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MULTIVARIATE STATISTICAL APPROACHES FOR INVESTIGATING DIVERSITY AMONG SORGHUM (*SORGHUM BICOLOR* L. MOENCH) HYBRIDS

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ABSTRACT

An experimental set comprising of 36 sorghum hybrids were developed through a Line × Tester fashion involving six females and six males at the Centre for Millets Research (CMR), Sardarkrushinagar Dantiwada Agricultural University, Deesa, during the summer 2023. The hybrids were evaluated in a randomized block design with three replications during the *kharif* 2023 season. Observations were recorded on key agronomic and quality traits including days to flowering, days to maturity, plant height, panicle length, panicle weight, 1000 grain weight, grain yield per plant, dry fodder yield per plant, protein content and pollen fertility. Genetic divergence analysis indicated that protein content contributed the highest to total divergence (17.90%), followed by panicle weight (15.70%) and days to flowering (13.10%). Cluster analysis grouped hybrids into distinct clusters, with cluster II consisted the largest group of thirteen hybrids, cluster III comprising of seven hybrids and cluster I consisted five hybrids. Greater inter cluster distances compared to intra cluster distances indicated substantial genetic divergence among clusters. Principal component analysis revealed that the first three components (eigenvalues > 1) accounted for 69.99 per cent of the total variation, with Dim1 and Dim2 explaining 40.3 per cent and 17.4 per cent, respectively. The PCA biplot provided an effective visualization of genetic diversity, trait relationships and phenotypic trends among hybrids, demonstrating the utility of multivariate analyses for identifying promising genetic groupings in sorghum breeding programs.

Key words : Sorghum, Hybrids, Cluster analysis, Mahalanobis distance, Principal component analysis and Diversity.

Introduction

Climate change is profoundly impacting agricultural crops worldwide by altering temperature, precipitation and increasing the frequency of extreme weather events, which disrupt crop growth, reduce yields and threaten food security. Major staples like wheat, maize and rice faces significant yield declines, with climate stress causing losses as high as 40 per cent in some regions by mid-century. Sorghum [*Sorghum bicolor* (L.) Moench], more resilient than other cereals, often referred to as a "camel crop," is increasingly attracting global attention due to its remarkable resilience, versatility and role in sustainable agriculture. On a global scale, sorghum holds the fifth position among the major cereals, following the production and significance levels of wheat, paddy, maize and barley. The semi-arid tropical regions, extending from Asia and Africa to Central America, are major areas for sorghum cultivation (Mengistu *et al.*, 2020). The term "Sorghum" generally denotes the cultivated species, a member of the *Poaceae* (*Gramineae*) family within the

Andropogoneae tribe. This species is defined by a diploid chromosome number of 2n = 2x = 20, an estimated nuclear DNA content of 1.6 picograms and a genome size of around 730 megabase pairs. Across the world, it is known by many regional names, such as great millet, guinea corn, broomcorn, kaffir corn, durra, mtama, milo, jowar and kaoliang (OECD, 2017). It is mainly a self-pollinating plant, but it shows an outcrossing rate estimated between 7 to 30 per cent (Dje *et al.* 2004). Cultivated sorghum has been classified into groups based on morphology. Researchers later developed a system dividing it into five primary races *viz.*, *bicolor*, *kafir*, *caudatum*, *durra*, *guinea* and ten intermediate races distinguished by floral traits (Harlan and de Wet, 1972). Its rich genetic diversity makes it a valuable candidate for climate smart agriculture, making sorghum one of the most attractive crops of the modern era.

The success of plant breeding and crop improvement programs is fundamentally anchored in the genetic diversity found within crop species (Motlhaodi *et al.*, 2017). Assessing crop genetic diversity is vital for managing germplasm and identifying superior genotypes. Selecting genetically diverse parents is crucial for breeding programs to maximize recombination and improve yield. The utilization of genetically diverse parents increases the probability of recovering superior recombinants through hybridization (Srivastava *et al.*, 2020). Genetic diversity assessment in plants often employs statistical tools such as metroglyph analysis, D^2 clustering and PCA to capture variance patterns.

