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ASSESSING GENETIC DIVERGENCE IN PEARL MILLET HYBRIDS THROUGH PRINCIPAL COMPONENT AND CLUSTER ANALYSES

RAJKUMAR PATEL[1], R. A. GAMI[2]*, K. G. KUGASHIYA[2], MANSHI J. PARMAR[2], H. N. ZALA[1], N. N. PRAJAPATI[3], R. N. PATEL[4] AND D. R. CHAUDHARY[5]

[1]Department of Genetics and Plant Breeding, C. P. College of Agriculture, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India
[2]Centre for Millets Research, S. D. Agricultural University, Deesa, Gujarat, India.
[3]Centre for Crop Improvement, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India
[4]Department of Seed technology, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India
[5] Agricultural Research Station, S. D. Agricultural University, Sardarkrushinagar, Ladol, India
*(e-mail : *ramangami@sdau.edu.in*)

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SUMMARY

An experimental material consists of twenty-one pearl millet F_1 hybrids developed through a Line × Tester design (3 lines × 7 testers) at SDAU, Deesa, during summer 2024 and evaluated at the Centre for Millets Research, SDAU, Deesa; Centre for Crop Improvement, SDAU, Sardarkrushinagar; SDAU, Ladol and Regional Research Station, SDAU, Kothara in a Randomized Block Design with three replications. The study involved the evaluation of nine traits, *viz.*, days to flowering, plant height, number of productive tillers per plant, earhead length, earhead girth, days to maturity, 1000 grains weight, grain yield per plant and dry fodder yield per plant. A multivariate analysis was carried out to indentify diverse genotype for breeding purpose. Mahalanobis D^2 analysis revealed substantial genetic diversity among the 21 pearl millet hybrids. Dry fodder yield per plant and earhead length contributed the most to genetic divergence, followed by grain yield per plant and 1000-grain weight. Cluster analysis grouped the hybrids into nine distinct clusters, with the highest inter-cluster distance observed between Clusters IV and VII, indicating that hybrids from these clusters could be used as promising parents for hybridization to obtain superior recombinants. Principal component analysis showed that the first three principal components explained 73.11% of the total variation. The variation was mainly associated with earhead length, earhead girth, grain yield per plant, dry fodder yield per plant, plant height, days to flowering, days to maturity and number of productive tillers per plant. Overall, Mahalanobis D^2 analysis, cluster analysis and principal component analysis effectively revealed the extent of genetic diversity and identified promising hybrids for future pearl millet breeding programmes.

Key words: Pearl millet, multivariate analysis, Mahalanobis' D^2, cluster analysis and Principal Component Analysis

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a C_4 diploid (2n=2x=14) cereal having a high photosynthetic efficiency typically grown under harsh agro-climatic conditions. It is also suitable for short growing season to allow multiple cropping due to faster growth rate under better agronomic practices (Yadav and Rai, 2013). It is an annual summer crop originating from Africa, from where it was introduced into other regions of the world with diverse agro-climatic conditions, from the hot areas of Africa (tropical zone) to the hot areas of temperate zones.

The mode of pollination is extremely cross pollination which leads to heterozygosity through crossing over and random mating. It has a 1.79 GB genome with seven linkage group (Khandelwal *et al.*, 2024). The chief pollinating agent is wind. However, insects also affect cross pollination. The protogyny nature was exploited for controlled cross pollination without resorting to emasculation which permits several breeding techniques from population improvement to heterosis breeding.

In pearl millet, multivariate analysis is extensively used to assess genetic divergence among genotypes based on yield-attributing traits. Techniques such as Mahalanobis' D^2 statistics, principal component analysis (PCA) and cluster analysis identify genetically diverse parents for hybridization programmes aimed at improving grain yield, fodder

yield and earliness. Principal component analysis further aids in identifying the traits that contribute most to total variability and minimum number of components which explain maximum variability while cluster analysis groups genotypes according to their genetic similarity. The information generated through multivariate analysis is therefore valuable for selecting diverse parental lines and developing superior pearl millet hybrids with enhanced productivity and adaptation. A few references, Joshi *et al.* (1988), Vidyadhar *et al.* (2004), Shanmuganathan *et al.* (2006), Kumar *et al.* (2020) and Gupta *et al.* (2022) have focused on the multivariate analysis in pearl millet.

The present study employed Mahalanobis' D^2 statistics and principal component analysis (PCA) to investigate the extent of genetic diversity among pearl millet hybrids based on multiple agronomic traits. Mahalanobis' D^2 is a multivariate distance measure that estimates the degree of divergence among genotypes by considering the covariance among traits, thereby providing a reliable assessment of genetic relationships (Mahalanobis, 1928; Del Giudice, 2017). In plant breeding, multivariate statistical techniques are extensively used to quantify genetic diversity and classify genotypes into distinct groups, facilitating the identification of diverse parental lines for hybridization (Rao, 1952; Chandra, 1977). Tocher's clustering method groups genotypes according to their genetic distances, where genotypes belonging to different clusters or separated by greater inter-cluster distances are considered genetically more diverse. Principal component analysis complements cluster analysis by reducing the dimensionality of multivariate data while retaining most of the original variability. The transformed principal components summarize the contribution of different traits to genetic variation, whereas the associated eigenvalues indicate the proportion of total variance explained by each component. Together, these multivariate approaches provide an effective framework for understanding genetic relationships and identifying promising breeding materials in pearl millet (Mazid *et al.,* 2013; Donde *et al.,* 2019).

