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Assessment of Genetic Diversity of Inbred Lines in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract

Using the Mahalanobis D^2 Statistics, 70 pearl millet inbred lines were examined to determine the extent of genetic diversity for 21 traits. According to Tocher's technique, the total genetic resource set was divided into twelve unique, non-overlapping clusters indicating the presence of ample amount of genetic diversity. Cluster I was the largest one comprising of 33 genotypes followed by cluster IX with 12 genotypes, cluster V with ten genotypes and cluster VI contains seven genotypes. Whereas, clusters II, III, IV, VII, VIII, X, XI and XII were monotypic indicating high degree of heterogeneity among the lines. Clusters VI and XII had the greatest inter-cluster distance between them showing that these genetic resources could be used in inter-varietal hybridization. The trait with highest contribution to overall divergence was 1000 grain weight followed by days to 50% flowering, number of productive tillers plant^{-1}, panicle weight, green fodder yield plant^{-1}, plant height, grain yield plant^{-1}, spike girth, threshing percentage and harvest index demonstrating the potential of the material for further improvement through those traits.

Key words : *Cluster, D^2 statistic, Tocher's technique, pearl millet.*

Introduction

Pearl millet (*Pennisetum glauccum* (L.) R. Br.) is a an annual, C_4 cereal crop which belongs to the family Gramineae native to Africa, from which it spread to other parts of the world with varying agro-climatic conditions, ranging from the hot region of Africa (the tropical zone) to the hot region of temperate Asia zones. In World serious and widespread human health problems have been emerged due to dietary deficiency of mineral micronutrients. Pearl millet is commonly known as a poor man's food and grown as major staple food crop in developing and under-developed countries of Africa and Asia, which these regions are greatly affected by malnutrition. The genetic potential of pearl millet needs to be further explored in order to increase the micronutrients like iron, zinc, copper, and manganese in the grain. Pearl millet evolved from several independent domestication events and a wide range of stressful environmental conditions in which it was traditionally cultivated, owing to its highly out-crossing breeding behaviour. Development of high-yielding hybrids is an important goal for pearl millet worldwide.

The availability, evaluation, and utilisation of genotype distance and genetic variety contribute to the formation of heterotic groups that facilitate the choice of parents for further crossing. Furthermore, in order to develop knowledge pertaining to germplasm conservation and breeding programmes, it is crucial to evaluate the degree of diversity for the economically significant traits and identify promising germplasm in untapped genetic resources of pearl millet. In addition to it, evaluation, characterization and classification of genotypes based on estimates of genetic divergence can assist in identifying various parental lines that can be employed in a hybridization procedure to create potential hybrids or varieties. Estimating the genetic distance between parents is critical for parent selection. High heterosis will result from greater genetic distance between parents. Different multivariate analyses are used to calculate genetic distance. Among the various multivariate analyses, Mahalanobis generalised distance estimated by D^2 statistics (1) serves as a potential and unique tool for evaluating phenotypic diversity, identifying genetically distant clusters of genotypes, and selecting important traits contributing to total variation in the germplasm.

Materials and Methods

The experiment was carried out during *Rabi,* 2021 at Agricultural Research Station, Perumallapalle, Tirupati located at 13°N latitude and 79°E longitude from an altitude of 182.9 m above mean sea level, and situated in southern agro-climatic zone of Andhra Pradesh. The experimental material utilized for the present study comprised of 70 pearl millet inbred lines (S_6 generation) developed at Agricultural Research Station,

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Table-1 : Distribution of 70 pearl millet inbred lines into 12 clusters based on Tocher's method.

