

Genetic Variability, Trait Association and Multivariate Analysis of Romanian Einkorn Wheat (*Triticum monococcum* L.) Germplasm

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ABSTRACT

Einkorn wheat (*Triticum monococcum* L.) is an ancient cereal species that has regained interest due to its adaptability to marginal environments and high nutritional value. The present study aimed to evaluate the genetic variability of yield-related traits and grain quality parameters in 17 local einkorn populations under the pedoclimatic conditions of Ezăreni, Iași, Romania, during the 2024-2025 growing seasons. Significant variability was observed among genotypes for all quantitative traits. High genotypic and phenotypic coefficients of variation were recorded for grain number per plant and grain weight per plant, indicating substantial genetic diversity. Broad-sense heritability was high for most traits, particularly for grain number per plant and dough strength, suggesting a strong genetic control and high selection efficiency. Climatic differences between the two years influenced trait expression, with the drier conditions of 2024 increased phenotypic differentiation among genotypes, whereas higher rainfall in 2025 reduced phenotypic variation but strengthened positive associations among yield traits. Correlation analysis revealed a trade-off between yield and quality traits in 2024, whereas in 2025 these relationships became more favorable. Principal component analysis and hierarchical clustering confirmed the presence of genetic diversity and allowed the differentiation of genotypes into distinct groups. The identified variability highlights the potential of local einkorn populations as valuable genetic resources for breeding programs aimed at improving both productivity and grain quality. These results contribute to a better understanding of the genetic potential of einkorn wheat and support its utilization in sustainable agriculture under changing environmental conditions.

Keywords: genetic variability, phenotypic traits, grain quality, einkorn wheat, principal component analysis.

INTRODUCTION

Cereal crops play a fundamental role in global food security, providing a major source of energy and nutrients for the human population (FAO, 2021). In the context of climate change and increasing environmental constraints, there is a growing interest in identifying crop species and genetic resources that combine adaptability with high nutritional value (Ceccarelli, 2015).

Among ancient wheat species, einkorn wheat (*Triticum monococcum* L.) has gained renewed attention due to its resilience to marginal growing conditions and its valuable nutritional properties (Brandolini and Hidalgo, 2011; Zrcková et al., 2019; Tătaru-Farmuş et al., 2021). As a diploid species, einkorn is characterized by a relatively simple genome and has been recognized for its tolerance to

abiotic stresses such as drought and low soil fertility (Zaharieva et al., 2010). In addition, einkorn grains are known for their high protein content and favorable biochemical composition, which make them suitable for functional foods and for sustainable agricultural systems (Suchowilska et al., 2009).

Einkorn wheat has attracted increasing interest in recent years due to its high nutritional value and adaptability to climate changes (Hidalgo and Brandolini, 2014; Brandolini et al., 2018). Moreover, the considerable genetic variability observed in einkorn germplasm represents an important resource for breeding programs aimed at improving both yield and grain quality (Badaeva et al., 2022).

Despite these advantages, einkorn wheat generally exhibits lower yield potential

compared to modern bread wheat, which limits its widespread cultivation (Damania et al., 1998). However, the existence of significant genetic variability within local populations represents an important resource for crop improvement (Hammer et al., 2003). The evaluation of genetic variability through parameters such as genotypic and phenotypic coefficients of variation, heritability, and genetic advance is essential for identifying traits with high selection potential and for supporting breeding programs (Falconer and Mackay, 1996; Sârbu et al., 2023; Simioniuc et al., 2024). Moreover, modern selection techniques can improve the genetic gain by leveraging molecular markers and high statistical methods in wheat breeding programs (Gabur et al., 2022).

Recent studies have highlighted einkorn and emmer as promising crops for sustainable and low-input agriculture, particularly for pesticide-free production systems, although their agronomic potential remains insufficiently exploited in modern farming (Bencze et al., 2025).

In Romania and southeastern Europe, local cereal landraces represent important reservoirs of genetic diversity that can contribute to future breeding efforts (Simeonovska, et al., 2013).

Therefore, the aim of this study was to evaluate the variability of yield-related and grain quality traits in 17 Romanian einkorn populations and to estimate key genetic parameters, trait associations, and multivariate relationships under the environmental conditions of Ezăreni, Iași, during the 2024-2025 growing seasons.

