

Genetic diversity among germplasm lines of maize studied under coastal ecology

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Abstract: The research work was undertaken to identify the extent of genetic diversity in 112 maize inbred lines for eighteen characters in alpha lattice design with two replications at Agricultural Research Station, Peddapuram, Kakinada during *rabi*, 2022-23. An analysis of variance of the data revealed significant genetic differences among the inbred lines for 18 characters under study. D² analysis highlights the significant contribution of kernel yield per plant, ear yield per plant, kernel rows per ear, number of kernels per row, ear height, days to 50% silking, ear position, tassel extrusion and tassel size towards overall divergence. This emphasizes the importance of these traits in selecting appropriate parents for hybridization programs. It was found that inbred lines from clusters III (CML 451), VI (PI 415, PI 424), VII (CL 02450) and VIII (PI 416) could be vital for such programs. The inbred lines PI 403, PL22398, PI 415, PI 424, PI 426, CML 451, CL 02450 are far apart from each other in the two dimension and three-dimension diagrams of principal component analysis. Hence, CML 451, PI 415, PI 424 and CL 02450 were found to be with sufficient genetic diversity in the studied lines as they have shown high inter cluster distances in D² and they are also far apart from each other in the two-dimensional and three-

dimensional graphs based on PCA scores may result in good F₁ combinations to explore the heterosis or to produce transgressive segregants in their respective F₂ and subsequent segregating generations.

Keywords: Cluster means · D² analysis · Genetic divergence · PCA

Introduction

Maize (*Zea mays* L.), ‘queen of cereal’, is one of the major cereals cultivated throughout the world after rice and wheat. Maize kernels contain 60 to 68 per cent starch and 7 to 15 per cent protein. Maize production in India has steadily increased over the years, especially with the development and release of heterotic hybrids at the beginning of the 21st century. Further, the focus on high-yielding hybrids has resulted in significant advancements in productivity. However, there is a need to develop varieties/hybrids suitable to varied environmental challenges *i.e.*, climate change and emergence of varied biotic and abiotic stresses and agro ecosystems. Evaluating the germplasm/inbreds for new traits along with important yield traits is need of the hour to select the parents and their utilization in plant breeding programmes.

The ability to pick genetically diverse parents is made possible by the quantification of genetic diversity using biometrical procedures. Crosses between genetically dissimilar parents yield more heterosis than crosses between closely related parents (Moll and Stuber, 1971). As a result, crop improvement programmes require genetically diverse parents for the initiation of breeding programmes. For the examination of genetic diversity, Mahalanobis D² statistic is one of the finest ways for

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measuring genetic diversity in a crop species. This assists in the grouping of germplasm/inbred lines into appropriate groups for future breeding operations, as genetically diverse genotypes are known to exhibit high hybrid vigour. Principal component analysis (PCA) is a multivariate statistical method used to identify and extract the most significant axes of variation in a data set. It calculates canonical vectors or roots, which represent these axes, along with their corresponding values, indicating the amount of variance accounted by each axis. The term “principal component” roots from the fact that the technique highlights the primary contributors to the overall variation.

Genetic diversity is essential for maize inbred lines to facilitate the development of improved varieties with desirable traits. Maize breeders rely on genetic diversity within inbred lines to select and combine alleles that confer traits such as high yield and other morphological traits. In essence, genetic diversity serve as fundamental tools for maize breeders to develop cultivars that meet the diverse needs of farmers and consumers while addressing the challenges of modern agriculture. Therefore this experiment was conducted to understanding the relationships among maize inbred lines based on morphological and molecular levels helps to identify genotypes with maximum diversity and their exploitation in breeding programmes can significantly contribute to improving maize productivity sustainably.

Materials and methods

The field experiment was conducted at Agricultural Research Station, Peddapuram, Kakinada during *rabi*, 2022-23 to study the genetic divergence among 112 maize inbred lines in alpha lattice design with two replications. Each plot consisted of two rows of four meters each genotype, spaced 60 cm row to row and 20 cm plant to plant distance. Software R statistics was used to analyze the data collected on eighteen traits. The data was recorded on five plants taken from each replication on Days to 50% anthesis, Days to 50% silking, Anthesis silking interval, Plant height (cm), Tassel height (cm), Tassel extrusion, Tassel size, Ear height (cm), Ear position, Ear length (cm), Ear girth (cm), Days to maturity, Kernel rows per ear, Number of kernels per row, Shelling percentage, 100-kernel weight (g), Ear yield per plant (g) and Kernel yield per plant (g). D^2 analysis as per

Mahalanobis (1936) by Tocher's method, Principal component analysis by ward's minimum variance method through INDOSTAT software.

