

Starch Content in a Wheat Genotypes Collection – Comparative Study

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Abstract. Wheat is an important crop for food safety and security. Starch is the main component by weight in the structure of wheat grains, with high importance for the processing process and the quality of the finished products. The present study evaluated the starch content in wheat grains in a collection of 25 genotypes. The field experiment was organized within ARDS Lovrin, in the agricultural year 2023 – 2024. The wheat genotypes were noted L5026 to L5050. The wheat crop was located on a chernozem type soil, with medium fertility, in a non-irrigated system. The previous crop was field peas, and uniform crop technology was applied for wheat. The starch content (STA, %) was determined in the Wheat Breeding Laboratory, ARDS Lovrin. Values between STA = 61.80±0.83% (L5045) and STA = 82.50±0.83% (L5042) were recorded, with normal distribution of the data series. In the case of the L5045 genotype, the outlier value was confirmed, at the $p < 0.05$ level. Mann-Kendall trend test showed a decreasing trend in the data series, and regression analysis described the trend of the values in statistical safety conditions. In relation to the average value at the experiment level ($\overline{STA} = 77.62\%$), 14 genotypes were recorded that presented differences in statistical safety conditions. Nine genotypes were recorded with positive differences, and five genotypes were recorded with negative differences. Genotype L5042 presented an increase in starch content $\Delta STA = 4.876\%$, under $p < 0.001$ conditions. Six genotypes (L5026, L5028, L5030, L5031, L5036, L5050) presented values above the average, with differences at the $p < 0.01$ level, and two genotypes (L5027, L5037) presented differences at the $p < 0.05$ level. Cluster analysis grouped the genotypes based on similarity, in relation to the starch content of the grains, under conditions of statistical safety (Coph.corr. = 0.960).

Keywords: breeding programme, clustering, model, regression analysis, wheat quality indices

INTRODUCTION

Wheat has had an important place in human nutrition throughout time and is the crop with a high contribution to food safety and security in the world (Shewry and Hey, 2015; de Sousa et al., 2021; Bentley et al., 2022; Deyalage et al., 2024).

Wheat grains have a complex content of compounds (carbohydrates, proteins, fats, mineral salts, vitamins, fibers) that give wheat a high advantage for human nutrition (Shewry and Hey, 2015; Khalid et al., 2023; Shewry, 2024). More and more studies are addressing wheat in relation to functional foods, and reconsidering ancient wheat varieties (Cooper, 2015; Aweya et al., 2025).

The content of wheat grains varies in relation to the genotype, but also in relation to environmental conditions, cultivation technology, and the interaction of these factors (Zhang et al., 2022; Ghafoor et al., 2024; Akman et al., 2025; Mutanda and Figlan, 2025).

Mineral fertilization differentially influenced the quality of wheat grains, in relation to the nutritional elements, the applied dose (Lin et al., 2015; Wu et al., 2022; Agapie et al., 2025).

Wheat improvement programs aim to improve wheat quality indices and balance quality indices, in relation to the destination of production for human consumption and the quality of finished products (e.g. bakery, pastry), for animal feed, for industrialization (Sissons et al., 2020; Subedi et al., 2023; Rezaei et al., 2024; El Hassouni et al., 2025).

Variations in wheat quality indices have been recorded in relation to climatic conditions, which have an increasingly strong impact on agricultural crops (Poggi et al., 2022; Zhang et al., 2022; Han et al., 2025; Zhou et al., 2025). To obtain genotypes with a high degree of adaptability to climatic conditions, models for estimating the variation of wheat quality indices in relation to climatic factors are of interest for wheat breeding programs (Zhang et al., 2022; Fradgley et al., 2023; Babalola et al., 2025).

Starch is the main component in the structure of wheat grains, with high importance in relation to the processing and quality of finished products (Zhang et al., 2017; Kim et al., 2021; Li et al., 2025). The variation in starch content was recorded in relation to wheat genotype, environmental factors, but also to the interaction effect (Rhazi et al., 2021; Hou et al., 2024; Huang et al., 2024).

