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Genetic diversity study among maize (*Zea mays* L.) inbred lines

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Abstract: Twenty-five maize inbred lines were evaluated during *rabi* 2020-21 at AICRP on maize, Dharwad to assess the genetic diversity for different quantitative characters. The principal component analysis (PCA) identified first two principle components with more than 1.0 eigenvector and cumulatively explained 73.0 % of the total variance. The Tocher's cluster analysis was worked out wherein; the 25 inbred lines were grouped into eight different clusters. Cluster IV had a maximum of five inbred lines, while cluster VIII had only one entry. Mahalanobis D^2analysis was performed to know inter and intra cluster distances. Cluster VII displayed maximum intra cluster distance of 133.59 among the clusters.The inter cluster distance was maximum with D^2 value of 649.90 between cluster III (VL 105554, KL 154690, VL 162563 and VL 18329) and cluster VIII (CI-4) followed by cluster V (VL 143906, VL 1110195, VL 109126 and VL 174449) and VIII (CI-4). It was suggested to intercross inbred lines from diverse cluster III, V, and VIII in order to develop superior hybrids with maximum heterosis. Among the eleven quantitative traits studied, days to 50 per cent silking contributed maximum of 24 % to the total divergence followed by grain yield and hundred grain weight.

Key words: Cluster, Genetic diversity, Hybrids

Introduction

Maize (*Zea mays* L.) is one of the leading food, feed and industrial crop in the world as well as in India and has shown wider adaptability across majority of the environments, because of its broad genetic base. Because maize is a highly cross-pollinated crop, breeders have been able to exploit heterosis commercially. They have also demonstrated heterosis benefits for increasing corn yield.The development of high-yielding single cross hybrids resistant to biotic and abiotic stresses is the main priority area of maize breeding and it is a continuous process. The hybrids generated from the diverse parents exhibit high heterosis (Makumbi *et al*., 2011). Thus, the selection of diverse inbred lines is a fundamental step in the development of hybrids. Knowledge of diversity in the germplasm lines has a significant impact on crop improvement (Hallauer *et al*., 2010).

The genetic diversity helps to identify diverse inbred lines and assist maize breeders to formulate the hybrid breeding programme to maximize the heterosis. Multivariate analysis such as cluster analysis and principal component analysis has been extensively used in diversity study. Genetic clustering of germplasm lines helps breeders to identify diverse lines. Usually, maximum diversity is expected between the lines which belong to different clusters, while inbred lines from the same cluster are expected to have low diversity (Nyombayire *et al*., 2016). D^2 analysis is the most useful technique in the analysis of genetic divergence among the inbred lines,which provides the estimation of inter and intra-cluster distances.

In Principle Component Analysis (PCA), observations are analyzed by several inter-correlated quantitative variables. The aim of PCA is to extract the essential details from the table, to represent it as a set of new orthogonal variables called the principal components, and to display the pattern of similarity of the observations and of the variables as points. PCA is a method of describing the pattern of variation among characters in an individual. This method eliminates inter-correlation among variables in multivariate data and plots a multi-dimensional relationship on two or three principal axes (Nwangburuka *et al*., 2011). The present investigation was carried out to estimate the magnitude of the genetic diversity among 25 maize inbred lines using cluster and the principle component analysis.

Material and methods

The material for the present study consisted of 23 lines selected based on their resistant reaction to turcicum leaf blight from TLB screening nursery along with two inbred checks CM-111 (flint type) and CM-202 (dent type) which have been described in Table 1. These 25 lines were evaluated in randomized complete block design (RCBD) with three replications during *rabi,* 2020-21 at All India Co-ordinated Maize Improvement Project, MARS, Dharwad. Each entry was raised in two rows of four-meter length with a spacing of 60 x 20 cm and following all recommended package of practice.

The observations were recorded from each inbred lines for eleven quantitative traits *viz.,* days to 50 per cent tasseling, days to 50 per cent silking, days to 75 per cent dry husk, plant height (cm), ear height (cm), number of kernel rows per cob, number of kernels per row, cob girth (cm), cob length (cm), hundred grain weight (g) and grain yield (q/ha). The data was analyzed using Mahalanobis D^2 statistic using dist function in R studio. All the genotypes were grouped into different clusters with the help of Tocher's method using toc function. The prcomp function was used to perform,the PrincipleComponent Analysis (PCA) and the biplot function was used to plot PC1 *vs* PC2.

