

RESEARCH PAPER

Discrimination of maize (*Zea mays* L.) inbreds for morpho-physiological and yield traits by D² statistics and principal component analysis (PCA)

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To discriminate maize inbred lines based on morpho-physiological and yield traits using D² statistics and principal component analysis (PCA), fifty five genotypes available at Department of Genetics and Plant breeding, Sam Higginbottom Institute of Agriculture Science and Technology, Allahabad were tested during *Rabi* 2013-14 under irrigation condition in Randomized Block Design with three replications. Analysis of variance revealed significant differences for 18 characters studied among the genotypes. The D² statistics displayed that cluster I, being largest group, comprises 37 maize genotypes followed by cluster II (13), III (3), IV (1) and V (1). The maximum intra-cluster distance was obtained for cluster I (624.35) while cluster IV and V showed Null values for it indicating homogeneous nature of the genotypes within the cluster. Further more, maximum inter-cluster distance was recorded between cluster IV and V (22331.82) followed by clusters III and V (15174.83) and clusters II and IV 14480.99). D² statistics and principal component analysis (PCA) emphasized that days to 50 per cent tasseling, silking and maturity, anthesis-silking interval, seed fill duration, cob weight, 100 seed weight and grain yield/plant were major contributing traits for total genetic diversity. Research results indicated that the inbreds under study are highly diversified and hence, high heterotic hybrids would be resulted through the crossing of diverse lines clubbed under different clusters as parent in any breeding programme aimed to enhance grain yield.

Key words : D² statistics, Heritability, Genetic advance, PCA

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INTRODUCTION

Maize (*Zea mays* L.) is an importance staple crop for the rural agrarian economy of the country. Being C4 and high fertilizer responsive plant plus highly cross pollinated crop, it offers tremendous scope for plant breeders to improve the crop at genetic level. Despite this there is still huge gap exist between the potential yield and actual yield harvested by the farmer at the field level. In India, the productivity of maize at present is

about 2,451 kg/ha (FAOSTAT, 2015). To boost up the yield, quality seed is most important in farming. Now-a-days hybrid seed is overwhelming farming system and displacing locally available seed of several crops including maize throughout the country.

Hybrid seed industry is using inbred lines of maize very sparingly depending on availability of lines from diverse source populations and gene pools and evaluating their *per se* performance as well as performance in

various cross combinations (Hallauer, 1990; Troyer, 2004; Koutsika-Sotiriou and Karagounis, 2005). The explicit knowledge of genetic diversity presents among the genotypes is paramount for selecting parents in hybridization programme, especially in a cross pollinated crops like maize. Genetic diversity among the inbred lines can be quantified at the biochemical, morphological and molecular levels and also by means of heterosis or through lines specific combining ability (SCA), expressed in a series of cross combinations (Ajmone-Marsan *et al.*, 1998; Paterniani *et al.*, 2000; Menkir *et al.*, 2004 and Laborda *et al.*, 2005). As the crossing between two extremely diverse parents results in significant heterosis (Duvick, 1999), it's not always hold true (Moll *et al.*, 1965). Due to presence of linkage and epistasis, poor hybrids may be resulted even-though genetically dissimilar parents were crossed. Some line produces out-yielded progenies on crossing, while other, allegedly equally important, proved to be poor. So that the information on genetic diversity, heterosis and combining ability is essential for effective breeding programme through selection of best parents (Beck *et al.*, 1990).

