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GENETIC DIVERSITY AND ASSOCIATION STUDY IN PEARL MILLET HYBRID PARENTS FOR GENETIC IMPROVEMENT OF HYBRIDS

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ABSTRACT

Twenty seven hybrid parents of pearl millet containing B and R lines were evaluated at Corteva Agriscience research farm, Aurangabad, Maharashtra to determine the extent of genetic diversity and association among yield and yield contributing traits. Multivariate techniques such as Principal component analysis (PCA) and cluster analysis were used to analyze the data. The results of PCA revealed three principal components with Eigen values greater than one, explaining 40.97%, 24.11% and 14.74% of the total variability, respectively, with a cumulative effect of 79.82%. The study identified the traits such as plant height; panicle length and grain yield as excellent selection indicators that exhibit positive loading toward genetic diversity. Cluster analysis sorted all the parental lines into five distinct clusters, with cluster I having the highest number of lines followed by cluster III and cluster IV due to similar ancestry of parental lines. The parental lines MOPT-24, MOPT-25, MOPT-27, MOPT-26, MIT-23 and MIT-24display diversity in both cluster and PCA analyses, indicating the utilization of these lines for hybridization programme to harness heterosis and to select superior segregants in pearl millet.

Keywords: Pearl Millet, Hybrid parents, Cluster analysis, Trait Association, Grain yield

Introduction

Pearl millet occupies 6.93 million ha with an average production of 8.61 million tonnes and productivity of 1243 kg/ha during 2018-19 (Directorate of Millets Development, 2020; Project Coordinator Review, 2020). In India, pearl millet hybrids cover a significant portion (70-75%) of the total land area, with open-pollinated varieties (OPVs) or land races accounting for the remaining area (Patil *et al.*, 2020). Pearl millet has a notable capacity to act in response to favourable environments because of its short duration and capacity for fastgrowth rate, thus making it an outstanding crop for short growing seasons underimproved crop management (Yadav and Rai,2013).

Deciphering the genetic diversity of grain yield and yield contributing traits will be useful to identify contrasting parents, to maximize heterozygosity and achieve yield stability along with nutrient value in variable and changing climates (Haussmann *et al.*, 2007 and Hausmann *et al.*, 2012). The pattern of genetic relationships between and within accessions can be studied by multivariate analysis methods. Principal component analysis (PCA) and clustering are the two useful multivariate statistical tools for studying the relationship among the related genotypes. Cluster

analysis is used to study the association between land races while relationships between traits are statistically analyzed using PCA. These techniques have been previously applied for genetic diversity study in many crops such as finger millet (Lule *et al.*, 2012), rice (Gana *et al.*, 2013), and pearl millet (Pucher *et al.*, 2015 and Kumari *et al.*, 2016). Therefore, the present study was aimed to characterize and evaluate 27 pearl millet parental lines for grain yield and yield contributing traits by clustering and PCA approach.

Materials and Methods

Twenty seven pearl millet parental lines were sown during rainy 2019 at Corteva Agriscience research farm, Aurangabad (19.87° North latitude: 75.34° East longitude and 568 m meters above mean sea level) in Maharashtra. The region falls under Agro Climatic Zone B with rainfall greater than 600 mm, heavy soils and mild temperature conditions. The parental lines were of diverse origin which represented the B zone (Aurangabad, Maharashtra, India) of the three agro-ecological zones (A, A₁ and B) of pearl millet in India (Table 1). During rainy season 2019, manual planting was done using seed dibbling method at Aurangabad. The plots were thinned up to spaced 12-15 cm apart at 15th day of planting. A basal dose of 100 kg of di-ammonium phosphate

(18% N and 46% P) was applied at the time of field preparation and 100 kg of urea (46% N) was applied as top dressing in two split doses, once at three weeks and other at five weeks after sowing (Fig. 3). Trials were irrigated considering moisture level of the field. All the recommended agronomic practices were followed for raising healthy

crops. Five plants were randomly selected and tagged for taking observations. The observations were recorded for quantitative morphological traits such as days to 50% flowering, plant height (cm), number of tillers, panicle length (cm), panicle girth (cm), grain yield (kg ha⁻¹) and 1000 seed weight (gm).

