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Exploring the Genetic diversity studies in little millet (*Panicum sumatrense*) using multivariate tools - Principal component analysis and Cluster analysis

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ABSTRACT

The depletion of biodiversity not only distracts the process of plant development designed for genetic enrichment but also disrupts the ultimate services that the ecosystem offered to humanity. Evaluation of variability is a multidimensional problem. The multivariate statistical tools aids in a comparative evaluation of genetic variability. The availability of access to diverse genetic material is important to be successful in any plant breeding effort. Sixty-three little millet genotypes were evaluated for determining the genetic divergence. Observations were recorded on days to fifty percent flowering, days to maturity, plant height, flag leaf length, flag leaf width, peduncle length, peduncle exertion, length of inflorescence, and grain yield per plant. Analysis of variance imparted significant differences for most of the characters studied. The first three principal components having Eigen value more than one are cumulatively contributing 67.97% to the total variability. PC1 has the contribution from the traits viz., days to fifty percent flowering (0.39), days to maturity (0.39), flag leaf width (0.22), and grain yield (0.33) which accounted for 36.90 % of total variability indicating these traits contributed more to the total variance. Cluster analysis revealed that the little millet genotypes were grouped into four clusters based on hierarchical clustering. Cluster II comprised the highest number of (21) genotypes whereas Cluster III consisted of the lowest number of (10) genotypes. This analysis reveals the presence of wide genetic variance in little millet breeding lines.

Keywords: *Little millet,genetic diversity, Principal Component Analysis, and Cluster Analysis*

INTRODUCTION

The place of origin of Little millet is not well recognized and is considered as Indian origin. Little millet was cultivated in India and Sri Lanka and interrelated wild species have been seen outside India which helps to suggest India as a place of origin. In the archaeological excavations of Gujarat dating to 2000- 1500 BC presence of little millet seeds was evinced (Venkatesh Bhat *et al.* 2018). The little millet is mainly grown in the states of Karnataka, Andhra Pradesh, Madhya Pradesh, Odisha, Tamil Nadu, Gujarat, and Maharashtra. Like other small millets, little millet is also enriched with high nutrients. Nutritional insecurity is a crucial threat to the growing world's population that is highly reliant on a cereals-based diet, deficient in micronutrients. Apart from ecological and agronomic benefits, millets can provide other benefits such as nutritional security for smallholders. Millets are considered as Nutri-cereals because they are nutritionally superior to other cereals. Each 100 g of little millet grain comprises nutrients of 65.5 g carbohydrate, 10.1 g protein, 3.89 g fat, 7.7 g dietary fiber, 16.1 mg calcium, 130 mg phosphorus, 91 mg magnesium, 1.8 mg zinc, 1.2 mg iron, 0.26 mg thiamin, 0.05 mg riboflavin, 1.3 mg niacin and 362µg folic acid [5]

The success of a breeding program relies on the variability that exists in the breeding population and the efficiency of the selection technique. The success of hybridization is based on using a desirable parent, which will exploit better heterosis and produce promising segregants. The existence of an adequate amount of genetic variability and diversity in a base population is essential to choose highly diverged parents in a breeding programme. In general, a valuable source of variability exists in the available germplasm. Hence, the right parents can be chosen with the help of information on the nature and degree of genetic divergence. The crosses between parents with extreme genetic divergence are usually the most responsible for genetic improvement.

Several methods of multivariate analyses are available nowadays to establish the pattern of genetic divergence among genotypes, which helps in analyzing of multiple measurements simultaneously on each and every individual under investigation. Among the various multivariate techniques, cluster analysis and principal component analysis will be widely utilized in choosing the genotypes to meet the aim of a plant breeder in their breeding program. In cluster analysis, the core subset is formed for grouping germplasm accessions with similar characters in one homogenous category. The resultant dendrogram from cluster analysis displays clear information of

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high heterogeneity between clusters and high homogeneity within clusters through their pictographic representation. Model-based and distance-based clustering are the two major classifications of clustering. In that distance-based clustering methods are supplementarily classified into two groups: hierarchical clustering and non-hierarchical clustering. Hierarchical clustering methods are used in grouping of similar individuals and further pooling of these groups is done based on their kinship. UPGMA is the most commonly used hierarchical method. In non-hierarchical clustering, a similar threshold or chronological threshold forms the basis for the pooling of individuals to desirable clusters [2]. Data reduction can be possible through principal component analysis and a few uncorrelated variables called principal components which explains and splits the total variance of related characters. The initial step in the principal component analysis is an estimation of eigen value which defines the total dissimilarity revealed on PC axis. Maximum variability is recorded for the first principal component. The remaining unchecked and uncorrelated of the first PC variance is occupied by the second PC. The foremost objective of a plant breeder is to sort out the desirable number of plant characters that are responsible to contribute maximum variability in the crop growth starting from sowing to till harvest. An attempt was initiated in the present study in little millet genotypes to study principal component analysis and cluster analysis which would facilitate the researcher to categorize the existing germplasm into separate groups based on the genetic diversity.