The present investigation was conducted to evaluate the genetic diversity among sorghum hybrids using Mahalanobis distance (D^2) and Principal component analysis (PCA), enabling a comprehensive assessment of their genetic relationships. Mahalanobis generalized distance, also known as Mahalanobis distance (D^2), measures the standardized difference between two groups along a discriminant axis (Del Giudice, 2017). Plant breeders use multivariate analysis (Rao, 1952) to measure genetic diversity, which helps categorize complex data into separate clusters for more straightforward interpretation (Chandra, 1977). Clustering can be achieved through Tocher's method, where genotypes with larger inter-cluster distances, or those placed in different clusters, are considered more genetically diverse. Principal component analysis (PCA) is a multivariate statistical tool that extracts valuable insights from complex datasets by transforming the original variables into a reduced set of new variables, known as principal components, which retain most of the variation in the data. These principal components are linearly uncorrelated, representing distinct sources of variation, while the eigenvalues reflect the proportion of total variation explained by each component. PCA classifies genotypes on the basis of genetic relatedness, with strongly dissimilar genotypes forming isolated clusters (Donde *et al.*, 2019; Mazid *et al.*, 2013).

Materials and Methods

Site of experiment

The experimental set comprised 36 sorghum hybrids generated by a Line × Tester fashion involving six female lines (2297A, 3060A, 1190A, 415A, 8914A and 2295A) and six male testers (CSV 20, CSV 27, CSV 39, CSV 41, GJ 43 and GJ 44). These hybrids were developed through the three-line breeding technique (A, B and R line), which utilizes cytoplasmic male sterility (CMS) to facilitate the efficient production of hybrid seeds. Hybridization was carried out at the Centre for Millets Research (CMR), Sardarkrushinagar Dantiwada Agricultural University, Deesa, during the summer of 2023. The research site is situated in North Gujarat Zone IV at 24°15' N latitude, 72°12' E longitude and an elevation of 146 meters above mean sea level. The soil is loamy sand, with a pH ranging from 7.5 to 9.3. All entries were evaluated using a Randomized block design (RBD) with three replications during the *kharif* 2023 at CMR, Deesa. Each entry was sown in a 4.0 m row, with an inter-row spacing of 45 cm and an intra-row spacing of 15 cm with standard agronomic practices.

Measures of traits

Hybrid performance, evaluated important traits such as number of days to flowering, days to maturity, plant height (cm), panicle length (cm), panicle weight (g), 1000 grain weight (g), grain yield per plant (g), dry fodder yield per plant (g), protein content (%) and pollen fertility (%).

Statistical data analysis

Replicated data were utilized for divergence study by calculating Mahalanobis' distance (D^2) (Mahalanobis, 1928) in accordance with the methodology delineated by Rao (1952). Hybrids were classified into distinct clusters based on D^2 values via Tocher's clustering scheme (Jadhav *et al.*, 2023). The mean values of the hybrids were incorporated into the larger dataset to facilitate dimensionality reduction through principal component analysis (PCA) by Hotelling (1936) across multiple traits. The dataset was further analyzed in R Studio (version 2025.05.1+513), where data visualization and interpretation were performed using specialized R packages including biotools, factorextra, FactoMineR and ggplot2.

Results and Discussion

Mean performance

Fig. 1 displays a violin plot that indicates the mean performance of sorghum hybrids in terms of grain yield and its associated traits. Each violin plot represents the distribution of trait values across thirty-six hybrids. The width and shape of the violins reveal the variation within the data: broader sections suggest that many hybrids have similar trait values, indicating a higher degree of consistency in performance, while narrower sections point to greater variation or fewer hybrids sharing similar values. These variations in the violin shapes offer a visual summary of the central tendency and variability of each trait, enabling a direct comparison of hybrid performance. Stretched-out or wider violins reflect more diverse trait expression, while narrower violins denote a more uniform and stable trait performance among hybrids. This graphical representation offers a clear perspective on genetic diversity across multiple traits (Rout *et al.*, 2025).

