

GENETIC VARIABILITY, TRAIT ASSOCIATION, AND YIELD PERFORMANCE OF
ADVANCED WHEAT LINES USING MULTIVARIATE STATISTICAL ANALYSIS

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Abstract

Wheat (*Triticum aestivum* L.) productivity in semi-arid regions is constrained by climatic stress and genetic limitations, necessitating the identification of high-performance and adaptable genotypes. The present study was conducted to evaluate the genetic variability, trait associations, and yield potential of fifteen wheat genotypes under semi-arid conditions at The Islamia University of Bahawalpur, Pakistan. The experiment was carried out in a randomized complete block design with three replications. Data were recorded on yield-related traits, including plant height, thousand-grain weight, number of tillers per plant, days to heading, days to maturity, number of grains per spike, peduncle length, and yield. Analysis of variance revealed highly significant differences among genotypes for all studied traits, indicating substantial genetic diversity. Correlation analysis showed that yield was positively and significantly associated with plant height ($r = 0.906^{**}$), thousand grains weight ($r = 0.897^{**}$), number of tillers per plant (0.921^{**}), days to heading ($r = 0.951^{**}$), days to maturity ($r = 0.950^{**}$), number of grains per spike ($r = 0.990^{**}$) and peduncle length ($r = 0.985^{**}$). Cluster analysis grouped the genotypes into two distinct clusters, with Cluster-2 exhibiting superior yield, high thousand-grain weight, number of tillers per plant, number of grains per spike, and days to maturity. Principal component analysis explained 98.74% of the total variability through the first two principal components, highlighting yield and yield-contributing traits as major sources of genetic divergence. Wheat genotypes FWP-

11, SSW-10, and ABW-1 demonstrated superior performance and can be exploited in future breeding programs aimed at improving wheat productivity under a semi-arid environment.

Introduction

Wheat (*Triticum aestivum* L.), commonly referred to as Bread wheat, is the major staple food that overcomes hunger (Joshi et al., 2020). It is recognized as the foremost cereal crop and an essential component of the global economy (Ammar et al., 2023). Globally, wheat is cultivated on 220 million hectares with a production of 799 million tons (Morgounov et al., 2025). In Pakistan, wheat remains a key component of agriculture sector, cultivated on 9.1 million hectares primarily across Punjab and Sindh (Khan et al., 2021). Major wheat-producing nations are China, India, Russia, the European Union, the United States, Canada, Australia, and Pakistan, while Pakistan accounts 4% of global production (Shahzad et al., 2022).

Wheat is an important cereal crop that contains carbohydrates, protein, fiber, and minerals like iron, zinc, magnesium, etc. (Sharma et al., 2020). Globally, Pakistan ranks 8th in wheat production with 31.44 million tons (2% of the world wheat area) (Shoaib et al., 2022). The average productivity of wheat in Pakistan is (3.23 t/ha) when compare to the world average (6.01 t/ha) some of the leading producers of wheat are namely, China (5.94 t/ha), India (3.56 t/ha), Russia (2.94 t/ha), European Union (5.37 t/ha), United States (3.44 t/ha) (Zakharova & Zakharov, 2024). Pakistan's wheat production for 2024/25 is 3.23 t/ha, marking a 3.5% increase from 2023/2024 (Muhammad Islam, 2025). But in previous years, the yield per hectare is subsequently low due to insect-pest infestation (mainly Aphid) and rust (mainly yellow and brown rust). Climate change and water scarcity are also showing a negative impact on yield (Arshad et al., 2019).

Many approaches exist to enhance wheat production; among these, the evaluation of cultivated germplasm is more efficient. Following systematic evaluation, high-performing genotypes can be identified. These superior genotypes may subsequently be advanced through a variety release program. The experiment was therefore designed to assess the performance of existing germplasm at The Islamia University of Bahawalpur, Pakistan. At

the end, the best performing genotypes or varieties will be selected for further utilization in breeding and variety development programs.

Materials and Methods

Site and Research Material

This research was conducted at the Islamia University of Bahawalpur, Pakistan. The site is characterized by a semi-arid climate. Fifteen genotypes were used in this research shown as in, Table 1); the study was laid out in a Randomized Complete Block Design (RCBD) with three replications. Sowing was carried out by the dibbler, @2 seeds/hill. All practices, including agronomic and plant protection, were carried out for all the treatments.

