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GLUTEN AND PROTEIN CONTENT IN WHEAT GENOTYPES – COMPARATIVE ANALYSIS

Gabriela GORINOIU[1], Marinel Nicolae HORABLAGA[1, 2], Alina Laura AGAPIE[1], Petru RAIN[1], Cerasela PETOLESCU[2], Florin SALA[1, 2]

[1]Agricultural Research and Development Station Lovrin, 307250, Lovrin, Romania
[2]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, Timişoara, Romania

Corresponding author email: florin_sala@usvt.ro

Abstract

Gluten and protein content were analyzed in 25 wheat genotypes (Wg5026 to Wg5050). The study was conducted at ARDS Lovrin, during the agricultural year 2023 – 2024. The comparative crops were organized in randomized replicates. The gluten content (Glt) varied between Glt = 46.00±0.48% (Wg5027, Wg5029) and Glt = 54.00±0.48% (Wg5042). The protein content (Pro, %) varied between Pro = 24.20±0.56% (Wg5041) and Pro = 35.90±0.56% (Wg5035). A comparative analysis was used to find out the differences between genotypes in relation to the quality indices studied. The gluten increase (ΔGlt) was between ΔGlt = 0.92% and ΔGlt = 3.92% (Wg5036, Wg5042), and eight genotypes showed statistical safety. The protein increase (ΔPro) ranged from ΔPro = 0.04% to ΔPro = 7.24% (Wg5035), and nine genotypes showed statistical safety. According to PCA, PC1 explained 53.466% of variance, and PC2 explained 46.534% of variance. Cluster analysis grouped the genotypes based on similarity, and genotypes ranking was done, based on the quality indices considered. The results are valuable for genotype selection in the wheat breeding program, as well as for agricultural practice.

Key words: *breeding program, genetic potential, gluten, protein, wheat genotypes.*

INTRODUCTION

Gluten and protein in wheat grains are quality indices of high importance for the quality of flour, for the food industry, and the quality of finished products (Zilić et al., 2011; Schopf et al., 2021; Schuster et al., 2023).
Improving the nutritional properties of wheat grains is a major objective in wheat breeding programs (Guzman et al., 2016; Fradgley et al., 2023; Petrović et al., 2024).
The performance of wheat genotypes for yield, for the main quality indices, essential in relation to the finished products, but also the "genotype x environment" interaction proven for different cultivation areas, are important criteria for selecting valuable genotypes (Tanin et al., 2022; Petrović et al., 2024).
Testing wheat genotypes in multiple locations, with varying climate and soil conditions, is important to understand and explain the "genotype x environment" interaction, and for selecting performing genotypes for specific locations (Tanin et al., 2022; Petrović et al., 2024; Temizgul et al., 2024).
The need to identify wheat genotypes adaptable to environmental conditions and climate change has been analyzed and communicated in various studies (Gebrewahid et al., 2020; Takač et al., 2021; Javed et al., 2022; Dimitrov et al., 2023).
For certain "key traits" differentiated variability was recorded in wheat, in relation to genotype, crop location, and "genotype x location" interactions (Gebrewahid et al., 2020).
Agronomic traits, yield and quality indices were studied in different collections of wheat genotypes, and valuable genotypes, or groups of genotypes, were identified (Amiri et al., 2018; Thungo et al., 2020; Alemu et al., 2021; Mahdavi et al., 2022; Gheorghe and Nicolae, 2023; Temizgul et al., 2024).
In response to environmental and technological conditions, variations in quality indices and wheat yield have been recorded, in relation to water, nutrient supply, crop rotation or different inputs (Sala et al., 2016; Attafy et al., 2023; Hao et al., 2023; Ceclan et al., 2024; Yordanova et al., 2024). Associated with environmental conditions, interactions between