In order to evaluate the quality of wheat production, various comparative analysis methods were applied, in relation to different wheat genotypes (old and modern genotypes), experimental variants based on

agrotechnical works, fertilization, irrigation, sanitary treatments, etc. (Rani et al., 2021; Khalid et al., 2022; Preiti et al., 2022; Cetiner and Koksels, 2024; Zhang et al., 2025).

The present study aimed to comparative evaluate some wheat genotypes based on the starch content of the grains and group the genotypes based on similarity in relation to starch content.

MATERIAL AND METHODS

The study was conducted at the Wheat Breeding Laboratory, ARDS Lovrin, in the pedoclimatic conditions specific to the Western Plain of Romania. The climatic conditions recorded during the experimental period are presented in Figure 1.

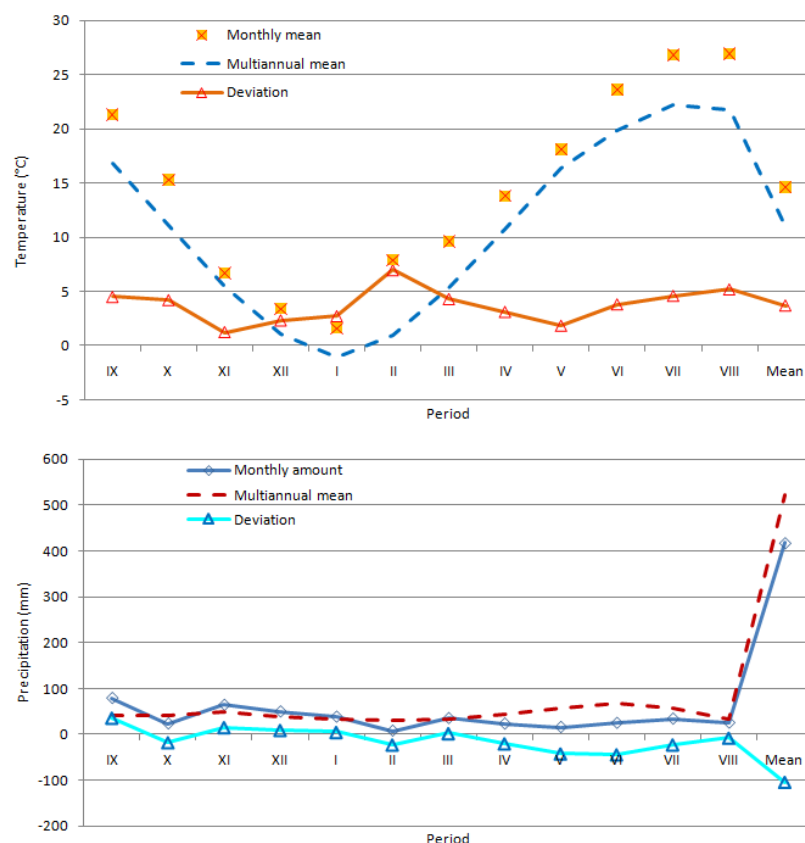


Figure 1. Climatic conditions in the study area, agricultural year 2023-2024

Twenty-five wheat genotypes, designated L5026 to L5050, were evaluated. The comparative crop was placed on a plot with chernozem soil, medium fertility, in a non-irrigated system. The preceding crop was field peas. The land was prepared in a classic system (plowing, discing, combine).

In the fall, fertilization was done with complex fertilizers (15:15:15; 180 kg/ha). Sowing was done in the second decade of October, on October 19, 2023. Starting with November 10, the emergence of wheat plants was recorded. In the spring, urea was applied (118 kg/ha). By spraying with Omnera (1 L/ha), weed control was ensured. On April 15, 2024, treatment with Ianazuma was applied.

The comparative evaluation of wheat genotypes was made based on starch content (STA, %). Partial subsamples were taken from the grain production, for each experimental variant and repetitions, which constituted the average sample for the evaluation of starch content (STA, %). The starch determination was made in the Wheat Breeding Laboratory, within ARDS Lovrin. The calculation module in EXCEL, and the PAST software (Hammer et al., 2001) were used for data analysis and graph generation.

