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Research Article

Genetic diversity analysis of indigenous collection of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm

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Abstract
Thirty pearl millet landraces along with two male sterile lines were evaluated to estimate the genetic distances and to identify the desired cross combinations for the development of high yielding hybrids. Based on the Mahalanobis' D^2 analysis, the 32 germplasm lines were grouped under eight clusters. A high range of variation for trait contribution to the total diversity was observed and grain yield (19.14%) contributed the maximum, followed by dry fodder yield (11.69%). Cluster I was the largest with 25 genotypes and the remaining seven were solitary clusters. Clusters VI had a maximum mean performance for most of the traits, followed by clusters III and VIII. The cluster distances among these were also high, hence, the genotypes from these clusters can be used in hybrid development as well as in the development of new inbred lines.

Keywords: D^2 analysis, clusters, landraces and pearl millet

INTRODUCTION

Pearl millet is a dual-purpose millet with C_4 mechanism having a high capacity of photosynthetic efficiency and dry matter production. It originated in Africa and cultivated widely in arid and semi-arid regions with low rainfall conditions of Africa and India. The grains are nutritionally good with 73% carbohydrates, 11% proteins, 8.4% fat and rich source of minerals such as iron (6-7mg/100g) and zinc (3.4 mg/100g) (Malik, 2015). Globally, India occupies first place in the production of pearl millet with 10.36 million tons from 7.54 million hectares of area and 1373.86kg/ha of productivity (Ministry of Agriculture, 2019-2020). In India, Rajasthan is the leading state in the cultivation of pearl millet in 4287.17 thousand hectares of acreage with 4685.88 thousand tons production and with a unit production of 1093kg/ha (Ministry of Agriculture, 2019-2020).

The protogynous and highly cross-pollinated nature of pearl millet along with the availability of several male sterility sources facilitates the successful development of hybrid cultivars at the field level (Barathi and Reddy, 2022). Development of superior hybrids requires the selection of diverse parents. Genetic diversity studies help in the identification of genetically diverse genotypes for use in hybrid breeding programmes. Among different biometrical techniques available for divergence studies, Mahalanobis' D^2 statistic (1936) as described by Rao (1952) is a powerful tool for quantifying the degree of divergence among all possible pairs of genotypes in a population. As a result, the present study was conducted for classification and to understand the nature and magnitude of the genetic diversity of 32 genotypes.

MATERIALS AND METHODS

Thirty landraces of pearl millet explored from two different zones of India *i.e.*, Madhya Pradesh of A zone and Tamil Nadu, Maharashtra and Andhra Pradesh of B zone along with two male sterile lines developed by ICRISAT,

Hyderabad were evaluated in alpha lattice design as per Patterson and Williams (1976) with three replications during *Kharif*, 2019 at IIMR, Hyderabad. The spacings followed were 45 × 15 cm with two rows for each genotype. Data was recorded on 18 quantitative traits *viz*., days to 50% flowering, plant height, leaf length, leaf width, stem width, tillers/plant, productive tillers/plant, panicle length, panicle width, panicle weight, 1000-grain weight, grain number/panicle, grain number per unit structural panicle mass, fresh fodder yield, dry fodder yield, total biomass, harvest index and grain yield; and four qualitative traits *viz*., iron content, zinc content, protein and rancidity. Iron and zinc contents were estimated from the extract of flour digested with a diacid mixture (HNO3 and HClO4 in 9:4 ratio) by the use of Atomic Absorption Spectrophotometry (AAS), (Lindsay and Norvell, 1978) as described by Tandon (1999). Protein content was determined by estimating the nitrogen content (%) following the Micro Kjeldhal method (Tandon, 1999) and multiplying it with the conversion factor (6.25) as given by Sadasivam and Manickam (1996). Rancidity was measured in terms of alcoholic acidity as per the IS 12711:1989 method of determination of alcoholic acidity. For this 5 g of bajra flour was mixed with 50 ml of neutral ethyl alcohol (90%) and allowed to stand for 24 hours with occasional swirling. Titrated the 10 ml of the alcoholic extract with standard sodium hydroxide solution (0.05N) to a pink endpoint using a phenolphthalein indicator. Days to 50% flowering, panicle weight, fresh fodder yield, dry fodder yield, total biomass, harvest index, grain yield, iron content, zinc content, protein content and rancidity were recorded on a plot basis; while the remaining traits were recorded on five competitive plants selected randomly in each replication. The 32 genotypes for all the 22 studied characters were subjected to Mahalanobis (1936) D^2 statistics.

