

RESEARCH ARTICLE

Exploring Genetic Diversity and Yield-Contributing Traits in Sorghum (*Sorghum bicolor*) using D² Statistics and Principal Component Analysis

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Abstract

Sorghum is a vital cereal crop with immense potential for grain and fodder, especially in semi-arid regions. This study evaluated the genetic diversity among 96 sorghum genotypes using Mahalanobis D² statistics and principal component analysis to identify key traits contributing to yield and other agronomic characteristics. The field experiment was conducted at the Millet breeding station, TNAU, Coimbatore, with two replications. The genotypes were grouped into 10 clusters, with distinct clusters showing unique trait combinations valuable for targeted breeding goals. For example, genotypes such as IS 28517, IS 29114, and TNSS 223 are ideal for biomass production because of their tall, late-maturing traits, whereas IS 10939 and AS 3883 are promising for high seed weight and high yield, respectively. Principal component analysis (PCA) identified traits such as leaf width, panicle weight, plant height, and flag leaf length as major contributors to genetic variability. The distribution of genotypes in the PCA biplot highlighted specific associations between yield-related traits and genotype groupings, offering insights for selecting parents for hybridization. This research provides valuable information for sorghum breeding programs aimed at enhancing productivity and resilience in dryland regions, thereby supporting food security and sustainable agriculture.

Keywords: Genetic diversity, Genetic resources, Hybridization, Multivariate analysis, Sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], the fifth most vital cereal crop worldwide, originated and was domesticated in northeastern Africa, with some of the earliest evidence of its domestication found in eastern Sudan around the 4th millennium BC. From there, it spread to other regions like India, China and eventually to North America. This widely cultivated species has been classified into five primary races—bicolor, durra, kafir, guinea, and caudatum—and ten intermediate races, reflecting its broad genetic diversity and adaptability (Harlan and de Wet, 1972). In India, sorghum is grown during both the *kharif* and *rabi* seasons, serving a dual purpose by providing both grain and fodder to farming communities (Swamy *et al.*, 2018). *Rabi*-season sorghum is particularly valued for its superior grain quality, as it is primarily grown for human consumption and has a higher market price than *kharif* sorghum (Prasad *et al.*, 2020). Sorghum is the fifth most significant cereal crop in India, after wheat, rice, maize and pearl millet and sorghum is cultivated extensively in semiarid, rainfed

regions. The major sorghum-producing states include Maharashtra, Karnataka, Tamil Nadu, Rajasthan, and Madhya Pradesh. Sorghum displays substantial variability in plant morphology, panicle and grain characteristics, and physiological responses to selection pressures and is strongly influenced by environmental factors (Ezeaku *et al.*, 1997). Given that rice and wheat are staple foods for much of the global population, sorghum, as a C4 crop, holds great potential for dryland agriculture, optimizing dryland usage and increasing farmer incomes (Dhar *et al.*, 2024). Known as "the king of coarse cereals" and a "nutri-cereal," sorghum has a remarkable nutritional profile and is rich in unsaturated fats, proteins, fibre, and minerals such as potassium, phosphorus, calcium, and iron (Thant *et al.*, 2020).

Understanding the genetic diversity of a crop is crucial for breeders, as it allows for the selection of appropriate parental lines for breeding programs and the introduction of genes from more distantly related germplasms (Bucheyeki *et al.*, 2009). In general, parents that exhibit greater genetic divergence are expected to produce hybrids with greater heterotic potential and create a broad spectrum of variability in segregating generations (Prasad and Sridhar, 2019). To analyse genetic variation, D^2 statistics are used to classify genotypes and measure genetic distance among clusters. Principal component analysis (PCA) further aids in identifying the most yield-contributing agronomic traits, which can then be emphasized in breeding efforts. Consequently, the present study aimed to evaluate genetic diversity among sorghum genotypes and identify the key traits that contribute to grain yield.

