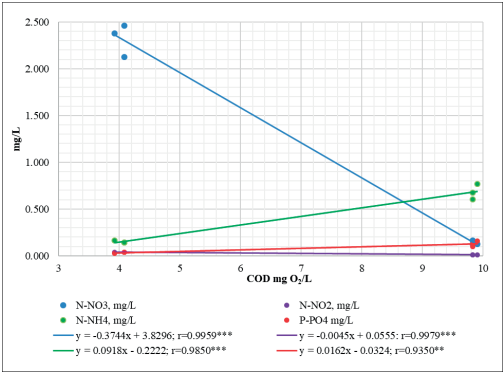


Figure 5. Relationships between chemical oxygen demand (COD) and nutrient concentrations: nitrate (N-NO_3^-), ammonium (N-NH_4^+), nitrite (N-NO_2^-), and phosphate (P-PO_4^{3-})

The observed relationships between COD and nutrient concentrations highlight important shifts in nitrogen and phosphorus dynamics within the aquatic system. The strong negative correlations of nitrate (N-NO_3^-) and nitrite (N-NO_2^-) with increasing COD suggest that these oxidized nitrogen forms are being depleted, possibly due to enhanced microbial consumption or conversion processes under higher organic load conditions. Meanwhile, the positive correlation between ammonium (N-NH_4^+) and COD indicates accumulation of reduced nitrogen species, which may result from increased organic matter decomposition or inhibited nitrification. The moderate increase in phosphate (P-PO_4^{3-}) with COD further suggests that organic enrichment may contribute to nutrient release or reduced uptake, potentially exacerbating eutrophication risks. Together, these patterns reflect how elevated organic pollution can alter nutrient cycling, impacting water quality and ecosystem health. As organic pollution rises (higher COD), oxygen is used up, shifting the nitrogen cycle from nitrate/nitrite forms toward ammonium, and phosphorus is released into the water. This change in nutrient forms can affect water quality and contribute to problems like eutrophication, where excessive nutrients cause algae blooms and harm aquatic life.

Although temporally limited, the water quality analysis allows a clear differentiation between sectors S1 and S2 and provides a plausible interpretative framework for the analysis of the growth patterns observed in fish.

The study thus contributes baseline data for a poorly documented sector of the Ialomița River and supports the design of subsequent ecological monitoring programs.

IV. Microbiological analysis

The microbiological analysis revealed the presence of microorganisms belonging to the main microbial groups: bacteria, yeasts, filamentous fungi (Figures 6 and 7).

In S1, bacterial colonies with morphology corresponding to Gram-positive cocci were observed, suggestive of genera such as *Micrococcus luteus* but also Gram-positive bacilli, possibly belonging to the genus *Bacillus*. Filamentous micromycetes were also identified: *Aspergillus* sp., *Rhizopus* sp., *Trichoderma* spp. as well as yeasts with morphology similar to *Saccharomyces* sp.

In S2, in addition to the previously mentioned bacterial types in S1 (*Micrococcus luteus* and the *Bacillus* spp.), bacterial colonies formed by Gram-positive cocci arranged in short chains were identified, possibly suggesting the presence of enterococci (e.g. *Enterococcus* spp.). Also, in S2, exclusively yeasts were highlighted, without the development of filamentous fungi.



Figure 6. Macroscopic appearance of the colonies developed on nutrient medium (S1 and S2)

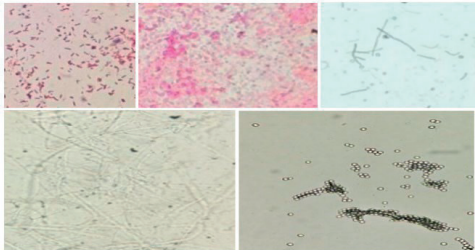


Figure 7. Macroscopic appearance after staining - bacteria and fungal structure

CONCLUSIONS

The microbiological results highlighted in this study are consistent with the physico-chemical ones, indicating a reduced degree of pollution in S1, respectively a higher organic load in S2. This is relevant, as the fish samples come from S1, a section of the Ialomița River where a positive allometric growth pattern was recorded for both species, considering the LWR calculation based on TL.

The analysed water samples exhibited variability in their chemical and physical characteristics, allowing their classification into different water quality classes in accordance with established regulatory standards. Overall, most parameters including pH, EC, TH, and nutrient concentrations fell within ranges considered suitable for freshwater ecosystems and aquaculture. However, nitrite levels in sample S1 were notably elevated, resulting in a lower water quality classification (class III) and highlighting the need for targeted monitoring and management measures. Physicochemical profiling and PCA analysis clearly distinguished S1 from S2, with robust correlations linking TH-EC-pH positively and COD inversely to oxidized nitrogen forms. These findings emphasize the importance of continuous water quality monitoring to protect aquatic life and to maintain the ecological status of water bodies in accordance with national and EU legislation. Furthermore, the results emphasize that maintaining water quality within safe ecological limits is essential for the sustainability of aquatic ecosystems and the protection of fish populations.

As for microbiological analysis, different distribution of microorganisms is observed between samples, indicating variations in microbial load, probably due to local environmental conditions (variations in organic matter input and local ecological conditions), an aspect that requires additional observations for confirmation and correlation with the physico-chemical parameters of the water.

The chemical and microbiological data from the samples indicate an input of biodegradable organic matter (e.g. domestic wastewater, manure, food waste).

S1 was predominantly in class I and presented typical microbiota (*Micrococcus*, *Bacillus*, *Aspergillus*), which suggests an area with low anthropogenic impact or well purified at the time of sampling. Therefore, S1 most likely corresponds to a section with better flow and self-purification conditions or located further from urban sources of household contamination.

The limitations of the present study are explained by the fact that catches from a single sampling point do not allow firm conclusions about the growth patterns of fish in relation to the quality of their habitat, but rather indicate potential correlations. For the herein report, LWR is interpreted only as preliminary observations. If in the future approach, White bream and Prussian carp were also sampled from S2, where the water has a higher organic load, it is very likely that *B. bjoerkna* would exhibit isometric or even hypoallometric growth, while *C. gibelio*, a species resistant to chemical and microbiological stress, would maintain its previously observed dynamics, and could benefit from the increase in organic matter, which gives it a competitive advantage in such habitats.

Overall, the results obtained can contribute to the development of local ecological monitoring and fisheries management strategies.

Future investigations should expand both the spatial and temporal coverage of monitoring by including seasonal sampling, assessments under variable hydrological conditions, and fish collections from both analyzed river sectors. Such an approach would enable a more rigorous evaluation of the relationships between organic load, nutrient dynamics, and fish growth parameters, thereby strengthening the ecological interpretation of habitat-biometry interactions.

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THE INFLUENCE OF HORMONAL COMBINATIONS WITH AUXINS ON POTATO VITRO PLANTS DEVELOPMENT

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Abstract

*In 2024, at NIRDPSB Brasov, Romania in Laboratory of Plant Tissue Culture was undertaken a study with whose main objective was to follow the evolution of the development of potato microplants under the influence of various concentrations of auxins. Bifactorial experiment, of the type: 4*12 with 48 variants, divided into 3 repetitions included: the experimental factor a, variety with four gradations: a1 - Azaria, a2 - Braşovia, a3 - Cosiana, a4 - Cezarina (considered control variety) and the factor b the culture medium with different amounts of auxin, with 12 gradations. Braşovia variety stood out with the highest average value of plant height (10.86 cm), followed by Cosiana variety (10.39 cm); for the other analysed parameters (number of leaves, root length and number of internodes), Cosiana variety records the highest values (leaves: 8.36; root length: 5.14 cm; number of internodes: 5.53). Significant differences existed among hormonal treatment, cultivars for all determinations. The differences in data across interaction between hormonal treatment and cultivar was not significant.*

Key words: auxins, growth regulators, microplants, potato, variety.

INTRODUCTION

Meristem culture was possibly the first biotechnological approach used to eliminate viruses from systemically infected potato clones. Over the years, this technique has been successfully combined with micropropagation to produce disease free potato seed. Plant tissue culture is a specialized technology used for plant propagation. It operates on the principle of growing disease-free plant tissues under sterile conditions in artificial plant growth medium (Shoyeb et al., 2017).

Tissue culture has been applied to improve production of potato by germplasm conservation, pathogen free potato plants and micropropagation (Khalafalla, 2001).

Previous studies show that micro propagation of seed potatoes depends on the biological value of cultivars, explant type (leaf, node, shoot tip, etc.), type of culture medium, season, temperature, photoperiod, and a balanced combination of plant growth regulators (PGRs) in the culture media (Akhtar et al., 2006; Dhital et al., 2010 quoted by Makau et al., 2022)

Addition of growth regulators in culture media has been reported to improve the number, growth and development of shoots (Ahmed et

al., 1993; Rabbani et al., 2001), though they are genotype dependant.

To establish tissue culture protocols for a plant species, exogenous growth regulators are used to evoke certain responses. Indole-3-acetic acid (IAA), a-naphthalene acetic acid (NAA), indole-3-butyric acid (IBA) are commonly used auxins to promote root initiation (Salem et al., 2022).

MATERIALS AND METHODS

In 2024, at National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania through Research Laboratory of Vegetal Tissue Culture was undertaken a study with whose main objective was to follow the evolution of potato microplants development under the influence of various concentrations of auxins. Minicuttings obtained *in vitro* by uninodal multiplication were inoculated immediately after cutting on Murashige-Skoog medium (1962) to which combinations of hormonal treatments were added. Bifactorial experiment, of the type: 4*12 with 48 variants, divided into 3 repetitions included: the experimental factor a, variety with four gradations: a1 - Azaria, a2 - Braşovia, a3 - Cosiana, a4 - Cezarina (considered control

variety) and the factor *b* culture medium with different amounts of auxin, with 12 graduations (according to the treatment scheme from Table 1). All 4 potato varieties are Romanian genotypes created at NIRDPSB Brasov. The

purpose of this study was to establish for each genotype which hormonal treatment has a beneficial effect for the four analysed parameters: leaves number, plantlets height, root length, number of internodes.

Table 1. Treatment scheme: combination of naphthyl acetic acid (in 3 concentrations) and indole-3-butyric acid (in 4 concentrations)

Auxine	Concentration of auxine introduced into the culture medium (mg/l)											
Naphthyl acetic acid (NAA)	0				0.25				0.5			
Indole-3-butyric acid (IBA)	0 b ₁ (Ct)	0.25 b ₂	0.5 b ₃	1 b ₄	0 b ₁	0.25 b ₂	0.5 b ₃	1 b ₄	0 b ₁	0.25 b ₂	0.5 b ₃	1 b ₄

RESULTS AND DISCUSSIONS

Table 2 shows the calculated F values for the differences in leaves number, plantlets height, root length and number of internodes between hormonal treatment, cultivars and their combination. Significant differences existed among hormonal treatment, cultivars for all determinations. The differences in data across interaction between hormonal treatment and cultivar was not significant for internodes number.

Under the influence of treatment with different concentrations of auxins, Braşovia variety (Table 3) stands out with the highest average value of plant height (10.86 cm), followed by the Cosiana variety (10.39 cm). For this parameter there were no significant differences for the varieties Braşovia, Cosiana and Cezarina, instead they differed significantly from variety Azaria.

For the other analysed parameters (number of leaves, root length and number of internodes), Cosiana variety records the highest values (leaves: 8.36; root length: 5.14 cm; number of

internodes: 5.53), followed by variety Braşovia (leaves 7.50; root length: 4.61 cm; number of internodes: 5.11 cm). Varieties Cosiana and Braşovia formed a well-developed root system, the values of root differing very significantly, positively from the control variety.

Hormonal combinations had different effects on the studied elements (Table 4). Thus, the treatment composed of 0.5 mg/l NAA - 0 mg/l IBA was beneficial in obtaining a high number of leaves (9.00), followed by the combination 0.25 mg/l NAA - 0 mg/l IBA, which resulted in 8.42 leaves. The treatment 0.25 mg/l NAA - 0.5 mg/l IBA was favourable to increase in height of plantlets (11.46 cm), followed by the combination of the hormonal regulators NAA: IBA 0.5: 0.0 mg/l, which determined the height evolution of plantlets to 10.92 cm. Higher values of root length were recorded for NAA - IBA treatments of: 0.25 - 0.5 mg/l; 0.25 - 1 mg/l and 0.5 - 0.0 mg/l (5.29, 4.83 and 4.79 cm). The combination 0.5 - 0.0 mg/l NAA-IBA was conducive to the achievement of a high number of internodes (5.92 internodes), followed by the combination 0.5 - 0.5 mg/l (5.75 internodes).

Table 2. Analysis of variance in F values for determined parameters

Source of variation	df	Leaves number	Plantlets height	Root length	Number of internodes
Variety (a)	3	5.465 * (4.76; 9.78)	36.778 **	82.438 **	7.041 *
Hormonal treatment (b)	11	3.274 ** (1.95; 2.55)	7.739 **	4.946 **	2.796 **
Variety (a) * Hormonal treatment (b)	33	2.244 ** (1.60; 1.84)	2.319 **	1.778*	1.56 ns

df, degrees of freedom.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
ns Not significant at the 0.05 probability level

Table 3. The variety influence on studied parameters under the effect of two auxins in culture medium

Variety	Leaves number	Diff. / Sign.	Plantlets height (cm)	Diff. (cm) / Sign.	Root length (cm)	Diff. (cm) / Sign.	Number of internodes	Diff. / Sign.
Azaria (a ₁)	7.22 A	0.11 ns	6.14 B	-3.58 ^{ooo}	2.86 C	0.09 ns	4.22 B	-0.75
Braşovia (a ₂)	7.50 A	0.39 ns	10.86 A	1.14 ns	4.61 B	1.85 ***	5.11 A	0.14
Cosiana (a ₃)	8.36 A	1.25 *	10.39 A	0.67 ns	5.14 A	2.38 ***	5.53 A	0.56
Cezarina (Ct) a ₄	7.11 A	-	9.72 A	-	2.76 C	-	4.97 A	-

LSD (p 5%) = 0.84; (p 1%) = 1.27; (p 0.1%) = 2.04.
 LSD (p 5%) = 1.23; (p 1%) = 1.86; (p 0.1%) = 2.99.
 LSD (p 5%) = 0.46; (p 1%) = 0.70; (p 0.1%) = 1.13.
 LSD (p 5%) = 0.71; (p 1%) = 1.08; (p 0.1%) = 1.73.

Table 4. The influence of hormonal treatment applied on the studied parameters

Hormonal treatment applied (mg/l)		Leaves number	Diff. / Sign.	Plantlets height (cm)	Diff. (cm) / Sign.	Root length (cm)	Diff. (cm) / Sign.	Number of internodes	Diff. / Sign.
NAA	IBA								
10	0 (Ct) b ₁	6.75 CD	-	6.33 G	-	2.53 F	-	4.08 CD	-
	0.25 b ₂	6.25 D	-0.50 ns	7.75 FG	1.42 ns	2.76 EF	0.23 ns	4.00 D	-0.08 ns
	0.5 b ₃	7.92 ABC	1.17 ns	9.00 CDEF	2.67 ***	3.83 BCDE	1.31 *	5.08 ABCD	1.00 *
	1 b ₄	7.17 BCD	0.42 ns	9.13 CDEF	2.79 ***	3.21 DEF	0.68 ns	5.00 ABCD	0.92 ns
00.25	0 b ₅	8.42 AB	1.67 **	9.71 BCDE	3.38 ***	4.08 BCD	1.56 **	5.00 ABCD	0.92 ns
	0.25 b ₆	7.42 BCD	0.67 ns	8.71 DEF	2.38 **	3.29 DEF	0.77 ns	4.58 CD	0.50 ns
	0.5 b ₇	8.00 ABC	1.25 *	11.46 A	5.13 ***	5.29 A	2.77 ***	5.17 ABC	1.08 *
	1 b ₈	7.08 BCD	0.33 ns	10.54 ABC	4.21 ***	4.83 AB	2.31 ***	5.17 ABC	1.08 *
00.5	0 b ₉	9.00 A	2.25 ***	10.92 AB	4.58 ***	4.79 ABC	2.27 ***	5.92 A	1.83 ***
	0.25 b ₁₀	6.92 CD	0.17 ns	8.08 EF	1.75 *	3.59 CDEF	1.07 ns	4.67 BCD	0.58 ns
	0.5 b ₁₁	8.00 ABC	1.25 *	10.33 ABCD	4.00 ***	4.17 ABCD	1.64 **	5.75 AB	1.67 ***
	1 b ₁₂	7.67 ABC	0.92 ns	9.38 BCDEF	3.04 ***	3.75 BCDE	1.23 *	5.08 ABCD	1.00 *

LSD (p 5%) = 1.20; (p 1%) = 1.58; (p 0.1%) = 2.05.
 LSD (p 5%) = 1.47; (p 1%) = 1.94; (p 0.1%) = 2.51.
 LSD (p 5%) = 1.07; (p 1%) = 1.41; (p 0.1%) = 1.83.
 LSD (p 5%) = 0.96; (p 1%) = 1.27; (p 0.1%) = 1.64.

