

MULTIVARIATE ANALYSIS OF PEARL MILLET DATA TO DELINEATE GENETIC VARIATION

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SUMMARY

Loss of biodiversity not only disturbs the process of plant development aimed at genetic amelioration but also disrupts the fundamental services that ecosystems provided to humanity. Assessment of variability is a multidimensional problem. The multivariate statistics can help in a comparative assessment of genetic variability. A set of 66 lines of pearl millet was analyzed for cluster and principal component analysis (PCA). PCA identified six principal components which explained 77.7 per cent of total variability among the 66 genotypes. The PC1 characters –main ear weight, dry fodder weight, total ear weight, grain yield, growth rate and plant height, the major characters of plant biomass and the basis for grain yield contributed maximum 35.94 per cent variability among the lines. The remaining PCs accounted for progressively lesser and lesser amount of variability. The lowest contribution 5.27 per cent was recorded by PC 6, the characters grain starch, starch recovery and ear girth. Only grain starch contributed positively to all the six components. The genotypes 50 (77/371), 3 (IPC-115), 41 (204/2 MP), 12 (IPC-1462), 37 (TCH-37-1), 22 (TCH-10-1), 61 (1307), 14 (862-P₂), 20 (TCH-3-2), 40 (204-2-3) were found to be better performers and diverse on the basis of principal factor scores with regard to grain yield and yield contributing characters. Hierarchical cluster analysis grouped 66 genotypes into six clusters, cluster 1 included maximum number of 21 genotypes and clusters 3 and 6 had the lowest number of 6 genotypes. The results on hierarchical cluster analysis almost mimicked the PCA. The grouping pattern of genotypes obtained by cluster analysis and PCA plots was almost similar. A wide range of diversity for most of the traits observed would enable to pick lines with suitable traits to be used in a breeding programme. Genetic diversity was not essentially associated with geographic diversity.

Key words : Pearl millet, germplasm lines, growth rate, quality-starch, protein, cluster, principal factor, 3-dimensional plot

Pearl millet [*Pennisetum glaucum* (L.) R. Br. emend Stanz] is a coarse grain crop hospitable to millions of people and animals over the world and grown under most inhospitable conditions of environment. The crop is more important for India and Africa where it is a means of livelihood of human and cattle population providing food, feed and fodder. Globally, it is cultivated over an area of about 26 million hectares. Rajasthan accounts for more than 50 per cent cultivation of the country's 9 million ha including desert areas. Importance of the crop thus assumes a central place in poor resource farmer agriculture and animal husbandry dominated

economy sustained marginal and under rainfed conditions with very low/no fertilizer inputs.

The commercial cultivation of pearl millet in India is subjected to environmental vagaries and in fact pearl millet is still mainly a rain dependent crop of dry lands where nothing else successfully grows. Patterns of rainfall in India are such as may lead to the conditions of terminal stress during grain formation period. Therefore, the yields greatly depend on the ability of the varieties to mature before the exhaustion of soil moisture. The extra early maturing wonder hybrid HHB 67 (60 days) developed at HAU (Kapoor *et al.*, 1989) has not

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only laid new direction in development of superior hybrids but has significantly increased productivity of pearl millet in Haryana which is now more than 2 t/ha and also increased productivity of Rajasthan.

For development of superior hybrids, assessment of nature and extent of genetic variation available among the parental lines is essential. Utilization of genetic resources in developing sustainable solutions to basic crop constraints has been suggested, but difficulty arises due to large number of variation effects and lack of sufficient evaluation and classification techniques.

Various first degree and second degree statistics are available for grouping of the germplasm to select for diverse types, but sometimes the recorded data become unmanageable and complicated to interpret. Often PCA and cluster analysis (Peeters *et al.*, 1989) have been used to reduce large number of germplasm lines up to a manageable source to identify the characters of significance to be used in a fresh programme with a greater degree of reliance. Very few reports (Bhattacharjee *et al.*, 2002; Yadav *et al.*, 2013) for grouping of pearl millet germplasm using dendrogram are available.

The present investigation was undertaken on

66 pearl millet lines, newly developed, old germplasm lines maintained at HAU and lines obtained from ICRISAT for evaluation, categorization and classification for similarity and degree of diversity based on data recorded on 19 morphological, physiological and biochemical characters using PCA.