Table 1 : List of pearl millet parental lines analyzed

Sr. No	Tret Nos	Pedigree	Source
1	MBL-1	MBL-1-S18-IC-BAMU-1	ICRISAT, Hyderabad
2	MBL-2	MBL-1-S18-IC-BAMU-2	ICRISAT, Hyderabad
3	MBL-3	MBL-1-S18-IC-BAMU-3	ICRISAT, Hyderabad
4	MBL-4	MBL-1-S18-IC-BAMU-4	ICRISAT, Hyderabad
5	MBL-5	MBL-1-S18-IC-BAMU-5	ICRISAT, Hyderabad
6	MBL-6	MBL-1-S18-IC-BAMU-6	ICRISAT, Hyderabad
7	MBL-7	MBL-1-S18-IC-BAMU-7	ICRISAT, Hyderabad
8	MBL-8	MBL-1-S18-IC-BAMU-8	ICRISAT, Hyderabad
9	MBL-9	MBL-1-S18-IC-BAMU-9	ICRISAT, Hyderabad
10	MBL-10	MBL-1-S18-IC-BAMU-10	ICRISAT, Hyderabad
11	MBL-11	MBL-1-S18-IC-BAMU-11	ICRISAT, Hyderabad
12	MBL-12	MBL-1-S18-IC-BAMU-12	ICRISAT, Hyderabad
13	MBL-13	MBL-1-S18-IC-BAMU-13	ICRISAT, Hyderabad
14	MBL-14	MBL-1-S18-IC-BAMU-14	ICRISAT, Hyderabad
15	MBL-15	MBL-1-S18-IC-BAMU-15	ICRISAT, Hyderabad
16	MBL-16	MBL-1-S18-IC-BAMU-16	ICRISAT, Hyderabad
17	MBL-17	MBL-1-S18-IC-BAMU-17	ICRISAT, Hyderabad
18	MBL-18	MBL-1-S18-IC-BAMU-18	ICRISAT, Hyderabad
19	MBL-19	MBL-1-S18-IC-BAMU-19	ICRISAT, Hyderabad
20	MBL-20	MBL-1-S18-IC-BAMU-20	ICRISAT, Hyderabad
21	MIT-21	MBL-1-S18-IC-BAMU-21	ICRISAT, Hyderabad
22	MIT-22	MBL-1-S18-IC-BAMU-22	ICRISAT, Hyderabad
23	MIT-23	MBL-1-S18-IC-BAMU-23	ICRISAT, Hyderabad
24	MOPT-24	MBL-1-S18-IC-BAMU-24	ICRISAT, Hyderabad
25	MOPT-25	MBL-1-S18-IC-BAMU-25	ICRISAT, Hyderabad
26	MOPT-26	MBL-1-S18-IC-BAMU-26	ICRISAT, Hyderabad
27	MOPT-27	MBL-1-S18-IC-BAMU-27	ICRISAT, Hyderabad

MBL – Millet B line; MIT – Millet inbred tester; MOPT – Millet open pollinated variety as tester

Genetic diversity among the genotypes was assessed through a cluster diagram, principal component analysis (PCA) following Davis (1986) and with a correlation matrix in the XLSTAT software. The PCs with Eigen value >1.0 were considered for determining the agro-morphological variability in the genotypes (Kaiser, 1960). Before PC Analysis, data was normalized using square root transformation and PC-1 and PC-2 scores were used to create a scatter plot of traits. To determine the association among yield and its contributing traits Pearson's correlation coefficients were computed for the seven individual traits using XLSTAT software.

