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ASSESSMENT OF WET GLUTEN CONTENT BASED ON THE INTERACTION BETWEEN NITROGEN LEVEL OF FERTILIZATION AND WINTER WHEAT VARIETY CULTIVATED AT DUDEȘTII NOI, AN IMPORTANT AGRICULTURAL AREA OF ROMANIA

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Abstract

The objective of this study was to analyse the effect of variety, nitric, amoniacal nitrogen and level of fertilization for values of wet gluten content on winter wheat. The research was carried out in 2021-2023 and the method of planting was carried out in subdivided randomized blocks with three repetitions. The subject of the experiment consisted in testing twenty-seven modern winter wheat varieties with the experimental variants: 120, 150, 170 kg ha^{-1} a.s. nitric and amammoniacal N. Compared with the mean of the experience of 32.83% the highest value was obtained of Ciprian variety – 35.64%. The application of treatments on a chernozem soil weakly acidic with nitric N positively influenced this index were the highest values of 34.54%, 33.86% and 34.73% were obtained. In those fertilizated with ammonium N, the values obtained were below the experience. The results were discussed in view of classical statistics using ANOVA and The Student`s t-tests.

***Key words**: wheat quality, fertilization levels, nitrogen types, wet gluten content.*

INTRODUCTION

The history of wheat cultivation is as long as the history of civilization. Historical data tells us that wheat is the oldest cultivated plant. It is believed that its domestication took place in the Fertile Crescent about 10,000 years ago and was part of the Neolithic Revolution, when sapiens acquired enough knowledge of the surrounding world and discovered this plant in the spontaneous flora that could serve as food, a fact which represented "*a radical change*", "*full of revolutionary consequences for whole species*", a plant that then spread to all parts of the world through the first farmers, who adapted local populations to different climates (Venske et al., 2019; Dubcovsky et al., 2007).
Therefore, it has transformed from a simple wild herbaceous plant, apparently insignificant, to a ubiquitous one and continues to be the staple food, like a *Panis caelestis* for humanity. The first ancient forms of wheat, einkorn, emmer and spelt, not only played an important role as a food source, but became the ancestors of the modern species currently cultivated around the world, with *Triticum aestivum* L. which now accounts for about 95% of world production. The success of this plant is inextricably linked to the ability of the gluten protein fraction that allows flour to be processed to produce bread, other pastries, noodles and pasta (Ozkan et al., 2002).
For wheat, maximum nitrogen (N) uptake occurs at the elongation of the first internode and until the heading period. N accumulated during these growth stages is used primarily for production elements. N accumulated after tillering has little effect on yield, but increases protein content (Tabak et al., 2020).
Increased levels of nitrogen before the emergence of the first internode, at BBCH 30-31 growth stage, lead to increased number of spikelet's/ear and fertile flowers/spike (Guarda et al., 2004). Increasing nitrogen levels also has a positive effect on the number of fertile tillers/m^2 (Abedi et al., 2011). Applications at sowing lead to increased grain dry matter. Nitrogen uptake during grain filling is closely related to the amount of N applied and has no

substantial effect on filling duration (Brennan et al., 2014; Foulkes et al., 2009). Excess nitrogen applications, above the optimum level, can delay the growth and development of the ear and lead to drop because the internodes are greatly elongated and the leaf mass has a lush but useless development, also the ripening time of the wheat is delayed and leads to grain germination in the ear (Kadar et al., 2018). The application of high doses of N fertilizer does not always imply an increase in production yields; but on the contrary, it even leads to a decrease (Yin et al., 2019; Liu et al., 2021).

Nitrogen is absorbed from the soil mainly in two forms of ions, ammoniacal nitrogen (NH_4^+) and nitric nitrogen (NO_3^-), and their absorption constitutes approximately 70% of the total of cations and anions. Most of the nitrogen uptake is in the form of NO_3^- which moves from the soil solution into the plant root cells along with the water absorbed by it. NO_3^- is then either stored in vacuoles or reduced in the cytosol and plastids as NH_4^+ by nitrate and nitrite reductase activity. NH_4^+ can then be assimilated to produce more complex nitrogen-containing compounds. These compounds include chlorophyll which captures light during the photosynthesis process. Nitric nitrogen is first converted to ammonium because it is the only reduced form of N that plants can use to assimilate it into N-bearing amino acids. The assimilation process consists of two steps to convert nitrates to NH_4^+; in the first step, nitrate reductase converts NO_3^- to nitrite in the cytoplasm, and in the second step, nitrite is converted to NH_4^+ by nitrite reductase in the plastids (Chen et al., 2024; Wang et al., 2024). Nitric nitrogen is absorbed by plants and then becomes mobile in the xylem vessels. Xylem is the main element for long-distance transport of N from roots to N-metabolizing organs (Liu et al., 2021).

