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UTILIZING PRINCIPAL COMPONENT AND DIVERSITY ANALYSIS FOR CHARACTERIZATION OF EARLY INBRED MAIZE LINES

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ABSTRACT

The basic aim of a crop enhancement program is to generate varieties that produce higher yields than existing cultivars. Therefore, the evaluation and creation of inbreds that form high yielding hybrids in maize is a crucial feature for a breeding program. To efficiently utilize the material for hybrid development, it is important to identify the degree of diversity present among the inbred maize lines.

A field study involving 17 inbred lines and the check variety PAC 751 Elite was undertaken using a Randomized Block Design with two replications for thirteen quantitative characters. The study was conducted to determine the level and type of genetic variability exhibited by each line, which will help in the selection of parents in hybridization programmes.

Mahalanobis diversity analysis grouped the genotypes into seven clusters, showing considerable genetic divergence. The biggest contribution to genetic divergence was made by days to 50% pollen shed followed by 100 kernel weight and tassel length. Consequently, these characters can be focused on in maize breeding studies. Principal component analysis found that the five principal components accounted for 82.25% of the overall variability. The genotypes PAC 751 Elite, IMPCH 35, and IMPCH 110 have been recommended as potential choices for hybridization studies.

Key words : Maize, Inbred, Diversity, Principal component.

Introduction

Maize is one of the most important cereal crops globally due to its diverse use as food for humans, industrial raw material and feed for livestock. It occupies third place after wheat and rice and is referred to as the Miracle Crop and Queen of the Cereals because of its exceptional productivity as compared to other members of the Poaceae family (Sinha *et al.*, 2019). Over 170 countries produce around 1231.13 million mt (metric tons) of maize on 208.01 million ha (hectares) with an average yield of 5.92 mt/ha (USDA, 2023-24). In India, the productivity of maize is 3.19 mt/ha and occupies a total area of 9.89 mha and contributes around 9% to the country's food supply (India AgriStat.com, 2020-21). The growing utilization of maize in the livestock and poultry

sectors is expected to significantly increase the demand for this commodity in the imminent future (India-Maize-Summit, Agri Vision, 2022). Subsequently, enhancing maize production across all maize growing areas across India will become a major priority to meet the rising demand and ensure food and feed security. The primary objective of a crop improvement programme is to create varieties with greater yields than existing cultivars. Therefore, the evaluation and production of inbreds that produce high yielding hybrids in maize is an important area of investigation for a breeding program (Saleh *et al.*, 2022). Strong heterotic effects have been shown to result from genetically diverse parents (Sharma *et al.*, 2020), thus a thorough understanding of genetic divergence is essential for a successful breeding

programme. To use the material in hybrid development, it is important to determine the level of variation existing among the inbred lines of maize (Kumar *et al.*, 2018). Multivariate analysis is generally used to assess the level of genetic variation in maize germplasm and to determine the relationships of genetic variability in a breeding program (Saleh *et al.*, 2022). Statistical methods for analysing genetic variation within germplasm have made it possible to quantify genetic diversity between populations. These include several multivariate techniques such as Mahalanobis D² statistic (1936) and principal component analysis (PCA), developed by Pearson (1901). In view of the aforesaid, 17 inbred lines were evaluated for thirteen quantitative characters, along with the hybrid PAC 751 Elite, in order to measure the extent and type of genetic diversity exhibited by each line, which will aid in the selection of parents in hybridization programmes.

Materials and Methods

The experiment was conducted at the Instructional-cum-Research (ICR) Farm of Assam Agricultural University during the rabi season of 2019-20 for evaluation of inbred progenies and during the *kharif* season of 2018-19 for selfing in composited company-bred maize hybrids and local maize germplasm. In a station breeding programme undertaken at the Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat during *rabi* 2017-18, equal quantities (100 g each) of seeds from nineteen maize hybrids developed by different seed companies were bulked together and grown to facilitate intermating among the hybrids. Seeds collected from the resulting open-pollinated plants constituted the base population for the development of inbred progenies through selfing during *kharif* 2018-2019.