Means of each parameter designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

When examining the combined influence of variety and hormonal treatment, it can be seen that the difference of 6 leaves (Table 5) for the 0.5 mg NAA:0 mg IBA combination, compared to the control, is very significant and positive for Azaria variety. For Braşovia variety, there were no significant differences for the hormonal combinations compared to the hormonal variant considered as a control, regarding leaves number. For varieties Cosiana and Cezarina, combination 0.25 mg NAA:0 mg IBA and respectively 0.5 mg NAA:0.5 mg IBA led to obtaining positive, distinctly significant and significant differences in leaves number, compared to the control (4 and 2.67 leaves). For Azaria, Braşovia and Cezarina varieties, higher values (10.50, 14.17 and 14 cm) of microplants height (Table 6), with very significant positive differences compared to the control (7.83, 6.17 and 6.83 cm), were obtained for the following hormonal variants: 0.25 mg NAA:1 mg IBA, 0.25 mg NAA:0.5 mg IBA, 0.5

mg NAA:0 mg IBA. For Cosiana variety, 0.25 mg NAA:0 mg IBA combination showed a beneficial effect on microplants height (11.67 cm), with a distinctly significant positive difference (4.17 cm).

The growth regulators 0.25 mg NAA:0.5 mg IBA variant showed a strong beneficial effect on the root system (Table 7) for Cosiana and Braşovia varieties (7.17 and 6.83 cm) with distinctly significant and very significant differences compared to control (3 and 4.33 cm). For Azaria variety, the most beneficial hormonal treatment was 0.25 mg NAA: 1 mg IBA, determining the highest microplant root value (5.33 cm), with a highly significant positive difference compared to the control treatment (5.23 cm). For the Cezarina variety there were no significant differences, for treatments applied.

Regarding the internodes number (Table 8), the most pronounced effect for Azaria variety, in obtaining high values (5.67) showed the

combinations: 0 mg NAA-0.5 mg IBA and 0.25 mg NAA:1 mg IBA. For the Cezarina variety, the highest internodes number (7.33) was recorded for 0.5 mg NAA - 0.5 mg IBA.

Braşovia and Cosiana varieties did not show significant differences in the hormonal variants compared to the control variant.

Table 5. Combined influence of cultivar and hormone treatment on leaf number

Variety/Hormonal treatment applied (mg/l)		Azaria (a ₁)		Braşovia (a ₂)		Cosiana (a ₃)		Cezarina (a ₄) (Ct)		a ₁ -a ₄	a ₂ -a ₄	a ₃ -a ₄
NAA	IBA	Leaves number	Diff. / Sign.	Leaves number	Diff. / Sign.	Leaves number	Diff. / Sign.	Leaves number	Diff. / Sign.			
0	0 (Ct)	4.33	-	7.33	-	8.33	0.00	7.00	-	-2.67 o	0.33 ns	1.33 ns
	0.25	4.67	0.33 ns	6.00	-1.33 ns	7.67	-0.67 ns	6.67	0.33 ns	-2.00 ns	-0.67 ns	1.00 ns
	0.5	9.00	4.67 ***	8.00	0.67 ns	7.67	-0.67 ns	7.00	0.00 ns	2.00 ns	1.00 ns	0.67 ns
	1	7.00	2.67 *	8.00	0.67 ns	7.67	-0.67 ns	6.00	-1.00 ns	1.00 ns	2.00 ns	1.67 ns
0.25	0	6.67	2.33 ns	7.33	0.00 ns	12.33	4.00 **	7.33	0.33 ns	-0.67 ns	0.00 ns	5.00 ***
	0.25	6.67	2.33 ns	8.00	0.67 ns	8.33	0.00 ns	6.67	-0.33 ns	0.00 ns	1.33 ns	1.67 ns
	0.5	8.67	4.33 ***	8.67	1.33 ns	8.67	0.33 ns	6.00	-1.00 ns	2.67 *	2.67 *	2.67 *
	1	7.00	2.67 *	6.00	-1.33 ns	8.33	0.00 ns	7.00	0.00 ns	0.00 ns	-1.00 ns	1.33 ns
0.5	0	10.33	6.00 ***	7.33	0.00	9.00	0.67 ns	9.33	2.33 ns	1.00 ns	-2.00 ns	-0.33 ns
	0.25	6.33	2.00 ns	7.67	0.33 ns	7.00	-1.33 ns	6.67	-0.33 ns	-0.33 ns	1.00 ns	0.33 ns
	0.5	6.67	2.33 ns	7.67	0.33 ns	8.00	-0.33 ns	9.67	2.67 *	-3.00 o	-2.00 ns	-1.67 ns
	1	9.33	5.00 ***	8.00	0.67 ns	7.33	-1.00 ns	6.00	-1.00 ns	3.33 **	2.00 ns	1.33 ns

LSD (p 5%) = 2.39;
(p 1%) = 3.17;
(p 0.1%) = 4.09.

LSD (p 5%) = 2.44;
(p 1%) = 3.27;
(p 0.1%) = 4.34.

Table 6. Combined influence of cultivar and hormone treatment on plantlets height (cm)

Variety/Hormonal treatment applied (mg/l)		Azaria (a ₁)		Braşovia(a ₂)		Cosiana (a ₃)		Cezarina (a ₄) (Ct)		a ₁ -a ₄	a ₂ -a ₄	a ₃ -a ₄
NAA	IBA	Plantlets height (cm)	Diff. (cm) / Sign.	Plantlets height (cm)	Diff. (cm) / Sign.	Plantlets height (cm)	Diff. (cm) / Sign.	Plantlets height (cm)	Diff. (cm) / Sign.			
0	0 (Ct)	2.67	-	8.00	0.00	7.50	-	7.17	-	-4.50 ^{oo}	0.83 ns	0.33 ns
	0.25	2.33	-0.33 ns	9.50	1.50 ns	11.33	3.83 *	7.83	0.67 ns	-5.50 ^{oo}	1.67 ns	3.50 *
	0.5	6.00	3.33 *	11.33	3.33 *	9.67	2.17 ns	9.00	1.83 ns	-3.00 ns	2.33 ns	0.67 ns
	1	5.33	2.67 ns	11.17	3.17 *	10.67	3.17 *	9.33	2.17 ns	-4.00 ^o	1.83 ns	1.33 ns
0.25	0	4.83	2.17 ns	11.83	3.83 *	11.67	4.17 **	10.50	3.33 *	-5.67 ^{ooo}	1.33 ns	1.17 ns
	0.25	4.00	1.33 ns	11.50	3.50 *	9.33	1.83 ns	10.00	2.83 ns	-6.00 ^{ooo}	1.50 ns	-0.67 ns
	0.5	9.50	6.83 ***	14.17	6.17 ***	11.00	3.50	11.17	4.00**	-1.67 ns	3.00 ns	-0.17 ns
	1	10.50	7.83 ***	12.17	4.17 **	10.83	3.33 *	8.67	1.50 ns	3.50 *	2.17 ns	
0.5	0	7.83	5.17 ***	11.33	3.33 *	10.50	3.00*	14.00	6.83 ***	-6.17 ^{ooo}	-2.67 ns	-3.50 ^o
	0.25	5.00	2.33 ns	8.33	0.33 ns	10.67	3.17 *	8.33	1.17 ns	-3.33 ^o	0.00 ns	2.33 ns
	0.5	6.50	3.83 *	11.00	3.00 *	10.50	3.00 *	13.33	6.17 ***	-6.83 ^{ooo}	-2.33 ns	-2.873 ns
	1	9.17	6.50 ***	10.00	2.00 ns	11.00	3.50 *	7.33	0.17	1.83 ns	2.67 ns	3.67 *

LSD (p 5%) = 2.94;
LSD (p 1%) = 3.89;
LSD (p 0.1%) = 5.02.

LSD (p 5%) = 3.06;
LSD (p 1%) = 4.13,
LSD (p 0.1%) = 5.53.

Table 7. Combined influence of cultivar and hormone treatment on root length (cm)

Variety/Hormonal treatment applied (mg/l)		Azaria (a ₁)		Braşovia(a ₂)		Cosiana (a ₃)		Cezarina (a ₄) (Ct)		a ₁ -a ₄	a ₂ -a ₄	a ₃ -a ₄
		Root length (cm)	Diff. (cm) / Sign.	Root length (cm)	Diff. (cm) / Sign.	Root length (cm)	Diff. (cm) / Sign.	Root length (cm)	Diff. (cm) / Sign.			
NAA	IBA											
0	0 (Ct)	0.10	-	2.50	-	4.17	-	3.33	-	-3.23 oo	-0.83 ns	0.83 ns
	0.25	0.83	0.73 ns	3.53	1.03 ns	5.17	1.00 ns	1.50	-1.83 ns	-0.67 ns	2.03 ns	3.67 ***
	0.5	3.33	3.23 **	4.00	1.50 ns	4.00	-0.17 ns	4.00	0.67 ns	-0.67 ns	0.00 ns	0.00 ns
	1	3.17	3.07 **	3.67	1.17 ns	3.67	-0.50 ns	2.33	-1.00 ns	0.83 ns	1.33 ns	1.33 ns
0.25	0	2.17	2.07 ns	5.17	2.67 *	5.83	1.67 ns	3.17	-0.17 ns	-1.00 ns	2.00 ns	2.67 *
	0.25	1.50	1.40 ns	5.17	2.67 *	4.17	0.00 ns	2.33	-1.00 ns	-0.83 ns	2.83 **	1.83 ns
	0.5	4.67	4.57 ***	6.83	4.33 ***	7.17	3.00 **	2.50	-0.83 ns	2.17 *	4.33 ***	4.67 ***
	1	5.33	5.23 ***	4.17	1.67 ns	7.00	2.83 **	2.83	-0.50 ns	2.50 *	1.33 ns	4.17 ***
0.5	0	3.83	3.73 ***	5.33	2.83 **	6.67	2.50 *	3.33	0.00 ns	0.50 ns	2.00 ns	3.33 **
	0.25	1.70	1.60 ns	4.83	2.33 *	4.67	0.50 ns	3.17	-0.17 ns	-1.47 ns	1.67 ns	1.50 ns
	0.5	3.33	3.23 **	5.50	3.00 **	5.33	1.17 ns	2.50	-0.83 ns	0.83 ns	3.00 **	2.83 **
	1	4.33	4.23 ***	4.67	2.17 *	3.83	-0.33 ns	2.17	-1.17 ns	2.17 *	2.50 *	1.67 ns

LSD (p 5%) = 2.14;
LSD (p 1%) = 2.82;
LSD (p 0.1%) = 3.65.

LSD (p 5%) = 2.09;
LSD (p 1%) = 2.79;
LSD (p 0.1%) = 3.64.

Table 8. The combined influence of variety and hormone treatment on the number of internodes

Variety/Hormonal treatment applied (mg/l)		Azaria (a ₁)		Braşovia(a ₂)		Cosiana (a ₃)		Cezarina (a ₄) (Ct)		a ₁ -a ₄	a ₂ -a ₄	a ₃ -a ₄
		Number of internodes	Diff. / Sign.	Number of internodes	Diff. / Sign.	Number of internodes	Diff. / Sign.	Number of internodes	Diff. / Sign.			
NAA	IBA											
0	0 (Ct)	1.67	-	5.00	-	5.67	-	4.00	0.00	-2.33 o	1.00 ns	1.67 ns
	0.25	2.00	0.33 ns	3.67	-1.33 ns	5.33	-0.33 ns	5.00	1.00 ns	-3.00 oo	-1.33 ns	0.33 ns
	0.5	5.67	4.00 ***	5.33	0.33 ns	4.67	-1.00 ns	4.67	0.67 ns	1.00 ns	0.67 ns	0.00 ns
	1	4.33	2.67 **	5.67	0.67 ns	5.33	-0.33 ns	4.67	0.67 ns	-0.33 ns	1.00 ns	0.67 ns
0.25	0	3.67	2.00 *	5.00	0.00 ns	7.00	1.33 ns	4.33	0.33 ns	-0.67 ns	1.00 ns	2.67 **
	0.25	4.00	2.33 *	5.33	0.33 ns	4.67	-1.00 ns	4.33	0.33 ns	-0.33 ns	1.00 ns	0.33 ns
	0.5	5.00	3.33 ***	5.33	0.33 ns	6.00	0.33 ns	4.33	0.33 ns	0.67 ns	1.00 ns	1.67 ns
	1	5.67	4.00 ***	4.67	-0.33 ns	5.33	-0.33 ns	5.00	1.00 ns	0.67 ns	-0.33 ns	0.33 ns
0.5	0	5.33	3.67 ***	6.00	1.00 ns	5.67	0.00 ns	6.67	2.67 **	-1.33 ns	-0.67 ns	-1.00 ns
	0.25	3.67	2.00 *	5.00	0.00 ns	5.00	-0.67 ns	5.00	1.00 ns	-1.33 ns	0.00 ns	0.00 ns
	0.5	4.67	3.00 **	5.00	0.00 ns	6.00	0.33 ns	7.33	3.33 ***	-2.67 oo	-2.33 o	-1.33 ns
	1	5.00	3.33 ***	5.33	0.33 ns	5.67	0.00 ns	4.33	0.33 ns	0.67 ns	1.00 ns	1.33 ns

LSD (p 5%) = 1.92;
LSD (p 1%) = 2.54;
LSD (p 0.1%) = 3.28.

LSD (p 5%) = 1.97;
LSD (p 1%) = 2.64;
LSD (p 0.1%) = 3.52.

CONCLUSIONS

Each growth regulator and each of its concentrations presented its own effect on *in vitro* regeneration.

The development potential of the micropropagated plantlets was dependent on the genotype.

In this study, Braşovia variety stood out with the highest average value of plant height (10.86 cm), followed by Cosiana variety (10.39 cm); for the other analysed parameters (number of leaves, root length and number of internodes), Cosiana variety records the highest values (leaves: 8.36; root length: 5.14 cm; internodes

number: 5.53), followed by the Braşovia variety (leaves 7.50; root length: 4.61 cm; internodes number: 5.11 cm).

The treatment composed of 0.5 mg/l NAA:0 mg/l IBA was beneficial in obtaining a high number of leaves (9.00), followed by the combination 0.25 mg/l NAA:0 mg/l IBA, which resulted in 8.42 leaves.

The hormonal treatment applied with 0.25 mg/l NAA:0.5 mg/l IBA was favourable for plantlets growth (11.46 cm), followed by the combination of hormonal regulators NAA: IBA 0.5:0.0 mg/l, which determined the height evolution of microplants (10.92 cm).

NAA - IBA treatments of: 0.25-0.5 mg/l; 0.25-1 mg/l and 0.5-0.0 mg/l led to obtaining higher values of root length (5.29, 4.83 and 4.79 cm). The combination 0.5:0.0 mg/l NAA: IBA was conducive to achieving a high number of internodes (5.92 internodes), followed by the combination 0.5: 0.5 mg/l (5.75 internodes). The treatment composed of 0.5 mg/l NAA - 0 mg/l IBA was beneficial in obtaining a high number of leaves (9.00); the hormonal treatment applied with 0.25 mg/l NAA - 0.5 mg/l IBA was favourable for plantlets growth (11.46 cm); NAA - IBA treatments of: 0.25- 0.5 mg/l; 0.25-1 mg/l and 0.5-0.0 mg/l led to obtaining higher values of root length (5.29, 4.83 and 4.79 cm); combination 0.5 - 0.0 mg/l NAA - IBA was conducive to achieving a high number of internodes (5.92 internodes).