Grain yield, a complex quantitatively inherited trait, is resulted from the interaction between vital processes such as photosynthesis, transpiration and storage of food material (Naushad *et al.*, 2007) and an orderly interaction of attributing characters *viz.*, number of cobs/plant, number of grain rows/cob, number of grains/row and 100 seed weight (Viola *et al.*, 2003). Existence of significant amount of variation in maize germplasm for various quantitative and qualitative traits has been reported by earlier workers (e.g. Marker and Krupakar, 2009; Zaman and Alam, 2013 and Kage *et al.*, 2013). Considerable genetic variability, a heritable difference among cultivars, within population is always desirable to facilitate and sustain plant breeding programme in a long term. Quantification of magnitude of genetic diversity among the germplasm has become possible with the help of advance biometrical technique; *viz.*, multivariate analysis (Rao, 1952) based on Mahalanobis (1936) D² statistics and principal component analysis (PCA) (Pearson, 1901). Crossa *et al.* (1995) used a combination of cluster analysis and principal component analysis to classify maize accessions. Principal component analysis assigned each genotype to only one group and also reflects significance of largest contributor to total variability at each axis of differentiation (Sharma, 1998). Genetic variation for agro-morphological and physiological traits

has been measured using cluster analysis and principal component analysis (Ganesan *et al.*, 2010; Azad *et al.*, 2012 and Mustafa *et al.*, 2015). A number of genotypes available at present are necessary to assess variation among them for evaluation of their potentiality. The present study was done to differentiate maize inbred lines based on morpho-physiological and yield traits using D² statistics and principal component analysis (PCA).

RESEARCH METHODOLOGY

Experimental site :

The study was conducted at Field Experimentation Center, Department of Genetics and Plant Breeding, Sam Higginbottom Institute of Agriculture Science and Technology (Deemed-to-be-University), Allahabad, India during *Rabi* 2013-14 under irrigated condition.

Plant materials and experimental design :

Fifty five maize inbred lines were grown in a Randomized Block Design with three replications. The seeds of each entry were sown in single row plot of 3 m long with a spacing of 70×30 cm. After thinning, one plant per hill was maintained.

Data recorded :

Data on traits like days to 50 per cent tasseling, days to 50 per cent silking and days to 50 per cent maturity were recorded on visual observations of plants on plot basis while data for plant height (cm), ear height (cm), leaf area index (LAI), number of cobs/plant, cob weight (g), cob length (cm), cob girth (cm), number of grain rows/cob, number of grains/ row, 100 seed weight (g), grain yield/plant (g), biological yield/plant (g) and harvest index (HI) were taken from five randomly selected plants from middle of row of each entry in each replication. The difference between days to 50 per cent silking and tasseling of each entry was reported as anthesis-silking interval (ASI) (days) while seed fill duration (SFD) (days) was computed as the difference between days to 50 per cent maturity and silking for each entry.

Statistical analysis :

Statistical analysis of mean data over replications were subject to analysis of variance as suggested by Panse and Sukhatme (1967), genetic divergence was computed by using D² statistics of Mahalanobis (1936),

and clustering of genotypes was done according to Tocher’s optimization method as described by Rao (1952). Relative contribution of characters towards genetic divergence was calculated as per the guideline of Singh and Chaudhary (1985). The principal component analysis was done as described by Pearson (1901).

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Analysis of variance :

The analysis of variance (Table 1) reflected highly significant differences among the genotypes for all the characters studied indicating that the experimental materials were genetically divergent to each other. This shows that there is sufficient space for selection of promising lines amongst the available genotypes aimed to enhance the genetic yield potential of maize. Marker and Krupakar (2009) also found significant variation among the 16 maize genotypes for the different characters viz., days to 50 per cent tasseling and silking, anthesis-silking interval, plant height, ear height, number of grain rows/cob, number of grains/row, 100 seed weight

and grain yield/plant. Existence of significant genetic variability among the treatments for all the quantitative characters studied was also noticed by Vashistha *et al.* (2013); Zaman and Alam (2013) and Kage *et al.* (2013).