Xylem therefore transports NO_3^- from roots to leaves, and the phloem is the main transport pathway for N stored or assimilated in leaves and transported to other parts of the plant (eg from leaf to caryopses) (Lemaire et al., 2008). Nitrate is the substance that is mostly absorbed by wheat plants through transporters located on the plasma membrane of the root cells. The rate of this process is influenced by several factors, such as soil nitrogen availability, pH, and ion competition. N-assimilating enzymes that are closely related to the process of N uptake in roots include nitrate reductase (NR), nitrite reductase (NRi), glutamine synthetase (GS) and glutamate aminotransferase (GA). NO_3^- entering the cell is reduced by the process of NR to NO_2. NO_2 is toxic to cells and is rapidly transported into the plastid for further re-duction to ammonium by NRi. Ammoniacal nitrogen bypasses the process of conversion into amino acids and enters directly into the plant's metabolism (Chauhan et al., 2022).

Wheat is one of the most used crops in human nutrition. The quality wheat is deter-mined by a combination of several parameters. To determine the final quality of the product must be evaluated multiple characteristics of the grain, flour and the dough. The future of wheat production and quality must be coordinated by a considerable reduction of nitrogen losses. Reducing the use of nitrogen-based fertilizers without loss of quality and quantity of production might be achievable. Only one direction will provide the significant increase in productions and sustainability, is the effective management of nutrients applied to the wheat crop. Nitrogen in particular is a factor that has generated a lot of interest also due to the increase in its production cost in recent years. In addition, having a negative impact on the environment when the application is not carried out correctly and efficiently. Improving the efficient use of nitrogen, maintaining high wheat yields and im-proving baking indices are global aspirations, aspirations that are also the purpose of this research.

MATERIALS AND METHODS

The study was conducted in 2021-2023 in the Dudeștii Noi experimental fields (45°50′51″N 21°06′30″E, 87 m altitude, located in the Western Plain of Romania. The experimental field was located on a cambic chernozem soil, wet phreatic, slightly levigated, medium clay loam, developed on fluvial, carbonate, medium fine materials with a well-defined profile and with insignificant differences regarding the physical, hydric and chemical properties (Roman et al., 2011). The soil profile is characterized by the sequence of the following horizons: Ap (bioaccumulative mineral horizon

- anthropogenically disturbed), Am (bioacumultive mineral horizon – mollic), Bv (subsurface horizon - with accumulation of clay formed in situ and through weathering processes, Ck (mineral horizon not significantly affected by pedogenetic processes and not consolidated - with calcium carbonate accumulation). Ap horizon: 0-15 cm, gradual transition, dark gray brown clayey texture, small glomerular structure, plastic, weak adhesive, small pores, frequent roots, dry. Am horizon: 15-35 cm, gradual transition, dark brown loamy texture, medium glomerular structure, well developed, plastic, adhesive, small pores, weak compact, thin roots frequent. Bv horizon: 35-60 cm, gradual transition, dark gray-brown loamy texture, medium subangular polyhedral structure, plastic, adhesive, fine porous, moderately compact, sparse thin roots, dry. Ck horizon: 60-100 cm, gradual transition, yellowish brown light gray loamy texture, subangular and medium angular polyhedral structure well developed, plastic, adhesive, dry (Sala, 2008).

Physical and hydraulic properties: the loamy-clay texture - dusty in the first 70 cm and loamy-dusty in the rest, makes the apparent density (AD) medium throughout the profile of the soil, with 1.46 g/cm3 in the Ap horizon and 1.58 g/cm3 in Am. Total porosity (TP) is medium in Ap (48.8%) and high in Am (58.2%). Air porosity (AP) is medium in Ap (15.2%), high in Am (23.8%), medium in Bv (21.7%) and low in Ck (12.2%). The ranges of variation for clay are between 33.4-40.3%. The processed Ap horizon has a clay content of 36% compared to the Am horizon with a content of 40.3%. In horizons Bv and Ck, the amount of clay shows lower values (33.8%) and 33.4%, respectively. The soil is weakly to moderately compacted on the horizons below the tilled horizon. The values of the hydro-physical indices show different values, correlated with the physical properties. A medium value of the withering coefficient is noted in the Ap and Am horizons (11.7%), high in Bv (13.2%) and medium in Ck (12%) (Imbrea, 2014).

The field capacity (FC) is medium over the entire soil profile, with values of 23.3% in Ap, Am, Bv and 22.4% in Ck. Chemical properties were determined using the Kjeldahl and Olsen et al. methods (Dumitru et al., 2011) The soil has a humus content of 3.04% in the Ap horizon and 1.78% in the Am horizon, decreasing by 1.24% in the Bv horizon. It is medium supplied in total nitrogen, with 0.172% in Ap, very well supplied in Am with mobile phosphorus (168 ppm) and well supplied with mobile potassium (248 ppm). The pH was tested using the distilled glass electrode method, 1:2.5 soil and water. The reaction of the soil on the profile is weakly acid in Ap horizon 5,58, weakly alkaline 7,66. The amount of exchangeable bases is medium (23.2 in Ap, 24.8 in Am), and the degree of saturation in bases shows values of 92.62% which gradually increases on the Bv horizon. Trace element content is high for zinc (176 ppm) in the Ap and extremely low in the Bv horizon (0.40 ppm).