Table 2 : Variations in quantitative traits of pearl millet parental lines

Character	Mean	Standard Deviation	Std. Error	Variance
Days to 50% flowering	56.8	4.2	0.56	18.15
Plant height (cm)	137.5	36.0	3.07	1297.58
Number of tillers	2.0	0.5	0.36	0.26
Panicle length (cm)	23.5	3.2	0.67	10.63
Panicle girth (cm)	30.3	3.3	0.61	11.49
Grain yield (kg ha ⁻¹)	10.0	2.1	0.69	4.80
1000 seed weight (gm)	1,525.6	816.8	20.91	667191.64

Results and Discussion

In present investigation, the analysis of variation showed considerable level of variability among different genotypes of pearl millet for most of the traits (Table 2). The largest variation was observed for grain yield and plant height. However, low variation was found for number of tillers and 1000 seed weight. This variability among the pearl millet parental lines could attribute to the inherent genetic differences or adaptation to local environmental condition.

Association Analysis

In the present study, a correlation matrix was used to determine the interdependence of several variables and their association with grain yield in pearl millet. Correlation coefficient for different quantitative characters (Table 3) revealed that grain yield was significantly and positively correlated with plant height, panicle length, panicle girth and 1000 seed weight. This finding is in agreement with previous studies by Manga (2002), Annamalai *et al.* (2020) and Narasimhulu *et al.* (2021a, 2022), which also found a favorable connection between grain yield and these traits. However, days to 50% flowering and number of tillers were

non-significantly correlated with grain yield. These findings are in agreement with the results of Rasitha *et al.* (2019) and Narasimhulu *et al.* (2021a). The length of the panicle was found to be proportionate to the plant height. These findings agree with the results of Narasimhulu *et al.* (2021b). Hence, the correlation matrix analysis conducted in this study identified several traits that were positively associated with grain yield in pearl millet. The findings of this study are consistent with previous research and provide valuable insights for developing trait-specific parental lines for future breeding programs.

Table 3: Correlation coefficients among 7 quantitative traits in pearl millet parental lines

Character	DF	PH	NOT	PL	PG	GY	TSW
Days to 50% flowering	1	-0.271	-0.568	0.083	0.187	0.041	-0.118
Plant height(cm)		1	0.289	0.534	0.335	0.756	0.477
Number of tillers			1	-0.050	-0.008	0.162	0.447
Panicle length (cm)				1	0.096	0.477	0.135
Panicle girth (cm)					1	0.501	0.346
Grain yield (kg ha ⁻¹)						1	0.524
1000 seed weight (gm)							1

Values in bold are different from 0 with a significance level $\alpha=0.05$

DF: Days to 50 % flowering, PH: Plant Height (cm), NOT: Number of tillers, PL: Panicle Length (cm), PG: Panicle Girth (cm), GY: grain yield (kg ha⁻¹), TW: 1000 - grain weight (g)

Cluster analysis

Cluster analysis was employed to investigate the genetic diversity among the 27 pearl millet parental lines based on different traits. The method classified the entities into different groups (Table 4) and further divided into sub-clusters (Fig. 1). Cluster I was the largest with eight lines (six B lines, one R lines and one OPV as R line) followed by cluster III and IV with five lines each respectively. The study suggests that selection of parents from cluster I, III, and IV

could serve as potential breeding material to improve yield attributing traits in hybridization programs. The results are consistent with studies by Kaushik *et al.* (2018), Kumar *et al.* (2020), and Narasimhulu *et al.* (2022), who also used quantitative data to identify prospective parents for heterotic expression of yield components. Singh *et al.* (2017) and Kumari *et al.* (2016) also reported similar results where clustering was used to identify diverse parental lines for developing high-yielding hybrids in pearl millet..