MATERIAL AND METHODS

Experimental Site

The experiment was conducted during the 2024-2025 growing seasons at the Ezăreni Research Station of the “Ion Ionescu de la Brad” University of Life Sciences, Iași, Romania, located in Northeastern Romania at the following geographical coordinates: 47°05.16'N latitude and 27°28.40'E longitude, at an altitude of 130 m.

The soil at the experimental site is classified as a cambic chernozem, with medium fertility and a pH ranging between 6.2 and 6.4. Standard agronomic practices for cereal cultivation in the region were applied uniformly across all experimental variants, including soil preparation and fertilization.

Daily climatic data, including air temperature and precipitation, were recorded using a meteorological station located within 500 m of the experimental field.

Plant Material

A total of 17 einkorn wheat (*Triticum monococcum* L.) genotypes, including local landraces, were selected from the collection of the “Mihai Cristea” Plant Genetic Resources Bank, Suceava, Romania, which stores approximately 56 accessions, in total.

Experimental Design and Crop Management

The field experiment was arranged in a randomized complete block design (RCBD) with three replications. Each genotype was sown in 1 m² plots consisting of four rows, with a row spacing of 0.25 m.

Standard agronomic practices, including soil preparation, fertilization, weed control, and plant protection, were applied uniformly throughout the experimental period, following recommended local agricultural guidelines.

Phenotypic Characterization

At full maturity, ten plants from each genotype were randomly selected from each plot for morphological evaluation. Phenotypic characterization was conducted using descriptors established by the International Board for Plant Genetic Resources (IBPGR) and the Food and Agriculture Organization (FAO).

The evaluated quantitative traits included plant height, number of spikelets per spike, number of tillers per plant, number of grains per plant, and grain weight per plant.

Qualitative traits, including spike density, awn presence, glume color, and presence of hairs on glumes, were assessed using FAO scoring scales.

Grain Quality Analysis

Grain quality traits, including protein content, wet gluten content, dough strength, and Zeleny sedimentation index, were determined using near-infrared spectroscopy (NIRS) with an Inframatic 9500 grain analyzer (Pertin Instruments, PerkinElmer Inc., Sweden).

The instrument operates within a spectral range of 570-1100 nm and was calibrated against reference wet chemistry methods according to the manufacturer's standard procedures. Spectral measurements were performed on whole grain samples collected from both experimental years.

Statistical Analysis

Statistical analyses were performed to assess genetic variability among einkorn genotypes based on data collected over the two experimental years.

Genotypic variance (GV) and phenotypic variance (PV) were estimated based on variance components derived from the experimental data. Phenotypic variance was considered as the sum of genotypic and environmental components.

The genotypic coefficient of variation (GCV%) and phenotypic coefficient of variation (PCV%) were calculated according to Burton and DeVane (1953):

$$GCV\% = (\sqrt{GV} / \text{mean}) \times 100$$

$$PCV\% = (\sqrt{PV} / \text{mean}) \times 100$$

Broad-sense heritability (H^2) was estimated following Robinson et al. (1949):

$$H^2 = (GV / PV) \times 100$$

Genetic advance as percentage of the mean

(GAM%) was calculated according to Johnson et al. (1955):

$$GAM\% = (K \times \sqrt{PV \times H^2} / \text{mean}) \times 100$$

where $K = 2.05$ represents the selection intensity at 5%.

Pearson's correlation coefficients were calculated to assess relationships among traits. Principal component analysis (PCA) and hierarchical clustering based on standardized two-year mean values were performed to evaluate multivariate relationships among genotypes.

All statistical analyses were performed using R software version 4.3.3 (R Core Team, 2023).

RESULTS AND DISCUSSION

Climatic Conditions during the Experimental Years

The climatic data recorded during the two experimental years showed noticeable differences, which influenced the expression of the traits studied (Table 1).

Total rainfall during the 2024-2025 growing season (670 mm) was considerably higher compared to the 2023-2024 season (456 mm), indicating a more humid year. In terms of temperature, the 2023-2024 season was characterized by a wider thermal amplitude, with minimum temperatures reaching -19.4°C and maximum temperatures up to 39.9°C . In contrast, the 2024-2025 season recorded milder temperature extremes, with a minimum of -14.2°C and a maximum of 36.5°C . However, the average temperature in the 2023-2024 season was 2 degrees higher compared to the 2024-2025 season.