RESULTS AND DISCUSSION

The correlated mean data from the three replications for all 22 traits were transformed into uncorrelated means using the pivotal condensation method to estimate the D^2 values. The D^2 values were estimated for all possible 496 pairs of genotypes. Analysis of variance for dispersion from Wilk's test (Wilk, 1932) revealed the presence of significant variation among the genotypes with enough amount of variability. Mahalanobis D^2 analysis (Tocher's method) was employed in 32 germplasm lines (**Table 1**) and grouped into eight different clusters (**Table 2**), indicating the presence of divergence among the germplasm lines. Cluster I was the largest and comprised of 25 genotypes. The remaining seven clusters were solitary. Monogenotypic clusters were obtained may be due to the geographic barriers preventing gene flow or intense natural and human selection for diverse and adaptable gene complexes (Arunachalam and Ram, 1967). Regarding the male sterile lines ICMA 04999 and ICMA 97111 developed at ICRISAT were grouped under two different clusters. In case of land races also the lines originated and collected from different states grouped same clustering pattern and vice-versa (**Table 1 and Table 2**). One male sterile line (ICMA 04999) and 24 landraces (2381, 2342, 2396, 2327, 2311, 2349, 2306, 2386, 2364, 2352, 2331, 2365, 2370, 2332, 2394, 2325 and 2346) explored and collected from different states were grouped under one cluster and the genotypes collected from same state were distributed in various diverse clusters (Genotypes explored from Maharashtra were grouped in 3 different clusters *i.e*., in cluster I, IV and VIII). This indicates that geographical diversity may not necessarily represent genetic diversity (Murty and Arunachalam, 1966). Grouping of genotypes into different clusters by using Mahalanobis D^2 statistics was earlier done by Sumathi *et al*. (2016), Kamble *et al*. (2022) and Shashibhushan *et al*. (2022).

Average intra- and inter-cluster distances are presented in **Table 3**. Intra cluster distances varied from 0.00 (Clusters II, III, IV, V, VI & VII) to 312.73 (Cluster I). The D^2 distances for all the cluster pairs in the present study were more than 300, indicating there are high inter cluster distances among the cluster. The highest inter-cluster distance was exhibited between cluster VII and cluster VIII (3353.07), followed by cluster III and cluster VIII (2962.07) indicating wider genetic divergence between these clusters. The lowest inter cluster distance was observed between cluster IV and cluster V (342.74) (**Fig.1**).

The cluster mean performance of the eight clusters (**Table 4**) was assessed and revealed the existence of a wide range of variation among the clusters. The cluster with the highest mean performance for most of the traits was registered by cluster VI for the traits *viz*., plant height (235.00cm), tillers per plant (7.00), productive tillers per plant (5.00), test weight (13.22g), green fodder yield (41.11t/ha), dry fodder yield (16.67t/ha) and total biomass (17.33) followed by the cluster III for leaf width (4.10cm), stem width (0.95cm), panicle length (23.89cm), panicle weight (5.98), grain number per panicle (2124.06) and grain yield (4.16t/ha). Cluster VIII recorded maximum mean values for the traits leaf length (80.00cm), panicle width (3.00cm) and grain number per unit structural panicle mass (103.43). Cluster VII for the number of days to 50% flowering (47.00 days), harvest index (0.65) and rancidity (0.07); cluster IV for zinc (3.87g/100g) and iron (6.58) contents; and cluster I for protein (10.95%) recorded highest mean values for the respective traits. Based on mean values, clusters VI, III and VIII exhibited desirable performance, hence the genotypes grouped under these clusters can be used to develop new and improved inbreds further, these can be used to generate high yielding hybrids. Similar kind of results in pearl millet with a high degree of diversity was reported by Kumar *et al.* (2017), Sharma *et al*. (2020) and Kumar *et al*. (2022). For the selection of desired parental combinations from different clusters for hybridization, the characters with a maximum contribution towards genetic divergence should be given more importance. Among