Table 4 : Grouping of pearl millet parental lines into different clusters

Cluster No	Number of genotypes	Parental Lines
I	8	MBL-3, MBL-4, MBL-10, MBL-14, MBL-17, MBL-19, MIT-22, MOPT-24
II	4	MBL-5, MBL-8, MBL-9, MBL-11
III	5	MBL-7, MBL-12, MBL-13, MBL-15, MBL-20
IV	5	MBL-18, MIT-21, MIT-23, MOPT-25, MOPT-27
V	1	MOPT-26

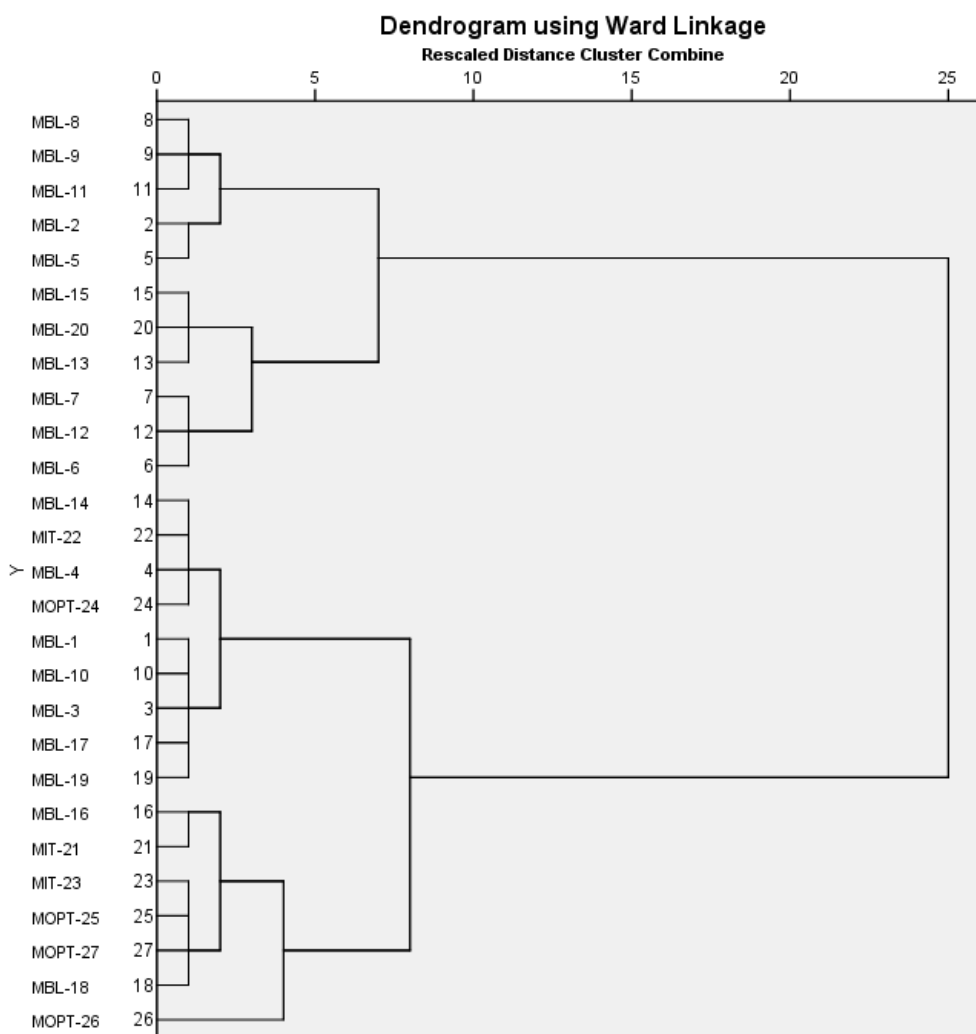


Fig. 1 : Dendrogram depicting clustering pattern of 27 parental lines analyzed in pearl millet.

Recent studies have continued to use clustering analysis to understand the genetic diversity of pearl millet germplasm. For example, a study by Singh *et al.*, (2021) identified 118 diverse germplasm accessions that were clustered into six groups based on 18 yield and quality traits. Hence clustering analysis was useful for identifying diverse parental lines and improving the yield potential of pearl millet.

Principal Component Analysis (PCA)

The results of principal component analysis (PCA) showed that the first three components had Eigen values greater than one and accounted for approximately 79.82% of the cumulative variability among the seven different morphological traits evaluated in 27 parental lines (Table 5).

