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Multivariate Principal Component Analysis and Clustering Methods for Assessing Genetic Diversity in Bread Wheat (*Triticum aestivum* L.) Genotypes

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ABSTRACT

The present experiment was conducted during *rabi* season (November, 2019–May, 2020) at Agricultural Research Farm of Banaras Hindu University, Varanasi, Uttar Pradesh, India to assess the genetic diversity and phenotypic variation among 50 bread wheat accessions. The genotypes were grown in a randomized complete block design with three replications, and data was collected on 14 quantitative traits. The multivariate techniques, including Principal Component Analysis (PCA) and K-means clustering were employed to identify traits contributing the most to phenotypic variation and to classify the genotypes into distinct groups based on their characteristics. PCA identified 13 principal components, with the first five explaining 65.2% of the total variation. The first two principal components accounted for 37% of the total phenotypic variation, with grain yield plant^{-1}, thousand seed weight, harvest index, and canopy temperature as major contributors to PC1, while total biomass and biomass contributed primarily to PC2. K-means clustering grouped the genotypes into five distinct clusters, with clusters 2 and 5 having the highest number of genotypes (14 each). Cluster 1 exhibited the highest mean values for germination percentage, days to maturity, and vegetative index. Hierarchical clustering further confirmed the genetic diversity, delineating five distinct clusters using Ward's method with Euclidean distance. This study provides valuable insights for breeders aiming to enhance traits such as yield and other morphological characteristics through heterosis and transgressive breeding.

KEYWORDS: Wheat, genetic diversity, phenotypic variation, PCA, clustering

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Bread wheat (*Triticum aestivum* L.), also known as common wheat, is an annual, predominantly autogamous species belonging to the Triticeae tribe of the grasses (Poaceae) family (Antim et al., 2022). The origin of these species is closely related to the development of human civilization (Strejcková et al., 2023). It originated from South-Western Asia and Central Asia and the Mediterranean and Ethiopian regions are centres of diversity for wheat and its related species (Liu et al., 2022). It has been the staple food of the major civilizations of Europe, West Asia, and North Africa for 8000 years (Zhao et al., 2023). It is an allohexaploid species, composed of 21 chromosome pairs organized in three sub genomes, A, B, and D, Genome BBAADD, 2n=6x=42 (Feldman and Levy, 2012). It is one of the most widely grown cereal crops, contributing to the global food supply and economic security (ARYA et al., 2017). It covers 17% of the total cultivated land in the world (Mitura et al., 2023). It has been described as the king of cereals (Dvivedi et al., 2023). It is the second-largest cereal crop in the globe after maize, having area, production, and productivity in the amount of 214.29 m ha, 734.05 mt and 3.43 t ha^{-1} globally, respectively (Akbarzai et al., 2023). India is the world's second-largest wheat producer, exporting 0.2 million tons each year and accounting for 13% of the global wheat supply (Zaveri and Lobell, 2019). It is the world's largest famous energy-rich cereal crop (Dubey et al., 2015). It plays a crucial role in human nutrition Wheat grain has a high nutritional value with 70–75% starch, 14% water, 8–20% proteins, 2–3% non-starch polysaccharides, 2% lipids, 1.6% minerals, antioxidants (Khalid et al., 2023). Plant breeding programs mainly focus on genetic diversity, inheritance, conservation and evolution (Turkoglu et al., 2023). "Grain yield is a complex trait and highly influenced by many genetic factors and environmental fluctuations. In plant breeding programme, direct selection for yield as such could be misleading (Herr et al., 2023). Therefore, study of genetic variability of grain yield and its component characters among different varieties provides a strong basis for selection of suitable genotypes for expansion of yield and other agronomic characteristics (Chaudhary et al., 2022). It is vital to investigate the relationships between genotype variation and yield components to make the best use of wheat genetic resources in breeding program initiatives (Abdelghany et al., 2023). Genetic diversity analysis of genetic resources is a prerequisite for their more efficient exploit in plant breeding program (Fouad, 2020). Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization (Mecha et al., 2017). Estimation of genetic diversity based on genetic distance is useful for wheat breeding as one of tools for parental selection to enhance the new genetic recombination for increase yield (Khodadadi et al., 2011). Multivariate analysis by principal component and cluster analysis can be effective to determine genetic diversity and parental selection (Poudel et al., 2017). Therefore, the present investigation is carried out to find out the diverse parental lines for future breeding programmes. The objectives of the study were to analyse the genetic diversity of 50 bread wheat genotypes, determine the genetic relationships among different traits which contribute more towards grain yield and determine the promising excellent genotypes which could be parents in wheat breeding program.