MATERIALS AND METHODS

Site of experiment

The experiment material comprised of twenty-one pearl millet hybrids developed by Line × Tester fashion involving three lines (ICMB 1901, ICMB 0233 and ICMB 15555) and seven testers (ICMB 13222, ICMB 3283, ICMB 3317, ICMB 4127, ICMB 5649, ICMB 5755 and ICMB 5760). The seeds of 21 F_1 hybrids were produced during the summer, 2024 at the Centre for Millets Research, Sardarkrushinagar Dantiwada Agricultural University, Deesa. The F_1 were evaluated at four different locations *viz.*, Centre for Millets Research, SDAU, Deesa; Centre for Crop Improvement, SDAU, Sardarkrushinagar; SDAU, Ladol and Regional Research Station, SDAU, Kothara. All the hybrids were evaluated using Randomized Block Design with three replications at each location. Each entry was sown in a 4.0 m row, with an inter row spacing of 45 cm and intra row distance of 10-15 cm with standard agronomic practices. (The genotype and environment codes are presented in Table 1)

Measure of traits

The study involved the evaluation of nine traits, *viz.*, days to flowering, plant height, number of productive tillers per plant, earhead length, earhead girth, days to maturity, 1000 grains weight, grain yield per plant and dry fodder yield per plant.

Statistical data analysis

Pooled replicated observation's data were used to assess genetic divergence by estimating Mahalanobis' D^2 statistics following the procedure described by Mahalanobis (1928) and further elaborated by Rao (1952). Based on the calculated D^2 values, the hybrids were grouped into different clusters using Tocher's clustering method (Jadhav *et al.*, 2023). Subsequently, the mean performance of each hybrid for all recorded traits was subjected to principal component analysis (PCA) according to the approach proposed by Hotelling (1936) to summarize multivariate variation and identify the major sources of diversity. Statistical analyses and graphical representations were carried out using R Studio (version 2025.05.1+513). Data processing, multivariate analysis and visualization were performed using the R packages biotools, FactoMineR, factoextra, and ggplot2.

RESULTS AND DISCUSSION

Mean performance

Fig. 1 shows the violin graph for the mean performance of the twenty-one hybrids for yield and associated traits. It represents the distribution, spread

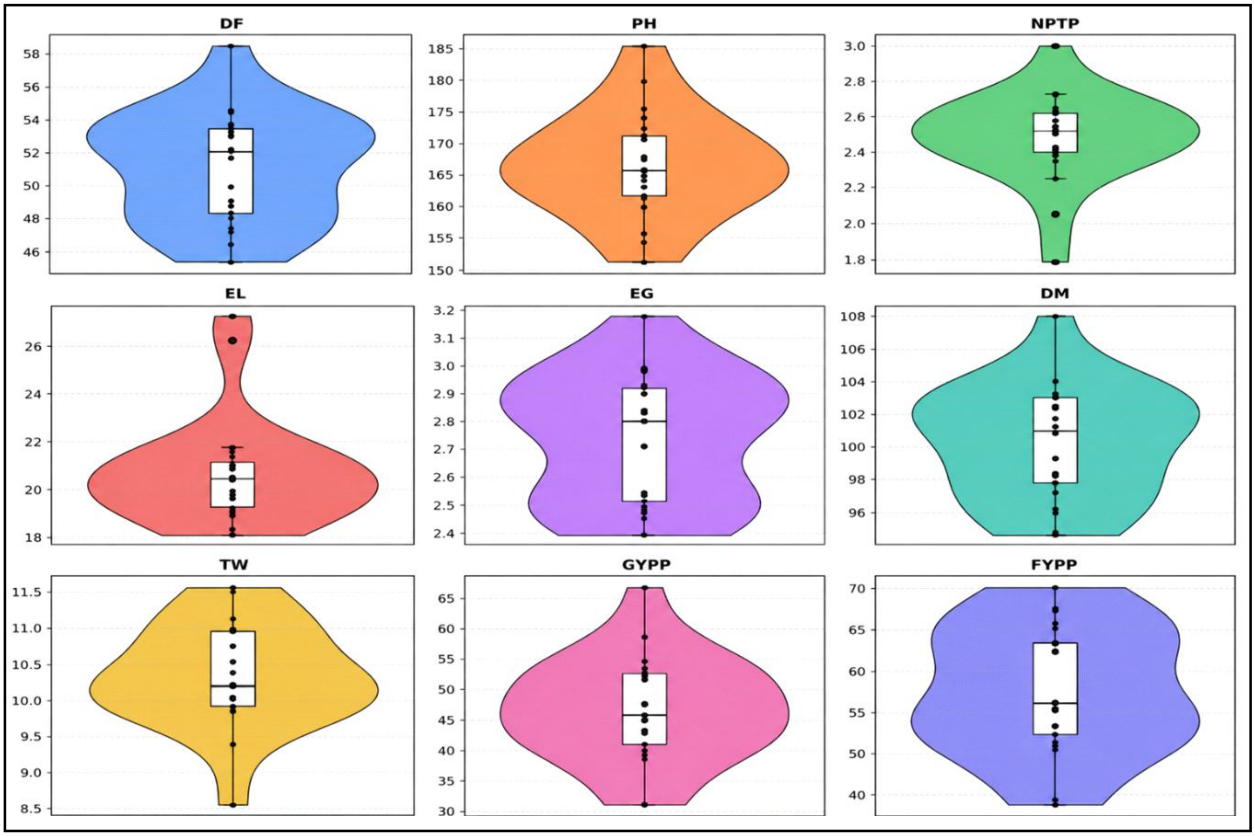

Fig. 1. Violin plots for mean performance of twenty-one hybrids of pearl millet. (Where, DF: Days to flowering; PH: Plant height (cm); NPTP: Number of productive tillers per plant; EL: Earhead length (cm); EG: Earhead girth (cm); DM: Days to maturity; TW: Test weight; GYPP: Grain yield per plant (g); FYPP: Fodder yield per plant (g).