Results and discussion

D² cluster analysis

The 112 inbred lines were grouped into eight clusters by using Tocher's method. Clustering pattern of inbred lines under this study reveals that the inbred lines showed considerable genetic diversity among themselves by occupying eight different clusters. The distribution of inbred lines into eight clusters is presented in Table 1 and illustrated in Figure 1. Cluster I had the highest number i.e., 96 inbred lines showing those lines are genetically similar while, cluster II had seven inbred lines showing high genetic resemblance among genotypes of this group. The clusters, III, V, VII and VIII had single inbred line i.e., solitary clusters, The formation of distinct solitary clusters may be due to geographic barriers preventing gene flow otherwise intensive natural and artificial selection for diverse and adaptable gene complexes while cluster IV had three inbred lines. The cluster VI had two inbred lines. Similar results pertaining to a grouping of maize germplasm were reported by Ivy *et al.* (2007); Talukder *et al.* (2012); Kumari *et al.* (2017).

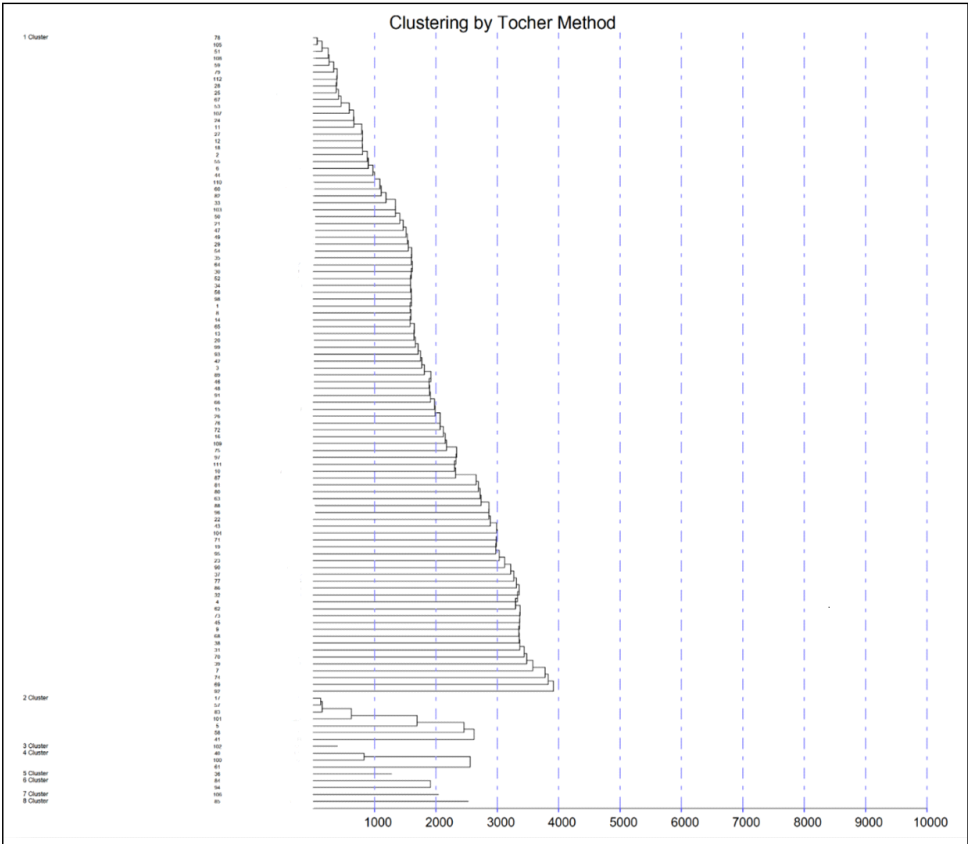
The eight clusters intra and inter-cluster distances are presented in Table 2. Intra-cluster average D^2 values ranged from 0 to 53.24 (Figure 2). Among the clusters, cluster IV had the maximum intra-cluster distance (53.24) while, the minimum was recorded in cluster II (46.08). The intra cluster distance was zero in clusters III, V, VII and VIII as they had single inbred line each. The inbred lines of these solitary clusters may be unique and useful in plant breeding programme. The minimum inter-cluster D^2 values were recorded between the clusters III and IV (38.15) followed by II and VII (74.83). The highest D^2 value was recorded in-between the clusters VI and VII (273.04) followed by the clusters II and VI (236.25) (Table 3).