Climatic data during the period of the experiment were collected from the meteorological station of Dudeștii Noi and are represented in Figure 1. The climate of this area is temperate - continental with slight Mediterranean influences. The average annual temperature is 10.8°C and the warmest month was July. Temperatures were also extremely high for September and October. As can be seen, during the entire experimental period and especially during the vegetation months, the average monthly temperatures exceeded the multiannual averages, with the highest values during the vegetation period being recorded between June and September in all two experimental years.

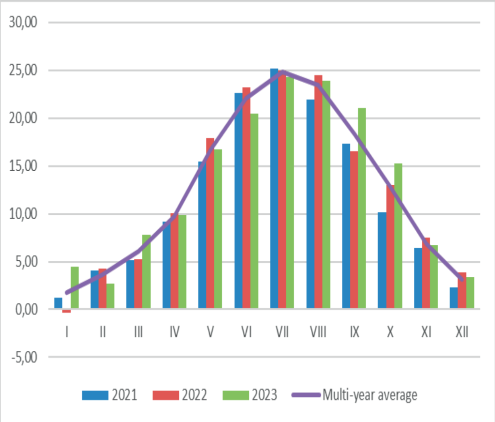

Figure 1. The average monthly temperatures (°C) recorded in Dudeștii Noi (2021-2023)

The average annual precipitation was frequently around 540 mm. In May and July, as a rule, the maximum pluviometric occurs (Figure 2). Sowing was carried out late, after successful application of a watering to help the suitable preparation of the field. The months from August until October were dry. These were registered against the background of acute lack of precipitation. The last three years, especially, have positioned the western area of Romania in an unprecedented position. The Western Plain of Romania, an important region of the country for wheat cultivation, has recently had to deal with long periods of very hot and dry weather and very wet and rainy periods with heavy rainfall in a short period of time. These climate events often exceed the relevant meteorological observations, some of which vary strongly from the multiyear mean values recorded to date.

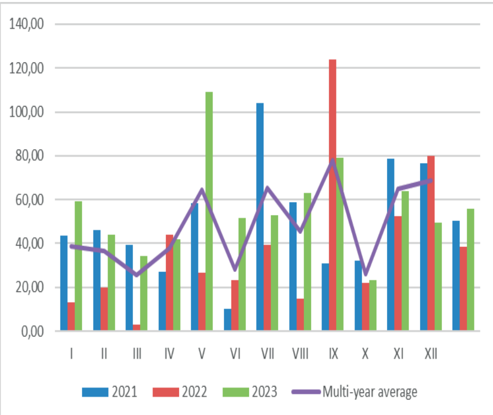

Figure 2. The average monthly rainfall (mm) recorded in Dudeștii Noi (2021-2023)

The layout of the experimental plan was done using the stratified randomization block method. Cultivars were factorially combined and arranged in completely randomized blocks. This experimental method was chosen in order to avoid the interfering effects of various environmental factors and to adequately and accurately estimate nitrogen utilization. The size of each plot was 1.2 m × 7 m, the distance between plots and adjacent blocks was kept at 0.5 m and 1.5 m, respectively, and the buffer strip was 2.5 m. Each block consisted of 27 plots with three replicates. The study is based on a trifactorial experiment, in subdivided plots, on the 27 × 3 type, with the following grading of the experimental factors:

FACTOR A - cultivated wheat variety:

a1 - Dacic, a2 - Miranda, a3 - Alex, a4 - Litera, a5 - Ciprian, a6 - Crişana, a7 - Biharia, a8 - Glosa, a9 - Bohemia, a10 - Sothys, a11 - Sacramento, a12 - Rubisko, a13 - Certiva, a14 - Aurelius, a15 - Aspect, a16 - Papillon, a17 - Activus, a18 - Centurion, a19 - Tika Taka, a20 - Chevignon, a21 - Sosthene, a22 - Vivendo, a23 - Sophie, a24 - Solindo, a25 - Tiberius, a26 - Arrezo and a27 - Apexus.

FACTOR B - types of fertilizers:

b1 - Ammoniacal nitrogen, b2 - Nitric nitrogen.

FACTOR C - fertilization level:

c1 - 120 kg/ha N a.s., c2 - 150 kg/ha N a.s., c3 - 170 kg/h N a.s.

The fertilizer consisted of two types of nitrogen (nitric and ammoniacal) that were applied in the form of ammonium sulfate and calcium nitrate, in three contrasting fertilization doses and administered to the plants in - a single dose, unfractionated, at the stage of growth BBCH 30-31. In both years of study, the sowing rate for each variety was established considering aspects related to the sowing rate such as purity, germination and the 1,000 kernel weight, and the density at sowing was 550 germinating grains/m^2. Wheat was sown at a depth of 4 centimeters with a row spacing of 12.5 cm. The treatments applied were V1 - nitric N dose of 120 kg/ha N a.s., V2 - nitric N dose of 150 kg/ha N a.s, V3 - nitric N dose of 170 kg/ha N a.s, V4 - ammoniacal N dose of 120 kg/ha N a.s., V5 - ammoniacal N dose of 150 kg/ha N a.s, V6 - ammoniacal N dose of 170 kg/ha N a.s. and the Cv - control variant was the average of the experimental field.

