

Multivariate Analysis of Grain Physical Architecture and Yield Performance in Bread Wheat Genotypes

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ABSTRACT

Grain physical characteristics play an important role in determining wheat yield potential, milling quality, and end-use performance. Understanding the relationships among grain architectural traits and yield components can improve selection efficiency in wheat breeding programs. In the present study, 30 bread wheat genotypes were evaluated to identify superior genotypes with desirable grain physical and yield characteristics. Significant genotypic differences ($P \leq 0.01$) were observed for all studied traits, confirming substantial phenotypic diversity within the evaluated germplasm. Mean values for grains per spike (GPS), thousand grain weight (TGW), grain yield per plant (GYP), grain length (GL), grain width (GW), grain thickness (GT), grain sphericity (GS), and grain surface area (GSA) were 50.81, 41.41 g, 18.14 g, 6.41 mm, 3.13 mm, 2.74 mm, 59.30, and 38.38 mm², respectively. Correlation analysis revealed strong positive associations among major grain dimensional and yield traits, particularly between grain surface area and grain length ($r = 0.82^{**}$), thousand grain weight and grain length ($r = 0.81^{**}$), and thousand grain weight and grain surface area ($r = 0.73^{**}$). Principal component analysis showed that the first two principal components explained 78.85% of the total variation, while PCA biplot and hierarchical cluster heatmap analyses separated genotypes into superior, intermediate, and poor-performing groups. Genotypes G5, G11, G20, and G28 consistently exhibited superior performance for grain dimensions, grain surface area, and yield-associated traits, whereas G3, G9, G17, and G24 showed comparatively lower performance. TGW, GL, GW, GT, and GSA emerged as useful indirect selection traits for improving yield potential, whereas GS showed a weak association with yield-related traits and may be less effective for direct yield-based selection. These findings provide valuable insights for future wheat breeding programs focused on yield improvement and grain quality enhancement.

Keywords: phenotyping, multivariate, germplasm, variability, yield.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is an important staple cereal crop worldwide and is central to global food security, human nutrition, and agricultural sustainability. Improving wheat productivity remains a great challenge as the world grapples with rapid population increase, climate variability and shrinking agricultural resources. In modern wheat breeding programs it is no longer

sufficient to improve just grain yield, and more emphasis is being placed on grain physical architecture since grain dimensional traits strongly impact grain weight, milling quality, grain filling efficiency, and end-use performance (Atsbeha et al., 2023; Mahnoor et al., 2025). In addition to yield improvement, understanding grain physical attributes has become increasingly important for developing wheat cultivars with improved processing quality and market value. Variation in grain

dimensions among genotypes provides useful information for breeders to select materials with desirable grain characteristics under diverse production environments (Ahmed et al., 2025b; Naveen et al., 2026).

Knowledge of the relationships between grain physical metrics and yield traits will therefore aid in the breeding of high-yielding and quality wheat (Yücel et al., 2009; Ahmed et al., 2018). Grain yield in wheat is a quantitative trait whose expression depends on the overall interaction between several yield components, such as grains/spike, thousand grain weight, and grain morphology traits. Grain length, grain width and grain thickness determine kernel size and grain density. Derived traits, such as grain sphericity and grain surface area, provide further insights into grain shape and patterns of seed development (Arshad et al., 2025; Mahnoor et al., 2025). Multiple investigations have shown that larger and better structured grain morphology typically leads to greater accumulation of stored assimilates, greater grain filling, and greater potential for overall productivity (Aniskina et al., 2024; Baloch et al., 2024). Therefore, grain morphology is becoming increasingly used as an indirect selection tool in wheat breeding programs.

The advent of multivariate statistical methods has profoundly aided the efficiency of genotype characterization and trait association in wheat. Recent studies have highlighted the importance of integrating diverse biological, structural, and analytical information to better understand complex traits and their underlying responses (Abubakar et al., 2023; Tong et al., 2026). Descriptive statistics and analysis of variance are used to ascertain the level of interaction or variability for phenotypic traits and the extent of genetic effect produced by differences among genotypes (Habbadi et al., 2025; Kumarbayeva et al., 2025). The use of Pearson correlation analysis identifies yield and other grain architectural traits with either a positive or negative relationship, for use in indirect selection when breeding (Khan et al., 2022; Ahmed et al., 2023). Principal component analysis (PCA) has also become

an attractive multivariate tool for reducing dimensionality and identifying major sources of phenotypic variation in collections of wheat germplasm, and PCA biplot analysis facilitates visualization of genotype-trait associations and grouping of genotypes (Fouad, 2020; Bibi et al., 2022).