Table 1. Climatic conditions during the experimented years

Weather data							
Rain falls (mm)		Temperatures ($^\circ\text{C}$) 2023-2024			Temperatures ($^\circ\text{C}$) 2024-2025		
2023-2024	2024-2025	Min.	Mean	Max.	Min.	Mean	Max.
456	670	-19.4	13.1	39.9	-14.2	11.1	36.5

These climatic differences likely contributed to the variation observed in yield-related traits and grain quality parameters. The higher rainfall in 2025 may have supported vegetative growth but also

increased environmental influence, which is reflected in the lower genotypic variability and reduced genetic advance for several traits compared to 2024.

Conversely, the more contrasting

temperature conditions and lower precipitation in 2024 may have enhanced the expression of genetic differences among genotypes, resulting in higher values of genotypic coefficient of variation and genetic advance, particularly for yield-related traits such as number of grains per plant and grain weight per plant.

Overall, the results suggest that climatic variability between the two years played a significant role in shaping both phenotypic expression and the estimation of genetic parameters in *Triticum monococcum* populations.

These findings are consistent with previous studies indicating that environmental conditions, particularly rainfall and temperature, significantly influence the expression of agronomic traits and the estimation of genetic parameters in cereals (Ceccarelli, 2015; Bencze et al., 2025). Increased moisture availability generally enhances vegetative growth but may reduce the expression of genetic variability due to stronger environmental effects.

Analysis of Genetic Parameters

Qualitative traits assessed according to FAO descriptors showed no variation among the studied genotypes across both

experimental years. All populations exhibited very dense spikes (score 9), the presence of awns (score 7), white glume color (score 1), and absence of hairs on glumes (score 0).

This uniformity suggests a high level of similarity among the evaluated einkorn populations with respect to these morphological descriptors, and consequently, these traits were not included in further statistical analysis.

The analysis of genetic parameters for quantitative traits, revealed a considerable variability among the studied traits in both experimental years (2024 and 2025), indicating the presence of a significant genetic potential within the evaluated einkorn populations (Table 2).

In 2024, high values of phenotypic (PCV) and genotypic (GCV) coefficients of variation were observed for most yield-related traits. The number of grains per plant and grain weight per plant exhibited particularly high variability (GCV = 54.94% and 69.62%, respectively), suggesting a strong genetic diversity for these traits. Similarly, the number of tillers per plant showed a high GCV value (54.45%), indicating substantial variation among genotypes.

Table 2. Analysis of genetic parameters in the studied einkorn wheat germplasm

Experimental Years	Descriptors analyzed	GV	PV	GCV%	PCV%	H ² %	GAM%
2024	Plant height (cm)	82.18	91.24	9.32	9.83	90.07	17.21
	Number of spikelets/spike	14.94	18.8	14.73	16.54	79.46	23.85
	Number of tillers/plant	5.13	7.39	54.45	65.5	69.41	77.03
	Grain number/plant	1925.35	1969.22	54.94	55.57	97.77	109.24
	Grain weight/plant (g)	4.87	7.07	69.62	84.14	68.88	98.47
	Protein content (%)	15.65	19.6	22.83	25.59	79.84	37.44
	Wet gluten content (%)	14.66	18.48	16.6	18.69	79.32	26.89
	Dough strength (J)	785.25	813.27	21.55	21.93	96.55	42.85
	Zeleny index (ml)	22.18	26.89	12.05	13.29	82.48	20.3
2025	Plant height (cm)	68.75	77.04	7.42	7.86	89.23	13.6
	Number of spikelets/spike	12.32	15.83	13.05	14.84	77.82	20.93
	Number of tillers/plant	0.66	1.47	24.47	36.62	44.89	22.07
	Grain number/plant	636.82	662.05	36.06	36.77	96.18	70.97
	Grain weight/plant (g)	1.16	2.24	52.97	74.09	51.78	56.99
	Protein content (%)	6.34	8.85	13.71	16.25	71.63	20.24
	Wet gluten content (%)	1.1	2.14	4.48	6.3	51.4	4.69
	Dough strength (J)	418	438	16.62	17.01	95.43	31.66
	Zeleny index (ml)	1.66	2.95	3.3	3.36	56.27	3.79

GV - Genotypic variance; PV - Phenotypic variance; GCV - Genotypic Coefficient of Variance; PCV - Phenotypic Coefficient of Variance; H² - heritability; GAM% - Genetic advance as percentage of the mean.