MATERIALS AND METHODS

The field experiment was carried out by using sixty-three little millet germplasm in Randomized Block Design with three replications at Regional Research Station, Paiyur (Table 1.). Proper agronomic practices have been carried out in order to get a healthy crop. Five plants were randomly selected in each genotype and observations were recorded on traits *viz.*, days to fifty percent flowering, days to maturity, plant height, flag leaf length, flag leaf width, peduncle length, peduncle exertion, length of inflorescence and grain yield per plant. The principal component analysis and cluster analysis were performed by using STAR Package.

RESULTS AND DISCUSSION

Principal Component Analysis

The aim of the PCA is to obtain a small number of factors that contribute for maximum variability out of the total variability. Factor loadings were presented in Table 2. Eigenvector values, percentage of variance, and the cumulative percentage are presented in Table 3. The first three components accounted for 36.90, 54.25, and 67.97 percent of cumulative variation progressively. The proportion of variation contributed by the individual PC1 and PC2 were 36.90 and 17.35 percent respectively. The eigenvalue was found greater than one for the principal components PC1, PC2, and PC3. The Eigenvalues are gradually declined from PC1 to PC9 (Table 3.). The Eigenvalues for left-out principal components were 0.9754,0.8043,0.5029,0.3543, 0.2458, and 0 respectively (Fig.1). PC1 recorded high positive loadings for days to fifty percent flowering (0.39), days to maturity (0.39), flag leaf width (0.22) and grain yield (0.33) and the remaining parameters showed negative loadings. The results were similar to the findings of [1] and [3]. With regard to PC 2, the high positive factor loadings were observed for the traits *viz.,* panicle exertion (0.23), flag leaf width (0.43), peduncle length (0.14), and grain yield (0.20). In PC3, plant height (0.30) showed positive loadings and the remaining factors showed negative loadings. The spreading of the scores for the nine quantitative traits in the Principal Component Analysis was widespread showing the large diversity. The analysis described the variance structure through a linear combination of the variables and the percentage of variability. The exact picture of the component traits that are contributing to maximum variability is identified from factor loadings. The shaping of breeding strategies and heterotic grouping of genotypes is possible by knowing the genetic diversity. The information of the nature and distribution of diversity for both variables and genotypes are obtained from the biplot diagram (Fig. 2 to 4).

Cluster analysis

To study the nature and magnitude of genetic divergence the genotypes were grouped into four clusters. In the present study, hierarchal cluster analysis was carried out using distinct 63 genotypes which were grouped into four distinct clusters and cluster I consisted 16 genotypes (Figure 5.). They were found divided into two separate -sub-groups. Subgroup one comprised of nine genotypes. The sub-group two was composed of 7 genotypes. Cluster II also had 21 genotypes. They in turn comprised of two subgroups having 11 and 10 genotypes respectively. Cluster III was formed by 9 individuals. Seventeen genotypes placed together in cluster IV had two subgroups. Group 2 contained the maximum number of individuals (9) followed by group 1 (8). Cluster analysis in little millet was performed by [4]

The clusters formed were non-overlying in nature. The second cluster was the largest with twenty-one accessions and cluster three was the smallest with ten accessions. The clustering pattern was represented using the dendrogram (Figure 5). Hybridization can be better exploited between accessions of distinct clusters.

CONCLUSION

Sixty-three genotypes were grouped into four distinct clusters on the basis of Euclidean distance. The first principal component had positive loading for days to fifty percent flowering, days to maturity, flag leaf width, and grain yield. The second principal component had positive loading for four characters *viz.,* panicle exertion, flag leaf width, peduncle length, and grain yield, while the third principal component had positive loading values for plant height.

Acknowledgements

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Conflict of interest

There is no conflict of interest in the research work on little millet.

Table 1. List of little millet accessions used for diversity studies

S.No.	Little millet accessions
1	TNAU 7
2	TNAU 23
3	PM 142
4	TNAU 4
5	PM 306
6	TNAU 12
7	MS 509
8	MS 1826
9	TNAU 9
10	MS 4700/1
11	IPM 221
12	PMR 762
13	TNAU 18
14	TNAU 3
15	IPM 1211
16	MS 3969
17	TNAU 24
18	RPM 81
19	PM 41
20	MS 4527
21	PM 296/1
22	TNAU 7/79
23	PM 272
24	MS 4735
25	PM 42
26	TNAU 25
27	TNAU 26
28	IPM 231
29	TNAU 16
30	MS 1003/1
31	CO 3
32	TNAU 22
33	MS 4729
34	TNAU 6
35	TNAU 23
36	TNAU 10
37	TNAU 21
38	TNAU 296
39	PM 307
40	MS 4684
41	TNAU 24/79
42	TNAU 17
43	PM 295
44	Paiyur 2
45	TNAU 11/79
46	Paiyur 1
47	TNAU 15
48	TNAU 102
49	RPM 11
50	TNAU 13
51	IPM 226
52	TNAU 2
53	PM 29
54	IPM 221/4
55	IPM 231
56	MS 4779
57	TNAU 5
58	TNAU 14
59	TNAU 1678
60	MS 662
61	IPM 232
62	CO 2
63	TNAU 1/79