morphological parameters of wheat grains and certain quality indices, e.g. starch and protein, have also been recorded (Mahdavi et al., 2022).
Under current crop and technological conditions, comparative studies have been conducted between old and modern wheat genotypes (De Santis et al., 2017; Brouns et al., 2022). In relation to the quality indices considered, similarities and differences were recorded in wheat genotypes (e.g. in gluten index, protein content), and in relation to the quality of the finished products and certain dietary diets, interest was shown, and different genotypes were selected (De Santis et al., 2017; Abdelaleem and Al-Azab, 2021; Brouns et al., 2022)
Comparative studies have facilitated the selection of appropriate wheat genotypes in relation to the cultivation location (climate and soil conditions), the agricultural system (conventional, organic) and the quality of the finished products (Takač et al., 2021).
Various data analysis methods have been used to differentiate valuable wheat genotypes, producers, or other associated elements, in relation to specific quality and yield objectives (Alemu et al., 2021; Schopf et al., 2021; Javed et al., 2022; Tanin et al., 2022).
This study analyzed the gluten and protein content of wheat grains, in a collection of 25 wheat genotypes, to identify performing genotypes for the considered quality indices, with utility for the wheat breeding program, and for their recommendation in agricultural practice.

MATERIALS AND METHODS

The study, research, and field experiments were organized and conducted within the ARDS Lovrin. The field experiments were organized in the agricultural year 2023-2024. The climatic conditions during the study period are presented in Figure 1.

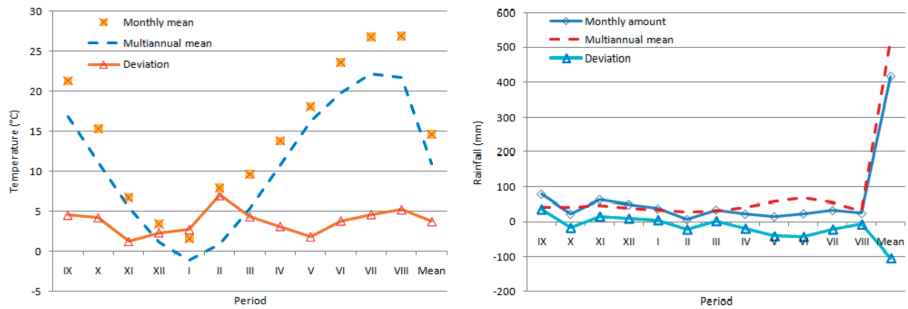


Figure 1. Climatic conditions in experimental area; temperature and precipitation values

Twenty-five wheat genotypes were considered and tested in comparative crops. The genotype names were in the format Wg5025 to Wg5050. Each genotype was cultivated in three replicates.
The field experiments were located in flat terrain conditions, medium fertility soil, chernozem type. The previous crop was field peas. The land was prepared for sowing by plowing, disking (two works), and combinator (two works). Sowing was done in the second decade of October 2023. Emergence was recorded in the first decade of November 2023.
Fertilization was done in the fall with complex fertilizer 15N:15P:15K at a dose of 180 kg/ha. In the spring, fertilization was completed with urea at a dose of 118 kg/ha.
Treatments were made with Omnera at a dose of 1 L/ha for weed control, and Inazuma for phytosanitary control in wheat crops.
Harvesting was done on each experimental variant (genotype and repetition) at maturity (Meier, 2001).
Subsamples were taken from the grain production of each genotype to determine the gluten (Glt, %) and protein (Pro, %). The analyses were made in the Wheat Breeding Laboratory, ARDS Lovrin.
According to the purpose of the study, the experimental data were analyzed in order to compare the genotypes tested for the two quality indices. The Anova Test (EXCEL),

Descriptive Statistical Analysis, t Test, Wilcoxon tests, Multivariate analysis, and Ranking were applied (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The mean values of the quality indices (Glt, Pro), and the calculated ratios (Glt/Pro, Pro/Glt) for the 25 studied wheat genotypes were analyzed by Anova Test (Alpha 0.05) and Descriptive Statistical Analysis for the general characterization of the experimental data. The presence of variance and statistical safety of the data were confirmed (Table 1).