Several S₁ lines were developed from this base population and utilized in the present investigation. These progenies were designated as IMPCH (Intermated Progenies from Company-bred Hybrids), with different numerical suffixes representing individual S₁ lines. Sixteen IMPCH lines along with one S₁ line derived from local maize germplasm and the hybrid check PAC 751 Elite were evaluated in a randomized block design (RBD) with two replications during rabi 2019-20. Each genotype was planted in a single row of 4 meters in length, with row

spacing of 60 cm between rows and 20 cm between plants. All the package of practices recommended for maize crop production in Assam were adopted to provide optimal growth and development.

The Mahalanobis D² (Mahalanobis, 1936) and principal component analysis (PCA) were evaluated using R statistical software. PCA and graphical representation were evaluated using the FACTOEXTRA package (Kassambara and Mundt, 2020), while the Mahalanobis D² statistics were calculated using the BIOTOOLS package (da Silva, 2021). Biplot analysis and graphical representations were created by using the GGplot2 package (Wickham, 2016). Euclidean distances of the genotypes and their visualization through cluster analysis were also calculated using FACTOEXTRA.

Results and Discussion

Multivariate analysis of variance (MANOVA)

Multivariate analysis of variance (MANOVA) based on Pillai's trace showed a highly significant effect of genotype on the combined set of traits, indicating significant multivariate genetic variability among the evaluated genotypes (Table 1). Conversely, the effect of replication was not significant, suggesting that environmental variation among replications did not significantly affect the multivariate response of the measured traits.

Cluster and diversity analysis of the maize genotypes

The 18 maize genotypes were grouped into 7 major clusters based on Mahalanobis distance using tocher's method (Table 2). The distribution patterns of the genotypes across the clusters indicated significant levels of genetic divergence in the inbred lines under evaluation. Cluster I was the largest comprised 10 maize genotypes, and Cluster II contained three (3) genotypes. Clusters III, IV, V, VI and VII were each considered ungrouped and contained one (1) genotype each. These results were supported by those of Jakhar *et al.* (2018), who observed seven clusters from thirty genotypes of maize; Antony *et al.* (2021), who identified eight clusters from twenty-five inbred lines of maize; and Borkhatariya *et al.* (2023), who reported nine clusters from forty-eight distinct genotypes of maize. Due to their distinct traits,

Table 1 : Multivariate analysis of variance.

Source of variation	Df	Pillai's trace	Approx F	Numerator Df	Denominator Df	p-value
Genotype	17	8.61	1.96	221	221	3.42 x 10 ^{-7***}
Replication	1	0.91	3.83	13	5	0.07
Residuals	17					

Table 2 : Distribution of 16 maize inbred lines and 2 hybrids among 7 clusters.

Cluster no.	No. of genotypes	Name of genotypes included
I	10	IMPCH 26,32, 55, 39, 62, 9, 35, 40, 66 and 110
II	3	IMPCH 19, 88 and Manipur Black
III	1	IMPCH 20
IV	1	IMPCH 111
V	1	IMPCH 70
VI	1	PAC 751(Elite)
VII	1	IMPCH 73

Table 3 : Average intra (diagonal) and inter cluster (off-diagonal) D² value among the cluster for 16 maize inbred lines and 2 hybrids.

	I	II	III	IV	V	VI	VII
I	111.55	194.50	272.10	209.03	177.94	304.70	219.06
II		99.01	250.01	354.93	388.40	415.96	192.71
III			0.00	243.58	643.02	460.49	290.59
IV				0.00	414.17	162.00	492.14
V					0.00	418.61	383.75
VI						0.00	734.80
VII							0.00

each of these ungrouped genotypes was categorized independently (Kumawat *et al.*, 2020).

The D² values of intra-cluster and inter-cluster ranged from 0.00 to 111.55 and 162.00 to 734.80, respectively indicating variation among the maize inbred lines (Table 3). The intra-cluster distances were smaller than the inter-cluster distances, indicating that the inter-cluster distances were more diverse than the intra-cluster distances (Khan *et al.*, 2022). The highest intra-cluster distance was for cluster I (111.55) followed by cluster II (99.01). Clusters III, IV, V, VI and VII were monogenotypic, with no intra-cluster distance (0.00). Maximum inter-cluster D² value was 734.80 between cluster VI and VII followed by 643.02 between cluster III and cluster V, 492.14 between cluster IV and VII and 460.49 between III and cluster VI indicating genetic distance among different clusters. The distances between clusters were larger than intra-cluster distances indicating that the diversity between clusters was larger than within clusters. Selection of inbred lines from these clusters will have advantage due to higher chances of getting high heterotic hybrids and wide range of variability in segregating generations due to crossing among more genetically divergent parents