Table 2. Factor loading by different morphological traits

Principal components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Days to 50 % flowering	**0.3948**	**-0.4548**	**-0.3151**	0.1933	-0.0044	0.0152	0.0031	0.0185	-0.7071
Days to maturity/duration	**0.3948**	**-0.4548**	**-0.3151**	0.1933	-0.0044	0.0152	0.0031	0.0185	0.7071
Plant height	**-0.1924**	**-0.0736**	**0.3034**	0.755	0.4372	-0.2469	-0.19	-0.0855	0
Flag leaf width	**0.2153**	**0.4268**	**-0.028**	0.495	-0.4919	0.4676	-0.094	-0.2369	0
Flag leaf length	**-0.3883**	**-0.4366**	**0.0177**	-0.1511	-0.1102	0.2097	-0.2066	-0.7325	0
Peduncle length	**-0.3464**	**0.1407**	**-0.5182**	0.2068	0.1175	-0.0475	0.7059	-0.1858	0
Panicle exertion	**-0.256**	**0.2269**	**-0.6576**	-0.0145	0.1521	-0.0096	-0.638	0.1419	0
Length of inflorescence	**-0.403**	**-0.3126**	**0.0833**	0.1196	0.0101	0.6488	0.0748	0.5404	0
Grain yield	**0.3258**	**0.1976**	**0.0178**	-0.1735	0.7195	0.5027	0.0381	-0.2293	0

Table 3. Principal component analysis for different morphological traits recorded in little millet

Principal Components	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Standard deviation	1.8223	1.2497	1.1112	0.9876	0.8968	0.7092	0.5952	0.4958	0
Proportion of variance	0.369	0.1735	0.1372	0.1084	0.0894	0.0559	0.0394	0.0273	0
Cumulative proportion	0.369	0.5425	0.6797	0.7881	0.8774	0.9333	0.9727	1	1
Eigen Values	**3.3207**	**1.5617**	**1.2349**	0.9754	0.8043	0.5029	0.3543	0.2458	0