2. MATERIALS AND METHODS

The experiment was carried out during *rabi* (November, 2019–May, 2020) at the agricultural farm of Banaras Hindu University of Varanasi (221005), Uttar Pradesh, India. Geographically, Varanasi, Uttar Pradesh, India, lay at a latitude of 25° 19' in the North and a longitude of 83° 46' to the east, with an altitude of 264 meters above mean sea level (MSL), representing the Eastern Plain Zone (EPZ). The soil in the experimental field was rich and sandy loam in texture. The experiment was laid out in Randomized Complete Block Design (RCBD) in 2 replications. In each replication, genotypes were sown in 6 rows of 5-meter length. The row to row and plant to plant distance was maintained at 25 and 10 cm, respectively. Experimental materials comprised 50 wheat genotypes, including the local check variety The Karan Vandana (DBW 187), which was released for irrigated timely sown conditions of North Eastern Plains Zones comprising of Eastern Uttar Pradesh, Bihar, Jharkhand, Assam, and West Bengal. The names and sources of the fifty bread wheat genotypes were given in supplementary Table 1. Observations were recorded for 13 morphological traits: germination percentage (GP), days to 50 percent flowering (DFF), days to maturity (DM), canopy temperature (CT), vegetative index (VI), biomass (BM), chlorophyll content (CC), grain yield per plot (GYPP), plant height (cm) (PH), number of productive tillers per meter (TM), length of the spike (SL), 1000 seed weight (g) (TSW), and harvest index (%) (HI). The data were subsequently analysed using standard statistical methods to ensure rigorous assessment of the traits. Germination percentage was determined in the field by observing the proportion of seeds that successfully sprouted from the total number of seeds planted. Days to maturity, days to 50% flowering, plant height, and the number of tillers per meter were recorded at maturity for each experimental plot. Three measurements of the Normalized Difference Vegetation Index (NDVI) were taken using a Green Seeker device from the vegetative stage to the dough stage. Chlorophyll content was measured at the heading and anthesis phases using a Minolta SPAD-502 Chlorophyll meter. Canopy temperature was measured from the vegetative stage to the dough stages using a handheld infrared thermometer. The

1000 seed weight was determined by weighing a sample of 1000 wheat grains. At maturity, the biomass was measured by harvesting the total above-ground biomass, while the grain yield plot^{-1} was measured by threshing grains from spikes harvested from each experimental plot. The harvest index was calculated by dividing the grain yield plot^{-1} by the total above-ground biomass harvested from the same plot at maturity. PCA and K-mean clustering analysis were carried out using R-software.

3. RESULTS AND DISCUSSION

3.1. Principal component analysis

Principal Component Analysis (PCA) was applied to assess the relative contributions of different morphological traits to the overall variability among the 50 bread wheat accessions (Figure 1). This method was used to analyze the average data, serving as a robust multivariate tool for identifying principal components that separately influenced plant characteristics (Chen et al., 2020). PCA reduces the dimensionality of the dataset while retaining as much of the original variability as possible (Maawali et al., 2021). This approach was particularly valuable for breeders who aim to genetically improve traits with low heritability, such as yield (Amin et al., 2024).

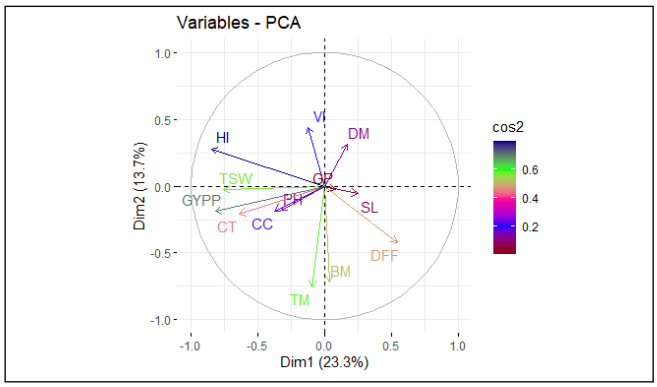

Figure 1: Direction and contributions of variables in influencing major principal components

The PCA partitioned the total variation contributed by traits into thirteen principal components (PCs). The proportions of the total variability accounted for by each PC, listed in decreasing order of importance, were presented in Table 1.