Cluster analysis of maize genotypes :

All the 55 maize inbred lines formed five major clusters following Tocher’s method (Table 2 and Fig. 1) based on the Mahalanobis distance. The pattern of distribution of genotypes among various clusters indicated the considerable genetic variability exists in the inbred lines under study. Cluster I consisted of 37 maize inbred lines, forming largest cluster, followed by cluster II (13),

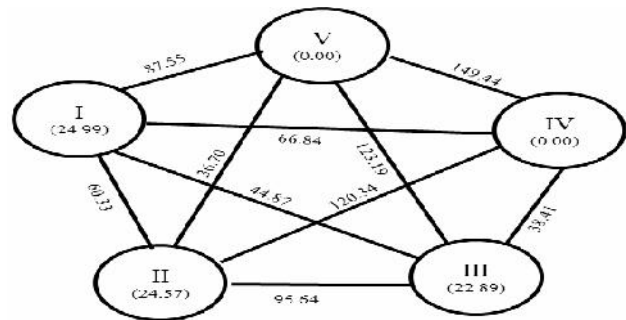


Fig. 1: Intra and inter-cluster distance diagram for different morpho-physiological and yield attributing characters in maize

Sources of variation	Replication (df = 2)	Genotypes (df = 54)	Error (df = 108)	CV (%)
Days to 50% tasseling	2.13	187.45**	1.36	1.29
Days to 50% silking	0.48	199.33**	1.75	1.40
ASI	0.92	1.86**	0.36	13.28
Days to 50% maturity	0.66	109.53**	3.69	1.45
SFD	0.70	63.22**	2.26	3.95
Plant height	27.89	2268.14**	142.80	7.40
Ear height	245.31	643.87**	82.44	15.15
LAI	0.27	1.62**	0.06	7.47
Cobs/plant	0.07	0.55**	0.08	20.60
Cob weight	532.88	3512.67**	106.64	10.21
Cob length	1.96	17.08**	0.79	5.95
Cob girth	7.12	6.59**	0.15	2.93
Grain rows/cob	3.57	5.13**	0.95	7.35
Grains/row	2.40	94.63**	3.68	9.27
100 seed weight	0.03	78.56**	0.03	0.59
Grain yield/plant	293.18	6154.12**	487.33	20.28
Biological yield/plant	1065.77	40679.72**	981.82	10.10
Harvest index	20.21	115.29**	43.08	18.64

ASI = Anthesis-Silking interval; SFD = Seed fill duration; LAI = Leaf area index; * and ** indicate significance of values at P=0.01 and 0.05 respectively

cluster III (3). Cluster IV and V were ungrouped consisting of single genotypes each. The uniqueness of these latter two varieties placed them separately. The result is favoured by the experimental results of Zaman and Alam (2013). The clustering pattern of inbred lines studied revealed that the inbred lines had significant genetic divergent among themselves comprising five different clusters. The genotypes which are within the clusters by and large exhibit a narrow range of genetic variability.

The morphological data on maize approved that substantial variation was existed between and within groups of maize inbred lines. The intra and inter-cluster D^2 values varied between 0.00 to 624.35 and 1346.62 to 22331.82, respectively, pointed out the existence of variation among the maize inbred lines (Table 3 and Fig. 1). The maximum intra-cluster distance was recorded for cluster I (624.35) followed by cluster II (603.91) and cluster III (523.74). Owing to be solitary cluster, the cluster IV and V showed no intra-cluster distance (0.00) indicating comparatively homogeneous nature of the genotype within the cluster. The maximum inter-cluster D^2 value was observed between clusters IV and V (22331.82) followed by clusters III and V (15174.83)

and clusters II and IV 14480.99), recommending that selection of inbred lines fell under these clusters as parent for hybridization would result desirable type since greater chances of obtaining high heterotic hybrids and a broad spectrum variability in segregating generations will results from crossing between more genetically diverse parents (Arunachalam, 1981). Selection of contrasting genotypes possessing desirable traits would made a significant result in any breeding programme as suggested by earlier results of Senshu *et al.* (2014) and Synrem *et al.* (2015).