(Pandey *et al.*, 2026). Inter-cluster distances were low between cluster IV and cluster VI (162.00) followed by cluster I and cluster V (177.94) and cluster II and cluster VII (192.71) indicating comparatively less diversity among the genotypes present in these clusters. Therefore, crossing genotypes from these clusters may not result in a high level of heterotic expression (Hussain *et al.*, 2021).

The mean values of cluster for yield and its different contributing characters were depicted in the Table 4. Cluster IV (98.50, 102.00) and cluster III (101, 105) recorded early anthesis and silking. The lowest plant height was recorded in cluster VI (131.50) followed by cluster IV (150.25). Maximum ear length was observed in cluster III (13.43) followed by cluster VI (12.90). Maximum ear diameter, kernel rows per ear and kernel per rows were found in cluster VI (5.42, 17.07 and 28.08). Cluster IV showed highest mean values for 100 kernel weight (36.91) and cluster VI for shelling percentage (85.61). The maximum yield was obtained by cluster V (54.64) followed by cluster VI (52.43). The mean values indicated a good performance of cluster VI and IV for cob traits and early anthesis and silking. Hence, these clusters can be used to select inbred lines for improving yield and cob traits (Antony *et al.*, 2021). The inbreds in these clusters showed short stature plants, less days to anthesis and silking, more number of kernels rows per ear, kernel per rows, 100 kernel weight, maximum ear diameter and reasonable yielding ability. Chandel and Gularia (2019), Matin *et al.* (2017) and Lenka *et al.* (2020) reported similar results. Identification of genotypes used as parents in hybridization program to develop high yielding varieties with several desirable traits and belonging to different clusters is important (Bhusal *et al.*, 2016).

Table 4 and Fig. 1 show the contribution of studied traits to the genetic diversity of maize. The traits contributing the most to genetic divergence were days to

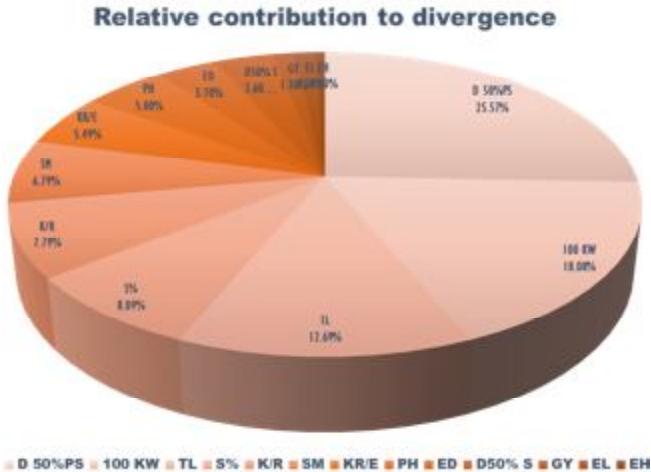


Fig. 1 : Relative contribution to divergence.

Table 4 : Cluster mean for the traits and their contribution to total divergence.

Traits	I	II	III	IV	V	VI	VII	Contribution (%)
D50%PS	108.40	103.00	101.00	98.50	115.50	102.50	112.00	25.60
D50%S	111.45	106.66	105.00	102.00	117.50	106.00	116.50	3.60
PH	156.77	188.25	173.75	150.25	151.50	131.50	191.50	5.00
EH	41.62	61.91	56.00	31.50	34.00	40.25	76.00	0.60
TL	32.32	33.83	42.75	28.50	26.00	28.75	42.50	12.70
EL	12.41	11.66	13.43	12.55	12.60	12.90	11.28	1.30
ED	4.70	4.48	4.72	4.72	4.81	5.42	4.25	3.70
KR/E	13.78	13.65	14.90	13.75	13.57	17.07	14.13	5.50
K/R	22.37	22.86	19.28	19.55	23.00	28.08	21.52	7.80
100 KW	31.54	24.96	32.64	36.91	34.70	33.69	24.93	18.10
S%	81.50	83.90	80.03	78.57	81.41	85.61	77.38	8.10
SM	26.15	25.93	24.85	26.85	26.10	27.40	25.55	6.80
GY/P	47.57	48.63	48.86	42.92	54.64	52.43	45.24	1.30