Data were collected for different key agronomic traits, including plant height (PH), thousand-grain weight (TGW), number of tillers per plant (NOTP), days to heading (DTH), days to maturity (DTM), number of grains per spike (NOGPS), peduncle length (PL), and yield per hectare. These traits were assessed at physiological maturity, excluding days to heading (Table 3).

Data Recording and Analysis

The collected data were subjected to analysis of variance (ANOVA) using RCBD design to determine significant differences among genotypes, principal component analysis (PCA), correlation coefficient, cluster analysis, and biplot analysis (Phougat et al., 2022). Correlation analysis was conducted to explore the relationship between agronomic traits. Statistix 8.1, XLSTAT, R/R Studio, as well as Microsoft Excel, were used to analyze the data and illustrate the graphs.

Results

Analysis of Variance (ANOVA)

The data were subjected to analysis of variance (ANOVA), which unveiled the differences among studied plant traits, which were highly significant, significant, as well as non-significant (Gerema et al., 2020). Highly significant differences were found for the studied plant traits i.e., Plant height (PH; 86.74**), thousand grains weight (TGW; 17.24**), number of tillers per plant (NOTP; 17.24**), days to heading (DTH; 45.61**), days to maturity (DTM;

11.56**), number of grains per spike (NOGS; 201.26**), peduncle length (PL; 53.40**), and yield (Yield; 1469121**) under wheat genotypes (Table 2) (Ahmed et al., 2022).

The highly significant ANOVA results for all studied traits confirm the presence of substantial genetic variability among the evaluated wheat genotypes, indicating strong potential for selection and crop improvement. Significant differences in plant height, thousand-grain weight, number of tillers per plant, days to heading, days to maturity, number of grains per spike, peduncle length, and yield suggest variation in genotypes (Israr Qureshi et al., 2025). In wheat, thousand grains weight is closely associated with yield. Likewise, the highly

significant differences observed for days to heading and days to maturity indicate the existence of diverse maturity groups among the studied genotypes, which can be exploited for developing cultivars adapted to different agro-climatic conditions (Krishna et al., 2023). Significant variation in thousand-grain weight and grain yield further demonstrates that yield performance is strongly genotype-dependent and governed by several interacting yield components. Therefore, these genotypes can be effectively utilized in future wheat breeding programs aimed at improving yield potential, adaptability, and agronomic performance (Pradesh et al., 2020).

Table 1: *Fifteen advanced wheat genotypes evaluated for quantitative and yield-related traits under semi-arid environmental conditions.*

Sr No	Genotypes
1	AZW-2
2	EWP-2
3	ARW-11
4	MW-22
5	MNW-3
6	ARW-2
7	FWP-4
8	MRW-5
9	NMW-3
10	HBW-1
11	HBW-2
12	ABW-1
13	AAW-9
14	SSW-10
15	FWP-11

Table 2: *Analysis of variance (ANOVA) indicating highly significant genetic differences among the fifteen wheat genotypes for all studied agronomic and yield-related traits.*

SOV	Replication	Genotype	Error	Total
Df	2	14	28	44
PH	6.35	86.74**	0.18	
TGW	2.33	17.24**	0.07	
NOTP	2.33	17.24**	0.07	
DTH	10.66	45.61**	0.03	
DTM	10.00	11.56**	0.14	
NOGS	13.53	201.26**	0.014	
PL	11.70	53.40**	0.037	

Yield	36260	1469121**	10
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Table 3: Morphological and yield-related traits recorded for the evaluation of genetic variability and agronomic performance among the fifteen wheat genotypes.

Trait Designation	Code	Trait Description
Plant Height (cm)	PH	Height of the wheat plant measured from the soil surface to the tip of the spike (excluding awns) at maturity, expressed in centimeters.
Thousand grains weight	TGW	Weight of one thousand grains, used as an indicator of grain size and grain quality.
Number of tillers per plant	(NOTP)	Total number of productive tillers produced by a single wheat plant.
Days to heading	(DTH)	Number of days taken from sowing to the emergence of spikes in 50% of the plants.
Days to maturity	(DTM)	Number of days taken from sowing until 90–95% of the plants reach physiological maturity.
Number of grains per spike	(NOGPS)	Average number of grains produced in a single spike of wheat.
Peduncle Length	(PL)	Length of the uppermost inter-node of the wheat stem from the flag leaf base to the base of the spike, measured in centimeters
Yield per hectare	Yield	Total grain yield produced per hectare, usually expressed in kilograms or tons per hectare.