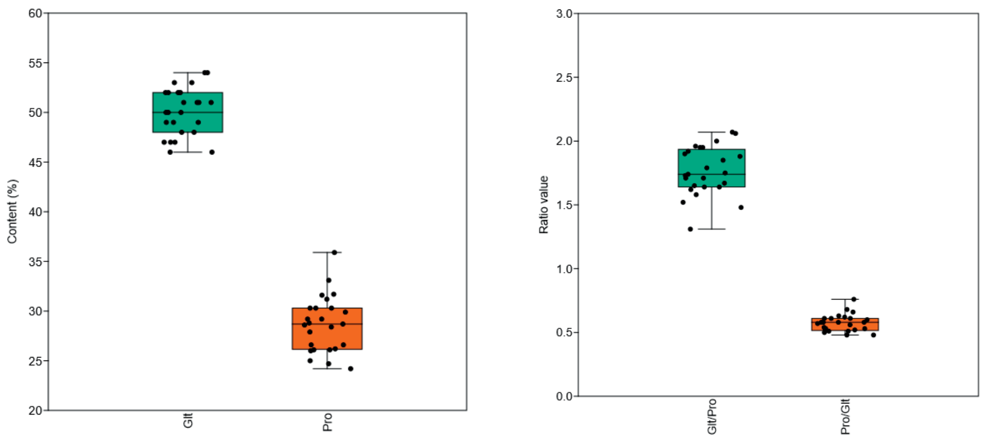
Table 1. Anova Test results

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	42238.15	3	14079.38	4073.143	5E-101	2.6994
Within Groups	331.8373	96	3.456638			
Total	42569.99	99				

In the case of gluten content, values between Glt = 46.00±0.48% were recorded for the Wg5027 genotype, and Glt = 54.00±0.48% for the Wg5029 and Wg5042 genotypes. In the case of protein content, values between Pro = 24.20±0.56% for the Wg5041 genotype, and Pro = 35.90±0.56% for the Wg5035 genotype were recorded (Table 2). In the case of the Glt/Pro ratio, the recorded values were between Glt/Pro = 1.31±0.04 in the Wg5035 genotype, and Glt/Pro = 2.07±0.04 in the Wg5041 genotype. In the case of the Pro/Glt ratio, the recorded values were between Pro/Glt = 0.48±0.01 in the Wg5041 genotype, and Pro/Glt = 0.76±0.01 in the Wg5035 genotype (Table 2). The graphical distribution in box-plot format of the recorded values for the indices and the calculated ratios is presented in Figure 2.

Table 2. Descriptive statistical analysis values for quality indices and calculated ratios for the studied wheat genotypes

Statistical Parameters	Glt	Pro	Glt/Pro	Pro/Glt
N	25	25	25	25
Min	46.00	24.20	1.31	0.48
Max	54.00	35.90	2.07	0.76
Sum	1252.00	716.60	44.08	14.34
Mean	50.08	28.66	1.76	0.57
Std. error	0.48	0.56	0.04	0.01
Variance	5.8267	7.9591	0.0363	0.0044
Stand. dev	2.41	2.82	0.19	0.07
Median	50.00	28.70	1.74	0.58
25 prcntil	48.00	26.15	1.64	0.52
75 prcntil	52.00	30.30	1.94	0.61
Skewness	-0.14031	0.53459	-0.31474	0.85446
Kurtosis	-0.98621	0.29252	-0.18694	1.07475
Coeff. var	4.82	9.84	10.80	11.63



(a) (b)

Figure 2. Distribution of data series for quality indices (a), and calculated ratios (b), for the studied wheat genotypes

The results regarding gluten content showed variability at the level of CV = 4.82, and protein content showed variability at the level of CV = 9.84. In the case of calculated ratios, the variability was at the level of CV = 10.80 for the Glu/Pro ratio, and CV = 11.63 for the Pro/Glu ratio respectively.

To compare the performance of wheat genotypes for grain gluten and protein content, the mean value at the experiment level, for each index, was calculated, and the results of each genotype were compared against the mean value. In the case of gluten content, the mean value at the experiment level was $\overline{Glt}$ = 50.08±0.48%. Compared to the mean value, a number of 12 genotypes showed positive differences, and 13 genotypes showed negative differences (Table 3).