RESULTS AND DISCUSSIONS

Under the study conditions, the wheat genotypes grown comparatively showed variable starch content, with values ranging from STA = 61.80±0.83% (L5045) to STA = 82.50±0.83% (L5042). The data series showed a normal distribution, with $r = 0.841$, Figure 2.

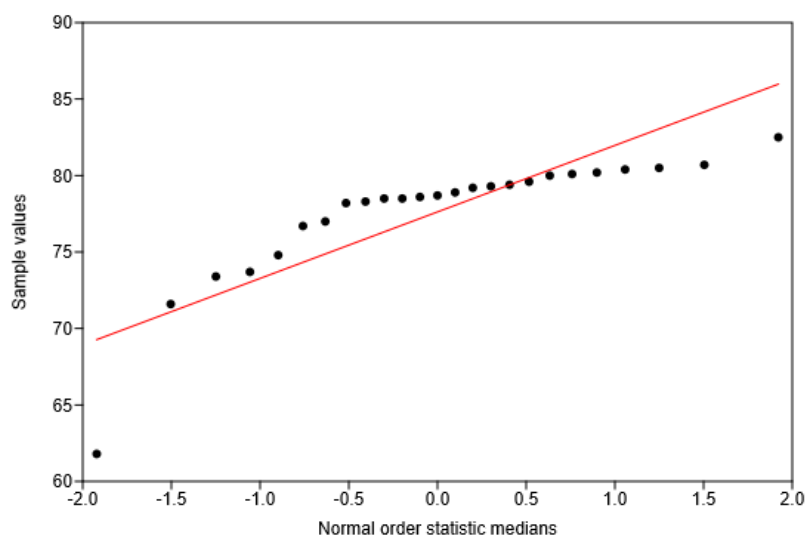


Figure 2. Probability plot for starch content values of the tested wheat genotypes

Outlier test highlighted 20% of the extreme values, Table 1. Among the identified cases, for the L5045 genotype the outlier value was confirmed, at the $p < 0.05$ level ($G = 3.805$; $Q(r_{22}) = 0.6203$).

Table 1. Multiple outliers at the level of wheat genotypes, based on starch content

Genotype	R	Rcrit	Outlier
L5045	3.805	2.822	Yes
L5046	2.58	2.802	No
L5035	2.338	2.78	No
L5043	2.622	2.758	No
L5049	2.627	2.734	No

Mann-Kendall trend test showed a decreasing trend in the data series regarding starch content in the tested wheat genotypes, with $S = -115$, $Z = -2.6632$, $p = 0.0077$.

Regression analysis (Ordinary Least Squares Regression) of the data series described the trend of starch content values, with the statistical results in Table 2, the graphical distribution in Figure 3, and the distribution of the test residual values in Figure 4.

Table 2. Results of regression analysis for the starch content data series of the studied wheat genotypes

Statistical parameters	Statistical parameter value		
Slope a	-0.2309	Std. error a:	0.1076
t	2.1471	p (slope):	0.0426
Intercept b	80.6260	Std. error b:	1.5989
95% bootstrapped confidence intervals (N=1999)			
Slope a	(-0.41037, 0.0090194)		
Intercept b	(78.668, 82.141)		
Correlation			
r	-0.4086		
r ²	0.1670		
t	-2.1471		
p (uncorr.)	0.0426		
Permutation p	0.0339		