The table presents the morphological traits recorded in 15 wheat genotypes used for the evaluation of genetic variability and yield performance.

Correlation Coefficient Analysis

The correlation coefficient analysis revealed the relationship among traits as, plant height (PH), thousand grain weight (TGW), number of tillers per plant (NOTP), days to heading (DTH), days to maturity (DTM), number of grains per spike (NOGS), peduncle length (PL), and yield (Yield) in the evaluated wheat genotypes (Lee et al., 2024). Yield exhibited strong and positive correlations with number of grains per spike ($r = 0.990^{**}$), peduncle length ($r = 0.985^{**}$), days to heading ($r = 0.951^{**}$), days to maturity ($r = 0.950^{**}$), number of

tillers per plant ($r = 0.921^{**}$), plant height ($r = 0.906^{**}$), and thousand grain weight ($r = 0.897^{**}$) (Table 4) (Kumar et al., 2024). Similarly, the number of grains per spike showed strong positive correlations with peduncle length ($r = 0.984^{**}$), days to heading ($r = 0.978^{**}$), days to maturity ($r = 0.962^{**}$), plant height ($r = 0.932^{**}$), thousand grain weight ($r = 0.927^{**}$), and number of tillers per plant ($r = 0.930^{**}$). A strong positive association was also observed between spike length and days to maturity ($r = 0.973^{**}$), days to heading ($r = 0.972^{**}$), and number of tillers per plant ($r = 0.956^{**}$). Furthermore, thousand-grain weight was highly correlated with plant height ($r = 0.980^{**}$), number of tillers per plant ($r = 0.980^{**}$), and days to maturity ($r = 0.978^{**}$) (Table 4) (Khan et al. 2022).

Table 4: Pearson Correlation Matrix among agronomic and yield-related traits of fifteen wheat genotypes evaluated under semi-arid conditions.

Variables	PH	TGW	NOTP	DTH	DTM	NOGS	PL	Yield
PH								
TGW	0.980							
NOTP	0.972	0.980						
DTH	0.974	0.978	0.961					
DTM	0.959	0.978	0.977	0.979				
NOGS	0.932	0.927	0.930	0.978	0.962			
PL	0.949	0.946	0.956	0.972	0.973	0.984		
Yield	0.906	0.897	0.921	0.951	0.950	0.990	0.985	

Principal Component Analysis

The principal component analysis (PCA) provided a comprehensive understanding of the variability among the fifteen wheat genotypes under a semi-arid climate, as demonstrated by eigenvalues, a scree plot, a correlation circle, variable contributions to PC-1 and PC-2, a combined PCA biplot, and an individual observation plot. The scree plot indicated that PC-1 and PC-2 together

accounted for a significant portion of divergence, with PC-1 explaining 96.40% and PC-2 2.33%, totaling 98.74% of the divergence, and these components represent the primary source of trait differentiation among wheat genotype (Abdi et al. 2023). But all other eight PC collectively explain 1.26% of the variation, which is non-significant (Figure 1) (Table 5).

