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Genetic divergence study in maize inbred lines (*Zea mays*)

PRAVEEN SINGH[1], A K SINGH[2] and M SHARMA[3]

Maize Breeding Research Sub-Station (SKUAST-J), Poonch, J & K 185 101

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ABSTRACT

The genetic divergence study in 46 maize inbred lines was conducted based on Mahalanobis D^2 statistics by using Tocher's canonical (vector) and Euclidean methods at Maize (*Zea mays* L.) Breeding Research Sub-Station, Poonch during 2009-2011. The genotypes were grouped into seven clusters by both the methods of divergence study. The grouping of genotypes in different clusters was beyond their geographic origins. This indicated appreciable amount of diversity present in the inbred lines under study. Canonical (vector) analysis suggested that plant height, stem girth and number of leaves per plant had contributed maximum towards total variability in the lines. 3D plotting of individual genotypes, based on principal component analysis scores and Euclidean distance matrix, showed that the PMS134 was the most diverse inbred with CML324 followed by M8-3, CML158, 6152 and CML399. These diversely related inbred lines can be utilized as parents in maize breeding programme to isolate desire hybrids for yield and component characters.

Key words: Cluster analysis, Genetic divergence, Maize, inbred line, PCA

Maize (*Zea mays* L.) is highly cross pollinated species maintained their heterozygous balance in population. It show heterosis in recombinants, particularly when inbreds differ for many genes affecting yield or some other character of importance are used as parents. In any crop improvement programme, genetic diversity is prerequisite (Naik *et al.* 2006), as it play important role because hybrids between genotypes of diverse genetic background, generally display a great heterosis and throw more recombinants than those between closely related parents. Maize inbred lines of different origin and genetic background serve as a valuable source material, and provide scope for building of genetic variability. Study of genetic divergence in the maize inbreds will help to ascertain the real potential value of the genotype. Statistical methods for measure the diversity based on multiple characters quantify the genetical distance among the breeding materials and reflect the contribution of characters towards diversity. Genetic diversity arises due to geographical separation or due to genetical barrier to crossability or due to different pattern of evolutions. Thus the study of diversity in inbreds of different origin may either complement or high light new features of the variation in maize breeding programme. The present study was, therefore undertaken to assess the extent of genetic diversity in 46 maize in bred lines which will help to select prospective parents to develop transgressive segregants.

[1] Junior Scientist (e mail: prof.praveen@gmail.com), Division of Plant Breeding and Genetics

MATERIALS AND METHODS

The present investigation was carried out at Maize Breeding Research Sub-station, Poonch (India) situated between 33 degree-25' to 34 degree-01' north latitude and 73 degree-58' to 74 degree-35' east longitude at height of 980 m above MSL and bounded by Kashmir valley and line of control with Pakistan. The experiment material consist of 46 maize inbred lines developed from different source materials carried out from local collections and different research stations of India and abroad (Table 4). All these inbreds were developed through repeated selfing followed by selection for last four years and are well stable in the prevailing agro-climatic condition. The present experiment was conducted in RBD design for three years, i.e. 2009, 2010 and 2011 following the recommended agronomical practices for maize cultivation in the region. The physiological data were recorded from mean of five randomly selected plants from each inbred per replication. The pooled data of three years were used to study the genetic divergence among the genotype under present investigation. The data were analysed following principal component analysis (PCA) and Mahalanobis (1936) generalised distance (D^2) by using Tocher's method extended by Rao (1952). The non hierarchical Euclidean cluster analysis was calculated following the method described by Beale (1969) and Katyal *et al.* (1985). All the statistical analysis were carried out using computer software Windostat version 8.5.