TABLE 1
Genotype and character codes used for analysis

S. No.	Code	Name of genotypes	S. No.	Code	Name of genotypes
1.	G1	ICMB 15555 × ICMB 13222	16.	G16	ICMB 0233 × ICMB 3283
2.	G2	ICMB 15555 × ICMB 3283	17.	G17	ICMB 0233 × ICMB 3317
3.	G3	ICMB 15555 × ICMB 3317	18.	G18	ICMB 0233 × ICMB 4127
4.	G4	ICMB 15555 × ICMB 4127	19.	G19	ICMB 0233 × ICMB 5649
5.	G5	ICMB 15555 × ICMB 5649	20.	G20	ICMB 0233 × ICMB 5755
6.	G6	ICMB 15555 × ICMB 5755	21.	G21	ICMB 0233 × ICMB 5760
7.	G7	ICMB 15555 × ICMB 5760	22.	DF	Days to flowering
8.	G8	ICMB 1901 × ICMB 13222	23.	PH	Plant height (cm)
9.	G9	ICMB 1901 × ICMB 3283	24.	NPTP	Number of productive tillers per plant
10.	G10	ICMB 1901 × ICMB 3317	25.	EL	Earhead length (cm)
11.	G11	ICMB 1901 × ICMB 4127	26.	EG	Earhead girth (cm)
12.	G12	ICMB 1901 × ICMB 5649	27.	DM	Days to maturity
13.	G13	ICMB 1901 × ICMB 5755	28.	TW	1000 grains weight (g)
14.	G14	ICMB 1901 × ICMB 5760	29.	GYPP	Grain yield per plant (g)
15.	G15	ICMB 0233 × ICMB 13222	30.	FYPP	Fodder yield per plant (g)

and density of the statistical data. It combines the box plot and density plot. The width of the violin at any point represents the density of the data, where wider sections indicate a higher concentration of observations and narrower sections indicate fewer observations. The overall height of the violin represents the range of the data, extending from the minimum to the maximum value. This graphical representation offers a clear perspective on genetic diversity across multiple traits (Rout *et al.*, 2025).

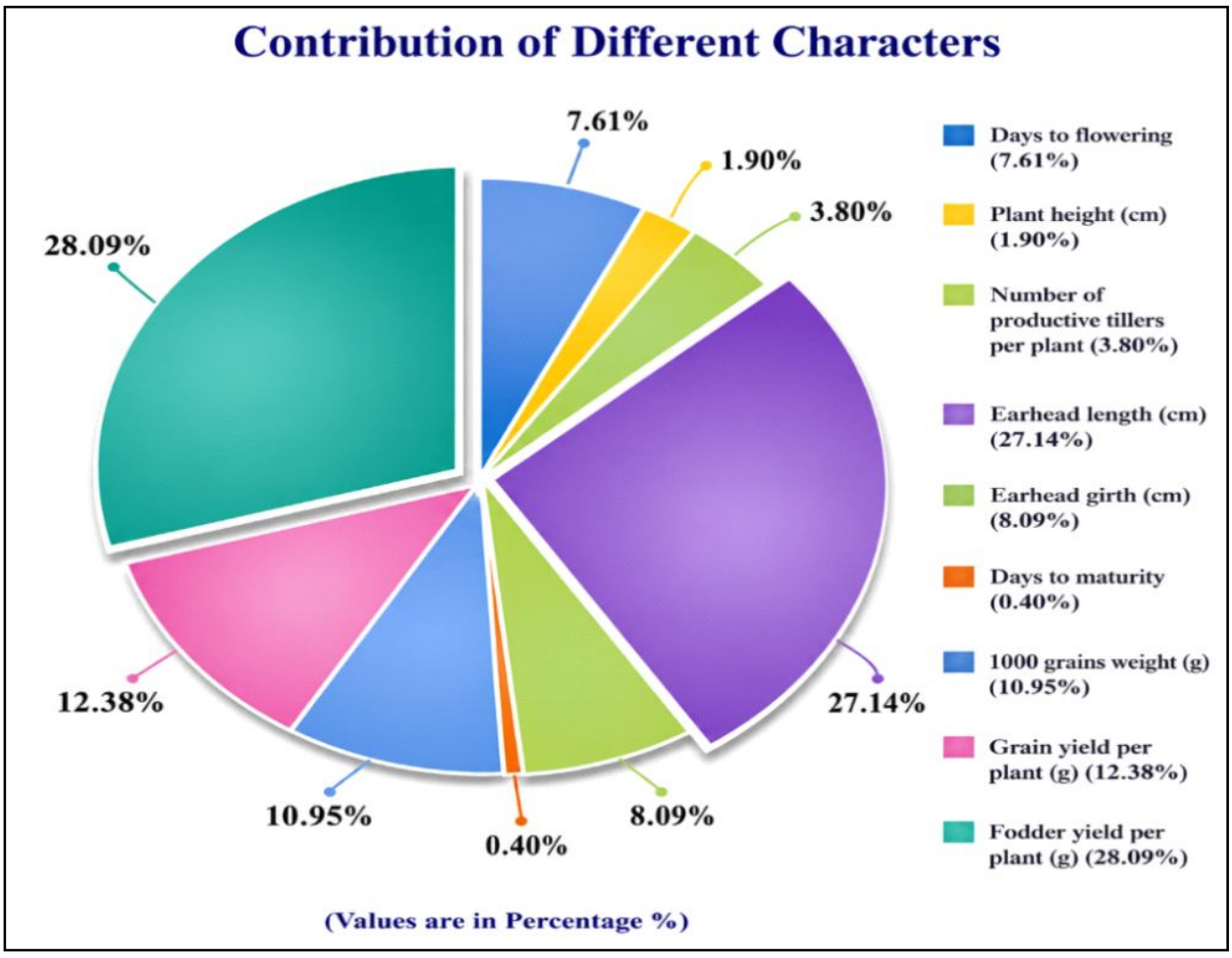

Fig. 2. Visual representation of the per cent contribution of individual characters to genetic divergence in pearl millet hybrids.