MATERIALS AND METHODS

A number of 66 lines, having/including 19 germplasm lines from collection maintained at ICRISAT, 25 advanced inbreds and 22 newly developed lines of CCSHAU, Hisar (Table 1) were sown in randomized block design with three replications in 1R x 3M x 0.5 M size plots. Recommended package of practices to raise a good crop was followed. Data were recorded on five plants of each genotype in each replication on 19 characters viz., plant height (PH) (cm), effective tillers per plant (ET), ear length (EL) (cm), ear girth (EG) (cm), ear weight per plant (EW) (g), grain yield per plant (GY) (g), grain weight of main tiller ear MEG (g), grain number (GN) of main tiller ear, 500-grain weight (GW) (g), seed density (SD) (g/cc) calculated as ratio of weight of seed (g) to volume of seed (cc), dry fodder yield per plant (DFW) (g), days to 50 per cent flowering

TABLE 1
List of 66 pearl millet lines evaluated in the study

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes
1.	IPG-1363	23.	TCH-10-2	45.	77/29-2
2.	IPG-998	24.	TCH-14-1	46.	VCF -2359
3.	IPG-1115	25.	TCH-15-1	47.	INB7-74-3-2
4.	IPG-765	26.	TCH-15-2	48.	78/711
5.	IPG-1523	27.	TCH-19	49.	CSSC-46-2
6.	IPG-1304	28.	TCH-20-1	50.	77/371
7.	IPG-1336	29.	TCH-20-2	51.	1250
8.	IPG-324	30.	TCH-24-1	52.	92AC93
9.	IPG-449	31.	TCH-24-2	53.	IH-8 77/371 × BSEC-TCP-1-1
10.	IPG-1071	32.	TCH-26-1	54.	1-1
11.	IPG-1134	33.	TCH-29-1	55.	G73-107
12.	IPG-1462	34.	TCH-29-2	56.	G73-10 × BSEC-TCP-1
13.	841B-P3	35.	TCH-29-3	57.	ICMR-01004
14.	863B-P2	36.	TCH-31-1	58.	ICR-48
15.	BKM-1163	37.	TCH-37-1	59.	ICR-159
16.	BG-32	38.	193-1-1	60.	HTP-31
17.	ICMB-94222	39.	198-5-1	61.	1307
18.	ICMB-88004	40.	204-2-3	62.	1660 (MT)
19.	ICMB-88006	41.	204/2MP	63.	1305
20.	TCH-3-2	42.	HTP-94/54	64.	ICR-161
21.	TCH-10	43.	ICMR-01007	65.	77/273
22.	TCH-10-1	44.	H-90/4-5	66.	77/833-2

(DF), days to maturity (DM), grain filling rate (GFR) (mg/grain/day) were calculated according to formula (Mashiringwani *et al.*, 1992).

$$\frac{\text{Grain weight/ear (g)} \times 100}{\text{Grain number/ear} \times \text{Grain filling duration (days)}}$$

Grain filling duration (GFD) as the difference of days to maturity and days to flowering, growth rate (GR) was calculated as :

$$\frac{\text{Dry fodder yield (g/plant)} + \text{Total ear weight (g/plant)}}{\text{Days to 50 per cent flowering}}$$

For grain starch (GS) – total sugar other than starch was extracted according to procedure (Cerning and Guithot, 1973) and starch from sugar for free pellets was extracted by the method (Clegg, 1956) and estimated by the method (Yamen and Wills, 1954), starch recovery (SR) was calculated as :

$$\frac{\text{Starch yield (g/100 g flour)}}{\text{Grain yield/plant (g)}}$$

Crude protein content (PC) was estimated by Micro-Kjeldahl's method (AOAC, 1990) . Means of five plants in each replication were used for analysis of variance using model :

$$Y_{ij} = \mu + t_i + b_j + e_{ij}$$

PCA and cluster analysis were carried out on 66 genotypes and 19 characters using SPSS package. Ward clustering method of hierarchical cluster analysis was used with squared distances to classify 66 genotypes and dendrogram was prepared using the scaled distances (Romesburg, 1984). The first three PCs were plotted in both three dimensional and biplot in various combinations. Only the biplots of the first three most informative components (PCAs) are presented.

RESULTS AND DISCUSSION

Analysis of variance revealed significant genotypic differences for 18 characters at 1 per cent level of significance and for seed density at 5 per cent

level of significance indicating large variability present among the 66 genotypes. It was thus worth while to proceed further for multivariate diversity analysis.

