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Genetic Diversity Studies in Wheat (*Triticum aestivum* L.) Germplasm in Doon Valley

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Authors' contributions

This work was carried out in collaboration among all authors. Author AST conceived and designed the experimental study, conducted the field trials, recorded observations and maintained a detailed observation diary. She performed all laboratory experiments and carried out comprehensive analyses of phenotypic and genotypic traits. Quality traits were also evaluated and analyzed by the same author. Author GS was present throughout the experiment to guide the author AST. Author SP read and approved the manuscript.

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Abstract

The present research study envisages genetic variability, correlation and multivariate diversity analysis in bread wheat (*Triticum aestivum* L.). 75 germplasm lines plus 5 checks (HD 2851, HD 3086, RAJ 3765, DBW-187 and PBW 343) were evaluated in an Augmented Complete Block Design. Fifteen quantitative traits were studied. ANOVA indicated significant variation among genotypes for all traits, supporting the presence of exploitable diversity. Significant Variation was

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observed for biological yield per plant and grain yield per plant whereas phenological traits exhibited less variation. Grain yield per plant showed significant positive correlations with biological yield, productive tillers, grains per spike and 1000-grain weight, and a significant negative correlation with days to 50% flowering. Principal component analysis retained six components explaining 64.27% of total variation. Hierarchical clustering grouped genotypes into seven clusters; the maximum inter-cluster distance occurred between clusters IV and VI (D = 4.48), indicating wide divergence. Overall, the results illustrate how variability, correlation, PCA and clustering can guide trait prioritization and parent choice in wheat breeding.

Keywords: Wheat; genetic variability; correlation; principal component analysis; cluster analysis; augmented design.

1. Introduction

Wheat (*Triticum aestivum* L.) is among the most widely cultivated cereals and a major contributor to food and nutritional security (Mir et al., 2025). Sustained improvement in wheat productivity is required to meet increasing demand while coping with constraints such as limited arable land, fluctuating water availability, and increasing temperature variability (Pillay & Kumar, 2018; Sharada et al., 2025; Shrungarpawar et al., 2025; Puri et al., 2025). Grain yield improvement is challenging because yield is a complex quantitative trait governed by many loci of small effect and strongly influenced by the environment. For this reason, wheat breeding programs typically combine direct selection for yield with selection for relatively heritable yield components and adaptive traits (Kumar et al., 2022).

Variability studies attempt to separate genetic causes of variation from environmental component and to identify traits that can respond to selection. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) are widely used to compare the magnitude of variability across traits because they standardize variance by the trait mean (Singh et al., 2025; Ali et al., 2021; Wei, 2021; Ambati et al., 2020). When PCV is substantially higher than GCV, environmental influence is considered stronger, and selection for such traits becomes less reliable. Heritability and genetic advance further help interpret whether a trait is likely to respond to selection, high heritability coupled with high genetic advance generally indicates predominance of additive gene action and better prospects for improvement through simple selection (Kumawat et al., 2023).

Trait association analysis complements variability studies because selection is rarely practiced on a single trait in isolation. Correlation analysis provides a first understanding of how traits change together across genotypes. A positive association of a component trait with grain yield suggests that selection on the component may indirectly improve yield, while a negative association may indicate biological trade-offs (for example, between grain number and grain size) or linked physiological constraints (Verma et al., 2024; Babalola et al., 2025; Yang et al., 2022; Singh et al., 2019). Correlation results also help breeders design selection indices and decide which traits should be measured early in a breeding pipeline. Although path coefficient analysis can further partition correlations into direct and indirect effects, correlation itself remains a practical starting point for identifying promising indirect selection criteria.

When many traits are recorded simultaneously, multivariate methods help to summarize overall diversity patterns beyond univariate variability and pairwise correlations (Sabhyata et al., 2024). Principal component analysis (PCA) reduces a large set of correlated traits into a smaller number of orthogonal components, each capturing a portion of total variance. PCA is useful for identifying the traits that contribute most to overall diversity and for visualizing genotype relationships in reduced-dimensional space (Unnipillai et al., 2022). In parallel, genetic divergence analysis groups genotypes into clusters to facilitate the choice of parents for hybridization. Mahalanobis D^2 statistics provide a multivariate distance measure that accounts for correlations among traits and is widely used to quantify divergence among genotypes (Ujjainkar, 2025). Crosses between genotypes drawn from highly divergent clusters are often proposed to maximize the chance of obtaining transgressive segregants.