In 2025, although the overall variability was slightly reduced for some traits, relatively high GCV values were still recorded for grain number per plant (36.06%) and grain weight per plant (52.97%). The reduction in variability observed in 2025 may be attributed to increased environmental uniformity under higher rainfall conditions, which reduced the phenotypic expression of genetic differences among genotypes.

For all studied traits, PCV values were generally higher than GCV values, indicating the influence of environmental factors on phenotypic expression. However, the relatively small differences between PCV and GCV for traits such as plant height and number of spikelets per spike suggest a lower environmental influence and greater genetic control.

The higher values of PCV compared to GCV observed in this study agree with the findings of Johnson et al. (1955) and Falconer and Mackay (1996), who reported that environmental factors often contribute significantly to phenotypic expression. Similar results have been reported in einkorn and other wheat species, where yield-related traits such as grain number and grain weight exhibited high variability and heritability, indicating their importance for selection (Zaharieva et al., 2010; Badaeva et al., 2022).

The combination of high heritability and high genetic advance observed for grain number per plant and grain weight per plant suggests the predominance of additive gene action, making these traits reliable targets for selection in breeding programs, as also reported by Robinson et al. (1949) and Baye et al. (2020).

Broad-sense heritability estimates were high for most traits in both years. In 2024, very high heritability values were recorded for number of grains per plant (97.77%), dough strength (W) (96.55%), and plant height (90.07%). Similarly, in 2025, high heritability values were observed for number of grains per plant (96.18%) and dough strength (95.43%), indicating that these traits are largely controlled by genetic factors and can be effectively improved through selection.

Genetic advance as percentage of the mean (GAM) further supported these findings. In 2024, high GAM values were recorded for grain number per plant (109.24%), grain weight per plant (98.47%), and number of tillers per plant (77.03%), indicating that selection for these traits would result in substantial genetic gain. In 2025, although GAM values were generally lower, grain number per plant (70.97%) and grain weight per plant (56.99%) still exhibited considerable potential for improvement.

Grain quality traits also showed moderate to high heritability values. Protein content exhibited relatively high heritability in both years (79.84% in 2024 and 71.63% in 2025), suggesting that selection for improved nutritional quality is feasible. Similarly, wet gluten content and Zeleny sedimentation index showed moderate to high heritability, although lower variability and genetic advance values in 2025 indicate a stronger environmental influence.

The relatively high heritability observed for grain quality traits such as protein content, wet gluten, and Zeleny index is consistent with previous reports on einkorn wheat, which highlighted its superior nutritional and technological properties compared to modern wheat species (Hidalgo and Brandolini, 2014; Brandolini et al., 2018). These traits are known to be influenced by both genetic and environmental factors, with protein content being particularly sensitive to environmental conditions.

Overall, traits such as grain number per plant, grain weight per plant, and dough strength (W) exhibited high heritability coupled with high genetic advance, suggesting that these traits are predominantly governed by additive gene effects and are suitable targets for selection in einkorn breeding programs.

The results confirm the existence of significant genetic variability within local einkorn populations and highlight their potential as valuable genetic resources for improving both productivity and grain quality under local environmental conditions.

Correlation analyses

Pearson's correlation analysis revealed contrasting patterns of association among the traits studied in the two experimental years, emphasizing the influence of environmental conditions on trait relationships.

In 2024, strong positive correlations were identified among yield components, particularly between the number of tillers per plant (TPL) and number of grains per plant (GNPL) ($r = 0.94$), indicating that increased tillering significantly contributed to grain production. Plant height (PH) showed a moderate positive correlation with grain weight per plant (GWPL) ($r = 0.61$), suggesting a partial contribution of vegetative growth to yield formation (Figure 1a).

However, grain weight per plant exhibited moderate negative correlations with grain quality traits, including protein content (PC) ($r = -0.39$), wet gluten (WG) ($r = -0.30$), dough strength (W) ($r = -0.35$), and Zeleny index (ZI) ($r = -0.32$), indicating a clear trade-off between yield and quality under the climatic conditions of 2024.

Grain quality traits were strongly and positively correlated among themselves. Protein content showed very high correlations with wet gluten ($r = 0.92$), dough strength ($r = 0.77$), and Zeleny index ($r = 0.93$). Similarly, wet gluten was almost perfectly correlated with Zeleny index ($r = 1.00$) and strongly correlated with dough strength ($r = 0.91$), confirming the close physiological and biochemical relationships among these quality parameters.