Relative contribution to genetic divergence :

The relative contribution of characters under study towards the genetic diversity of maize is presented in Table 4. The maximum contribution to genetic divergence was made by days to 50 per cent tasseling (45.18%), followed by days to 50 per cent silking (31.02%), seed fill duration (18.11%), anthesis-silking interval (3.03), 100 seed weight (1.72%), days to 50 per cent maturity (0.83%) and grain yield/plant (0.02%). Therefore, these characters should be given priority while selecting diverse parents in maize breeding programme aimed to harness earliness and higher yield. These findings are also at par with the result of Alom *et al.* (2003); Marker and

Table 2 : Distribution of 55 maize inbred lines among 5 clusters

Cluster no.	No. of genotypes	Name of genotypes included
I	37	HKI 34(H2)-1, HY-10RN-10235-462, HY-10RN-10235-105, R-13-1-17, CM 136, S995-T4QBBB-3-BBB, HY-10RN-10235-477, CML 40, HY-10RN-10235-535, HY-10RN-10235-322, EC 598475, TSK 197, CML 470 BX15, BBB-B2-NBB, CML 224-1, CML41, Tarun 83-1-3-2, Early White, POP 31Q-18211, CML304, HKI 193-1, CML 358, CML439, UMC-4, JP25-W95, TEMP×TROP QPM, HKI193-1-1, DMR 9071, CML165/AMTCo 571-1-1-1-2-1-1-1, HY-10RN-10235-274, CM13, YHP Pant, TEMP×TROP (HQ) QPM-BBB-45-BB, TSK 194, UMC-12, TSK 195, CM 600
II	13	CM13-1, R 13-1-1, CML 27, CIM 44, DMR N4, DMR N-21, Vivek 333vF2, R-13-1-10, POP31 Q, LM13, TSK 196, HKI 193-2, CM 138
III	3	CML 359, HY-10RN-10235-4, DMR 9047
IV	1	Tarun 83-1-3-3
V	1	CML 224

Table 3 : Average intra (diagonal) and inter cluster (off-diagonal) D^2 value among the cluster for 55 maize inbred lines

Clusters	I	II	III	IV	V
I	624.35 (24.99)	3639.65 (60.33)	2013.46 (44.87)	4467.18 (66.84)	7665.43 (87.55)
II		603.91 (24.57)	9147.62 (95.64)	14480.99 (120.34)	1346.62 (36.70)
III			523.74 (22.89)	1475.38 (38.41)	15174.83 (123.19)
IV				0.00 (0.00)	22331.82 (149.44)
V					0.00 (0.00)

Table 4 : Cluster mean values of different clusters for 18 traits in maize inbred lines

Clusters	50% Tasseling (days)	50% Silking (days)	ASI (days)	50% maturity (days)	SFD (days)	Plant height (cm)	Ear height (cm)	LAI	Cobs/plant (no.)	Cob weight (g)	Cob length (cm)	Cob girth (cm)	Grain rows Cob (no.)	Grams/row w (no.)	100 seed weight (g)	Grain yield/plant (g)	Biological yield/plant (g)	Harvest index
I	89.80	94.33	4.53	131.86	37.53	162.42	59.91	3.25	1.36	95.09	14.90	12.93	13.18	20.74	26.73	103.77	308.54	34.09
II	89.28	93.77	4.49	134.15	40.38	172.91	65.82	3.52	1.31	130.77	15.76	14.40	13.49	22.03	35.38	135.33	352.23	33.39
III	101.22	106.10	4.78	138.56	32.55	102.62	36.98	2.61	1.67	56.67	13.86	11.25	12.78	14.89	20.65	72.66	196.67	34.63
IV	77.67	82.00	4.33	124.33	42.33	172.10	72.00	3.01	1.67	75.00	14.93	11.79	13.33	24.67	17.03	92.16	271.50	33.89
V	89.33	93.00	3.67	130.33	37.33	139.37	41.67	2.66	1.00	98.33	14.10	11.63	13.67	14.33	40.00	78.51	203.83	33.54
Mean	89.46	93.82	4.36	131.85	38.03	149.88	55.28	3.01	1.40	91.17	14.71	12.40	13.29	19.33	27.56	96.49	266.55	35.91
Relative contribution towards total divergence																		
%	45.18	31.02	3.63	0.83	18.11	0.01	0.00	0.01	0.00	0.01	0.01	0.02	0.00	0.30	1.72	0.02	0.01	0.01