Table 3. Gluten content of the studied wheat genotypes

Wheat genotypes	Gluten content (Glt)	Differences compared to the mean value	Percentage expression of differences (100%)	Sig
Wg5026	51	0.92	101.84	ns
Wg5027	46	-4.08	91.85	ooo
Wg5028	47	-3.08	93.85	ooo
Wg5029	46	-4.08	91.85	ooo
Wg5030	49	-1.08	97.84	o
Wg5031	51	0.92	101.84	ns
Wg5032	50	-0.08	99.84	ns
Wg5033	53	2.92	105.83	***
Wg5034	52	1.92	103.83	***
Wg5035	47	-3.08	93.85	ooo
Wg5036	54	3.92	107.83	***
Wg5037	52	1.92	103.83	***
Wg5038	51	0.92	101.84	ns
Wg5039	51	0.92	101.84	ns
Wg5040	50	-0.08	99.84	ns
Wg5041	50	-0.08	99.84	ns
Wg5042	54	3.92	107.83	***
Wg5043	48	-2.08	95.85	ooo
Wg5044	53	2.92	105.83	***
Wg5045	48	-2.08	95.85	ooo
Wg5046	52	1.92	103.83	***
Wg5047	52	1.92	103.83	***
Wg5048	49	-1.08	97.84	o
Wg5049	47	-3.08	93.85	ooo
Wg5050	49	-1.08	97.84	o
Mean	50.08	-	100.00	-
SE	±0.48	-	-	-

In the case of genotypes with values above the mean, the increase in gluten content (ΔGlt) was between ΔGlt = 0.92% (four genotypes), and ΔGlt = 3.92% (two genotypes). In the case of genotypes with values above the mean gluten content, in eight genotypes the increase in gluten content (ΔGlt) presented statistical safety, at the $p<0.001$ level (Table 3).

In the case of genotypes with values below the mean of the experiment, the differences in gluten content (ΔGlt) ranged between ΔGlt = - 4.08% (two genotypes), and ΔGlt = - 0.08% (three genotypes).

In the case of genotypes with gluten content values lower than the mean of the experiment, in ten genotypes the negative increase in gluten content (ΔGlt) presented statistical safety, at the $p<0.05$ level (three genotypes), and at the $p<0.001$ level (seven genotypes) (Table 3). The differences in gluten content, in relation to the mean value, recorded in the wheat genotypes studied, are presented in Figure 3.

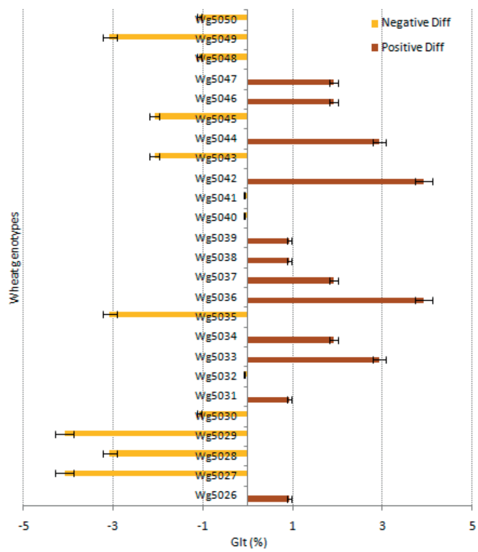


Figure 3. Gluten content differences in wheat genotypes

In the case of protein content, the mean value at the experiment level was $\overline{Pro}$ = 28.66±0.56%. Compared to the mean value, a number of 13 genotypes showed positive differences, and 12 genotypes showed negative differences (Table 4). In the case of genotypes with above-mean values, the increase in protein content (ΔPro) was between ΔPro = 0.04% (genotype Wg5049), and ΔPro = 7.24% (genotype Wg5035).

In the case of genotypes with above-mean protein content values, in nine genotypes the protein increase (ΔPro) presented statistical safety, at $p<0.05$ level (one genotype), at $p<0.01$ level (three genotypes), and at $p<0.001$ level (five genotypes) (Table 4).

In the case of genotypes with lower values compared to the mean, the negative increase in protein content (ΔPro) was between ΔPro = -4.46% (genotype Wg5041), and ΔPro = -0.06% (genotype Wg5050). In the case of genotypes with below-mean protein content values, in nine genotypes the protein increase (ΔPro) presented statistical safety, at the $p<0.01$ level (two genotypes), and at the $p<0.001$ level (seven genotypes) (Table 4).

