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Genetic Diversity Analysis among Inbred Lines of Pearl millet [*Pennisetum glaucum* (L.) R. Br.] Based on Grain Yield and Yield Component Characters

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A B S T R A C T

K e y w o r d s Genetic divergence, Pearl millet, Maintainer (B-) lines, Restorer (R-) lines, Principal component analysis, Hierarchical cluster analysis.	An experiment was conducted to assess genetic divergence among 60 inbred lines included 27 maintainer (B-) and 33 restorer (R-) lines of pearl millet based on quantitative data of grain yield and its ten component traits using hierarchical cluster and principal component analysis (PCA). The PCA identified four principal components (PCs) with Eigen value greater than 1.00 and accounted for 70.97 per cent of total variation. Most important traits in PC1 are days to 50 per cent flowering, plant height, ear length, ear diameter, grain yield per plant, fresh stover yield per plant, dry matter yield per plant and grain harvest index and captured 26.85 per cent of total variation. PC2 was represented by ear diameter and dry matter yield per plant and contributed 18.06% of total variation. Two characters, grain yield per plant and grain harvest index contributed positively on all the first four PCs. Cluster analysis grouped the inbred lines into eight clusters and the characters, plant height, 1000 grain weight, dry matter yield per plant and productive tillers per plant contributed maximum towards genetic divergence. The grouping patterns of parental lines in PCA and cluster analysis were almost in agreement with each other with minor deviations. The study noticed maximum inter cluster distance between lines of cluster I and II with cluster VII, indicating that lines included in these clusters may have high heterotic response and produce better seggregants when used in Pearl millet hybridization programme.
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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] Is one most important cultivated cereals in the world, ranking after rice, wheat, maize, barely and sorghum in terms of area planted to these crops (Khairwal *et al.,* 2007). It is grown on about 30 m ha in more than 30 countries with the majority of this area in Asia (>10 m ha), Africa (about 18 m ha), and Americas (>2 m ha) (Gupta *et al.,* 2015). It exhibits tremendous amount of genetic diversity

because its wide distribution across the world, well adoption under harsh environmental conditions and cross pollinated mechanism with protogynous flowering (Satyavathi *et al.,* 2013 and Singh *et al.,* 2013). Genetic diversity is the basic requirement for any crop improvement programme. Several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives, *viz.,* Mahalanobis D^2 analysis,

Principal component analysis and hierarchical cluster analysis based on Ward's minimum variance method. Evaluation, characterization and classification of genotypes based on estimates of genetic diversity will help to identify diverse parental lines which can be used in hybrid breeding to develop potential hybrids or varieties. Therefore, the present investigation was undertaken to study the nature and magnitude of genetic divergence for grain yield and its component characters among the inbred lines to provide a basis for selection of parents for hybridization in Pearl millet hybridization programme.

Materials and Methods

Experimental material

The material used in the experiment comprised of 60 inbred lines selected on the basis of genetic distance obtained from 88 SSR polymorphic markers of 343 inbred lines of Pearl millet. The selected parental lines were procured from Pearl millet Breeding unit, ICRISAT, Patancheru, Telangana, India is given in table 1.

Evaluation of parental lines

The parental lines were evaluated during *rabi*, 2015 at Agricultural college farm, Naira, ANGRAU, Andhra Pradesh in a Randomized block design with two replications. The planting was done on ridges which were 45 cm apart. Each entry was planted in two rows of 2 m length with a spacing 15 cm between plant to plant, at a uniform depth. Standard agronomic management practices were followed throughout the entire growing period as required. The data on 11 quantitative traits were recorded, out of 11 traits, observations on days to 50 per cent flowering, productive tillers per plant, head yield per plant (g plant^{-1}), grain yield per plant (g plant^{-1}), fresh stover yield per plant (g plant^{-1}), dry matter yield per

plant (g plant^{-1}), 1000-grain weight (g) and grain harvest index (%) were recorded on plot basis. The data on remaining quantitative traits *viz.*, plant height, ear length and ear diameter were recorded on five randomly selected representative plants in a plot. Average values of these five plants were computed and mean values were used for statistical analysis.

Statistical analysis

The data were subjected to statistical analysis using software Windostat Version 9.2. Principal component analysis (PCA) was performed for dimensional reduction and to know the importance of different traits in explaining multivariate polymorphism. Hierarchical cluster analysis was done following the minimum variance method of Ward (1963) based on squared Euclidean distances.