S. No.	Cluster number	Number of Genotypes	Genotypes
1.	I	33	PPBi-4,7,8,13,17,19,22,26,27,28,30,32,33,35,36,37,41,42,45,48,49,51,54,56,58,59,60,61,63,64,65,66,69
2.	II	1	PPBi-67
3.	III	1	PPBi-1
4.	IV	1	PPBi-52
5.	V	10	PPBi-2,10,11,29,31,34,38,39,55,62
6.	VI	7	PPBi-6,9,14,16,18,24,53
7.	VII	1	PPBi-5
8.	VIII	1	PPBi-68
9.	IX	12	PPBi-3,12,15,20,21,23,25,40,44,46,57,70
10.	X	1	PPBi-43
11.	XI	1	PPBi-47
12.	XII	1	PPBi-50

Table-2 : Average Inter (above diagonal) and Intra cluster (diagonal) D^2 and D values (in parenthesis) for 12 clusters in 70 inbred lines of pearl millet.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	47.73 (6.91)	61.42 (7.84)	73.34 (8.56)	72.72 (8.53)	66.57 (8.16)	106.58 (10.32)	62.69 (7.92)	79.60 (8.92)	79.27 (8.90)	97.61 (9.88)	100.94 (10.05)	190.99 (13.82)
II		0.00 (0.00)	101.39 (10.07)	118.20 (10.87)	75.37 (8.68)	154.20 (12.42)	67.04 (8.19)	37.90 (6.16)	60.48 (7.78)	105.99 (10.30)	161.43 (12.71)	161.27 (12.70)
III			0.00 (0.00)	17.55 (4.19)	72.53 (8.52)	41.27 (6.42)	105.21 (10.26)	119.29 (10.92)	125.09 (11.18)	65.05 (8.07)	143.57 (11.98)	296.42 (17.22)
IV				0.00 (0.00)	72.28 (8.50)	43.88 (6.62)	123.13 (11.10)	139.98 (11.83)	131.49 (11.47)	64.43 (8.03)	147.66 (12.15)	289.85 (17.02)
V					54.96 (7.41)	104.45 (10.22)	76.70 (8.76)	84.61 (9.20)	100.91 (10.05)	103.78 (10.19)	154.78 (12.44)	247.42 (15.73)
VI						65.36 (8.08)	138.75 (11.78)	172.28 (13.13)	170.20 (13.05)	112.04 (10.58)	146.29 (12.10)	337.24 (18.36)
VII							0.00 (0.00)	60.14 (7.75)	74.54 (8.63)	173.02 (13.15)	99.46 (9.97)	200.1 (14.15)
VIII								0.00 (0.00)	80.74 (8.99)	157.95 (12.57)	194.89 (13.96)	272.26 (16.50)
IX									88.81 (9.42)	142.53 (11.94)	163.01 (12.77)	178.44 (13.36)
X										0.00 (0.00)	197.90 (14.07)	304.13 (17.44)
XI											0.00 (0.00)	196.70 (14.02)
XII												0.00 (0.00)

Perumallapalle, Tirupati. 21 morphological traits on five randomly tagged plants from each entry in each replication were recorded as per National test guidelines for Distinctiveness, Uniformity and Stability (DUS) testing given by Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA, 2001). The traits include days to 50% flowering, days to maturity, SPAD chlorophyll meter reading, specific leaf area (cm^2 gm^{-1}), leaf sheath length (cm), leaf blade length (cm), leaf blade width (cm), number of nodes $plant^{-1}$, spike length (cm), spike girth (cm), number of productive tillers $plant^{-1}$, plant height (cm), 1000 grain weight (g), panicle weight (g), green fodder yield $plant^{-1}$ (g), dry fodder yield $plant^{-1}$ (g), threshing (%), harvest index (%) and grain yield $plant^{-1}$ (g). The data was analyzed through software – INDOSTAT and genetic divergence analysis was done using Mahalonobis D^2 (1936) statistics. The genotypes were grouped into different clusters by Tocher's method described by (1).

Results and Discussion

All the 70 genotypes of pearl millet were grouped into 12 distinct and non-overlapping clusters using Tocher's method (1) such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The distribution of genotypes into various clusters was presented in Table-1.