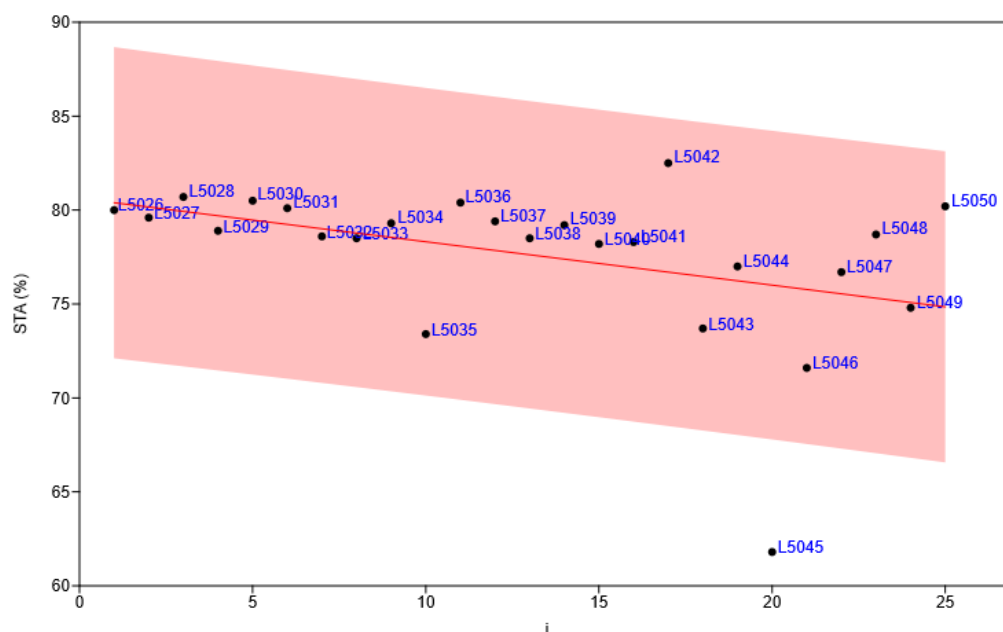


Figure 3. Distribution of wheat genotypes in relation to starch content, as a result of OLSR regression analysis