Table 4. Protein content of the studied wheat genotypes

Wheat genotypes	Protein content (Pro)	Differences compared to the mean value	Percentage expression of differences (100%)	Sig
Wg5026	26.10	-2.56	91.07	ooo
Wg5027	30.30	1.64	105.72	**
Wg5028	26.20	-2.46	91.42	ooo
Wg5029	28.40	-0.26	99.09	ns
Wg5030	26.10	-2.56	91.07	ooo
Wg5031	26.00	-2.66	90.72	ooo
Wg5032	29.20	0.54	101.88	ns
Wg5033	27.90	-0.76	97.35	ns
Wg5034	29.90	1.24	104.33	*
Wg5035	35.90	7.24	125.26	***
Wg5036	29.20	0.54	101.88	ns
Wg5037	26.60	-2.06	92.81	oo
Wg5038	26.60	-2.06	92.81	oo
Wg5039	24.70	-3.96	86.18	ooo
Wg5040	25.00	-3.66	87.23	ooo
Wg5041	24.20	-4.46	84.44	ooo
Wg5042	31.20	2.54	108.86	***
Wg5043	28.80	0.14	100.49	ns
Wg5044	30.30	1.64	105.72	**
Wg5045	30.30	1.64	105.72	**
Wg5046	31.60	2.94	110.26	***
Wg5047	31.70	3.04	110.61	***
Wg5048	33.10	4.44	115.49	***
Wg5049	28.70	0.04	100.14	ns
Wg5050	28.60	-0.06	99.79	ns
Mean	28.66	-	100.00	-
SE	±0.56			

The differences in protein content compared to the mean value, recorded by the studied wheat genotypes, are presented in Figure 4.

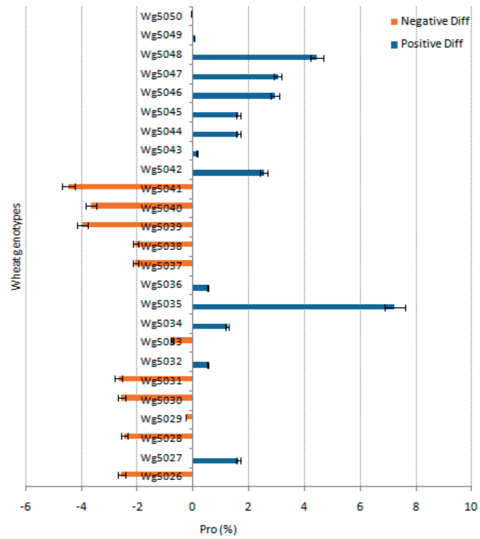


Figure 4. Protein content differences in wheat genotypes

The ratio between the quality indices considered (gluten, protein) was calculated as a result of the importance of these indices in wheat quality and the way of capitalizing on wheat production.

In the case of the gluten to protein ratio, the values varied between Glt/Pro = 1.31±0.04 in the Wg5035 genotype, and Glt/Pro = 2.07±0.04 in the Wg5041 genotype (Table 5). In the case of the protein to gluten ratio, values between Pro/Glt = 0.48±0.01 in the Wg5041 genotype, and Pro/Glt = 0.76±0.01 in the Wg5035 genotype were recorded (Table 5).

In the case of the gluten to protein ratio, the value GLT/PRO = 2.00 indicated a double gluten content relative to the protein content. Higher values of the ratio indicated a higher proportion of gluten relative to the protein content in the wheat grain.

Values lower than the identified threshold indicated a higher share of protein content in the grains, in relation to gluten. These values can be useful for the direction of grain production valorization, depending on the final products, or industrialization processes.

In the case of the Wg5040 genotype, Glt/Pro = 2.00 was recorded. In the wheat genotypes

Wg5039 and Wg5041, Glt/Pro>2.00 was recorded, and in all other genotypes studied, Glt/Pro<2.00 was recorded. The lowest value of the Glt/Pro ratio was recorded in the Wg5035 genotype, which recorded the highest protein content.