Likewise, a hierarchical cluster heatmap approach has shown efficacy in the assessment of genetic diversity and the identification of superior groups of genotypes for integrated traits performance. Heatmap visualization facilitates simultaneous interpretation of multivariate trait distributions and genotype similarity patterns, thus aiding efficient parental selection in wheat breeding programs (Ali et al., 2021; Singh et al., 2025). Therefore, correlation analysis, PCA and hierarchical clustering can be used together for a comprehensive understanding of grain physical architecture and yield relationships in wheat (Naveen et al., 2026). In modern wheat breeding, the simultaneous evaluation of multiple traits provides a more effective strategy for identifying superior genotypes than selection based on individual traits alone. Multivariate approaches integrate information from several correlated attributes, allowing breeders to understand trait contributions, genotype performance patterns, and potential selection criteria for yield improvement (Manzoor et al., 2026). Such approaches are particularly useful for complex traits like grain yield, where the combined expression of yield components and grain physical characteristics determines overall breeding value (Chen et al., 2022; Ahmed et al., 2025a; Naveen et al., 2026).

The identification of stable and superior genotypes requires an integrated evaluation approach because individual traits may not fully represent overall breeding potential. Therefore, combining phenotypic characterization with statistical approaches provides a comprehensive framework for understanding genetic diversity and selecting promising materials for future wheat improvement (Naveen et al., 2026; Rani et al., 2026). The experiment was, therefore, conducted to decipher grain physical

architecture and grain yield potential in bread wheat genotypes using descriptive statistics, correlation analysis, principal component analysis and hierarchical clustering approaches. The data presented would be useful in identifying superior wheat genotypes and may also assist future wheat breeding and selection efforts.

MATERIAL AND METHODS

This experiment was conducted during the Rabi season 2024-2025 at the research field of the Department of Plant Breeding and Genetics (PBG), Faculty of Agriculture and Environment (FA&E), The Islamia University of Bahawalpur (IUB), Bahawalpur, Punjab, Pakistan. The experimental site is located at approximately 29°24' N latitude and 71°47' E longitude, with an altitude of about 117 m above sea level. The region is characterized by an arid to semi-arid climate with hot summers, cool winters, low relative humidity, and limited rainfall. During the wheat growing season, temperature ranged from approximately 11 to 21°C during early crop growth, with increasing temperatures toward crop maturity. The annual rainfall of the region is generally low and variable, while the experimental soil is mainly characterized as alluvial loamy soil suitable for wheat cultivation. Thirty bread wheat genotypes were used as breeding material, obtained from the Department of PBG, The Islamia University of Bahawalpur. These genotypes represented diverse breeding materials developed and maintained for wheat improvement purposes and were evaluated to investigate variability in grain physical architecture and yield potential through phenotypic and multivariate approaches.

These genotypes were evaluated under field conditions in a randomized complete block design (RCBD) with three replications during a normal wheat growing season. Sowing was done manually by the dibbling method under uniform agronomic conditions. Row-to-row and plant-to-plant distances were 30 cm and 15 cm, respectively. Only healthy seedlings were retained while

thinning was done to maintain one healthy plant per hill. The crop was irrigated four times at critical growth stages including crown root initiation, tillering, booting, and grain filling stages. All recommended agronomic and crop management practices were applied uniformly during the experimental period to maintain consistency in the experimental procedure.

Traits Measurement

At maturity, representative plants from each replication were harvested and the grains assessed for physical and yield characters. Grains per spike (GPS) was assessed as the total number of grains counted from randomly selected spikes. Thousand grain weight (TGW) was determined as the weight of 1000 healthy and fully matured grains recorded in grams using a digital analytical balance. Grain yield per plant (GYP) was assessed by threshing individual plants and recording the grain weight per plant. Grain dimensional or physical traits like grain length (GL), grain width (GW), and grain thickness (GT), were estimated in millimeters (mm) using the digital Vernier caliper as described by (Ahmed et al., 2018), while grain sphericity (GS) and grain surface area (GSA) were calculated from grain dimensional parameters, using the following equations (Mohsenin, 1986; Yücel et al., 2009)

Grain sphericity calculate as

$$GS = \left(\frac{(GL \times GW \times GT)^{1/3}}{GL} \right) \times 100$$

where: GL = grain length, GW = grain width, and GT = grain thickness.

Grain surface area calculate as

$$B = \sqrt{GW \times GT}, GSA = \frac{\pi \times B \times GL^2}{2GL - B}$$

where: B represents the geometric mean of grain width and grain thickness.