Plots were harvested mechanically at ripening, after reaching full grain maturity at 12% moisture content and the production for each plot was calculated automatically by the harvest machine by weighing the amount of grain yield. Harvesting was a complex process that was carried out in the shortest possible time without loss of product. The experimental lots were harvested mechanically, after reaching full maturity (BBCH 89-92), in which on the one hand the losses were the lowest, and on the other hand there were no difficulties in terms of keeping until performing quality tests. More specifically, it was not necessary to consume energy to bring the storage moisture. The harvest quality indices were monitored throughout the process and referred to grain losses, grain breakage and grain purity.

Wet gluten content was measured using the Perten Glutomatic System ac-cording to ISO 21415-2:2015, an apparatus system consisting of the Perten Glutomatic 2000 gluten washer, the Gluten Index 2010 centrifuge and the Glutork 2020 gluten dryer. The samples were grounded with a laboratory steel hammer mill fitted with a 200 µm sieve to produce a flour of the appropriate particle size for testing, and the flour was al-lowed to cool for one hour before being analyzed.

Principle of the method for obtaining wet gluten content: for each sample, 10 g of flour were grounded with a laboratory mill with a precision of 0.01 g. Then a dough was formed from the flour mass and 4.8 ml of NaCl solution which was mixed with the help of the aluminium paddles of the device for 20 seconds in the washing vessel equipped with a sieve with 88µ holes. The separation of protein substances in the form of wet gluten con-sists in washing with sodium chloride solution 20 g/l (2%), at a temperature of 22°C, used as a reagent of the formed dough. For the preparation of NaCl, distilled water was used so that the results would not suffer changes due to minerals or other substances with which the tap water could have been contaminated. The formed dough is placed in the washing vessel equipped with a fine sieve with holes of 88 microns and washed for 5 minutes with sodium chloride solution with a flow rate of 50-56 ml/minute. The gluten obtained in this way is placed in specially designed boxes in the centrifuge for whipping. The samples were processed for one minute, the centrifuge operating at 6,000 revolutions/ minute. The dough is removed with the help of a spatula and weighed with an accuracy of 0.01g and then the wet gluten is calculated with the formula *Wet gluten = mass of gluten × 10* (Hellemans, 2018).

In parallel, two determinations were performed for each analyzed sample.

Based on the results of two years' field experiment, wet gluten content parameter was calculated for optimal nitrogen dose and maximum wet gluten content of winter grain for that dose. First of all, we used the Analysis of variance (ANOVA), Student t tests which is an analysis tool used in statistics that splits an observed aggregate variability found inside a data set into two parts: systematic factors and random factors (Boldea et al., 2010).

RESULTS AND DISCUSSIONS

Gluten consists of 90% protein, 8% fat and 2% carbohydrate (Pronin et al., 2020). The gluten content is directly correlated with the protein content which is strongly influenced by pedoclimatic conditions and nitrogen fertilization. Wheat genotype is considered the most important factor influencing gluten quality characteristics (Simic et al., 2006). Total protein content of wheat correlates positively with gluten content (Shewry et al., 2002).

Gluten can be defined as the "*cohesive, visco-elastic protein material*" resulting as a by-product obtained by isolating starch from wheat flour. The gluten content of a flour depends on the climatic and technological conditions of the culture (especially the ad-ministration of nitrogen fertilizers during the vegetation period), as well as on the degree of extraction of the flour. A higher degree of extraction means a higher gluten content, because gliadins and glutelins are located in the endosperm, and through extraction the seed coats are removed. From a qualitative point of view, the gluten must be agglomerated, quite resistant and elastic. In no case should it deform, be soft, stringy or sticky. The quality of gluten is genetically determined, but it can be affected by excess moisture or the presence of pests. The minimum content of wheat gluten's flour should be about 24% - wet and 9% - dry (Singh et al., 2006). F test (Table 1, p column), shows that: A factor (variety), B factor (level of fertilization) and their interaction A × 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 × 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.

Table 1. Variation analysis

Variation source	SSP (SP)	Degree of Freedom	Weighed Least of Squares WSL (s^2)	F test for s^2 error		
				Value	P	Signification
A (variety)	851.56	26	32.8	9.2	0.000000	***
B (agrofund)	1254.28	5	250.9	70.3	0.000000	***
A×B	773.15	130	5.9	1.7	0.000152	***
Error	1155.89	324	3.6			
Total	4034.89					

*, **, 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 2. Student test for factor A (variety) - witness (Cv), average of the field