The first five principal components, each with eigenvalues greater than one as shown in the scree plot (Figure 2), accounted for 65.2% of the total variation. Specifically, PC1 explained 23.3% of the variation, PC2 contributed 13.7%, PC3 accounted for 11%, PC4 explained 9.4%, and PC5 contributed 7.8%. Together, the first two principal components explained 37% of the total phenotypic variation in the data, with PC1 and PC2 explaining 23.3% and 13.7% of this variance, respectively (Figure 1). In the study conducted by Rufati and Manasievska (2022), PCA identified three principal components that collectively accounted for 85.75% of the total variation among the genotypes. The factor loadings presented in Table 1 and Figure 3 illustrate the relationships between traits and their grouping across different components, offering insights into the underlying structure of the data (Adilova et al., 2020; Al Lawati et al., 2021). Germination percentage significantly influences PC3 and PC6, indicating its impact on early plant vigour. Days to 50% flowering and days to maturity were closely associated with PC11 and PC4/5, respectively, highlighting their roles in controlling reproductive timing. Canopy temperature negatively correlates with vegetative growth traits in PC1 and PC10, suggesting its role in stress response mechanisms, as supported by the findings of Srivastava et al. (2017) and Lepekhov (2022).

The vegetative index and biomass show complex interactions with growth and yield across several PCs, while chlorophyll

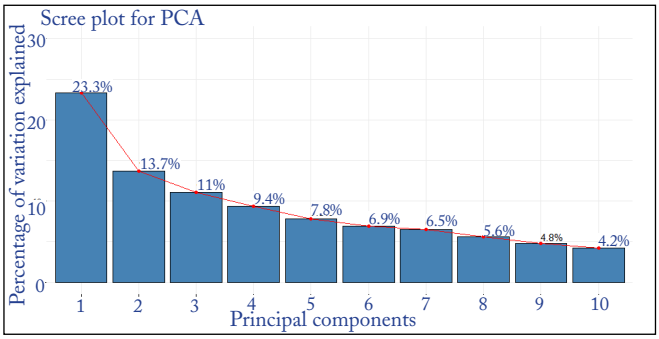

Figure 2: Scree plot showing the percentage of variation explained by the principal components

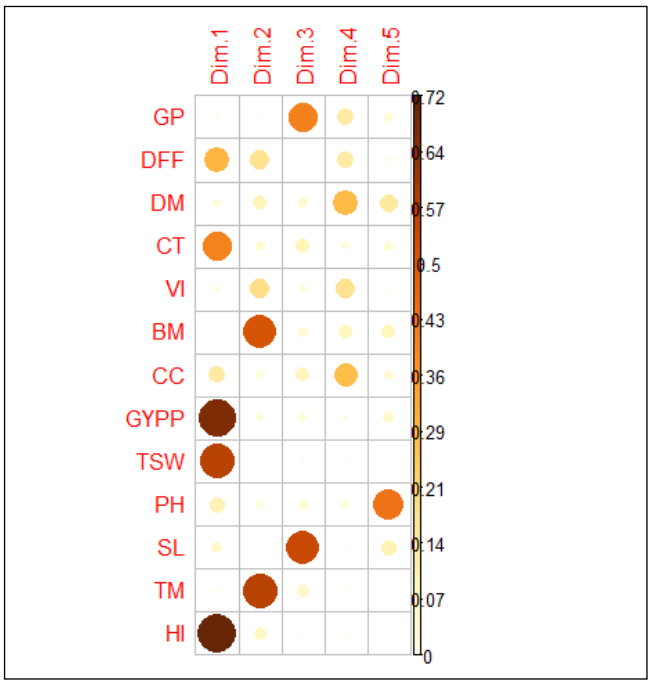

Figure 3: Corr plot showing the relationship between variables and individual principal components (PCs)