Statistical Analysis

Descriptive statistics, coefficient of variation, and analysis of variance were computed to evaluate phenotypic variability and genotypic differences among the evaluated genotypes, while Pearson correlation analysis was used to determine

associations between traits (Pearson, 1895; Fisher, 1954; Gomez and Gomez, 1984). Principal component analysis was performed to summarize major sources of variation among grain physical and yield traits (Jolliffe, 2022). Hierarchical cluster heatmap analysis was performed using standardized trait values and Euclidean distance. All statistical analyses and graphical representations such as correlation matrix, scree plot, PCA biplot, hierarchical heatmap and violin distribution plots were performed using R software (version 4.3.0) through different statistical and visualization packages namely *ggplot2*, *corrplot*, *factoextra*, *pheatmap* and *tidyverse* (Wickham et al., 2019).

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) indicated highly significant ($P \leq 0.01$) differences among bread wheat genotypes for all studied grain physical and yield traits suggesting that sufficient variability exists among the evaluated genotypes (Table 1). In contrast, the replication effect remained non-significant for all traits implying no significant variation among replications (Mahnoor et al., 2025).

Distribution Pattern and Descriptive Variability

There was considerable phenotypic diversity observed for all grain physical traits and yield traits among the bread wheat genotypes evaluated in this study (Figure 1), indicating considerable phenotypic variation potentially available in these traits for improved architecture and yield. The wider the regions of the violin components, the greater the data density, indicating greater phenotypic dispersion, while the narrower the region, the more stable the observations in that specific trait. The X-axis showed the list of studied traits and the Y-axis exhibited the observed values while the coloured annotations indicate CV%, maximum, minimum, mean and SD values of each trait studied. Grains per spike (GPS) showed a mean value of 50.81 (41.67-62.00; SD = 5.25; CV = 10.33%). The general variation in GPS reflects differences among genotypes in

spike fertility and sink capacity, which together determine grain accumulation and yield performance. Similar results have been obtained in studies of germplasm in bread wheat (Aniskina et al., 2024; Mahnoor et al., 2025).

Thousand grain weight (TGW) ranged from 31.95 to 49.78 g, with a mean of 41.41 g and CV of 9.72%. These differences in TGW indicated differences in assimilate transport and grain filling capacity among the genotypes. However, higher TGW reflects more effective deposition of assimilates during grain filling as also reported in previous studies in wheat (Ahmed et al., 2023; Aniskina et al., 2024). Grain yield per plant (GYP) ranged from 14.74 to 21.45 g with a mean of 18.14 g and CV of 9.10%, indicating significant differences in productivity potential among the studied genotypes. Given that grain yield is regulated by many interacting components, the variation may be attributed to the number of grains and grain dimensional traits. Similar variability has been reported for diverse bread wheat populations (Ali et al., 2020; Khan et al., 2020).

Grain length (GL) ranged from 5.74 to 7.28 mm with a mean of 6.41 mm and a relatively low CV (5.96%), being fairly stable in terms of seed elongation among the genotypes. In contrast, grain width (GW) and grain thickness (GT) showed moderately high ranges of variation of 2.75-3.68 mm and 2.34-3.18 mm with CV values of 8.25% and 7.66%, respectively. Variation in these traits reflects differences in grain compactness, starch accumulation, and grain dimensions that affect grain weight and milling quality (Wang et al., 2025). Grain sphericity (GS) ranged from 53.75 to 63.80, with a mean value of 59.30 and the lowest CV (3.18%) across all traits of interest in this research, indicative of relatively stable grain shape architecture across genetic backgrounds. Given that GS depends on grain-dimensional traits, its lower degree of variability may reflect coordinated grain development. Previously, similar trends have been reported in wheat morphological studies (Ahmed et al., 2018; Mahnoor et al., 2025).

Grain surface area (GSA) ranged from 29.36 to 48.42 mm² with a mean value of 38.38 mm² and a CV of 13.08%. The wider range of GSA indicates considerable differences in grain size and external morphology among the genotypes. Since

GSA is influenced by the size of the grain, its variation may reflect differences in grain development among genotypes. Similar findings have been observed in recent wheat morphometric studies (Ali et al., 2020; Aniskina et al., 2024; Arshad et al., 2025).