Out of 12 clusters, cluster I was the largest one comprising of 33 genotypes followed by cluster IX with 12 genotypes, cluster V with ten genotypes and cluster VI contains seven genotypes. Whereas, clusters II, III, IV, VII, VIII, X, XI and XII had only one genotype indicating high degree of heterogeneity among the genotypes. The

Table-3 : Cluster means with respect to yield, yield attributes and overall character wise score in 70 inbred lines of pearl millet.

Clusters	Characters											
	DF	DM	SCMR 45	SCMR 65	SLA 45	SLA 65	LSL	LBL	LBW	NN	SL	SG
I	56.89 (5)	88.88 (5)	37.41(4)	48.83 (9)	143.81 (6)	158.46 (6)	13.59 (5)	45.78 (5)	3.09 (5)	5.65 (4)	19.90 (8)	2.54 (5)
II	58.50 (7)	90.00 (6)	35.25 (8)	35.25 (12)	106.63 (2)	148.41 (4)	10.79 (11)	32.90 (12)	2.55 (12)	4.70 (10)	15.00 (11)	2.39 (9)
III	70.00 (11)	101.00 (11)	30.25 (12)	51.55 (4)	177.63 (12)	173.34 (10)	12.61 (10)	47.05 (4)	2.95 (7)	4.80 (9)	27.90 (1)	2.88 (2)
IV	68.00 (10)	99.50 (10)	32.35 (11)	47.40 (10)	174.54 (11)	203.11 (12)	14.50 (3)	50.00 (3)	3.65 (1)	5.40 (7)	27.30 (2)	2.87 (3)
V	60.70 (8)	92.85 (8)	32.91 (9)	49.65 (8)	153.23 (8)	160.75 (7)	13.56 (6)	44.47 (6)	2.84 (9)	5.44 (6)	20.54 (7)	2.06 (12)
VI	72.57 (12)	104.21 (12)	32.56 (10)	50.40 (7)	157.88 (9)	170.77 (9)	15.05 (1)	52.26 (1)	3.54 (3)	5.51 (5)	25.71 (4)	2.89 (1)
VII	52.50 (1)	85.50 (1)	35.55 (7)	56.60 (1)	162.15 (10)	200.00 (11)	14.97 (2)	41.40 (9)	3.13 (4)	6.30 (2)	22.50 (5)	2.41 (7)
VIII	52.50 (1)	85.50 (1)	35.95 (5)	51.05 (5)	116.92 (3)	155.74 (5)	10.40 (12)	33.60 (11)	2.58 (11)	4.30 (12)	14.80 (12)	2.37 (10)
IX	54.83 (4)	86.79 (3)	35.95 (5)	50.49 (6)	148.29 (7)	163.68 (8)	13.26 (7)	43.42 (7)	2.95 (7)	5.32 (8)	18.43(10)	2.40 (8)
X	63.50 (9)	94.50 (9)	38.15 (2)	46.05 (11)	105.71 (1)	115.16 (1)	13.00 (8)	39.55 (10)	3.59 (2)	4.60 (11)	19.20 (9)	2.32 (11)
XI	58.50 (6)	91.50 (7)	53.60 (1)	55.60 (2)	129.10 (5)	128.13 (3)	14.45 (4)	42.55 (8)	2.71 (10)	7.30 (1)	22.20 (6)	2.87 (3)
XII	54.00 (3)	87.50 (4)	37.90 (3)	52.70 (3)	121.16 (4)	128.03 (2)	13.00 (8)	51.15 (2)	2.98 (6)	6.20 (3)	26.80 (3)	2.51 (6)

Table-3 : Contd…..