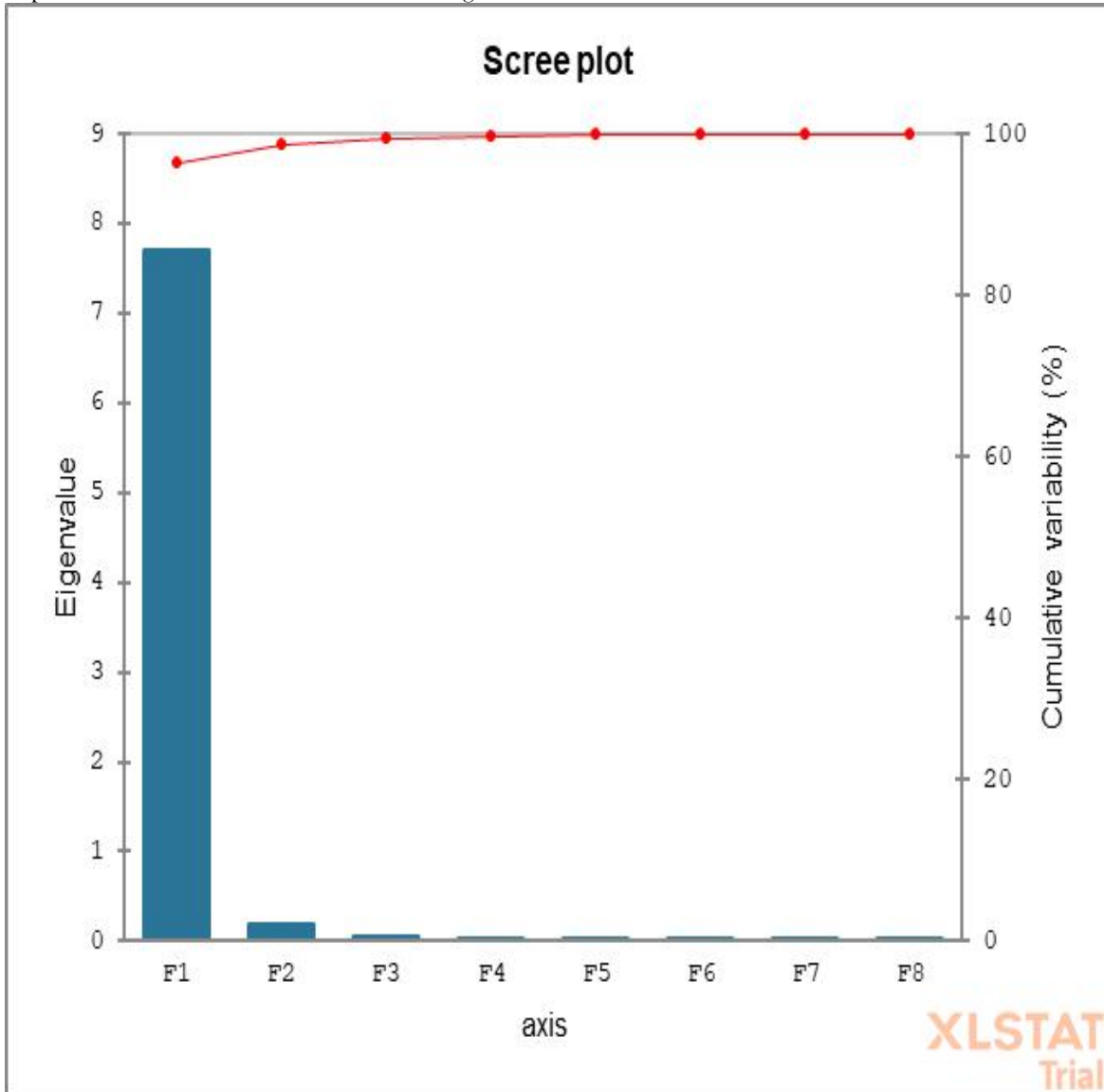


Figure 1: Scree plot showing the eigenvalues and cumulative percentage variability explained by the principal components in fifteen wheat genotypes.

The correlation circle demonstrated that all studied traits were positively associated with PC1, indicating strong contributions to the overall

variability. Yield, number of grains per spike, and peduncle length were strongly aligned along the positive side of PC1 (Lv et al. 2024). Similarly, days

to maturity, days to heading, number of tillers per plant, thousand grain weight, and plant height also exhibited positive associations with PC1, reflecting

their importance in determining overall agronomic performance (Figure 2) (Zulfiqar et al., 2024).