Table 1. Analysis of Variance (ANOVA) for Grain Physical and Yield Traits in Bread Wheat Genotypes

Source	df	GPS	TGW	GYP	GL	GW	GT	GS	GSA
Replication	2	2.011 ^{ns}	3.179 ^{ns}	0.329 ^{ns}	0.003 ^{ns}	0.005 ^{ns}	0.001 ^{ns}	3.384 ^{ns}	2.365 ^{ns}
Genotype	29	82.682 ^{**}	48.633 ^{**}	25.991 ^{**}	0.438 ^{**}	0.200 ^{**}	0.130 ^{**}	21.898 ^{**}	25.504 ^{**}
Residuals	58	3.207	1.189	0.412	0.018	0.006	0.004	0.915	0.844
Total	89								

Note: ** and * indicate significance at $P \leq 0.01$ and $P \leq 0.05$, respectively, whereas ns indicates non-significant differences. df = Degree of freedom, GPS = Grains per Spike; TGW = Thousand Grain Weight; GYP = Grain Yield per Plant; GL = Grain Length; GW = Grain Width; GT = Grain Thickness; GS = Grain Sphericity; GSA = Grain Surface Area.

Mean Performance of Grain Physical and Yield-Related Traits

A significant level of variation in physical grain and yield traits among the various bread wheat genotypes evaluated was found (Table 2). The evaluated bread wheat genotypes exhibited considerable variation in grain physical and yield-related traits. For grains per spike (GPS), the highest value obtained was for genotype G28 (62.00), followed by G20 (59.67), and G5 (57.67) while the lowest GPS values were recorded for G4 (42.33), G9 (41.67), and G24 (41.67). Greater output of GPS suggests greater sink capacity and higher ability to produce grains. Similar trends in spike fertility and wheat productivity were reported by many scientists. They also reported that stress-responsive genes contribute to the regulation of important agronomic traits in wheat (Khan et al., 2020; Sarfraz et al., 2025). For thousand grain weight (TGW), higher values were recorded for G5 (49.78 g), G28 (49.04 g), and G20 (48.06 g) while lower values were recorded for G9 (31.95 g), G17 (35.40 g), and G12 (35.73 g). Higher TGW suggests better grain filling and accumulation of assimilates, which directly affecting final grain yield. Comparable findings were reported by (Ahmed et al., 2023; Aniskina et al., 2024). For GYP, the highest value was recorded in genotype G20 (21.45 g), followed by G28 (20.66 g), G11 (20.65 g) and G19 (20.02 g);

conversely lower GYP values were recorded for G9 (14.74 g), G3 (14.85 g) and G16 (16.08 g) genotypes. The higher yielding genotypes generally exhibited favorable grain dimensional traits, indicating possible interrelatedness between morphological and yield components of grains. Similar observations were described by (Nageshwar et al., 2021; Shirdelmoghanloo et al., 2022).

A considerable variation was also observed for GL where G28 recorded the highest value of 7.28 mm followed by G20 (7.21mm), G5 (6.92mm) and G10 (6.88mm). The lowest grain length was recorded for G24 (5.74 mm), followed by G9 (5.76 mm). Long grains are often associated with good milling and better grain development (Khan et al., 2020). In terms of grain width (GW), genotype G28 scored the highest (3.68 mm) followed by G11 (3.55 mm), G5 (3.52 mm) and G10 (3.51 mm), while the lowest GW values were found in G16 (2.75 mm) G24 (2.75) and G17 (2.77 mm). Like GW, the value of grain thickness (GT) varied from 2.34 mm for G24 to 3.18 mm for G20 and G18 (3.13 mm) and G5 (3.05 mm). Improved grain width and thickness, enhance the grain plumpness which is an important parameter of grain quality (Shirdelmoghanloo et al., 2022; Mahnoor et al., 2025).

Grain sphericity (GS) ranged from 53.75 in G8 to 63.80 in G18. Higher values were also found in G12 (62.84) and G17 (62.64),

indicative of a relatively more regular geometry of the grains. Since GS is a mathematical derivation of the dimensions of the grain, the exhibited variation indicates differences in shape and structure of the kernel among the genotypes. Similarly, grain surface area (GSA) ranged from a high of 48.42 mm² in G20 to a low of 29.36 mm² in G24. Other high values were obtained in G28

(48.16 mm²), G11 (46.74 mm²) and G5 (46.66 mm²) while lower values were obtained in G9 (31.00 mm²) and G17 (32.00 mm²). Higher GSA reflects greater grain dimensions and overall kernel size. Similar associations between grain dimensions and grain surface characteristics have been reported in wheat (Ahmed et al., 2018; Arshad et al., 2025).

