

Genetic Variability and Multivariate Analysis of Bread Wheat Genotypes Evaluated in North-Eastern Romania

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ABSTRACT

Genetic diversity represents a fundamental resource for wheat improvement under changing environmental conditions. The present study aimed to evaluate genetic variability, trait associations, and multivariate patterns among fifty bread wheat (*Triticum aestivum* L.) genotypes stored at the Suceava Gene Bank. The experiment was conducted over two growing seasons (2021/2022 and 2022/2023) at the Ezareni Research Station, Iași, Romania, using a randomized complete block design with three replications. Seven quantitative agronomic traits were evaluated, including plant height, spike density, spikelets per spike, seeds per spike, tillers per plant, seeds per plant, and seed weight per plant. Genetic parameters such as genotypic and phenotypic coefficients of variation, heritability, and genetic advance were estimated. Pearson correlation analysis was performed to assess relationships among traits, while principal component analysis (PCA) and hierarchical clustering were applied to investigate genetic diversity and genotype $\times$ year interaction. Moderate to high phenotypic variability was observed for most yield-related traits, with higher phenotypic than genotypic coefficients of variation, indicating environmental influence. Moderate heritability and genetic advance were recorded for tiller number/plant and plant height. Strong positive correlations were detected between seed number per plant and seed weight per plant, as well as between tiller number/plant and yield components. PCA revealed that the first two components explained 71.92% of the total variation, with productivity-related traits being the major contributors to genetic divergence. Hierarchical cluster analysis grouped genotypes primarily according to the year of evaluation, indicating a pronounced genotype $\times$ year interaction. The results demonstrate that the studied germplasm possesses valuable genetic diversity and identify key traits for indirect selection. These findings provide useful information for the development of high-yielding and stable wheat cultivars.

Keywords: wheat, genetic variability, principal component analysis, correlation analysis, yield components.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, providing a major source of calories, proteins, and essential micronutrients for human nutrition. Continuous population growth and the increasing impact of climate change have intensified the demand for higher wheat productivity and improved adaptation to environmental stresses. It is estimated that global wheat production must increase by nearly 50% by 2050 to meet future food requirements. Consequently, the development of high-yielding and climate-resilient cultivars represents a major challenge for modern wheat breeding programs.

Genetic diversity constitutes the fundamental basis for crop improvement and adaptation to changing environments. However, during recent decades, the replacement of traditional landraces with genetically uniform, high-yielding cultivars has led to a gradual reduction in genetic variability in bread wheat. This narrowing of the genetic base increases vulnerability to biotic and abiotic stresses and limits future breeding progress. Therefore, the characterization and efficient utilization of available genetic resources are essential for sustainable wheat production.

Phenotypic evaluation remains a key approach for assessing genetic variability and identifying superior genotypes adapted to specific agroclimatic conditions. Estimation

of genetic parameters such as genotypic and phenotypic coefficients of variation, heritability, and genetic advance provides valuable information on the inheritance of economically important traits and the potential response to selection. In addition, understanding the relationships among agronomic traits through correlation analysis facilitates indirect selection for grain yield and related characteristics.

Multivariate statistical methods, particularly principal component analysis (PCA) and cluster analysis, have proven to be powerful tools for exploring complex patterns of variation in germplasm collections. These methods allow the identification of major traits contributing to diversity, classification of genotypes into distinct groups, and detection of genotype $\times$ environment interactions. Moreover, multiyear evaluations are essential for assessing the stability of genotypic performance under variable environmental conditions.

Previous studies have reported considerable genetic variability in wheat germplasm for yield-related traits (Peng et al., 2011; Manda and Săulescu, 2018; Winfield et al., 2018; Baye et al., 2020; Sansaloni et al., 2020; Ahmad et al., 2022; Fernandes et al., 2022; Sârbu et al., 2023; Pochkolina et al., 2024; Simioniuc et al., 2024; Săulescu et al., 2025). These studies emphasized the importance of tiller number, spike architecture and seed-related traits as key determinants of grain yield. Similarly, Grassini et al., (2013) and Tsonev et al. (2021) highlighted the relevance of multivariate analyses for characterizing wheat diversity, while Raoufi and Abdul Saleem (2025) assessed the impact of climate change on winter wheat cultivars.