The statistical summary of the nine quantitative traits (Table 2) recorded for the twenty-one pearl millet hybrids demonstrated a wide range of variation, indicating the existence of considerable genetic diversity among the experimental material. Days to flowering (DF) varied from 44.50 days in ICMB 1901 × ICMB 13222 to 54.42 days in ICMB 0233 × ICMB 3317. Plant height (PH) ranged between 151.19 cm in ICMB 1901 × ICMB 5760 and 185.41 cm in ICMB 1901 × ICMB 5649. The number of productive tillers per plant (NPTP) was lowest in ICMB 1901 × ICMB 5649 (1.78) and highest in ICMB 0233 × ICMB 13222 (3.00). Earhead length (EL) ranged from 17.85 cm in ICMB 15555 × ICMB 13222 to 27.48 cm in ICMB 15555 × ICMB 5755, while earhead girth (EG) varied from 2.31 cm in ICMB 15555 × ICMB 3283 to 3.18 cm in ICMB 1901 × ICMB 5755. Days to maturity (DM) ranged from 92.17 days in ICMB 15555 × ICMB 5760 to 103.25 days in ICMB 0233 × ICMB 3317. The 1000-grain weight (TW) ranged from 7.94 g in ICMB 15555 × ICMB 13222 to 12.06 g in ICMB 15555 × ICMB 5755. A substantial variation was also observed for grain yield per plant (GYPP), which ranged from 28.07 g in ICMB 15555 × ICMB 3283 to 71.16 g in ICMB 15555 × ICMB 5760. Likewise, fodder yield per plant (FYPP) ranged from 45.83 g in ICMB 1901 × ICMB 3283 to 70.26 g in ICMB 1901 × ICMB 5755. The broad range observed for these traits reflects sufficient genetic variability among the hybrids, which can be effectively utilized for selecting superior genotypes and developing high-yielding pearl millet hybrids in future breeding programmes.

Mahalanobis distance (D^2)

The nine yield and yield attributing traits were assessed for their relative per cent contribution towards genetic divergence among the hybrids (Table 3). The results showed that fodder yield per plant (28.09%) contributed the most to genetic divergence, followed by earhead length (27.14%). Also, grain yield per plant (12.38%) and 1000 grains weight (10.95%)

TABLE 2
Statistical summary of various characters for twenty-one pearl millet hybrids

Characters	General mean	Minimum		Maximum		S.Em.	CD at 5%	CV
		Values	Hybrids	Values	Hybrids			
DF	49.24	44.50	G8	54.42	G17	1.41	3.96	4.88
PH	168.06	151.19	G14	185.41	G12	5.86	16.40	6.07
NPTP	2.41	1.78	G12	3.00	G15	0.21	0.60	15.12
EL	21.08	17.85	G1	27.48	G6	0.96	2.69	7.95
EG	2.73	2.31	G2	3.18	G13	0.10	0.30	6.91
DM	98.17	92.17	G7	103.25	G17	2.60	7.30	4.55
TW	9.94	7.94	G1	12.06	G6	0.68	1.91	11.73
GYPP	45.55	28.07	G2	71.16	G7	3.55	9.94	13.35
FYPP	57.60	45.83	G9	70.26	G13	2.53	7.10	7.91

TABLE 3
Character-wise per cent contribution towards genetic divergence among hybrids.

Characters	Proportion (%)
Days to flowering	7.61
Plant height (cm)	1.90
Number of productive tillers per plant	3.80
Earhead length (cm)	27.14
Earhead girth (cm)	8.09
Days to maturity	0.4
1000 grains weight (g)	10.95
Grain yield per plant (g)	12.38
Fodder yield per plant (g)	28.09

TABLE 4
Grouping of hybrids into nine clusters

Cluster	Number of hybrid(s)	Name of hybrid(s)
I	5	G1, G2, G3, G5 and G20
II	5	G4, G10, G16, G21 and G18
III	1	G6
IV	1	G7
V	3	G8, G19 and G9
VI	3	G11, G17 and G14
VII	1	G12
VIII	1	G13
IX	1	G15

emphasizing noted influence on the genetic divergence. The traits days to maturity (0.4%) reported lowest contribution towards genetic divergence among the traits studied. The contribution of each measured character to the total genetic divergence is presented in Fig. 2 through a pie chart, offering a clear visual representation of their relative importance.

The twenty-one hybrids are grouped into nine distinct clusters (Table 4) through assessment of multiple characters. The largest clusters are I and II which compromised five genotype each. The cluster I include genotypes G1, G2, G3, G5 and G20. While, the cluster II include G4, G10, G16, G18 and G21. The clusters V (G8, G19 and G9) and VI (G11, G17 and G14) include three genotype each. Clusters III, IV, VII, VIII and IX were each represented by a single hybrid, namely G6, G7, G12, G13 and G15, respectively. The formation of solitary clusters suggests that these hybrids possess distinct genetic constitutions and phenotypic attributes, resulting in marked divergence from the other hybrids included in the study. Such a clustering pattern reflects the presence of considerable genetic variability and emphasizes the broad distribution of pearl millet hybrids based on the evaluated traits. The identification of these unique hybrids may provide valuable genetic resources for future breeding programmes aimed at broadening the genetic base and improving desirable agronomic characteristics. The clustering pattern observed in the present study are agreement with the findings of Yadav *et al.* (2005) and Choudhary *et al.* (2015), who also reported considerable genetic diversity and distinct cluster formation among pearl millet genotypes based on multivariate analysis.