Table 1: Principal components (PCs) and their features

PC	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9	PC_{10}	PC_{11}	PC_{12}	PC_{13}
Standard deviation	1.74	1.33	1.19	1.10	1.00	0.94	0.91	0.85	0.78	0.73	0.67	0.52	0.3.7
Proportion of variance	0.23	0.13	0.11	0.09	0.07	0.069	0.064	0.05	0.047	0.041	0.03	0.02	0.01
Cumulative proportion	0.23	0.37	0.48	0.57	0.65	0.72	0.78	0.84	0.89	0.93	0.96	0.98	1.00
Eigen value	3.03	1.78	1.43	1.21	1.01	0.89	0.84	0.72	0.61	0.54	0.45	0.27	0.14
Factor loadings													
GP	0.05	-0.02	0.53	0.34	-0.20	0.55	0.05	0.21	0.02	-0.23	0.37	0.11	-0.04
DFF	0.31	-0.32	0.02	-0.34	0.11	-0.20	-0.16	-0.32	0.10	-0.32	0.62	-0.10	0.06
DM	0.10	0.24	0.19	-0.49	-0.39	-0.32	0.30	0.49	-0.18	-0.06	0.09	-0.11	-0.14
CT	-0.37	-0.16	0.26	-0.16	0.21	-0.19	-0.02	0.13	0.37	-0.56	-0.33	0.30	-0.03
VI	-0.07	0.33	0.15	0.39	0.10	-0.38	0.59	-0.41	-0.05	-0.14	0.15	0.05	-0.02
BM	0.02	-0.54	-0.17	0.26	-0.31	-0.19	0.34	0.25	0.29	0.10	0.02	-0.05	0.46
CC	-0.22	-0.14	0.25	-0.48	-0.22	0.29	0.30	-0.47	0.11	0.36	-0.07	0.21	0.10
GYPP	-0.47	-0.14	-0.15	0.12	-0.25	-0.09	-0.07	-0.04	0.29	0.12	0.26	-0.23	-0.65
TSW	-0.43	-0.02	0.08	0.06	0.02	-0.30	-0.30	0.13	-0.35	0.26	0.37	0.47	0.23
PH	-0.19	-0.14	-0.18	-0.17	0.66	0.26	0.44	0.32	-0.08	0.11	0.25	-0.07	-0.07
SL	0.14	-0.04	0.62	0.06	0.32	-0.26	-0.15	0.09	0.28	0.47	-0.05	-0.30	-0.01
TM	-0.06	-0.57	0.22	0.08	-0.01	-0.07	0.06	-0.12	-0.66	-0.12	-0.26	-0.22	-0.20
HI	-0.49	0.21	0.06	-0.08	-0.05	0.11	-0.11	-0.07	-0.06	-0.19	0.06	-0.64	0.48

content was crucial for photosynthetic efficiency, particularly in PC4 and PC8. Grain yield per plot, 1000 seed weight, and plant height were key determinants of yield components, reflected in their strong contributions to PC1, PC12, and PC5 (Javed et al., 2024). Spike length and the number of productive tillers per meter were important for shaping reproductive and productivity traits, while the harvest index reflects the balance between yield and vegetative growth, especially in PC1 and PC13 (Farokhzadeh et al., 2022; Kaswan et al., 2018). These findings underscore the complex genetic and physiological trade-offs that breeders must manage to optimize both yield and growth traits in breeding programs. Principal Component Analysis (PCA) had been employed in various studies as a multivariate technique to analyze diversity in bread wheat (Adilova et al., 2020; Chen et al., 2020; Boudersa et al., 2021; Ahmed et al., 2020)

Correlation coefficients were utilized to depict the relationships and contribution of each trait to the main PCs (Figure 3). The major contributors for variation observed in first principal component were GYPP, TSW, HI, CT respectively (Degewione and Alamerew, 2013). The major contributors for variation observed in second principal component were TM, BM respectively (Bennani et al., 2016; Samita et al., 2022; Reddy and Babariya, 2020). PCA- Biplot analysis (Figure 4) also depicted that there was scattered distribution of studied traits among all bread wheat genotypes utilized in this study. Histograms (Supplementary Figure 1) depicting distributions of all traits were also in accordance with the variation observed through PCA (Figure 4).