RESULTS AND DISCUSSION

The result of divergence analysis through Tocher

method (Fig 1) revealed that all the genotypes under study were grouped in seven clusters, where cluster 2 was largest with 15 inbred lines and cluster 3 and cluster 5 were smallest as they had only one genotype in each cluster. The high numbers of clusters evidence the wide range of variability among the genotypes under study. More *et al.* (2006) and Singh *et al.* (2005) also found more numbers of clusters and genotypes within the clusters in their experiments on maize. The diverse parents showed high heterotic effects in their combinations. The result also showed the maximum inter cluster distance between cluster 7 and cluster 6, where as the minimum cluster distance was observed between cluster 1 and cluster 5 (Table 2). The maximum intra cluster distance was found between inbreds of cluster 7 whereas minimum intra cluster distance was found in inbreds of cluster 1. The result indicated that the inbreds in different clusters differing largely and marginally in their genetic architecture as per their maximum and minimum intra cluster distance, respectively. Similar experimental findings were also observed by Castanon *et al.* (1999).

The non-hierarchical Euclidean cluster analysis provides more critical observations on genetic diversity than Tocher's method of divergence analysis. The Euclidean analysis observes the sub clusters of major groups and

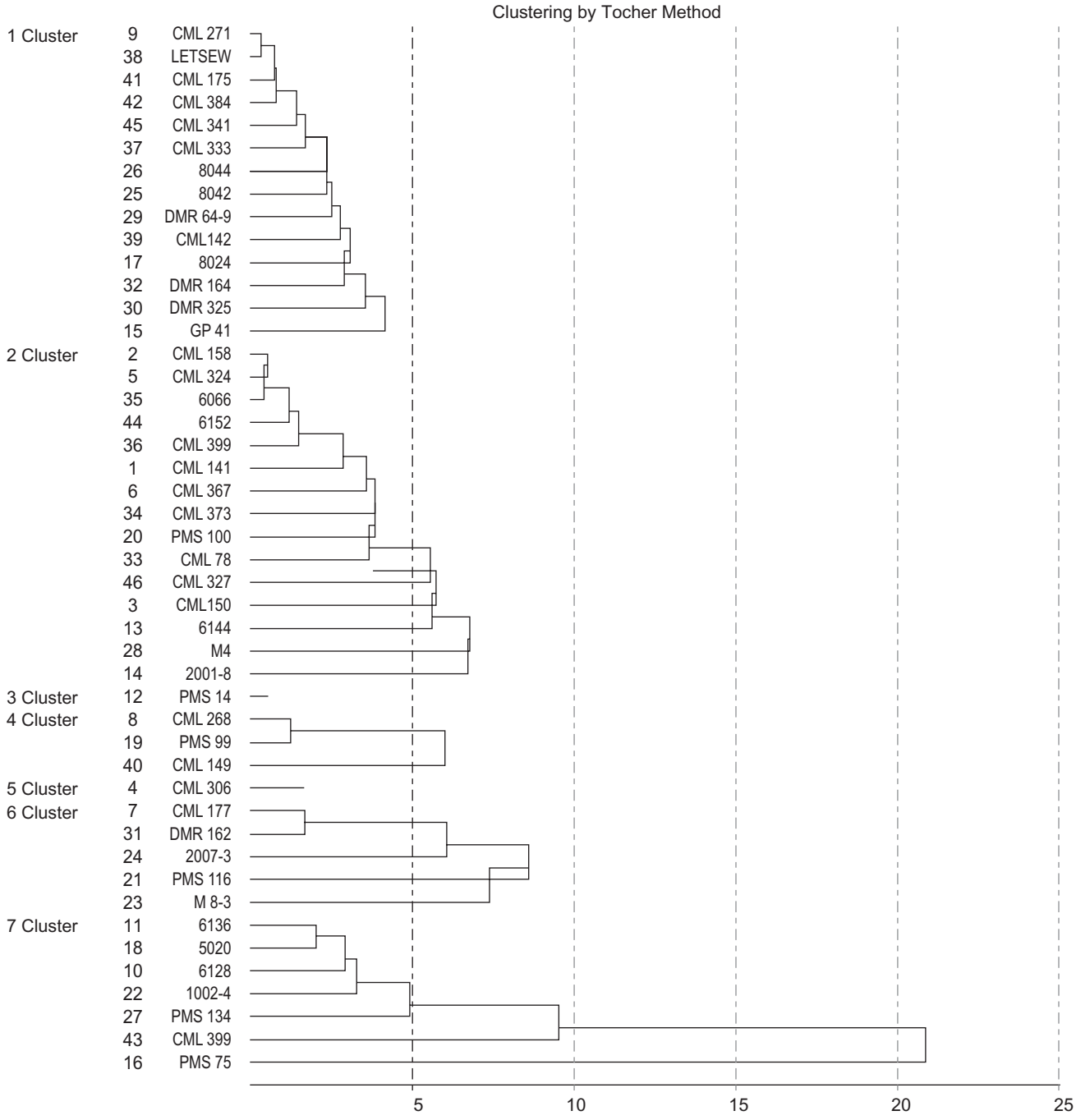

Fig 1 Clustering pattern of maize inbred lines by Tocher method

Table 1 Distribution of 46 maize inbred lines in to seven clusters based on tocher method and Euclidean cluster analysis.

Cluster	Method	No. of inbred	Name of inbred
I	Tocher	14	CML271, LETSEW, CML175, CML384, CML341, CML333, 8044, 8042, DMR-64-9, CML142, 8024, DMR164, DMR325, GP41
	Euclidean	8	CML141, CML367, CML78, CML373, DMR64-9, PMS100, CML268, PMS99
II	Tocher	15	CML158, CML324, 6066, 6152, CML399, CML141, CML367, CML373, PMS100, CML78, CML327, CML150, 6144, M4, 2001-8
	Euclidean	6	CML158, CML324, 6066, CML399, 6152, CML149
III	Tocher	1	PMS14
