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Research Article

Study of genetic variability and diversity analysis in maize (*Zea mays* L.) by agglomerative hierarchical clustering and principal component analysis

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Abstract

The most comprehensive study was undertaken to investigate genetic variability, character association studies and diversity analysis for yield and yield attributing traits in sixty-five maize genotypes along with five checks raised in Augmented block design II and observations were recorded on fourteen morphological traits. For all the traits, the analysis of variance revealed significant variance. PCV and GCV was high for anthesis silking interval. High genetic advance as per cent of mean along with heritability was recorded for anthesis silking interval, number of tassel branches, cob length, plant height and tassel height. This showed the effectiveness of selection for these traits. Character association analysis confirmed that grain yield per plant had a positive significant association with number of kernel rows per cob, number of kernels per row, cob breadth, cob length, 100 seed weight, shelling percentage and plant height. This implies that by selecting for these traits, grain yield per plant can be improved significantly. Path analysis for the attributed traits showed the direct effect of shelling percentage and plant height on single plant yield. Consequently, an emphasis on these traits could lead to the successful identification of higher yielding genotypes. Based on agglomerative hierarchical clustering, genotypes UMI1113, UMI1153, UMI 653-2-3, UMI1131-5 and UMI1076-5-4-2 can be used as parental lines in the hybridization programme. PCA biplot showed that number of kernel rows per cob, number of kernels per row, cob placement height, plant height and single plant yield mostly contributed towards variability. Furthermore, these parental lines should be assessed for their general combining ability and specific combining ability to generate stable and superior hybrids for better yield performance.

Keywords: Genetic variability, character association, diversity analysis.

INTRODUCTION

Maize (*Zea mays* L.) is the major cereal, also referred as the “queen of cereals,” belonging to the grass family Poaceae’s Maydeae tribe (Dhaka *et al.*, 2010). India is the world’s fourth-largest maize producer, with 10.2 Mha area, 26.264 MT production and a productivity of 2.58 t/ha (FAOSTAT, 2016). With an area of 193.7 Mha and an annual productivity of 5.75 t/ha, about 1147.7 MT of

maize are already being grown by more than 170 nations (FAOSTAT, 2020). By 2050, the developing world’s demand for maize is expected to have doubled, and therefore by 2025, maize is expected to produce the most both in the world wide and in the industrialized regions (Rosegrant *et al.*, 2009). Understanding the genetic structure of maize requires knowledge of its genetic diversity, which in turn

aids breeders in selecting suitable parents for breeding programmes (Al Badeiry *et al.*, 2014). Superior hybrids and lines can be created by segregating populations with more variability and the introduction of desirable traits/genes using genotypes with more diversity. Hence, it is necessary to assess the level of genetic diversity using the genotypes that are now available.

Knowing the genetic relationships between the attributes, the existence of genetic variability, and the heritability for the trait of interest among the germplasm lines is advantageous for any plant breeding effort that attempts to produce inbreds based on specified objectives. The emphasis on performance in maize breeding strategies is to maximise the traits with adequate amount of variability. Through the selection of yield component characters, yield can be improved effectively. Therefore, correlation analysis gives information on the type and strength of the relationship between various component characters and seed yield. A considerable level of genetic diversity, or a heritable difference between cultivars, is needed in a population for a long-term plant breeding programme to be successful. According to reports, the degree of genetic diversity in the population has a direct correlation with the rate of selection (Tabanao and Bernardo, 2005). Furthermore, heritability and Genetic Advance (GA) of each individual characteristic determine how effectively genotypic variability can be utilised by selection (Bilgin *et al.*, 2010). Making choices during plant breeding programmes requires a fundamental understanding of the direction and strength of associations between characteristics. The significance rises, particularly if some of the desirable features, like grain production, are challenging to evaluate, difficult to choose, or have low heredity (Cruz *et al.*, 2014). As a result, the current experiment was done to understand the degree and extent of genetic diversity and variability among germplasm in order to identify features to increase maize production and to select diverse parents to produce heterotic hybrids.

MATERIALS AND METHODS

Using 65 germplasm lines that were obtained from the maize unit, Department of Millets, Tamil Nadu Agricultural University between 2021 and 2022, the study was carried out to investigate the genetic variability, character association, and path analysis for yield and yield component characteristics in maize. In Rabi 2021–2022, the experiment was set up using an augmented block design II, with five blocks of 18 rows and five checks *viz.*, UMI-1200, UMI-1201, UMI-1205, UMI-1230, and CO6 and checks were replicated five times. Each germplasm was seeded in a single row with a 60 x 20 cm spacing, all crop cultivation aspects were followed as recommended. The observations on plant height (cm), days to 50 percent silking, days to 50 percent tasseling, tassel length, anthesis silking interval, number of tassel branches, cob length (cm), cob breadth (cm), number of kernel rows per cob, number of kernels per row, cob placement height,

shelling percentage, 100 seed weight (g) and single plant yield (g) were recorded. Using the TNAU STAT statistical software, the recorded data were analysed to examine the variability and character association. Agglomerative hierarchical clustering (AHC) analysis was performed using XLSTAT software based on Pearson's coefficient with the Unweighted Pair Group method and dendrograms were constructed. Principal component analysis was performed using GRAPES software.