3.2. K–means clustering

Cluster analysis was widely used to assess genetic diversity and to group genotypes with similar parental backgrounds into distinct clusters (El-Esawi et al., 2022). In this study, K-means clustering (Figure 5) classified the genotypes into five clusters: Cluster 1, Cluster 2, Cluster 3, Cluster 4, and Cluster 5 (Fouad, 2020; Neha et al., 2022). The highest number of genotypes was found in Clusters 2 and 5, each containing 14 genotypes, followed by Cluster 4 with 11 genotypes, and Cluster 3 with 10 genotypes. Cluster 1 contained the fewest genotypes, with only one. Notably, Cluster 1 had the highest mean values for germination percentage (GP), days to maturity (DM), and vegetative index (VI) among all clusters (Mohanty et al., 2017; Degewione and Alamerew, 2013). Cluster 2, with 14 genotypes, had the highest mean value for days to 50% flowering (DFF). Cluster 3, which included 10 genotypes, had the highest mean values for biomass (BM), plant height (PH), grain yield per plot (GYPP), number of productive tillers per meter (TM), and vegetative index (VI). Cluster 4, containing 11 genotypes, had the highest mean values for spike length (SL), canopy temperature (CT), germination percentage (GP), number of productive tillers per meter

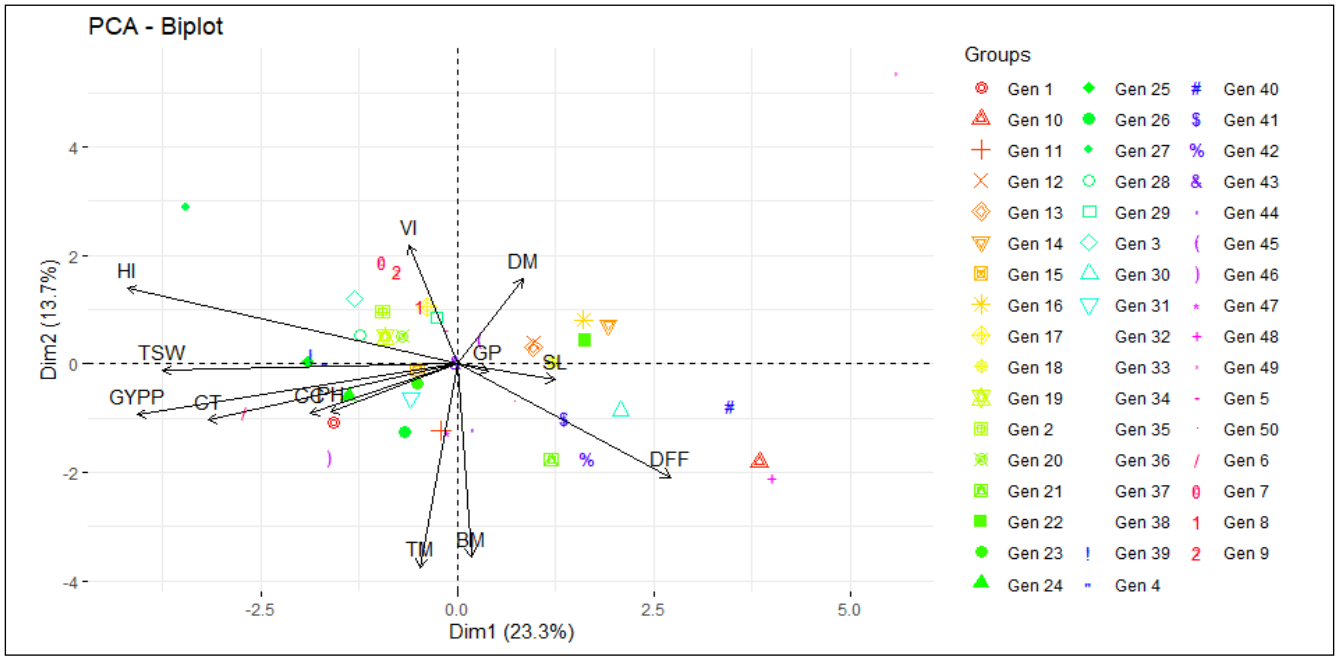

Figure 4: PCA- Biplot analysis showcasing the distribution of bread wheat genotypes across various traits

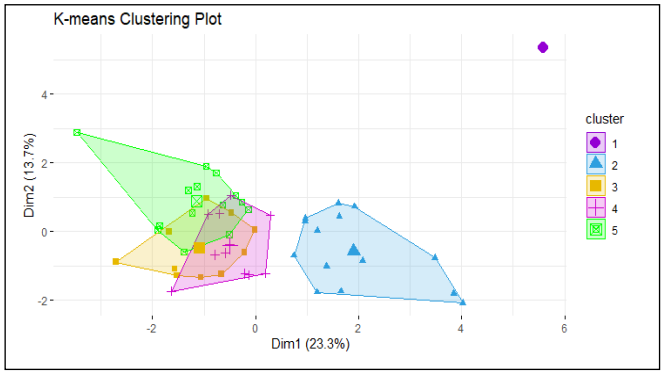

Figure 5: Partitioning K-means cluster plot showing distribution of genotypes into different clusters