Table 1 summarizes the results of the descriptive statistical analysis performed on the various characteristics of the hybrids. The hybrids had an average flowering time of 77.14 days. The earliest flowering was noted in hybrid SH34, which flowered in 65.00 days, while SH21 took the longest to flower. On average, the hybrids matured in 113.27 days. The earliest maturity was observed in hybrids SH20 and SH34 at 109.33 days, while SH4 showed the latest maturity at 122.33 days. The average plant height was 192.19 cm, with SH16 being the shortest at 116.67 cm and SH29 the tallest at 237.67 cm. The average panicle length was 23.40 cm, with SH17 exhibiting the shortest panicle at 17.56 cm and SH34 the longest at 29.87 cm. Mean panicle weight was 63.17 g, ranging from a minimum of 22.00 g in SH17 to a maximum of 92.53 g in SH32. The average 1000 grain weight stood at 29.07 g, with SH17 recording the lowest value (22.40 g) and SH36 the highest (33.56 g). Grain yield per plant averaged 43.81 g, varying from 21.17 g in SH3 to 78.80 g in SH29. The mean dry fodder yield per plant was 151.59 g, with SH16 having the lowest yield (95.32 g) and SH2 the highest (257.39 g). The hybrids showed an average protein content of 7.82 per cent, with SH18 recording the lowest (6.52%) and SH25 the highest (9.40%). The mean pollen fertility was 91.53 per cent, ranging from a minimum of 80.76 per cent in SH23 to a maximum of 97.39 per cent in SH5.

Mahalanobis distance (D^2)

The ten characters were assessed for their per cent contribution to genetic divergence among the hybrids (Table 2). The analysis revealed that protein content had the highest impact on genetic divergence with a contribution of 17.90 per cent. Panicle weight (15.70%) and days to flowering (13.10%) followed closely, emphasizing their major influence. Dry fodder yield per plant (12.30%), 1000 grain weight (11.20%) and grain yield per plant (10.10%) exhibited moderate effects, while pollen fertility (8.1%), panicle length (4.8%) and plant height (4.60%) contributed comparatively less. Days to

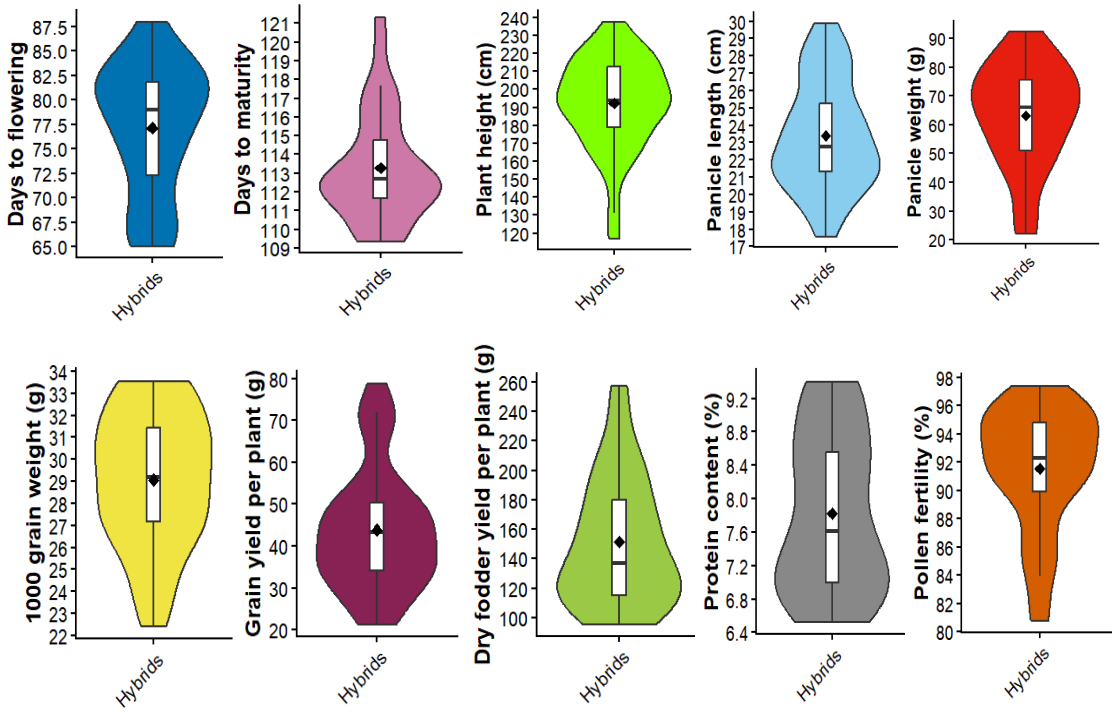

Fig. 1 : Violin plots for mean performance of thirty-six hybrids of sorghum.