ASI = Anthesis-Silking interval; SFD = Seed fill duration; LAI = Leaf area index

Krupaker (2009) and Synrem *et al.* (2015) who stated that per cent contribution to genetic divergence was higher from 50 per cent tasseling, days to 50 per cent silking, days to 50 per cent maturity, anthesis-silking interval, cob girth, number of cobs/plant, 100- seed weight, grain yield/plant, protein content. Kage *et al.* (2013) also reported that the plant height, days to 75 per cent brown husk maturity, ear height, grain yield/ha, number of grains/row, days to 50 per cent tasseling, 100 seed weight, number of grain rows/ear and ear girth contributed maximum towards total genetic diversity.

Clusters mean comparison :

Mean performance of genotypes for various characters clubbed in five clusters (Table 4) expressed substantial variation among the clusters for grain yield and its related components. Highest mean value for grain yield/plant (135.33g), cob girth (14.40cm), cob length (15.76cm), cob weight (130.77g) and LAI (3.52) were recorded from Cluster II, while highest mean for 100 seed weight (40.00g), number of grain rows/cob (13.69), shorter anthesis-silking interval (3.67 days) were found in cluster V. cluster IV showed high mean value for number of grains/row (24.67), number of cobs/plant (1.67) and seed fill duration (42.33 days). This indicates the presence of wide range of variation for grain yield and its related components in maize as reported by Singh and Choudhary (2001). Greater variation for grain yield and yield contributing characters in maize has been reported by several maize breeders like Marker and Krupaker (2009); Ranawat *et al.* (2013) and Synrem *et al.* (2015). It is always advisable to look for genotypes used as parents in hybridization programme to develop high yield varieties having more than one desirable trait and belong to different clusters as aforementioned.

Principal component analysis :

Using quantitative data of eighteen morpho-physiological traits of fifty five maize inbred lines, the Eigen vectors and Eigen values for principal components were derived and presented in Table 5. Out of total 18, five principal components (PC) extracted had Eigen value >1 and about 78.12 per cent variation among the lines is attributable to these first five components (Table 5 and Fig. 2). The traits contributing most heavily to variation were cob weight (-0.34), grain yield/plant (-0.33), biological yield/plant (-0.30), grains/row (-0.30) and cob girth (-0.30) with negative loadings in PC1. Similarly, the

traits like days to 50 per cent tasseling (-0.41), days to 50 per cent silking (-0.42), days to 50 per cent maturity (-0.38) and cobs/plant (-0.32) contributed significantly towards total variation with negative loadings in PC2. In PC3, 100 seed weight (-0.47) and days to 50 per cent maturity (-0.41) with negative loadings and cobs/plant (0.44) with positive loadings were causing variability among genotypes. Traits like harvest index (0.56), anthesis-siking interval (ASI) (0.39) and cobs/plant (0.29) with positive loadings and again harvest index (0.44) with positive loadings

and anthesis-siking interval (-0.59) with negative loading played crucial role for creating genetic variability on maize genotypes in PC4 and PC5, respectively.

The PCA boxplot of Fig. 3 depicted that the variables and genotypes were superimposed in the plot as vectors. It also reflected that traits such as days to 50 per cent tasseling, silking and maturity, ASI, SFD, cobs/plant, cob weight, 100 seed weight, grain yield/plant and biological yield/plant as whole contributed most strongly towards variability among genotypes, indicating that first emphasis