Table 5. The values of the calculated ratios between quality indices in wheat genotypes

Wheat genotype	Glt/Pro	Pro/Glt	Wheat genotype	Glt/Pro	Pro/Glt
Wg5026	1.954	0.512	Wg5039	2.065	0.484
Wg5027	1.518	0.659	Wg5040	2.000	0.500
Wg5028	1.794	0.557	Wg5041	2.066	0.484
Wg5029	1.620	0.617	Wg5042	1.731	0.578
Wg5030	1.877	0.533	Wg5043	1.667	0.600
Wg5031	1.962	0.510	Wg5044	1.749	0.572
Wg5032	1.712	0.584	Wg5045	1.584	0.631
Wg5033	1.900	0.526	Wg5046	1.646	0.608
Wg5034	1.739	0.575	Wg5047	1.640	0.610
Wg5035	1.309	0.764	Wg5048	1.480	0.676
Wg5036	1.849	0.541	Wg5049	1.638	0.611
Wg5037	1.955	0.512	Wg5050	1.713	0.584
Wg5038	1.917	0.522			
SE	±0.04	±0.01	SE	±0.04	±0.01

The multivariate analysis generated the diagram in Figure 5, in which the wheat genotypes were distributed in relation to the quality indices and the calculated ratios, as biplot. PC1 explained 76.124% of variance, and PC2 explained 23.582% of variance. Correlated with gluten was the Wg5036 genotype, which presented the highest gluten content. The Wg5035 genotype, which recorded the highest protein content, was associated with the Pro/Glt ratio.

The parameter loadings, as factors, were analyzed in relation to the principal components within the PCA (Table 6).

In relation to PC1, gluten showed a value of r = -0.500, protein showed a value of r = 0.897, the Glt/Pro ratio showed a value of r = -0.996, and the Pro/Glt ratio showed a value of r = 0.999. In relation to PC2, gluten showed a value of r = 0.866, protein showed a value of r = 0.440, the Glt/Pro ratio showed a value of -0.025, and the Pro/Glt ratio showed a value of r = 0.014.

In relation to PC3 and PC4, the correlation values recorded for the parameters considered were insignificant (Table 6).

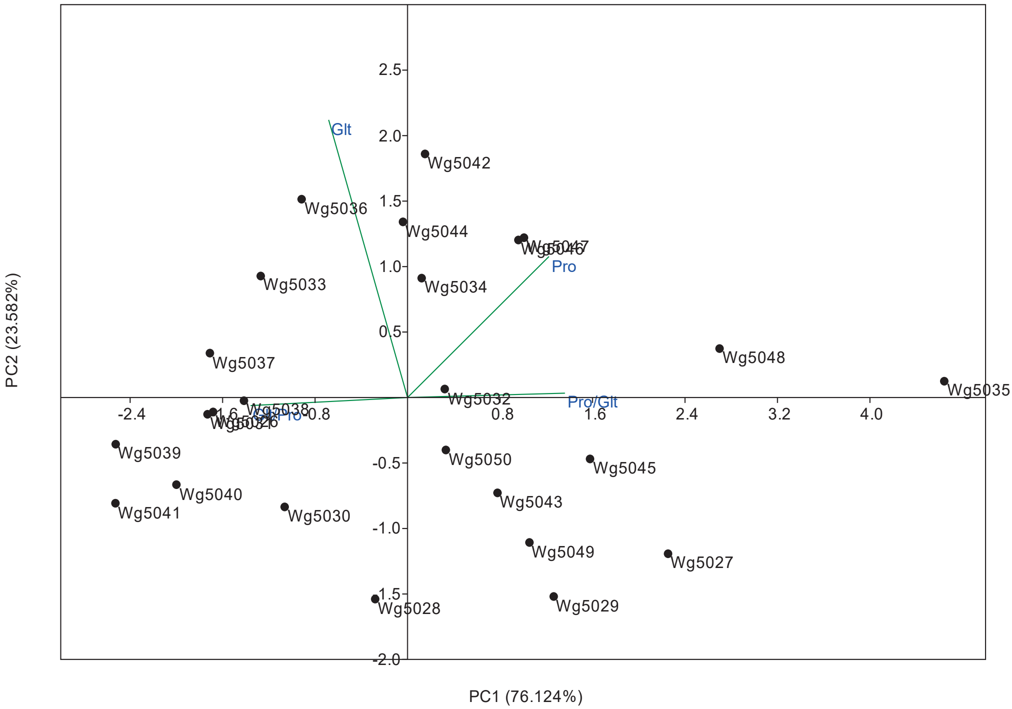


Figure 5. PCA diagram of the distribution of wheat genotypes in relation to quality indices and calculated ratios