In this study, fifty bread wheat genotypes from the Suceava Gene Bank were evaluated under the soil and climatic conditions of the IULS Ezareni Research Station during the 2022 and 2023 growing seasons. The objectives were to (i) assess phenotypic and genotypic variability for major agronomic traits, (ii) estimate genetic parameters and trait correlations, (iii) analyze genetic diversity using PCA and hierarchical clustering, and (iv) investigate the influence of year-to-year environmental variation on genotype performance. The results provide valuable information for the selection of superior and stable genotypes for future wheat breeding programs.

MATERIAL AND METHODS

Experimental Site and Plant Material

The experiment was conducted during the two consecutive growing seasons (2021/2022 and 2022/2023) at the Ezareni Research Station of the “Ion Ionescu de la Brad” University of Life Sciences, Iași, Romania. The experimental field is located at an altitude of 130 m on cambic chernozem soil. Detailed information on the geographical location and climatic conditions of the experimental site is presented in Table 1.

A total of 50 bread wheat (*Triticum aestivum* L.) genotypes, including commercial cultivars, advanced breeding lines, and landraces, were selected from the collection of the “Mihai Cristea” Vegetal Genetic Resources Bank, Suceava, which maintains approximately 2600 accessions. Monthly precipitation and air temperature data were obtained from the Romanian National Meteorological Administration (www.meteoromania.ro).

Table 1. Geographical location and climate of the experimental site (www.meteoromania.ro)

Location	Altitude (m)	Geographical location		Soil type	Weather data					
		Latitude	Longitude		Rainfall (mm)		Temperatures (C°)			
					2021-2022	2022-2023	2021-2022		2022-2023	
							Min.	Max.	Min.	Max.
Ezareni Farm	130	47°05' - 47°10'	27°28' - 27°33'	Cambic chernozem	341.8	512.0	-12.1	37	-16.4	38.8

Experimental Design

The field trial was established using a randomized complete block design with three replications. Each experimental plot covered an area of 1 m² and consisted of four rows, 3 m in length, with 0.25 m spacing between rows. All genotypes were randomly assigned within each block.

Phenotypic Evaluation

At physiological maturity, ten representative plants from each genotype and replication were harvested manually for phenotypic evaluation. Trait characterization was performed

following the descriptors developed by the International Board for Plant Genetic Resources and the Food and Agriculture Organization.

The evaluated quantitative traits included plant height, number of spikelets per spike, number of seeds per spikelet, number of fertile tillers per plant, average number of seeds per plant, and average seed weight per plant.

Qualitative traits were assessed using FAO scoring scales and included spike density, awn presence, glume color, and presence of hairs on glumes. The detailed description and scoring of these traits are presented in Table 2.

Table 2. IBPGR Descriptors Used for the Phenotypic Characterization of the Wheat Cultivars Studied

Descriptor	Description / Significance
Growth type (FAO scores)	1 - winter; 2 - facultative (intermediate); 3 - spring
Plant height (cm)	The height of the plant is measured at maturity from the soil surface to the tip of the spike, excluding the awns, using 10 plants per sample.
Spike density (FAO scores)	1 - very lax; 3 - lax; 5 - intermediate; 7 - dense; 9 - very dense (visual observations using a 1-9 scale).
Awn presence (FAO scores)	0 - awnless; 1 - short awns; 7 - fully awned.
Glume color (FAO scores)	1 - white; 2 - red to brown; 3 - violet to black.
Presence of hairs on glumes (FAO scores)	0 - absent; 3 - low; 7 - high.
Number of spikelets per spike	Average number of spikelets per spike, based on five spikes selected from each sample.
Number of grains per spikelet	Average number of seeds per spikelet, obtained from the central portion of the spike. Five spikes from each sample are used.
Seed color (FAO scores)	1 - white; 2 - red; 3 - violet (tested using 5% NaOH solution).
	Seeds are placed in Petri dishes and covered with a 5% NaOH solution (60-90 seeds are used). Red seeds turn dark orange-brown, while white seeds turn straw-yellow.