Table 1 : Statistical summary of various characters for thirty-six sorghum hybrids.

Characters	General mean	Minimum		Maximum		S.Em.	CD at 5%	CV
		Values	Hybrids	Values	Hybrids			
DF	77.14	65.00	SH34	88.00	SH21	1.26	3.54	2.82
DM	113.27	109.33	SH20, SH34	121.33	SH4	1.66	4.69	2.54
PH	192.19	116.67	SH16	237.67	SH29	8.53	24.05	7.68
PL	23.40	17.56	SH17	29.87	SH34	0.96	2.70	7.09
PW	63.17	22.00	SH17	92.53	SH32	2.94	8.29	8.06
TGW	29.07	22.40	SH17	33.56	SH36	0.60	1.69	3.58
GYP	43.81	21.17	SH3	78.80	SH29	3.04	8.59	12.04
DFYP	151.59	95.32	SH16	257.39	SH2	7.92	22.34	9.05
PC	7.82	6.52	SH18	9.40	SH25	0.14	0.39	3.03
PF	91.53	80.76	SH23	97.39	SH5	0.94	2.66	1.78

DF: Days to flowering, DM: Days to maturity, PH: Plant height (cm), PL: Panicle length (cm), PW: Panicle weight (g), TGW: 1000 grain weight (g), GYP: Grain yield per plant (g), DFYP: Dry fodder yield per plant (g), PC: Protein content (%) and PF: Pollen fertility (%); S.Em.: Standard error of mean; CV: Coefficient of variation; CD: critical difference.

Table 2 : Character-wise per cent contribution towards genetic divergence among hybrids.

Characters	Proportion (%)
Days to flowering	13.10
Days to maturity	2.20
Plant height (cm)	4.60
Panicle length (cm)	4.80
Panicle weight (g)	15.70
1000 grain weight (g)	11.20
Grain yield per plant (g)	10.10
Dry fodder yield per plant (g)	12.30
Protein content (%)	17.90
Pollen fertility (%)	8.10

maturity, at 2.20 per cent, accounted for the smallest proportion of variation. Patel *et al.* (2024) reported that similar results for protein content and days to flowering contribute most significantly to genetic divergence, while Varma and Biradar (2022) identified panicle weight as a major contributing factors. Swamy *et al.* (2018) found that days to flowering, panicle length and dry fodder yield per plant exhibited the highest contribution toward genetic divergence in sorghum. The proportion of the various measured characters to the overall genetic divergence is visually depicted using a pie chart, as illustrated in Fig. 2, providing a clear and concise graphical summary of their relative influence.

The hybrids were grouped into ten distinct clusters following a comprehensive assessment of multiple characters (Table 3). The largest group, cluster II consisted of thirteen hybrids, namely SH12, SH28, SH20, SH27, SH15, SH22, SH26, SH13, SH14, SH19, SH6, SH31 and SH11. The seven hybrids categorized under cluster III included SH1, SH24, SH8, SH23, SH30, SH25 and SH33. Cluster I comprised five hybrids: SH32, SH36, SH35, SH34 and SH10. Cluster IV consisted of four hybrids: SH18, SH21, SH17 and SH3, while cluster V included only two hybrids (SH4 and SH5). Each of the remaining clusters VI, VII, VIII, IX and X contained a single hybrid: SH2, SH7, SH9, SH16 and SH29, respectively and these findings reveal unique genetic and phenotypic features that substantially distinguish these hybrids from the remaining studied hybrids. This clustering arrangement highlights the diversity and distribution of sorghum hybrids according to their evaluated characters.