Clusters	Characters									Productivity Traits	
	NPT	PH	1000GW	PW	GFY	DFY	TH	HI	GYP	Total score	Final Rank
I	1.51 (6)	144.41 (7)	10.18 (9)	37.19 (9)	79.68 (8)	23.91 (8)	53.12 (4)	32.35 (5)	19.44 (5)	128	4
II	2.15(3)	99.70 (11)	11.06 (5)	35.00 (10)	56.40 (10)	16.92 (10)	46.50 (7)	31.46 (6)	16.10 (9)	175	11
III	1.20 (12)	152.90 (5)	10.87 (6)	51.60 (3)	116.60 (4)	34.98 (4)	33.43 (11)	19.92 (11)	17.25 (8)	157	8
IV	1.40 (9)	160.50 (3)	8.84 (11)	42.60 (7)	122.20 (3)	36.66 (3)	49.78 (6)	26.74 (9)	21.20 (4)	138	7
V	1.49 (8)	130.39 (10)	8.87 (10)	24.21 (11)	53.07 (11)	15.92 (11)	46.03 (9)	27.75 (8)	11.10 (11)	183	12
VI	1.26 (11)	172.00 (2)	11.10 (4)	47.41 (4)	108.23 (5)	32.47 (5)	40.90 (10)	24.20 (10)	19.18 (6)	131	6
VII	1.70 (5)	144.10 (8)	14.46 (1)	40.10 (8)	72.70 (9)	21.81 (9)	46.07 (8)	29.78 (7)	18.22 (7)	122	3
VIII	1.30 (10)	95.10 (12)	11.41 (3)	16.60 (12)	24.60 (12)	7.38 (12)	66.38 (1)	45.82 (1)	11.28 (10)	161	10
IX	2.36 (2)	134.04 (9)	10.73 (7)	44.56 (5)	83.23 (7)	24.97 (7)	52.86 (5)	33.81 (4)	22.53 (3)	129	5
X	1.90 (4)	148.10 (6)	5.58(12)	43.90 (6)	106.60 (6)	31.98 (6)	24.82 (12)	14.29 (12)	10.80 (12)	160	9
XI	1.50 (7)	192.50 (1)	12.98 (2)	54.10 (2)	129.00 (2)	38.70 (2)	58.16 (2)	33.89 (3)	31.45 (2)	79	2
XII	4.20 (1)	156.90 (4)	10.24(8)	101.80 (1)	165.30 (1)	49.59 (1)	57.40 (3)	38.61 (2)	58.42 (1)	68	1

Note : Numbers in the parenthesis indicates the ranks based on cluster mean. Total score is the summation of rank numbers for all characters, based on which final rank indicated. Bold numbers indicated highest mean values for each character.

DF	Days to 50% flowering	LBL	Leaf blade length (cm)	1000 GW	1000 grain weight (g)
DM	Days to maturity	LBW	Leaf blade width (cm)	PW	Panicle weight (g)
SCMR 45	SPAD Chlorophyll Meter Reading at 45 DAS	NN	No of nodes plant^{-1}	GFY	Green fodder yield plant -1(g)
SCMR 65	SPAD Chlorophyll Meter Reading at 65 DAS	SL	Spike length (cm)	DFY	Dry fodder yield plant -1(g)
SLA 45	Specific leaf area at 45 DAS (cm^2 g^{-1})	SG	Spike girth (cm)	TH	Threshing (%)
SLA 65	Specific leaf area at 65 DAS (cm^2 g^{-1})	NPT	Number of productive tillers plant^{-1}	HI	Harvest index (%)
LSL	Leaf sheath length (cm)	PH	Plant height (cm)	GYP	Grain yield plant -1(g)

Table-4 : Relative contribution of various characters towards genetic divergence in 70 inbred lines of pearl millet.

S. No.	Characters	Number of times ranked first	Contribution (%)
1.	Days to 50% flowering	430	17.81%
2.	Days to maturity	6	0.25%
3.	SPAD chlorophyll meter reading at 45 DAS	8	0.33%
4.	SPAD chlorophyll meter reading at 65 DAS	4	0.17%
5.	Specific leaf area at 45 DAS (cm^2 g^{-1})	29	1.20%
6.	Specific leaf area at 65 DAS (cm^2 g^{-1})	9	0.37%
7.	Leaf sheath length (cm)	10	0.41%
8.	Leaf blade length (cm)	16	0.66%
9.	Leaf blade width (cm)	40	1.66%
10.	No of nodes plant^{-1}	35	1.45%
11.	Spike length (cm)	27	1.12%
12.	Spike girth (cm)	118	4.89%
13.	Number of productive tillers plant^{-1}	308	12.75%
14.	Plant height (cm)	207	8.57%
15.	1000 grain weight (g)	523	21.66%
16.	Panicle weight (g)	238	9.86%
17.	Green fodder yield plant^{-1}(g)	123	5.09%
18.	Dry fodder yield plant^{-1} (g)	0	0.00%
19.	Threshing (%)	101	4.18%
20.	Harvest index (%)	64	2.65%
21.	Grain yield plant^{-1} (g)	119	4.93%