In the present study as maximum inter-cluster distance was found between cluster VI and VII (273.04) followed by cluster II and VI (236.25) indicating their usefulness in the exploitation of heterosis. Similar trend of inter cluster and intra-cluster distances were reported by Singh *et al.* (2020) and Deepak *et al.* (2023).

Table 1. Clustering pattern by Tocher’s method in inbred lines of maize (*Zea mays* L.)

Cluster Group	No. of inbred lines	List of inbred lines
I	96	PI UK, PL 22424, PL 22444, PL 22392, PI 404, PL 22439, PI 417, PL 22429, PL 22440, PL 22397, PI 405, PL 22409, PI 418, PL 22394, PL 22389, PL 22448, PL 22435, PL 22441, PI 419, PL 22396, PL 22408, PL 22399, PL 22398, PL 22411, PI 420, PL 22427, PL 22426, PL 22393, PL 22395, PL 22437, PI 421, PL 22421, PL 22449, PL 22417, PL 22414, PL 22430, PI 422, PL 22407, PL 22445, PL 22432, PL 22413, PL 22450, PI 423, PI 399, PL 22422, PL 22404, PL 22425, PL 22442, PI 425, PI 400, PL22428, PL 22416, PL 22420, PL 22447, PI 426, PI 401, PL 22431, PL 22402, PL 22400, PL 22410, PI 427, CML 451, PL 22434, PI 394, PL 22406, PL 22446, PI 428, CML 474, PL 22415, PI 395, PL 22433, PL 22403, CML 581, PL 22405, PI 1, PL 22443, PL 22438, CML582, LM 13, PI 397, PL 22419, PI 406, LM14, PL 22418, PI 407, BML 6, PL 22391, PI 408, BML 7, PI 409, BML 45, PI 410, CAL1812, PI 411, PI 412, PI 413
II	7	PL 22401, PL 22390, PI 414, CML 425, PL 22436, PL 22412 & PI 403
III	1	CML 451
IV	3	PI 402, UMI 1200 & PL 22423
V	1	PI 398
VI	2	PI 415, PI 424
VII	1	CL 02450
VIII	1	PI 416

Figure 1. Dendrogram showing relationship based on Mahalanobis’ D² values in eight clusters among 112 inbred lines of maize (*Zea mays* L.)