Materials and Methods

The experimental material consisted of 96 diverse sorghum genotypes, including two checks (CO 32 and K 13) collected from the Department of Millets, TNAU, Coimbatore and ICRISAT Hyderabad (Table 1). These genotypes were selected to represent wide genetic variability in terms of geographical origin, agromorphological traits, maturity duration, and yield potential. The inclusion of landraces, breeding lines, exotic accessions, and two standard checks ensured adequate diversity for assessing genetic divergence and identifying superior parents for breeding programmes. The field experiment was carried out in a randomized block design (RBD) with two replications at Millets Breeding Station, TNAU, Coimbatore, between 11°N latitude and 77°E longitude during Rabi 2023-24. Each line was sown in 4-meter rows with 45 cm x 15 cm spacing. All the recommended agronomical practices for sorghum were followed to raise a good crop during the season. Five randomly selected plants of each genotype were observed for fourteen yield and yield-contributing traits, i.e., days to 50% flowering (DFF), number of leaves per plant (NL), leaf length (LL), leaf width (LW), flag leaf length (FLL), flag leaf width (FLW), plant height (PH), stem girth (SG), panicle length (PL), panicle width (PW), panicle weight (PWT), days to maturity (DM), hundred seed weight (HSW), and single-plant yield (SPY). The recorded data were then subjected to statistical analysis. To assess the genetic diversity present in the sorghum accessions, Mahalanobis' D^2 statistics were performed in WINDOSTAT ver. 7.1, and multivariate principal component analysis was performed in the AGRICOLAE package of R software.

Table 1: List of the ninety-six sorghum genotypes evaluated in the study

| Entries | Origin | Entries | Origin |
|----------------------|-----------------------------------|----------|----------|
| T Kallupatti local | India | SD 2033 | - |
| IS 22794 | Ethiopia | IS 23460 | Yemen |
| IS 29640 | USA | IS 30383 | China |
| IS 10718 | Ethiopia | IS 30450 | India |
| IS 29690 | India | IS 30507 | Korea |
| IS 20594 | Ethiopia | IS 1212 | China |
| CO 4 | India | IS 1233 | China |
| IS 1052 | India | IS 10477 | USA |
| Erichanampatti local | India | IS 3937 | India |
| IS 1141 | Sudan | IS 3971 | India |
| ICSB 23 | India | IS 5286 | India |
| SH 92 | India | IS 5603 | India |
| SH 29 | India | IS 10304 | India |
| SH 25 | India | IS 10472 | Ethiopia |
| N 14 | Sudan (also referred as IS 18331) | IS 10939 | USA |

| | | | |
|-----------------|------------------------|----------|-------------------|
| SH 24 | Kenya | IS 19003 | Ethiopia |
| IS 1137 | India | IS 19534 | Sudan |
| SH 16 | India | IS 19824 | India |
| E 96 | Ethiopia | IS 21770 | Sudan |
| SEB 12002 | India | CO 32 | India |
| A 6072 | Ethiopia | APK 1 | India |
| AS 3883 | India | K 13 | India |
| IS 9283 | India | M35-1 | India |
| CO 26 | India | IS 1219 | China |
| IS 3076 | Deccan region of India | SD 2033 | Sudan |
| Kottathur local | India | IS 23460 | India |
| IS 30536 | Korea | IS 30383 | China |
| SD 12885 | USA | IS 30450 | China |
| CS 113 | Sudan | IS 30507 | Republic of Korea |
| SD 8375 | Sudan | IS 1212 | China |
| Paiyur 2 | India | IS 1233 | China |
| IS 2883 | Italy | IS 10477 | USA |
| IS 12804 | Turkey | IS 3937 | USA |
| IS 24578 | Lebanon | IS 3971 | India |
| IS 24617 | Lebanon | IS 5286 | India |
| IS 24629 | Lebanon | IS 5603 | India |
| IS 24632 | Lebanon | IS 10304 | USA |
| IS 28517 | Yemen | IS 10472 | USA |
| IS 29114 | Yemen | IS 10939 | USA |
| IS 511 | Mexico | IS 19003 | Sudan |
| IS 869 | USA | IS 19534 | Sudan |
| IS 932 | Sudan | IS 19824 | India |
| IS 2940 | USA | IS 21770 | Sudan |
| IS 3932 | USA | CO 32 | India |
| RS 29 | India | APK 1 | India |
| RC 585 | - | K 13 | India |
| IS 20575 | Sudan | M35-1 | India |
| IS 27786 | Morocco | IS 1219 | China |

Results and Discussion

*D*² analysis

According to the Mahalanobis *D*² analysis, the *D*² values measure the degree of diversity and determine the pertinent contribution of each constituent character to total divergence. The genotypes studied were grouped into different clusters on the basis of genetic distance among the genotypes.