Correlation matrix based principal component (PC) method was used to extract the principal factors (PF) as it does not require the assumption of normal distribution of populations and the PCs with eigen values greater than one were retained. Latent root i. e. variance on each axis, the percentage of total variance that represents the coefficients used in weighted sum (eigenvectors or loadings) are presented in Table 2. The first six components were with eigen values more than one and they together explained 77.7 per cent of the total variability amongst the 66 lines evaluated for 19 characters. Only starch content contributed positively to all the six PCs. The characters main ear weight, dry fodder weight, total ear weight, grain yield, growth rate and plant height, the major characters of plant biomass and the basis for grain yield, contributed maximum 35.94 per cent variability to the first principal component (PC 1) indicating that the high yielding lines were differentiated on the basis of these characters. The remaining PCs accounted for progressively lesser and lesser amount of variation. Similar trend using PC analysis in pearl millet was reported (Bhattacharjee *et al.*, 2002; Yadav *et al.*, 2013). 500-grain weight, grain filling rate and days to flower were the characters

TABLE 2
Total variance explained by various principal components

Component	Eigen value	Variance explained (%)	Cumulative (%)
1	6.8320	35.9411	35.9411
2	2.4678	12.9824	48.9235
3	2.0564	10.8180	59.7415
4	1.2410	6.5283	66.2698
5	1.1712	6.1614	72.4311
6	1.0011	5.2666	77.6978
7	0.9142	4.8094	82.5072
8	0.7585	3.9900	86.4972
9	0.6038	3.1763	89.6735
10	0.5555	2.9224	92.5959
11	0.5053	2.6580	95.2538
12	0.3232	1.7000	96.9538
13	0.2429	1.2776	98.2315
14	0.1811	0.9525	99.1840
15	0.1002	0.5272	99.7112
16	0.0410	0.2159	99.9271
17	0.0100	0.0525	99.9797
18	0.0038	0.0199	99.9996
19	0.0001	0.0004	100.0000

contributing positively to the second principal component (PC 2) accounting for 12.98 per cent of the variability explained. It shows that bolder seeds, high GFR and late flowering plants had less effective tillers, less seed density, poor growth rate and less ear weight (Chaudhary, 2005). PC 3 represented positively by days to maturity, days to flower, grain filling duration showing that high biomass and later maturing types had low grain filling rate, smaller grains and thinner heads and explained 10.82 per cent variation. The PC 4 accounting for 6.53 per cent variability included characters such as grain starch, seed density, effective tillers, ear girth and grain filling duration. The 6.16 per cent variance explained by PC 5 was contributed by characters seed density, grain starch and grain filling duration. The PC 6's contribution to 77.7 per cent variance was lowest, the 5.27 per cent by characters grain starch, starch recovery and ear girth.

The failure of PCA without rotation to draw useful conclusions prompted to go for analysis with rotation. Factors loading of different variables obtained through varimax rotation are presented in Table 3. All the 19 variables showed high loading on different PCs and none of them was left rotation of the factor axes. Moreover, it already grouped the similar type of variables by loading them together as a common PC. The first PC showed high loading for all the six variables

TABLE 3
Factor loadings of different characters with respect to different PC's (Principal components)

Character	1	2	3	4	5	6
PH	0.119	-0.022	0.07	-0.064	0.065	-0.056
ET	0.043	-0.187	-0.026	0.353	-0.315	-0.265
EL	0.08	0.094	0.182	-0.051	-0.007	0.174
EG	0.036	0.169	-0.227	-0.278	0.079	0.269
DFW	0.125	-0.027	-0.007	0.126	0.046	0.056
EW	0.123	-0.07	0	0.129	-0.077	0.142
GN	0.107	-0.128	0.14	-0.165	-0.161	0.193
GY	0.123	-0.079	-0.065	-0.044	-0.035	-0.077
DF	0.066	0.243	0.263	-0.13	-0.098	-0.014
GFD	-0.01	0.019	0.222	0.251	0.287	-0.529
DM	0.06	0.236	0.31	-0.055	-0.015	-0.153
GS	0.015	0.009	0.114	0.448	0.398	0.506
SD	0.026	-0.127	-0.026	-0.37	0.487	-0.203
MEG	0.134	0.026	-0.024	-0.042	-0.076	0.052
500GW	0.063	0.266	-0.239	0.195	0.067	-0.225
GFR	0.063	0.263	-0.271	0.144	0.013	-0.124
GR	0.122	-0.108	-0.075	0.158	0.046	0.08
PC	-0.065	0.093	0.052	0.026	-0.448	0.057
SR	-0.107	0.11	0.061	0.17	0.088	0.279

supporting the component analysis i. e. the important characters of grain yield. Similarly, the loadings of PC 2 and PC 3 were high for the characters observed important for bolder grains and grain development period, respectively. The loadings of PC 4 were positive for grain starch, effective tillers and grain filling duration but negative for ear girth. The trend was similar with minor deviation of ear girth which could be due to some other character like grain density.