Using the methodology of Singh and Chaudhary (2010), the average intra and inter cluster D^2 measures were obtained. The intra and inter cluster distances for all possible pairs among the ten clusters were calculated in Table 4. The analysis revealed that inter cluster (off diagonal) D^2 values ranged from 93.43 to 530.35. The highest inter cluster distance was found between clusters I and V (D^2 = 530.35), followed by clusters I and IV (D^2 = 488.42), cluster I & VI (D^2 = 458.83), cluster IV & X (D^2 = 438.48), cluster IX & X (D^2 = 414.49), cluster V & X (D^2 = 403.49), cluster VI & IX (D^2 = 387.46) and cluster V & VIII (D^2 = 387.33). The lowest inter cluster distance occurred between clusters VII & VIII (D^2 = 93.43), with other closely related pairs being cluster VIII & X (D^2 = 101.01), cluster I & II (D^2 = 109.05), cluster II & IX (D^2 = 109.54), cluster VI & VII (D^2 = 111.33), cluster III & VII (D^2 = 112.72), and cluster VII & X (D^2 = 116.36). The intra cluster distances (diagonal) ranged from 00.0 to 79.04, with the highest intra cluster divergence observed in cluster IV (79.04), followed by cluster III (D^2 = 73.28), cluster II (D^2 = 67.99), cluster I

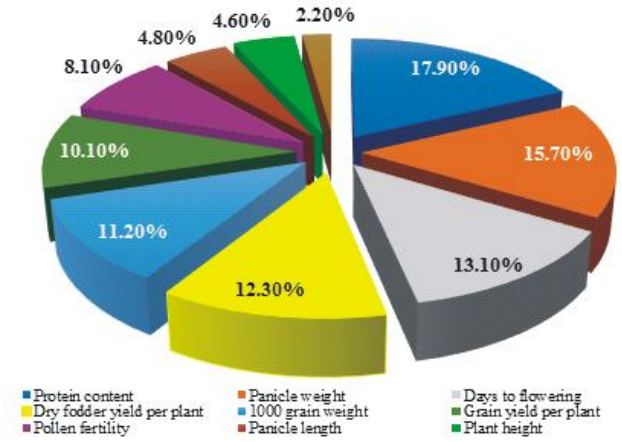

Fig. 2 : Visual representation of the per cent contribution of individual characters to genetic divergence in sorghum hybrids.

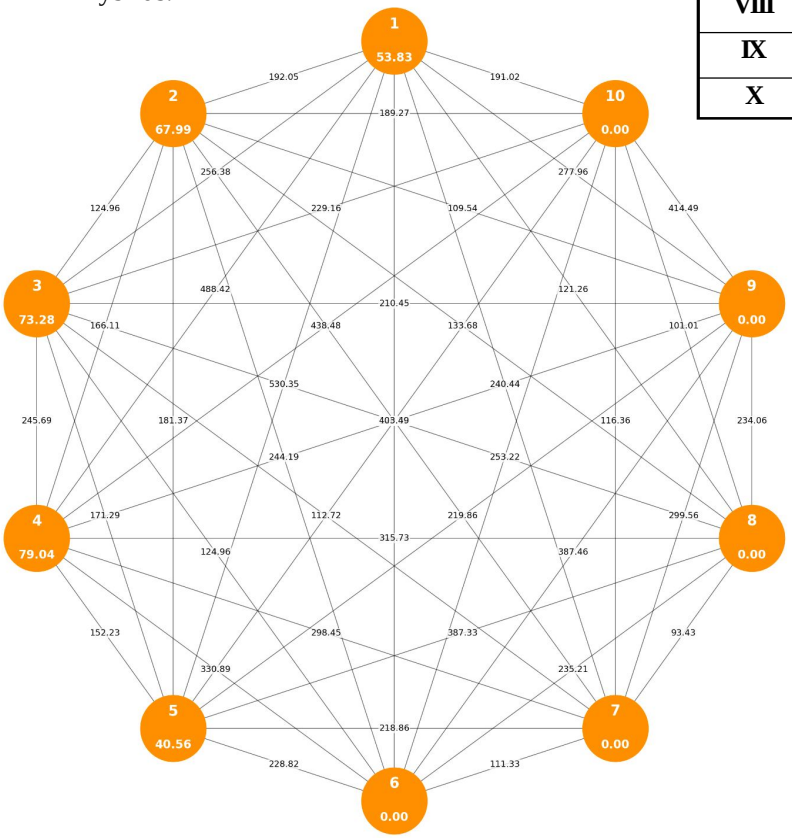

Fig. 3 : Inter and intra cluster distance of sorghum hybrids in ten clusters based on euclidean distance.