In this study, 96 genotypes, including two checks, were grouped into 10 different clusters using Tocher's method (Fig. 1) on the basis of their relative distances, i.e., *D*² values. The dendrogram can be a useful visualization tool for understanding the structure of the data and identifying natural groupings among the observations. Among the 10 clusters, a large number of genotypes (64) fell into Cluster I, implying greater similarity among these genotypes, which indicates a lack of sufficient divergence within this group. Cluster II consisted of 16 genotypes, followed by cluster V with 7

genotypes and cluster III with 3 genotypes. In contrast, the presence of solitary clusters (III, IV, VI, VII, VIII, and X) containing only one genotype in each cluster suggested that these genotypes are quite distinct from the others, potentially harbouring unique traits that could be valuable for breeding programs (Table 2).

Table 2: Clustering of ninety-six sorghum genotypes by Tocher's method into 10 different clusters

| Clusters | Number of genotypes | Genotypes |
|-----------|---------------------|--|
| Cluster I | 64 | IS 2883, IS 23460, IS 22794, SEB 12002, IS 20575, IS 19003, IS 19824, ICSB 23, Erichanampatti local, IS 3971, IS 1141, Kottathur local, CO 4, T Kallupatti local, IS 20594, E 96, SD 12885, IS 1052, CO 32, IL 46, A 6072, IS 27786, MR 99, IS 10477, IS 511, IS 10472, M35-1, IS 3932, ICS PIRMER, IS 2940, K |

| | | |
|--------------|----|---|
| | | 13, ART 3015, IS 29690, SH 16, IS 24629, IL 497, IS 19534, IS 869, COS 28, IS 932, IS 10304, SH 92, SH 25, Paiyur 2, CO 30, IS 3937, IS 5603, SD 8375, IS 1219, IS 30450, IS 30507, IS 5286, IS 21770, SH 24, IS 1212, ISHAT 2001, SD 2033, IS 24617, IS 24632, IS 24578, AKZ 354, IS 1233, IS 3076, N 14 |
| Cluster II | 16 | TNS 695, TNS 704, CO 26, TNS 702, IS 29322, APK 1, TNSS 225, TNSS 227, IS 1137, IS 9283, SAR 39, SH 29, CS 113, A 404, RS 29, SB 103 |
| Cluster III | 1 | IS 30536 |
| Cluster IV | 1 | IS 30383 |
| Cluster V | 7 | IS 29640, ICSB 29, IS 12804, CO 27, TNFS 230, TNFS 239, IS 10718 |
| Cluster VI | 1 | IS 10939 |
| Cluster VII | 1 | RS 627 |
| Cluster VIII | 1 | RC 585 |
| Cluster IX | 3 | IS 28517, IS 29114, TNSS, 223 |
| Cluster X | 1 | AS 3883 |

The mean values of ten clusters for 96 genotypes (Table 3) revealed notable genetic diversity across traits, which is valuable for breeding objectives. Cluster IX presented the highest means for plant height (252.85 cm), days to 50% flowering (75.83 days), number of leaves per plant (10.67), flag leaf length (64.43 cm), and days to maturity (113.83 days). Thus, the genotypes present in this cluster, such as IS 28517, IS 29114, and TNSS 223, were late-maturing, tall, and had more leaves, making them promising for improving the biomass of forage and silage crops. Cluster VI had the highest hundred-seed weight (4.25 g), indicating that genotype IS 10939 has strong grain yield potential. Cluster X presented the greatest means for leaf length (87.12 cm), stem girth (2.15 cm), panicle weight (102.30 g), and single-plant yield (78.24 g), identifying genotype AS 3883 as ideal for high-yield, dual-purpose varieties for grain and fodder (Greater plant height- 224.5 cm). Cluster VII presented low values for days to 50% flowering, panicle length, panicle width, and single-plant yield, indicating early maturity and suitability for short-duration crops. Cluster VIII exhibited maximum mean values for leaf width (10.50 cm) and flag leaf width (9.47 cm), which indicated the potential for increased photosynthesis and biomass. Cluster IV had the highest mean panicle length (32.67 cm), and the solitary nature of this cluster suggested that the genotype within this cluster was quite distinct and could possess unique traits not found in the other clusters. Hence, the genotypes linked with these clusters can be utilized as parents in a breeding plan, as observed by Navya et al., (2021) in a similar study.