PCA mainly contributes towards consideration of several traits simultaneously in the selection of materials with the added advantage of selectively rejecting traits by virtue of their duplication leads to not only greater labour but also causes loss of precision in the selection process when large number of characters are considered together. Since the principal components are based on correlation matrix, it stands to reason that only a few of the characters are considered providing the same extent of information as well as precision (Datta and Mukherjee, 2004).

The PC 1 and PC 2 involving characters of major economic importance i. e. grain yield and explaining 48.9 per cent variance were used to study the clustering/divergence pattern of the 66 genotypes evaluated.

Assessment of workable diversity in an available set of germplasm assumes greater significance, while initiating any breeding programme. The prime objective in the present study was to identify good lines for grain yield, yield contributing characters and combining modification of early maturity, good nutritional quality like protein and starch content. While the PCA grouped the genotypes into six clusters showing wide diversity. Ward's method of hierarchical clustering used with squared Euclidian distances classified the 66 genotypes using dendrogram prepared using the rescaled distances also into six clusters (Table 4).

The pattern of distribution of lines among various clusters reflected considerable genetic variability present among the lines. The cluster 1 comprised 21 lines, followed by cluster V with 14 lines, cluster II had 10 lines and cluster IV 9 lines and clusters III and VI included 6 lines each. The association among the different lines was prepared using rescaled distances. The resemblance coefficient between two lines is the value at which their branches join. The dendrogram also showed the relative magnitude of resemblance among the different clusters (Fig. 1).

The mean performance of different clusters

TABLE 4
Grouping of pearl millet inbreds in different clusters

Cluster	No. of inbreds	Inbreds/genotypes
I	21	IPC 1336, IPC 324, 841B-P3, BG32, TCH-15-2, TCH-19, TCH-20-1, TCH-20-2, TCH-26-1, TCH-29-1, TCH-29-2, TCH-29-3, TCH-31-1, ICMR-01007, H 90/4-5, G73-107, ICMR-01004R, ICR 48, ICR 159, HTP 31, 1660 (MT)
II	10	TCH-3-2, TCH-10-2, TCH-14-1, TCH-15-1, TCH-24-2, HTP94/54, 77/371, 96AC93, 1305, ICR 161
III	6	IPC 765, BKM 1163, ICMB 94222, ICMB 88006, 77/371 x BSEC-TCP-1-1, 1307
IV	9	IPC 998, IPC 449, IPC 1134, 863 B-P2, ICMB 88004, TCH-10-1, TCH-24-1, TCH-37-1, 1H8
V	14	IPC 1363, IPC 1304, TCH-10, 193-1-1, 198-5-1, 77/29-2, VCF ₄ 2359, INB 7/74-3-2, 78/711, CSSC 46-2, 1250, G73-107 x BSEC-TCP-1, 77/273, 77/833-2
VI	6	IPC 1115, IPC 1523, IPC 1071, IPC 1462, 204-2-3, 204/2MP

calculated for different characters revealed wide range of variation among the clusters with respect to these characters (Table 5). The perusal of the cluster means and general means for different characters (Table 6) showed that cluster VI including 4 of ICRISAT lines (IPC-1115, IPC-1523, IPC-1071, IPC-1462) and two newly developed lines (204-2-3 and 204/LMP) exhibited superior performance for most of the characters under study including grain yield and its contributing characters as envisaged in PC 1 and PC 2. It was followed by cluster III also including ICRISAT and newly developed lines for four characters including grain quality and early maturity characters. Cluster I contained lines inferior for most of the characters. The lines grouped in clusters II, IV and V included lines performing better for seed density, ear girth and longer grain filling period, respectively.