Table 1. List of studied pearl millet germplasm

S. No.	Experimental material	Source of origin	
Male sterile lines			
1.	ICMA 04999A	ICRISAT, Hyderabad	
2.	ICMA 97111A	ICRISAT, Hyderabad	
Landraces			
S. No.	**Experimental material**	**Germplasm Collection Number**	**Source of origin**
1.	2306	ERP 10	Tamil Nadu
2.	2309	ERP 30	Tamil Nadu
3.	2310	ERP 34	Tamil Nadu
4.	2311	ERP 38	Tamil Nadu
5.	2318	ERP 107	Tamil Nadu
6.	2325	ERP 127	Tamil Nadu
7.	2327	ELSG 1	Maharashtra
8.	2328	ELSG 2	Maharashtra
9.	2329	ELSG 3	Maharashtra
10.	2330	ELSG 66	Maharashtra
11.	2331	ELSG 89	Maharashtra
12.	2332	ELSG 99	Maharashtra
13.	2333	SEJ 143	Andhra Pradesh
14.	2337	ESD 28	Maharashtra
15.	2342	EN 6	Tamil Nadu
16.	2346	EN 28	Tamil Nadu
17.	2348	EN 34	Tamil Nadu
18.	2349	EN 36	Tamil Nadu
19.	2352	EN 47	Tamil Nadu
20.	2364	SEA 6	Andhra Pradesh
21.	2365	SEA 17	Andhra Pradesh
22.	2368	SEA 45	Andhra Pradesh
23.	2370	SEA 61	Andhra Pradesh
24.	2381	ER 72	Tamil Nadu
25.	2382	ER 74	Tamil Nadu
26.	2386	ER 86	Tamil Nadu
27.	2387	ER 87	Tamil Nadu
28.	2394	EUK 34	Madhya Pradesh
29.	2395	EUK 42	Madhya Pradesh
30.	2396	EUK 43	Madhya Pradesh

Table 2. Clustering pattern of 32 pearl millet genotypes by Tocher's method

Cluster Number	Number of Genotypes	Name of genotypes
I	25	2381,2342,2396,2327,2311,2349,2306,2386,2364,2352,2331,2365,2370,2332,2394,2325,2346, ICMA 04999
II	1	2382
III	1	2348
IV	1	2329
V	1	2309
VI	1	2368
VII	1	ICMA 97111
VIII	1	2328

Table 3. Average intra and inter-cluster distances (D^2 values) among eight clusters of 32 pearl millet genotypes

Cluster Number	I	II	III	IV	V	VI	VII	VIII
I	**312.73**	620.19	659.57	673.26	801.26	628.95	1013.40	2038.69
II		**0.00**	1068.51	1121.19	750.87	757.35	1479.91	629.79
III			**0.00**	1872.18	1813.59	1283.64	634.57	2962.07
IV				**0.00**	342.74	1125.53	1976.68	2146.87
V					**0.00**	1015.85	1733.56	1491.84
VI						**0.00**	1516.96	2282.14
VII							**0.00**	3353.07
VIII								**0.00**