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THE BIOCHEMICAL COMPOSITION OF BIOMASS FROM INTERMEDIATE WHEATGRASS (*Agropyron intermedium*) AND POTENTIAL USES IN MOLDOVA

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Abstract

This research was conducted to determine biomass performances of intermediate wheatgrass (*Agropyron intermedium*) and its potential uses. We found that the dry matter content, biochemical composition and nutritive value of intermediate wheatgrass whole plants was characterized by the following indices: 13.1% CP, 39.0% CF, 67.2% NDF, 40.7% ADF, 3.6% ADL, 7.5% TSS, 9.1% ash with nutritive energy values 57.2% DMD, RFV = 79, 11.37MJ/kg DE, 9.34MJ/kg ME and 5.55MJ/kg NEL. The dry matter of prepared hay from intermediate wheatgrass contained 122g/kg CP, 94 g/kg ash, 398 g/kg CF, 412 g/kg ADF, 682g/kg NDF, 37 g/kg ADL with 55.8% DMD, 11.30 MJ/kg DE, 9.28 MJ/kg ME, 5.30 MJ/kg NEL. The obtained silage is characterized by pleasant smell and dark green colour, the nutrient content and feed energy values of dry matter were: 144 g/kg CP, 120g/kg ash, 395g/kg CF, 399 g/kg ADF, 654 g/kg NDF, 21 g/kg ADL, 57.8% DMD, 11.48MJ/kg DE, 9.43MJ/kg ME, 5.45MJ/kg NEL. The biochemical methane potential of the intermediate wheatgrass substrates ranged from 320 to 333 L/kg volatile solids.

Key words: *Agropyron intermedium*, biochemical composition, biomethane potential, green mass, hay, nutritive value, silage.

INTRODUCTION

The Poaceae family (grasses) comprises over 10,000 annual and perennial species, predominantly herbaceous, and largely distributed across tropical and warm-temperate regions. Numerous species within this family hold significant agricultural value, serving as sources of food, forage, and ornamental plants. Perennial grasses are hypothesized to provide a broader spectrum of supporting and regulating ecosystem services compared to annual species, including reduced soil disturbance, enhanced carbon sequestration over time, and the maintenance of biodiversity.

Intermediate wheatgrass, *Agropyron intermedium* (Host) Beauv. (syn. *Elymus hispidus* (Opiz) Melderis; *Elytrigia intermedia* (Host) Nevski; *Thinopyrum intermedium* [Host] Barkworth & D.R. Dewey; *Thinopyrum intermedium* spp. *barbulatum*), is a long-lived C₃ perennial grass native to Central Europe, the Balkans, and Asia Minor. It has been extensively introduced and cultivated as a forage species in the USA, Canada, Australia, and South America. The species exhibits weakly to

strongly creeping rootstocks that form a dense sod under moist conditions, with generally short rhizomes, although these traits vary among ecotypes and cultivars. The stems are robust, erect, and typically reach 90-150 cm in height. Foliage coloration ranges from bright green to dull blue-green, while leaf margins, inflorescences, and seeds can be glabrous or pubescent. The inflorescence is a narrow spike, 15-25 cm in length, with spikelets arranged alternately along the rachis. Each spikelet contains 2-6 florets, with glumes that are pointed and approximately half the length of the spikelet. Seeds mature by August, with an estimated 194,000 seeds per kilogram. Seedling vigor is rated from good to excellent, and establishment occurs relatively quickly compared to other meadow grasses. The species performs poorly in wet, poorly drained, or moderately saline to alkaline soils. It exhibits moderate cold tolerance, can withstand occasional spring flooding, and is highly fire-tolerant. The pubescent form is more resilient to low-fertility, alkaline soils, higher elevations, and drier conditions than the intermediate form. Intermediate wheatgrass is well-suited for the

stabilization of disturbed soils and can be employed in urban and critical environments with limited irrigation, including ditch banks, dikes, and roadsides.

Recently, it has been proposed as a dual-purpose perennial cereal, capable of providing both forage and grains for human consumption. Its integration into agricultural systems offers environmental benefits such as soil protection and water-quality regulation, attributable to its permanent soil cover and extensive belowground biomass. While domestication is at an early stage and grain yield remains low compared to annual cereals, the seeds can be utilized in baked products or beverages, potentially supplementing or substituting wheat, rye, or barley. Forage production presents an additional avenue for agricultural valorization. The species is compatible with other crops, notably alfalfa (*Medicago* spp.), with mixed stands often enhancing both longevity and productivity.

Intermediate wheatgrass has been the subject of extensive study and breeding across diverse geographical regions. New cultivars are being developed for multiple applications, including grain production, grazing pasture, hay, and silage (Wills et al., 1998; Ogle et al., 2011; Hybner & Jacobs, 2012; Culman et al., 2013, 2023; Jungers et al., 2017, 2018, 2022; De Oliveira et al., 2020; Lanker et al., 2020; Bowden, 2023; Bugayov et al., 2023; Locatelli et al., 2023; Rusch et al., 2025; Fagnant, 2024; Pan et al., 2025; Gutknecht et al., 2026; Pinto et al., 2026).

This research was conducted to evaluate the biomass performance of intermediate wheatgrass (*Agropyron intermedium*) and to assess its potential applications as livestock fodder, as well as a feedstock for biogas reactors in the production of biomethane as a renewable energy source.

MATERIALS AND METHODS

The study focused on a local ecotype of intermediate wheatgrass (*Agropyron intermedium* (Host) P. Beauv.) cultivated in a pure stand on the experimental plot of the “Alexandru Ciubotaru” National Botanical Garden (Institute), Moldova State University, Chişinău. Local ecotypes of tall oatgrass

(*Arrhenatherum elatius*), tall fescue (*Festuca arundinacea*), and meadow fescue (*Festuca pratensis*) were used as control species. Samples were collected at the first cut, consisting of whole plants harvested manually. The freshly cut biomass was chopped into pieces of approximately 1.5-2.0 cm using a laboratory forage chopper. The dry matter content was determined by drying the samples at 105°C until constant weight was achieved.

Silage was prepared from the chopped green biomass, which was compacted in tightly sealed glass containers and stored at ambient temperature (18-20°C). After 45 days of fermentation, the containers were opened and the sensory characteristics and fermentation parameters of the silage were evaluated according to standard laboratory procedures outlined in the Moldovan standard SM 108. Hay samples were obtained by drying the biomass directly in the field.

For biochemical analyses, plant samples were dried in a forced-air oven at 60°C and ground in a beater mill fitted with a 1 mm sieve. The main biochemical parameters - crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), and total soluble sugars (TSS) - were determined using near-infrared spectroscopy (NIRS) with a PERTEN DA 7200 analyser at the Research and Development Institute for Grasslands, Braşov, Romania. Hemicellulose (HC), cellulose (Cel), digestible dry matter (DDM), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEL), and relative feed value (RFV) were calculated using standard equations.

The carbon content of the substrates was estimated using the empirical equation proposed by Badger et al. (1979), while the biochemical methane potential was calculated according to the equations described by Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

The analysis of biological characteristics of the studied grass species (Table 1) indicated that at the time of the first cut, the tested local ecotype of *Agropyron intermedium* reached a height of 128 cm, whereas the control grass species, ranged from 89 to 114 cm. The fresh biomass

yield of the local ecotype of *Agropyron intermedium* was 4.99 kg/m², corresponding to 1.15 kg/m² of dry matter. In comparison, the control species produced the following yields: *Arrhenatherum elatius* - 4.18 kg/m² fresh mass (1.01 kg/m² dry matter), *Festuca arundinacea* - 4.25 kg/m² fresh mass (1.02 kg/m² dry matter),

and *Festuca pratensis* - 3.89 kg/m² fresh mass (1.04 kg/m² dry matter). The proportion of leaves and inflorescences in the fresh biomass of *Agropyron intermedium* was higher than that in *Arrhenatherum elatius*, but lower than in the *Festuca* species.

Table 1. Some agrobiological peculiarities of the studied grass species

Species	Plant height, cm	Yield, kg/m ²		Content of leaves and inflorescences in fodder, %
		fresh mass	dry matter	
<i>Agropyron intermedium</i>	128	4.99	1.15	60.00
<i>Arrhenatherum elatius</i>	114	4.18	1.01	58.76
<i>Festuca arundinacea</i>	109	4.25	1.02	62.20
<i>Festuca pratensis</i>	89	3.89	1.04	67.00

Previous studies support these observations. Culman et al. (2013) reported vegetative biomass yields of intermediate wheatgrass ranging from 12,202 kg/ha under organic management to 13,083-17,131 kg/ha under conventional management. Sakiroglu et al. (2020) mentioned that, in Minnesota, summer aboveground biomass from dual-use Kernza intermediate wheatgrass in the third growing year averaged 1.06 kg/m². Pototskaya et al. (2022) observed that the wheatgrass cultivar ‘Sova’ produced 21.0 t/ha of green mass or 7.1 t/ha of hay. Bugayov et al. (2024) reported that fodder productivity of the cultivar ‘Kernza’ of intermediate wheatgrass across two cuts ranged from 1.49 to 2.65 kg/m² dry matter, while the cultivar ‘Horse’ ranged from 1.18 to 1.79 kg/m² dry matter. The quality indices of the harvested biomass from the studied grass species are presented in Table 2. The comparative analysis of the

biochemical composition of the dry matter indicated that the green forage of *Agropyron intermedium* contained higher crude protein (131 g/kg) and mineral content (91 g/kg) than *Arrhenatherum elatius* (80 g/kg and 83 g/kg, respectively) and the *Festuca* species (107-124 g/kg protein and 83-89 g/kg minerals). Neutral detergent fibre (NDF) and acid detergent lignin (ADL) concentrations in *Agropyron intermedium* were lower than in *Arrhenatherum elatius* but higher than in the *Festuca* species. The total soluble sugar content in *Agropyron intermedium* forage was lower than in the control grass species. The cellulose content in *Agropyron intermedium* was comparable to that of *Arrhenatherum elatius* but exceeded that of the *Festuca* species. Hemicellulose levels in *Agropyron intermedium* were higher than in *Festuca pratensis* but lower than in *Arrhenatherum elatius* and *Festuca arundinacea*.

Table 2. The biochemical composition and the nutritive value of the green mass from studied grass species

Indices	<i>Agropyron intermedium</i>	<i>Arrhenatherum elatius</i>	<i>Festuca arundinacea</i>	<i>Festuca pratensis</i>
Crude protein, g/kg DM	131	80	107	124
Crude fibre, g/kg DM	390	382	354	315
Minerals, g/kg DM	91	83	89	83
Acid detergent fibre, g/kg DM	407	411	388	339
Neutral detergent fibre, g/kg DM	672	710	666	574
Acid detergent lignin, g/kg DM	36	38	34	30
Cellulose, g/kg DM	371	373	354	309
Hemicellulose, g/kg DM	265	299	278	235
Total soluble sugars	75	118	122	108
Digestible dry matter, g/kg DM	572	561	587	625
Relative feed value	79	74	87	110
Digestible energy, MJ/ kg DM	11.37	11.18	11.64	12.20
Metabolizable energy, MJ/ kg DM	9.34	9.18	9.56	10.01
Net energy for lactation, MJ/ kg DM	5.55	5.31	5.57	6.13

Regarding feed value, *Agropyron intermedium* forage exhibited favourable dry matter digestibility, relative feed value (RFV), and energy concentration compared to *Arrhenatherum elatius*, although these values remained lower than those observed in the *Festuca* species.

The nutrient composition of biomass harvested from *Agropyron* species has been extensively documented in the literature. Wills et al. (1998) reported that intermediate wheatgrass forage contained 2.7% nitrogen, 13.6% soluble carbohydrates, 3.6% lipids, 44.0% NDF, 24.8% ADF, and 683 g/kg organic matter digestibility (OMD), whereas tall oatgrass exhibited 1.8% nitrogen, 13.6% soluble carbohydrates, 2.6% lipids, 44.0% NDF, 28.6% ADF, and 709 g/kg OMD. Glover et al. (2004) found that intermediate wheatgrass grown on saline soils yielded 3,320 kg/ha dry matter, containing 10.4% crude protein (CP), 0.24% phosphorus (P), 55.4% NDF, and 29.8% ADF, while reed canary grass produced 11.0% CP, 0.25% P, 57.7% NDF, and 32.9% ADF. Zhao et al. (2008) reported crude protein concentrations of 135 g/kg dry matter (DM) in intermediate wheatgrass forage, compared with 111 g/kg DM in tall fescue and festulolium. Phillips et al. (2009) observed that forage from intermediate wheatgrass contained 349 g/kg DM, with 2.09% nitrogen, 66.94% NDF, and 31.72% ADF. Albayrak et al. (2011) documented that *Agropyron intermedium* forage contained 9.90-10.42% CP, 57.72-58.86% NDF, 41.88-44.29% ADF, 44.18-47.28% total digestible nutrients (TDN), and a relative feed value (RFV) of 86.00-92.51, whereas *Bromus inermis* contained 10.45-11.30% CP, 57.85-59.70% NDF, 41.75-43.50% ADF, 45.16-47.45% TDN, and RFV of 85.57-88.11. Jensen et al. (2017) reported that the nutritive value of intermediate wheatgrass at the late reproductive stage included 10.0% CP, 39.4% ADF, 63.6% NDF, and 630 g/kg neutral detergent fiber digestibility (NDFD). Newell & Hayes (2017) indicated that first-year intermediate wheatgrass forage contained 18.97% CP, 80.3% digestible dry matter (DMD), 12.3 MJ/kg metabolizable energy (ME), 32.0% NDF, 12.3% ADF, 21.2% water-soluble carbohydrates (WSC), and 5.0% ash. Favre et al. (2019) reported seasonal variation in forage quality, with spring forage containing

22.5% CP, 45.6% NDF, 24.9% ADF, and a relative forage quality (RFQ) of 175; summer forage showed 5.1% CP, 70.2% NDF, 42.7% ADF, RFQ = 65; and fall forage had 11.9% CP, 59.0% NDF, 33.7% ADF, RFQ = 116. Gürsoy et al. (2021) reported that green biomass of *Agropyron intermedium* contained 9.14% ash, 19.56% CP, 3.06% ether extract (EE), 20.88% crude fiber (CF), 57.82% NDF, 26.49% ADF, 5.45% acid detergent lignin (ADL), 71.40% TDN, 65.92% true dry matter digestibility (TDMD), 56.02% total NDF digestibility (TNDFD), 97.75% total organic matter digestibility (TOMD), RFQ = 138.29, 8.82 MJ/kg ME, and 5.42 MJ/kg net energy for lactation (NEI). By comparison, green mass from *Dactylis glomerata* contained 7.84% ash, 13.47% CP, 1.46% EE, 27.49% CF, 66.23% NDF, 34.14% ADF, 8.52% ADL, 68.79% TDN, 67.85% TDMD, 58.40% TNDFD, 97.70% TOMD, RFQ = 122.24, 6.85 MJ/kg ME, and 3.73 MJ/kg NEI. Pinto et al. (2022) observed that intermediate wheatgrass harvested during the vegetative period contained 17.3% CP, 55.1% NDF, 30.0% ADF, RFV = 111, whereas the forage collected during the reproductive stage of plants had 5.6% CP, 69.6% NDF, 42.6% ADF, RFV = 75. Puka-Beals et al. (2022) reported that first-cut forage at the boot stage contained 10.05% CP, 37.45% ADF, and 64.53% NDF, while the harvest at anthesis stage contained 63.2% CP, 43.27% ADF, 73.28% NDF, and at the dough stage contained 4.62% CP, 40.11% ADF, and 69.68% NDF. Bowden (2023) reported that non-fertilized intermediate wheatgrass forage contained 7.08-9.50% CP, RFV = 95-117, whereas manure-treated variants contained 9.49-11.20% CP, RFV = 93-120. Locatelli et al. (2023) documented that in Uruguay, winter-harvested intermediate wheatgrass contained 19.73% CP, 50.07% NDF, 30.47% ADF, RFV = 108.5, while summer-harvested plants contained 8.20% CP, 70.58% NDF, 42.99% ADF, RFV = 73.2. Yavuz and Kır (2023) observed that intermediate wheatgrass forage contained 11.5-12.0% CP, 54.2-57.4% NDF, 41.2-43.5% ADF, 6.1-7.6% ADL, whereas smooth brome forage contained 12.3-12.5% CP, 53-56% NDF, 39.0-42.6% ADF, 5.3-6.5% ADL. Fagnant et al. (2024) highlighted that forage quality of intermediate wheatgrass varied with phenological stage, with ash content