The grouping of pearl millet lines in different clusters gives an opportunity to select them to serve our objective in developing high grain and fodder yield combining good nutritional quality and early maturity. To assess the diversity, inter- and intra-cluster distances were calculated and are presented in Table 5. The maximum inter cluster distance (9.466) was noted between clusters III and VI, followed by 6.925 between clusters II and III, 6.575 between clusters V and VI and 6.232 between clusters III and IV indicating that the lines grouped in these clusters were diverse and selected lines could be intercrossed to prepare a base population to combine the desirable characteristics. Best lines from these clusters can be used as pollinators for development of hybrids after testing for latter's *per se* performance and specific combining ability. The line grouping in different clusters inspite of their place of development and geographical distribution showed that geographical isolation was not directly related to genetic diversity.

This observation is in concordance with earlier reports in pearl millet (Murthy and Arunachalam, 1966; Murti and Tiwari, 1967; Mukherjee *et al.*, 1981) and in other crop species (Sagar *et al.*, 1976; Katiyar and Singh, 1979; Yadav *et al.*, 2004). However, a few genotypes related by their place of origin along with ICRISAT genotypes, showed tendency to group in the same cluster, which may be because of dependence upon the directional selection pressure that leads to well evolved homeostatic mechanism that would favour consistency of the associated character and thus resulting in indiscriminate clustering as reasoned (Katiyar and Singh, 1979; Marchais and Pernes, 1985; Marchais and Tostain, 1985; Singh *et al.*, 2010).

It shows that geographic diversity does not essentially lead to genetic diversity, the factors of original domestication and environmental conditions at the time of development play an important role in perpetuation, adaptation and stabilization of similar genotypes.

The results of hierarchical cluster analysis and PCA confirmed the finding of each other. Principal factor scores (PF scores) for all the 66 genotypes were estimated for all the six PCs using Anderson-Rubin method as given in SPSS. These scores may be utilized to construct precise selection indices based on P. variability explained by each of the principal factor. The genotypes were plotted for PC 1 and PC 2 which together explained 48.9 per cent variability and included the major grain yield characters (Fig. 2). Positive side of PC 1 indicated the genotypes giving high yield were 50 (77/371), 3 (IPC-1115), 41 (204/2MP), 10 (IPC-1071) and 12 (IPC-1462). The genotypes 17 (ICMB-94222), 61 (1307), 15 (BKM-1163), 19 (ICMB-88006), 4 (IPC 765), 6 (IPC 1304) and 14 (863B-P2) represented by positive values of PC 2 were the genotypes combining bolder beads, high grain filling rate and late maturity.