Variety	Wet gluten content (%)	Difference (%)	Signification
a1 - Dacic	34.42	1.60	*
a2 - Miranda	32.78	-0.04	
a3 - Alex	33.33	0.50	
a4 - Litera	33.64	0.81	
a5 - Ciprian	35.64	2.81	***
a6 - Crișana	34.20	1.37	*
a7 - Biharia	33.06	0.24	
a8 - Glossa	33.38	0.55	
a9 - Boema	33.91	1.09	
a10 - Sothys	31.39	-0.43	0
a11 - Sacramento	31.94	-0.88	
a12 - Rubisko	30.63	-2.20	000
a13 - Certiva	31.71	-1.12	
a14 - Aurelius	34.48	1.66	**
a15 - Aspekt	31.60	-1.23	
a16 - Papillon	30.94	-1.89	00
a17 - Activus	32.32	-0.51	
a18 - Centurion	30.47	-2.36	000
a19 - Tika Taka	34.69	1.86	**
a20 - Chevignon	33.48	0.65	
a21 - Sosthene	31.50	-1.33	0
a22 - Vivendo	32.14	-0.69	
a23 - Sophie	32.58	-0.25	
a24 - Solindo	32.55	-0.27	
a25 - Tiberius	34.21	1.38	*
a26 - Arrezo	33.59	0.76	
a27 - Apexus	31.72	-1.10	
Average	32.83	Cv	
LSD 5% = 1.240; LSD 1% = 1.632; LSD 0.1% = 2.081			

*, **, 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.

In conclusion, the null hypothesis H_0 is rejected for factor A (variety), factor B (level of fertilization) and the A × B interaction. To examine the differences between the different varieties, the differences between the agrofunds, as well as the interaction A × B (variety × agrofund) we apply the t-test and we will use as the error variant: $s^2 = 3.6$, degrees of freedom = 324.

The wet gluten content achieved using the 27 winter wheat varieties is shown in Table 2. Compared to the control - the average of the experience, the following values were obtained: significant in the varieties: Dacic, Crișana and Tiberius; distinctly significant in Aurelius and Tika Taka varieties; very significant in the Ciprian variety. It is worth noting that there were also varieties that obtained negative increases, that is, the values of the wet gluten content obtained in these varieties were lower than the average of the experience, the varieties Sothys and Sosthene having significant differences, Papillon with a distinctly significant difference and Rubisko and Centurion with very significant differences. The other varieties did not register differences. The highest values of this index were obtained by the varieties Ciprian - 35.64% and Tika Taka - 34.69%. Below the experience average, with the lowest values, the Rubisko - 30.63% and Centurion - 30.47% varieties were ranked.

Table 3. Student test for factor B (level of fertilizer) – witness (Cv), average of the field

Variant	Wet gluten content (%)	Difference (%)	Signification
V1 - 120 kg/ha N a.s nitric N	34.54	1.72	***
V2 - 150 kg/ha N a.s nitric N	34.73	1.91	***
V3 - 170 kg/ha N a.s nitric N	33.86	1.04	***
V4 - 120 kg/ha N a.s ammoniacal N	30.74	-2.09	000
V5 - 150 kg/ha N a.s ammoniacal N	31.26	-1.56	000
V6 - 170 kg/ha N a.s ammoniacal N	31.81	-1.02	000
Average	32.83	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. Student test for A × B interaction (variety × level of fertilization) - witness (Cv), average of the field