Genetic Parameters Estimation

Genotypic variance (GV), environmental variance (EV), and phenotypic variance (PV) were estimated according to standard quantitative genetic procedures. Phenotypic variance was calculated as:

$$PV = GV + EV$$

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed following (Burton and DeVane, 1953):

$$GCV(\%) = \frac{\sqrt{GV}}{\bar{X}} \times 100$$

$$PCV(\%) = \frac{\sqrt{PV}}{\bar{X}} \times 100$$

where $\bar{X}$ represents the trait mean; GCV and PCV values were classified as low (<10%), moderate (10-20%), and high (>20%).

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

$$H^2(\%) = \frac{GV}{PV} \times 100$$

Heritability estimates were categorized as low (0-30%), moderate (31-60%), and high (>60%).

Genetic advance (GA) under selection was calculated following (Johnson et al., 1955):

$$GA(\%) = \frac{K \times H^2 \times \sigma_p}{\bar{X}} \times 100$$

where $K = 2.05$ represents the selection intensity at 5% and σ_p is the phenotypic standard deviation.

Genetic advance values were classified as low (<10%), moderate (10.20%), and high (>20%).

Statistical Analysis

Analysis of variance was performed to estimate phenotypic and genotypic components of variance. Genotypic and phenotypic coefficients of variation, broad-sense heritability, and genetic advance were calculated following standard quantitative genetic procedures.

Genotypic and phenotypic correlation coefficients among traits were estimated to assess relationships between yield and its components (www.agrianalyze.com).

Principal component analysis (PCA) and hierarchical clustering on principal components (HCPC) were applied to evaluate genetic diversity and classify genotypes based on multiple traits. PCA and cluster analyses were performed using the FactoMineR and factoextra packages in R software. Separate PCA analyses were performed for each growing season to evaluate genotype $\times$ year interactions and environmental effects on trait expression.

RESULTS AND DISCUSSION

Genetic Variability and Parameters

Morphological descriptors evaluated using FAO scoring scales revealed substantial phenotypic diversity within the studied germplasm. Spike density varied from lax (score 3) to dense (score 7). Regarding awn presence, 30 genotypes were fully awned, 11 exhibited short awns, and 9 were without awns. Most genotypes (46) had white glumes, while four displayed red to brown coloration. Hair presence on glumes was absent in 42 genotypes, low in six, and high in two genotypes.

Estimation of genetic parameters indicated considerable phenotypic variability for several agronomic traits (Table 3). The phenotypic coefficient of variation (PCV) showed moderate values (10-20%) for plant height and seeds per spikelet, whereas spikelets per spike, tillers per plant, seeds per plant, and seed weight per plant exhibited high PCV values (>20%). In all traits, PCV values were higher than GCV values, indicating the influence of environmental factors on trait expression.

Table 3. Genotypic, phenotypic, and environmental variation for wheat genotypes

Trait	Mean	Maximum	Minimum	Geno. Var.	Pheno. Var.	Env. Var.
Plant Height	83.6690	120.4	50.6	81.7400	156.6757	74.9358
Spikelets/Spike	17.1910	65.4	11	3.9947	26.2788	22.2841
Seed/Spikelets	3.0800	6	1	0.1171	0.3129	0.1957
Tillers/Plant	6.6228	16.4	2.6	1.0678	2.8729	1.8050
Seed/Plant	261.3256	745.8	53.4	1273.1526	4790.2454	357.0928
Seed Weight/Plant	10.8240	26.042	1.516	0.4956	6.3361	5.8406

Genotypic coefficients of variation (GCV) ranged from 6.50% to 15.60%, with most traits exhibiting moderate values, indicating the presence of exploitable genetic variability for selection (Table 4).

Broad-sense heritability estimates ranged from low to moderate. Plant height (52.17%), seeds per spikelet (37.44%), and tillers per plant (37.17%) showed moderate heritability, indicating a relatively higher genetic control compared with the other traits. In contrast, seed weight per plant exhibited very low

heritability (7.82%), suggesting strong environmental influence.

Genetic advance expressed as percentage of mean was moderate only for tillers per plant (19.59%) and plant height (16.07%), while the remaining traits recorded low values. The combination of moderate heritability and moderate genetic advance for tillers per plant indicates the potential effectiveness of selection for this trait. Conversely, traits with low heritability and low genetic advance may require multi-

environment testing to improve selection efficiency.