Table 2. Mean performance of grain physical and yield-related traits in bread wheat genotypes

Genotype	GPS	TGW	GYP	GL	GW	GT	GS	GSA
G1	49.33	41.47	16.92	6.47	3.12	2.77	59.10	38.66
G2	53	43.36	18.84	6.39	3.26	2.73	60.18	39.06
G3	44.33	36.9	14.85	6.24	2.84	2.62	57.60	34.22
G4	42.33	41.29	18.12	6.57	3.34	2.92	60.91	42.28
G5	57.67	49.78	19.2	6.92	3.52	3.05	60.75	46.66
G6	49.33	41.69	18.59	6.34	3.08	2.68	59.00	36.99
G7	56	40.81	18.85	6.4	3.16	2.73	59.50	38.32
G8	50	40.67	18.54	6.68	2.84	2.44	53.75	34.40
G9	41.67	31.95	14.74	5.76	2.93	2.38	59.46	31.00
G10	47.33	43.78	19.97	6.88	3.51	2.74	58.79	43.27
G11	55.33	47.5	20.65	6.88	3.55	3.05	61.16	46.74
G12	47	35.73	16.16	5.78	3.07	2.7	62.84	34.81
G13	54.67	42.14	18.76	6.27	3.04	2.79	59.98	37.36
G14	47	40.07	18.43	6.24	2.81	2.51	56.58	33.07
G15	55.33	39.14	19.1	5.92	3.03	2.48	59.85	33.17
G16	52.67	43.96	18.13	6.32	2.75	2.75	57.42	34.89
G17	48	35.4	17.73	6.04	2.77	2.51	57.55	32.00
G18	46.67	43.04	16.67	6.21	3.2	3.13	63.80	41.43
G19	56.33	39.79	20.02	6.51	3.08	2.98	60.05	40.37
G20	59.67	48.06	21.45	7.21	3.42	3.18	59.36	48.42
G21	49	39.11	17.32	6.15	2.99	2.69	59.69	35.61
G22	54.67	42.35	17.08	6.55	3.33	2.74	59.69	40.39
G23	45	39.66	16.74	6.16	2.88	2.58	58.07	33.87
G24	41.67	36.63	16.41	5.74	2.75	2.34	58.02	29.36
G25	52	41.35	17.56	6.47	3.44	2.81	61.35	41.59
G26	49	44.9	17.97	6.64	3.26	2.83	59.37	41.08
G27	51.67	38.86	16.08	6.31	3.08	2.8	60.06	37.93
G28	62	49.04	20.66	7.28	3.68	2.9	58.61	48.16
G29	55	41.19	19.04	6.46	3.04	2.81	58.93	38.33
G30	50.67	42.75	19.68	6.59	3.08	2.68	57.50	38.03

Pearson Correlation among Grain Physical and Yield-Related Traits

Pearson correlation analyses indicated significant positive correlations among most grain physical and yield-related traits in bread wheat genotypes (Figure 2). Red colour indicated positive correlations and blue indicated negative correlations among traits. *, ** and ns indicate significant ($P \leq 0.05$), highly significant ($P \leq 0.01$) and non-significant correlations respectively, and larger pie segments indicate stronger correlation

strength. Grains per spike (GPS) showed significant positive associations with thousand grain weight (TGW; $r = 0.64^{**}$), grain yield per plant (GYP; $r = 0.52^{**}$), grain length (GL; $r = 0.58^{**}$), grain width (GW; $r = 0.48^{**}$), grain thickness (GT; $r = 0.48^{**}$), and grain surface area (GSA; $r = 0.53^{**}$). Such positive associations suggest that genotypes with higher GPS tended to have greater grain dimensions and yield potential. Similar positive correlations between spike

traits and grain yield parameters have also been reported in wheat breeding populations (Khan et al., 2020; Nageshwar et al., 2021). Thousand grain weight (TGW) was positively correlated with GL ($r = 0.81^{**}$), GSA ($r = 0.73^{**}$), GW ($r = 0.68^{**}$) and GT ($r = 0.65^{**}$). These results indicated a strong

association between grain dimensions and grain weight. The positive correlations between TGW and grain dimensions may reflect greater assimilate accumulation during grain filling. Comparable findings were reported by (Ullah et al., 2021; Ahmed et al., 2023).