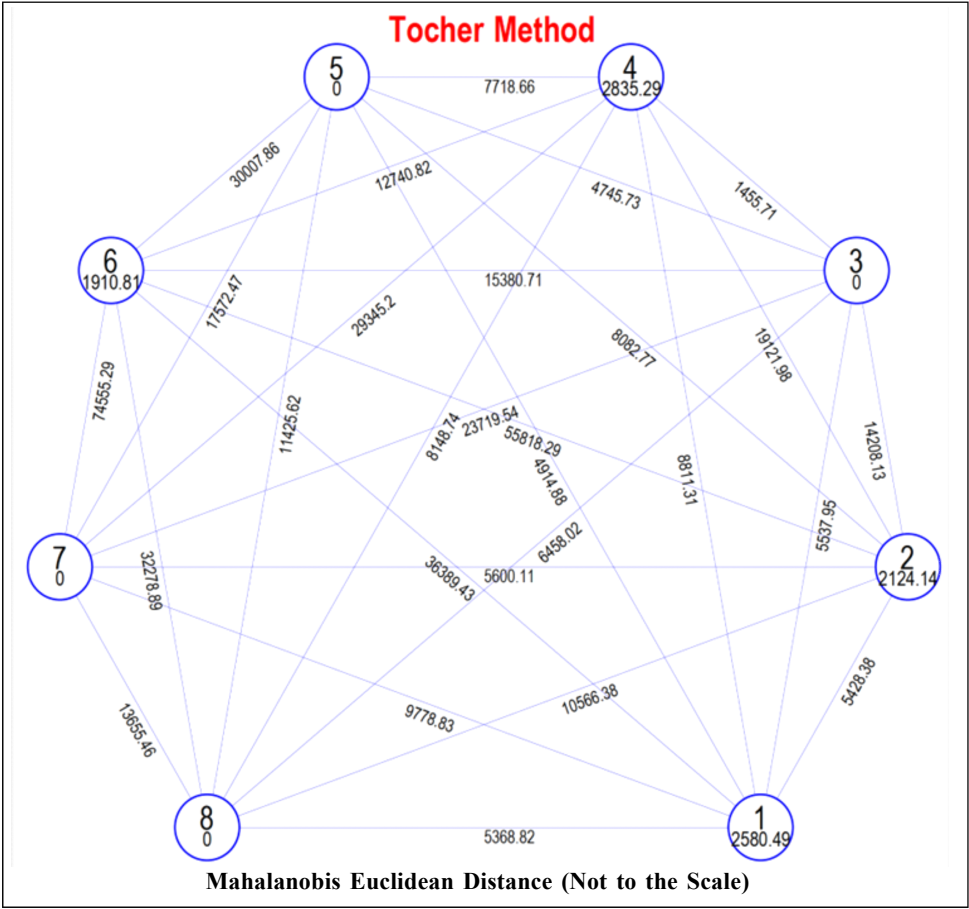
The cluster means for each of 18 characters are furnished in Table 4. The cluster means for days to 50% anthesis ranged from 61 (Cluster VI) to 69.50 (Cluster VII). Days to 50% silking ranged from 62 (Cluster VI) to 72.5 (Cluster V). Anthesis-silking interval ranged from

0.50 (Cluster VII) to 5.00 (Cluster V). Plant height ranged from 122.86 (Cluster II) to 191.25(Cluster VI). For tassel height, cluster means ranged from 29.50 (Cluster V) to 36 (Cluster III). Similarly, Tassel extrusion ranged from 1.87(Cluster II) to 2.35(Cluster III, V, VI, VII, VIII), for

Table 2. Average intra (bold) and inter-cluster D2 distance among 8 clusters of 112 maize inbred lines (*Zea mays* L.)

Cluster Distances								
	I	II	III	IV	V	VI	VII	VIII
I	2580.49 (50.79)	5428.38 (73.67)	5537.95 (74.41)	8811.31 (93.86)	4914.88 (70.10)	36389.43 (190.76)	9778.83 (98.88)	5368.82 (73.27)
II		2124.14 (46.08)	14208.13 (119.19)	19121.98 (138.28)	8082.77 (89.89)	55818.29 (236.25)	5600.11 (74.833)	10566.38 (102.79)
III			0.00 (0.00)	1455.71 (38.15)	4745.73 (68.88)	15380.71 (124.01)	23719.54 (154.00)	6458.02 (80.36)
IV				2835.29 (53.24)	7718.66 (87.855)	12740.82 (112.87)	29345.20 (171.304)	8148.74 (90.27)
V					0.00 (0.00)	30007.86 (173.22)	17572.47 (132.56)	11425.62 (106.89)
VI						1910.81 (43.71)	74555.29 (273.04)	32278.89 (179.66)
VII							0.00 (0.00)	13655.46 (116.85)
VIII								0.00 (0.00)

Figure 2. Intra and inter-cluster distances in eight clusters based on Tocher’s method in maize (*Zea mays* L.)



tassel size, the cluster means ranged from 1.58 (Cluster II and III) 1.58. Whereas the ear height ranged from 42.93 (Cluster II) to 83.75 (Cluster VI) and ear position ranged from 0.33 (Cluster V) to 0.50 (Cluster VIII). The cluster mean for ear length ranged from 8.57(Cluster II)

to 15.34 (Cluster VI). The cluster mean for ear girth ranged from 11.0 (Cluster VII) to 15.88(Cluster VI). The cluster mean for Days to maturity ranged from 89 (Cluster VII) to 127.50 (Cluster V) and for Kernel rows per ear ranged from 12.00 (Cluster VIII) to 14.85 (Cluster

Table 3. The nearest and farthest clusters from each cluster based on D values in Maize inbred lines (*Zea mays* L.)