Table 5 : Principal components, Eigen values, proportion of variation and total variation across axis

Principal component axis	1	2	3	4	5	6	7
Eigenvalue	2.86	1.68	1.03	0.57	0.37	0.30	0.16
Variability (%)	40.97	24.11	14.74	8.22	5.28	4.29	2.37
Cumulative %	40.97	65.08	79.82	88.04	93.33	97.63	100.00

PC1 was the greatest contributor to variability, accounting for 40.97% of the overall variation, followed by PC2 and PC3 which contributed 24.11% and 14.74% of the total variability, respectively. The study suggested considering traits with high PC1 scores to capture the variability of particular traits. The study suggested considering traits with high PC1 scores to capture the variability of particular traits.

PC1 was largely influenced by plant height (0.88), grain yield (0.87) and 1000 seed weight indicating that these traits were important contributors to variation among the breeding material (Table 6). The presence of positive and

negative loading in a single component indicated the presence of positive and negative correlation trends between the components and the variables. Traits with high positive or negative loading contributed more to the diversity. For instance, PC1 had a negative factor loading for the days to 50% flowering (-0.21), indicating a negative correlation with the trait. Similarly, PC2 contributed 29.8% of the total variation through days to 50% flowering (0.84), panicle girth (0.39), panicle length (0.36) and grain yield (0.27). Grain yield was found to increase with days to 50% flowering, panicle length and panicle girth. PC3 showed that grain yield was enhanced by a greater panicle length and plant height.

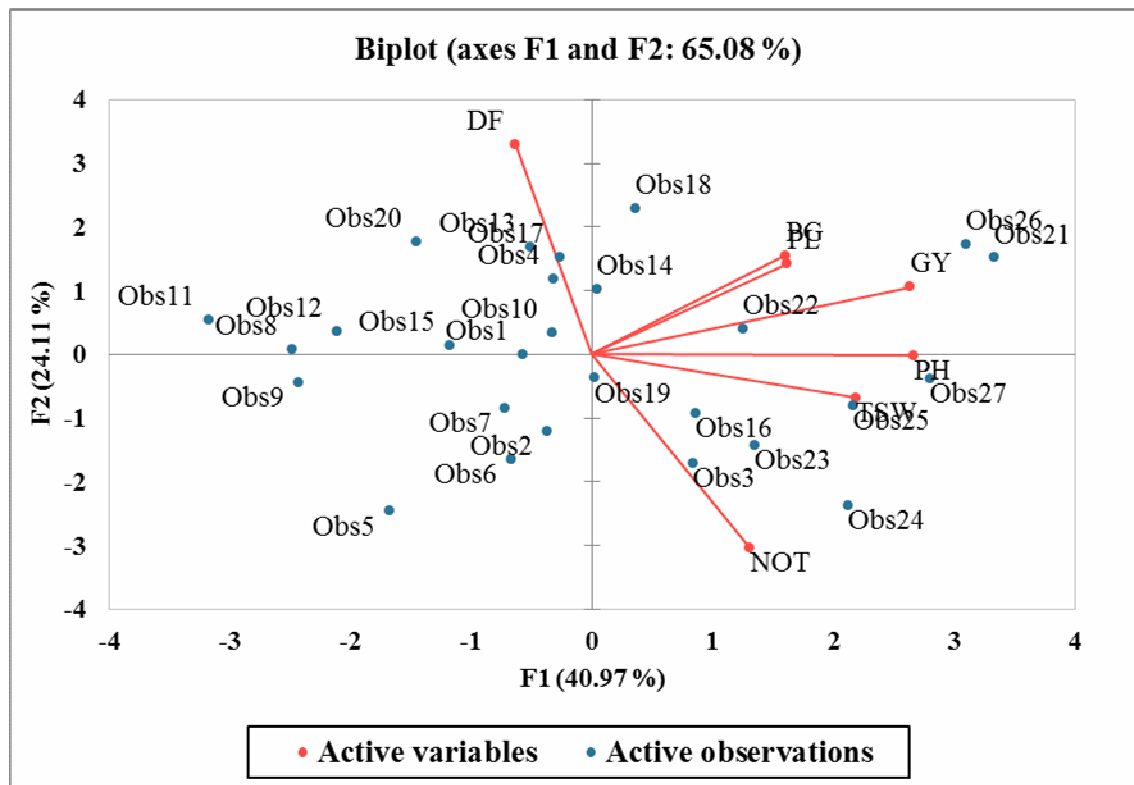
Table 6 : Factor loading of three important principal components for the traits studied