Variety	V 1		V 2		V 3		V 4		V 5		V 6	
	WG	Diff	WG	Diff	WG	Diff	WG	Diff	WG	Diff	WG	Diff
a1 - Dacic	36.32	3.49*	35.85	3.02	35.59	2.76	31.94	-0.89	31.84	-0.99	35.02	2.19
a2 - Miranda	36.01	3.18*	36.02	3.19*	32.70	-0.13	30.04	-2.79	31.62	-1.21	30.31	-2.52
a3 - Alex	35.48	2.65	36.00	3.17*	35.44	2.61	27.36	-5.47	31.98	-0.85	33.72	0.89
a4 - Litera	35.19	2.36	34.26	1.43	35.29	2.47	29.03	-3.80^{0}	35.89	3.06*	32.16	-0.67
a5 - Ciprian	38.32	5.49***	36.62	3.79*	36.40	3.58*	32.44	-0.39	34.92	2.09	35.12	2.29
a6 - Crișana	36.03	3.20*	34.40	1.57	36.34	3.52*	33.43	0.60	31.07	-1.76	33.93	1.10
a7 - Biharia	36.49	3.66*	37.41	4.58**	33.42	0.60	30.89	-1.94	30.06	-2.77	30.10	-2.73
a8 - Glossa	37.57	4.74**	34.96	2.13	35.47	2.64	29.94	-2.89	31.56	-1.26	30.77	-2.06
a9 - Boema	36.12	3.29*	35.73	2.90	35.81	2.98	32.30	-0.53	30.98	-1.85	32.56	-0.27
a10 - Sothys	34.04	1.21	33.95	1.12	34.09	1.27	29.67	-3.16^{0}	27.77	-5.06^{00}	28.83	-4.00^{00}
a11 - Sacramento	32.83	0.00	33.37	0.54	33.45	0.62	30.33	-2.50	30.63	-2.20	31.05	-1.78
a12 - Rubisko	31.58	-1.25	33.96	1.13	32.04	-0.79	29.62	-3.21^{0}	28.21	-4.62^{00}	28.38	-4.45^{00}
a13 - Certiva	33.40	0.57	35.39	2.56	30.19	-2.63	29.65	-3.18^{0}	30.60	-2.23	31.03	-1.80
a14 - Aurelius	34.68	1.85	36.45	3.62*	36.77	3.94*	32.56	-0.27	33.04	0.21	33.42	0.59
a15 - Aspekt	32.05	-0.78	32.62	-0.21	32.48	-0.34	30.27	-2.56	30.48	-2.35	31.71	-1.12
a16 - Papilon	33.75	0.92	33.16	0.33	32.05	-0.78	29.44	-3.39^{0}	29.28	-3.55^{0}	27.97	-4.86^{00}
a17 - Activus	34.47	1.64	32.88	0.05	33.06	0.23	31.43	-1.40	30.39	-2.44	31.71	-1.12
a18 - Centurion	31.79	-1.04	34.15	1.32	29.57	-3.25^{0}	28.28	-4.55^{00}	28.25	-4.58^{00}	30.77	-2.06
a19 - Tika Taka	36.18	3.35*	35.29	2.46	32.83	0.00	34.91	2.08	33.37	0.54	35.55	2.72
a20 - Chevignon	36.80	3.97*	34.65	1.82	34.00	1.17	32.11	-0.72	32.61	-0.22	30.73	-2.10
a21 - Sosthene	31.89	-0.94	32.90	0.07	35.74	2.91	28.33	-4.50^{00}	28.08	-4.75^{00}	32.08	-0.75
a22 - Vivendo	33.47	0.64	33.52	0.69	30.73	-2.09	32.95	0.12	31.02	-1.81	31.16	-1.67
a23 - Sophie	30.28	-2.55	34.31	1.48	34.12	1.29	32.23	-0.60	32.71	-0.12	31.84	-0.99
a24 - Solindo	33.44	0.61	35.14	2.31	32.65	-0.18	29.33	-3.50^{0}	32.68	-0.15	32.09	-0.74
a25 - Tiberius	37.34	4.51**	36.19	3.36*	36.79	3.96*	31.82	-1.01	31.49	-1.34	31.64	-1.19
a26 - Arrezo	34.49	1.66	35.16	2.33	34.15	1.32	31.43	-1.40	32.42	-0.41	33.89	1.06
a27 - Apexus	32.67	-0.16	33.51	0.68	33.20	0.38	28.30	-4.53^{00}	31.24	-1.59	31.41	-1.42
Average	32.83											
LSD 5% = 3.036; LSD 1% = 3.997; LSD 0.1% = 5.097												

*, **, 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 wet gluten content achieved using the nitric and ammoniacal nitrogen fertilization is shown in Table 3. Compared to the control variant (Cv)- the average of the experience, very significant increases were obtained at all of the treatments, regardless of the level or type of N fertilizer. The highest value of this index was obtained at V2 - 34.73, followed by V1 -

34.54% and V3 - 33.86%. It should be noted, however, that in the treatments applied with nitric nitrogen, the wet gluten content values were ranked above the experience mean, and in those performed with ammonium nitrogen, the values obtained are below the experience average of the two years of study.

From Table 4 it can be seen that compared to the control – the average of the experiment, the statistically assured wet gluten content values were both positive and negative, i.e. the values were both below and above the average of the experiment. The varieties whose wet gluten content values are higher than the average of the experiment are classified as follows:

- V1: Dacic, Miranda, Ciprian, Crișana, Biharia, Glossa, Boema, Tika Taka, Chevignon, Tiberius;
- V2: Miranda, Alex, Ciprian, Biharia, Aurelius, Tiberius;
- V3: Ciprian, Crisana, Aurelius, Tiberius;
- V5: Litera.

Influence of variety on wet gluten content: The wet gluten content values vary between 30.7% (Centurion variety) and 35.7% (Ciprian variety). The wet gluten content values for all other 25 varieties vary between 30.8 and 34.8%. The differences between varieties are highly significant ($p<0.001$), according to the F-test value.

Influence of agrofund on wet gluten content: The wet gluten content values vary between 30.8% (120 kg/ha a.s. ammoniacal nitrogen) and 34.7% (150 kg/ha a.s. nitric nitrogen). The differences between agrofunds are highly significant ($p<0.001$), according to the F-test value. The wet gluten content value has an upward trend from the first fertilization level of 120 kg/ha a.s. nitric nitrogen, at the second of 150 kg/ha a.s. nitric nitrogen, after which the trend is downward until the fertilization level of 170 kg/ha a.s. nitric nitrogen. In the case of fertilization with ammoniacal nitrogen, the trend is upward until the last fertilization level with ammoniacal nitrogen of 170 kg/ha a.s.