Results and discussion

Several studies on maize have shown that inbred lines from diverse genetic bases to be more productive than crosses of inbred lines derived from closely related stocks. Genetic

Table 1. Description and source of maize inbred lines used for the investigation

Sl. No.	Inbreds	Source
1	IMIC-02	CIMMYT
2	IMIC-40	CIMMYT
3	IMIC-68	CIMMYT
4	IMIC-69	CIMMYT
5	IMIC-73	CIMMYT
6	IMIC-87	CIMMYT
7	CTLB-01	CIMMYT
8	CTLB-02	CIMMYT
9	CML-451	CIMMYT
10	CI-4	AICRP on maize, MARS, UAS Dharwad
11	CM-202	AICRP on maize, MARS, UAS Dharwad
12	CM-111	AICRP on maize, MARS, UAS Dharwad
13	VL 109126	CIMMYT
14	ZL 153493	CIMMYT
15	VL 105554	CIMMYT
16	VL 143906	CIMMYT
17	VL 18448	CIMMYT
18	ZL 14501	CIMMYT
19	VL 18321	CIMMYT
20	VL 174449	CIMMYT
21	VL 18329	CIMMYT
22	VL 1110195	CIMMYT
23	KL 154690	CIMMYT
24	VL 19190	CIMMYT
25	VL 162563	CIMMYT

diversity is a prerequisite for any crop improvement programme because hybrids between genotypes with diverse genetic backgrounds show greater heterosis and produce a greater proportion of recombinants than those between closely related parents (Laude and Carena, 2015). A genetic diversity assessment for inbred lines of maize is important for the development of high-yielding hybrids, as genetically diverse parents have been shown to produce high heterotic effects. Further, the study of genetic divergence in the maize inbred lines will help to determine the real potential of the genotype.

The ANOVA for different quantitative traits among the inbred lines differed significantly (Table 2) indicating that there was genetical difference among the germplasm material chosen. Among the different traits cob girth showed lower variation followed by number of kernel rows per cob. Whereas, plant height followed by grain yield recorded higher variation. The diversity analysis among these twenty-five genotypes was able to group these genotypes into 8 different clusters. Cluster IV consists maximum of 5 inbred lines, followed by clusters II, III and V of 4 inbred lines each, cluster VI (3), clusters I and VII consisted two inbred lines. Whereas, cluster VIII was solitary (Table 3). The study found that inbred lines possessed considerable genetic diversity among themselves, as they were distributed among eight different clusters. These eight clusters were differing genetically among themselves, even within the cluster inbred lines were varying genetically which is indicated by their inter and intra cluster distance calculated by Mahalanobis D^2 statistics and discussed briefly below.

The inter-cluster distance varied from 0.00 (Cluster VIII) to 133.59 (Cluster VII).Intra cluster distance is maximum in cluster VII (D^2= 133.59) followed by cluster VI (D^2= 99.51) and cluster IV (D^2= 99.32). These higher intra cluster values indicate the presence of divergence among the inbred lines in the same cluster. The inter-cluster D^2 values also ranged widely with a minimum value of 128.31 between clusters II and VII to a maximum value of 649.90 between clusters III and VIII indicating high diversity among the genotypes of different clusters (Table 4). It is desirable to select the genotypes from the cluster showing the high inter-cluster distance in the breeding programme for obtaining more heterosis. Based on the inter-cluster values nearest and farthest clusters were worked out and listed in Table 5. The inbred CI-4 in cluster VIII was most divergent with many inbred lines, especially with inbred lines of cluster III (649.90), cluster V (543.50) and cluster II (351.55),