ranging 3.1-13.3%, CP 28.0-31.3%, NDF 27.0-79.9%, ADF 16.4-51.4%, ADL 1.1-11.2%, cellulose 14.4-52.8%, and OMD 280-930 g/kg. Culman et al. (2023) reported that spring-harvested intermediate wheatgrass contained 15.1-21.1% CP, 51.2-55.7% NDF, 26.1-28.7% ADF, 9.6-10.6% ash, and RFV = 112-126.8, whereas summer-harvested forage contained 4.1-4.8% CP, 67.2-70.2% NDF, 38.2-42.6% ADF, 6.0-6.5% ash, and RFV = 75.0-80.9. Fagnant (2024) reported that the forage quality of intermediate wheatgrass at the first harvest in early May was characterized by 18.2% CP, 25.5% Cel, 51.9% NDF, 26.2% ADF, and 2.2% ADL, with an OMD of 785 g/kg and a NEL of 1524 kcal/kg. When harvested later, at the end of May, forage quality declined markedly, with CP decreasing to 9.8%, while fiber fractions increased to 34.7% Cel, 61.7% NDF, 32.5% ADF, and 3.2% ADL. At the same time, OMD decreased to 654 g/kg and NEL to 1281 kcal/kg. Similarly, Pan et al. (2025) reported that the

chemical composition of fresh intermediate wheatgrass biomass included 264.3 g/kg DM, 14.31% CP, 61.81% NDF, 31.79% ADF, 14.17% WSC, 3.58% EE, and 8.91% ash. Pinto et al. (2026) observed seasonal variation in forage quality, reporting CP concentrations of 10.6-21.6%, NDF of 45.3-67.7%, and ADF of 24.9-37.2% during the spring period. Hay represents the principal dried forage source for ruminants (cattle, sheep, and goats), horses, and small herbivores such as rabbits, providing essential structural fiber necessary for maintaining digestive health. It is a fundamental component of livestock diets, particularly during winter or periods, when grazing is limited. The nutritional value of hay depends primarily on plant species, stage of maturity at harvest, and the effectiveness of the drying process. The biochemical composition and nutritive value of hay produced from the studied grass species are presented in Table 3.

Table 3. The biochemical composition and the nutritive value of the hay from studied grass species

Indices	<i>Agropyron intermedium</i>	<i>Arrhenatherum elatus</i>	<i>Festuca arundinacea</i>	<i>Festuca pratensis</i>
Crude protein, g/kg DM	122	77	100	122
Crude fibre, g/kg DM	398	414	393	350
Minerals, g/kg DM	94	80	89	80
Acid detergent fibre, g/kg DM	412	436	427	385
Neutral detergent fibre, g/kg DM	682	740	720	621
Acid detergent lignin, g/kg DM	37	40	35	34
Cellulose, g/kg DM	375	396	372	351
Hemicellulose, g/kg DM	270	304	292	236
Total soluble sugars	49	98	55	102
Digestible dry matter, g/kg DM	558	541	556	589
Relative feed value	76	68	74	93
Digestible energy, MJ/ kg DM	11.30	10.82	11.09	11.68
Metabolizable energy, MJ/ kg DM	9.28	8.88	9.10	9.59
Net energy for lactation, MJ/ kg DM	5.30	5.02	5.13	5.61

The hay prepared from *Agropyron intermedium* contained 122 g/kg CP, 94 g/kg ash, 398 g/kg CF, 412 g/kg ADF, 682 g/kg NDF, 37 g/kg ADL, 375 g/kg cellulose, 270 g/kg hemicellulose, and 49 g/kg TSS. The nutritive and energy value of this hay was characterized by a DMD of 55.8%, DE of 11.30 MJ/kg, ME of 9.28 MJ/kg, and NEL of 5.30 MJ/kg. During the haymaking process, the concentration of structural carbohydrates increased, whereas the content of total soluble sugars decreased in hay obtained from all investigated species. Compared with the control grass species, *Agropyron intermedium* hay exhibited a crude

protein concentration similar to that of *Festuca pratensis* hay, but considerably higher than that of *Arrhenatherum elatus* and *Festuca arundinacea* hay. The hay from *Agropyron intermedium* contained lower levels of total soluble sugars and minerals. The concentration of cell wall fractions in *Agropyron intermedium* hay was lower than that observed in *Arrhenatherum elatus* hay, but higher than in *Festuca pratensis* hay. Furthermore, the dry matter digestibility, relative feed value, and energy concentration of *Agropyron intermedium* hay were more favorable compared with those

of *Arrhenatherum elatius* and *Festuca arundinacea* hay.

Several studies have reported the nutritive characteristics and quality indices of hay derived from *Agropyron* species. Moore et al. (1995) indicated that intermediate wheatgrass hay harvested during the spring vegetative stage contained approximately 20% CP, 50.0% NDF, and exhibited a DMD ranging from 700 to 800 g/kg. Maheri-Sis et al. (2008) evaluated the chemical composition of hay obtained from *Agropyron repens* (quackgrass) harvested at a late stage of maturity and reported values of 950 g/kg DM and 887 g/kg OM. The hay contained 8.9% CP, 1.44% EE, 34.30% CF, 66.0% NFE, 8.96% NFC, 69.60% NDF, 38.30% ADF, 5.70% ADL, and 11.2% ash. In addition, the authors reported an OMD of 43.54% and a ME concentration of 6.58 MJ/kg. Similarly, Phillips et al. (2009) reported that baled intermediate wheatgrass hay contained 2.08% N, 67.21% NDF and 32.42% ADF. More recent studies by Hunter et al. (2020) highlighted the strong seasonal variability in the nutritive value of intermediate wheatgrass hay. Spring-harvested hay exhibited high protein concentrations (22.0-28.0% CP) and a relative feed value (RFV) of 148-161, whereas hay harvested in autumn showed a more reduced protein content (12.8-13.2% CP) and lower RFV values (89-107). Miron et al. (2023) investigated the nutritive composition of hay obtained from *Elytrigia repens* (syn. *Agropyron repens*) grasslands and reported CP concentrations ranging from 6.62 to 11.79%, EE from 1.31 to 2.47%, CF from 30.78 to 37.17%, and NFE from 40.64 to 53.01%. The hay also contained 4.31-6.82% soluble sugars, 1.40-1.79% starch, and 7.12-7.69% ash. Mineral concentrations ranged from 0.27 to 0.49% Ca and 0.16 to 0.21% P. Energy values were reported as 18.22-19.94 MJ/kg GE, 8.05-9.73 MJ/kg ME, and 4.39-5.50 MJ/kg NEI. More recently, Nazare et al. (2026) reported that the hay produced from *Elytrigia repens* grasslands contained 8.5% CP, 42.4% CF, 75.3% NDF, 45.0% ADF, and 4.4% ADL, with structural carbohydrates composed of 41.6% cellulose and 30.3% hemicellulose. The hay also contained 8.6% ash and demonstrated a dry matter digestibility of 54.3%, with energy concentrations of 8.84 MJ/kg ME and 4.86 MJ/kg NEI.

Silage production represents an important preservation strategy that stabilizes the nutritional value of fresh forage while ensuring a continuous supply of high-quality feed for ruminants throughout the year. By maintaining the biochemical integrity of harvested biomass through controlled anaerobic fermentation, silage helps bridge seasonal forage shortages and prevents fluctuations in feed quality.

The fermentation characteristics, biochemical composition, and nutritional value of silage produced from the investigated grass species are presented in Table 4. The silage prepared from *Agropyron intermedium* was characterized by a pleasant smell and dark-green color, with a pH of 3.97 and a total organic acid concentration of 37.1 g/kg, including 33.8 g/kg lactic acid. Butyric acid was not detected, indicating a well-preserved fermentation process. In addition, the concentration of acetic acid in *Agropyron intermedium* silage was lower than that observed in the control silages. The nutrient composition and energy value of *Agropyron intermedium* silage dry matter were as follows: 144 g/kg CP, 120 g/kg ash, 395 g/kg CF, 399 g/kg ADF, 654 g/kg NDF, and 21 g/kg ADL. The digestibility and energy indices were 57.8% DMD, 11.48 MJ/kg DE, 9.43 MJ/kg ME, and 5.45 MJ/kg NEI. During the ensiling process, the concentration of ADL decreased. Compared with the initial green biomass, the silage exhibited increased concentrations of crude protein and minerals, while the contents of crude fiber, ADF, and ADL decreased, contributing to improved digestibility and higher energy value of the resulting forage.

In comparison with silages obtained from the control grass species, *Agropyron intermedium* silage was distinguished by a higher crude protein concentration. Moreover, the dry matter digestibility and energy content of this silage were superior to those recorded for silages produced from *Arrhenatherum elatius* and *Festuca arundinacea*.

Similar findings were reported by Pan et al. (2025), who described intermediate wheatgrass silage with a pH of 4.90, containing 4.46% lactic acid and 0.68% acetic acid. The silage had a dry matter content of 289.9 g/kg and included 13.43% CP, 45.20% NDF, 30.20% ADF, 8.61% WSC, 1.68% EE, and 10.56% ash.

Table 4. The quality indices of the silage from studied grass species

Indices	<i>Agropyron intermedium</i>	<i>Arrhenatherum elatius</i>	<i>Festuca arundinacea</i>	<i>Festuca pratensis</i>
pH index	3.97	4.26	4.05	4.11
Organic acids, g/kg DM	37.1	24.2	36.7	39.6
Total acetic acid, g/kg DM	3.3	5.9	6.7	10.0
Total butyric acid, g/kg DM	0	0	1.3	0
Total lactic acid, g/kg DM	33.8	18.3	28.7	29.6
Acetic acid, % of organic acids	8.9	24.4	18.3	25.3
Butyric acid, % of organic acids	0	0	3.5	0
Lactic acid, % of organic acids	91.1	75.6	78.2	74.7
Crude protein, g/kg DM	144	88	106	130
Crude fibre, g/kg DM	395	405	380	320
Minerals, g/kg DM	120	100	105	91
Acid detergent fibre, g/kg DM	399	420	400	339
Neutral detergent fibre, g/kg DM	654	724	690	562
Acid detergent lignin, g/kg DM	21	26	19	24
Cellulose, g/kg DM	378	394	381	315
Hemicellulose, g/kg DM	255	304	290	223
Digestible dry matter, g/kg DM	578	565	577	625
Relative feed value	82	73	82	112
Digestible energy, MJ/ kg DM	11.48	11.25	11.46	12.32
Metabolizable energy, MJ/ kg DM	9.43	9.19	9.41	10.12
Net energy for lactation, MJ/ kg DM	5.45	5.21	5.43	6.13

Perennial species of the Poaceae family are increasingly recognized as valuable feedstock for renewable energy production due to their high biomass productivity, relatively low input requirements, and adaptability to marginal lands. These grass species can be utilized for the production of biogas, solid biofuels, and bioethanol, thereby contributing to the development of sustainable bioenergy systems and reducing greenhouse gas emissions (Dandikas et al., 2015; Muntean et al., 2018; Țiței & Roșca, 2021; Cozari et al., 2023; Țiței, 2024).

Efficient anaerobic digestion requires an optimal carbon-to-nitrogen (C/N) ratio, generally ranging from 20:1 to 30:1. Ratios exceeding 30:1 may slow microbial degradation due to nitrogen limitation, whereas ratios below 10:1 can lead to ammonia accumulation, reduced pH, and inhibition of methanogenic activity. A balanced ratio of approximately 25:1 is therefore considered optimal for maximizing methane production efficiency. The quality indices of the prepared substrates from the studied grass species and their biochemical methane production potential are presented in Table 5. The C/N ratio of both green biomass and silage substrates from *Agropyron intermedium* was within a favorable range for anaerobic digestion. Hemicellulose content in these substrates varied between 255 and

265 g/kg, while acid detergent lignin ranged from 21 to 36 g/kg. The biochemical methane potential ranged from 352 to 378 L/kg volatile solids (VS). The highest methane yield was obtained from the silage substrate, which also exceeded the methane production potential of silage derived from *Festuca pratensis*.

Previous studies have reported comparable methane production potentials for biomass from *Agropyron* species. Dickeduisberg (2017) reported methane yields ranging from 204 to 282 L/kg for substrates derived from tall wheatgrass herbage. In earlier investigations, Țiței (2021) reported biochemical methane potentials of 328 L/kg VS for green biomass substrates from both *Agropyron cristatum* and *Agropyron desertorum*. Lalak et al. (2022) noted that substrates derived from *Agropyron elongatum* contained 46.24% cellulose, 28.51% hemicellulose, and 28.27% lignin, achieving a biogas production potential of 398 L/kg in treated substrates. Miron et al. (2023) reported that the specific methane yield of biomass substrates from *Elytrigia repens* (syn. *Agropyron repens*) grasslands ranged between 242 and 282 L/kg VS. Similarly, Nazare et al. (2026) indicated that substrates derived from *Elytrigia repens* grasslands had a C/N ratio of 37.34, 4.4% ADL, and 30.3% hemicellulose, with a biomethane potential reaching 333 L/kg VS.

Table 5. The biochemical biomethane production potential of the investigated substrates from studied grass species

	<i>Agropyron intermedium</i>		<i>Arrhenatherum elatius</i>		<i>Festuca arundinacea</i>		<i>Festuca pratensis</i>	
	green mass	silage	green mass	silage	green mass	silage	green mass	silage
Crude protein, g/kg DM	131.00	144.00	80.00	88.00	107.00	106.00	124.00	130.00
Nitrogen, g/kg DM	20.96	23.04	12.80	14.10	17.12	16.96	19.84	20.80
Ash, g/kg DM	91.00	120.00	83.00	100.00	89.00	105.00	83.00	91.00
Carbon, g/kg DM	505.00	488.89	509.00	500.00	506.11	497.22	509.44	505.00
Ratio carbon/nitrogen	24.09	21.22	39.80	35.50	29.42	29.32	25.68	24.28
Acid detergent lignin, g/kg DM	36.00	21.00	38.00	26.00	34.00	19.00	30.00	24.00
Hemicellulose, g/kg DM	265.00	255.00	299.00	304.00	278.00	290.00	235.00	223.00
Biomethane potential, L/kg VS	352.00	378.00	341.00	361.00	351.00	376.00	360.00	369.00
Biomethane potential, L/kg DM	320.00	333.00	313.00	325.00	320.00	337.00	330.00	336.00

CONCLUSIONS

The local ecotype of intermediate wheatgrass (*Agropyron intermedium*) is well-suited for establishing temporary pastures, either in pure stands or in mixtures with other grasses and legumes, as well as for the restoration of eroded lands and the reintegration of degraded soils into agricultural use.

The harvested biomass of intermediate wheatgrass can be utilized as fresh forage, hay, or silage for livestock, and it also represents a viable feedstock for biogas production, contributing to biomethane generation as a renewable energy source.

Moreover, the evaluated local ecotype of *Agropyron intermedium* represents a valuable genetic resource for breeding programs aimed at developing new cultivars for forage production and other applications in bioeconomy.

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BIOMETHANE: PRODUCTION, USES AND ENVIRONMENTAL IMPACT

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Abstract

The climate changes of last decades require more and more the finding of alternative solutions for the provision with electrical and thermal energy of at the farm level. The first biomethane plant from biogas was put into operation in Germany in 2006, thus offering an alternative to fossil fuels. Biomethane is used in liquid form as bio-LNG or in gaseous form as bio-CNG. In addition to its use in the electricity and heating markets, biomethane is particularly suitable for freight transport, while bio-CNG is more suitable for passenger cars, delivery vehicles, public transport and tractors. At the same time, at the farm level, biomethane offers a decarbonisation tool by providing electricity and heat with a closed carbon cycle, being a renewable gas that can replace fossil fuels. At present, depending on European and national legislation, each of the European countries has its own means to support the biomethane market. This paper aims to present the current situation of biomethane production technologies, the possibilities of their use at farm level and the impact on the environment.

Key words: biomethane, biogas upgrading, climate change, renewable energy, carbon cycle.