(D^2 = 53.83) and cluster V (D^2 = 40.56). Notably, the minimum intra cluster distance (0.00) was recorded for clusters VI, VII, VIII, IX and X.

Based on the above results, inter cluster distances being larger than intra cluster distances confirms notable genetic divergence, implying that hybrids in separate clusters are genetically distinct. Demelash *et al.* (2024) performed an eight clusters analysis, while Mengistu *et al.* (2020) generated five clusters using various sorghum

Table 3 : Grouping of hybrids into ten clusters.

Clusters	No. of hybrid(s)	Name of hybrid(s)
I	5	SH32, SH36, SH35, SH34 and SH10
II	13	SH12, SH28, SH20, SH27, SH15, SH22, SH26, SH13, SH14, SH19, SH6, SH31 and SH11
III	7	SH1, SH24, SH8, SH23, SH30, SH25 and SH33
IV	4	SH18, SH21, SH17 and SH3
V	2	SH4 and SH5
VI	1	SH2
VII	1	SH7
VIII	1	SH9
IX	1	SH16
X	1	SH29

accessions. Various researchers, including Swamy *et al.* (2018), Navyashree *et al.* (2024), Bhadouriya *et al.* (2024) and Patel *et al.* (2024), have employed the Mahalanobis distance to evaluate genetic divergence among sorghum genotypes.

Principal Component Analysis (PCA)

In PCA, components are computed and their eigenvalues are placed in decreasing order to reflect explained variance. The first three principal components, each having eigenvalues greater than one, together explained 69.99 per cent of the total variability in the dataset, emphasizing their significant contribution to understanding the overall data structure (Table 5). Principal components with eigenvalues less than one were not considered. Among them, the first principal component (PC1) alone accounted for 40.34 per cent of the total variance, indicating the maximum contribution with positive loadings. The key characters influencing PC1 were grain yield per plant (0.439), panicle weight (0.421), 1000 grain weight (0.398), panicle length (0.377), pollen fertility (0.067), dry fodder yield per plant (0.047) and protein content (0.046). Several scientists, including Mengistu *et al.* (2020), Bhadouriya *et al.* (2024), Kumari *et al.* (2024) and Jain and Patel (2016), have documented the positive loading effects of panicle length, panicle weight and 1000 grain weight on PC1.

Accounting for 17.40 per cent of total variance, PC2

Table 4 : Intra cluster and inter cluster distance of ten clusters of sorghum.

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	**53.83**	109.05	256.38	488.42	530.35	458.83	240.44	121.26	277.96	191.02
II		**67.99**	124.96	166.11	181.37	244.19	145.05	133.68	109.54	189.27
III			**73.28**	245.69	171.29	124.96	112.72	180.02	210.45	229.16
IV				**79.04**	152.23	330.89	298.45	315.73	120.49	438.48
V					**40.56**	228.82	218.86	387.33	219.86	403.49
VI						**0.00**	111.33	235.21	387.46	253.22
VII							**0.00**	93.43	299.56	116.36
VIII								**0.00**	234.06	101.01
IX									**0.00**	414.49
X										**0.00**

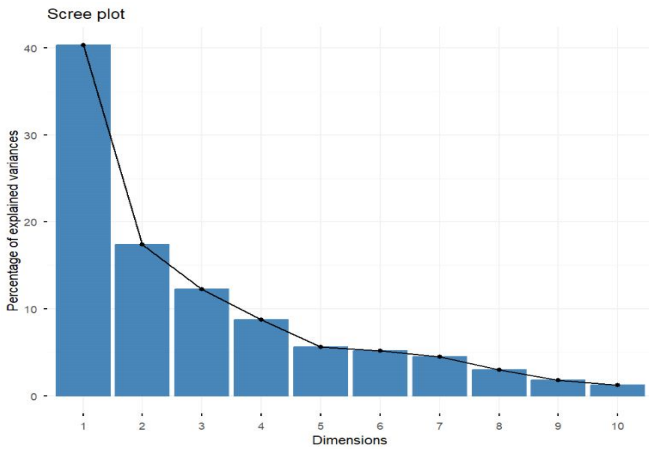

Fig. 4 : Visualization of eigenvalues and principal components through a scree plot.