Augmented designs are often a good choice when there is constraint on the amount of seed of germplasm and entries are large. In such trials, checks provide an estimate of experimental error and allow comparison of test entries across blocks. Many test germplasm lines are distributed across blocks, with a consistent set of checks repeated in each block. By working through the analysis of variance, variability parameters, correlation matrix, PCA and clustering on this type of dataset, one can build competence in interpreting results and translating them into breeding decisions, such as identifying trait priorities and selecting divergent, high-performing parents for crossing (Sharma et al., 2025).

Phenological traits (e.g., heading, flowering and maturity time) also deserve attention because they determine crop duration and stress escape. Earlier flowering or maturity can help avoid terminal heat in some environments, while longer grain filling may increase yield when moisture and temperature are favorable (Chaulagain et al., 2024). Understanding how these traits correlate with yield components within a given germplasm set helps breeders balance adaptation and productivity. Therefore, integrating variability measures with correlation and multivariate diversity analysis provides a more complete picture than any single method alone.

The objectives of the present study were: (i) to quantify genetic variability for phenological, morphological and yield-related traits; (ii) to study the association of grain yield with key component traits; and (iii) to characterize multivariate diversity using PCA and cluster analysis, following approaches commonly reported in wheat germplasm studies.

2. Methodology

2.1 Experimental Material and Design

The experiment comprised of 80 germplasm of wheat including 5 checks (HD 2851, HD 3086, RAJ 3765, DBW-187 and PBW 343). The experiment was laid in Augmented Complete Block Design (ACBD) with 5 blocks. Single row of 3 m of individual germplasm was sown in blocks and the checks were replicated in each block.

Fifteen quantitative traits viz. days to emergence, days to 50% booting, days to 50% heading, days to 50% flowering, days to maturity, grain filling duration, plant height, peduncle length, flag leaf area, productive tillers per plant, spike length, grains per spike, 1000-grain weight, biological yield per plant and grain yield per plant were studied.

2.2 Analysis of Variance and Coefficient of Variation

For each trait, an additive linear model was fitted with block and genotype effects:

$$Y_{ij} = \mu + B_i + G_j + e_{ij},$$

where Y_{ij} is the observation of genotype j in block i, μ is the overall mean, B_i is the block effect, G_j is the genotype effect and e_{ij} is the residual. ANOVA was used to test the significance of genotype differences. Experimental precision was described using the coefficient of variation (CV%), computed as:

$$CV\% = (\sqrt{MSe} / \bar{x}) \times 100,$$

where MSe is the error mean square and $\bar{x}$ is the trait mean.

2.3 Variability Parameters

Genotypic variance ($\sigma^2 g$) and phenotypic variance ($\sigma^2 p$) were estimated from ANOVA mean squares as:

$\sigma^2 g = (MSg - MSe) / r$ and $\sigma^2 p = \sigma^2 g + \sigma^2 e$,

where MSg is the genotype mean square, $\sigma^2 e = MSe$ and r is the effective replication factor. For this. augmented design, r was taken as the number of blocks ($r = 5$) to reflect that precision and error were derived from the replicated checks. GCV and PCV were computed as:

$GCV\% = (\sqrt{\sigma^2 g} / \bar{x}) \times 100$ and $PCV\% = (\sqrt{\sigma^2 p} / \bar{x}) \times 100$

Broad-sense heritability was computed as $H^2 = \sigma^2 g / \sigma^2 p$ (reported in %). Expected genetic advance (GA) under 5% selection intensity was computed as:

$GA = k \times \sqrt{\sigma^2 p} \times H^2$,

where $k = 2.06$, and GA was also expressed as GA% of mean.

2.4 Correlation Analysis

Phenotypic correlation coefficients among traits were computed using genotype-wise means across the 80 genotypes. The significance of correlations was tested using a t test:

$t = r \times \sqrt{(n - 2)/(1 - r^2)}$,

with $(n - 2)$ degrees of freedom, where n is the number of genotypes.