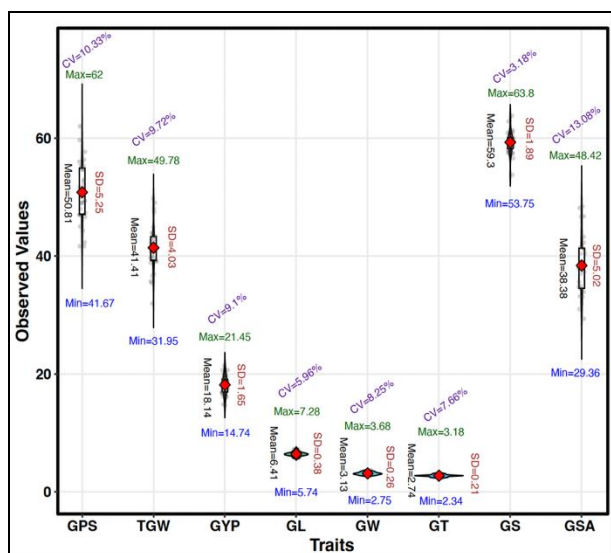


Figure 1. Distribution pattern and descriptive variability of grain physical and yield-related traits in bread wheat genotypes

Grain yield per plant (GYP) also showed significant positive correlations with TGW ($r = 0.56^{**}$), GL ($r = 0.47^{**}$), GW ($r = 0.42^{**}$), and GSA ($r = 0.44^{**}$), suggesting that grain dimensional architecture is associated with grain productivity. Relationships between yield and grain morphology have previously been observed for bread wheat (Ali et al., 2020; Arshad et al., 2025). Among grain dimensional traits, GL showed strong positive correlations with GW ($r = 0.70^{**}$), GT ($r = 0.60^{**}$), and GSA ($r = 0.82^{**}$). GW also showed strong associations with GT ($r = 0.65^{**}$) and GSA ($r = 0.82^{**}$). Furthermore, grain sphericity (GS) was not significantly correlated with any of the grain-related traits, including GYP ($r = -0.02^{ns}$), TGW ($r = 0.04^{ns}$) and GSA ($r = 0.01^{ns}$).

Principal Component Analysis of Grain Physical and Yield-Related Traits

Principal component analysis (PCA) demonstrated considerable variation among the

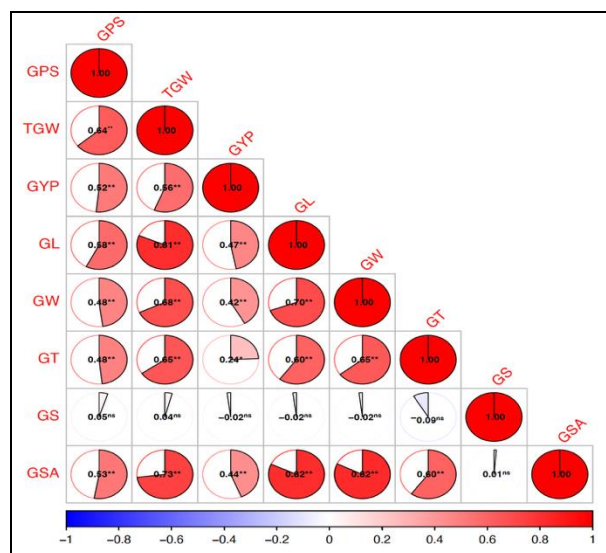


Figure 2. Pearson correlation matrix among grain physical and yield-related traits in bread wheat genotypes

assessed bread wheat genotypes (Figure 3). Bars in this figure indicate the variance accounted for by individual principal components while the line indicates the cumulative variance accounted for by successive components. Eigenvalues (Eig) above the bars corresponding to each principal component indicate the strength of contribution to the variation detected. The first two principal components accounted for 78.86% of the total variation, indicating that these components accounted for most of the phenotypic variation present in the germplasm explored. PC1 had an eigenvalue of 5.21 accounting for 65.16% variation whereas PC2 accounted for 13.69% variation with an eigenvalue of 1.1. The relatively high eigenvalue of PC1 suggests that yield and grain quantitative traits (dimension indices) cumulatively played a significant role in contributing to overall genotypic variation, similar to the dominance of the first principal component for yield associated traits in bread wheat diversity (Ali et al., 2021; Bibi et al., 2022).

The cumulative variance reached 86.95% following PC3, but lesser variation was observed for subsequent components indicating that there was relatively little additional information beyond the first handful of principal components. PC3, PC4, and PC5 contributed 8.10%, 4.91%, and 3.63% variation, respectively, whereas PC6–PC8 each explained <3% variation. The data suggest that most of the shared phenotypic diversity was harvested within major grain architectural and yield components. Upgraded eigenvalues and variation contributions associated with the early components signifies tight organization across grain dimensional traits, grain surface area and yield components. Similar PCA patterns highlighting the importance of grain morphology and yield components in driving wheat genotypic variation have been reported by (Gao et al., 2021; Shirdelmoghanloo et al., 2022; Zewdu et al., 2024).