	Euclidean	11	CML177, DMR162, CML271, LETSEW, CML341, CML333, CML175, CML354, 8042, 8044, CML142
IV	Tocher	3	CML268, PMS99, CML149
	Euclidean	3	PMS14, 2001-8, CML150
V	Tocher	1	CML306
	Euclidean	10	6144, M4, GP41, CML306, 8024, DMR164, DMR325, PMS116, M8-3, 2007-3
VI	Tocher	5	CML177, DMR162, 2007-3, PMS116, M8-3
	Euclidean	7	6128, 1002-4, CML327, 6136, 5020, PMS134, CML399
VII	Tocher	7	6136, 5020, 6128, 1002-4, PMS134, CML399, PMS75
	Euclidean	1	PMS75

Table 2 Mean inter and intra cluster distance among seven clusters in maize inbred lines on the basis of D2 statistics (Tocher's Method and Euclidian method)

Tocher Euclidean	1 Cluster	2 Cluster	3 Cluster	4 Cluster	5 Cluster	6 Cluster	7 Cluster
1 Cluster	8.220	23.749	36.132	43.615	13.795	17.727	81.029
	34.751	54.386	57.107	88.644	94.88	153.059	470.486
2 Cluster		14.135	22.674	31.414	22.559	41.412	44.798
		20.289	127.201	87.986	142.171	79.948	377.193
3 Cluster			0.000	67.387	21.333	57.188	29.599
			25.867	112.274	54.194	224.528	546.328
4 Cluster				17.072	57.370	67.807	89.971
				15.839	68.617	85.065	194.389
5 Cluster					0.000	26.135	58.952
					28.109	167.464	378.977
6 Cluster						24.328	103.397
						53.914	200.477
7 Cluster							31.973
							0.000

provides more information about diversity of genotypes which are very useful in crop breeding programme. The results on Euclidean cluster analysis (based on ward minimum variance dendrogram) showed the relative association among the genotypes which was prepared using rescale distance (Fig 2). Out of the seven clusters made by this method, cluster 3 had maximum number of genotypes, where as cluster 7 had only one genotype (Table 1). Williams and Hallarver (2000) also observed mono genotypic clusters in their experiment. The mono genotypic clusters genotypes (PMS14 and CML306) of Tocher's method were grouped in cluster 4 and cluster 5 of Euclidean clusters, which seem to be more similar with genotypes of respective clusters. The inter cluster distance was found maximum between cluster 7 and cluster 3, where as

Table 3 Canonical vectors which supply best linear function of variates, value of canonical roots and percentage of variation absorbed by respective roots.