Influence of the A × B interaction on the wet gluten content: The highest value of this index was recorded for the Ciprian variety - 34.7% at the fertilization level of 150 kg/ha a.s. nitric nitrogen. And the lowest value was obtained by the Sosthene variety - 27.9% at the fertilization level of 150 kg/ha a.s. ammoniacal nitrogen.

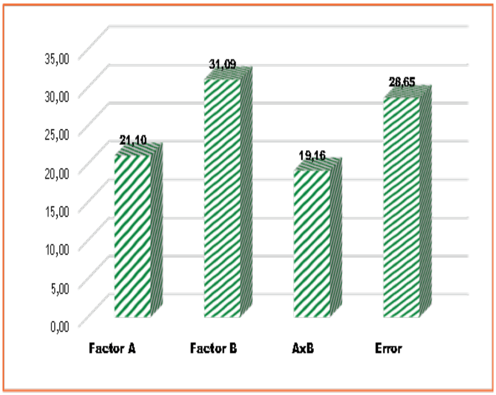

Figure 3. The contribution of factors and the interaction of factors on wet gluten content

Factor A - variety contributes to the formation of the wet gluten content index by 21.10%, factor B - fertilization system by 31.09%, and the interaction of the two mentioned factors A×B by 19.16%. The greatest contribution to the formation of the wet gluten content is made by factor B - fertilization system, followed by other factors that were not taken into account in this study, then by factor A - variety and by the interaction of factors A × B and with a very small difference.

CONCLUSIONS

The average of the two years of study for the wet gluten content is 32.83%. The highest values of this index were obtained by the varieties Ciprian - 35.64% and Tika Taka - 34.69%. Below the average of the experience, with the lowest values, the varieties Rubisko - 30.63% and Centurion - 30.47% were ranked. Regarding the fertilization levels, the highest value of 34.73 was obtained at agrofond 2, followed by agrofond 1 - 34.54% and agrofond 3 - 33.86%. The agrofonds in which fertilization was carried out with ammoniacal nitrogen were ranked below the average of the experience. Influence of the A × B interaction on the wet gluten content: The highest value of this index was recorded at the Ciprian variety - 34.7% at the fertilization level of 150 kg/ha a.s. nitric nitrogen. And the lowest value was obtained by the Sosthene variety - 27.9% at the fertilization level of 150 kg/ha of ammoniacal nitrogen.

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 pyrophosphorylase (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).

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REFERENCES

Abedi, T, Alemzadeh, A, Kazemeini, S.A. (2011) Wheat yield and grain protein response to nitrogen amount and timing. *Australian Journal of Crop Science*; 5:330-336.

Boldea, M., Sala, F., Radulov, I., Crista, F. (2010) A Mathematical Model on the Dependence between the Agricultural Production and Chemical Fertilizer. *AIP Conf. Proc*., 1281, 1363–1366.

Brennan, J., Hackett, R., McCabe, T., Grant, J., Fortune R.A., Forristal, P.D. (2014). The effect of tillage system and residue management on grain yield and nitrogen use efficiency in winter wheat in a cool Atlantic climate, *European Journal of Agronomy*; 54: 61-69, 1161-0301.

Chauhan, J., Chachad, S., Shaikh, Z., Khan, J., & Ali, A. (2022). Nitrate in Plant Physiology. *In Nitrate Handboo*k; (pp. 73–90). CRC Press.

Chen, J., Li, J., Li, W., Li, P., Zhu, R., Zhong, Y., Zhang, W., & Li, T. (2024). The optimal ammonium-nitrate ratio for various crops: A Meta-analysis. *Field Crops Research*; 307, 109240.

Dubcovsky, J, Dvorak, J. (2007). Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science*. 29;316(5833):1862-6.

Dumitru, M., Manea, A., et al. (2011). Chemical and microbiological analysis methods used in the soil monitoring system, 2nd ed.; Sitech Publishing House, Craiova, Romania; pp. 54-89.

Foulkes, M.J., Hawkesford, M.J., Barraclough, P.B., Holdsworth, M.J., Kerr, S., Kightley, S., Shewry, P.R. (2009). Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crops Research*, 114, 329–342.

Guarda, G., Padovan, S., Delogu, G. (2004). Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. *European Journal of Agronomy*; 21:2, 181-192.

Hellemans, T. (2018). Impact of crop husbandry practices and environmental conditions on wheat composition and quality: a view. *Journal of Agricultural and Food Chemistry*; 66:2491–2509.

Imbrea, F. (2014). Crop Science 1st ed.; Eurobit Publishing House, Timisoara, Romania, 2014; pp: 225-237

Kadar, R., Muntean, L., Racz, I., Ona, A., Ceclan, A., Hirişcău, D. (2018). The Effect of Genotype, Climatic Conditions and Nitrogen Fertilization on Yield and Grain Protein Content of Spring Wheat (*Triticum aestivum* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(2), 515–521

Kong, L., Zhang, Y., Zhang, B., Li, H., Wang, Z., Si, J., Fan, S., & Feng, B. (2022). Does energy cost constitute the primary cause of ammonium toxicity in plants? *Planta*; 256(3), 62.