Results and Discussion

The analysis of variance for 60 inbred lines of Pearl millet for eleven quantitative traits is given in table 2. The results showed significant differences between the inbred lines for the characters studied (p≤0.01), indicating a considerable amount of genetic variability and hence divergence analysis was carried out.

In principal component analysis, the number of variables is reduced to linear functions called canonical vectors which accounts for most of the variation produced by the characters under study. The Eigen values, per cent variance, per cent cumulative variance and factor loading of different characters studied are presented in table 3. The study identified four Principal Components (PCs) with Eigen value greater than 1.00 which accounted for 70.97 per cent of the total variation for discriminating the inbred lines of

Pearl millet based on grain yield and its ten component traits. The percentages of total variability accounted by each of the first four principal components were 26.85, 18.06, 15.61 and 10.45 per cent, respectively. The traits, grain yield per plant and grain harvest index had positive contribution towards all the four PCs. The highest loading displayable variables on four PCs were grain yield per plant, grain harvest index, 1000 grain weight and productive tillers per plant. The PC1 classified inbred lines based on days to 50 per cent flowering, plant height, ear length, grain yield per plant, fresh stover yield per plant and grain harvest index. PC2 separated the material based on ear diameter and dry matter yield per plant. On the basis of head yield per plant and 1000 grain weight, PC3 separated the lines and PC4 separated the parental material based on productive tillers per plant. The results indicated the role of traits (specific to each PC) which contributed more towards divergence in discriminating inbred lines of pearl millet.

The first two principal components PC1 and PC2 with most of the desirable traits namely, days to 50 per cent flowering, plant height, ear length, ear diameter, grain yield per plant, fresh stover yield per plant, dry matter yield per plant and grain harvest index accounting for 44.92 per cent of total variation were considered to study grouping pattern of material under study.

The three dimensional scatter plot of PC1 and PC2 axes is represented in figure 1. The inbred lines represented by 5, 9, 16, 17, 24, 25, 30, 34, 36, 39, 42, 45, 46, 56, 58 and 60 were accumulated on positive side of PC1 axis which accounted for high grain and stover yield characters.

The inbred line, 38 is represented on positive side of PC2 axis where the line has thicker ears and high stover yield character. The remaining lines were represented on positive

side of both PC1 and PC2 axes indicating that these parental lines are characterised by high grain and stover yield with related traits (earliness, longer and thicker ears, high harvest index).

The hierarchical clustering pattern of parental lines of Pearl millet based on Mahalanobis squared Euclidean distance matrix obtained from quantitative data using Ward method is depicted in figure 2.

The experimental material was assigned into eight clusters at an average D^2 value of 398.08, revealing the existence of variability among parental lines for the traits under study.

Cluster V was the largest with 18 lines followed by cluster II, cluster III, cluster VI, cluster I and cluster IV with 13, 12, 7, 5 and 4 lines, respectively. While, remaining clusters VII and VIII were solitary demonstrating the impact of selection pressure in increasing the genetic diversity. The cluster I comprised of four R-lines and single B-line, while cluster II had ten R-lines and three B-lines, cluster III had eight B-lines and four R-lines, cluster V had each of nine B- and R-lines, cluster VI had four R-lines and two B-lines. These results suggested clear differentiation of R-lines and B-lines with minor exceptions. The preliminary evaluation of breeding material to identify potent parents for hybridization programme based on phenotypic data is fast, simple and can be considered as a general approach for assessing genetic diversity among genetically diverse lines. Likewise, grouping of genetic material based on quantitative data in pearl millet was reported by Shanmuganathan *et al.,* (2006), Vidhyadhar and Devi (2007), Govindaraj *et al.,* (2011), Drabo *et al.,* (2013), Sathya *et al.,* (2013), Upadhyaya *et al.,* (2013), Sankar *et al.,* (2014), Chaudhary *et al.,* (2015), and Kumar *et al.,* (2015).