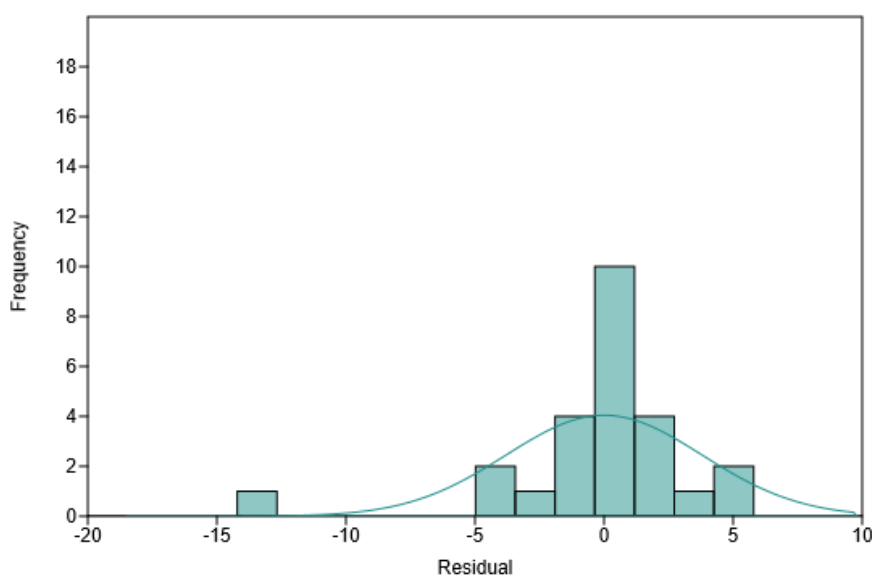


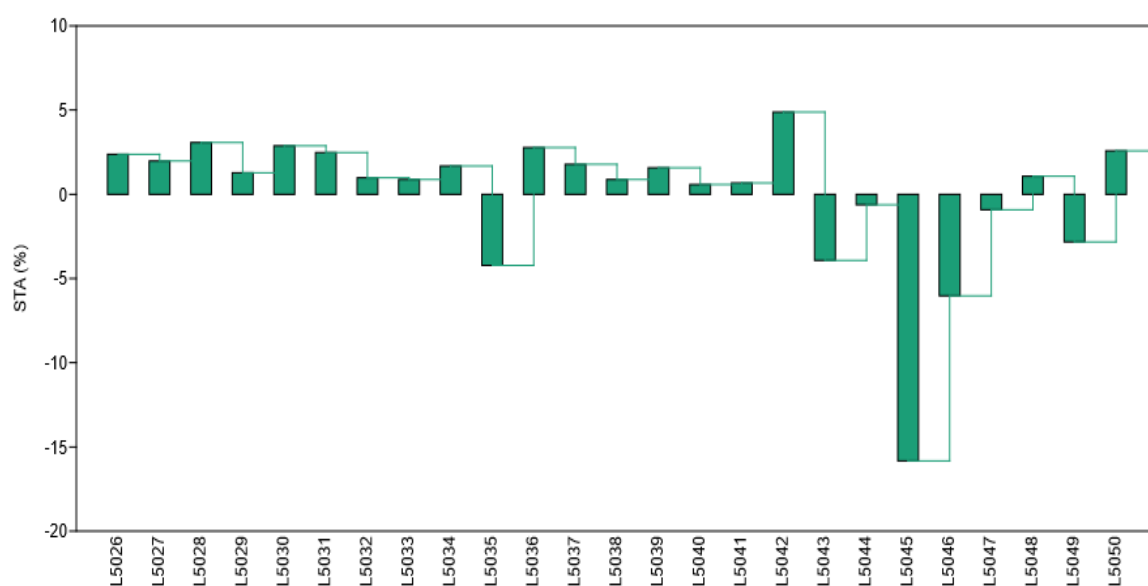
Figure 4. Distribution of residual values

The comparative analysis of wheat genotypes based on starch content (STA, %), in relation to the average value at the experiment level ($\overline{STA} = 77.62\%$), led to the results in Table 3. In relation to the average value, 14 genotypes were recorded that presented differences in statistical safety conditions. Nine genotypes were recorded with positive differences, and five genotypes were recorded with negative differences.

In the case of genotypes with values above the experimental average, the L5042 genotype showed an increase in starch content $\Delta STA = 4.876\%$, in conditions $p < 0.001$. With a statistical confidence level of $p < 0.01$, the genotypes L5026 ($\Delta STA = 2.376\%$), L5028 ($\Delta STA = 3.076\%$), L5030 ($\Delta STA = 2.876\%$), L5031 ($\Delta STA = 2.476\%$), L5036 ($\Delta STA = 2.776\%$), and L5050 ($\Delta STA = 2.576\%$). With a confidence level of $p < 0.05$, the genotypes L5027 ($\Delta STA = 1.976\%$), and L5037 ($\Delta STA = 1.776\%$) were recorded.

Table 3. Results of comparative analysis of wheat genotypes based on starch content

Wheat genotype	Given mean	Sample mean	95% conf. interval	Difference	95% conf. interval	t	p (same mean)	Sig
L5026	80.00			2.376	(0.65914 4.0929)	-2.8563	0.0087	**