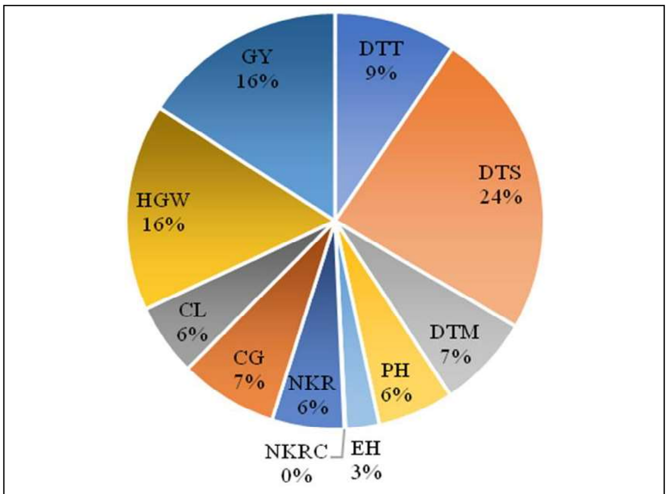

Fig 1. Percent contribution of each character to divergence

Table 2. Analysis of variance for yield and yield attributes in maize inbred lines

Traits	df	DTT	DTS	DTM	PH	EH	NKRC	NKR	CG	CL	HGW	GY
Replications	2	4.96	0.85	0.57	59.92	74.89	0.13	1.38	0.03	0.74	2.09	9.26
Genotypes	24	111.87**	93.56**	21.63**	1090.53**	535.23**	4.84**	59.51**	0.51**	10.41**	54.78**	878.25**
Error	48	3.62	1.08	0.74	95.22	34.3	0.43	2.08	0.01	0.43	1.54	17.05
CV (%)	-	2.64	1.42	0.82	7.35	9.65	4.17	6.24	3.41	4.86	4.08	13.91
C.D. @ 5%	-	3.12	1.71	1.41	15.85	8.49	0.94	2.54	0.21	1.09	2.04	10.59

*- Significant at 5 % and **- Significant at 1 % level of probability

DTT - Days to 50 per cent tasseling
DTS -Days to 50 per cent silking
DTM - Days to 75 per cent dry husk
PH - Plant height
EH - Ear height
NKRC Number of kernel rows per cob
NKR - Number of kernels per row
CG - Cob girth
CL - Cob length
HGW -Hundred grain weight
GY - Grain yield

Table 3. Distribution of 25 maize inbred lines into eight clusters based on D^2 statistics (Tocher's Method)

Cluster	No. of genotypes	Genotypes
I	2	VL 18448, VL 174449
II	4	CM-111, ZL 14501, CM-202, CTLB-02
III	4	VL 105554, KL 154690, VL 162563, VL 18329
IV	5	IMIC-02, VL 19190, IMIC-69, IMIC-40, IMIC-87
V	4	VL 143906, VL 1110195, VL 109126, ZL 153493
VI	3	CTLB-01, VL 18321, IMIC-68
VII	2	IMIC-73, CML-451
VIII	1	CI-4

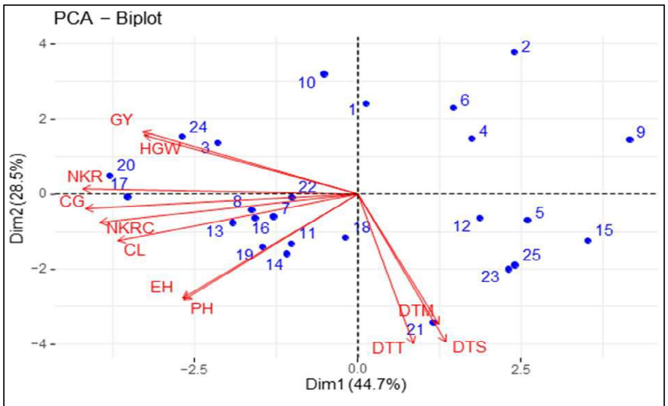

Fig 2. Scatter diagram of 25 genotypes of maize inbred lines

Table 4. Intra (bold face) and Inter cluster distance of 25 maize inbred lines

Cluster	I	II	III	IV	V	VI	VII	VIII
I	14.88	216.21	402.40	166.04	137.66	138.02	371.69	299.22
II		68.86	144.06	270.33	256.44	153.66	128.31	351.55
III			66.37	448.28	276.01	309.05	183.22	649.90
IV				99.32	291.49	170.28	268.14	149.98
V					99.00	235.95	384.64	543.50
VI						99.51	218.77	221.62
VII							133.59	344.78
VIII								0.00