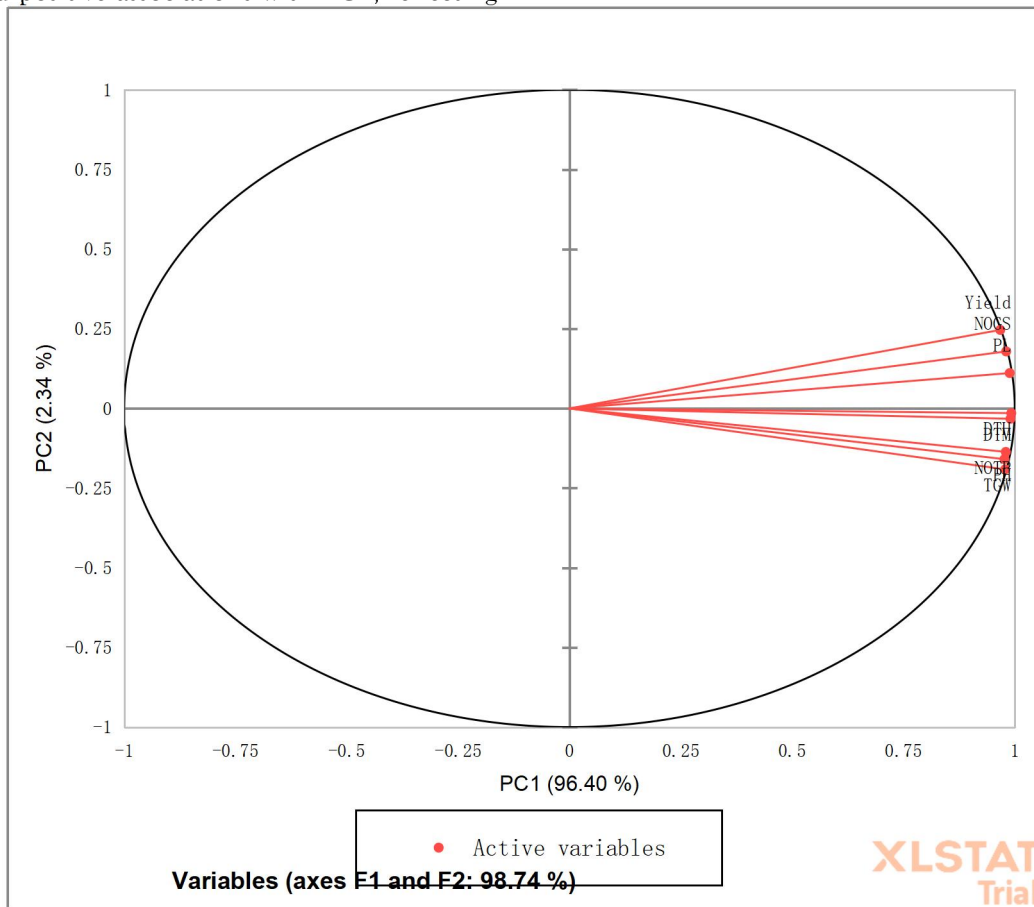


Figure 2: Variable Plot/Correlation circle showing the contribution and association of agronomic and yield-related traits with the first two principal components in fifteen wheat genotypes.

The combined PCA biplot positioned genotypes like FWP-11, SSW-10, ABW-1 along the positive PC-1 axis, close to yield, number of grains per spike, and peduncle length, indicating their high yielding

potential, while MRW-5, MNW-3, and HBW-1 exhibited high thousand grain weight, number of tillers per plant, and plant height (Phougat, Verma, and Choudhary 2022) (Figure 3).