	1 Vector	2 Vector	3 Vector
Eigene Value (Root)	3.154	1.426	0.984
% Var. Exp.	45.051	20.366	14.064
Cum. Var. Exp.	45.051	65.417	79.480
Days to tasseling	0.143	0.629	0.141
Days to maturity	0.128	–0.430	0.771
Plant height (cm)	–0.531	0.111	0.145
Ear height (cm)	–0.111	–0.607	–0.447
Stem girth (cm)	0.521	0.085	–0.122
No. of leaves	0.510	–0.051	–0.262
Gain yield/ plant (gm)	–0.371	0.167	–0.286

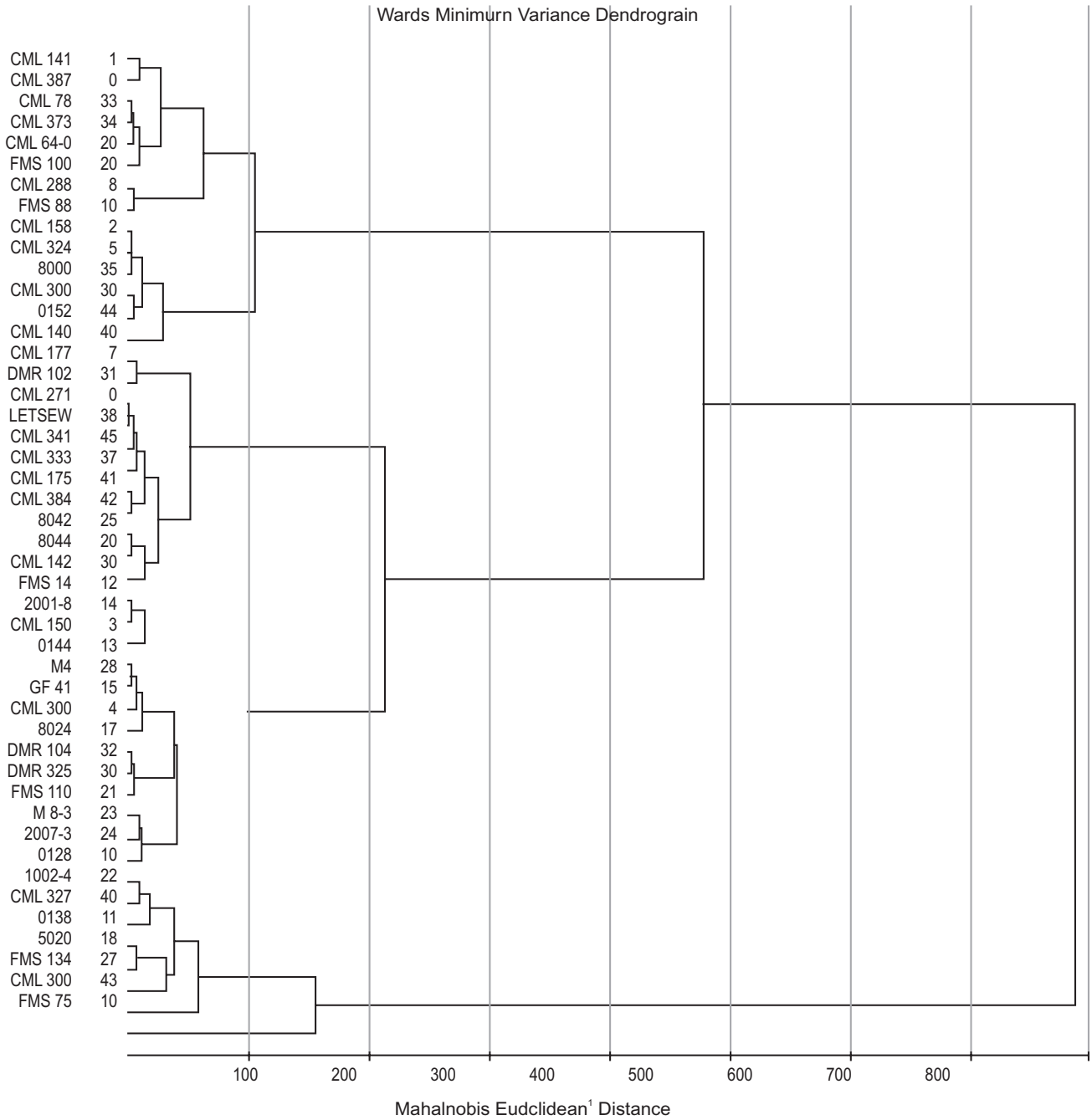

Fig 2 Clustering pattern of maize inbred lines by Euclidean method

minimum inter cluster distance was observed between cluster 5 and cluster 3 (Table 2). The intra cluster distance was maximum in inbreds of cluster 6 whereas it was minimum in inbreds of cluster 4. The result showed that there was wide range of diversity present between the clusters as well as within the clusters, which has immense value in varietal improvement programme. Such genetic diversity present in the available parental lines for character of interest is most important.

Both the methods of genetic diversity study showed the present of high degree of diversity among inbreds of present investigation. The results also showed that the genotypes were random and independent for clustering. Most of the genotypes of same geographical origin were grouped in different clusters. Sharma *et al.* (2008) and Chen *et al.* (2007) also observed similar findings in their experiments. This may be due to genetic architecturer of the genotypes which caused greater diversity than the geographic origin alone (Yin Zhin Tang *et al.* 2004).

Principal component analysis (PCA) is very useful in measuring the genetic diversity in multivariate scale (Liu *et al.* 2006). In the present investigation, the first component of variance explained 45.05 percent of variation where as second and third principal components explained 20.37 and 14.05 percent variation, respectively. Cumulatively, 79.48% percent variability was explained by first three principal

Table 4 Details of inbred lines and their mean values of vectors calculated through canonical (vector) method