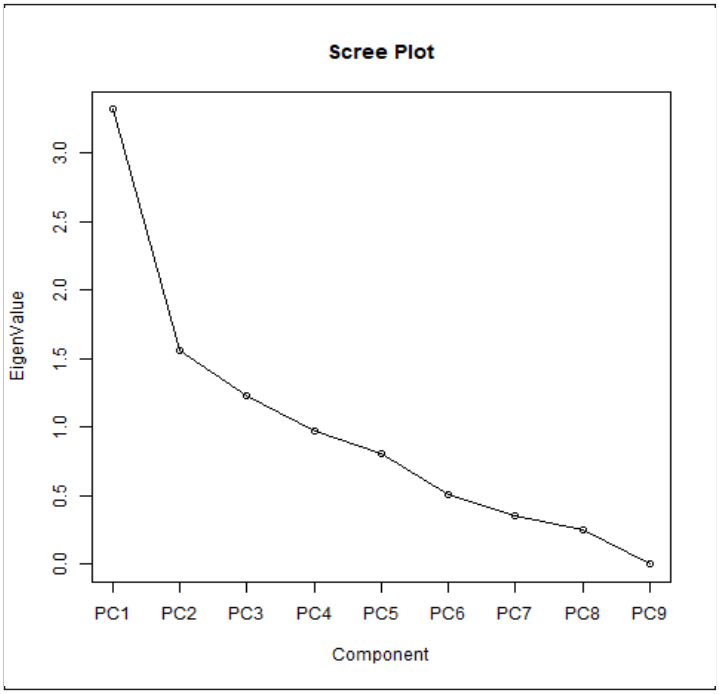

Fig. 1. Scree plot for Eigen values and principal components

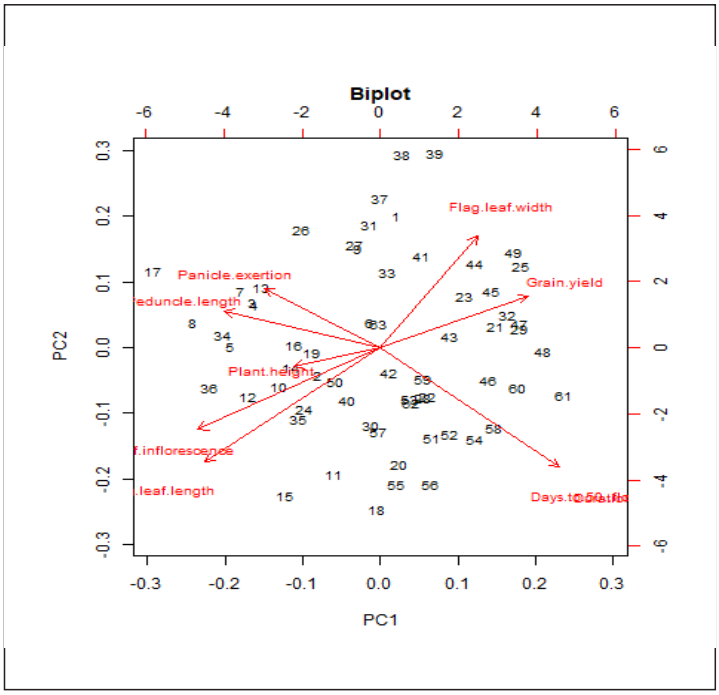

Fig. 2. Distribution of genotypes across first two components based on PCA

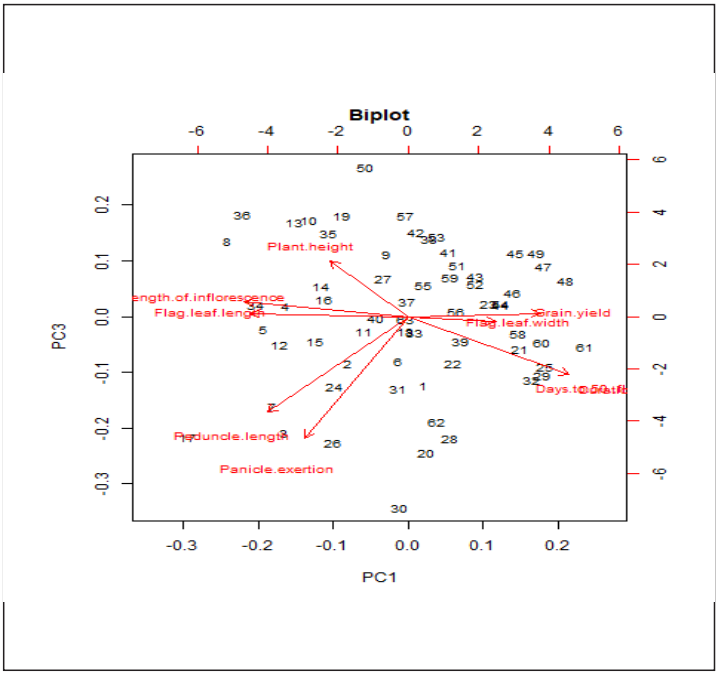

Fig. 3. Distribution of genotypes across first and third components based on PCA

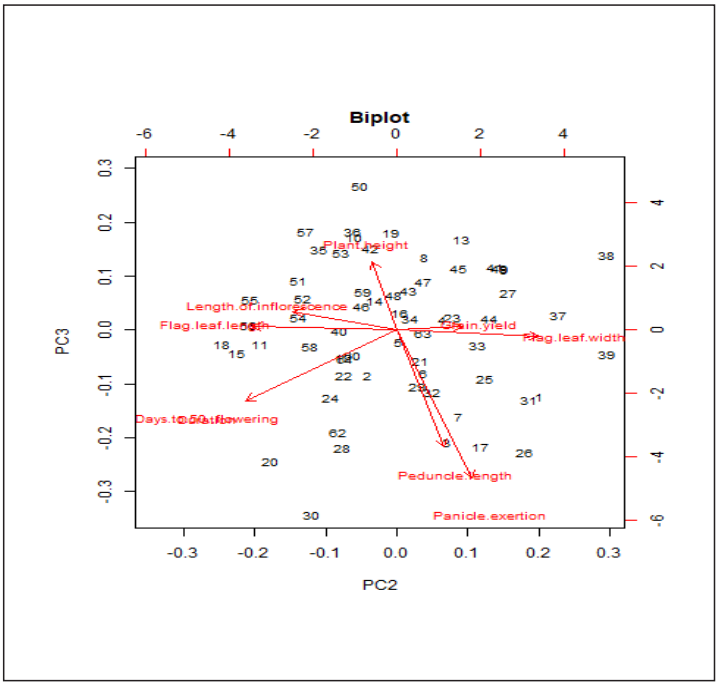

Fig. 4. Distribution of genotypes across second and third components based on PCA

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