L5027	79.60			1.976	(0.25914 3.6929)	-2.3754	0.0259	*
L5028	80.70			3.076	(1.3591 4.7929)	-3.6978	0.0011	**
L5029	78.90			1.276	(-0.44086 2.9929)	-1.5339	0.1381	ns
L5030	80.50			2.876	(1.1591 4.5929)	-3.4574	0.0020	**
L5031	80.10			2.476	(0.75914 4.1929)	-2.9765	0.0066	**
L5032	78.60			0.976	(-0.74086 2.6929)	-1.1733	0.2522	ns
L5033	78.50			0.876	(-0.84086 2.5929)	-1.0531	0.3028	ns
L5034	79.30			1.676	(-0.040856 3.3929)	-2.0148	0.0553	ns
L5035	73.40			4.224	(2.5071 5.9409)	5.0778	p<0.001	ooo
L5036	80.40			2.776	(1.0591 4.4929)	-3.3371	0.0028	**
L5037	79.40			1.776	(0.059144 3.4929)	-2.1350	0.0432	*
L5038	78.50	77.62	(75.907 79.341)	0.876	(-0.84086 2.5929)	-1.0531	0.3028	ns
L5039	79.20			1.576	(-0.14086 3.2929)	-1.8946	0.0703	ns
L5040	78.20			0.576	(-1.1409 2.2929)	-0.6924	0.4953	ns
L5041	78.30			0.676	(-1.0409 2.3929)	-0.8127	0.4244	ns
L5042	82.50			4.876	(3.1591 6.5929)	-5.8616	p<0.001	***
L5043	73.70			3.924	(2.2071 5.6409)	4.7172	p<0.001	ooo
L5044	77.00			0.624	(-1.0929 2.3409)	0.7501	0.4605	ns
L5045	61.80			15.824	(14.107 17.541)	19.0230	p<0.001	ooo
L5046	71.60			6.024	(4.3071 7.7409)	7.2417	p<0.001	ooo
L5047	76.70			0.924	(-0.79286 2.6409)	1.1108	0.2777	ns
L5048	78.70			1.076	(-0.64086 2.7929)	-1.2935	0.2082	ns
L5049	74.80			2.824	(1.1071 4.5409)	3.3948	0.0024	oo
L5050	80.20			2.576	(0.85914 4.2929)	-3.0967	0.0049	**