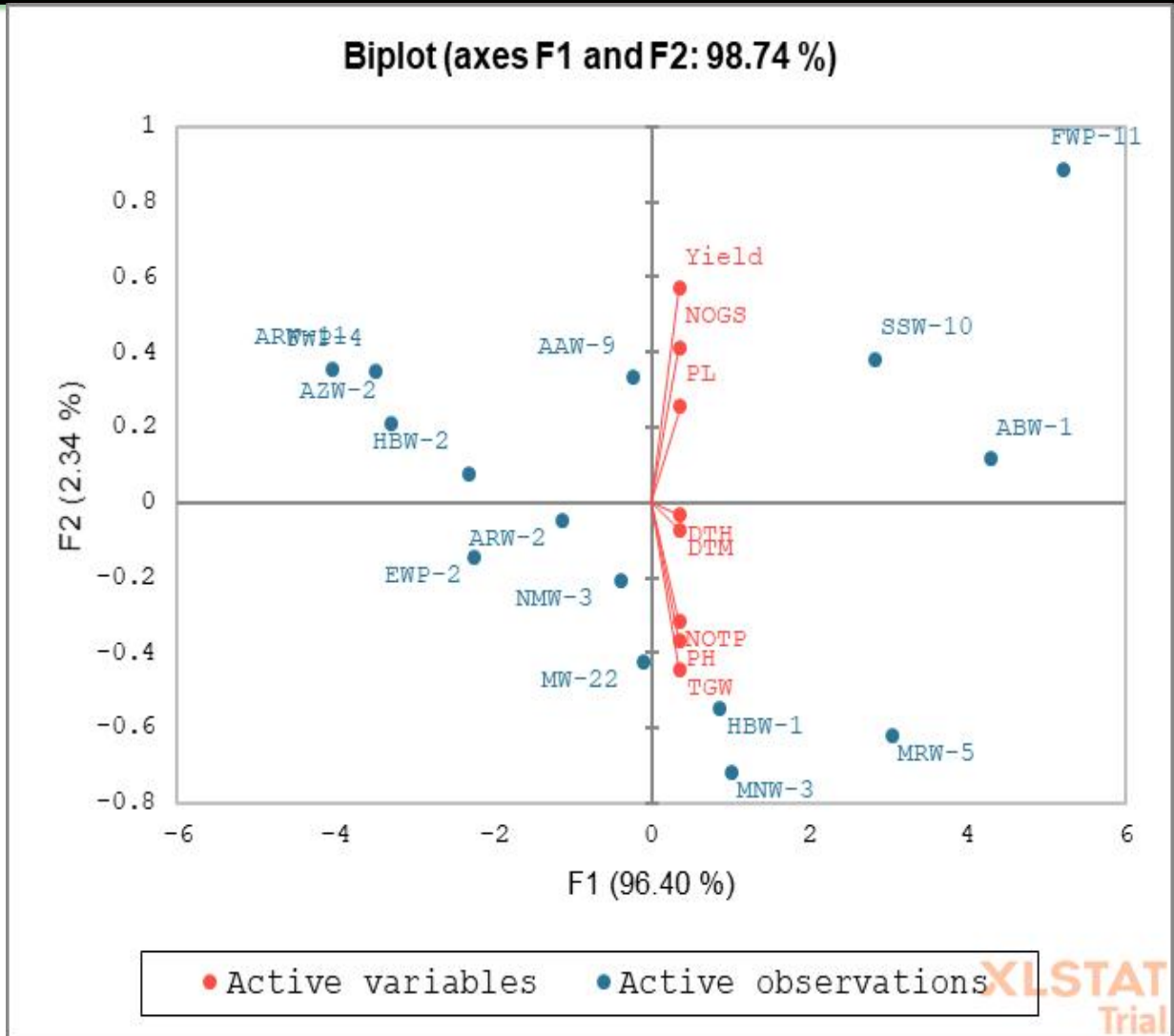


Figure 3: PCA biplot showing the distribution of wheat genotypes and their association with agronomic and yield-related traits based on the first two principal components.

Table 5: Eigenvalues, percentage variability, and cumulative variability explained by the principal components in fifteen wheat genotypes.

	Eigenvalue	Variability %	Cumulative %
PC1	7.712	96.403	96.403
PC2	0.187	2.336	98.740
PC3	0.045	0.568	99.308
PC4	0.032	0.406	99.714
PC5	0.012	0.148	99.862
PC6	0.008	0.148	99.966
PC7	0.002	0.020	99.986
PC8	0.001	0.014	100.000

Cluster Analysis

The cluster analysis categorized fifteen wheat genotypes into two distinct clusters based on their

performance across plant height (PH), thousand grain weight (TGW), number of tillers per plant (NOTP), days to heading (DTH), days to maturity

(DTM), number of grains per spike (NOGS), peduncle length (PL), and yield (Table 7). The dendrogram, using Euclidean distance and Ward's method, divided the genotypes into two clusters, where Cluster-1 consisted of 12 genotypes, and Cluster-2 consisted of 3 genotypes, as shown in Figure 4 and Table 7. Cluster-2 showed superior performance for most of the studied traits, including higher plant height (104.083), thousand grain weight (45.667 g), number of tillers per plant (14.806), days to heading (103.417), days to

maturity (143.750), number of grains per spike (68.083), peduncle length (42.389), and yield (6258.333 kg/ha) (Al-Ashkar et al. 2023). In contrast, Cluster-1 exhibited comparatively lower mean values for these traits, with plant height (96.132), thousand grain weight (42.007 g), number of tillers per plant (12.313), days to heading (96.549), days to maturity (140.389), number of grains per spike (51.840), peduncle length (34.264), and yield (4834.375 kg/ha) (Table 6).