Lemaire, G., Jeuffroy, M.H., Gastal, F. (2008). Diagnosis tool for plant and crop N status in vegetative stage. Theory and practices for crop N management. *European Journal of Agronomy*; 28:614–624.

Liu, D, Song, C, Fang, C, Xin, Z, Xi, J, Lu, Y. (2021). A recommended nitrogen application strategy for high crop yield and low envi-ronmental pollution at a basin scale. *Sci Total Enviro*n. 20;792:148464.

Liu, Y., Li, Y., Li, Y., Tian, Z., Hu, J., Adkins, S., & Dai, T. (2021). Changes of oxidative metabolism in the roots of wheat (*Triticum aestivum* L.) seedlings in response to elevated ammonium concentrations. *Journal of Integrative Agriculture*; 20(5), 1216–1228.

Lyu, X., Liu, Y., Li, N., Ku, L., Hou, Y., Wen, X. (2022). Foliar applications of various nitrogen (N) forms to winter wheat affect grain protein accumulation and quality via N metabolism and remobilization. *The Crop Journal;* 10(4),

Munteanu, I., Florea, N. (2012). Romanian System of Soil Taxonomy. 2nd ed.; Sitech Publishing House, Craiova, Romania, pp. 84-129.

Ozkan, H, Brandolini, A, Schäfer-Pregl, R, Salamini, F. (2002). AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey. *Mol Biol Evol*.; 19(10):1797-801.

Pronin, D., Börner, A., Weber, H., Scherf, K.A. (2020). Wheat (*Triticum aestivum* L.) Breeding from 1891 to 2010 Contributed to Increasing Yield and Glutenin Contents but Decreasing Protein and Gliadin Contents. *Journal of Agricultural and Food Chemistry*, 68, 13247–13256.

Ravier, C., Meynard, J.M., Cohan, J.P., Gate, P., Jeuffroy, M.H. (2017). Early nitrogen deficiencies favor high yield, grain protein content and N use efficiency in wheat. *European Journal of Agronomy*, 89, 16–24.

Roman, G. V., Tabără, V., Robu, T., Pârşan, P., Axinte, M., Ştefan, M., Morar, G., Cernea, S. (2011) Cereals and legumes for grains Crop Vol. I. 1st ed.; Editura Universitara Publishing House, Bucharest, Romania; pp: 45-48

Sala, F. (2008). Agrochemistry 2nd ed.; Eurobit Publishing House, Timisoara, Romania, pp: 123-145

Shewry, P.R., Halford, N.G., Belton, P.S., Tatham, A.S., (2002). The structure and properties of gluten: an elastic protein from wheat grain. *Philos Trans R Soc Lond B Biol Sci.* 28;357(1418):133-42.

Simic, G., Horvat, D., Jurkovic, Z., Drezner, G., Novoselovic, D., Dvojkovic, K., (2006). The genotype effect on the ratio of wet gluten content to total wheat grain protein. *Journal of Central European Agriculture*;7(1):13–18.

Singh, B., Singh, N. (2006). Physico-chemical, water and oil absorption and thermal properties of gluten isolated from different Indian wheat cultivars. *J. Food Sci Technol.* 43(3):251–255

Tabak, M., Lepiarczyk, A., Filipek-Mazur, B., Lisowska, A. (2020). Efficiency of nitrogen fertilization of winter wheat depending on sulfur fertilization. *Agronomy*, 10, 1304.

Venske, E., Dos Santos, R.S., Busanello, C., Gustafson, P., Costa de Oliveira, A. (2019). Bread wheat: A role model for plant domestication and breeding. *Hereditas*; 156:16;

Wang, F, Wang, Q, Yu, Q, Ye, J, Gao, J, Liu, H, Yong, JWH, Yu, Y, Liu, X, Kong, H, He, X, Ma, J .(2022). Is the NH4+-induced growth inhibition caused by the NH4+ form of the nitrogen source or by soil acidification? *Frontiers in Plant Science* 9;13:968707

Wang, C., Wu, G., Wang, H., Wang, J., Yuan, M., Guo, X., Liu, C., Xing, S., Sun, Y., Talpur, M.M.A. (2024). Optimizing Tomato Cultivation: Impact of Ammonium–Nitrate Ratios on Growth, Nutrient Uptake, and Fertilizer Utilization. *Sustainability*, 16, 5373.

Wang, F., Gao, J., Yong, J. W. H., Liu, Y., Cao, D., & He, X. (2020). Glutamate over-accumulation may serve as an endogenous indicator of tricarboxylic acid (TCA) Front. Plant Sci., Sec. Crop and Product Physiology, Volume 13 https://doi.org/10.3389/fpls.2022.968707

Yin, W., Xinyue, Z., Jian, C., Anji, C., Liying, W., Xiaoying, G., Yali, N., Shuoran, L., Guohua, M., Qiang, G. (2019). Reducing basal nitrogen rate to improve maize seedling growth, water and nitrogen use efficiencies under drought stress by optimizing root morphology and distribution, *Agricultural Water Management*, 212, 328-337