**Figure 5.** Graphical distribution of starch content differences in wheat genotypes

In the case of genotypes with values lower than the average of the experiment, L5035 ($\Delta\text{STA} = -4.224\%$), L5043 ($\Delta\text{STA} = -3.924\%$), L5045 ($\Delta\text{STA} = -15.824\%$), L5046 ($\Delta\text{STA} = -6.024\%$) were recorded at the $p < 0.001$ level, respectively the genotype L5049 ($\Delta\text{STA} = -2.824\%$) at the $p < 0.01$ level.

In the case of the other genotypes, the differences from the average value of the experiment did not present statistical significance.

Cluster analysis led to the grouping of genotypes based on similarity in relation to starch content values (STA, %), under conditions of $\text{Coph.coph.} = 0.960$, Figure 6.

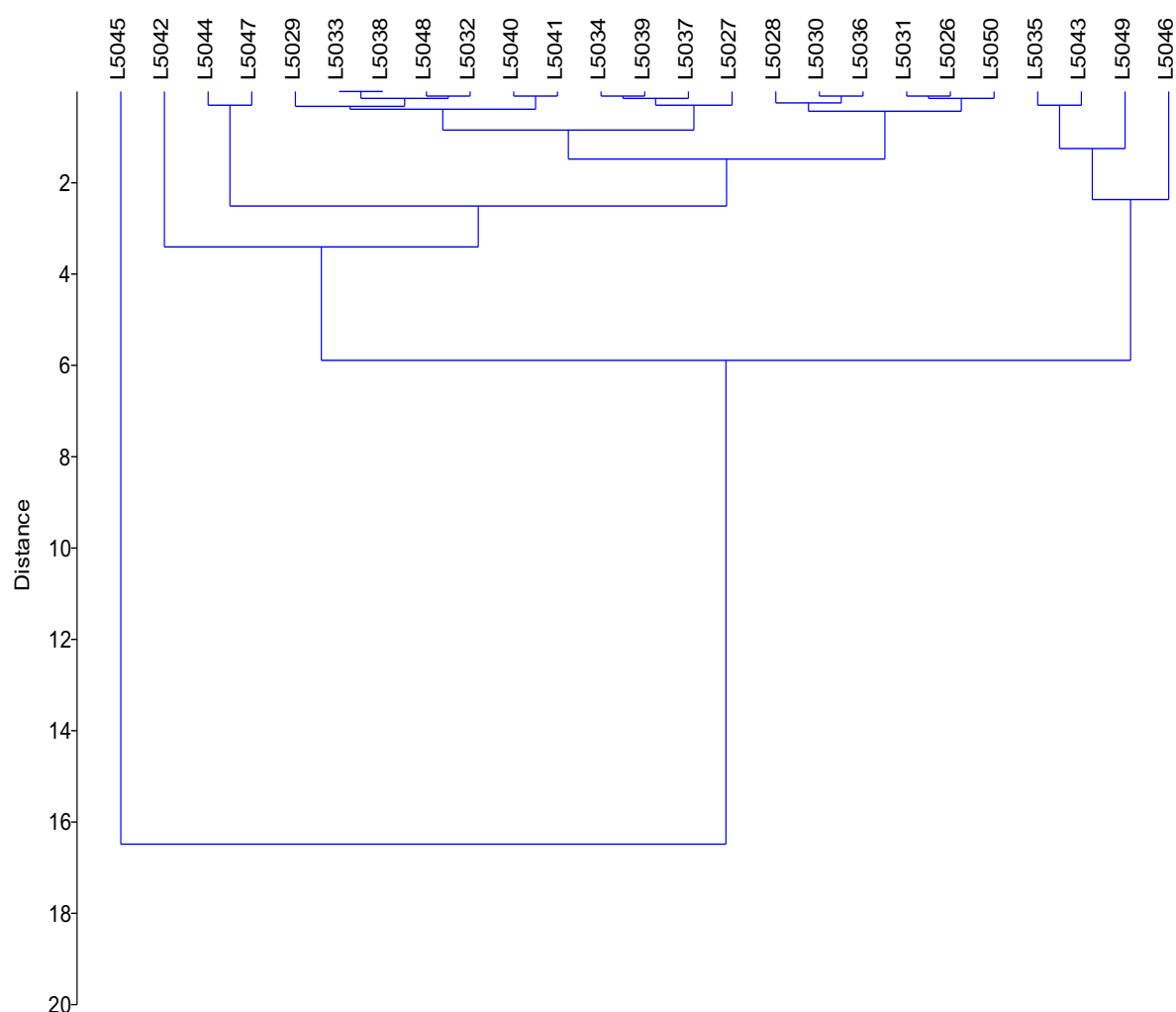


Figure 6. Cluster diagram with wheat genotypes based on starch content

The genotype L5045, with the lowest starch content at the experiment level ($\text{STA} = 61.80\%$), was positioned independently. The other genotypes were grouped within a single cluster, with several subclusters. The genotype L5042 was positioned within a subcluster, with an independent position, with the highest starch content ($\text{STA} = 82.50\%$). Different levels of similarity were recorded between the cultivated wheat genotypes, with the highest level between the genotypes L5033 and L5038 ($\text{SDI} = 0.00$).

Based on the thresholds generated by Descriptive Statistical Analysis for quartiles, and the values recorded by each wheat genotype for starch content (STA, %), the tested genotypes were grouped into quartiles based on performance in relation to starch content, Figure 7.

Six wheat genotypes (L5045, L5046, L5035, L5043, L5049, L5047) were grouped in the lower quartile (lower than 76.85). Thirteen wheat genotypes (L5044, L5040, L5041, L5033, L5038, L5032, L5048, L5029, L5039, L5034, L5037, L5027, L5026) were grouped in the middle quartile (76.85 - 80.05). Six wheat genotypes (L5031, L5050, L5036, L5030, L5028, L5042) were grouped in the upper quartile (higher than 80.05). These results, correlated with those from the cluster analysis and comparative analysis, help select the best wheat

genotypes, in relation to the starch content in the grains, both for the wheat breeding program and for promotion in agricultural practice.