Table.1 List of 60 (27 B-lines and 33 R-lines) parental lines of pearl millet with pedigree details

S. No.	Parental lines	Pedigree
1	R1	(AIMP 92901 S1-480-1-1-1-2-B-2 x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-11-1-1-B
2	R2	[(IPC 1268×ICMV 91059 S1-58-2-2-2-1)×AIMP 92901 S1-296-2-1-1-1-B-B]-2-2-3-2-3
3	R3	((ICMV IS 94206 S1-15-2)×{(SRC II C3 S1-19-3-2 x HHVBC)-5-3-1})-B-13-4-2-1-1-1-1-3-2
4	R4	MDMRRC S1-329-1
5	R5	RCB-2 S1-33-1-3-3-2-3-B-B-B-B-B-B-B
6	R6	ICTP 8202 S1-25-1
7	R7	JBV 3 S1-257-1-4-1-B
8	R8	{[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-28-3-2-2-2}×{GB 8735-S1-15-3-1-1-3-4-2-2-2-1}-B-30-2-2-1-B-B-B-1
9	R9	[(IPC 337×SDMV 90031-S1-84-1-1-1-1)×RCB-2-S1-144-2-2-2-1-1-1]-1-1-3-1
10	R10	ICMS 7704-S1-127-5-1-5-1-1-3-3-2-B-B
11	R11	[(((ICMV-IS 94206-15)×B-Lines)-B-6) × (MRC S1-156-2-1-B)]-B-13-1-3-3-2-B
12	R12	MRC HS-219-2-1-2-B-B-B-B
13	R13	[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-B
14	R14	MRC HS-130-6-1-1-B-B-B-B-B-B
15	R15	[(((ICMV-IS 94206-15)×B-Lines)-B-6) × (MRC S1-156-2-1-B)]-B-38-3-1-B-7-B
16	R16	LaGrap C2-S1-81-1-2-1-4-2
17	R17	(RCB-2-S1-43-3-4 × MRC)-B-2-1-1-B-1-B
18	R18	(EERC-HS-32)-B-8-1-1-B
19	R19	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-4-1-2
20	R20	MDMRRC S1-1-278-2-5-3-B
21	R21	{(((MC 94 S1-34-1-B × HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-28-3-1-2-2}×{MRC HS 225-3-5-2-B-B-B-B}-B-4-2-2-1-B-B
22	R22	ICMV 91059 S1-4-2-3-2-1-1-4-B-1-5-B-B
23	R23	(MC 94 C2-S1-3-2-2-2-1-3-B-B x AIMP 92901 S1-488-2-1-1-4-B-B)-B-30-3-4-2
24	R24	MC 94 C2-S1-47-1-1-3-B-1-B-B
25	R25	[MC 94 C2-S1-3-1-3-1-4-B-B x LaGrap C2-S1-97]-B-11-1-1-2-B
26	R26	SDMV 95045 S1-7-2-4-2-3-2-1-B-B-B-B-8-1-1
27	R27	Jakhrana × SRC II S2-215-3-2-1-B-3
28	R28	ICMS 8511 S1-17-2-1-1-4-1-B-3-2-2-B
29	R29	[(IPC 1617×SDMV 90031-S1-84-1-1-1-1)×GB 8735-S1-25-4-4-1-1-3-1-1]-1-1-3-2-1-B-B
30	R30	MDMRRC S1-1-276-1-2-1-1
31	B31	[(ICMB 95111 x 9035/S92-B-3)-17-1-B-B-B-B