Table 6: Cluster centroids showing mean performance of agronomic and yield-related traits in two wheat genotype clusters.

Variables	Cluster 1	Cluster 2
PH	96.132	104.083
TGW	42.007	45.667
NOTP	12.313	14.806
DTH	96.549	103.417
DTM	140.389	143.750
NOGS	51.840	68.083
PL	34.264	42.389
YIELD	4834.375	6258.333



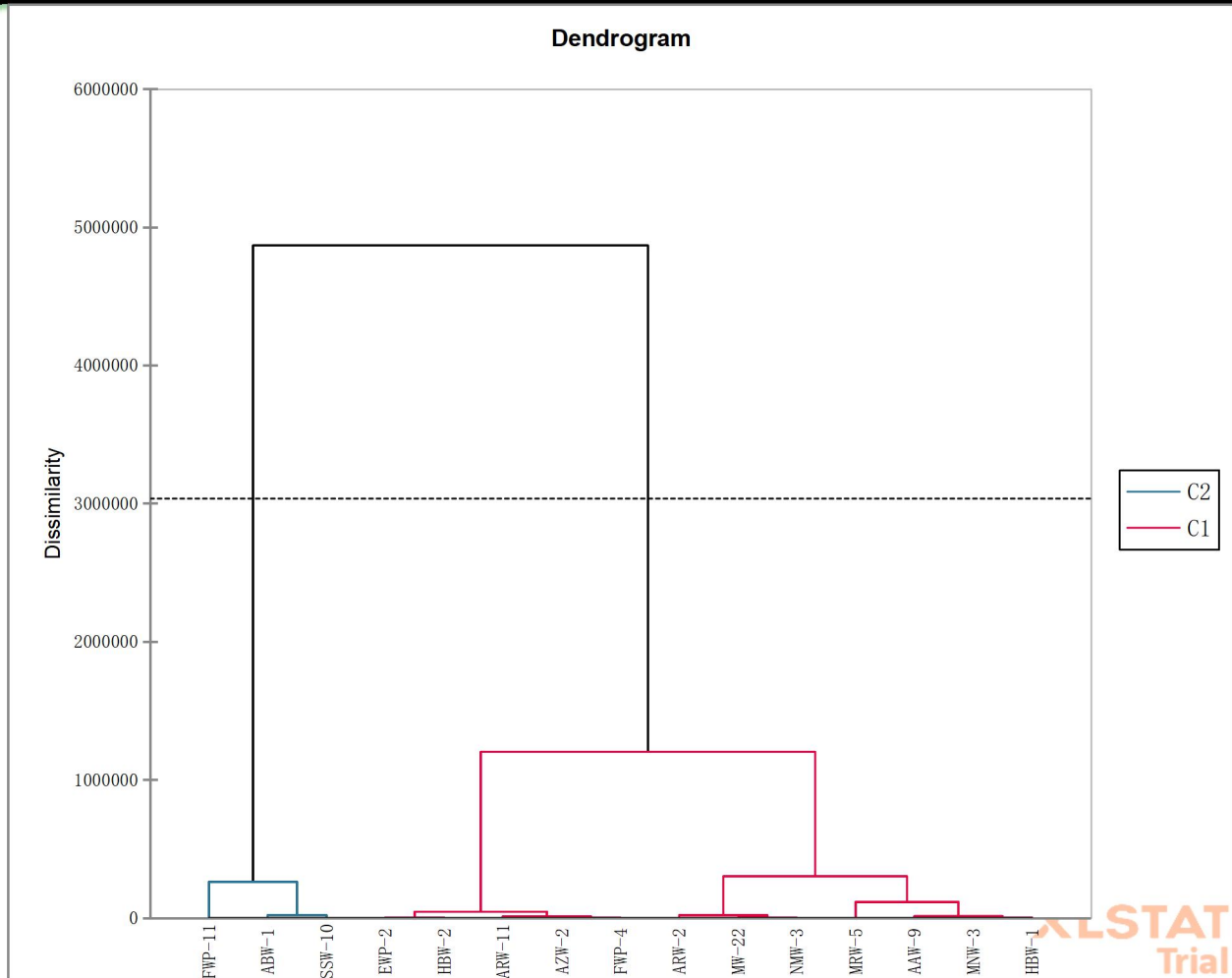


Figure 4: Dendrogram illustrating the hierarchical clustering of fifteen wheat genotypes based on agronomic and yield-related traits using Ward’s method and Euclidean distance.