Cluster No	Nearest cluster with D values	Farthest cluster with D values
I	V (70.10)	VI (190.76)
II	VII (74.833)	VI (236.25)
III	IV (38.15)	VII (154.00)
IV	III (38.15)	VII (171.304)
V	III (68.89)	VI (173.22)
VI	IV (112.88)	VII (273.04)
VII	II (74.83)	VI (273.08)
VIII	I (73.2)	VI (179.6)

VI). The 100-Kernel weight ranged from 24.8 (Cluster VI) to 35.85 (Cluster VIII). The cluster mean for number of kernels per row ranged from 18.15 (Cluster V) to 33.53 (Cluster VI). Similarly, cluster mean for shelling percentage ranged from 465.34(Cluster VIII) to 78.90 (Cluster III). Likewise, the cluster mean for ear yield per plant ranged from 62.70 (Cluster VII) to 233.00 (Cluster VI). The cluster mean for Kernel yield per plant ranged from 42.50 (Cluster VII) to 187.50 (Cluster VI).

Based on the cluster means (Table 5), important clusters were cluster III (CML 451) for tassel height, tassel extrusion and shelling percentage. Cluster VI (PI 415, PI 424) is important for desired days to 50% anthesis, days to 50% silking, plant height, tassel size, ear height, ear length, ear girth, kernel rows per ear, number of kernels per row, ear yield per plant and kernel yield per plant. As a result, inbred lines from clusters VI may be used as a parent in hybridization programme to enhance these traits. Cluster VIII (PI 416) should be chosen for the improvement of ear position and 100-kernel weight. Cluster VII (CL 02450) is important for anthesis-silking interval and days to maturity. This indicates that this genotype may be used in breeding for earliness. Similar kind of results were reported by Kumar *et al.* (2015); Dubey *et al.* (2001) and Deepak *et al.* (2023).

Relative contribution of individual characters towards divergence

The characters appeared in the first rank contribute more to towards diversity. Among all the characters, Kernel

Table 4. Cluster means of eight clusters estimated by Tocher's method in 112 maize inbred lines (*Zea mays* L.)

cluster	Days to 50% anthesis	Days to 50% silking	Anthesis silking interval	Plant height (cm)	Tassel height (cm)	Tassel extrusion	Tassel size	Ear height (cm)	Ear position
I	65.60	67.46	1.87	159.45	31.54	2.24	1.83	65.55	0.41
II	63.21	65.71	2.50	122.86	31.21	1.87	1.58	42.93	0.35
III	64.00	67.50	3.50	170.00	36.00	2.35	1.58	72.50	0.43
IV	64.33	65.33	1.00	171.67	30.33	2.12	1.83	83.33	0.48
V	69.50	72.50	5.00	145.00	29.50	2.35	1.82	47.50	0.33
VI	61.00	62.00	1.00	191.25	35.00	2.35	2.32	83.75	0.44
VII	67.00	68.00	0.50	152.50	34.50	2.35	2.12	60.00	0.40
VIII	63.00	64.00	1.00	167.50	31.50	2.35	2.12	82.50	0.50

Table 4. contd...

Cluster	Ear length (cm)	Ear girth (cm)	Days to maturity	Kernel rows per ear	No. of kernels per row	Shelling percentage	100 kernel weight (g)	Ear yield per plant (g)	Kernel yield per plant (g)
I	11.27	11.96	122.14	12.81	23.10	76.00	27.06	88.03	71.88
II	8.57	11.95	120.86	12.42	18.52	72.00	25.53	75.04	64.21
III	12.67	13.17	122.50	13.50	28.85	78.90	24.80	136.00	113.00
IV	12.14	12.83	120.17	12.62	25.50	74.79	33.22	148.33	121.17
V	10.09	12.17	127.50	12.35	18.15	69.20	26.50	99.35	74.00
VI	15.34	15.88	117.00	14.85	33.53	76.25	35.15	233.00	187.50
VII	11.00	11.00	89.00	13.50	20.50	77.80	26.20	62.70	42.50
VIII	10.84	12.75	119.00	12.00	22.70	65.34	35.85	128.00	48.50

Table 5. Mean performance of top four clusters with selected inbred lines for hybridization and promising characters in inbred lines using Tocher's method in maize (*Zea mays* L.)