32	B32	[EEDBC S1-452-3-1-2-3-B-B-B-1 x B-bulk (3981-3989/S06 G1)]-4-2-4-B
33	B33	[ICMB 97444 x (843B x 405B)-4]-1-2-B-B-B
34	B34	[IPC 1598 x (843B x DSA 105B)]-51-3-B-B
35	B35	(ICMB 96555 x IP 10437)-2-4-2-B-6-1
36	B36	(ICMB 89111 x IP 9554-9)-4-2-2
37	B37	[(MC 94 S1-34-1-B x HHVBC)-10-4-3-2-2-B-B-2 x (ICMR 312 S1-1-5-3-B x HHVBC)-7-1-1-1-B-B-B]-21-B-1-2
38	B38	NC D2 S1-2-2-2-3-2-B-2
39	B39	(B x B) F2 S1-109-2-3-3-1-1-4
40	B40	(ICMB 03111 x {(MC 94 S1-34-1-B x HHVBC)-16-2-1-1-1-1-B-B-5 x (MC 94 S1-34-1-B x HHVBC)-10-4-1-2-1-B-B-1-30-2-4-3-1)-13-2-3-3
41	B41	{[(81B x SRL-53-1) x 843B]-3-5-3 x [(843B x 111B)-10-1-2-2]}-226-B-2-B-B-B
42	B42	[{26B x (81B x SRL 50-1)}-1-1-2 x 852B]-69-1-1
43	B43	ARD-288-1-10-1-2 (RM)-5
44	B44	MC 94 C2-S1-3-1-3-3-1-1-2-B-B
45	B45	{(MC 94 S1-81-1-B x HHVBC)-4-4-1 x (MC 94 S1-81-1-B x HHVBC)-4-2-4-10-3-1--B-B-B x ICMB 02777}-24-3-2
46	B46	EEDBC S1-465-3-2-5-5
47	B47	(ICMB 93333 x ICMB 01222)-11-2-2-2-B-2-6
48	B48	NC D2 BC7F4-12-1-2-3-1-4-3-B-B
49	B49	690-93B
50	B50	(SRC II C3 S1-19-3-2 x HHVBC)-12-4-1-3-2-1-B-2-B-4-B-B
51	B51	[HHV-S1-24-3-B-3-2 x (ICMB 96333 x HHVBC)]-19-B-1-3-B-B-B-B
52	B52	(ICMB 03111 x {(MC 94 S1-34-1-B x HHVBC)-16-2-1-1-1-1-B-B-5 x (MC 94 S1-34-1-B x HHVBC)-10-4-1-2-1-B-B-1-30-2-4-2-1)-7-5-4-1-1
53	B53	(ICMB 03111 x {(MC 94 S1-34-1-B x HHVBC)-16-2-1-1-1-1-B-B-5 x (MC 94 S1-34-1-B x HHVBC)-10-4-1-2-1-B-B-1-30-2-4-3-1)-13-2-5-1
54	B54	[ARD-288-1-10-1-2 (RM)-3 x B-bulk]-14-B-1-1
55	843-22B	843-22B
56	843B	843B
57	ICMB 95222	ICMB 95222
58	ICMR 01004	ICMR 01004
59	ICMR 01029	ICMR 01029
60	ICMR 11003	ICMR 11003

Table.2 Analysis of variance for yield and its component traits in in Pearl millet

S. No.	Character	Mean sum of squares		
		Replications df (1)	Treatments df (59)	Error df (59)
1	Days to 50 % flowering	0.53	100.62**	11.75
2	Plant height (cm)	11.56	1333.05**	18.36
3	Ear length (cm)	0.02	65.38**	1.30
4	Ear diameter (cm)	0.00	0.54**	0.02
5	Productive tillers per plant	0.02	0.73**	0.05
6	Head yield per plant (g per plant)	10.84	402.78**	14.43
7	Grain yield per plant (g per plant)	10.29	162.95**	7.34
8	Fresh stover yield per plant (g per plant)	16.54	1231.74**	77.49
9	Dry matter yield per plant (g per plant)	0.77	145.31**	3.92
10	1000 Grain weight (g per plant)	0.00	15.19**	0.36
11	Grain harvest index (%)	35.70	271.29**	13.86

df: Degree of Freedom; ** Significant at P≤0.01

Table.3 The eigen values, per cent variation and per cent cumulative variation for four Principal Components (PCs) and factor loading between PCs and traits studied in Pearl millet

	PC1	PC2	PC3	PC4
Eigen Value (Root)	2.954	1.988	1.716	1.150
Per cent Variation	26.852	18.069	15.603	10.455
Per cent Cumulative variation	26.852	44.921	60.524	70.979
Character	**Factor Loading**			
Days to 50% flowering	**-0.377**	-0.093	-0.367	-0.330
Plant height (cm)	**0.320**	-0.243	-0.345	0.196
Ear length (cm)	**0.168**	-0.482	-0.371	-0.119
Ear diameter (mm)	-0.013	**0.284**	0.149	-0.723
Productive tillers per plant	-0.430	0.179	0.038	**0.332**
Head yield per plant (g plant[-1])	0.173	-0.438	**0.224**	-0.393
Grain yield per plant (g plant[-1])	**0.490**	0.100	0.134	0.107
Fresh stover yield per plant (g plant[-1])	**0.277**	0.263	-0.515	-0.027
Dry matter yield per plant (g plant[-1])	0.077	**0.441**	-0.358	-0.188
1000 grain weight (g)	0.229	-0.022	**0.334**	-0.039
Grain harvest index (%)	**0.371**	0.347	0.114	0.034

Table.4 Average intra (diagonal and bold) and inter cluster D^2 values for eight clusters in Pearl millet