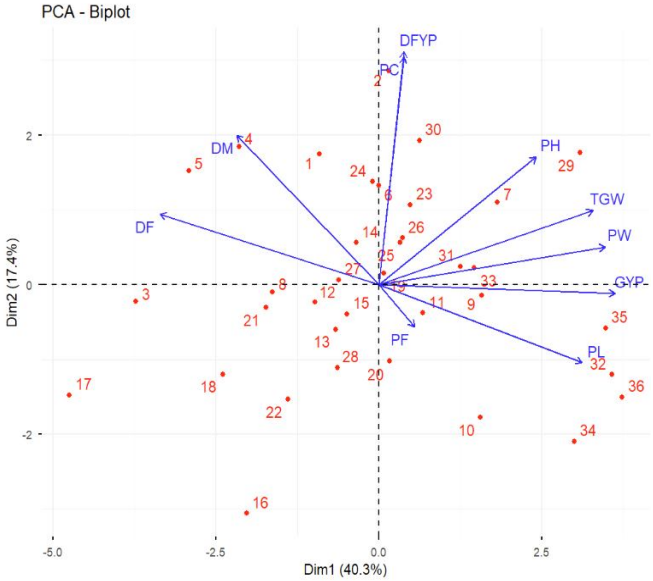

Fig. 5 : Biplot displaying the distribution of hybrids and characters within the principal component space.

was chiefly determined by positive loadings on dry fodder yield per plant (0.574) and protein content (0.561), along with days to maturity (0.368), plant height (0.314), 1000 grain weight (0.183), days to flowering (0.174) and panicle weight (0.093). Boratikar *et al.* (2025), Massaoudou *et al.* (2018) and Jain and Patel (2016) reported significant loadings for plant height for PC2. Additionally, Mengistu *et al.* (2024) reported significant loadings for plant height, panicle weight and 1000 grain weight for PC2.

The third principal component (PC3) explained 12.24 per cent of the total variation, with dry fodder yield per plant (0.242), 1000 grain weight (0.061) and panicle weight (0.045) emerging as the most influential traits based on their significant loadings. Boratikar *et al.* (2025), Kumari *et al.* (2024) and Jain and Patel (2016) similarly observed that the initial three components contributed 70 per cent to the total variance. The combined analysis of these principal components reveals a detailed picture of genetic diversity and character interactions in the hybrids, highlighting the contribution of multiple characters to the dataset's variability.

The scree plot suggests that the first principal component (PC1) explains the largest portion of the total variance, close to 40 per cent. The second component (PC2) contributes significantly less, about half of the first, while the third component (PC3) accounts for approximately half of the second. After the third component, the decrease in explained variance becomes more gradual (Fig. 4). This PCA biplot provides a simultaneous visualization of genetic diversity among hybrids (represented by red dots with numbers) and the relationships of various characters (represented by blue arrows) along two principal component axes: Dim1 (accounting for 40.3% of variance) on the X-axis and Dim2 (accounting for 17.4%) on the Y-axis (Fig. 5). The distribution of the 36 sorghum hybrids was effectively visualized using a 3D PCA scatter diagram, where the first three principal components (accounting for 69.99% of the total variance) were represented on the X, Y and Z axes. This multivariate statistical approach facilitated a clear and comprehensive visualization of essential