Tabelul 6. Loadings values of the parameters in the PCA analysis

Parameters	Components			
	PC 1	PC 2	PC 3	PC 4
Glt	-0.500	0.866	-0.015	0.010
Pro	0.897	0.440	0.034	-0.020
Glt/Pro	-0.996	-0.025	0.085	-0.002
Pro/Glt	0.999	0.014	0.047	0.021

Cluster analysis generated cluster dendrograms, based on similarity in relation to gluten content values, Coph.corr. = 0.752 (Figure 6) and protein, Coph.corr. = 0.797 (Figure 7).
In relation to the Glt parameter (Figure 6), the wheat genotypes were grouped into two distinct clusters, with several subclusters each. Cluster C1 included the genotypes with low gluten content values, and cluster C2 included the genotypes with high and medium gluten content. Within cluster C2, subcluster C2-1 grouped the genotypes with the highest gluten content (Wg5036,Wg5038) and (Wg5033, Wg5044).

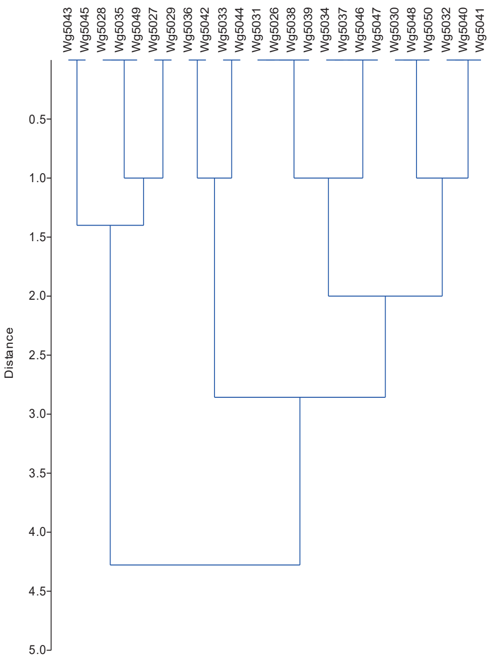


Figure 6. Cluster dendrogram of wheat genotypes based on gluten content

In relation to protein content (Figure 7), two clusters emerged, with three major subclusters.

Cluster C2 included two genotypes, with the highest protein content values (Wg5035, Wg5048).
Cluster C1 included the other genotypes, grouped into two major clusters, with several subclusters.