In 2025, the correlation pattern changed substantially. Yield-related traits exhibited stronger and more consistent positive associations. The number of grains per plant (GNPL) showed a very strong correlation with grain weight per plant (GWPL) ($r = 0.91$), as well as strong correlations with number of spikelet's per spike (SpSP) ($r = 0.76$) and number of tillers per plant (TPL) ($r = 0.85$), indicating a coordinated expression of yield components under more favorable environmental conditions (Figure 1b).

Plant height also showed moderate positive correlations with most yield

components, including GNPL ($r = 0.52$) and GWPL ($r = 0.47$), suggesting a more integrated growth and productivity response in 2025.

Unlike in 2024, the negative relationships between yield and quality traits were reduced or even reversed. Grain weight per plant showed positive correlations with protein content ($r = 0.34$), wet gluten ($r = 0.49$), and Zeleny index ($r = 0.41$), indicating that under improved moisture conditions, yield and quality traits may be simultaneously enhanced.

Among quality traits, the strong association between wet gluten and Zeleny index remained consistent ($r = 0.98$), highlighting the stability of this relationship across environments. However, correlations between protein content and other quality traits were weaker compared to 2024, suggesting a greater environmental influence on protein expression in 2025.

Overall, the results demonstrate that environmental conditions significantly influenced the relationships among traits. The drier conditions in 2024 accentuated the trade-off between yield and quality, whereas the more favorable conditions in 2025 promoted positive associations among yield components and reduced antagonistic relationships with quality traits, suggesting improved selection efficiency under optimal environments.

The observed trade-off between yield and quality traits under less favorable environmental conditions is widely reported in cereal crops and reflects the allocation of resources between biomass production and grain composition (Ceccarelli, 2015). Similar relationships have been reported in wheat, where higher yields are often associated with lower protein content under stress conditions.

The strong positive correlations among grain quality traits, particularly between wet gluten and Zeleny index, agree with previous studies that demonstrated the close physiological relationship among these parameters and their importance in determining technological quality (Suchowilska et al., 2009; Hidalgo and Brandolini, 2014).

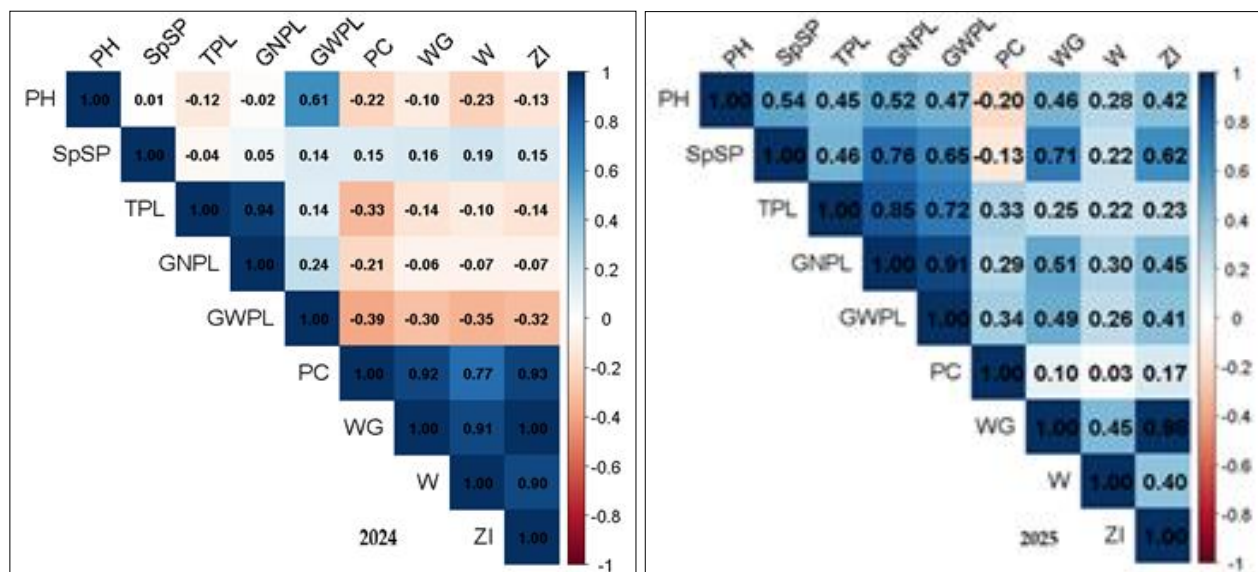


Figure 1. Pearson correlation coefficients among yield and quality traits in 2024 and 2025

Principal component analysis

Principal component analysis (PCA) was performed based on the mean values of the traits studied across the two experimental years to evaluate the relationships among traits and the differentiation of einkorn genotypes (Figure 2).