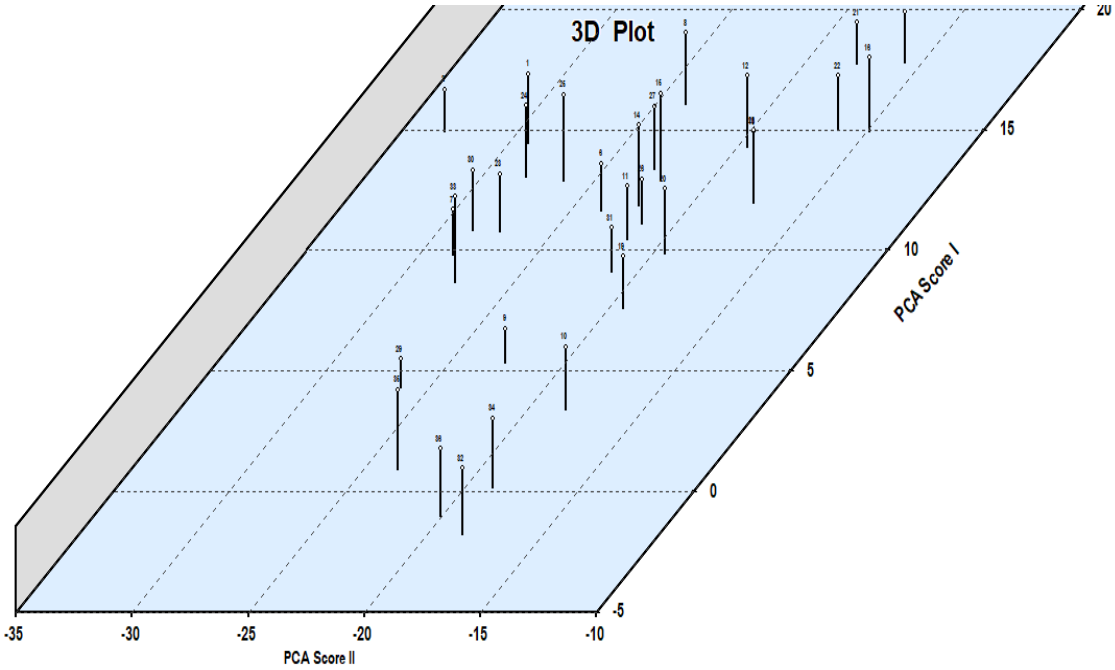

Fig. 6 : Positioning of thirty-six sorghum hybrids on the 3D scatter diagram reflects their PCA scores.

Table 5 : Eigenvalues, the share of total variance accounted for by each principal component and their loading coefficients across various sorghum traits.

Principal components	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**
Eigenvalues	4.034	1.741	1.225	0.873	0.562	0.52	0.447	0.299	0.18	0.12
Percentage of variance	40.344	17.408	12.246	8.726	5.622	5.199	4.472	2.985	1.802	1.196
Cumulative percentage of variance	40.344	57.752	69.998	78.724	84.347	89.545	94.017	97.002	98.804	100
Factor loading by multiple characters										
Days to flowering	-0.407	0.174	-0.119	0.146	-0.196	0.486	-0.323	0.142	0.202	-0.573
Days to maturity	-0.264	0.368	-0.286	-0.389	-0.501	-0.184	0.246	-0.464	0.014	0.026
Plant height (cm)	0.292	0.314	-0.353	0.343	0.284	-0.265	-0.431	-0.411	0.256	-0.042
Panicle length (cm)	0.377	-0.192	-0.032	-0.41	-0.25	-0.425	-0.171	0.33	0.324	-0.406
Panicle weight (g)	0.421	0.093	0.045	-0.104	-0.118	0.553	0.199	-0.059	0.597	0.287
1000 grain weight (g)	0.398	0.183	0.061	-0.06	-0.432	0.225	-0.492	0.042	-0.536	0.182
Grain yield per plant (g)	0.439	-0.021	-0.014	0.168	0.02	0.154	0.476	-0.272	-0.314	-0.596
Dry fodder yield per plant (g)	0.047	0.574	0.242	0.457	-0.247	-0.281	0.271	0.423	0.085	0.035
Protein content (%)	0.046	0.561	-0.02	-0.526	0.552	0.117	0.026	0.234	-0.151	-0.093
Pollen fertility (%)	0.067	-0.104	-0.845	0.114	-0.033	0.081	0.193	0.417	-0.126	0.155

genetic groupings and phenotypic trends, enhancing interpretation in genetics and plant breeding research (Fig. 6).

Conclusion

Mahalanobis generalized distance (D^2) analysis of the ten measured characters in sorghum hybrids demonstrated substantial genetic divergence, with protein content, panicle weight and days to flowering emerging as the primary contributors to this variation. The clustering analysis revealed clear genetic and phenotypic distinctions among the ten groups, with larger inter cluster distances than intra cluster distances, confirming that hybrids in different clusters are genetically diverse. Principal Component Analysis (PCA) of the hybrids revealed that the first three principal components, all with eigenvalues above one, accounted for 69.99 per cent of the total genetic variability, reflecting a strong and effective dimensional reduction in the dataset.

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