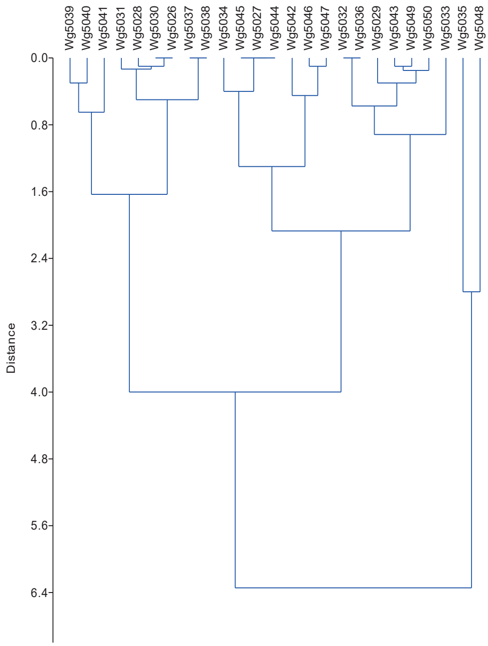


Figure 7. Cluster dendrogram of wheat genotypes based on protein content

Based on the two dendrograms, valuable wheat genotypes can be selected based on similarity, in relation to the genetic potential for gluten and protein production, respectively.
They can be used in the breeding process, as a source of germplasm. At the same time, they can be recommended for the economic sector, for farmers, in order to promote the level of agricultural crops in vegetable farms.
In relation to the two quality parameters (Glt, Pro) a value hierarchy of wheat genotypes was made (Figure 8).
The genotypes were ranked in descending order, from the top to the bottom of the diagram. This ranking facilitates the selection of genotypes in relation to their potential for the two quality indices considered.
The quality of wheat production is important for farmers, for the food industry and the quality of finished products, and for consumers

(Schopf et al., 2021; Schuster et al., 2023; Hoang et al., 2024).

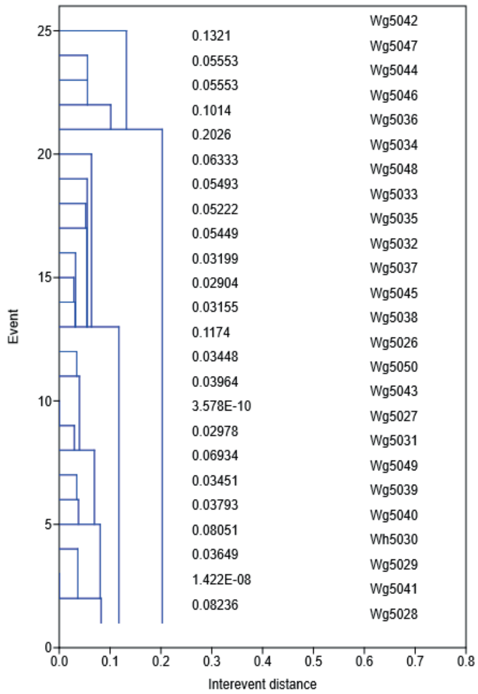


Figure 8. Ranking of wheat genotypes based on Glt and Pro indices

Wheat breeding programs place high importance on quality indices, in order to promote high-performing genotypes (De Santis et al., 2017; Abdelaleem and Al-Azab, 2021; Alemu et al., 2021).

Regarding protein and gluten, various values of content in wheat grains have been reported, in relation to the genotypes tested, environmental conditions, agricultural technologies, stress factors (Alemu et al., 2021; Temizgul et al., 2024).

In the case of the 25 genotypes tested in the present study, seven genotypes were identified with gluten content above the mean value of the experiment, with a gluten increase (ΔGlt) in conditions of statistical safety ($p<0.001$).

Regarding protein content, eight genotypes recorded values above the mean, in conditions of statistical safety, at different safety level ($p<0.05$ level – one genotype; $p<0.01$ level – three genotypes; $p<0.001$ level – five genotypes).

Parallel analysis of wheat genotypes, based on the two quality indices, showed that five genotypes ranked above the mean at the experiment level, in the case of both quality indices considered (Wg5034, Wh5042, Wg5044, Wg5046, and Wg5047). These genotypes can be considered as a valuable genetic source for the wheat breeding program. At the same time, they can be promoted for the crop, to the attention of farmers.

CONCLUSIONS

The wheat genotypes tested generated different gluten and protein content, under the study conditions, in relation to the specific genetic potential of each genotype for the quality indices considered.

Seven genotypes provided a gluten content higher than the mean of the experiment, with differences ranging between 0.92% and 3.92% (Wg5036 – the best tested genotype).

Nine genotypes provided a protein content above mean, with differences ranging between 0.04% and 2.54% (Wg5042 – the best tested genotype).

Multivariate analysis generated a PCA plot of genotypes with a scatter plot relative to the values recorded for the quality indices. The principal components fully explained the variation in the data set.

Grouping based on genotype similarity was obtained through cluster analysis, which facilitated the identification of groups of genotypes with similar results for each quality index considered.

The selection of wheat genotypes with high gluten and protein performance was possible based on the results recorded. Five genotypes were identified with above-mean values for both, gluten and protein. They will be important for the wheat breeding program, but also in the recommendation for farmers.

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