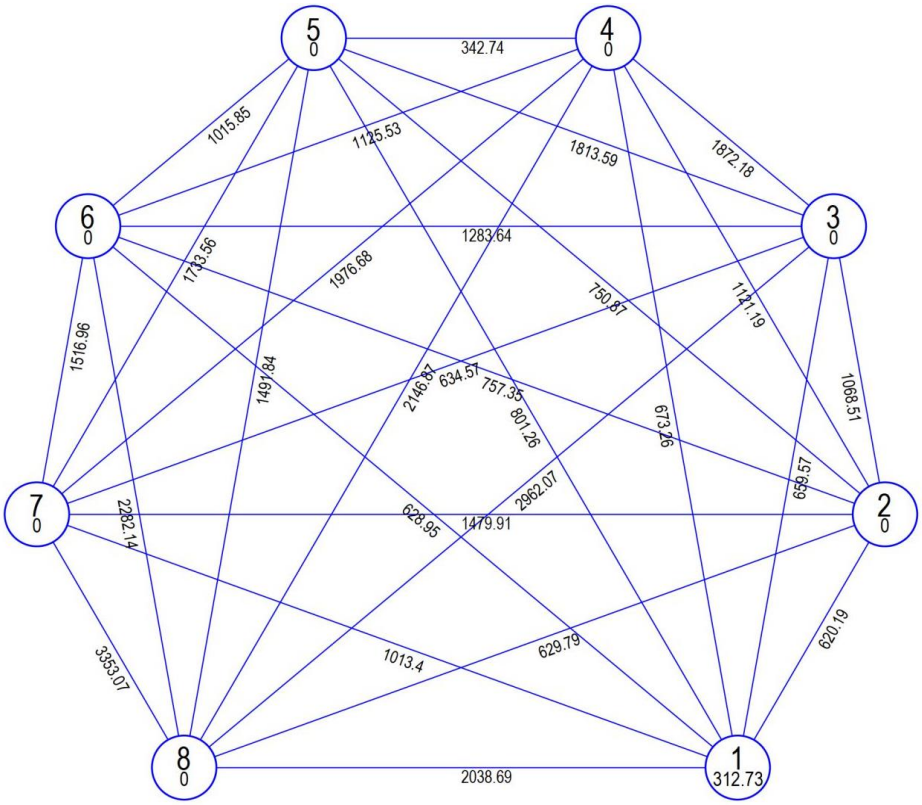

Fig. 1. Intra and inter-cluster distances of 32 pearl millet genotypes in eight clusters based on Tocher's method

the traits, grain yield (19.14%) contributed most of the diversity followed by dry fodder yield (11.69%), grain number per unit structural panicle mass (11.24%), rancidity (8.06%), leaf width (8.00%), harvest index (6.85%), leaf length (4.00%), panicle weight (3.83%), green fodder yield (3.63%), days to 50% flowering (3.00%) and total biomass(3.00%). While the remaining traits contributed with less than 3% contribution to the total diversity (**Table 5**). These results are in agreement with the reports of Swamynatham *et al*. (2020) and Athoni *et al*. (2016).

Diverse genotypes from different clusters along with the highest mean values for most of the characters can be selected as parents for the production of improved hybrids. All the cluster pairs recorded high cluster distances. The maximum genetic distance was observed between the solitary clusters VII and VIII, followed by clusters III and VIII, clusters VI and VIII and clusters IV and VIII. Among these clusters VI, III, VIII and VII recorded the highest mean values for majority of the traits. The two male sterile lines were falls under two clusters *i.e*., ICMA 04999

Table 4. Mean values of eight clusters estimated by Tocher's method in 32 pearl millet genotypes

Cluster Number	Days to 50% flowering	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Stem width (cm)	Tillers per plant	Productive tillers per plant	Panicle length (cm)	Panicle width (cm)	Panicle weight (t/ha)	Test weight (g)
I	54.95	172.10	60.59	3.50	0.87	3.48	2.27	21.61	2.46	3.72	8.96
II	63.33	220.00	66.28	4.07	0.89	3.44	2.33	22.78	2.48	0.94	7.66
III	56.00	190.00	63.85	**4.10**	**0.95**	3.89	2.78	**23.89**	2.57	**5.98**	9.47
IV	59.50	130.00	64.00	3.85	0.93	2.83	1.84	17.58	1.93	3.22	8.12
V	53.00	155.00	44.95	2.95	0.59	5.00	3.67	15.00	1.28	0.35	7.35
VI	60.00	**235.00**	60.00	3.20	0.80	**7.00**	**5.00**	20.50	1.80	0.67	**13.22**
VII	**47.00**	150.00	48.33	2.72	0.79	4.17	3.34	19.75	2.77	4.52	11.44
VIII	69.00	150.00	**80.00**	3.70	0.80	1.00	1.00	19.00	**3.00**	0.33	8.35