2.5 Principal Component and Cluster Analysis

PCA was performed on the standardized (mean 0, variance 1) genotype mean matrix so that traits with different units contributed comparably. Components with eigenvalue > 1 were retained (Kaiser criterion) and interpreted using loadings (eigenvectors). Genetic divergence was further summarized using hierarchical clustering (Ward's method) on the standardized data matrix. Inter-cluster distances were calculated from the Euclidean distance between cluster centroids in standardized space and reported as D (and D^2 as squared distance), consistent with multivariate distance concepts used in divergence studies.

3. Results and Discussion

ANOVA revealed highly significant differences among genotypes for all fifteen traits, indicating that the germplasms contained ample variability for both phenological and yield-related characters (Table 1). Block effects were also significant for several traits, which is expected in field situations and reinforces the usefulness of repeated checks for adjusting block-to-block variation in an augmented design. The coefficient of variation (CV%) ranged from 1.11 to 5.80. Lower CV values (<2%) for phenological traits such as days to heading and days to maturity indicate high precision in timing observations, whereas relatively higher CV values for grain yield per plant (5.80%) and productive tillers per plant (4.69%) reflect the inherently higher biological variability of yield traits.

3.1 Range of Variation and Genetic Variability Parameters

Wide range were observed among genotypes for key traits (Table 2). Days to 50% flowering ranged from 73.55 to 90.66 days and days to maturity ranged from 111.54 to 133.95 days, indicating meaningful diversity in crop duration. Grain yield per plant ranged from 10.52 to 21.51 g, while biological yield per plant ranged from 18.46 to 50.27 g, demonstrating substantial variation.

PCV exceeded GCV for all traits (Table 2), indicating that environmental variation contributed to phenotypic expression, as expected for quantitative characters. Among the traits, biological yield per plant recorded the highest GCV (7.82%) and PCV (8.99), followed by grain yield per plant (GCV 6.41%, PCV 8.65%) and productive tillers per plant (GCV 5.47%, PCV 7.20%). These higher coefficients suggested that these traits would show noticeable response to selection if genetic variance is effectively captured. Phenological traits (booting, heading, flowering and maturity time) had comparatively low coefficients (<2.0% GCV), indicating more uniformity for crop duration in the germplasm.

Table 1. ANOVA for fifteen quantitative traits

Trait	Mean	MS (Genotype)	MS (Error)	F value	CV%
Days to emergence	7.0	0.44	0.03	13.34	2.60
Days to 50% booting	67.4	10.66	1.87	5.71	2.00
Days to 50% heading	77.7	10.95	0.74	14.83	1.10
Days to 50% flowering	82.9	12.97	1.74	7.46	1.60
Days to maturity	121.9	18.23	1.89	9.64	1.10
Grain filling duration	39.1	8.28	0.73	11.38	2.20
Plant height (cm)	92.3	33.43	7.81	4.28	3.00
Peduncle length (cm)	21.5	4.53	0.61	7.42	3.60
Flag leaf area	33.9	13.97	2.37	5.88	4.50
Productive tillers per plant	6.3	0.69	0.08	7.82	4.70
Spike length (cm)	10.40	0.81	0.10	7.83	3.10
Grains per spike	48.40	25.58	2.56	9.99	3.30
1000-grain weight (g)	40.60	14.85	1.05	14.09	2.50
Biological yield per plant (g)	37.30	45.39	2.76	16.41	4.50
Grain yield per plant (g)	15.70	5.94	0.83	7.11	5.80

Table 2. Range and genetic variability parameters (GCV, PCV, heritability and genetic advance)

Trait	Range	Mean	GCV%	PCV%	H^2 (%)	GA (% of mean)
Days to emergence	5.73–8.61	7.00	4.08	4.84	71.16	7.10
Days to 50% booting	59.25–72.91	67.46	1.97	2.82	48.51	2.82
Days to 50% heading	69.17–85.58	77.70	1.84	2.15	73.44	3.25
Days to 50% flowering	73.55–90.66	82.91	1.81	2.41	56.36	2.80
Days to maturity	111.54–133.95	121.91	1.48	1.86	63.34	2.43
Grain filling duration	32.20–44.08	39.12	3.14	3.82	67.49	5.32
Plant height (cm)	74.57–102.79	92.38	2.45	3.89	39.60	3.18
Peduncle length (cm)	17.65–26.83	21.59	4.10	5.47	56.21	6.34
Flag leaf area (cm^2)	24.55–43.19	33.95	4.49	6.38	49.40	6.50
Productive tillers per plant	4.21–7.87	6.35	5.47	7.20	57.71	8.57
Spike length (cm)	8.20–12.40	10.47	3.60	4.74	57.72	5.64
Grains per spike	36.15–60.04	48.43	4.43	5.53	64.26	7.32
1000-grain weight (g)	29.46–49.76	40.66	4.09	4.80	72.36	7.16
Biological yield per plant (g)	18.46–50.27	37.36	7.82	8.99	75.50	13.99
Grain yield per plant (g)	10.52–21.51	15.76	6.41	8.65	55.01	9.80