Cluster	I	II	III	IV	V	VI	VII	VIII
I	**230.56**	287.19	390.31	581.60	353.22	546.62	2008.93	1264.30
II		**168.52**	312.72	645.57	261.62	583.86	1648.75	1296.03
III			**211.99**	509.60	278.77	523.85	1376.72	1073.05
IV				**382.29**	547.46	537.04	1737.60	1590.72
V					**147.90**	291.13	1210.81	857.88
VI						**231.14**	1388.42	909.20
VII							**0.00**	1234.81
VIII								**0.00**

Table.5 Cluster means of sixty inbred lines for eleven quantitative traits in Pearl millet

S. No.	Character	I	II	III	IV	V	VI	VII	VIII	Contribution %
1	Days to 50% flowering	57.80	53.31	54.00	51.75	52.06	53.67	42.50	56.50	0.40
2	Plant height (cm)	90.53	75.56	91.07	98.19	112.66	141.81	161.50	151.00	28.31
3	Ear length (cm)	11.90	9.80	14.01	16.81	16.54	20.63	25.33	41.00	6.33
4	Ear diameter (cm)	2.91	2.78	2.94	3.47	3.22	3.51	3.85	2.61	6.67
5	Productive tillers	2.09	1.36	1.60	2.28	1.30	1.22	2.15	1.10	10.06
6	Head yield per plant (g per plant)	16.21	18.03	26.11	44.72	25.05	26.65	97.50	23.39	5.03
7	Grain yield per plant (g per plant)	10.28	10.84	16.58	32.82	16.10	19.51	40.50	7.90	3.05
8	Fresh stover yield per plant (g per plant)	55.63	22.91	48.65	97.33	39.83	77.70	62.00	50.00	4.24
9	Dry matter yield per plant (g per plant)	19.54	6.35	13.01	34.33	9.91	22.58	10.98	9.00	11.47
10	1000 grain weight (g)	7.66	7.34	12.55	11.02	9.40	10.00	12.93	8.89	21.98
11	Grain harvest index (%)	26.90	42.99	42.68	41.61	44.61	38.32	37.33	24.68	2.49