The average intra and inter-cluster Mahalanobis (D^2) distances were estimated following the procedure described by Singh and Chaudhary (2010). The resulting intra and inter-cluster distances for all possible combinations of the nine clusters are presented in Table 5 and Fig. 3. Among the clusters, Cluster VI exhibited the highest intra-cluster distance (99.94), followed by Cluster I (88.82), Cluster II (85.63) and Cluster V (53.355), indicating the presence of comparatively greater genetic variability among the hybrids within these clusters. In contrast, Clusters III, IV, VII, VIII and IX recorded an intra-cluster distance of zero, as each consisted of only one hybrid,

Table 5
Intra cluster and inter cluster distance of nine clusters of pearl millet

	I	II	III	IV	V	VI	VII	VIII	IX
I	88.82	167.49	608.12	595.32	139.94	195.54	304.96	500.83	341.29
II		85.63	351.74	430.74	137.25	121.09	148.80	265.00	216.24
III			0	199.35	380.33	496.92	602.87	290.62	209.11
IV				0	384.25	613.55	738.87	337.28	269.57
V					53.355	249.16	337.94	498.12	280.43
VI						99.94	186.67	306.52	251.27
VII							0	332.28	365.58
VIII								0	155.07
IX									0

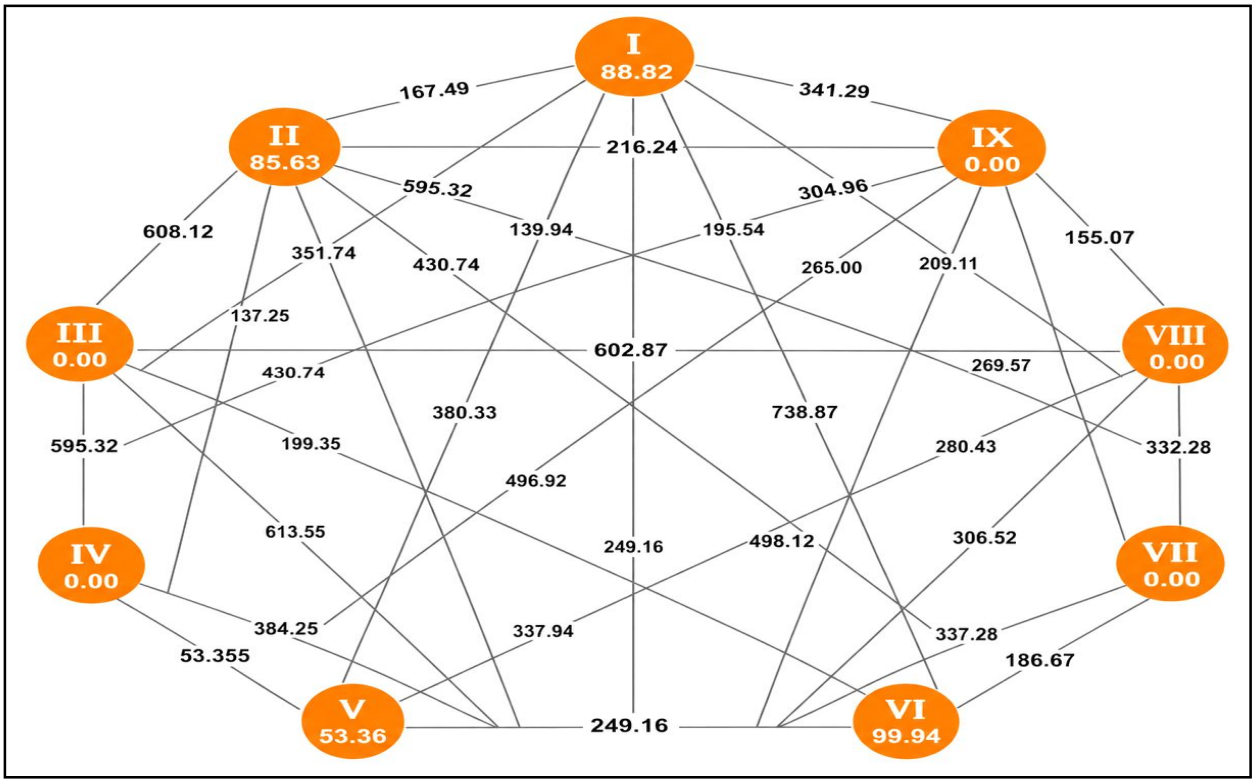

Fig. 3. Inter and intra cluster distance of pearl millet hybrids in nine clusters based on euclidean distance.

reflecting the absence of within-cluster variation. The inter-cluster distances varied considerably, suggesting different levels of genetic divergence among the clusters. The maximum inter-cluster distance was observed between Clusters IV and VII (738.87), followed by Clusters IV and VI (613.55), Clusters I and III (608.12), Clusters III and VII (602.87) and Clusters III and VIII (498.12) and such large distances indicate a high degree of genetic divergence between the hybrids belonging to these clusters. Therefore, crosses involving hybrids from these widely separated clusters may generate greater variability and improve the chances of obtaining superior recombinants in breeding programmes. On the other hand, the minimum inter-cluster distance was recorded between Clusters VI and II (121.09), followed by Clusters II and V (137.25) and Clusters I and V (139.94). The relatively small distances between these clusters indicate closer genetic relationships among their constituent hybrids. Overall, the wide range of intra- and inter-cluster distances confirms the existence of substantial genetic diversity among the pearl millet hybrids. The highly divergent clusters identified in this study provide useful parental combinations for hybridization aimed at broadening the genetic base and developing improved genotypes with desirable agronomic traits. Similar findings were reported by Govindaraj *et al.* (2011) and Meena *et al.* (2019), who also observed wide