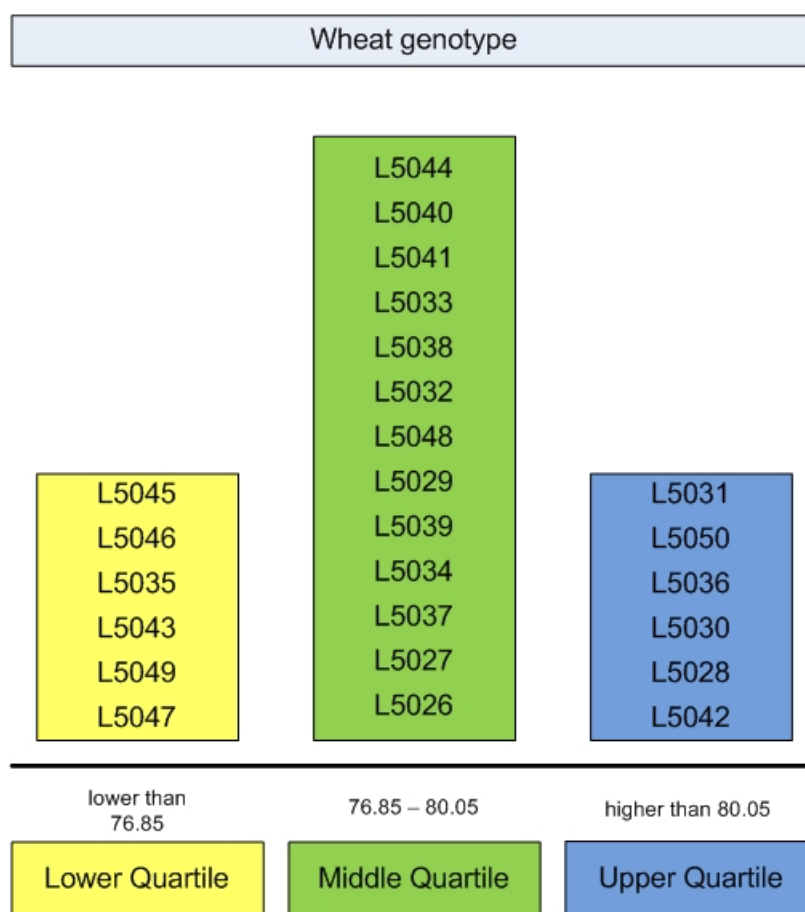


Figure 7. Grouping of wheat genotypes by quartile

Comparative studies at the level of agricultural crops have been carried out in relation to physiological indices, biomass production, yield, quality indices (Pirvulescu et al., 2015; Constantinescu et al., 2018; Sattar et al., 2021; Ahmed et al., 2025; Meena et al., 2025). Different methods, tests and models have been used to analyze and evaluate data series in wheat, but also in other agricultural crops (Boldea and Sala, 2010; Michel and Makowski, 2013; Shawon et al., 2025; Zeng et al., 2025). The identification of outlier values in experimental data is important for the calculations and interpretation of the results (Mouret et al., 2021; Pompeo et al., 2025; Zhou et al., 2025).

In the context of the present study, an outlier value was confirmed in the case of the L5045 genotype, at the $p < 0.05$ level ($G = 3.805$; $Q(r22) = 0.6203$). The experimental conditions ensured uniform conditions for all tested wheat genotypes, and no external causes were identified that would have affected the starch content in wheat grains of the respective genotype. Thus, it was considered that the occupied position was strictly associated with the low starch content, as a result of the genetic potential.

The comparative analysis between genotypes highlighted 14 genotypes with statistically significant differences from the average value of the experiment. Within these, nine genotypes presented values above the average. The cluster analysis grouped the genotypes based on similarity, in relation to the starch content values.

CONCLUSIONS

The experimental conditions ensured uniform conditions for the wheat genotypes, and the variation in starch content was generated by the genetic potential of each genotype for this grain quality index. The L5045 genotype was identified as an outlier in the average starch content data series, with the lowest value ($STA = 61.80 \pm 0.83\%$).

The decreasing variation trend was identified in the experimental data series (Mann-Kendall trend test), and linear regression analysis described the data distribution in statistical safety conditions.

The comparative analysis identified genotypes with values above the average of the experiment, genotypes that showed a genetic advantage for starch content. Nine genotypes showed values above the average in statistical safety conditions, and with the highest starch content was identified the genotype L5042, which showed an increase in starch content $\Delta STA = 4.876\%$, in conditions $p < 0.001$.

Cluster analysis facilitated the identification of genotypes with a high level of similarity, in relation to the starch content of the grains. The information is useful for the wheat breeding program, for the purpose of selecting the parents of the forms considered to have a genetic advantage for the gluten content.

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