Fig.1 Ward dendrogram of 60 inbred lines of pearl millet based on eleven quantitative traits
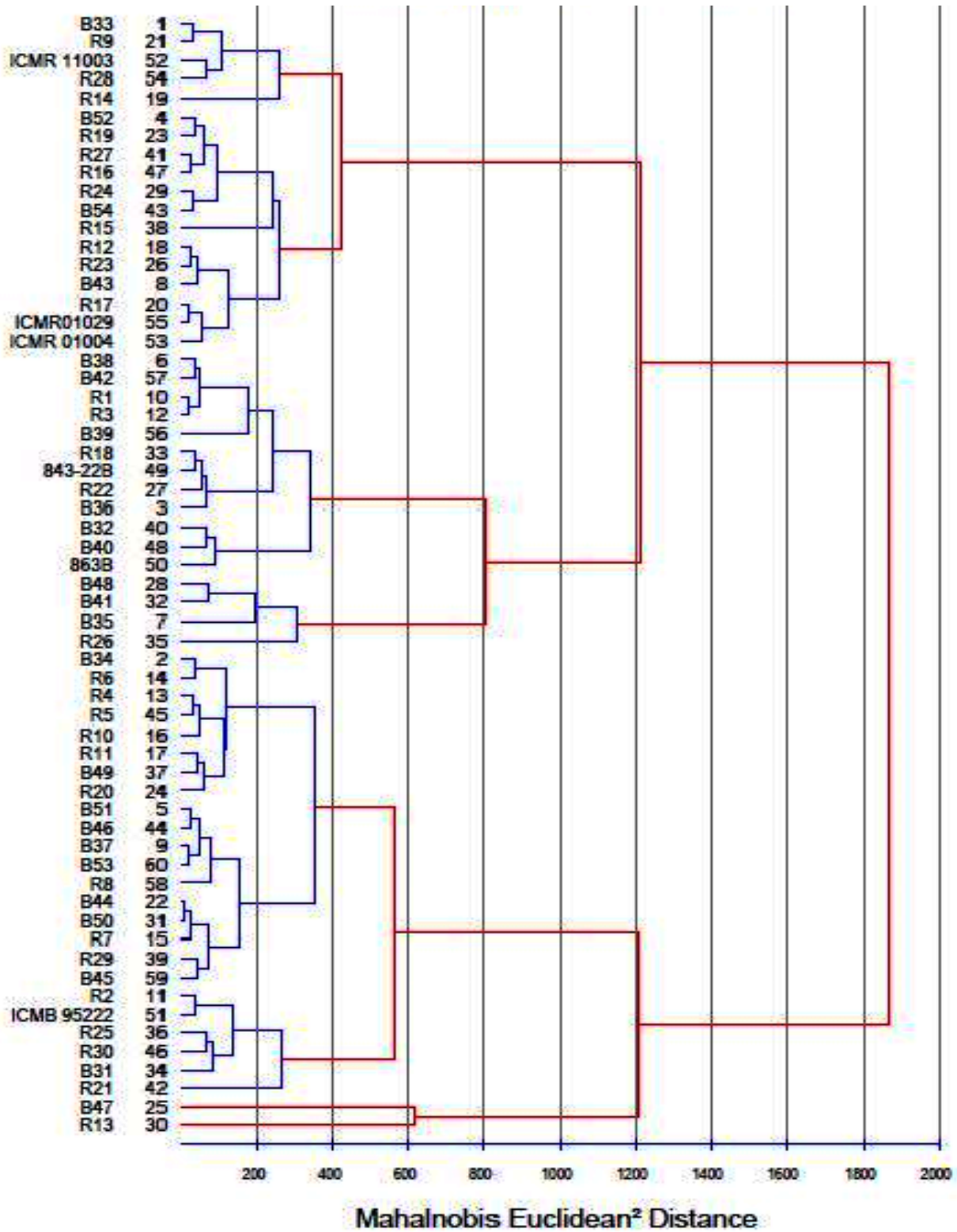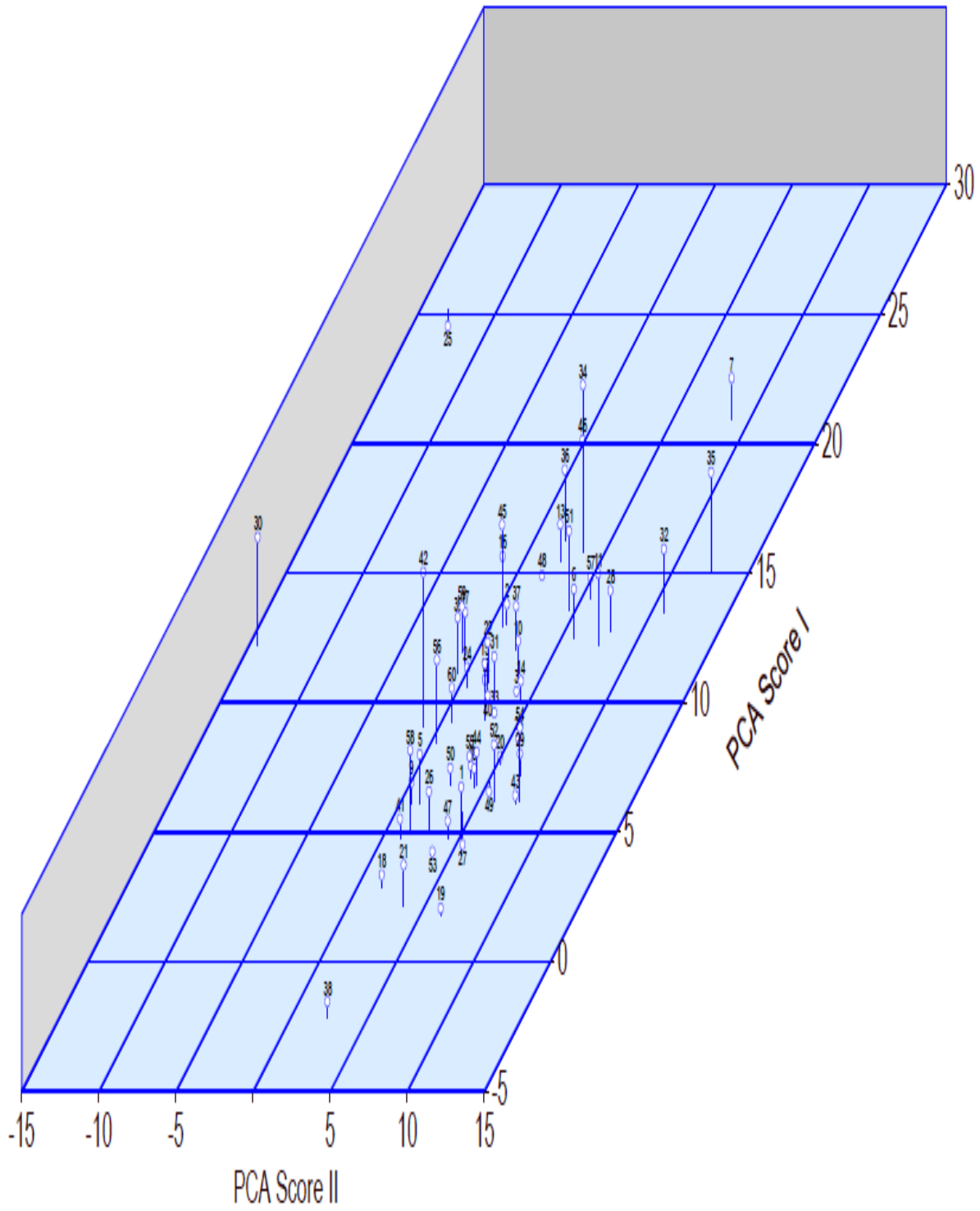(Scale on the bottom is squared Euclidean distance from D^2 analysis)