Cluster Number	Green fodder yield (t/ha)	Dry fodder yield (t/ha)	Grain number per panicle	Total biomass (t/ha)	Harvest index	Grain number per unit structural panicle mass	Grain yield (t/ha)	Zinc content (mg/100g)	Iron content (mg/100g)	Protein (%)	Rancidity
I	19.21	8.02	1346.48	11.74	0.19	12.61	1.88	3.21	5.83	**10.95**	0.16
II	24.82	12.60	1469.01	13.54	0.12	59.47	1.09	2.25	4.94	7.70	0.11
III	14.07	4.81	**2124.06**	10.79	0.38	13.84	**4.16**	2.53	5.14	8.90	0.26
IV	5.00	1.67	835.61	4.89	0.07	3.01	0.37	**3.87**	**6.58**	10.40	0.11
V	5.00	1.11	401.07	1.46	0.13	28.56	0.19	2.87	5.40	9.67	0.13
VI	**41.11**	**16.67**	567.32	**17.33**	0.01	12.61	0.17	2.87	5.60	9.73	0.13
VII	38.89	0.05	546.80	4.52	**0.65**	4.05	2.93	3.09	6.27	10.70	**0.07**
VIII	26.67	12.22	1137.72	12.56	0.02	**103.43**	0.21	2.70	5.49	8.60	0.16

Bold numbers indicate the maximum mean performance for the respective trait

Table 5. Contribution of different characters towards genetic divergence in 32 pearl millet genotypes

S. No.	Source	Per cent contribution	Number of times ranked first
1	Days to 50% flowering	3.00	15
2	Plant height	2.00	10
3	Leaf length	4.00	20
4	Leaf width	8.00	40
5	Stem width	2.00	10
6	Tillers per plant	0.81	4
7	Productive tillers per plant	1.81	9
8	Panicle length	2.00	10
9	Panicle width	1.61	8
10	Panicle weight	3.83	19
11	Test weight	0.20	1
12	Green fodder yield	3.63	18
13	Dry fodder yield	11.69	58
14	Grain number per panicle	2.82	14
15	Total biomass	3.00	15
16	Harvest index	6.85	34
17	Grain number per unit structural panicle mass	11.24	56
18	Grain yield	19.14	95
19	Zinc content	1.10	5
20	Iron content	2.00	10
21	Protein	1.21	6
22	Rancidity	8.06	40

under cluster I and ICMA 97111 under cluster VII. Hence, land races 2328 (cluster VIII), 2348 (cluster III) and 2368 (cluster VI); and male sterile line ICMA 97111 (cluster VII) can be used for the development of improved hybrids.

Further, the crossing of a male sterile inbred line with landraces may not maximize hybrid vigor but can increase yield potential in the resultant top cross progeny than open pollinated varieties and also provide a broad genetic base. Such resultant top cross progeny will have the agronomic characteristics of landraces preferred by farmers and provide more genetic diversity for diverse environmental conditions. These progenies may be explored for identifying superior segregants which can be utilized in different breeding programmes. In the current study, the landraces 2328, 2348 and 2368 from different clusters with a maximum *per se* performance for most of the traits may have the potential to generate superior transgressive segregants by crossing with the male sterile line ICMA 97111. Further, the identified landraces may also be used in population improvements for the development of improved inbred lines.