Particulars	PC1	PC2	PC3
Days to 50% flowering	-0.21	0.84	-0.21
Plant height (cm)	0.88	0.00	0.26
Number of tillers	0.43	-0.77	-0.12
Panicle length (cm)	0.53	0.36	0.66
Panicle girth (cm)	0.53	0.39	-0.57
Grain yield (kg ha ⁻¹)	0.87	0.27	0.00
1000 seed weight (gm)	0.72	-0.17	-0.38

Traits such as plant height, panicle length, panicle girth and 1000-seed weight contributed positively to the total variation in all the seven components. These traits could be effectively used for selection in crop improvement programs to obtain superior in bred with higher yields in pearl millet. Recent studies in pearl millet have also shown similar results. For instance, a study conducted by Govindaraj *et al.* (2019) reported that grain yield and its components, such as panicle weight, number of panicles per plant, and plant height, significantly contributed to the total variation observed in pearl millet germplasm.

PCA results are generally displayed as a biplot, in which axes correspond to the new system of coordinates (Fig. 2). The direction of arrow denotes the maximum change in quantity and the length could be related with the rate of change. The acute coordinate angle (<90°) between the traits

or principal component axis and trait shows the positive association between these traits, whereas obtuse angle (>90°) shows negative association; whereas right angle (=90°) indicates no correlation between the traits (Govindaraj *et al.*, 2020). Most of the traits were in acute angle with the PC1 coordinates except days to 50% flowering. The third quadrant did not have any trait plotted in the region. All most all the traits were in acute angle with grain yield. The lowest acute and adjacent angle with grain yield were observed for panicle length, panicle girth and plant height. Grain yield showed acute angle with most of the traits indicating positive correlation and exhibited the significance of the trait selection for improvement of yield attributing characters mainly like plant height, panicle length, panicle girth and 1000 seed weight, which were highly correlated (Kalagare *et al.*, 2021; Narasimhulu *et al.*, 2022).

**Fig. 3:** Biplot analysis for 27 pearl millet parental lines with 2 PCs

Principal component score for all the accessions were estimated and presented in Table 7. The high score of accessions can be utilized for precise selection of parent, whose intensity is determined by variability explained by each PCs. The high score of lines can be utilized for precise selection of parent, whose intensity is determined by variability explained by each PCs. MOPT-24 (2.118), MOPT-25 (2.160), MOPT-27 (2.797), MOPT-26 (3.100) and

MIT-21 (3.332) had high PCs score which is present in PC1. It means these parental lines are high PC score for plant height (cm), number of tillers, panicle length (cm), panicle girth (cm), grain yield (kg ha⁻¹) and 1000 seed weight (gm). MBL-20 (1.769), MBL-13 (1.682), MBL-17 (1.531), MBL-18 (2.291) and MOPT-26 (1.734) had high PC score for PC2 and highly loaded for panicle length and plant height. On the

basis of top 5 PC score, parental lines are selected and depicted in Table 7.

Table 7: Top five PC score selections in each PCs

PC1	PC2
MOPT-24 (2.118)	MBL-20 (1.769)
MOPT-25 (2.160)	MBL-13 (1.682)
MOPT-27 (2.797)	MBL-17 (1.531)
MOPT-26 (3.100)	MBL-18 (2.291)
MIT-21 (3.332)	MOPT-26 (1.734)

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