TABLE 6
Eigenvalues, the share of total variance accounted for by each principal component and their loading coefficients across various pearl millet traits

Principle components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigenvalues	3.16	2.11	1.31	0.78	0.75	0.47	0.21	0.20	0.00
Percentage of variance	35.07	23.48	14.56	8.63	8.38	5.23	2.33	2.27	0.05
Cumulative percentage of variance	35.07	58.55	73.11	81.74	90.12	95.35	97.68	99.95	100.00
Factor loading by multiple characters									
Days to flowering	-0.104	0.653	0.086	-0.178	0.169	0.079	-0.117	0.066	0.686
Plant height (cm)	0.381	0.031	-0.052	0.357	0.654	-0.454	-0.207	-0.217	0.003
Number of productive tillers per plant	0.016	-0.002	0.829	0.189	0.157	0.005	0.482	0.140	-0.022
Earhead length (cm)	0.489	0.068	0.198	0.117	-0.220	0.108	-0.513	0.610	-0.091
Earhead girth (cm)	0.363	0.287	-0.314	0.146	-0.386	-0.415	0.558	0.166	0.082
Days to maturity	-0.154	0.649	0.009	-0.162	0.126	-0.031	-0.019	0.007	-0.715
1000 grains weight (g)	-0.315	0.123	-0.268	0.782	0.087	0.385	0.074	0.201	-0.002
Grain yield per plant (g)	0.445	0.205	0.129	0.176	-0.276	0.444	-0.029	-0.661	-0.023
Fodder yield per plant (g)	0.389	-0.087	-0.277	-0.318	0.471	0.508	0.362	0.226	-0.041

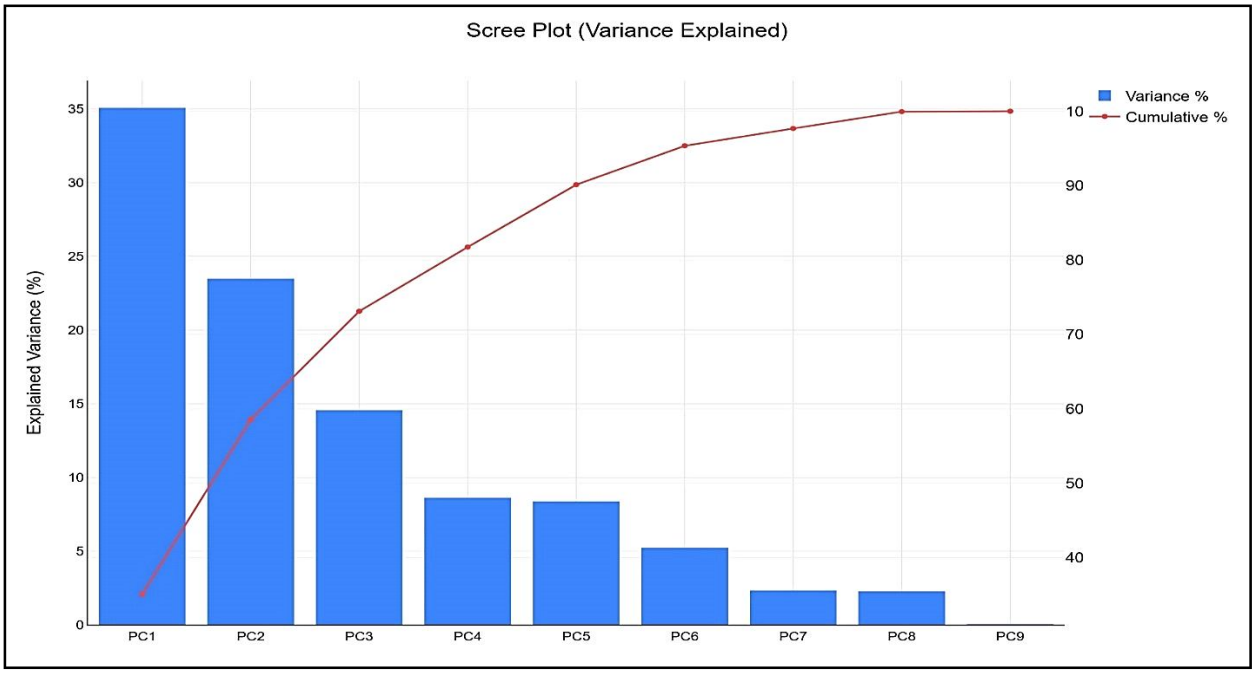

Fig. 4. Visualization of eigenvalues and principal components through a scree plot.

inter-cluster distances and suggested that crosses between genetically divergent clusters could be exploited to obtain superior recombinants in pearl millet.

Principal Components Analysis

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 73.11 per cent of the total variability in the dataset, emphasizing their significant contribution to understanding the overall data structure (Table 6). Principal components with eigenvalues less than one were not considered. Among them, the first principal component (PC1) alone accounted for 35.07 per cent of the total variance, indicating the maximum contribution with positive loadings. PC1 was predominantly influenced by traits with positive factor loadings. Earhead length (0.489) contributed the most, followed by grain yield per plant (0.445), fodder yield per plant (0.389), plant height (0.381) and earhead girth (0.363). Number of productive tillers per plant (0.016) showed the least positive contribution. These results indicate that PC1 mainly represents variation associated with yield and related morphological traits.