S. No.	Cluster no.	Selected inbred lines for hybridization	Promising characters in inbred lines
1	VI	PI415, PI 424	Days to 50% anthesis, days to 50% silking, plant height, tassel size, ear height, ear length, ear girth, kernel rows per ear, number of kernels per row, ear yield per plant, kernel yield per plant
2	III	CML451	Tassel height, tassel extrusion, shelling percentage
3	VII	CL 02450	Anthesis-silking interval and days to maturity
4	VIII	PI416	Ear position, 100-kernel weight

yield per plant (g) contributed the maximum (14.68%) to the diversity by taking the first rank in 920 times, followed by Ear yield per plant (g) (13.22%) with 828 times ranked first, Kernel rows per ear (9.78%) with 613 times ranked first and Ear height (cm) contributed 8.99% with 563 times ranked first. Number of kernels per row (6.26%) with 392 times ranked first (Table 6).

Other characters *viz.*, days to 50% anthesis, days to 50% silking, anthesis-silking interval, plant height (cm), tassel height (cm), tassel extrusion, tassel size, ear length (cm), ear position, ear girth (cm), days to maturity, shelling percentage, 100-kernel weight (g) contributed 3.18, 5.30, 2.95, 4.96, 6.00, 5.68, 5.67, 2.70, 4.50, 2.84, 1.36, 1.04 and 0.89 per cent, respectively, to the total genetic diversity. Similar kind of results were reported by Amin *et al.* (2013); Maruthi *et al.* (2015) and Lone *et al.* (2017).

Principal component analysis

In the present study principal component analysis (PCA) was used to validate the clustering pattern obtained by Mahalanobis D^2 statistic. Eigen values, variance (%) and cumulative variance (%) by the principal components (PCs) in maize inbred lines evaluated were given in Table 7. The results suggested the importance of the first seven PCs with Eigen values greater than or equal to 1.0 in discriminating the collection.

The results revealed that seven canonical roots accounted for 74.66 per cent of total divergence. PC_2 contributed maximum towards divergence (38.10%) with Eigen value of 2.93. The first, third and fourth canonical vectors contributed 23.44, 9.10 and 7.40 per cent to total divergence, respectively. The remaining canonical roots *viz.*, fifth, sixth and seventh, accounts for 7.03, 6.54 and 5.67 per cent, respectively to total variability.

The scores of PCA with respect to the studied 112 inbred lines for the 1st three PCs were listed in Table 7.

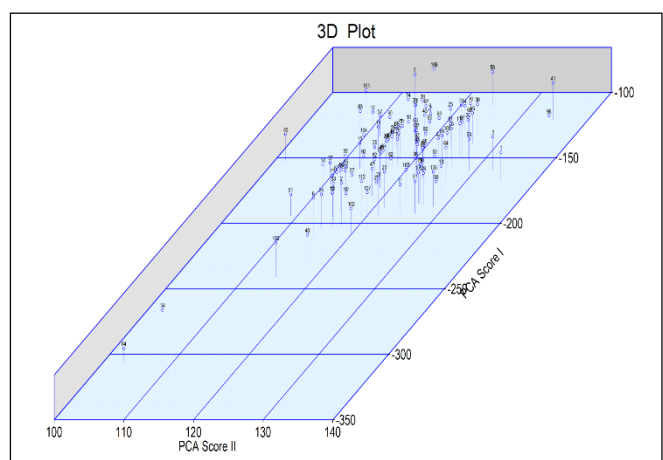
Table 6. The relative contribution of 18 characters to genetic diversity in 112 maize inbred lines (*Zea mays* L.)