The first two principal components explained a substantial proportion of the total variability, with PC1 accounting for 40.8% and PC2 for 26.3%, cumulatively explaining 67.1% of the total variation. This indicates

that the selected traits effectively describe the variability among the studied populations.

The distribution of variables on the biplot revealed two main groups of traits. Yield-related traits, such as number of grains per plant (GNPL), grain weight per plant (GWPL), and number of tillers per plant (TPL), were positively associated and oriented in the same direction along PC2, indicating a strong positive relationship among these productivity components.

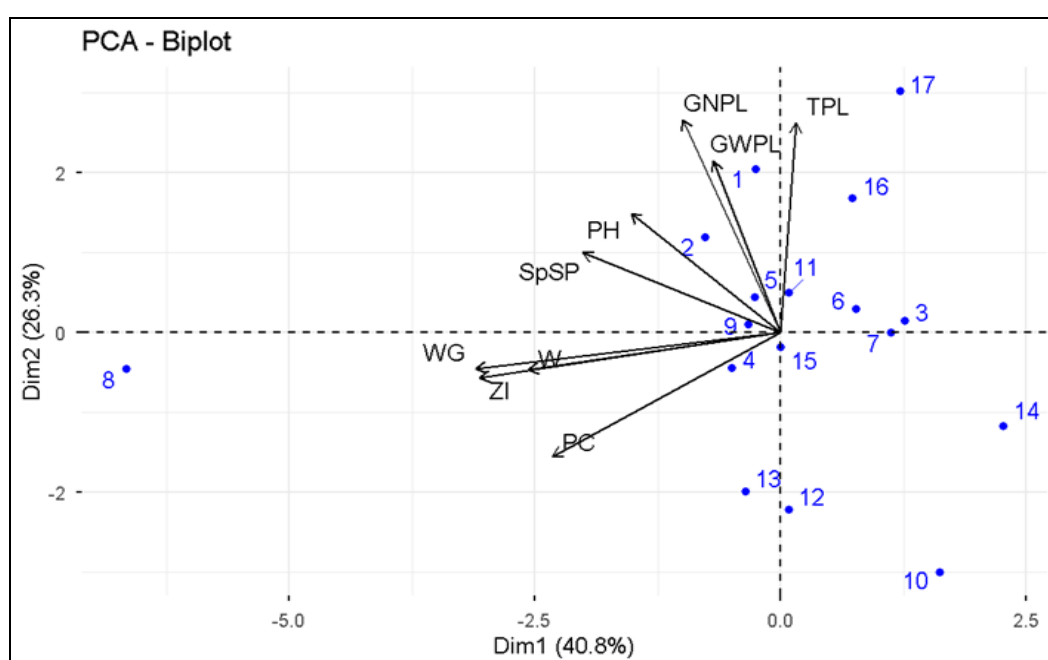


Figure 2. Principal component analysis (PCA) biplot showing the relationships among traits and the distribution of einkorn genotypes based on two-year mean values

In contrast, grain quality traits, including protein content (PC), wet gluten (WG), dough strength (W), and Zeleny index (ZI), were grouped together and oriented in the opposite direction along PC1, suggesting a negative association between yield and quality traits. This pattern confirms the trade-off observed in the correlation analysis.

Plant height (PH) and number of spikelets per spike (SpSP) showed intermediate positions, indicating moderate associations with both yield and quality traits.

The distribution of genotypes on the PCA plot highlighted a considerable level of differentiation among populations. Some genotypes were positioned closer to yield-related traits, indicating higher productivity potential, while others were associated with quality traits, suggesting superior grain quality characteristics.

Overall, the PCA results confirm the existence of significant variability among the studied einkorn populations and support the potential for selecting genotypes with desirable combinations of yield and quality traits.

The grouping of traits observed in the PCA biplot is consistent with previous multivariate analyses in wheat, where yield-related traits and quality traits tend to form distinct clusters, reflecting different genetic and physiological controls (Badaeva et al., 2022). The separation of genotypes along the principal components highlights the potential for selecting genotypes with specific trait combinations.