Table 5. Nearest and farthest cluster based on inter cluster distance (D^2)

Cluster Number	Nearest Cluster	Farthest Cluster
I	V (137.66)	III (402.40)
II	VII (128.31)	VIII (351.55)
III	II (144.06)	VIII (649.90)
IV	VIII (149.98)	III (448.28)
V	I (137.66)	VIII (543.50)
VI	I (138.02)	III (309.05)
VII	II (128.31)	V (384.64)
VIII	IV (149.98)	III (649.90)

- values in the parenthesis are inter cluster distances (D^2)

as the genotypes from these three clusters were farther from cluster VIII. Similarly, cluster III is the farthest cluster from cluster IV (448.28), cluster I (402.40) and cluster VI (309.05). While the genotypes from cluster VII and cluster V (384.64) were more diverse. Hence, crossing the inbred lines from these farthest clusters would result in higher heterosis. These results are in confirmatory with the findings of Kumar *et al.* (2015), Sood *et al.* (2017) and Singh *et al.* (2019).

Among the eleven traits days to 50 per cent silking contributed more to diversity (24%) followed by grain yield and hundred grain weight contributing at 16 % each. Whereas, number of kernel rows per cob contributed least for the divergence indicating the inbred lines were not varying much for this trait (Fig 1). The divergence of cluster VIII and cluster III would be explained to a certain extent by the cluster mean values of various traits (Table 6). The cluster mean values reveals that cluster VIII has the lowest mean value for days to 50 per cent silking and days to 50 per cent tasseling compared to other clusters which are contributing combinedly 33 % to the total divergence, hence this might be the reason for the CI-4 being a single genotype in cluster VIII and diverse from other clusters. Likewise, cluster III had the lowest mean value for grain yield and highest mean for days to 50 per cent silking and days to 50 per tasseling (Table 6) which made it to be more diverse from cluster VIII and other clusters. Considering the mean values, cluster I, V and VI had good performance for

Table 6. Cluster mean of eleven quantitative characters

Traits	I	II	III	IV	V	VI	VII	VIII
Days to 50 per cent tasseling	69.25	73.88	79.38	65.27	77.96	70.56	72.50	57.67
Days to 50 per cent silking	70.17	74.79	81.50	67.00	77.38	71.00	73.17	62.00
Days to 75 per cent dry husk	102.17	104.08	108.25	102.80	105.58	107.22	106.00	102.67
Plant height (cm)	162.93	140.20	132.04	113.75	138.98	131.62	112.07	114.93
Ear height (cm)	68.16	61.39	55.85	45.37	54.30	59.40	36.17	39.60
Number of kernels rows per cob	15.60	14.11	13.10	13.44	14.80	14.83	12.60	14.00
Number of kernels per row	30.50	27.11	21.12	23.91	28.10	30.08	20.75	28.90
Cob girth (cm)	4.29	3.95	3.49	3.66	4.11	4.22	3.23	3.88
Cob length (cm)	16.15	14.75	12.70	12.08	14.09	14.83	11.80	13.60
Hundred grain weight (g)	35.25	27.08	26.75	32.20	34.50	31.33	23.55	34.00
Grain yield (q/ha)	60.80	33.27	23.74	52.70	58.57	54.85	29.09	35.67

Table 7. Principal component analysis summary and Eigen values for various traits in maize inbred lines