Similar moderate GCV and PCV values for yield-related traits were reported by Javed et al. (2024), Baye et al. (2020) and, Fernandes et al. (2022) indicating the influence of environmental factors on phenotypic expression. The moderate

heritability observed for plant height and tiller number in the present study is consistent with findings of Ahmad et al. (2022) and Sârbu et al. (2023). These results indicate that traits such as tiller number and plant height may respond favorably to selection, while traits with low heritability require evaluation across multiple environments.

Table 4. Analysis of genetic parameters in the studied germplasm

Trait	GCV	PCV	ECV	Heritability (%)	Genetic advance (%)
Plant Height	10.8057	14.9602	10.3462	52.17	16.078
Spikelets/Spike	11.6262	29.8196	27.4597	15.2	9.337
Seed/Spikelets	11.1124	18.1603	14.3635	37.44	14.007
Tillers/Plant	15.6030	25.5927	20.2863	37.17	19.595
Seed/Plant	13.6539	26.4848	22.6939	26.58	14.500
Seed Weight/Plant	6.5037	23.2554	22.3274	7.82	3.746

GCV - Genotypic Coefficient of Variance; PCV - Phenotypic Coefficient of Variance; ECV - Environment Coefficient of Variance.

Correlation Analysis

Pearson correlation analysis revealed strong and significant relationships among yield-related traits (Table 5, Figure 1). A very strong positive correlation was observed between seed number per plant and seed weight per plant ($r = 0.972$), indicating that seed number is the main determinant of yield formation. Significant positive correlations were also found between tiller number per plant and seed number per plant ($r = 0.859$) and between tiller number and seed weight per plant ($r = 0.834$), highlighting the importance of tillering capacity in yield improvement.

The number of seeds per spike showed strong positive associations with seed number per plant ($r = 0.732$) and seed weight per plant ($r = 0.769$), suggesting that spike fertility contributes substantially to grain yield.

The strong positive association between seed number and seed weight observed in this study is in agreement with previous reports by Tayyar (2010) and Baye et al. (2020), who

also emphasized the role of these traits in yield formation.

Plant height exhibited moderate negative correlations with yield-related traits, particularly with seeds per spike ($r = -0.607$) and seed weight per plant ($r = -0.442$), indicating that shorter genotypes tended to be more productive under the studied conditions.

Moderate positive correlations were observed between spikelets per spike and yield components, reflecting their indirect contribution to productivity.

Overall, the observed correlations indicate that tiller number, seed number per plant, and seed number per spike can be considered reliable selection criteria for indirect improvement of grain yield in wheat breeding programs.

Therefore, these traits may serve as reliable indirect selection criteria for improving grain yield in wheat breeding programs, as also suggested by Baye et al. (2020).

Table 5. Pearson correlation coefficients among agronomic traits of wheat genotypes evaluated during 2022-2023

Correlated Traits	PIH	SPD	SpSP	SDSp	TilPI	SDPI	SDWPI
PIH	1	-0.160	-0.223	-0.607 ⁰	-0.264	-0.432 ⁰	-0.442 ⁰
SPD		1	0.290	0.147	0.241	0.253	0.247
SpSP			1	0.550*	0.448*	0.493*	0.510*
SDSp				1	0.427*	0.732**	0.769**
TilPI					1	0.859**	0.834**
SDPI						1	0.972**
SDWPI							1

* $p < 0.05$; ** $p < 0.01$; PIH - Plant height; SPD - Spike density; SpSP - Spikelets per spike; SDSp - Seeds per spike; TilPI - Tillers per plant; SDPI - Seeds per plant; SDWPI - Seed weight per plant.

The correlation heatmap (Figure 1) confirmed strong positive associations among

yield-related traits, particularly between seed number per plant and seed weight per plant.