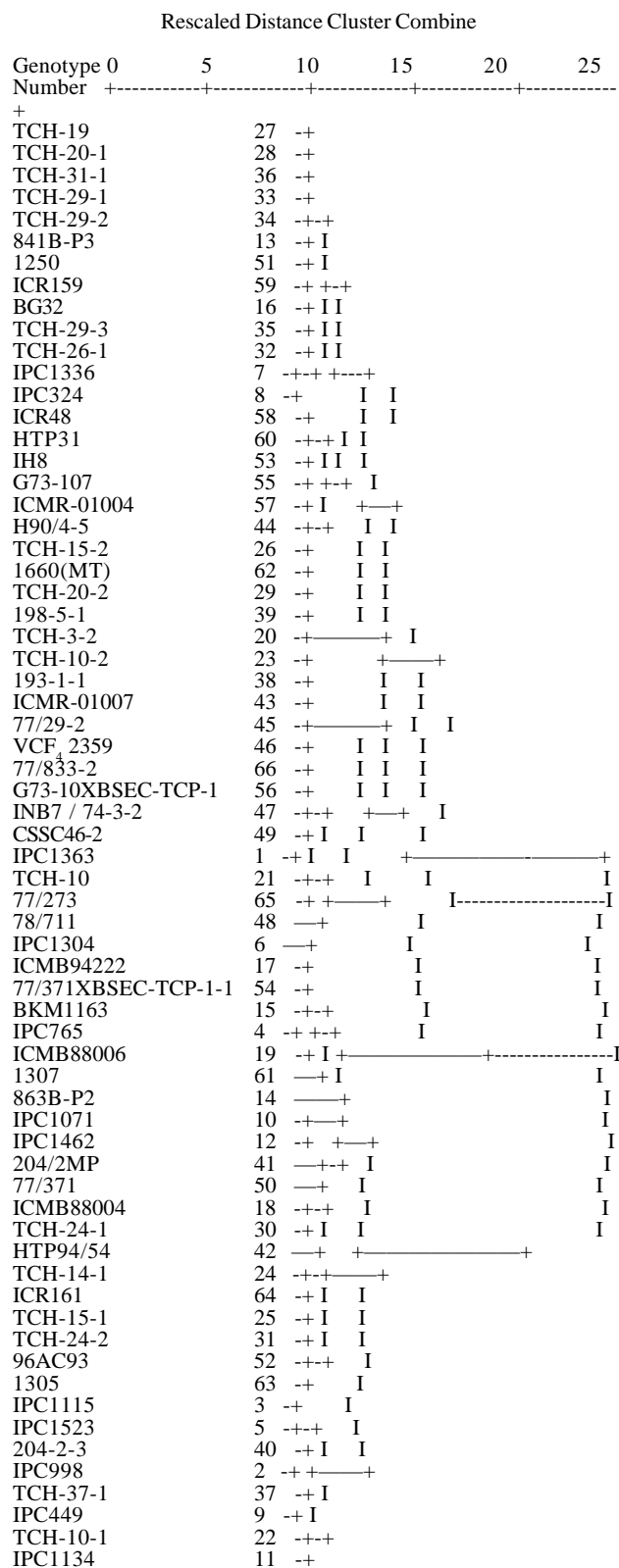


Fig. 1. Dendrogram showing relationship of 66 pearl millet lines.

TABLE 5
Average inter-and intra-cluster distances in pearl millet

Clusters	I	II	III	IV	V	VI
I	2.491	3.704	4.466	3.757	2.811	6.131
II		3.650	6.925	3.651	4.727	4.340
III			2.400	6.232	4.243	9.466
IV				3.285	4.553	4.547
V					3.252	6.575
VI						3.232

The genotypes 37 (TCH-37-1), 22 (TCH-10-1), 11 (IPC 1134), 40 (204-2-3), 18 (ICMB 89004), 30 (TCH24-1), 5 (IPC 1523) and 42 (HTP94/54), scored high and positively both for PC 1 and PC 2 i. e. the genotypes giving high yield and yield contributing characters, bolder seeds and high grain filling rate. The groupings obtained in 3-D plot of principal components (Fig. 3), illustrates the accession distribution in the first three principal components clearly showed the separation of 66 genotypes evaluated. The analysis separated the accessions into six different groups. The pattern of clustering obtained with PCA is comparable to the UPGMA based analysis and distinguished the groups clearly and effectively. Also the results of PCA were closely in consonance with those of the cluster analysis. Accessions from diverse group will maximize opportunities to obtain transgressive segregants as there is a higher chance from genotypes to contribute unique desirable alleles at various loci. It was possible to reduce large number of variables into only six principal factors and identify different lines better for different combinations of characters. Hence, indirect selection for seed yield based on component traits may lead to create better genetic recombinants for improving yield and quality *per se*.

The positive contribution of starch content to all the six PCs is very interesting and an encouraging information. The high starch content lines are expected to play a vital role in brewing industry. Chaudhary (2005) found no relationship of grain starch with any of the characters studied, which suggested that high grain yield and high grain starch were mutually exclusive traits. Further as she noted that the grain starch exhibited high genotypic coefficient of variation, high heritability, the improvement of these two traits is expected without loss for any one or the other by selection. Higher expected alcohol yield (516.5 l/t) of pearl millet grain from the material evaluated than the 422-488 l/t of sorghum grains