INTRODUCTION

Finding alternative sources to reduce carbon dioxide (CO₂) emissions and other polluting emissions with greenhouse effect was and is a constant and deep concern of farmers but also tractors and agricultural machinery manufacturers (KunakAIR, 2025). It has been known for decades that at the farm and municipal community level, manure, landfills and decomposing organic waste release methane (CH₄), a greenhouse gas 28 times more potent than carbon dioxide (CO₂) (Arnau, 2025a). Therefore, biogas plants collect it through controlled and sealed anaerobic digestion, producing biogas, thus preventing methane (CH₄) from evaporating into the air. According to research in the field, biogas consists of 50-60% methane (CH₄), depending on the nature of the substrate and pH of the reactor. The rest consists of carbon dioxide (CO₂) and minor accompanying gases such as hydrogen sulfide (H₂S), nitrogen (N₂), oxygen (O₂), siloxanes, volatile organic compounds (VOCs), carbon monoxide (CO), and ammonia (NH₃) (Adnan et al., 2019; Angelidaki et al., 2018). The transformation of biogas into biomethane (known as upgraded biogas) involves the separation of these non-combustible gases. After

processing raw biogas, biomethane typically contains only very small amounts of carbon dioxide (CO₂), usually less than 1-5%. While the original biogas still consists of about 30-50% CO₂, this is removed through refining processes to achieve methane (CH₄) content of over 95-99% (Kabeyi & Olanrewaju, 2024).

Although the first biomethane plant from biogas was put into operation in Germany in 2006, upgrading of biogas on an industrial scale in the EU developed a few years later. Founded in 2009 The European Biogas Association (EBA) counts today on a well-established network of over 350 national associations and other organisations covering the whole biogas and biomethane value chain throughout Europe and beyond (EBA, 2020). Another benefit of biomethane production is that after biogas upgrading, carbon capture, utilization, and storage (CCUS) is becoming a very popular decarbonisation solution. This means that the reductions of CO₂ emission into the atmosphere can only be achieved by either reducing the CO₂ emissions from the sources or increasing the usage of CO₂ for another purpose (Adnan et al., 2019). Once purified, CO₂ can be used in numerous industrial fields: carbon fertilization in greenhouses; wastewater treatment; food applications in the brewing and carbonated soft

drinks industry; food applications for remineralization of drinking water or obtaining dry ice that it can be used in both the food and non-food industry (Godoy et al., 2024; Arnau, 2025b).

According to statistics, biomethane production worldwide in 2023 was around 8 billion cubic metres (bcm), from which 4.9 bcm production in the EU, with the majority injected into natural gas grids for use in industry, transport and buildings. The remainder bottled as compressed natural gas (CNG) for use as a transport fuel. As of 2025, Europe has 1,678 biomethane plants in operation with an annual production capacity that has reached 7 bcm (IEA, 2025a). Europe significantly dominates the biomethane market share in the global market and has the most mature biomethane production ecosystem. France, Germany, Italy, United Kingdom, and the Denmark are leading, with Spain that also is in a phase of accelerated growth (Table 1) (Arnau, 2025a). At least 86% of the biomethane plants currently active in Europe are known to be connected to the gas grid. 7% of European biomethane plants do not have a grid connection, and for the remaining 7% of Europe's plants no information is available to the EBA database (European-biomethane-map-2025).

Table 1. Countries with the largest biomethane installed capacities (in Nm³/h - *Normal Cubic Meters/Hour*)

	2025		2024		2022
France	190,711	Germany	147,749	Germany	147,711
Germany	157,258	France	132,818	United Kingdom	107,029
Italy	99,658	United Kingdom	114,358	France	87,691
United Kingdom	93,161	Italy	97,757	Denmark	70,105
Denmark	85,142	Denmark	85,117	Sweden	45,421

(Source: Arnau, 2025a)

Therefore the European Commission fully recognises these benefits and in its recent REPowerEU plan set a target of 35 billion cubic meters (bcm) of annual biomethane production by 2030 (Alberici et al., 2022).

Romania has also a huge potential for developing biomethane production capacities. According to European Commission analyses Romania has ability to replace about 80% of current NG imports with biomethane. However, there is currently no such facility in Romania (Energy Magazine, 2024).

Biomethane provides new opportunities at different levels for the society because compares

to raw biogas, can be used both for energy production and vehicle fuel (Kabeyi et al., 2024; Godoy, 2024).

METHODS AND TECHNIQUES FOR UPGRADING BIOGAS INTO BIOMETHANE

As previously stated, biomethane or upgraded biogas is made by qualitative processing of raw biogas through several steps to remove impurities mainly CO₂, that causes environmental problems such as climate change (Mohammadpoura, 2024). Today, the use of biomethane as a fuel in the transport sector is also very promising, as it can be used in natural gas vehicles without further conversion. Biomethane is very valuable for use in transport sectors, e.g. in liquid form in heavy-duty transport, but also in agriculture sector, so that biomethane can contribute to the successful decarbonisation of these sectors (Figure 1) (Snam, 2025). Bio-Liquefied natural gas (or bio-LNG) is liquefied biomethane, a drop-in replacement for its fossil LNG counterpart. This is not only easily transportable but also guarantees greater efficiency when used as a fuel (Snam, 2025).

This renewable substitute for LNG offers the same advantages that LNG offers over Diesel: reduction of carbon dioxide (CO₂) emissions; quieter engine operation; reduction of nitrogen oxide (NO_x) emissions and significantly fewer emissions of polluting particles (Rehman et al., 2020). The main stages in obtaining biomethane are as follows: *biogas production* (raw material collection, methanization), *biogas purification*, *filtration-odorization* and *pressure regulation*. After these processes, biomethane is ready for the final stage: *reinjection into gas networks* (Alberici et al., 2022). After biogas purification (i.e. removal of residues of CO₂, H₂S, NH₄, water vapor), etc., the methane content increases from 55% to ≥96%. It should be noted that, like natural gas, for safety reasons, when introducing biomethane into the network it is filtered and odorized (Alberici et al., 2022). Figure 2 presents the technologies for obtaining biomethane from different sources of raw materials.

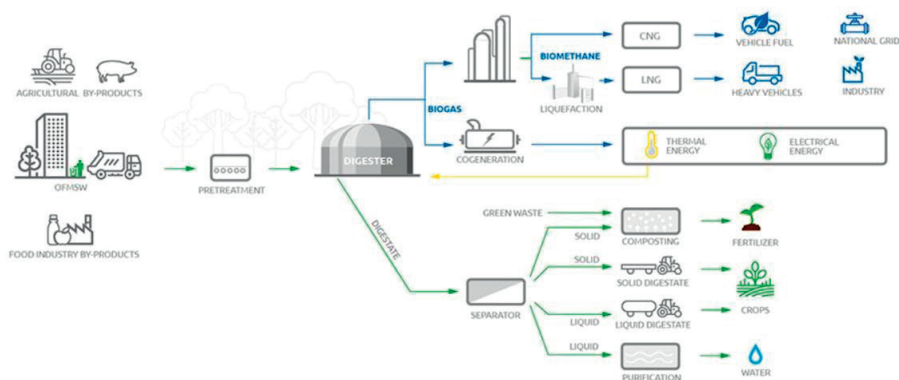
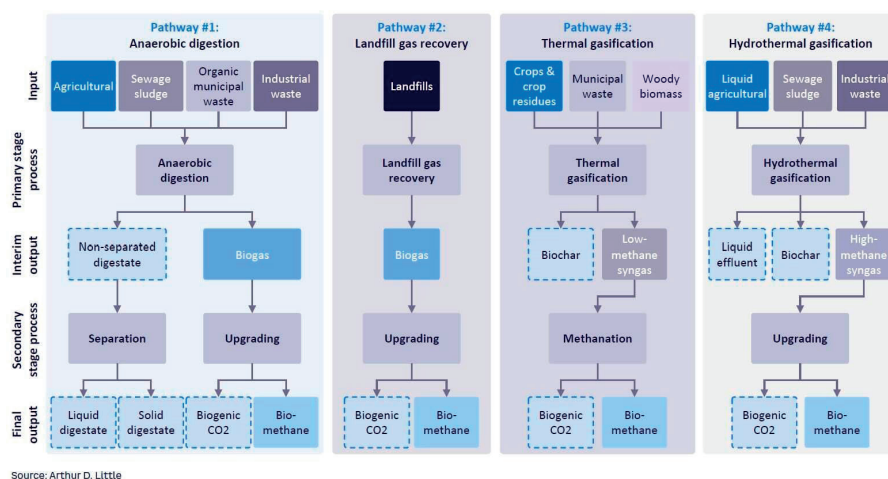


Figure 1. Biomethane production scheme through biogas upgrading
(Source: <https://www.snam.it/en/our-businesses/biomethane.html>)



Source: Arthur D. Little

Figure 2. Biomethane production pathways
(Source: <https://www.adlittle.com/mx-en/insights/viewpoints/future-biomethane>)

In order to eliminate unwanted components from biogas, such as CO_2 and H_2S , and to obtain an efficient fuel thus increasing its specific calorific value, several upgrading technologies have been proposed. All technologies for methane enrichment have in common, that the accompanying gas compounds besides to methane are removed by using physical, biological or chemical applications (Weiland, 2003; Ahrens & Weiland, 2007; Hurtig et al., 2025). Common technologies for *removal of H_2S* are: **biological desulphurisation** and **adsorption with activated carbon or iron and zinc oxide** (Ahrens & Weiland, 2007). For the removal of CO_2 the following strategies are well

known. Figure 3 presents a simplified schema of these currently applied technologies. The gas absorption method is divided into two categories: *physical and chemical purification*. The *adsorption method* is usually carried out in a process called Pressure Swing Adsorption (PSA). For the *CO_2 separation method*, membrane separation technology or cryogenic separation is applied. The cleaning process also captures biogenic CO_2 , which can replace fossil CO_2 in many processes or be removed permanently from the greenhouse gas emission cycle (Directorate-General for Energy, EU 2025).

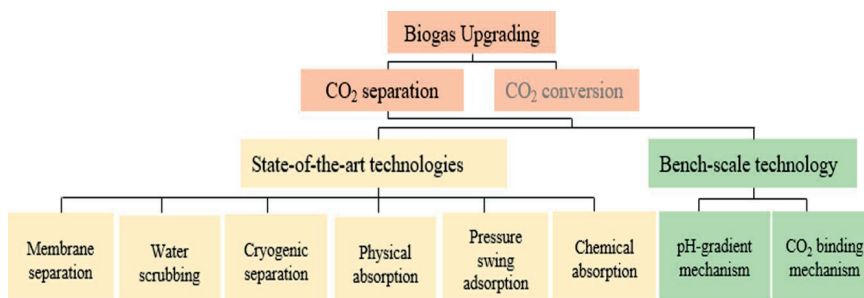


Figure 3. Technologies for biogas upgrading in to biomethan
(Source: Mohammadpoura et al., 2024)

Upgrading technology involves a significant investment and can vary substantially depending on the scale of the facility and the region where the biogas plant is located (IEA, 2025b). The cleaning and upgrading processes commonly represent approximately 20-40% of the total investment costs for a new biogas plant (Ammenberg et al., 2021). Each method of obtaining biomethane by upgrading biogas has a series of advantages and disadvantages. Among these we can list:

- **Water scrubbing**

The water scrubbing process is used to remove carbon dioxide CO₂ and hydrogen sulfide H₂S from the biogas composition, since these gases are more soluble than methane. One of the advantages of this biogas purification process is that no special chemicals are required to remove carbon dioxide CO₂ and hydrogen sulfide H₂S. The disadvantage is that it requires the use of a large amount of water (Zhao et al., 2010).

- **Chemical absorption**

Chemical absorption involves the formation of reversible chemical bonds between the solution and the solvent. Solvent regeneration involves the breaking of these bonds and brings a relatively high energy ratio. The advantages of this process are: complete removal of hydrogen sulfide H₂S, high efficiency and reaction rate compared to wet scrubbing, and the ability to operate at low pressure. The disadvantages are the use of additional chemicals and the need to treat the chemical waste resulting from this process (Zhao et al., 2010).

- **Pressure Swing Adsorption**

Pressure Swing Adsorption (PSA) is a technology used to separate certain types of gases from a mixture under pressure, depending on their molecular characteristics and affinity

for the adsorbent material. The main disadvantages are an additional step of removing hydrogen sulfide H₂S, the need for waste gas treatment, and high costs. The advantages of the pressure swing adsorption process are: reduced emissions, removal of nitrogen N and oxygen O, methane enrichment by over 97% and reduced energy requirements (Zhao et al., 2010).

- **Membrane Separation**

This technique uses polymeric membranes to separate the CO₂ from the methane in biogas while under high pressure (Green, 2018; Ardolino et al., 2021). The membrane separation process produces ultrapure carbon dioxide that can provide an additional source of income for plant operators, and can be subsequently used in a wide range of industries (food industry, horticulture, firefighting or refrigeration plants) (Laumann, 2025).

- **Cryogenic Separation**

Cryogenic separation of biogas is based on the fact that CO₂, H₂S and all other biogas contaminants can be separated from CH₄ based on the fact that each contaminant liquefies at a different temperature-pressure domain (Bauer et al., 2013). This separation process operates at low temperatures, near -100°C, and at high pressures, almost 40 bars. The main advantage of cryogenic separation is the high purity of the upgraded biogas (99% CH₄), as well as the large quantities that can be efficiently processed. The main disadvantage of cryogenic separation is that cryogenic processes require the use of considerable process equipment, mainly compressors, turbines and heat exchangers. The need for the equipment raises capital and operating costs relative to other options (de Hullu et al., 2008; Zhao et al., 2010).

• Amine Scrubbing

Amine scrubbing system uses a two-step approach to upgrading biogas. The first step is adsorption followed by a second step of stripping or desorption. Benefit claims of systems suppliers include: low electricity usage; thermal efficiency; greater than 99.5% CH₄ capture; low usage of water and amine solvents; wet process can minimize need for robust pretreatment; system handles VOCs, siloxanes and H₂S; fully automated to minimize operator attention; flexible system that can easily turn down capacity if needed; CO₂ off-gas is pure enough to recover for subsequent reuse (Green, 2018). Recently, advancements are being made towards new technologies such as *electrochemical CO₂ separation*. (Mohammadpoura et al., 2024).

WAYS TO USE BIOMETHANE

As previously described, in the case of biomethane production, it must be taken into account that, in addition to the methane used as fuel, biomethane plants produce useful, sustainable and indigenous by-products, such as digestate, biogenic CO₂ and biochar. (Directorate-General for Energy, EU 2025).

Usually, 1 m³ of biogas typically yields around 0.56 m³ to 0.62 m³ of biomethane when upgraded (Boglárká (Bogi), 2025). The digestate, or organic residue resulting from anaerobic digestion, is rich in nutrients (phosphorus, nitrogen and potassium) essential raw materials for EU agriculture. If correctly applied in agriculture, this biofertilizer can support sustainable agriculture and generate additional income for biomethane producers. (Directorate-General for Energy, EU 2025).

Purified form of CO₂, the so-called biogenic CO₂, can be captured, stored and used in various applications in other areas of agriculture or the food industry (for example as a process stimulating agent for greenhouse plants, for water treatment or in the food and beverage sector) (Directorate-General for Energy, EU 2025). At the same time, biochar can improve soil health and decompose raw materials, or it can be a terrestrial option for carbon removal because it locks carbon in the soil for the long term (Directorate-General for Energy, EU 2025). Year after year, the establishment of

biomethane stations constitutes a challenge for both industry and farmers due to the increasing demand for biofuels. In the European Union scenario for the years 2030 (Figure 4) and 2050 (Figure 5), one can observe both the trend of increasing the amount of biomethane and changes in the production technology (anaerobic digestion or thermal gasification) for each country.