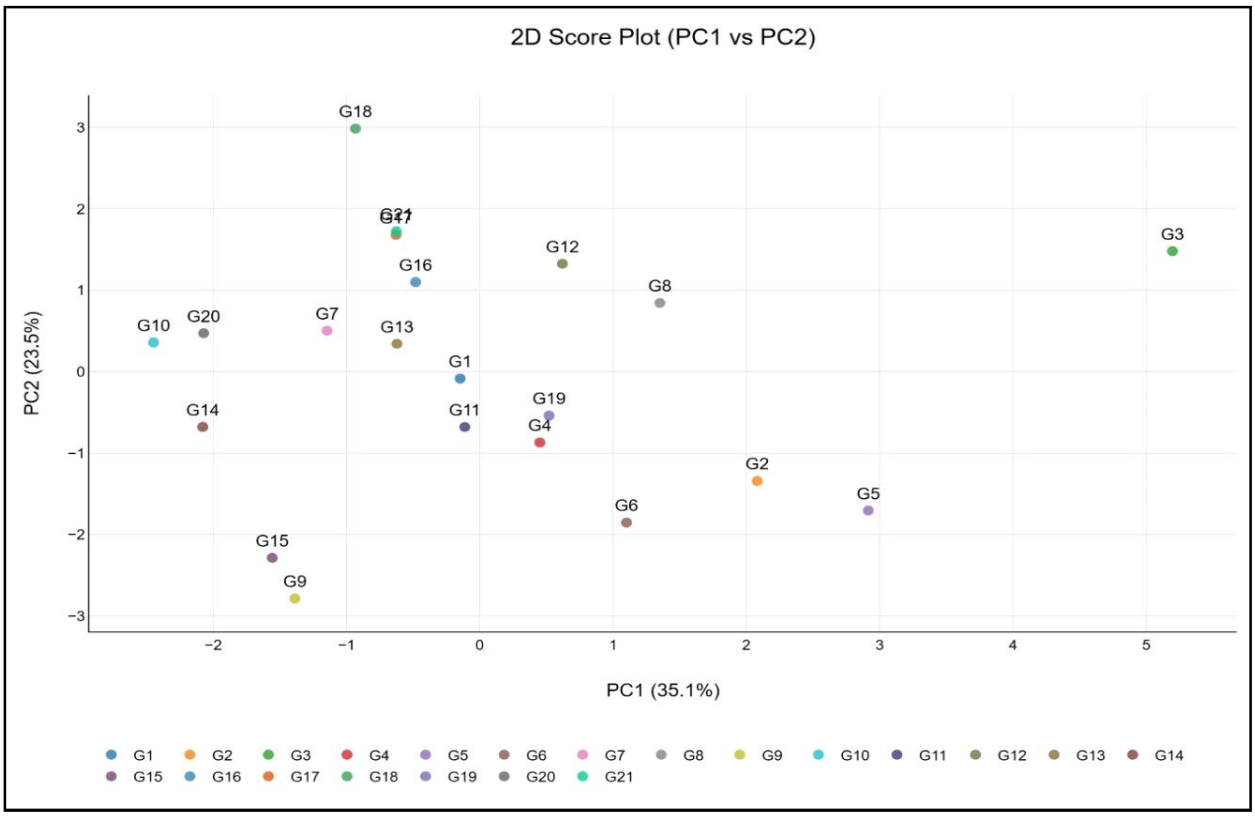

Fig. 5. Biplot displaying the distribution of hybrids within the principal component space.

PC2 accounted for 23.48% of the total variation and was primarily associated with days to flowering (0.653) and days to maturity (0.649), which showed the highest positive factor loadings. These were followed by earhead girth (0.287), grain yield per plant (0.205), 1000 grains weight (0.123), earhead length (0.068) and plant height (0.031), indicating relatively smaller positive contributions. In contrast, number of productive tillers per plant (-0.002) and fodder yield per plant (-0.087) exhibited negligible to slight negative loadings, suggesting minimal influence on this principal component. Thus, PC2 mainly reflects variation related to crop phenology, particularly flowering and maturity duration.

PC3 accounted for 14.56% of the total variation and was primarily characterized by the number of productive tillers per plant (0.829), which showed the highest positive factor loading. This was followed by earhead length (0.198), grain yield per plant (0.129), days to flowering (0.086) and days to maturity (0.009), indicating comparatively smaller positive contributions. These findings suggest that PC3 mainly represents variation associated with productive tillering, with minor contributions from earhead length, grain yield and phenological traits.

The scree plot (Fig. 4) revealed that the first principal component (PC1) explained 35.07% of the total variation, followed by PC2 (23.48%) and PC3 (14.56%), accounting for a cumulative variance of 73.11%. The inclusion of PC4 and PC5 increased the cumulative variance explained to 81.74% and 90.12%, respectively. The remaining principal components (PC6–PC9) together contributed only 9.88% of the total variation, with the cumulative variance reaching 100.00%. These results indicate that the first five principal components adequately captured the majority of the genetic variability among the pearl millet genotypes.

The score plot (Fig. 5) showed considerable genetic diversity among the 21 pearl millet hybrids. The hybrids ICMB 15555 × ICMB 3317 (G3), ICMB 15555 × ICMB 5649 (G5) and ICMB 15555 × ICMB 3283 (G2) were positioned on the positive side of PC1, indicating their association with traits such as earhead length, earhead girth, grain yield per plant, plant height and fodder yield per plant. Conversely, ICMB 1901 × ICMB 3317 (G10), ICMB 0233 × ICMB 5760 (G21), ICMB 0233 × ICMB 5755 (G20) and ICMB 1901 × ICMB 5760 (G14) were positioned on the negative side of PC1, reflecting contrasting trait combinations.