Table 7: Distribution of fifteen wheat genotypes into two clusters based on multivariate cluster analysis.

CLUTER 1	CLUSTER 2
AZW-2	ABW-1
EWP-2	SSW-10
ARW-11	FWP-11
MW-22	
MNW-3	
ARW-2	
FWP-4	
MRW-5	
NMW-3	
HBW-1	
HBW-2	
AAW-9	

Discussion

The significant variation observed among the

advanced wheat lines for all studied traits indicates the presence of substantial genetic diversity within

the experimental material. Such variability is essential for effective selection and provides opportunities for the development of superior genotypes adapted to semi-arid environments. The observed differences in plant height, tillering capacity, grain weight, maturity duration, number of grains per spike, peduncle length, and grain yield suggest that the evaluated genotypes possess diverse genetic backgrounds and varying levels of agronomic potential.

Grain yield is a complex trait influenced by several interrelated components. The strong positive associations of yield with plant height, thousand-grain weight, number of tillers per plant, days to heading, days to maturity, number of grains per spike, and peduncle length indicate that these traits play an important role in determining yield performance. Among them, the number of grains per spike and peduncle length exhibited the strongest relationships with grain yield, highlighting their importance as potential selection criteria for improving wheat productivity. The positive association among yield-contributing traits further suggests that simultaneous improvement of these characters may result in substantial gains in grain yield.

The principal component analysis effectively summarized the variability present among the wheat lines and identified the traits responsible for the majority of genetic divergence. The first two principal components explained 98.74% of the total variation, demonstrating that most of the observed variability was attributed to a limited number of agronomically important traits. The close association of grain yield with thousand-grain weight, number of grains per spike, peduncle length, and tiller number indicates that these characters were the major contributors to genotype differentiation. Furthermore, the PCA biplot facilitated the identification of superior genotypes by revealing their association with desirable yield-related traits.

Cluster analysis further confirmed the existence of genetic diversity among the evaluated wheat lines by grouping them into two distinct clusters. The superior performance of Cluster-2 for grain yield and most yield-related traits suggests that genotypes within this cluster possess favorable genetic

combinations for enhanced productivity under semi-arid conditions. The clear separation between clusters reflects considerable genetic divergence, which may be exploited in breeding programs through hybridization of genetically distinct parents to generate broad variability and desirable recombinants.

The combined use of correlation analysis, principal component analysis, and cluster analysis proved highly effective in characterizing the genetic diversity and yield potential of the evaluated wheat lines. Based on their superior performance and favorable association with key yield-contributing traits, the genotypes FWP-11, SSW-10, and ABW-1 emerged as the most promising candidates for future varietal development and breeding programs. These genotypes may serve as valuable genetic resources for improving wheat productivity and adaptation under semi-arid agro-ecological conditions.

Conclusion

The present study demonstrated substantial genetic variability or divergence among the evaluated wheat genotypes under semi-arid climatic conditions, providing valuable opportunities for selection and genetic improvement. Yield performance was primarily governed by yield-contributing traits such as thousand grain weight, number of tillers per plant, days to maturity, and number of grains per spike, emphasizing their importance as key selection criteria in breeding programs. Multivariate analyses, including cluster analysis and principal component analysis, effectively differentiated the genotypes and identified superior performers. Genotypes grouped in Cluster-2, particularly FWP-11, SSW-10, and ABW-1, exhibited higher yield potential, making them promising candidates for varietal release or use as parental material in hybridization programs. Overall, the findings provide a robust scientific basis for selecting high-yielding and adaptable wheat genotypes and contribute to the development of improved varieties suited for semi-arid regions.

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facilities and technical support for conducting this study.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Authors' Contribution

Muhammad Noman Manzoor conceived and designed the study, collected and analyzed the data, and prepared the manuscript. Muhammad Arham Rayan and Muhammad Hamza Saeed assisted in field experimentation and data collection. Sayeda Zunaira Kiran, Asma Mukhtar, Muhammad Raza, Shabana Naz, Sana Ashraf, Eesha Afshal, Zargham Abbas, Atufa Batool, and Noshaba Batool contributed to data interpretation, manuscript review, and editing. All authors read and approved the final manuscript.

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