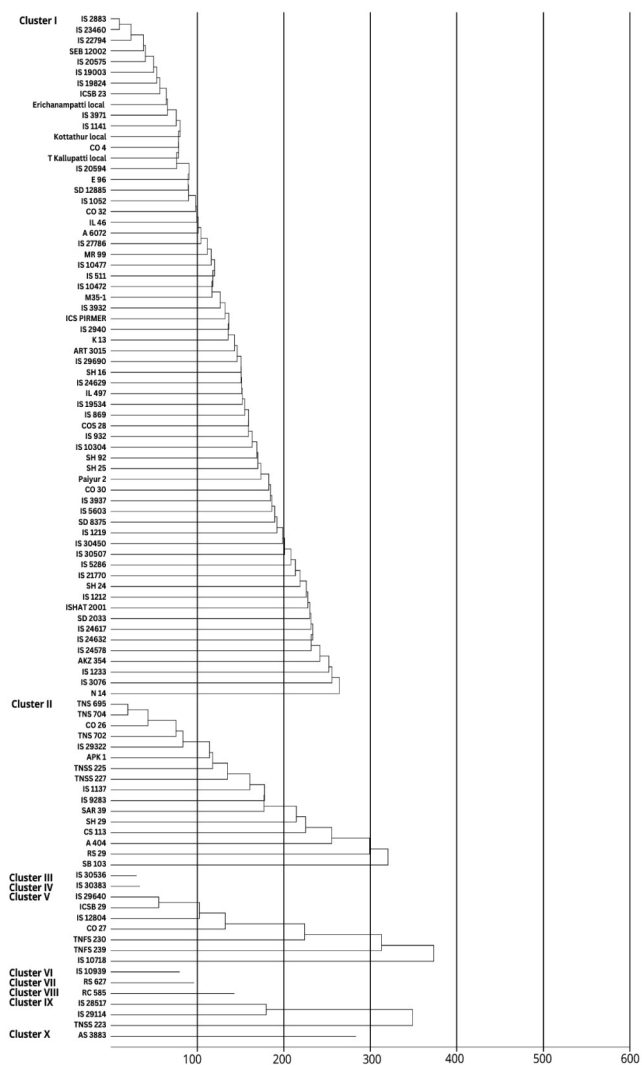


Fig. 1: Dendrogram showing the clustering pattern of 96 genotypes by Tocher's method.

The analysis of intracluster and intercluster distances offers valuable insights for breeding strategies (Table 4). The highest intracluster distance in Cluster IX (20.22) highlighted considerable variability, indicating potential for selection within this group containing the genotypes IS 28517, IS 29114, and TNSS, 223. Clusters with zero intracluster distances, such as III, IV, VI, VII, VIII, and X, represent solitary clusters. The largest intercluster distances were observed between Clusters VI and X (46.91) and between Clusters VII and X (44.71), suggesting significant genetic divergence. Therefore, IS 10939, AS 3883 and RS 627 could be selected as parental materials for hybridization programs. The overall larger intercluster distances than intracluster distances suggested substantial diversity across groups, which aligns with the findings of Prasad and Biradar (2018), Thant et al., (2020), Navya et al., (2021), Verma et al., (2022) and Bhadouriya and Bhadouriya (2024).

Table 3: Cluster mean values of 10 clusters for 14 different biometrical characters

| | DFF | NL | LL | LW | FLL | FLW | PH | SG | PL | PW | PWT | DM | HSW | SPY |
|--------------|-------|-------|-------|-------|-------|------|--------|------|-------|------|--------|--------|------|-------|
| CLUSTER I | 62.50 | 7.02 | 57.30 | 5.81 | 37.84 | 4.88 | 157.40 | 1.12 | 18.97 | 5.58 | 30.57 | 104.98 | 2.50 | 20.42 |
| CLUSTER II | 61.94 | 7.86 | 72.27 | 7.58 | 45.57 | 6.37 | 173.36 | 1.28 | 24.66 | 6.50 | 63.58 | 105.59 | 2.55 | 45.96 |
| CLUSTER III | 56.50 | 5.66 | 52.53 | 5.35 | 38.93 | 4.49 | 211.79 | 1.11 | 31.42 | 5.35 | 14.88 | 99.50 | 1.87 | 10.34 |
| CLUSTER IV | 66.50 | 7.16 | 56.13 | 5.22 | 40.90 | 3.80 | 191.75 | 1.18 | 32.67 | 5.15 | 30.33 | 109.50 | 2.05 | 22.93 |
| CLUSTER V | 55.64 | 6.69 | 56.41 | 4.89 | 38.99 | 4.58 | 198.11 | 1.10 | 28.05 | 8.15 | 19.73 | 97.36 | 1.64 | 13.89 |
| CLUSTER VI | 59.50 | 7.34 | 42.92 | 4.07 | 27.45 | 3.15 | 103.89 | 1.09 | 19.00 | 4.91 | 23.20 | 104.50 | 4.25 | 17.99 |
| CLUSTER VII | 51.50 | 8.66 | 78.92 | 7.40 | 48.30 | 7.37 | 106.33 | 1.12 | 17.78 | 3.68 | 17.25 | 91.50 | 1.45 | 9.00 |
| CLUSTER VIII | 73.50 | 8.84 | 77.05 | 10.50 | 59.19 | 9.47 | 118.89 | 1.38 | 27.05 | 9.52 | 92.90 | 113.50 | 2.46 | 57.70 |
| CLUSTER IX | 75.83 | 10.67 | 79.59 | 9.23 | 64.43 | 8.10 | 252.85 | 2.06 | 20.33 | 9.34 | 65.61 | 113.83 | 3.36 | 37.87 |
| CLUSTER X | 65.50 | 7.66 | 87.12 | 10.12 | 53.30 | 7.74 | 224.50 | 2.15 | 31.13 | 8.05 | 102.30 | 105.50 | 2.62 | 78.24 |