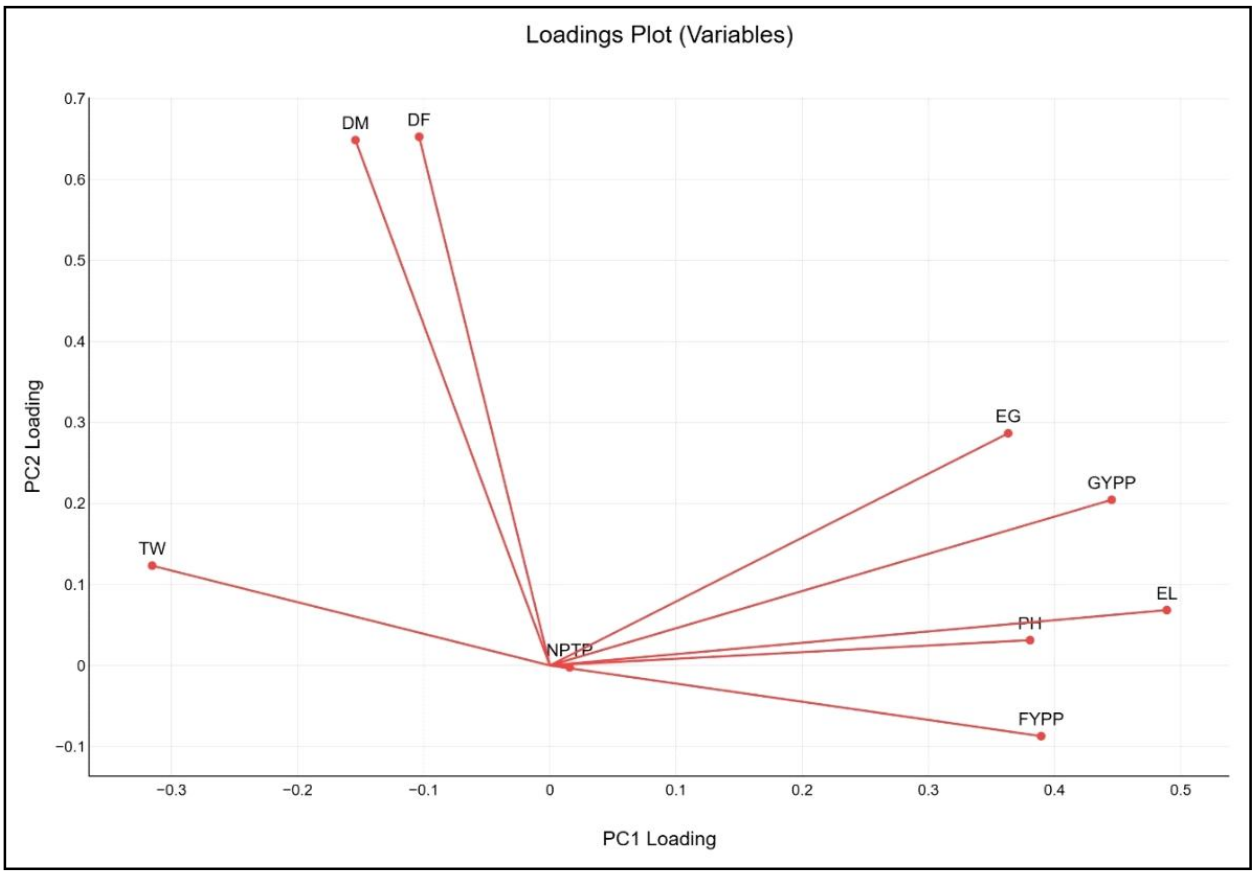

Fig. 6. Biplot displaying the distribution of characters within the principal component space. (Where, DF: Days to flowering; PH: Plant height (cm); NPTP: Number of productive tillers per plant; EL: Earhead length (cm); EG: Earhead girth (cm); DM: Days to maturity; TW: Test weight ; GYPP: Grain yield per plant (g); FYPP: Fodder yield per plant (g).

Along PC2, ICMB 0233 × ICMB 4127 (G18), ICMB 1901 × ICMB 5649 (G12) and ICMB 0233 × ICMB 3317 (G17) were located on the positive side and were associated with days to flowering and days to maturity, whereas ICMB 0233 × ICMB 13222 (G15), ICMB 1901 × ICMB 3283 (G9) and ICMB 15555 × ICMB 5649 (G5) were positioned on the negative side of PC2.

The loading plot (Figure 6) indicated that earhead length (EL), earhead girth (EG), grain yield per plant (GYPP), plant height (PH) and fodder yield per plant (FYPP) had positive loadings on PC1, suggesting that these traits contributed substantially to the variation represented by this component. Days to flowering (DF) and days to maturity (DM) exhibited high positive loadings on PC2, indicating their major contribution to the variability explained by the second principal component. In contrast, 1000-grain weight (TW) showed a negative loading on PC1, whereas the number of productive tillers per plant (NPTP) was located close to the origin, indicating a relatively lower contribution to the variation explained by the first two principal components. The present findings are in conformity with the reports of Govindaraj *et al.* (2011), Yadav *et al.* (2012), Rathod *et al.* (2017) and Meena *et al.* (2019), who also reported that the first few principal components explained most of the total variability and that yield and its component traits contributed significantly to genetic diversity in pearl millet.

Overall, the loading and score plots effectively distinguished the pearl millet hybrids based on their morphological traits and demonstrated substantial genetic variability among the genotypes. The first two principal components successfully summarized the trait relationships and genotype distribution, providing useful information for identifying diverse and promising hybrids for future breeding programmes.

CONCLUSION

The multivariate analysis confirmed the presence of considerable genetic diversity among the twenty-one pearl millet hybrids. Mahalanobis D^2

analysis identified fodder yield per plant, earhead length, grain yield per plant and 1000-grain weight as the major contributors to genetic divergence, while cluster analysis grouped the hybrids into nine distinct clusters, indicating a broad genetic base. Principal component analysis revealed that the first three principal components explained 73.11% of the total variation, with yield-related traits contributing most to the observed variability. Overall, the study identified genetically diverse and promising hybrids that can be effectively utilized as parents in future breeding programmes for the development of high-yielding pearl millet cultivars.

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