Fig.2 Three dimensional principal component scatter plot showing positions of sixty inbred lines of pearl millet

The average D^2 values within (intra cluster) distance and between (inter cluster) clusters are given in table 4. The average intra cluster distance ranged from 0.00 (cluster VII and VIII) to 382.29 (cluster IV). The maximum intra cluster distance was observed in cluster IV (382.29) followed by cluster VI (231.14), cluster I (230.56), cluster II (168.52) and cluster V (147.90). Therefore, selection within these clusters might be carried out on the basis of highest mean for desirable traits. Such intra cluster genetic diversity among the parental lines within the same group could be due to heterogeneity, pedigree and degree of general combining ability. The relative divergence of each cluster from other clusters (inter cluster distance) indicated high order of divergence between cluster I and cluster VII (2008.93) followed by that between cluster II and cluster VII (1648.75). Hence, the parents included in these clusters are genetically diverse and may have high heterotic response when used in hybridization programme. The selected lines could be used in inter crossing to develop base population with desirable characters. These findings were supported by reports of Vidhyadhar and Devi (2007) and Chaudhary *et al.,* (2015). The minimum inter cluster distance was observed between cluster II and cluster V (261.62) indicating narrow genetic diversity.

The cluster mean and per cent contribution of each character towards genetic diversity is presented in table 5. There was wide range of variation in the cluster mean values for most of the characters under study. Cluster VII had highest mean values for plant height (161.50 cm), ear diameter (3.85cm), head yield per plant (97.50 g), grain yield per plant (40.50 g) and 1000 grain weight (12.93 g) and also recorded least number of days to 50% flowering (42.50). Cluster IV had shown highest mean values for productive tillers per plant (2.28), fresh stover yield per plant (97.33 g) and dry matter yield per plant

(34.33 g), cluster V for grain harvest index (44.61%) and cluster VIII for ear length (41.00 cm). The characters contributing to most of the divergence should be given more importance for the purpose of effective selection and the choice of parents for hybridization. Plant height contributed maximum (28.31%) towards genetic divergence followed by 1000 grain weight (21.98%), dry matter yield per plant (11.47%) and productive tillers per plant (10.06%). The remaining characters contributed less genetic divergence indicating narrow genetic diversity for those characters. Shanmuganathan *et al.,* (2006) and Kumar *et al.,* (2015) reported similar results in Pearl millet. The distribution pattern of inbred lines on canonical graph matched mostly with the clustering pattern of hierarchical cluster analysis with few exceptions. This could be due to less contribution of first two principal components towards total variation. Such confirmatory results were also given by Gixhari *et al.,* (2014), Chaudhary *et al.,* (2015) and Kumar *et al.,* (2015).

In conclusion, this study differentiated the parental lines of Pearl millet into eight clusters. On the basis of genetic distances, the lines of cluster VII, I and II could be used as parents in crop improvement programme to develop promising hybrids. In addition, it is essential to have knowledge on the general combing ability of the selected parents in the hybridization programme. Therefore, the parents and hybrids generated should be evaluated over different locations or seasons to launch successful hybridization programme and also to test the correlation between genetic distance and hybrid performance for grain and stover yield characteristics in Pearl millet.

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