The PCA biplot demonstrated genetic diversity and clustering of the evaluated bread wheat genotypes based on grain

physical and yield related traits (Figure 4). Arrows indicate the direction and contribution of individual traits toward principal components, whereas the genotypes positioned closer together would be more similar in terms of traits. Coloured circles represent genotype groups determined through multivariate associations of traits and clustering patterns. The first two principal components, PC1 and PC2, explained 65.16% and 13.69% of the total variation, respectively, accounting for 78.85% of the total phenotypic variability. Most traits of grain dimension and yield, including GPS, TGW, GYP, GL, GW GT and GSA were positively associated and clustered on the positive side of PC1. The close and positive association between TGW, GYP, GW and GSA suggest close relationships among grain dimensions, grain weight, and yield performance. Similar associations among grain physical traits and yield components have been reported (Ahmed et al., 2018; Khan et al., 2020; Arshad et al., 2025).

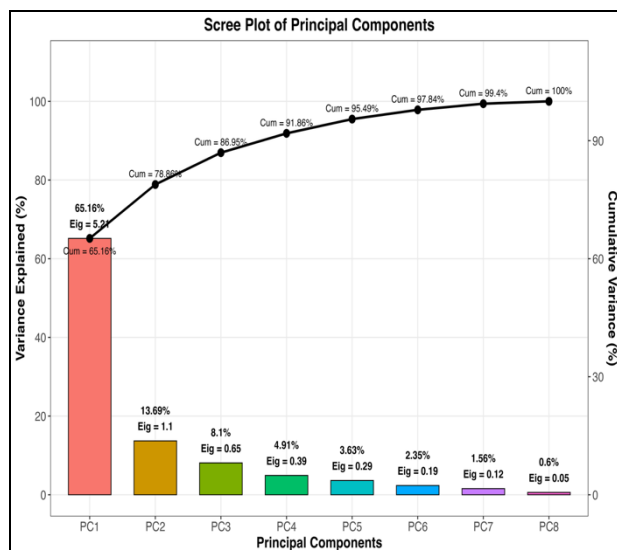


Figure 3. Scree plot and cumulative variance explained by principal components for grain physical and yield-related traits in bread wheat genotypes

Conversely, GS was situated separately in the negative direction of PC2, indicating a relatively distinct contribution of this trait from other grain dimensional traits, which is in accordance with the correlation analysis where GS weakly and non-significantly associated

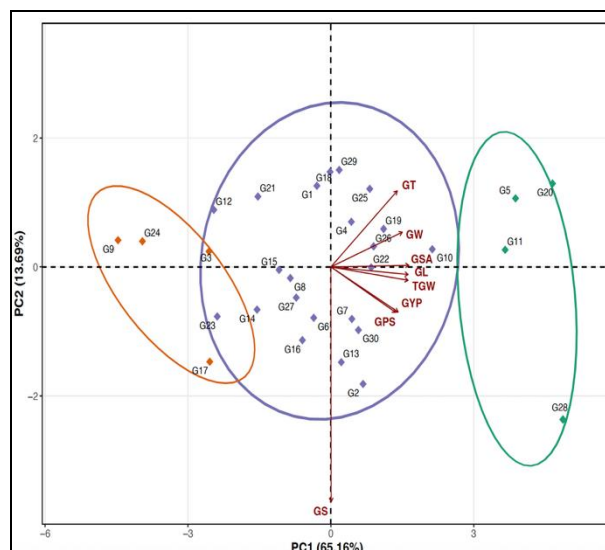


Figure 4. Principal component analysis (PCA) biplot illustrating the association among grain physical and yield-related traits and the clustering pattern of bread wheat genotypes

with most yield-related parameters. The biplot identified identified genotypes in distinct clusters as well, and indicated the superior performing genotypes G5, G11, G20 and G28 on the positive side of PC1 strongly associated with TGW, GYP, GL, GW, GT and GSA, GG

actively contributes to greater grain yield for the biplots of genotype coordinates. The poorly performing genotypes like G9, G17 and G24 on the negative side of PC1 were characterized by comparatively low values for important yield and grain dimensional traits. Mostly, intermediate genotypes were located the central region of the biplot representing moderate performance and balanced overall trait characteristics. The observed pattern of clustering indicated that PCA was effective in distinguishing genotypes based on integrated grain physical and yield related characteristics. Similar PCA-based genotype separation patterns have been reported in bread wheat germplasm evaluations (Khan et al., 2020; Ali et al., 2021; Rasool et al., 2021).