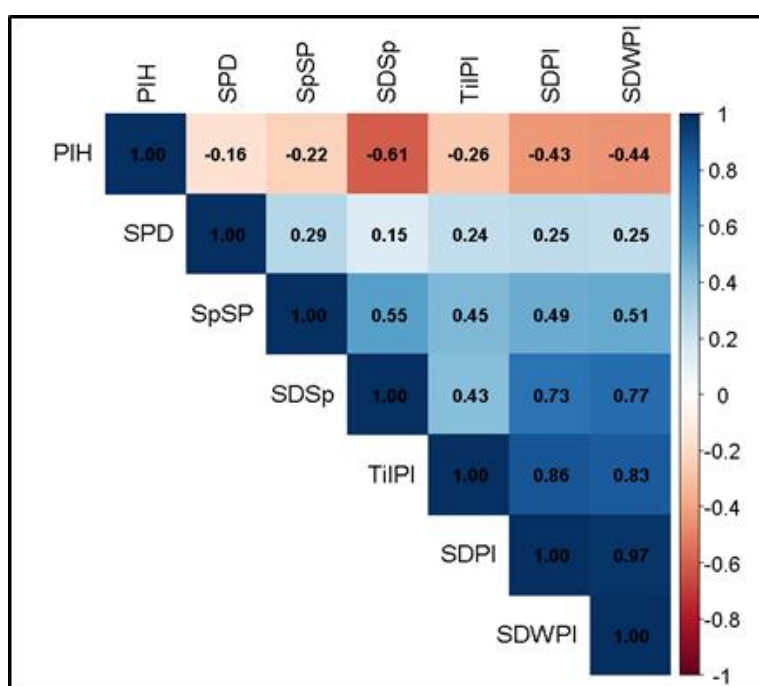


Figure 1. Heatmap of Pearson correlation coefficients among agronomic traits of wheat genotypes evaluated during 2022-2023

Principal Component Analysis

Principal component analysis revealed that the first two components explained 71.92% of the total variation among wheat genotypes (Table 6, Figure 2). Similar levels of explained variability have been reported in previous wheat diversity studies (Yang et al., 2020; Tsonev et al., 2021), confirming the efficiency of PCA in germplasm characterization. Similar associations between yield components and the first principal component were reported by Tsonev et al. (2021), indicating that productivity-related traits are the main drivers of genetic divergence in wheat.

The first principal component (PC1) accounted for 57.94% of the total variability and was mainly associated with yield-related traits, including seed number per plant, seed weight per plant, seeds per spike, and tiller number.

This indicates that PC1 represents the productivity potential of the genotypes. The second principal component (PC2), explaining 13.98% of the variation, was primarily related to plant height and spike density, reflecting differences in plant architecture and spike morphology.

Table 6. Eigenvalues and percentage of variance explained by principal components

Component	Eigenvalue	Variance (%)	Cumulative (%)
PC1	4.05	57.94	57.94
PC2	0.97	13.98	71.92
PC3	0.88	12.69	84.61

The PCA biplot (Figure 2) illustrates the relationships among wheat genotypes and agronomic traits. Yield-related traits, including seed number per plant, seed weight per plant, seeds per spike, and tiller number, were strongly associated with the positive side of PC1. Genotypes located in this region exhibited superior productivity. In contrast,

plant height was positioned on the negative side of PC1, indicating a negative association with yield components. Spike density and spikelet number contributed mainly to PC2, reflecting differences in plant architecture. Genotypes located near the origin showed intermediate and stable performance.