D50%PS: Days to 50% pollen shed

EL: Ear length

S%: Shelling percentage

D50%S: Days to 50% silking

ED: Ear diameter

SM: Seed moisture

PH: Plant Height

KR/E: Kernel rows per ear

GY/P: Grain yield per plant

EH: Ear height

K/R: Kernels per row

TL: Tassel length

100KW: 100 kernel weight

Table 5 : Eigen values, % variance and cumulative % variance.

Parameters	Eigenvalue	Variance percentage	Cumulative variance percentage
PC 1	3.83	29.48	29.48
PC 2	2.53	19.43	48.91
PC 3	1.84	14.16	63.06
PC 4	1.43	10.99	74.06
PC 5	1.06	8.19	82.25

50% pollen shed (25.6%), 100 kernel weight (18.1%), tassel length (12.7%), shelling percentage (8.1%), kernels per row (7.8%), seed moisture (6.8%), kernel rows per ear (5.5%), plant height (5%), ear diameter (3.7%), days to 50 % silking (3.6) and grain yield (1.3%). Hence, these characters should be preferred for selecting diverse parents in maize breeding programme aimed to exploit higher yield. These results are in agreement with Bhusal *et al.* (2016), Kumawat *et al.* (2020), who also reported the above mentioned characters as major components contributing maximum to the total variation in maize.

Principal Component analysis

Principal components (PC) are considered important when the Eigenvalue is >1 and PC accounts for at least 5% of the variation in the data (Wander and Bollero,

1999). Principal component analysis (PCA), among the thirteen traits of the 18 genotypes (17 inbred lines and 1 hybrid), only five principal components (PCs) had an Eigenvalue of more than one and explained 82.25% cumulative variability among the traits studied (Table 5). Hence, these five PCs were the main principal components of the study. These results were also confirmed by Saleh *et al.* (2022), where of the fifteen principal components (PC), five had EigenValues greater than one and accounted for of the overall 88.40% variability among the characteristics. Harish *et al.* (2026) identified four main PCs out of 11 PCs accounting for 75.11% of the total variability. The highest variability was obtained from PC1 (29.48%) followed by PC2 (19.43%), PC3 (14.16%), PC4 (10.99%) and PC5 (8.19%).

The first PC was positively correlated with the traits ear height (0.35), plant height (0.34), days to 50% silking (0.25) and tassel length (0.20) (Table 6). Whereas, ear diameter (-0.43), ear length (-0.33), 100 kernel weight (-0.32) and grain yield per plant (-0.24) had negative contribution to the first PC indicating the maximum contribution of these characters to the component. The traits days to 50% pollen shed (0.46), days to 50% silking (0.42) contributed positively while tassel length (-0.46), plant height (-0.38) and ear height (-0.32) were negatively associated with PC 2. Sinana *et al.* (2023) reported similar

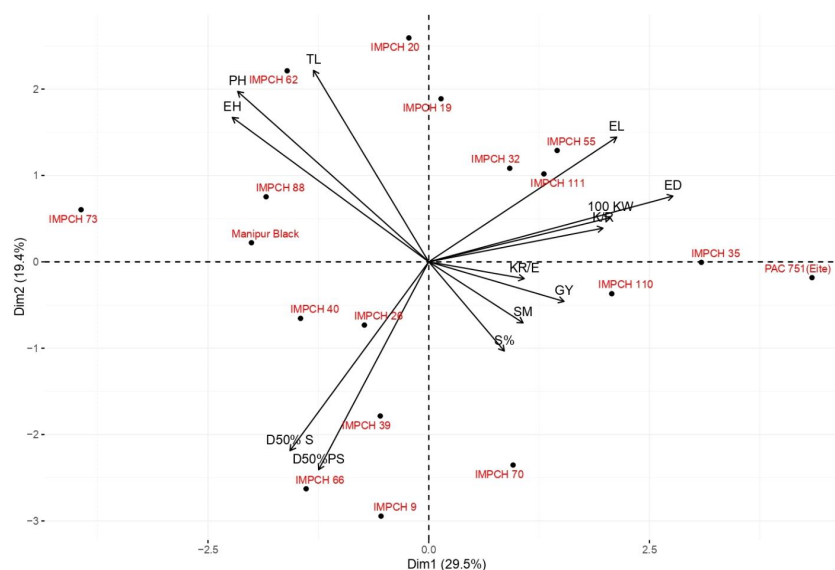


Fig. 2 : Biplot analysis for 18 maize genotypes and the traits measured.