TABLE 6
Cluster and general means for different characters in pearl millet

Character	Clusters					
	I	II	III	IV	V	VI
PH	148.16± 13.01	170.77± 18.07	110.07± 14.36	179.07± 27.49	146.92± 23.16	197.70± 20.36
ET	2.22± 0.44	2.40± 0.45	1.87±0.30	2.06±0.58	2.30± 0.41	2.75±0.72
EL	19.13±2.90	20.65±2.70	18.22±2.97	21.15±4.01	20.96±4.50	25.70±2.57
EG	7.26± 0.74	7.53±1.02	7.67±0.96	8.09±1.16	6.53 ± 0.89	7.41±1.37
DFW	53.13±10.25	99.60±21.41	33.69±12.33	67.04±10.69	52.27±10.21	118.84±21.46
EW	33.52±5.77	42.59±5.37	20.56±4.11	36.39±5.78	31.29±8.13	52.83±11.70
GN	1288.51±249.08	1355.57±359.06	615.39±179.33	1234.15±322.21	1173±36±331.81	1787.67±133.04
GY	19.06±3.42	22.32±5.75	7.70±1.42	22.10±5.39	14.11±4.17	29.94±9.03
DF	48.52±3.45	48.40±2.78	48.05±3.06	55.22±4.03	51.14±6.69	57.16±4.16
GFD	24.71±0.96	25.37±0.94	25.39±1.02	26.15±1.52	27.38±1.40	25.94±0.83
DM	73.24±3.36	73.77±2.47	73.44±2.59	81.30±4.06	78.52±6.37	83.11±4.74
GS	62.96±3.05	65.35±4.41	64.32±2.45	61.31±3.15	66.15±3.01	66.24±2.34
SD	1.11±0.09	1.53±0.53	1.01±0.09	1.19±0.19	1.16±0.11	1.06±0.15
MEW	10.18±1.97	12.97±2.51	5.44±0.99	13.95±2.75	8.31±2.23	18.57±3.15
500GW	4.01±0.51	4.95±1.39	4.59±0.74	5.97±1.06	3.64±0.52	5.25±0.78
GFR	0.32±0.05	0.39±0.11	0.36±0.07	0.46±0.08	0.27±0.04	0.41±0.06
GR	1.49±0.23	2.46±0.43	0.93±0.16	1.60±0.26	1.37±0.27	2.57±0.39
PC	11.96±1.40	10.07±1.12	12.63±2.01	11.25±1.27	12.00±1.60	11.70±1.76
RS	3.48±0.69	3.14±0.78	9.08±2.65	3.01±1.02	5.18±1.57	2.42±0.75

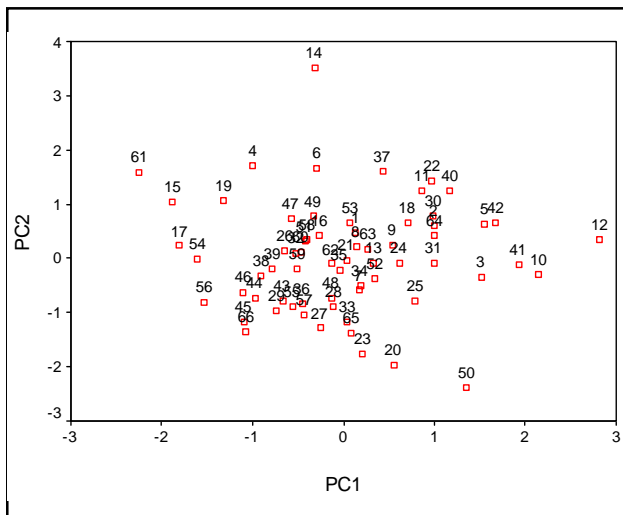


Fig. 2. Genotypes plotted for PC 1 and PC 2.

by Mandke and Kapoor (2004) is a big score for pearl millet. Pearl millet has better production potential under rainfed conditions and also the cost of grain is less than sorghum grain. The alcohol produced from grains is neutral alcohol and needs very less refinement to meet international standards, whereas that from molasses is rectified spirit and needs much refinement and would not be of high quality.

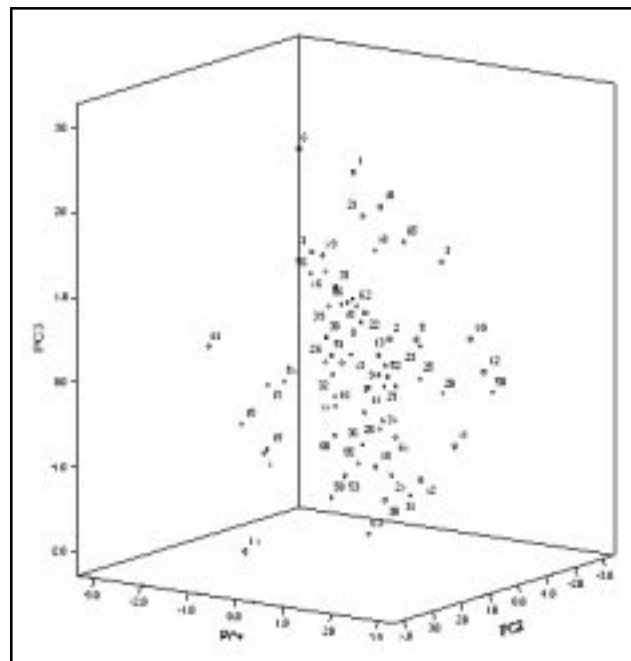


Fig. 3. 3D plot for first three principal component scores.

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