Cluster analysis

Hierarchical cluster analysis based on Euclidean distance and Ward's method grouped the 17 einkorn genotypes into three distinct clusters (Figure 3), highlighting different patterns of similarity among the studied populations.

Cluster I included genotypes 8, 16, 5, and 17, which were clearly separated from the rest, indicating a high degree of divergence. These genotypes may represent valuable genetic resources due to their distinct trait combinations.

Cluster II comprised genotypes 10, 12, and 13, which formed a compact group, suggesting a high level of similarity among them.

Cluster III included most of the genotypes (2, 4, 15, 3, 7, 14, 11, 1, 6, and 9), which were further structured into subgroups, indicating moderate variability within this cluster.

The clustering pattern is consistent with the PCA results, confirming the existence of genetic diversity among the einkorn populations studied. The identification of distinct clusters provides useful information for selection and breeding, as genetically divergent genotypes can be exploited to maximize variability and genetic gain.

The clustering pattern obtained in this study agrees with previous findings that reported considerable genetic diversity within einkorn germplasm (Hammer et al., 2003; Badaeva et al., 2022). The identification of distinct and divergent genotypes is particularly important for breeding programs, as it allows the selection of parents with complementary traits, leading to increased heterogeneity and genetic gain.

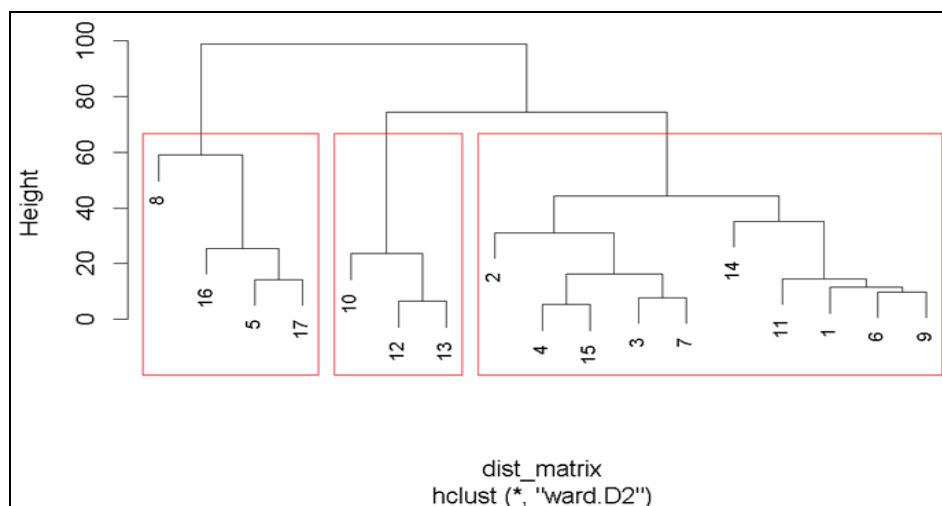


Figure 3. Hierarchical cluster dendrogram of einkorn genotypes based on Euclidean distance and Ward's method, showing the grouping into three main clusters

CONCLUSIONS

The present study highlighted the existence of significant genetic variability among the 17 einkorn (*Triticum monococcum* L.) populations evaluated under the environmental conditions of Ezăreni, Iași.

High values of genotypic coefficient of variation, heritability, and genetic advance were recorded for key yield-related traits, particularly grain number per plant and grain weight per plant, indicating substantial genetic variation and favorable prospects for selection. Grain quality traits, including protein content, wet gluten, dough strength, and Zeleny index, also exhibited moderate to high heritability, suggesting a significant genetic contribution to their expression and their potential use as selection criteria in breeding programs.

The results demonstrated a strong influence of environmental conditions on trait expression and trait relationships. Under the drier conditions of 2024, a trade-off between yield and quality traits was observed, whereas the more favorable climatic conditions of 2025 promoted positive associations among yield components and reduced antagonistic relationships with quality traits.

Multivariate analyses, including principal component analysis and cluster analysis, confirmed the existence of genetic diversity and allowed the identification of distinct groups of genotypes. Divergent genotypes identified in separate clusters represent valuable genetic resources for breeding, as they can be used to increase variability and selection efficiency.

Overall, the studied einkorn populations constitute an important source of genetic diversity for the development of improved cultivars combining productivity, grain quality and adaptation to variable environmental conditions.

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