results. PC3 was positively contributed by kernel weight (0.38) and ear length (0.25) while negatively contributed by shelling percentage (-0.56), grain yield (-0.39), ear height (-0.28), kernel rows per ear (-0.28), seed moisture (-0.27) and kernel per rows (-0.20). Kernel per rows (-0.45), days to 50% pollen shed, days to 50% silking (-0.43) and grain yield (-0.38) contributed negatively and shelling percentage (0.17) and kernel rows per ear (0.10) contributed positively for PC4. PC5 had a positive contribution of kernel rows per ear (0.76) and negative contribution of seed moisture (-0.54). These are in accordance with the results obtained by Mallikarjuna *et al.* (2021) and Sheela *et al.* (2023).

Biplot analysis : The PCA biplot (Fig. 2) was used to visualize the relationship between maize genotypes and morphometric traits based on the first two principal components, PC1 (29.5%) and PC2 (19.4%), which explained a total variation of 48.9% by the two components. The length of the vector in the PCA biplot is the magnitude of the contribution of a given trait to the principal components. The angle between trait vectors is a measure of how correlated traits are. A small angle ($<90^\circ$) between two vectors indicates positive correlation, an obtuse angle ($>90^\circ$) indicates negative correlation, while an angle of 90° indicates no correlation between the traits (Bhogal *et al.*, 2025). Traits in the first quadrant, such as, ear length (EL), ear diameter (ED), 100 kernel weight (100 KW) and kernels per row (K/R) were related to each other. The second quadrant showed high correlation among tassel length (TL), plant height (PH) and ear height (EH). Strong association was observed between days to 50% pollen shedding (D50%PS) and days to 50% silking (D50%S) in the third quadrant. In the fourth quadrant, kernel rows per ear (KR/E), grain

yield (GY), shelling percentage (S%) and seed moisture (SM) and showed positive correlations among themselves. Positive associations were also observed among traits in the first and fourth quadrants indicating that ear and kernel traits contributed positively towards grain yield. However, D50%PS and D50%S showed negative correlation with traits in the first quadrant, whereas vegetative traits in the second quadrant also showed negative association with yield related traits in the first quadrant. The present findings are supported by Singh *et al.*, (2024); Long *et al.*, (2024) and Mustafa, (2025). Among the genotypes IMPCH 26, IMPCH 39 and IMPCH 66 were projected near to the vectors representing D50%PS and D50%S

indicating positive association with flowering and maturity (Fig. 2). Similarly, IMPCH 32, IMPCH 55 and IMPCH 111 were highly associated with ear length while IMPCH 110 showed positive association with grain yield.

Genotype contribution : The genotypes with high negative scores in PC1 (Table 7), namely PAC 751 Elite (-4.22), IMPCH 35 (-3.00), IMPCH 110 (-2.01), IMPCH 55 (-1.41), and IMPCH 111 (-1.27), were strongly associated with superior yield-attributing traits such as grain yield, larger ear diameter, and 100 kernel weight. Genotypes with high negative PC2 scores, including IMPCH 20 (-2.52), IMPCH 62 (-2.15), IMPCH 19 (-1.83), IMPCH 55 (-1.25), and IMPCH 32 (-1.05),

Table 6 : Principal components for yield and yield attributing traits in maize genotypes.