Origin source	Inbred	Pedigree	Vector 1	Vector 2	Vector 3	Mean yield/ plant (gm)
CIMMYT	1	CML 141	–3.401	6.599	15.433	82.6667
CIMMYT	2	CML 158	–4.838	7.405	14.893	113.3333
CIMMYT	3	CML 150	–3.738	6.240	14.891	35.0000
CIMMYT	4	CML 306	–2.860	6.164	12.274	73.3333
CIMMYT	5	CML 324	–5.298	7.735	15.239	123.3333
CIMMYT	6	CML 367	–3.342	7.232	14.549	83.3333
CIMMYT	7	CML 177	1.934	6.991	14.162	43.3333
CIMMYT	8	CML 268	–0.577	9.781	15.653	53.3333
CIMMYT	9	CML 271	–0.907	6.548	13.719	46.6667
CIMMYT	10	6128	–7.558	5.399	14.275	130.0000
CIMMYT	11	6136	–8.355	6.511	14.744	100.0000
LOCAL	12	PMS 14	–5.543	4.608	14.599	46.6667
CIMMYT	13	6144	–3.600	4.866	13.211	90.0000
LOCAL (C8)	14	2001-8	–4.994	4.960	14.040	43.3333
LOCAL	15	GP 41	–2.708	4.314	13.288	56.6667
LOCAL	16	PMS 75	–10.864	2.701	13.351	40.0000
CIMMYT	17	8024	–1.537	4.308	13.614	53.3333
CIMMYT (QPM)	18	5020	–7.686	7.496	15.644	116.6667
LOCAL	19	PMS 99	–1.778	10.437	16.081	56.6667
LOCAL	20	PMS 100	–2.280	7.577	13.596	93.3333
LOCAL	21	PMS 116	–1.510	3.287	10.508	80.0000
K 517	22	1002-4	–8.441	4.664	13.163	100.0000
MEERTH (LOCAL)	23	M 8-3	–3.234	3.989	11.703	113.3333
SURYA	24	2007-3	–0.660	3.466	12.630	86.6667
CIMMYT	25	8042	–1.793	5.813	14.136	40.0000
CIMMYT	26	8044	–1.247	5.039	13.398	36.6667
LOCAL	27	PMS 134	–11.228	6.434	14.037	140.0000
MEERTH (LOCAL)	28	M 4	–3.390	4.991	12.374	73.3333
DMR	29	DMR 64-9	–2.593	6.818	13.797	60.0000
DMR	30	DMR 325	–0.770	4.130	13.227	28.3333
DMR	31	DMR 162	0.885	6.084	14.304	66.6667
DMR	32	DMR 164	–0.952	4.178	13.995	31.6667
CIMMYT	33	CML 78	–2.189	8.044	15.067	50.0000
CIMMYT	34	CML 373	–2.688	6.837	15.101	43.3333
CIMMYT	35	6066	–4.945	8.058	15.385	100.0000
CIMMYT	36	CML 399	–6.161	8.602	14.237	113.3333
CIMMYT	37	CML 333	–1.605	6.455	12.746	90.0000
LOCAL*	38	LETSEW	–0.721	6.633	13.502	63.3333
CIMMYT	39	CML 142	–1.192	6.419	11.643	56.6667
CIMMYT	40	CML 149	–5.727	10.274	16.859	100.0000
CIMMYT	41	CML 175	0.107	7.390	13.110	36.6667
CIMMYT	42	CML 384	0.324	7.846	13.241	53.3333
CIMMYT	43	CML 399	–6.725	7.400	11.656	180.0000
CIMMYT	44	6152	–5.045	8.353	15.307	113.3333
CIMMYT	45	CML 341	–0.217	5.882	13.748	50.0000
CIMMYT	46	CML 327	–6.072	6.390	12.604	100.0000

components (Table 3). Contribution of characters towards divergence was estimated through canonical vector method, where vectors of canonical roots represent the different axis of inbreds for graphical presentation. The important characters responsible for genetic diversity in the major axis of differentiation (vector I) were plant height, stem girth and number of leaves per plant with element value –0.531, 0.521 and 0.510, respectively. In vector II, which represent the second axis of differentiation, the important characters were days to tasseling and ear height with element value 0.629 and –0.607, respectively. The important character for vector III was days to maturity which had

element value 0.771. These characters played important role on respective axis and indicated as important component traits of genetic diversity (Azad *et al.* 2012). So, emphasis should be given to these contributing characters while selection of parents for hybridization programme.

Further, the vectors values were used as principal factor scores for 3D representation of individual inbred lines. The spatial distance between individual genotypes were measured as genetic divergence. Three principal factor scores were used to plot all the inbred lines in 3D diagram for most contributing characters for diversity (Table 4). Similar type of study was also carried out by More *et al.* (2006) in forage maize, who reported that leaf area per plant, plant height and days to 50% flowering were the major contributor towards divergence.

The results of present investigation suggested that hybridization between inbreds from diverse clusters will show good performance and may help in obtaining high yielding genotypes. As result showed, there was no association between geographical origin and clustering pattern of the genotypes, the hybridization programme need not necessarily be based on geographical diversity, the genetic diversity may play important role for the purpose. The inbreds which are diversely related can be utilized as parents in crossing programme to isolate desire hybrids for yield and component characters, like plant height, stem girth and number of leaves per plant. These component characters had contributed maximum towards total divergence in the present investigation. 3D plotting of different inbred lines based on PCA scores and Euclidean distant matrix suggested that PMS134 was most diverse parent with CML 324 followed by M 8-3, CML 158, 6152 and CML 399. Thus these diverse lines should be chosen for crossing in maize breeding programme which will likely to produce wide variability and transgresive segregations.

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