Heritability estimates ranged from moderate to high across all traits (Table 2). High heritability was observed for biological yield per plant (75.50%) and 1000-grain weight (72.36%), suggesting relatively stable genetic control and lower masking by environment. Grain yield per plant exhibited moderate heritability (55.01%), reflecting that yield is influenced by multiple component traits and environment. Genetic advance as percent of mean (GA%) provides a practical expectation of gain under selection. Biological yield per plant showed the highest GA% (13.99%), followed by grain yield per plant (9.80%) and productive tillers per plant (8.57%). High heritability together with high GA% (as seen for biological yield) is often interpreted as the action of additive gene effects, making such traits suitable for direct selection.

3.2 Correlation Analysis

Phenotypic correlation coefficients among traits revealed relationships that are useful for breeding objectives (Table 3). Grain yield per plant showed significant positive correlation with biological yield per plant ($r = 0.532$, $p < 0.01$), indicating that genotypes with greater total biomass tended to partition more biomass to grain. Grain yield also correlated positively with productive tillers per plant ($r = 0.445$, $p < 0.01$), grains per spike ($r = 0.304$, $p < 0.01$) and 1000-grain weight ($r = 0.228$, $p < 0.05$). These associations support the classical view that yield is primarily determined by sink size (grain number) and grain size, moderated by source strength (biomass production). A significant negative correlation was observed between grain yield and days to 50% flowering ($r = -0.220$, $p < 0.05$), suggesting a mild advantage of earlier flowering under the late sown scenario.

Other correlations supported strong biological relationships among traits. For instance, phenological traits were positively associated among themselves (booting–heading–flowering–maturity), reflecting shared developmental timing. Spike length showed a positive association with grains per spike, consistent with larger spikes accommodating more grains. From a selection standpoint, the positive correlations of yield with tillers, grains per spike and thousand grain weight indicate that these traits can be used as indirect selection criteria, especially when direct yield measurements are noisy. However, correlation values were moderate rather than extreme, reinforcing the need to consider multiple traits jointly.

3.3 Principal Component Analysis

PCA was conducted to identify the major axes of multivariate variation among the 80 genotypes (Table 4). Six principal components (PC1–PC6) had eigenvalues greater than 1 and together explained 64.27% of the total standardized variance. PC1 explained 14.90% of the variation and was largely associated with productivity-related traits, with high absolute loadings for grain yield and biological yield (Table 5). PC2 (11.98%) explained variation mainly for phenological timing, with large loadings for days to heading and days to flowering. PC3 (11.04%)

reflected spike architecture and canopy traits, dominated by spike length and flag leaf area loadings. These interpretations are typical in germplasm datasets where a few components separate yield potential from maturity patterns and from morphological architecture.

The screen plot (Fig. 1) showed a gradual decline in eigenvalues after PC6, suggesting that additional components contribute smaller, more specific sources of variation. In practical breeding terms, the PCA results emphasize that productivity traits and phenology behave as partially independent dimensions in the dataset. This is helpful because breeders often seek combinations such as high yield with appropriate maturity. Genotypes scoring favorably on the productivity axis (PC1) while maintaining suitable phenology (PC2) would be attractive candidates for parent selection.