Note: DFF=Days to 50% flowering (days count), NL=number of leaves per plant (numbers count), LL=leaf length (cm), LW=leaf width (cm), FLL=flag leaf length (cm), FLW=flag leaf width (cm), PH=plant height (cm), SG=stem girth (cm), PL=panicle length (cm), PW=panicle width (cm), PWT=panicle weight (g), DM=days to maturity (days count), HSW=hundred seed weight (g/100 seed), and SPY=single plant yield (g).

Table 4: Average intracluster and intercluster distances (D^2 values) among the 10 clusters

| | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII | Cluster IX | Cluster X |
|--------------|-----------|------------|-------------|------------|-----------|------------|-------------|--------------|------------|-----------|
| Cluster I | 13.54 | 21.09 | 17.48 | 16.86 | 20.88 | 18.88 | 17.95 | 29.49 | 33.14 | 40.44 |
| Cluster II | 21.09 | 15.09 | 24.62 | 19.87 | 27.01 | 27.32 | 25.67 | 19.74 | 28.46 | 27.34 |
| Cluster III | 17.48 | 24.62 | 0.00 | 9.12 | 15.82 | 25.65 | 17.77 | 35.45 | 38.73 | 42.29 |
| Cluster IV | 16.86 | 19.87 | 9.12 | 0.00 | 19.03 | 24.01 | 19.80 | 30.37 | 35.40 | 37.16 |
| Cluster V | 20.88 | 27.01 | 15.82 | 19.03 | 17.60 | 29.55 | 21.42 | 34.39 | 38.36 | 43.68 |
| Cluster VI | 18.88 | 27.32 | 25.65 | 24.01 | 29.55 | 0.00 | 27.73 | 35.69 | 36.04 | 46.91 |
| Cluster VII | 17.95 | 25.67 | 17.77 | 19.80 | 21.42 | 27.73 | 0.00 | 34.54 | 40.46 | 44.71 |
| Cluster VIII | 29.49 | 19.74 | 35.45 | 30.37 | 34.39 | 35.69 | 34.54 | 0.00 | 25.82 | 24.51 |
| Cluster IX | 33.14 | 28.46 | 38.73 | 35.40 | 38.36 | 36.04 | 40.46 | 25.82 | 20.22 | 29.65 |
| Cluster X | 40.44 | 27.34 | 42.29 | 37.16 | 43.68 | 46.91 | 44.71 | 24.51 | 29.65 | 0.00 |

Principal component analysis

A multivariate statistical approach known as principal component analysis (PCA) was performed for 96 sorghum genotypes across 14 biometric traits. PCA was used to assess the genetic diversity of the sorghum genotypes and their relationships with the observed attributes on the basis of the correlation between the traits and the pattern of variation in the genotypes. A scree plot displays the eigenvalues and percentage of variation associated with each principal component (Fig. 2). Three major principal components, i.e., PC1, PC2 and PC3, were extracted because they had eigenvalues greater than one (Brejda *et al.*, 2000), which collectively explained 67.38% of the total variation (Table 5). PC1 contributed 44.77% of the variance and was heavily influenced by traits such as leaf width, panicle weight, and plant height, indicating that these traits are key drivers of genetic diversity. PC2, accounting for 12.36%, was strongly associated with panicle length and width, indicating that panicle size plays a significant role in genotype differentiation. PC3 explained 10.25% of the variance and was influenced mainly by plant height, days to flowering, and panicle dimensions.