genotypes of the solitary clusters may be unique and very useful for breeding purpose. In the present study the genotypes identified in solitary clusters are cluster II, cluster III, cluster IV, cluster VII, cluster VIII, cluster X, cluster XI and cluster XII. Study of clustering pattern with respect to pedigree of genotypes revealed that genotypes with common parentage fell in either same cluster or in different clusters with low inter cluster distances which indicated presence of parallelism between genetic divergence and pedigree.

The average intra and inter-cluster D^2 and D values among 12 clusters were given in Table-2. The average intra cluster distance ranged from 0.00 to 88.81. Maximum intra cluster distance was observed in cluster IX (88.81), followed by cluster VI (65.36) and cluster V (54.96) indicating that some divergence still existed among the genotypes of same cluster, which could be made use in the yield improvement through recombination breeding. Such intra cluster genetic diversity among the inbred lines within the same cluster could be due to heterogeneity, degree of general combining ability (GCA) and their pedigree. Therefore, selection within these clusters could be based on highest mean performance for desirable traits. While the clusters II, III, IV, VII, VIII, X, XI and XII recorded zero values as they included only single genotype in each of them.

While, the inter-cluster D^2 values ranged from 17.55 to 337.24, the maximum inter cluster distance (337.24) was observed between cluster VI and XII followed by cluster X and XII (304.13), cluster III and XII (296.42), cluster IV and XII (289.85) and cluster VIII and XII (272.26). Whereas, minimum inter cluster distance of 17.55 was recorded between cluster III and IV, followed by cluster II and VIII (37.9) and between cluster III and VI (41.27) indicating that genotypes of these clusters were genetically close and had maximum number of gene complexes.

Inter-cluster distances were higher than intra-cluster distance indicating the presence of wider genetic diversity between the clusters rather than within the clusters. Based on inter cluster distances the cluster combinations *viz.,* VI×XII, X×XII, III×XII, IV×XII and VIII× XII were found to be more divergent with high mean performance.

The cluster means for 21 characters are presented in Table-3. Considerable variation among the cluster means for all the characters indicated the divergent nature of clusters formed.

Among all the characters studied, maximum contribution towards genetic divergence is recorded by 1000 grain weight (21.66%) by taking first rank in 523 times out of 2415 combinations, followed by days to 50% flowering (17.81%), number of productive tillers plant^{-1} (12.75%), panicle weight (9.86%), plant height (8.57%), green fodder yield plant^{-1} (5.09%), grain yield plant^{-1} (4.93%), spike girth (4.89%), threshing percentage (4.18%) and harvest index (2.65%). The relative contribution of the characters towards divergence was presented in the table-5.

In the present study, 1000 grain weight, days to 50% flowering, number of productive tillers plant^{-1}, panicle weight and plant height were found the best discriminatory characters for better selection of diverse genotypes and contributing maximum towards divergence in inbred lines of pearl millet, so these traits could be exploited maximum in order to get superior hybrids with higher yield.

Similar results were recorded by the earlier researchers for 1000 grain weight (2,3,4); for days to 50% flowering (3,4); for number of productive tillers plant^{-1} (2,5,6); for panicle weight (7,8); for plant height (2,5); and for spike girth (3,7,9,10).

Therefore, hybridization between genotypes from widely divergent clusters which showed higher inter cluster distances and high mean values for the respective traits to be improved would be beneficial in developing promising hybrids.

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