Traits	PC1	PC2	PC3	PC4	PC5
D50 % PS	0.19	0.46	0.13	-0.43	0.09
D50 % S	0.25	0.42	0.09	-0.43	0.09
PH	0.34	-0.38	-0.09	-0.15	-0.11
EH	0.35	-0.32	-0.28	-0.19	0.07
TL	0.20	-0.43	-0.15	-0.25	0.09
EL	-0.33	-0.28	0.25	-0.33	-0.11
ED	-0.43	-0.15	0.02	-0.07	0.18
KR/E	-0.17	0.04	-0.28	0.10	0.76
K/R	-0.31	-0.07	-0.20	-0.45	-0.15
100 KW	-0.32	-0.10	0.38	-0.08	0.04
S %	-0.13	0.20	-0.56	0.17	-0.14
SM	-0.17	0.14	-0.27	0.03	-0.54
GY	-0.24	0.09	-0.39	-0.38	0.07

Table 7: Contribution of genotypes to the principle components.

Genotypes	PC1	PC2	PC3	PC4	PC5
IMPCH20	0.22	-2.52	0.30	0.33	1.54
IMPCH 111	-1.27	-0.99	1.85	2.49	-0.77
IMPCH35	-3.00	0.01	-1.31	-0.84	-0.62
IMPCH 19	-0.14	-1.83	-0.86	0.19	0.02
IMPCH 110	-2.01	0.36	0.96	-0.10	1.42
IMPCH 40	1.41	0.64	-0.38	-2.07	-0.68
IMPCH 9	0.53	2.86	-1.26	1.28	0.69
IMPCH 66	1.35	2.55	1.61	1.13	0.83
IMPCH 70	-0.93	2.29	0.64	-1.89	0.08
PAC 751(Elite)	-4.22	0.18	-2.23	0.02	1.09
IMPCH 26	0.71	0.71	-0.17	0.75	-1.69
IMPCH 55	-1.41	-1.25	2.01	-0.74	-1.08
IMPCH 39	0.53	1.73	1.32	-0.12	-0.12
IMPCH 73	3.83	-0.59	0.01	-1.28	0.78
Manipur Black	1.95	-0.21	-2.06	1.84	-0.66
IMPCH 88	1.79	-0.73	-1.87	-0.40	-0.90
IMPCH 32	-0.89	-1.05	0.93	-0.39	-1.38
IMPCH 62	1.56	-2.15	0.52	-0.22	1.45

represented early maturing whereas genotypes with high positive PC2 scores such as IMPCH 9 (2.86), IMPCH 66 (2.55), IMPCH 70 (2.29) and IMPCH 39 (1.73) represented late flowering genotypes, which may be useful for selecting materials suited to different cropping systems and agro-climatic conditions. In PC3, genotypes such as PAC 751 Elite (-2.23), Manipur Black (-2.06), IMPCH 88 (-1.87), IMPCH 35 (-1.31) and IMPCH 9 (-1.26) were associated with higher grain yield, kernel rows per ear, shelling percentage, and ear placement. In PC4, genotypes with high positive values, IMPCH 111 (2.49) and Manipur Black (1.84), were associated with higher kernel rows per ear and shelling percentage. In the fifth principal component, genotypes such as IMPCH 20 (1.54), IMPCH 62 (1.45), IMPCH 110 (1.42), and PAC 751 Elite (1.09) were associated with increased kernel row number. Among all the principal components, PC1 and PC3 contributed predominantly towards yield and yield-attributing traits. Based on these components, PAC 751 Elite, IMPCH 35 and IMPCH 110 may be considered promising genotypes for yield improvement programmes. Thus, the genotypes with high scores could be used as future parents in hybridization programmes aimed at exploiting heterosis (Kushwah *et al.*, 2024).

Conclusion

This study revealed considerable genetic diversity among the inbreds through Mahalanobis D² and principal components analysis. The intra and inter cluster distance values reported the presence of significant genetic diversity among the genotypes evaluated. The traits days to 50 % pollen shedding, 100 kernel weight, tassel length, and shelling percentage were the main contributors to variance. These can be used as selection criteria for future breeding programmes.

The PCA analysis revealed that the five principal components contributed to 82.25% of the overall variability. The PCA biplot efficiently exhibited trait association and genotype performance. The genotypes PAC 751 Elite, IMPCH 35, and IMPCH 110 identified as potential candidates for hybridization experiments. The study confirms that the multivariate techniques such as PCA and D² analysis are useful methods for finding superior and divergent parents consequently helping in the development of high-yielding maize hybrids.

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