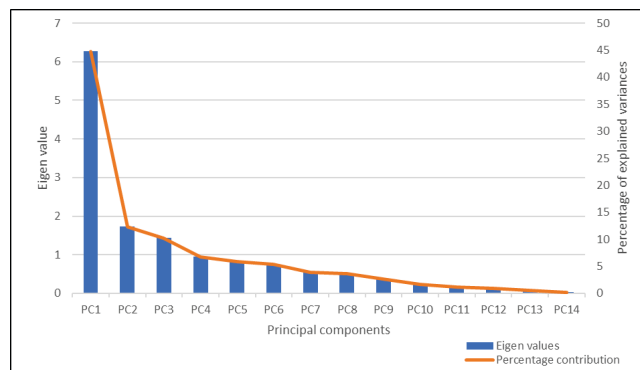


Fig. 2: Scree plot showing the eigenvalue and percentage contribution of each principal component towards divergence.

Similarly, Pugahendhi *et al.*, (2023) reported that the first three principal components accounted for 43.26% of the total variation and highlighted the key agro-morphological traits driving diversity among 96 kharif sorghum genotypes using 20 agro-morphological traits. However, the factor loadings of traits on each principal component (PC) provided insights into how each trait contributes to the observed variability among the genotypes (Table 5). The traits with positive loadings on PC1—such as leaf width, flag leaf width, panicle weight, flag leaf length, single plant yield, stem girth, days to 50% flowering, and plant height—were key drivers of genetic divergence among the sorghum genotypes, highlighting their importance for phenotypic variability. Genotypes with higher values

Table 5: Relative contribution and factor loading of each biometrical trait towards divergence in the 3 major principal components

| Variables | Principal components | | |
|-----------------------|----------------------|--------|--------|
| | Pc1 | Pc2 | Pc3 |
| Eigen values | 6.268 | 1.731 | 1.435 |
| Variance % | 44.773 | 12.364 | 10.253 |
| Cumulative variance % | 44.773 | 57.137 | 67.39 |

| Trait | Factor loadings | | |
|-------|-----------------|--------|--------|
| | Pc1 | Pc2 | Pc3 |
| DFF | 0.229 | -0.413 | 0.384 |
| NL | 0.221 | -0.210 | -0.054 |
| LL | 0.328 | 0.165 | -0.161 |
| LW | 0.359 | 0.043 | -0.159 |
| FLL | 0.335 | 0.095 | 0.143 |
| FLW | 0.346 | 0.120 | -0.089 |
| PH | 0.119 | 0.232 | 0.568 |
| SG | 0.287 | -0.05 | 0.088 |
| PL | 0.112 | 0.343 | 0.181 |
| PW | 0.206 | 0.339 | 0.288 |
| PWT | 0.341 | 0.044 | -0.295 |
| DM | 0.215 | -0.47 | 0.321 |
| HSW | 0.123 | -0.466 | -0.162 |
| SPY | 0.318 | 0.065 | -0.327 |

Note: DFF=Days to 50% flowering, NL=number of leaves per plant, LL=leaf length, LW=leaf width, FLL=flag leaf length, FLW=flag leaf width, PH=plant height, SG=stem girth, PL=panicle length, PW=panicle width, PWT=panicle weight, DM=days to maturity, HSW=hundred seed weight, and SPY=single plant yield.

for these traits showed strong associations with PC1 variance. The results were in accordance with the findings of Pugahendhi *et al.*, (2023) for traits such as days to 50% flowering, plant height, and leaf dimensions; by Boukrouh *et al.*, (2023) for grain yield, panicle weight, and plant height; and by Malini *et al.*, (2023) for flowering and maturity timing. Overall, flag leaf length, plant height, panicle length, and panicle width presented positive loadings across all three principal components, indicating that they are key contributors to genetic divergence and phenotypic differentiation among sorghum genotypes. The consistent influence of these genes suggested that they are valuable traits for selection in sorghum breeding programs.

PCA also provided valuable insights into the relationships among biometric traits (Fig. 3), as shown by vector angles. An acute angle between days to 50% flowering and days to maturity indicated a strong positive correlation, which is useful for selecting early-maturing types. Similarly, positive correlations were

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