Table 3. Phenotypic correlation of grain yield per plant with other traits

Trait	Correlation with grain yield (r)	Significance
Biological yield per plant (g)	0.532	** (p<0.01)
Productive tillers per plant	0.445	** (p<0.01)
Grains per spike	0.304	** (p<0.01)
1000-grain weight (g)	0.228	* (p<0.05)
Days to emergence	0.173	Ns
Spike length (cm)	0.152	Ns
Days to 50% booting	0.124	Ns
Flag leaf area (cm^2)	0.100	Ns
Peduncle length (cm)	0.091	Ns
Plant height (cm)	0.084	Ns
Days to maturity	0.083	Ns
Days to 50% heading	0.030	Ns
Grain filling duration	-0.113	Ns
Days to 50% flowering	-0.220	* (p<0.05)

Table 4. Eigenvalues and variance explained by principal components

PC	Eigenvalue	Explained_%	Cumulative_%
PC1	2.264	14.90	14.90
PC2	1.819	11.98	26.88
PC3	1.677	11.04	37.92
PC4	1.509	9.94	47.86
PC5	1.306	8.60	56.45
PC6	1.188	7.82	64.27
PC7	0.939	6.18	70.46
PC8	0.904	5.95	76.41
PC9	0.811	5.34	81.75
PC10	0.667	4.39	86.14

Table 5. Trait loadings (eigenvectors) for the first three principal components

Trait	PC1	PC2	PC3
Days to 50% booting	-0.339	-0.301	0.305
Days to 50% heading	-0.179	-0.634	0.092
Days to 50% flowering	0.161	-0.511	-0.139
Flag leaf area (cm^2)	-0.130	-0.310	-0.390
Productive tillers per plant	-0.248	0.091	-0.382
Spike length (cm)	-0.224	0.050	0.521
Biological yield per plant (g)	-0.460	0.102	-0.148
Grain yield per plant (g)	-0.546	0.172	-0.147

Table 6. Cluster size, representative genotypes and cluster means for key traits

Cluster	Size	Representative (high yield)	Representative (mid)	Representative (low)	Mean days to 50% flowering	Mean plant height (cm)	Mean productive tillers	Mean grains/ spike	Mean 1000-grain wt (g)	Mean biological yield (g)	Mean grain yield (g)
Cluster 1	12	G74	G31	G48	81.87	93.09	5.70	47.79	41.94	33.1	14.51
Cluster 2	14	G40	G05	G19	84.25	96.05	6.71	45.31	39.67	35.55	14.42
Cluster 3	07	G70	G16	G12	79.68	88.17	6.08	48.47	37.46	36.73	15.42
Cluster 4	06	RAJ 3765	G58	G13	83.57	89.59	6.67	46.36	41.53	47.40	16.56
Cluster 5	13	G10	G66	G17	79.88	92.78	6.82	51.34	42.85	43.28	18.44
Cluster 6	10	G63	G53	G68	82.78	93.39	6.25	47.11	40.85	38.07	16.18
Cluster 7	18	G34	G65	G39	85.28	92.48	6.16	50.34	40.58	34.20	14.88