(TM), and days to 50% flowering (DFF). Cluster 5, with 14 genotypes, showed the highest mean values for harvest index (HI), chlorophyll content (CC), and 1000 seed weight (TSW). Similar clustering approaches had been used by Al-Naggar et al. (2022), Msundi et al. (2021), Saeed et al. (2016), Singh et al. (2022), Zheng et al. (2015) to elucidate the distribution of genetic diversity in bread wheat.

3.3. Hierarchical clustering

Hierarchical clustering was conducted to examine and validate the genetic diversity among different genotypes. This analysis produced a dendrogram that identified five distinct clusters (Figure 6), each characterized by unique genetic traits (Almohisen, 2020; Maawali et al., 2021).

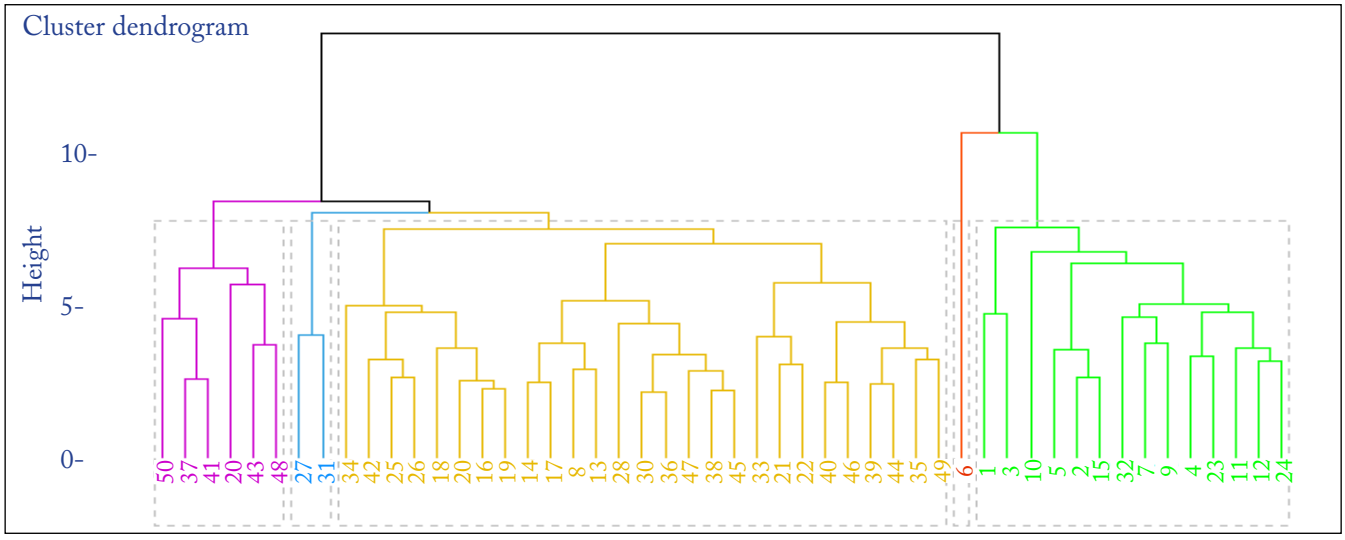

Figure 6: Cluster dendrogram developed using ward method showing divergence for genotypes within and among the clusters

The dendrogram was generated using Ward's method with Euclidean distance to measure dissimilarities between genotypes. Genotypes that were positioned closer together on the dendrogram were more genetically similar than those that were further apart (Kara et al., 2020). These findings were consistent with the results from the K-means clustering analysis, confirming the diversity patterns observed in the studied germplasm. However, some researchers had found that the results of these two clustering methods can be independent (Nisha and Kaur, 2015; Degewione and Alamerew, 2013).

4. CONCLUSION

Principal Component Analysis (PCA) and K-means clustering identified grain yield per plant, thousand seed weight, and canopy temperature as key contributors to genetic variation. K-means and hierarchical clustering grouped wheat genotypes into five distinct clusters, confirming significant genetic diversity.

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