Hierarchical Cluster Heatmap Analysis of Bread Wheat Genotypes

Hierarchical cluster heatmap demonstrated high variability among bread wheat genotypes in relation to grain physical and

yield-related traits (Figure 5), where the red color intensity indicates relatively high values and blue color intensity relatively low values among genotypes. Dendrogram and side color bars showing clustering and similarity of genotypes (Poudel et al., 2017; Zewdu et al., 2024). The heatmap clustered genotypes effectively into different clusters based on their performance across most multivariate traits, with each cluster exhibiting noticeably different architecture for grain traits/yield. Higher yielding genotypes like (G28, G20, G11, and G5) clustered separately as they have higher value of GPS, TGW, GYP, GL, GW, GT and GSA (indicated by dominance of red color). The clustering serves to validate the desirable grain dimension attributes and potential productivity pedigree of these genotypes. Similar clustering trend among the high yielding genotypes of wheat has been reported in recent multivariate diversity studies (Aniskina et al., 2024; Arshad et al., 2025; Mahnoor et al., 2025).

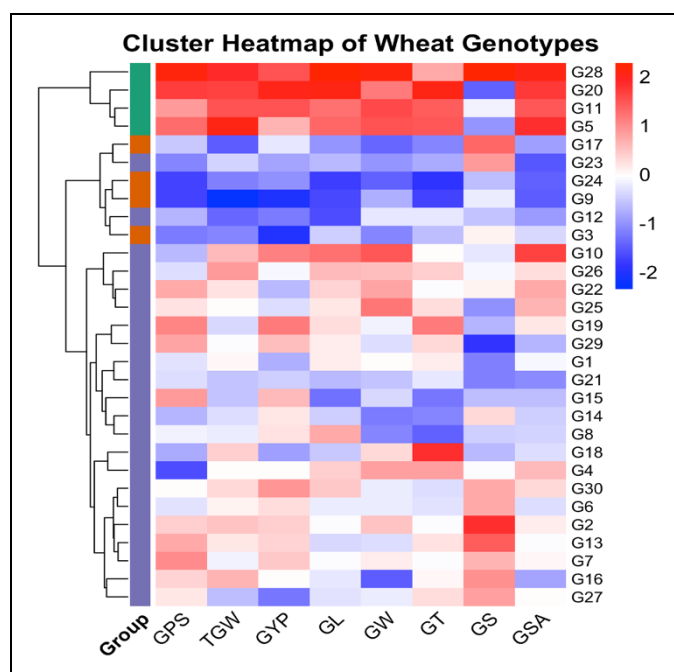


Figure 5. Hierarchical cluster heatmap showing the multivariate distribution and grouping pattern of bread wheat genotypes based on grain physical and yield-related traits

In contrast, poor genotypes, like G9, G24, G17, and G3, were grouped in another cluster associated with lower values of major yield and grain dimensional traits, characterized by

a blue color. The low expression of TGW, GYP, GL and GSA in these genotypes indicate relatively poor grain filling efficiency and kernel development. The

majority of intermediate genotypes were located within the central cluster and were of moderate expression for grain physical as well as yield related traits. The close linkage of TGW, GYP, GL, GW, GT and GSA reinforces the strong associations exhibited by correlation coefficients and PCA results. Meanwhile, GS displayed a comparatively distinct pattern of distribution thus revealing its lesser associated nature with the other traits examined (Ali et al., 2020; Gao et al., 2021; Bibi et al., 2022; Zewdu et al., 2024). Overall, the hierarchical heatmap underscored the utility of multivariate clustering in recognizing bread wheat genotypes according to integrated grain physical and yield characteristics. Similar heatmap based clustering of the likely wheat germplasm have been reported previously for wheat characterization studies (Yücel et al., 2009; Aniskina et al., 2024).

CONCLUSIONS

The present study revealed substantial phenotypic diversity among bread wheat genotypes for grain physical and yield-associated traits, confirming the availability of valuable genetic variation for wheat improvement. Strong positive associations among thousand grain weight, grain dimensions, grain surface area, and grain yield indicate that these traits can serve as reliable indirect selection criteria in breeding programs aimed at enhancing productivity. In contrast, grain sphericity showed weak relationships with yield-associated traits, suggesting limited value as a direct selection parameter. Multivariate analysis, including principal component analysis and hierarchical cluster, effectively classified genotypes into distinct superior, intermediate, and poor-performing groups. Superior genotypes including G5, G11, G20, and G28 consistently maintained favourable expression for major grain and yield traits. TGW, GL, GW, GT, and GSA emerged as useful indirect selection traits for improving yield potential, whereas GS showed weak association with yield-related traits and may be less effective for direct yield-based selection. The identified superior

genotypes may serve as valuable genetic resources for the development of high-yielding wheat cultivars with improved grain architecture and kernel quality under future breeding programs.

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