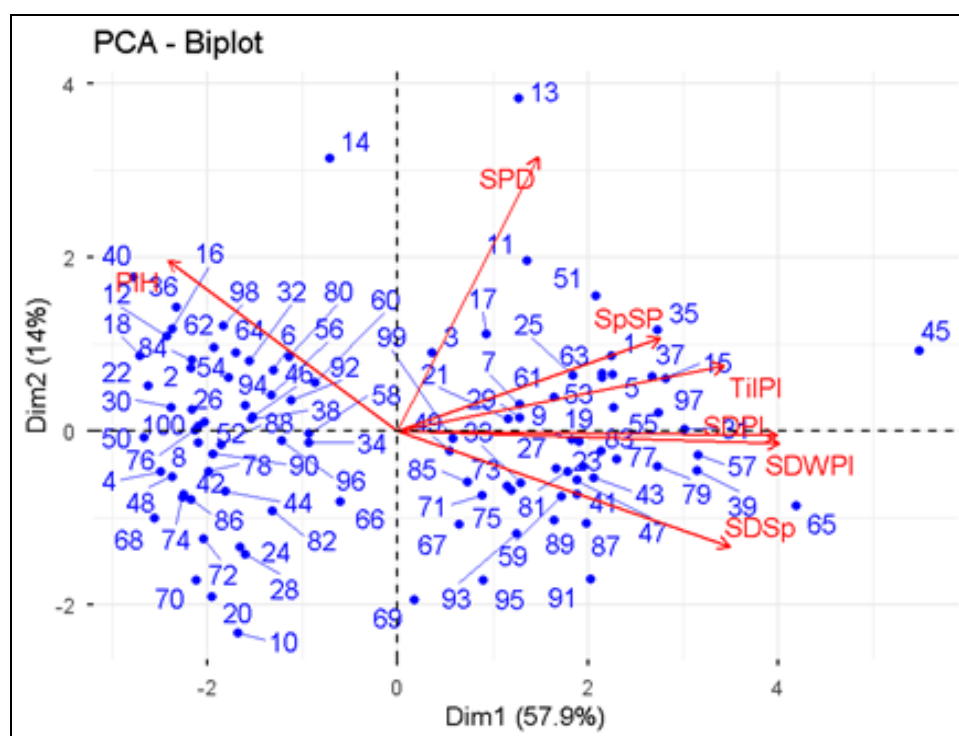


Figure 2. PCA biplot showing relationships among wheat genotypes and agronomic traits

PCA Analysis by Year

Separate PCA analyses were conducted each year to evaluate the influence of environmental conditions on trait expression (Figures 3 and 4). In 2022, the first two principal components explained 60.6% of the

total variation, with PC1 and PC2 showing a relatively balanced contribution. PC1 was mainly associated with yield-related traits, while PC2 reflected variation in spike architecture and plant morphology.

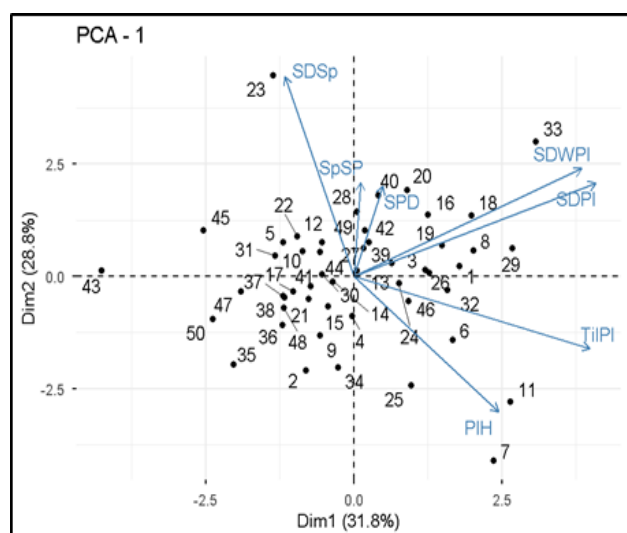


Figure 3. PCA biplot of wheat genotypes evaluated in 2022

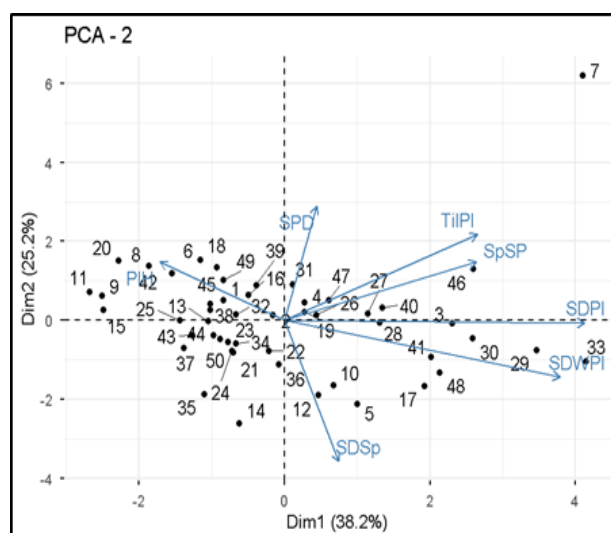


Figure 4. PCA biplot of wheat genotypes evaluated in 2023

In 2023, the first two components accounted for 63.4% of the total variability, with PC1 explaining a higher proportion of variation (38.2%). Yield-related traits, including seed number and seed weight per plant, were the major contributors to PC1, whereas PC2 was influenced by plant height and spike density.

The differences observed between years indicate a significant genotype $\times$ year interaction and highlight the strong influence of environmental conditions on phenotypic performance. Such variations may be attributed to differences in rainfall and temperature between years, emphasizing the importance of environmental conditions in determining genotype performance.