Source	Contribution %	Times ranked 1st
Days to 50% anthesis	3.18	199
Days to 50% silking	5.30	332
Anthesis silking interval	2.95	185
Plant height (cm)	4.96	311
Tassel height (cm)	6.00	376
Tassel extrusion	5.68	356
Tassel size	5.67	355
Ear height (cm)	8.99	563
Ear position	4.50	282
Ear length (cm)	2.70	169
Ear girth (cm)	2.84	178
Days to maturity	1.36	85
Kernel rows per ear	9.78	613
Number of kernels per row	6.26	392
Shelling percentage	1.04	65
100-kernel weight (g)	0.89	56
Ear yield per plant (g)	13.22	828
Kernel yield per plant (g)	14.68	920

These scores of PCA were employed in plotting two-dimensional (Figure 3) and three-dimensional scatter diagrams (Figure 4) for the studied inbred lines. The combination with the diverse inbred lines numbered as 41 (PI 403), 45 (PL22398), 84 (PI 415), 94 (PI 424), 96 (PI 426), 102 (CML 451), 106 (CL 02450) which are far apart from each other in the two dimension and three-dimension diagrams may result in good F_1 combinations to explore the heterosis or to produce transgressive segregants in their respective F_2 and subsequent segregation generation.

Similar usage of PCA for obtaining 2D & 3D diagrams and in turn to understand the genetic diversity was earlier employed in maize by Javaid *et al.* (2023); Yadesa *et al.* (2022) and Devaux *et al.* (2023).

	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅	PC ₆	PC ₇
Eigene Value (Root)	4.68983	2.93035	1.98031	1.48174	1.40767	1.30957	1.13438
Variability %	23.44916	14.65172	9.90157	7.40869	7.03835	6.54787	5.67188
Cumulative variability %	23.44916	38.10088	48.00244	55.41113	62.44947	68.99734	74.66923
Characters							
Days to 50% anthesis	0.02813	0.50269	0.10195	0.19072	0.19162	0.04122	0.14434
Days to 50% silking	0.05670	0.52890	0.10562	-0.02767	0.10543	0.17124	0.08280
Anthesis silking interval	0.07538	0.10801	0.01436	-0.54213	-0.21306	0.35098	-0.10385
Plant height (cm)	-0.33438	0.05300	0.28948	0.12415	0.00295	0.04971	0.00613
Tassel height (cm)	0.02088	0.21963	-0.36696	0.27223	-0.12900	-0.13026	-0.32444
Tassel extrusion	-0.14940	-0.01371	0.18450	-0.27318	0.24616	-0.42280	0.33085
Tassel size	-0.07785	0.04747	-0.38233	0.14787	-0.09863	0.32178	-0.24287
Ear height (cm)	-0.34331	0.08368	0.29949	-0.07282	0.12644	-0.00640	-0.38349
Ear position	-0.21086	0.08060	0.19325	-0.27081	0.17252	-0.06128	-0.60488
Ear length (cm)	-0.33992	0.08328	0.01609	0.27407	-0.18633	-0.13371	0.09470
Ear girth (cm)	-0.27036	-0.02528	-0.35943	-0.24318	0.26110	0.15696	0.08707
Days to maturity	-0.00689	0.37379	0.08548	-0.14997	-0.15744	0.32913	0.24632
Kernel rows per ear	-0.05810	0.14733	-0.45273	-0.33692	0.24570	-0.24113	0.11851
Number of kernels per row	-0.32924	0.14264	-0.13600	0.08601	-0.22956	-0.27354	0.00547
Shelling percentage (%)	-0.06527	-0.01214	0.13019	-0.18213	-0.61924	-0.07634	0.11490
100 kernel weight (g)	-0.22356	-0.23010	0.16889	0.21696	0.20342	0.39156	0.15769
Ear yield per plant (g)	-0.40866	-0.05836	-0.16761	0.00244	0.03333	0.12977	0.11088
Kernel yield per plant (g)	-0.38498	-0.04180	-0.13621	-0.02838	-0.10339	0.16049	0.18075



The research of the present study revealed that, the cluster VI and VII (273.04) followed by cluster II and VI (236.25) had the greatest inter cluster distance indicating that the inbred lines included in these cluster pairs have a more diverse genetic composition. The selection of parents for a future breeding programme from these

clusters would result with higher manifestation of heterosis in hybrids and broad heterogeneity in later. The genotypes CML 451, PI 415, PI 424 and CL 02450 were identified as high yielding and divergent genotypes based on cluster analysis and per se performance and they are also far apart from each other in the two-dimensional and three-dimensional graphs based on PCA scores. Hence, these varieties may result in good F_1 combinations to explore the heterosis or to produce transgressive segregants in their respective F_2 and subsequent generation.

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