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
DTT	0.08	-0.49	0.42	-0.01	0.20	-0.21	-0.08	0.02	0.05	0.29	-0.63
DTS	0.13	-0.48	0.34	0.06	0.31	-0.13	-0.10	0.00	0.05	-0.38	0.60
DTM	0.12	-0.42	0.15	-0.08	-0.62	0.56	0.26	-0.07	0.06	-0.02	0.02
PH	-0.26	-0.35	-0.35	0.46	0.09	0.00	0.07	-0.44	-0.12	0.46	0.21
EH	-0.26	-0.34	-0.43	0.24	-0.22	-0.19	-0.15	0.57	0.27	-0.23	-0.12
NKRC	-0.39	-0.09	0.11	-0.21	-0.40	-0.45	-0.04	-0.22	-0.55	-0.25	-0.04
NKR	-0.41	0.02	0.05	-0.38	-0.04	-0.12	-0.06	-0.38	0.72	0.02	0.05
CG	-0.41	-0.05	0.17	-0.36	0.08	0.19	-0.11	0.49	-0.19	0.50	0.30
CL	-0.36	-0.15	-0.20	-0.20	0.50	0.43	0.24	-0.07	-0.18	-0.40	-0.28
HGW	-0.32	0.19	0.36	0.45	-0.07	0.37	-0.59	-0.10	0.00	-0.15	-0.11
GY	-0.32	0.20	0.40	0.40	-0.01	-0.14	0.68	0.18	0.13	-0.04	0.02
Standard deviation	2.22	1.77	0.97	0.80	0.73	0.61	0.45	0.32	0.29	0.22	0.12
Proportion of variance	0.45	0.29	0.09	0.06	0.05	0.03	0.02	0.01	0.01	0.00	0.00
Cumulative proportion	0.45	0.73	0.82	0.88	0.93	0.96	0.98	0.99	0.99	1.00	1.00

DTT - Days to 50 per cent tasseling
DTS - Days to 50 per cent silking
DTM - Days to 75 per cent dry husk
PH - Plant height
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NKRC - Number of kernel rows per cob
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grain yield and other cob traits. Hence, for the improvement of yield and cob traits, inbred lines can be selected from these clusters.

The principal component reduces the dimensionality of multivariate data by removing interrelations among the variables and the Eigen values represent the variance of the principal component.The principal components formed were equal to number of characters (11) used in the study and the Eigen values of the all 11 principle components are listed in Table 7. It was noticed that first two principal components PC1 and PC2 had >1 standard deviation among 11PC's and exhibited 73% cumulative variation (Table 7). The PC1 was responsible for 45% of the variation of total variance due to number of kernels per row, cob girth, number of kernel rows per cob and cob length traits. The second principal component (PC2) could explain 29% to the total variance and was mainly related to days to 50 per cent tasseling, days to 50 per cent silking, days to 75 per cent dry husk and plant height. The dimensionality reduction through principal component analysis suggested that the population structure of the study material is explained by cob-related traits because, the cob traits *viz,* number of kernels per row, cob girth, number of kernel rows per cob and cob length poses higher eigen values in PC1 which is alone contributing to the extent of 45% of the total variation. These findings are similar to the results of Sandeep *et al.* (2015) and Mounika *et al.* (2018). While, few studies reported contrasting results, where grain yield and hundred grain weight are mainly contributed to the principal components (Dutta *et al.*, 2017 Al-Naggar *et al.*, 2020).

The scatter biplot of PC1 vs PC2 depicts the variability of each trait displayed as red lines (Fig 2). In the scatter plot the genotypes are distributed similarly as of clustering. The genotypes which are placed towards the betterend (*i.e.,* either positive end or negative end depends on the direction in which trait associated with the principle component)of the principal components issupposed to be good for those components.Therefore, inbred line 17 (VL 18448) and 20 (VL 174449) are good for cob traits and grain yield, since these traits have a maximum negative value in PC1. These two lines are grouped in cluster I, as discussed earlier mean performance of cluster I for yield attributes is also high. While, inbred line 2 (IMIC-40), 10 (CI-4), 1 (IMIC-02) and 6 (IMIC-87) belong to cluster IV and VIII are good for flowering and maturity traits along with yield attributes as per their position in scatter plot. Therefore, they serve as a promising line for these traits. These results of clustering and principal components of the current study would help in characterizing the genotypes used in the study and utilize them effectively in the further breeding programme.

Conclusion

There was presence of significant genetical difference among the genotypes under study. Further the diversity analysis was able to identify maxium genetic diversity among the genotypes and was able to group them into eight different clusters. The inbred lines belonging to clusters I, V and VI were found to be promising for grain yield and cob characters, since they had maximum mean values for these traits. Along with grain yield the genotypes in clusters IV and VIII also exhibited lower mean values for flowering and maturity traits which could be used for breeding for early maturity. The clusters III and VIII were farther from each other and crossing between genotypes from two clusters would produce maximum heterosis as these clusters exhibited maximum divergence. CI-4 was identified as highly genetically diverse inbred line and days to 50 per cent silking trait contributed maximum to the genetic divergence among the genotypes.

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