REFERENCES

Arunachalam, V. and Ram, J. 1967. Geographical diversity in relation to genetic divergence in cultivated sorghum. *Indian J. Genet. Plant Br.*, **27**: 369-380.

Athoni, B.K., Boodi, I., Pattanashetty, S.K. and Guggari, A.K. 2016. Genetic diversity for yield and its component traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Int. J. Sci Nat.*, **7**(4): 795-798.

Barathi, M.B. and Reddy, P.S. 2022. Genetic analysis and heterosis for quality traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Electronic Journal of Plant Breeding*, **13**(3):838 - 844. [Cross Ref]

Kamble, P.S., Barhate, K.K. and Andhale, G.R. 2022. Genetic diversity for zinc, iron and yeild contributing characters in pearl millet [*Pennisetum glaucum* (L.) R.Br.] genotypes. *Int. J. Multidiscip. Res. Dev.*, **5** (6): 1194-1199. [Cross Ref]

Kumar, M., Gupta, P. C., Sharma, N. and Narayan, S. 2017. Economic heterosis and genetic divergence in pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrids under rainfed conditions. *Res. Env. Life Sci.*, **10**(3): 243-245.

Kumar, R., Gupta, P.C., Sanadya, S.K. and Kumar, A. 2022. Genetic diversity analysis in pearl millet (*Pennisetum glaucum*) hybrids under arid region. *Biological Forum*, **14**(2): 446-449.

Lindsay, W. L. and Norvell, W. A. 1978. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Soc. Am. J.*, **41**: 421-428. [Cross Ref]

Mahalanobis, P.C. 1936. On the generalized distance in statistics. National Institute of Science of India. **2**: 49-55.

Malik, S. 2015. Pearl millet-nutritional value and medical uses. *Int. J.* Adv. Res. *Innov. Ideas Educ.*, **1**(3): 414-418. [Cross Ref]

Ministry of Agriculture, 2019-2020. Government of India. http:// *www.indiaagristat.com*

Murty, B.R. and Arunachalam, V. 1966. The nature of divergence in relation to breeding system in some crop plants. *Indian J. Genet. Plant Breed.*, **26**: 188-198.

Patterson, H. D and Williams, E. R. 1976. A new class of resolvable incomplete block designs. *Biometrika.* **63**(1): 83-90. [Cross Ref]

Rao, C.R. 1952. *Advanced Statistical Methods in Biometrical Research*. John Wiley and Sons, New York. 236-272.

Sadasivam, S. and Manickam, A. 1996. Biochemical Methods. New Age International Publishers, New Delhi. 12-34.

Sharma, V., Sharma, L.D., Jakhar, M.L., Govindaraj, M., Singh, R.V., Sharma, S.K., Get, S., Parashar, N. and Solanki, R.K. 2020. Genetic diversity in pearl millet inbred restorers for agro morphological and grain quality traits. *Electronic Journal of Plant Breeding,* **11**(1): 310-313. [Cross Ref]

Shashibhushan, D., Kumar, C.V.S. and Kondi, R.K.R. 2022. Genetic diversity analysis of pearl millet germplasm by cluster analysis. *Emer Life Sci Res.*, **8**(1): 70-74. [Cross Ref]

Sumathi, P., Lalithkannan, R. and Revathi, S. 2016. Genetic analysis and diversity studies in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Electronic Journal of Plant Breeding,* **7**(4): 1014-1019. [Cross Ref]

Swamynatham, S., Kumar, M.H., Reddy, D.M. and Latha, P. 2020. Genetic divergence studies in pearl millet [*Pennisetum glaucum* (L). R. Br]. *Electronic Journal of Plant Breeding,* **11**(1): 76-80. [Cross Ref]

Tandon, H.L.S. 1999. Methods of Analysis of Soils, Plants, Waters and Fertilizers. Fertilizer Development and Consultation Organization, New Delhi, India. 86-96

Wilk, S.S. 1932. Criterion of generalization in the analysis of variance. *Biometrika*. **24**: 471-484. [Cross Ref]