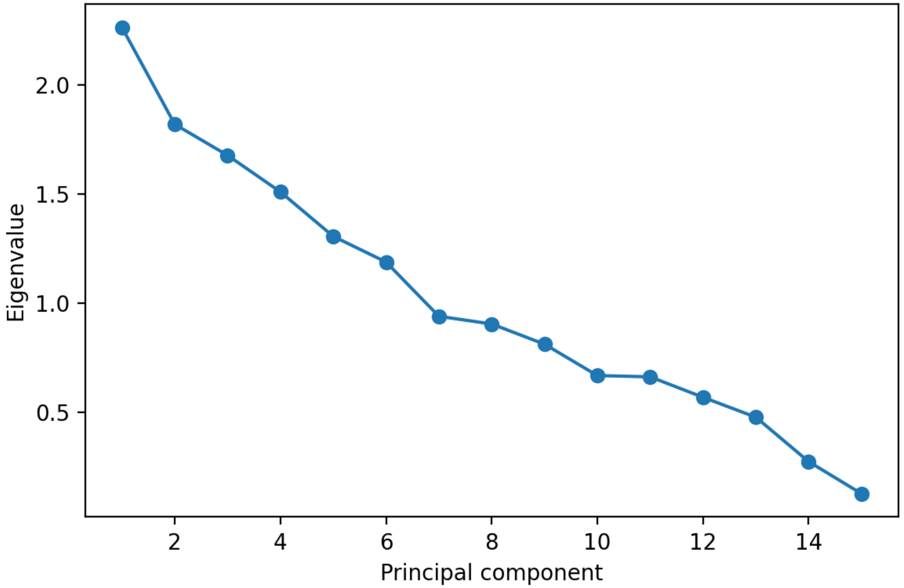

Fig. 1. Screen plot of PCA eigenvalues

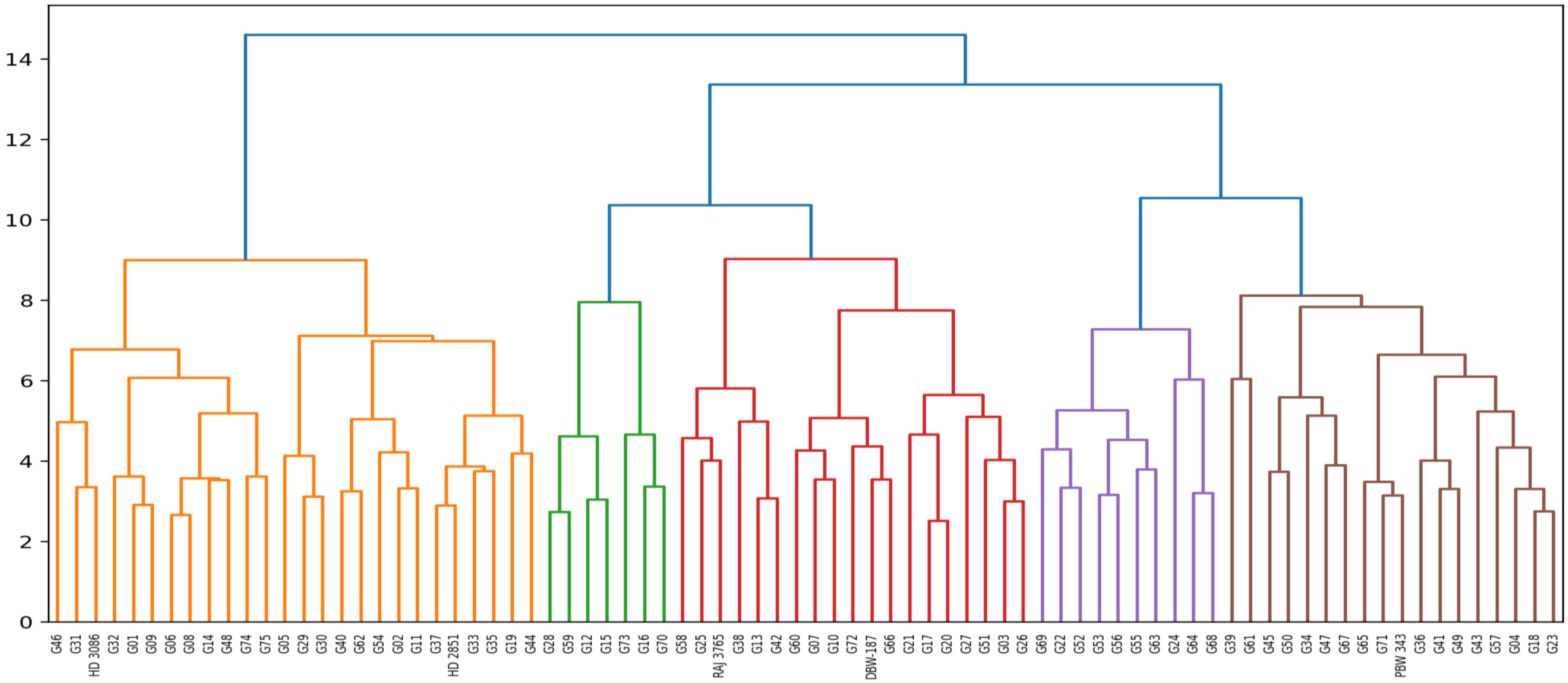

Fig. 2. Dendrogram from hierarchical clustering (Ward's method)

Table 7. Inter-cluster distances (D) between cluster centroids in standardized trait space

Cluster	C1	C2	C3	C4	C5	C6	C7
C1	0.00	2.50	3.18	4.02	3.49	3.68	3.27
C2	2.50	0.00	3.74	3.88	3.62	3.35	3.16
C3	3.18	3.74	0.00	3.95	3.36	3.97	3.28
C4	4.02	3.88	3.95	0.00	3.15	4.48	3.38
C5	3.49	3.62	3.36	3.15	0.00	3.09	3.44
C6	3.68	3.35	3.97	4.48	3.09	0.00	2.94
C7	3.27	3.16	3.28	3.38	3.44	2.94	0.00