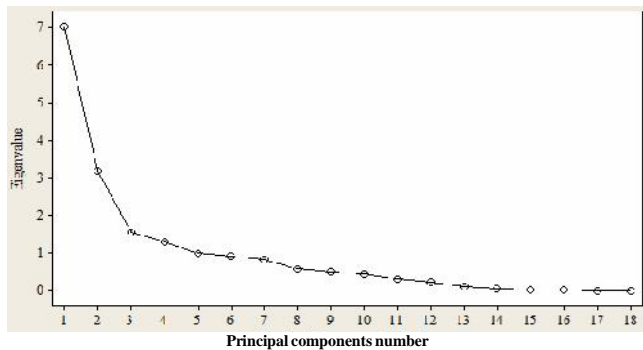


Fig. 2: Scree plots of eighteen principal components against eigen value

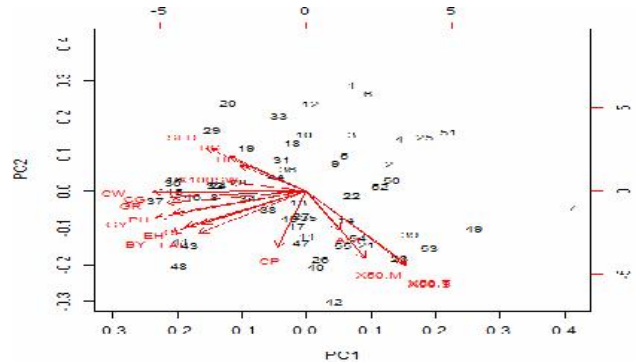


Fig. 3: Boxplot between PC1 and PC2 showing contribution of 18 characters in variability of maize genotypes

Table 5: Eigen vectors and Eigen value for principal components using fifty five maize lines and eighteen characters

Characters	PC1	PC2	PC3	PC4	PC5
Days to 50% tasseling	0.22	-0.41	-0.24	0.01	0.14
Days to 50% silking	0.22	-0.42	-0.23	0.04	0.08
ASI	0.07	-0.22	0.02	0.39	-0.59
Days to 50% maturity	0.13	-0.38	-0.41	-0.19	-0.05
SFD	-0.22	0.25	-0.13	-0.33	-0.21
Plant height	-0.29	-0.13	0.12	-0.26	0.05
Ear height	-0.27	-0.20	0.08	-0.29	0.12
LAI	-0.23	-0.24	0.12	-0.17	-0.09
Cobs/plant	-0.06	-0.32	0.44	0.29	0.03
Cob weight	-0.34	-0.01	-0.25	0.09	0.12
Cob length	-0.23	-0.19	-0.07	0.05	0.02
Cob girth	-0.30	-0.04	-0.29	0.01	-0.23
Grain rows/cob	-0.17	0.20	-0.16	0.22	-0.38
Grains/row	-0.30	-0.07	0.02	0.01	0.33
100 seed weight	-0.17	0.05	-0.47	0.04	-0.03
Grain yield/plant	-0.33	-0.15	0.08	0.26	0.01
Biological yield/plant	-0.30	-0.24	0.18	0.02	-0.19
Harvest index	-0.14	0.15	-0.19	0.56	0.44
Eigen values	7.03	3.18	1.55	1.30	1.01
Proportion (%)	39.03	17.65	8.63	7.22	5.59
Cumulative (%)	39.03	56.68	65.31	72.54	78.12

should pay in these traits while selecting the genotypes in maize improvement project. Azad *et al.* (2012) pointed out that the traits like days to maturity, ear height, ear diameter, ear length, grain rows/ear, grains/row and yield/plant had significant contribution to genetic variation of maize genotypes. Mustafa *et al.* (2015) also highlighted the usefulness of PCA in selection drought tolerance maize genotypes *via* choosing of traits that strongly contributing in genetic variation of maize germplasms.

Conclusion :

Biometrical technique has made ease to measure

the magnitude of variability exist among the breeding materials. Choose of contrasting parent lines for hybridization as reflected from D² statistics and principal component analysis (PCA) would ensure greater chances of obtaining high heterotichybrids and broad spectrum of variability in segregating progenies. Therefore, crossing between inbred lines fell under clusters II, III, IV and V would result in highly heterotic hybrids as they have higher mean value for yield and its important attributing traits, higher inter-cluster distance and greater variability and diversity.

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