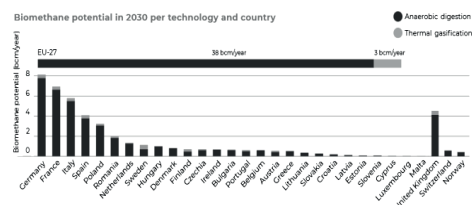


Figure 4. Biomethane potential in 2030 per technology and country (Source: Alberici et al., 2022)

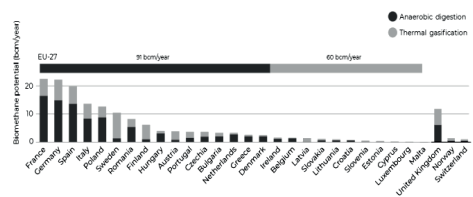


Figure 5. Biomethane potential in 2050 per technology and country (Source: Alberici et al., 2024)

At a global level, especially in the USA and China, the scenarios for 2030 are also optimistic, taking into account the growing demand for renewable natural gas and import activities (Figures 6 and 7). At the present time, at national level, many countries use dedicated support mechanisms for the development of the biogas and biomethane sector, including feed-in tariffs, premium feed-in tariffs and fiscal incentives. Feed-in tariffs are the minimum prices guaranteed, over a defined period, by the national governments for each kWh generated, either injected into a network or directly used (Prussi et al., 2019).

At European level, the gas network is generally structured in 3 layers: *high pressure, medium pressure and low pressure networks*.

Usually the biomethane produced is injected locally into the low pressure network, which is designed for unidirectional flow. This can be a disadvantage because - unlike the electricity network - it depends on local gas consumption

to create space for new inputs (Boglárka (Bogi), 2025). Therefore, in summer, when the demand for biomethane is lower and the gas supply exceeds local consumption, the delivery pressure decreases, which can cause congestion in the network. Therefore, during this period, the ability of producers to inject biomethane constantly is limited, especially in rural areas where there is low demand for gas for heating (Boglárka (Bogi), 2025).

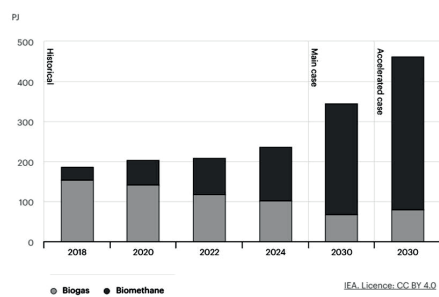


Figure 6. Production of biogas and biomethane in the United States, 2018-2030 in petajoules (PJ) (Source: IEA, 2025b)

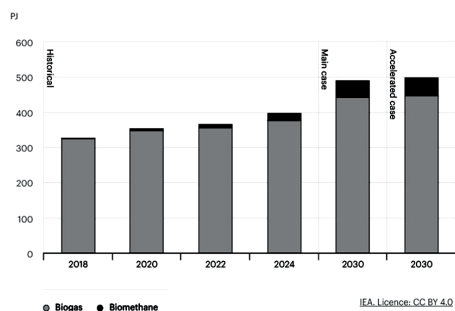


Figure 7. Production of biogas and biomethane in the China, 2018-2030 in petajoules (PJ) (Source: IEA, 2025b)

Biomethane as a vehicle fuels

Biomethane can be used as a green fuel for vehicles, referred to as **bioNGV** (Nagy & Princz-Jakovics, 2025). It produces no nitrogen oxides (NOx), no black smoke, and minimal greenhouse gas emissions and it is available in compressed (bioCNG) or liquefied (bioLNG) forms (<https://www.engie.com>). Vehicles which can be fueled using compressed natural gas, and as a result biomethane, include generally heavy duty vehicles (trucks and city, buses), passenger cars, and for a few years now also tractors.

Approximately 20–30 million vehicles globally are fueled by compressed natural gas or biomethane (Ammenberg et al., 2021). The use of biomethane as a fuel requires a series of costs. The main costs are due to: biogas upgrading, CH₄ liquefaction, distribution, filling station and the costs of vehicles using biomethane. Today, engines designed for methane propulsion are currently being produced by several truck and bus manufacturers, including Daimler (2018), Isuzu (2018), Iveco (2015), Scania (2017) and Volvo Trucks (2017) (Gustafsson & Svensson, 2021). In the Nordic countries, where gas grids are limited (except in Denmark), biomethane is primarily used in transportation (Finland and Sweden) (Boglárka (Bogi), 2025).

Research carried out in various projects has shown that in the case of biomethane used as a fuel for vehicles, the potential for reducing greenhouse gas emissions *depends mainly on the raw material used*. In addition to the fact that a reduction of 60-80% was found compared to gasoline, a greater potential for reducing these emissions was also observed when using organic waste and municipal waste, compared to biomethane obtained from energy crops (IRENA, 2018). This fact thus allows the expansion of biomethane stations in non-agricultural areas where there are large quantities of waste obtained from the retail or wholesale sector (expired food products). Under Central European conditions, it has been calculated that in general the total cost for biomethane production, meaning all stages from raw material supply to injection into the gas network, varies from 0.28 USD/m³ to 1.94 USD/m³ CH₄ (IRENA, 2018). The calorific value of biogas is between 20.7 and 27.8 MJ/m³, while the biomethane calorific value is higher (about 37.7-39.8 MJ/m³). This difference largely affects the respective costs, when used for energy production, ranging between 0.89 and 2.97 pence/kWh for the case of biogas and 1.49-3.30 pence/kWh for biomethane (Gkotsis et al., 2023). To be injected into a vehicle, biomethane must be compressed to at least 200 bars at the refilling station, in order to exceed the pressure in NGV fuel tanks. Vehicles can also be retrofitted for bi-fuel operation, knowing that methane delivers almost the same performance and efficiency as gasoline (IRENA, 2018). With the same levels of power and torque as its diesel

equivalent, farmers also benefit from up to 30% lower running costs. It produces 99% less particulate matter, cuts overall emissions by 80% and when using methane reduces CO₂

emissions by 10-15%. When running on biomethane near-zero CO₂ emissions are achievable (EIP-AGRI Project, 2023).



Figure 8. New Holland T7.270 Methane Power Tractor
(Source: <https://agriculture.newholland.com>)

In the transport sector, the EU has set a target of 14% by 2030 in the use of renewable energy sources. In addition, at least a share of 3.5% of final energy consumption in the transport sector by 2030 will have to come from advanced biofuels, generally defined as fuels derived from waste or non-food biomass, such as animal manure, the organic fraction of municipal solid waste or agricultural by-products (Tratzi et al., 2022). Alternative propulsion technologies in agriculture to reduce pollution and climate change impact are broadly investigated in research, especially electric batteries (for electric tractors) and liquefied gas or compressed gas (for diesel engines) (Gustafsson & Svensson, 2021). The New Holland T6.180 Methane Power is the world's first 100% methane powered production tractor and is key to CO₂ reduction without compromising performance (EIP-AGRI Project, 2023). The new series T7 270 Methane Power CNG tractor can run on biomethane derived from livestock manure, supporting on-farm sustainability (Figure 8).

Refilling can be performed directly from the gas grid network or at specific biomethane stations. Research on methane-powered tractors has shown that they not only have the potential to reduce pollutant emissions by 80%, but also significantly reduce fuel costs. This directly

contributes to a lower carbon footprint. The T7 Methane Power features all-new NEF 6.7L engine specifically developed for agricultural applications by FPT Industrial, a leader in natural gas power train technology (Vale, 2025). This engine model has 6 cylinders with 4 valves and stage V, with 3-way catalyst, 6728 capacity (cm³). The engine's peak torque is 1,160 Nm, and produces a maximum 199 kW (270 hp) and a rated engine speed - 2200 (rpm). The methane tank capacity at 200 bar and 25°C is 656/115 (litres/kg) along with an optional 480-litre range extender and that offers an incredible autonomy of higher than 11 hours at medium duty (Vale, 2025).

Environmental Impact

Through the studies undertaken and published technical reports, Edwards et al. (2014), Börjesson et al. (2016), Gustafsson et al. (2021), note that in addition to the advantage, the climate change impact of biomethane can even become negative if external effects of biogas production are included in the calculations. Thus, regarding the environmental impact, it must be taken into account that if the pollutants removed from biogas (CO₂, H₂S and even residual CH₄) during the upgrading are emitted into the atmosphere, the environmental contamination will be as high as in the case of

using current fossil fuels. Depending on each process used for biogas upgrading, there may be solutions to avoid this inconvenience. For example, in the case of the *chemical absorption method*, a catalyst is usually used. This can be either amines for CO₂ absorption or Fe/EDTA complexes for H₂S absorption. In the case of the *high-pressure water purification method*, especially the CO₂-enriched air is removed and traces of CH₄ are burned. The largest amount of CO₂ and H₂S is absorbed in the stripper during the gas phase. The *pressure swing adsorption method (PSA)* involves burning CH₄ from the residual flow in the heat engine of a generator. Sometimes the waste stream is recycled which leads to an increase in the CH₄ yield in the product stream. The *cryogenic separation process*, as an environmentally friendly technique, does not use chemicals, but consumes considerable energy. Here too the waste stream consisting of a high percentage of CO₂ with traces of H₂S and CH₄ must be treated carefully in order not to be harmful to the environment. In the case of the *membrane separation method*, the waste gas still contains CH₄, which is very polluting. It is recommended that part of this quantity be reintroduced into the intake or, as in pressure swing absorption, the waste gas be burned in a gas engine connected to a generator (Zhao et al., 2010).

Romania case

Unfortunately, at the present time, Romania represents one of the least developed biogas markets in the EU27, but has ability to replace about 80% of current NG imports with biomethane. In the field of road transport as infrastructure, in 2022 Romania has 12 CNG charging stations compared to Italy which has 1063, Germany 868 or Sweden 178 (<https://cngeurope.com/countries/romania/>). This means that, current number CNG filling stations are not sufficient to have a larger uptake of biomethane in transport. Industry estimates Romania’s sustainable biomethane potential as 2.0 bcm (1.9 bcm from anaerobe digestion and 0.1 from gasification) by 2030 (Figure 9 and Table 2). According to reports prepared by the European Commission, considering the sustainable potential, Romania could be among the top biomethane markets in the EU27, with

significant national benefits (BIOMETHANE FICHE - Romania, European Commission -2021). For all that weakness, in 2030 Romania is planning a 5% share of biomethane in its natural gas network and to double it by mid-century. The sectors of waste management and agriculture can produce an estimated 501.000 tons of oil equivalent in 2050 (Todorović, 2025).

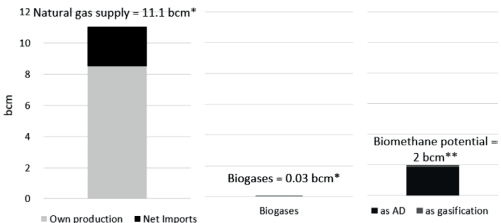


Figure 9. Comparison of current natural gas supply, biomethane production and potential in Romania (Eurostat: Energy Balances, 2022)

Table 2. Biogas/biomethane potential in bcm, by feedstock for Romania (Source: Guidehouse: Gas for Climate Report, 2022)

Feedstock	Production pathways	
	Thermal gasification	Anaerobic Digestion
Wood waste	0.05	-
Forestry residues	0.04	-
Landscape care wood	0.03	-
Municipal solid waste	0.02	-
Agricultural residues	-	0.8
Animal manure	-	0.4
Biowaste	-	0.3
Sewage sludge	-	0.02
Industrial wastewater	-	0.05
Roadside verge grass	-	0.01
Sequential crops	-	0.31

CONCLUSIONS

At the farm level, biogas and biomethane, produced locally from organic materials such as agricultural residues, manure, and sewage sludge, it can both decarbonization and energy security. At the same time, biomethane, produced from organic waste and residues, is a renewable and circular fuel that transforms waste into clean energy - delivering environmental, economic, and social benefits. It is a prime example of the circular economy applied to transport. A tailored national approach is needed in the future to maximise the

value of these resources. Differences between countries in strategic priorities for renewables resource availability and infrastructure need to be taken into account. The researches and studies demonstrated that a biomethane car, truck or tractor is less expensive than other renewable forms of propulsion (for example electric or H₂). The advantage is due first of all to compatibility with natural gas and this means that biomethane has an existing charging/ and vehicle infrastructure. On the other hand the fact that biomethane is nearly climate neutral, producing more than 90% fewer climate emissions. From an economic point of view, the production cost of LBM is much higher than in the case of LNG. This is due to the fact that natural gas has the advantage of that it exists in large volumes and requires relatively cheap processes to obtain a high-energy fuel and upgrading biogas to biomethane is produced at a much smaller scale with higher specific costs. At the farm level through biomethane production many countries, including Romania, could additionally reduce NG import dependency by including the synthetic fertiliser production capacities in improvement and marketing of digestate, or extract of macro-nutrients for bio-fertilizers

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INTEGRATED UAV AND GIS-BASED TECHNOLOGIES FOR EARLY DETECTION AND SITE-SPECIFIC MANAGEMENT OF WEEDS IN ARABLE CROPS UNDER CLIMATE CHANGE CONDITIONS

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Abstract

Climate change and increasing environmental constraints require more precise and adaptive approaches to crop protection. This study investigates the use of integrated unmanned aerial vehicle (UAV) imagery and Geographic Information Systems (GIS) for early weed detection and site-specific management in arable crops. Multispectral UAV data were collected during key crop development stages and processed to produce high-resolution RGB, NIR and vegetation index-based orthophotoplans. Spatial analysis techniques were applied to distinguish crop rows from weed-infested areas and to evaluate the intensity and distribution of infestations. The results show that combining UAV-derived data with GIS analysis allows accurate identification of weed patterns at field level and supports targeted management decisions. Field observations were used to validate remote sensing outputs and to improve interpretation accuracy. The proposed approach contributes to reducing unnecessary agrochemical applications while maintaining effective weed control. Overall, the study demonstrates that UAV-GIS integration can provide a reliable decision-support tool for sustainable weed management and climate-resilient arable farming systems.

Key words: precision agriculture, UAV, GIS, weed detection, climate change.

INTRODUCTION

Climate change, rising input costs, and tightening environmental constraints are increasing the need for weed-management strategies that are both agronomically effective and more selective in their use of herbicides. In arable farming systems, weeds remain one of the principal biological constraints to crop establishment and productivity because they compete with crops for light, water, nutrients, and space. In this context, precision weed management is increasingly regarded as an operational extension of integrated weed management, since it combines monitoring, spatial targeting, and reduced-input intervention while maintaining weed-control effectiveness (Monteiro & Santos, 2022; Gerhards et al., 2022).

Recent advances in unmanned aerial vehicle technology, multispectral sensing, and GIS-based spatial analysis have made it possible to detect within-field heterogeneity at a much finer scale than conventional whole-field scouting.

UAV-derived orthomosaics can reveal crop-row continuity, canopy gaps, border effects, and anomalous vegetation patches at centimeter-level resolution, while GIS provides the framework needed to organize, compare, and translate those raster products into management-oriented spatial layers. Recent studies show that UAV imagery can support both vegetation-index-based interpretation and AI-assisted weed classification, although performance still depends strongly on crop stage, image resolution, and the degree of spectral similarity between the crop and the weeds (Yu et al., 2022; Shahi et al., 2023; Alirezazadeh et al., 2024). The scientific rationale for this approach lies in the fact that weeds are rarely distributed uniformly across a field. Instead, infestations tend to form spatially explicit patches influenced by field margins, traffic lines, emergence variability, and local soil conditions. In such circumstances, uniform herbicide application may be less efficient than an approach that first identifies anomaly zones and only then applies targeted intervention. This logic is central to

site-specific weed management and has been repeatedly emphasized in recent reviews dealing with sustainable weed control and precision agriculture (Monteiro & Santos, 2022; Gerhards et al., 2022).

The present study evaluates an integrated UAV-GIS workflow for early weed screening and site-specific management in experimental arable plots under climate-variability conditions. The final update focuses on the Hârșova orthophotoplan set acquired in the Topzon-supported campaign and interpreted within the broader research context of INSAC-AGRI and ADER 2.1.4. The objective was to assess whether high-resolution orthomosaics, multispectral layers, and zonation products could reliably delineate probable weed-prone sectors, describe their spatial distribution at plot level, and support more precise intervention decisions than a uniform parcel-wide approach.