3.4 Cluster Analysis and Genetic Divergence

Hierarchical clustering grouped the 80 genotypes into seven clusters (Table 6), reflecting the multivariate similarity structure across the 15 traits. Cluster sizes ranged from 6 genotypes (Cluster IV) to 18 genotypes (Cluster VII). Inter-cluster distances (Table 7) indicated substantial divergence among clusters; the maximum distance was observed between Cluster IV and Cluster VI (*$D = 4.48$; $D^2 = 20.06$).* Large inter-cluster distance implies that genotypes from these clusters are genetically diverse with respect to the trait set, and crosses between them are expected to generate broader recombination and variability. Clusters V and VI also showed notable separation from the others, suggesting distinct trait combinations. Cluster means (Table 6) provide practical interpretation for planning a effective crossing programme. Cluster V recorded the highest mean grain yield (18.44 g plant^{-1}) together with higher grains per spike and 1000-grain weight, representing a high-productivity group. Cluster III exhibited the earliest mean flowering (79.68 days) with moderate yield, indicating potential for earliness and stress escape. Cluster IV had high mean biological yield (47.40 g plant^{-1}) and above-average grain yield, suggesting strong source capacity. Based on divergence and mean performance, crosses between high-yield Cluster V and the most divergent cluster (Cluster VI or Cluster IV) could be prioritized to combine productivity with diversity. Within-cluster representatives with high grain yield included G10 (Cluster V), G63 (Cluster VI) and RAJ 3765 (Cluster IV). The overall top-yielding genotypes in the dataset were G10, G07, G27, G21 and G26, each exceeding 19 g plant^{-1} grain yield.

Fig. 2 presents the dendrogram, illustrating the hierarchical relationship among genotypes and visually supporting the cluster solution. The germplasm analysis interpreted: significant variability provides selection opportunity; correlation identifies key yield drivers; PCA summarizes major axes of variation; and clustering highlights diverse parent combinations for hybridization.

4. Conclusion

In the present study significant variability among genotypes for phenological, morphological and yield-related traits was present indicating adequate diversity for selection and choice of parents for effective breeding programme. Traits particularly biological yield per plant, grain yield per plant and productive tillers per plant showed comparatively higher GCV and PCV and higher expected genetic advance, suggesting that meaningful selection response is feasible for these characters. Correlation analysis suggested biological yield, productive tillers, grains per spike and 1000-grain weight as the most important traits positively associated with grain yield, while days to 50% flowering showed a small but significant negative association, implying potential value of early flowering under the late sown condition. PCA partitioned the traits into different components and the first six components explained 64.27% of the total variation. Cluster analysis grouped genotypes into seven clusters and revealed high divergence between clusters IV and VI, while cluster V exhibited the highest mean grain yield. The result suggest that selection targeting tillering, grains per spike and grain weight, coupled with parent choice from divergent high-performing clusters (e.g., cluster V crossed with clusters IV/VI), would be a rational breeding strategy for wheat crop improvement in future.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

EIGENVECTORS

VARIABLE	PC1	PC2
DAYS TO EMERGENCE	-0.12	-0.126
DAYS 50 % BOOTING	-0.323	-0.37
DAYS 50 % HEADING	-0.315	-0.398
DAYS TO 50 % FLOWERING	-0.308	-0.364
DAYS TO 50 % MATURITY	-0.149	0.336
GRAIN FILLING DAYS	-0.065	0.433
NUMBER OF PRODUCTIVE TILLERS	0.216	0.13
PLANT HEIGHT	0.321	-0.17
PEDUNCLE LENGTH	0.307	-0.068
AWN LENGTH	-0.043	-0.067
SPIKE LENGTH	0.106	-0.094
SPIKE WEIGHT	0.335	-0.109
NUMBER OF GRAINS PER SPIKE	0.382	-0.128
1000 SEED WEIGHT	0.267	-0.292
SEED YIELD PER PLANT	0.273	-0.281

EIGENANALYSIS OF THE CORRELATION MATRIX

PCS	PC1	PC2
EIGENVALUE	5.025	2.514
PROPORTION	33.500	16.800
CUMULATIVE	33.500	50.300

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