Cluster Analysis

Hierarchical cluster analysis based on principal component scores grouped the wheat genotypes into three distinct clusters (Figure 5). Cluster I comprised mainly genotypes evaluated in 2023 and were

characterized by lower values of yield-related traits. Cluster II included most of the genotypes evaluated in 2022 and exhibited higher productivity potential. Cluster III consisted of a single genotype showing a distinct phenotypic profile. Similar clustering patterns based on multivariate analysis were reported by Yang et al. (2020) and Tsonev et al. (2021), confirming the usefulness of PCA-based clustering for wheat germplasm characterization.

The observed clustering pattern reflects the strong influence of environmental conditions and confirms the presence of genotype $\times$ year interaction. The separation of genotypes according to evaluation year highlights the importance of multi-year testing in wheat breeding programs. Similar clustering patterns based on multivariate analysis were reported by Yang et al. (2020) and Tsonev et al. (2021), confirming the usefulness of PCA-based clustering for wheat germplasm characterization.

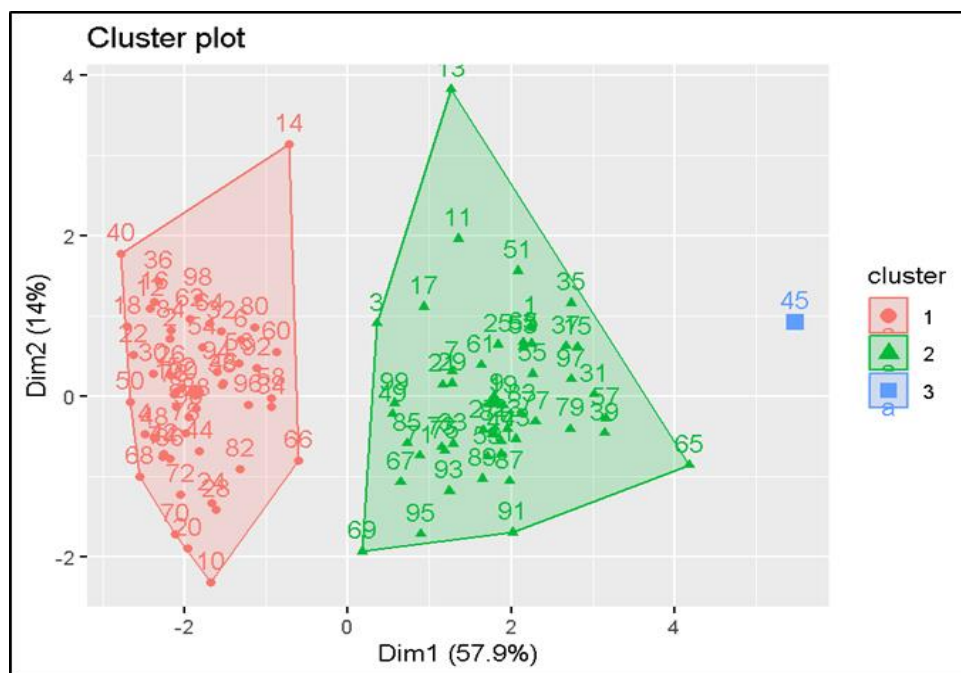


Figure 6. Hierarchical cluster analysis of wheat genotypes based on principal components

CONCLUSIONS

The evaluated wheat germplasm exhibited considerable phenotypic and genetic variability for major agronomic traits, confirming the presence of exploitable diversity for wheat improvement. Moderate heritability and genetic advance observed for tiller number and plant height suggest that these traits could be effectively improved through phenotypic selection.

Strong positive correlations among yield-related traits, particularly between seed numbers per plant and seed weight per plant, highlight the importance of these components as indirect selection criteria for improving grain yield. Principal component analysis demonstrated that productivity-related traits represent the main source of genetic divergence among genotypes. The grouping of genotypes according to evaluation year, revealed by PCA and cluster analysis, indicates the influence of genotype $\times$ year interaction and emphasizes the importance of multi-year testing under variable environmental conditions.

Overall, the results provide valuable information for identifying superior and stable wheat genotypes and support the efficient utilization of genetic resources in future breeding programs.

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