MATERIALS AND METHODS

The study was carried out in experimental arable plots from Romania, with the detailed image-based update referring to the Hârșova parcel (Figure 1). For the final interpretation, the image set included RGB orthomosaics dated 1 April 2024 and 29 September 2024, grayscale multispectral orthomosaics representing the green, red, red-edge, and near-infrared bands, as well as derivative products including TGI, VARI, and a parcel zonation map. The Hârșova orthophotoplans were acquired at approximately 20 m above ground level using nadir image capture, which provided sufficient spatial resolution to observe plot boundaries, canopy continuity, and localized anomaly patches at the scale required for experimental weed screening. UAV-based weed-detection studies similarly rely on high-resolution orthomosaics to separate crop, soil, and anomalous vegetation before or around early canopy development stages (Yu et al., 2022; Nikolić et al., 2021).

The image-processing workflow followed the logic of a Pix4D-GIS pipeline. Orthomosaics and spectral layers were generated and exported as georeferenced plot-level products, after which the raster layers were interpreted in a GIS environment together with experimental plot boundaries. RGB imagery was used to assess visible canopy continuity, parcel-edge effects,

and the geometry of the experimental strips. The multispectral layers and derived TGI, VARI, and zonation products were then compared in order to identify sectors with persistent canopy heterogeneity or abnormal vegetation signatures. This procedure follows the broader recommendation from recent precision-weed-management literature that spectral indices should be interpreted together with spatial structure and agronomic context rather than as stand-alone evidence of weed occurrence (Monteiro & Santos, 2022; Gerhards et al., 2022; Alirezazadeh et al., 2024).

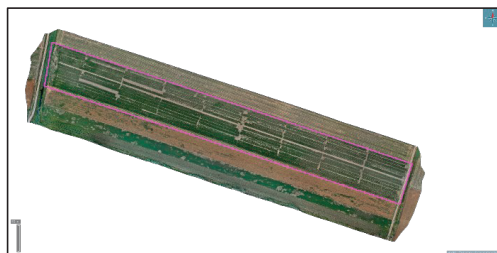


Figure 1. Experimental layout of the Hârșova parcel derived from UAV orthomosaic imagery, showing the delineated plots used for parcel-level interpretation and zonation

In the present update, weed analysis was performed as image-based anomaly mapping. Accordingly, low-vigour and heterogeneous zones were interpreted as probable weed-pressure areas only when comparable spatial signals were visible across several outputs, namely the dated RGB orthomosaics, the TGI/VARI layers, and the final zonation raster. Species-level weed identification was not inferred directly from the images alone. Instead, the workflow was designed as a screening and prioritization step for site-specific field inspection, which is consistent with both rule-based and AI-assisted weed-mapping studies that emphasize the importance of verification under real field conditions (Shahi et al., 2023; Alirezazadeh et al., 2024). For the zonation product, the 20 delineated experimental plots were treated as the basic analytical units. Pixel shares of the main vigour classes were summarized at parcel level to distinguish the more homogeneous sectors from the more heterogeneous ones. A conservative hotspot threshold was defined as the low-to-poor classes, while a broader scouting threshold also

included the moderate class. This classification logic is appropriate for site-specific weed management because image-derived maps are used first to direct scouting and then, if agronomically confirmed, to support localized mechanical or chemical intervention instead of blanket treatment (Gerhards et al., 2022; Monteiro & Santos, 2022; Nikolić et al., 2021).

RESULTS AND DISCUSSIONS

The uploaded orthophotoplans indicate that vegetation heterogeneity in the Hârşova parcel was spatially concentrated rather than uniformly distributed (Figures 2, 3). The dated RGB orthomosaics show stronger non-crop vegetation pressure on the north-western and western side of the experimental strip than along the opposite border. Inside the experimental layout, the central and lower sections of the parcel appeared visually more uniform, whereas the upper headland block and the transition zone between the narrow upper strips and the main plot sequence displayed the clearest canopy irregularities, border effects, and probable reinfestation pressure from adjacent unmanaged areas.

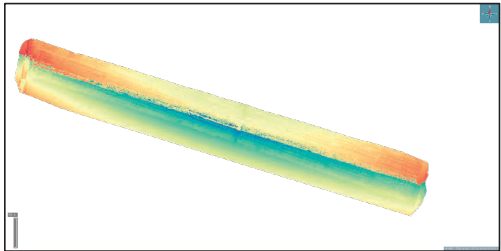


Figure 2. TGI-based visualization of canopy heterogeneity and border-related anomaly zones in the Hârşova experimental parcel

The parcel-based zonation provided the clearest quantitative summary of this pattern. Across the 20 delineated plot interiors, 45.4% of pixels were assigned to the high-vigour class and 32.5% to the good-vigour class. The moderate class represented 17.5% of the mapped interior, while the conservative hotspot classes (low, very low, poor, and very poor) summed to 4.6%. Under a strict interpretation, therefore, only a small fraction of the parcel behaved as a strong anomaly hotspot; under a scouting-oriented interpretation that also includes the moderate

class, 22.1% of the parcel should be treated as a priority zone for field checking.

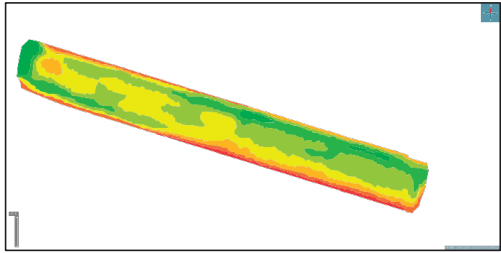


Figure 3. Parcel zonation derived from UAV imagery, highlighting high-, moderate-, and low-vigour sectors used to prioritize scouting and localized weed-management decisions

Table 1. Image-based zonation summary of the Hârşova experimental parcel

Indicator	Value	Interpretation
Delineated experimental plots	20	Parcel-level units used in the zonation analysis
High-vigour class	45.4%	Stable canopy sectors
Good-vigour class	32.5%	Generally acceptable crop performance
Moderate class	17.5%	Parcels/sectors that should be prioritized for scouting if a broader screening threshold is used
Low-to-poor classes	4.6%	Conservative hotspot estimate for probable weed/stress anomalies
High + good classes	77.9%	Majority of the parcel does not support blanket treatment logic
Plots with >80% high + good classes	11/20	Predominantly uniform plots
Plots with >5% low-to-poor share	7/20	Localized heterogeneity requiring closer field checking
Upper block high + good / moderate / low-to-poor	60.0% / 33.8% / 6.2%	More heterogeneous sector, likely influenced by headland and border effects
Main block high + good / moderate / low-to-poor	82.0% / 13.8% / 4.2%	More stable sector with fewer strong anomaly zones

The internal distribution of anomaly classes was also informative. The upper five plots were notably more heterogeneous than the remaining fifteen plots. In the upper block, the combined high-to-good classes accounted for 60.0% of the mapped interior, the moderate class accounted for 33.8%, and the low-to-poor classes accounted for 6.2%. In the main plot block, by contrast, the high-to-good classes reached 82.0%, the moderate class decreased to 13.8%, and the low-to-poor classes accounted for 4.2%(Table 1). At whole-plot level, 11 of the 20

plots were dominated by more than 80% high-to-good classes, and only one plot exceeded 10% low-to-poor classes. This indicates that the principal weed- or stress-prone sectors were concentrated in the upper headland and transition areas rather than across the entire parcel. The TGI, VARI, and zonation outputs supported the same agronomic interpretation. TGI emphasized stronger contrast along parcel borders and around the north-western external vegetation source. The zonation image showed that red-to-orange anomaly classes were concentrated mainly outside the parcel or immediately adjacent to parcel margins, while green classes dominated the interior of most plots. This is consistent with the broader literature showing that UAV-based weed screening performs best when crop–weed and crop–soil contrast remains visible and becomes more uncertain when crop and weed signatures start to overlap or when other stress factors produce similar spectral responses (Yu et al., 2022; Shahi et al., 2023; Alirezazadeh et al., 2024).

An important consequence for interpretation is that the mapped anomaly classes should not be equated automatically with botanical weed identity. Some discontinuities visible in the uploaded products may also reflect local emergence variability, traffic-induced compaction, moisture gradients, or border competition. Even so, the orthophotoplans clearly support a site-specific management logic, because they show that the parcel does not require uniform treatment. The image evidence instead prioritizes the upper parcel sector, the border-adjacent strips, and the transition zone as the most likely targets for intensified scouting and, if field confirmation supports it, localized intervention. This conclusion is fully aligned with the recent literature on precision weed management, which links spatial targeting to lower input consumption and more sustainable weed control (Monteiro & Santos, 2022; Gerhards et al., 2022).

The practical relevance of this interpretation is strengthened by comparable UAV-based studies that have moved from weed detection to prescription-map generation. In maize, for example, Nikolić et al. (2021) combined UAV imagery with VARI- and ANN-based classification and reported sprayed-area

reductions ranging from 42.43% to 87.82% for ANN and from 65.29% to 93.35% for VARI, depending on decision threshold. Although the present Hârşova dataset was interpreted conservatively and without claiming species-level classification, it points in the same direction: localized anomaly mapping can support targeted scouting and potentially reduce the need for blanket herbicide application.

The agronomic significance of these findings lies in the potential to support site-specific weed management rather than uniform field-wide spraying. By identifying where intervention is needed, the proposed workflow can reduce unnecessary agrochemical use, lower operational costs, and improve the environmental performance of crop protection strategies. This direction is consistent with both recent scientific literature and the goals of current precision agriculture demonstrative projects. At the same time, the results emphasize that vegetation indices should be interpreted within a broader agronomic and spatial framework, because weed pressure is only one of several drivers of spectral variability in arable fields. For that reason, the most robust decision-support strategy combines UAV imagery, GIS, and field validation in an iterative workflow.

CONCLUSIONS

The updated orthophotoplan analysis substantially strengthens the article because it replaces generic statements with parcel-specific evidence. In the Hârşova experimental parcel, image-based zonation showed that 77.9% of plot interiors belonged to high-to-good vigour classes, while the conservative low-to-poor hotspot classes represented only 4.6% of the mapped area. The strongest anomalies were concentrated in the upper headland block, the transition zone between plot groups, and the external parcel margins. Under these conditions, UAV-GIS integration clearly supports a site-specific management strategy rather than a uniform parcel-wide intervention.

The results also confirm that UAV products are most valuable when used as a first-line decision-support tool. The orthophotoplans, multispectral layers, and zonation output made it possible to rank sectors by probable weed or canopy-stress risk, thereby improving the efficiency of field

scouting. At the same time, the image evidence should be interpreted conservatively, because canopy anomalies may also arise from emergence variability, compaction, or local moisture effects. For that reason, the strongest scientific formulation is that the workflow identifies probable weed-prone zones and supports targeted agronomic verification, not that it alone provides definitive botanical diagnosis.

From an applied perspective, the present update supports the broader conclusion that UAV–GIS workflows can contribute to climate-resilient and lower-input crop protection. By concentrating attention on a relatively small proportion of the parcel, the method creates the basis for patch treatment, variable-rate application, or intensified local monitoring, all of which are central objectives of precision weed management and sustainable weed-control systems (Monteiro & Santos, 2022; Gerhards et al., 2022; Nikolić et al., 2021).

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USE OF GENERATIVE ARTIFICIAL INTELLIGENCE (GenAI)

The authors declare the use of Generative Artificial Intelligence (GenAI) tools in the preparation of this manuscript. GenAI was used exclusively for language editing and text refinement in the Introduction and Conclusions sections. All scientific content, data analysis, and interpretations were developed and verified by the authors.

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STUDY OF SENSITIVITY TO DIFFERENT CONCENTRATIONS OF KCl IN SAMPLES OF THE SPECIES *Aegilops geniculata* Roth

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Abstract

The study investigated the sensitivity of *Aegilops geniculata* Roth to salinity stress from varying concentrations of potassium chloride (KCl) during seed germination and early seedling development. The research utilized five accessions of *Aegilops geniculata* Roth sourced from the Genebank of Bulgaria's working collection. A completely randomized experimental design was employed, incorporating seven KCl concentrations (0 mM - control, 50, 100, 150, 200, 250, and 300 mM), with two replicates per treatment. Results revealed significant adverse effects on germination rates and seedling traits, except for an extended germination period under saline conditions. Notably, shoot growth was more hindered by high salinity than root development, particularly at 300 mM KCl. At this concentration, all genotypes except BGR43677 failed to germinate or sustain shoot and root growth. In contrast, the BGR43677 genotype exhibited exceptional salt tolerance, maintaining viable germination and promoting both shoot and root growth under extreme salinity. These findings underscore the variability in salt tolerance among *A. geniculata* accessions and highlight BGR43677 as a promising candidate for further study in saline environments.

Key words: *Aegilops geniculata* Roth, salinity, KCl, sensitivity, germination, seedling growth.

INTRODUCTION

Salinity is a major environmental stress factor affecting plant growth and productivity worldwide. It is estimated that over 20% of irrigated land globally is affected by salinity, resulting in significant economic losses (Machado et al., 2017; Singh et al., 2024). Salinization arises primarily from natural processes such as weathering of rocks and deposition of salts by wind and rain, but is often exacerbated by human activities like irrigation with saline water, inappropriate land management practices, and fertilizer overuse (Tarolli et al., 2024). The accumulation of salts in the soil disrupts plant water uptake, ionic balance, and nutrient acquisition, leading to physiological and biochemical imbalances that ultimately limit growth and survival (Balasubramaniam et al., 2023; Atta et al., 2023; Zhou et al., 2024).

Aegilops geniculata Roth, commonly known as ovate goatgrass, is an annual grass native to the Mediterranean region, now naturalized in many

parts of the world, including North America. It is often found in disturbed habitats, agricultural fields, and rangelands (Winston et al., 2014). Its adaptability to a wide range of environmental conditions suggests some level of tolerance to stress, including salinity (Guo et al., 2022). Understanding the salinity tolerance of *Aegilops geniculata* Roth is important for several reasons:

- ✓ Ecological Implications: Salinity levels can influence the distribution and abundance of *Aegilops geniculata* Roth in different environments. Knowledge of its tolerance limits can help predict how this species will respond to increasing salinization in various habitats;
- ✓ Agricultural Management: *Aegilops geniculata* Roth can be a problematic weed in wheat fields. Understanding its salinity tolerance relative to wheat can inform management strategies aimed at minimizing its impact;
- ✓ Genetic Resources: *Aegilops* species are closely related to wheat and represent a valuable source of genetic diversity for improving wheat's tolerance to abiotic

stresses, including salinity (Darko et al., 2020). Studying the salinity response of *Aegilops geniculata* Roth may provide insights into potential genes or mechanisms that can be transferred to wheat.

Salinity stress is complex, involving osmotic effects, ionic toxicity, and nutritional imbalances (Farooq et al., 2017; Zhao et al., 2020; Maryum et al., 2022). Sodium chloride (NaCl) is the most abundant salt in saline soils, but other salts, such as potassium chloride (KCl), magnesium sulfate (MgSO₄), and calcium chloride (CaCl₂), can also contribute significantly to the overall salinity (Waisel, 1972). While NaCl is often the focus of salinity studies, the specific effects of other salts, such as KCl, on plant growth and development are less well understood. Potassium (K⁺) is an essential macronutrient for plants, playing crucial roles in enzyme activation, osmoregulation, and protein synthesis (Hasanuzzaman et al., 2018; Papadakis et al., 2023; Islam et al., 2025). However, high concentrations of K⁺ can be toxic, disrupting nutrient uptake and cellular functions (Pantha et al., 2023).

This study aims to examine how varying concentrations of potassium chloride (KCl) influence the germination and early growth stages of *Aegilops geniculata* Roth, assessing the species' sensitivity to salinity stress under controlled conditions.

MATERIALS AND METHODS

The study was performed on five Bulgarian populations of *Aegilops geniculata* Roth collected in 2024. A fully randomized layout was adopted, comprising seven KCl treatments, each replicated twice. The KCl concentrations applied were 0 mM (control), 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 300 mM, prepared by dissolving analytical grade potassium chloride in distilled water.

Germination Test

For each treatment, two replicates of 50 seeds were surface sterilized (1 % NaOCl for 5 min, followed by rinses with distilled water) and placed on a double layer of filter paper moistened with 20 mL of the corresponding KCl solution. Petri dishes were sealed in plastic bags

to minimise evaporation and incubated in a growth chamber set at 20°C, 16 h light/8 h dark. Germination was recorded daily for 14 days; a seed was scored as germinated when the radicle extended ≥ 2 mm. The following germination parameters were calculated: Final Germination Percentage (GP), Germination Rate Index (GRI) and Mean Germination Time (MGT) (Kader, 2005).

Seedling Growth Assessment

After 14 day germination period, ten seedlings were randomly chosen from each replicate to evaluate the following growth traits: Root length (measured from the radicle emergence point to the tip of the longest root (mm) with a ruler), Shoot length (measured from the coleoptile emergence point to the tip of the longest leaf - mm), Fresh biomass (fresh weight of shoots and roots recorded immediately after harvest using an analytical balance - mg), Dry Biomass (shoots and roots were oven dried at 80°C for 24 h until constant weight, then weighed - mg). Relative dry weight and seedling height reduction was quantified by Kandil et al. (2012).

Statistical Treatment

All data were subjected to analysis of variance (ANOVA) with SPSS v.22. Treatment means were separated using Duncan's multiple range test at a 5 % significance level.

To elucidate genotype performance across the salinity gradient and to identify the most tolerant accession, the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI) were computed as described by Pour Aboughadareh et al. (2019).

RESULTS AND DISCUSSIONS

The germination of *A. geniculata* Roth seeds was profoundly inhibited by increasing concentrations of KCl, demonstrating a clear dose-dependent response that was statistically significant ($p < 0.05$) (Table 1). As KCl concentration rose, both the percentage and rate of germination declined markedly across all tested seed accessions, while the mean germination time (MGT) increased, indicating not only reduced germination success but also a substantial delay in the initiation and progression of germination under salt stress.

Table 1. Influences of KCl salinization on germination characteristics in five populations of *Aegilops geniculata* Roth

Accession number	Concentration of KCl, mM	GRI, % day ⁻¹	MGT, day	GE, %	G, %
BGR43663	0	35.83b	2.31a	80.00c	80.00b
BGR43677	0	38.50b	2.21a	80.00c	80.00b
BGR43704	0	35.00b	2.37a	80.00c	80.00b
BGR43496	0	43.06b	3.75b	60.00b	85.00b
BGR43682	0	20.83a	2.12a	40.00a	40.00a
Average		34.65	2.56	68.00	73.00
BGR43663	50	30.88b	3.19c	70.00b	80.00bc
BGR43677	50	24.17b	2.85bc	65.00b	65.00b
BGR43704	50	32.50b	2.62ab	80.00b	80.00bc
BGR43496	50	33.08b	3.29c	75.00b	85.00c
BGR43682	50	13.33a	2.33a	30.00a	30.00a
Average		26.79	2.86	64.00	68.00
BGR43663	100	18.33b	4.27b	70.00b	75.00c
BGR43677	100	20.12b	3.36a	50.00b	55.00b
BGR43704	100	31.67c	3.12a	60.00b	80.00cd
BGR43496	100	23.76b	4.54b	60.00b	85.00d
BGR43682	100	2.00a	5.00b	10.00a	10.00a
Average		19.18	4.06	50.00	61.00
BGR43663	150	14.91c	4.78b	60.00d	70.00d
BGR43677	150	8.84b	5.00b	15.00b	35.00b
BGR43704	150	20.45d	4.75b	50.00d	80.00e
BGR43496	150	10.66bc	4.91b	35.00c	50.00c
BGR43682	150	0.00a	0.00a	0.00a	0.00a
Average		10.97	3.89	32.00	47.00
BGR43663	200	4.00ab	7.66c	0.00a	30.00b
BGR43677	200	5.05ab	5.25b	10.00a	25.00b
BGR43704	200	17.68c	4.87b	30.00b	60.00c
BGR43496	200	9.82b	7.50c	10.00a	70.00c
BGR43682	200	0.00a	0.00a	0.00a	0.00a
Average		7.31	5.06	10.00	37.00
BGR43663	250	2.36b	8.50c	0.00	20.00b
BGR43677	250	2.80b	6.83b	0.00	20.00b
BGR43704	250	6.43c	6.25b	0.00	40.00c
BGR43496	250	0.00a	0.00a	0.00	0.00a
BGR43682	250	0.00a	0.00a	0.00	0.00a
Average		2.32	4.32	0.00	16.00
BGR43663	300	0.00	0.00	0.00	0.00
BGR43677	300	1.43	9.47	0.00	10.00
BGR43704	300	0.00	0.00	0.00	0.00

germination (G, %), germination rate index (GRI, % day⁻¹), mean germination time (MGT, day)

The mean values in the same column followed by the same letters do not differ significantly at $p \leq 0.05$ according to Duncan's test.

Under optimal, non-stressful control conditions (0 mM KCl), germination performance was strongest, with the BGR43496 accession achieving the highest germination percentage at 85% and a germination rate index of 43.06% per day - values significantly superior to those of the poorest-performing accession, BGR43682. However, as salinity intensified, this advantage diminished progressively. At 200 mM KCl, germination was severely suppressed, with BGR43682 exhibiting complete inhibition (0% germination), whereas BGR43496 still maintained a relatively resilient 70% germination rate. The inhibitory effects escalated at 250 mM KCl and approached near-

total suppression at 300 mM, where only a single accession, BGR43677, managed to germinate, and even then only at a minimal level of 10%. Concomitantly, salt stress drastically slowed the germination dynamics: MGT extended from a rapid 2.12 days in the control to a markedly delayed 9.47 days at 300 mM KCl, underscoring the physiological constraints imposed by high salinity. These findings highlight the sensitivity of *Aegilops geniculata* seeds to potassium chloride-induced osmotic and ionic stress and reveal notable inter-accession variability in salt tolerance, suggesting potential for selecting more resilient genotypes in saline environments (Prasad et al., 2018; Solangi et al., 2018; Tebini et al., 2022; Bibi et al., 2025).

Elevated concentrations of potassium chloride (KCl) exert a pronounced inhibitory effect on seedling development, operating in a dose-dependent manner ($p < 0.05$), with escalating salinity progressively impairing both root and shoot elongation. Shoot growth is consistently more vulnerable to KCl stress than root growth across all genotypes evaluated, reflecting a differential response to osmotic and ionic imbalances (Shoukat et al., 2020; Gul et al., 2024; Raneenga et al., 2024). As outlined in Table 2, varying KCl concentrations elicit substantial divergence in root height reduction (RHR), shoot height reduction (ShHR), and overall seedling height reduction (SHR), underscoring the complex interplay between salinity and genotype-specific resilience. At 50 mM KCl, a moderate stress threshold, genotype BGR43704 exhibits the smallest reductions in RHR, ShHR, and SHR, suggesting a relatively robust adaptation to early salinity stress. However, as KCl concentration escalates to 100 mM, root growth inhibition surges to an average of 42%, while shoot elongation plummets by 49%, with BGR43677 registering the highest reduction- albeit not significantly distinct from BGR43704. This dynamic shifts further under 150 mM KCl, where root growth inhibition spans 32% to 79% (mean: 59%), paralleled by a starkly broader shoot inhibition range of 31% to 100%. BGR43677 demonstrates the least suppression, signalling its emergent resilience, whereas BGR43682 emerges as the most sensitive genotype.

Table 2. Variation in the degree of reduction in the length of roots, shoots and seedlings at different levels of KCl salinity in populations of the species *Aegilops geniculata* Roth

Number of accession	Concentration of KCl, mM	RHR	ShHR	SHR
BGR43663	50	0.35c	0.38c	0.37c
BGR43677	50	0.14ab	0.04a	0.09a
BGR43704	50	0.09a	0.01a	0.05a
BGR43496	50	0.24bc	0.25b	0.25b
BGR43682	50	0.22c	0.44c	0.38c
Average		0.23	0.23	0.23
BGR43663	100	0.45b	0.49b	0.47b
BGR43677	100	0.27a	0.17a	0.22a
BGR43704	100	0.29a	0.32a	0.30a
BGR43496	100	0.40b	0.58b	0.50b
BGR43682	100	0.66c	0.88c	0.79c
Average		0.42	0.49	0.46
BGR43663	150	0.51b	0.54b	0.53b
BGR43677	150	0.32a	0.31a	0.32a
BGR43704	150	0.62c	0.48b	0.55b
BGR43496	150	0.67d	0.94c	0.82c
BGR43682	150	0.79e	1.00c	0.91d
Average		0.59	0.66	0.63
BGR43663	200	0.74a	0.64b	0.68b
BGR43677	200	0.72a	0.35a	0.52a
BGR43704	200	0.78a	0.75b	0.77c
BGR43496	200	0.72a	0.95c	0.85c
BGR43682	200	1.00b	1.00c	1.00d
Average		0.80	0.74	0.77
BGR43663	250	0.82ab	0.79b	0.80b
BGR43677	250	0.74a	0.75a	0.75a
BGR43704	250	0.85b	0.92c	0.89c
BGR43496	250	0.83ab	1.00d	0.92c
BGR43682	250	1.00c	1.00d	1.00d
Average		0.85	0.90	0.88
BGR43663	300	0.90b	1.00b	0.96b
BGR43677	300	0.76a	0.79a	0.78a
BGR43704	300	0.90b	1.00b	0.95b
BGR43496	300	1.00c	1.00b	1.00c
BGR43682	300	1.00c	1.00b	1.00c
Average		0.92	0.96	0.94

RHR-root length reduction rate, ShHR-shoot length reduction rate, SHR-shoot length reduction rate
Mean values in the same column followed by the same letters are not significantly different at $p \leq 0.05$, according to Duncan's test.

Under extreme stress at 200 mM KCl, both BGR43677 and BGR43496 experience a 72% root length reduction, while BGR43682 undergoes complete root growth cessation, reflecting its inability to withstand high salinity. Shoot growth inhibition at this concentration varies markedly: BGR43677 experiences a modest 35% decline, in contrast to total inhibition in BGR43682. At the highest tested concentrations (250-300 mM KCl), BGR43677 exhibits 75% shoot suppression at 250 mM, yet

retains marginal viability, whereas BGR43496 and BGR43682 face total growth collapse. Across all salinity thresholds and measured parameters, BGR43677 consistently displays minimal growth reduction, cementing its status as the most salt-tolerant genotype under KCl-induced stress (Table 2). These findings highlight the critical role of genotype-specific mechanisms in mitigating salinity effects (Sadiq et al., 2024), with BGR43677 serving as a potential model for enhancing crop resilience in high-salinity environments.

KCl-induced salinity exerts a pronounced detrimental effect on the biomass accumulation of *Aegilops geniculata* seedlings, markedly decreasing the relative dry weight of shoots, roots, and the whole plant (Figure 1 and Figure 2). This decline can be attributed primarily to the combined action of ionic toxicity - resulting from excessive accumulation of potassium and chloride ions - and the accompanying osmotic stress that impairs water uptake and cellular metabolism. Among the genotypes examined, BGR 43677 displayed the greatest resilience under severe KCl salinity, maintaining the highest relative dry weights for roots, shoots, and whole seedlings relative to the other genotypes. These findings are consistent with previous reports documenting the inhibitory influence of elevated KCl concentrations on dry matter production across a range of species (Solang et al., 2014; Gebremedhn et al., 2020; Valchinova et al., 2025).

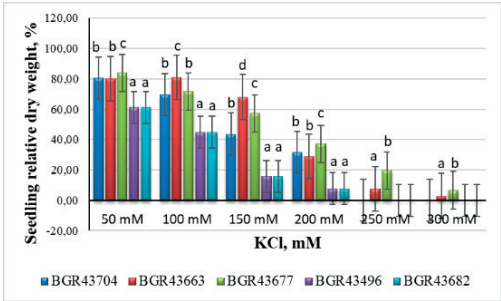


Figure 1. Variation of seedling relative dry weight in five accessions from *Aegilops geniculata* Roth at different levels of salinity stress

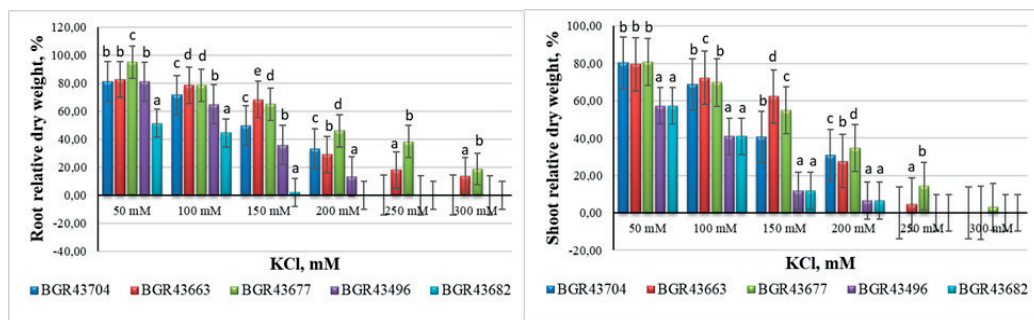


Figure 2. Variation of root and shoot relative dry weights in five accessions from *Aegilops geniculata* Roth at different levels of salinity stress

To pinpoint the genotype with the highest resilience to salinity, our analysis utilized two mathematical indicators: the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI).

Adapted from the research by Pour-Aboughadareh et al. (2019), these indices were calculated using shoot dry weight measurements obtained from both non-stress (normal) and salt-stress (saline) environments.

The SSI evaluates the degree of yield loss a genotype experiences under saline conditions, where smaller values denote superior tolerance. Conversely, the STI assesses a genotype's capacity to sustain high yields under stress, with larger values reflecting enhanced tolerance. Therefore, the genotypes demonstrating a combination of a low SSI and a high STI were identified as possessing the greatest capacity to withstand salinity.

According to the data presented in Table 3, which details the variation in these indices across different salinity levels, genotype BGR43663 emerged as particularly resilient under potassium chloride salinity concentrations ranging from 50 to 200 mM, demonstrating consistently low SSI and high STI values.

At more extreme salinity levels (250-300 mM), however, genotype BGR43677 exhibited the most favourable response, registering the lowest SSI values and relatively elevated STI values among the tested genotypes, suggesting a superior capacity to maintain physiological function and growth under intense salt stress.

Table 3. Variation in the Stress Susceptibility Index (SSI) and the Stress Tolerance Index (STI) at different levels of KCl salinity in populations of the species *Aegilops geniculata* Roth

Genotype	SSI	STI	Genotype	SSI	STI
50 mM KCl					
BGR43704	0.61	0.21	BGR43704	0.67	0.18
BGR43663	0.64	1.37	BGR43663	0.60	1.25
BGR43677	0.60	0.64	BGR43677	0.65	0.55
BGR43496	1.31	1.48	BGR43496	1.28	1.05
BGR43682	1.79	0.19	BGR43682	1.83	0.07
150 mM KCl					
BGR43704	0.90	0.11	BGR43704	0.85	0.08
BGR43663	0.57	1.08	BGR43663	0.89	0.48
BGR43677	0.68	0.44	BGR43677	0.80	0.27
BGR43496	1.34	0.31	BGR43496	1.15	0.17
BGR43682	1.52	0.00	BGR43682	1.23	0.00
250 mM KCl					
BGR43704	1.04	0.00	BGR43704	1.01	0.00
BGR43663	0.99	0.08	BGR43663	1.01	0.00
BGR43677	0.89	0.11	BGR43677	0.97	0.02
BGR43496	1.04	0.00	BGR43496	1.01	0.00
BGR43682	1.04	0.00	BGR43682	1.01	0.00
300 mM KCl					
BGR43704	1.04	0.00	BGR43704	1.01	0.00
BGR43663	0.99	0.08	BGR43663	1.01	0.00
BGR43677	0.89	0.11	BGR43677	0.97	0.02
BGR43496	1.04	0.00	BGR43496	1.01	0.00
BGR43682	1.04	0.00	BGR43682	1.01	0.00

CONCLUSIONS

This study provides valuable information on the sensitivity of *Aegilops geniculata* Roth to salinity stress, specifically to different concentrations of KCl, during the processes of germination and seedling growth. The findings indicate a significant negative correlation between the concentration of KCl and key growth metrics, including germination, germination speed, root development, and shoot elongation. In order to further elucidate the underlying physiological responses, future research endeavours should prioritise unravelling the molecular mechanisms driving the observed salt sensitivity, as well as assessing the species' performance under diverse saline conditions and composite salt regimes.

Furthermore, the identification of BGR43677 as a resilient genotype emphasises its potential as a prime candidate for salt-tolerant breeding initiatives, tailored to thrive in high-salinity environments.

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