RESULTS AND DISCUSSION

Analysis of variance based on fourteen quantitative characters showed significant variance among the evaluated genotypes of maize (Table 1). Significant variation across the genotypes showed that there were enough genotypic variations to improve the selection potential (Ghimire and Timsina, 2015). For all of the examined variables, genetic variability studies showed that the phenotypic coefficient of variation was larger than the genotypic coefficient of variation (Table 2). This demonstrated that environmental influences had a significant impact on the expression of these characters. In this present study, high Phenotypic coefficient of variation (PCV) and Genotypic coefficient of variation (GCV) were observed for the traits *viz.*, anthesis silking interval, number of tassel branches, cob length and plant height. The anthesis silking interval showed the highest PCV (28.14%) and GCV (23.95%) whereas shelling percentage showed the lowest PCV (3.34%) and GCV (3.02%) (Table 1). The similar results of high values of PCV and GCV for anthesis silking interval was reported by Bista *et al.* (2022). Moreover, moderate to high range of PCV and GCV estimates were observed for traits like plant height, cob placement height, grain yield per cob, cob length and number of kernels per row, similarly reported by Bhadru *et al.* (2020).

While examining the traits heritability, the broad sense heritability varied from 27.40 (single plant yield) to 97.04 (plant height). Genetic Advance over Mean (GAM%) for the traits was varied between 5.62% for shelling percentage and 42% for anthesis silking interval. The traits, plant height, days to 50% silking, days to 50% tasselling, cob placement height, cob length, cob breadth, 100 seed weight, number of tassel branches and tassel height were found to be highly heritable (Table 2). Similar outcomes was given by Bhiusal *et al.* (2016). Despite a high degree of heritability, selection for characteristics is mostly dependent on the combined effects of high genetic gain. In the current experiment, it was shown that plant height, anthesis silking interval, number of tassel branches, tassel height and cob length all had significant heritability with high genetic advance as a percent of mean. This shows that these attributes may be improved by selection and hybridization since they are governed by additive gene action (Muhammad Akbar *et al.*, 2006 and Ali *et al.*, 2010). Wali *et al.* (2019) reported similar outcomes. However, shelling percentage and single plant

Table 1. ANOVA for 14 yield attributing traits

Characters	Block (df:4)	Treatment (df:68)	Checks (df:3)	Total entries (df:64)	Checks vs Treatment (df:1)	Error (df:12)
PH	1728.86*	641.53*	2135.97*	530.89*	3239.66*	15.69
DFT	109.86*	7.81*	0.45*	6.09*	140.21*	0.37
DFS	96.62*	7.98*	0.40*	6.53*	123.25*	0.28
ASI	0.70*	0.54*	0.32*	0.54*	0.59*	0.15
TH	49.10*	45.04*	192.58*	38.60*	14.36*	4.25
NTB	5.22*	7.99*	8.18*	8.09*	1.63*	0.77
CPH	1179.91*	200.04*	579.15*	159.9*	1632.18*	8.91
NKR (per cob)	2.28*	2.53*	4.05*	2.45*	2.90*	0.97
NK (per row)	60.30*	6.78*	7.73*	5.54*	83.24*	2.73
CL	7.70*	7.34*	4.96*	6.73*	53.04*	0.58
CB	1.14*	3.38*	3.98*	3.35*	3.70*	0.28
SP	6.22*	7.23*	15.06*	6.88*	6.24*	1.26
HSW	16.12*	14.71*	29.37*	13.25*	64.01*	1.22
SPY	765.04*	142.79*	399.82*	112.53*	1308.56*	81.70

*Significance at 5% level, df: Degree of freedom

Table 2. Estimates of the yield and yield-contributing attributes of maize germplasm accessions

Traits	PCV	GCV	ECV	h ²	GA	GAM
PH	18.17	17.90	3.12	97.04	46.06	36.32
DFT	4.43	4.29	1.09	93.98	4.78	8.57
DFS	4.38	4.29	0.90	95.79	5.04	8.64
ASI	28.14	23.95	14.76	72.46	1.10	42.00
TH	18.01	16.99	5.98	88.99	11.39	33.02
NTB	19.16	18.23	5.90	90.52	5.30	35.73
CPH	14.89	14.47	3.52	94.42	24.60	28.96
NKR	12.88	10.03	8.09	60.60	1.96	16.08
NK	13.79	9.81	9.69	50.64	2.45	14.38
CL	18.31	17.51	5.36	91.43	4.89	34.48
CB	14.63	14.00	4.25	91.57	3.45	27.59
SP	3.34	3.02	1.43	81.61	4.41	5.62
HSW	15.63	14.89	4.75	90.77	6.81	29.23
GY	15.93	8.34	13.57	27.40	5.99	8.99

PH-Plant height, DFT- Days to fifty per cent tasselling, DFS- Days to fifty per cent silking, ASI- Anthesis – silking interval, TH- Tassel height, NTB- Number of tassel branches, CPH-Cob placement height, NKR- Number of kernel rows per cob, NK- Number of kernels per row, CL- Cob length, CB- Cob breadth, SP- Shelling percentage, HSW- 100 Seed weight, SPY- Single plant yield

yield had moderate heritability and lower genetic gain, indicating that non-additive gene action was responsible for these traits among the genotypes tested. As a result, breeding techniques like heterosis breeding and progeny selection methods can be used to enhance the traits (Bello *et al.*, 2012).

According to character association studies, single plant yield significantly and positively correlated with the

number of kernel rows per cob, number of kernels per row, cob breadth, cob length, weight of 100 seeds, the percentage of shelled seeds, and the plant height, and vice versa (Table 3). Among the yield attributing traits, cob breadth showed high significance with single plant yield. The similar association was reported by Prakash *et al.* (2019) and Keerthana *et al.* (2022). The flowering traits viz., days to 50 percent silking had positive and significant correlation but days to 50 percent tasseling

Table 3. Coefficients of phenotypic correlation (r) between the yield and its attributing traits

	PH	DFT	DFS	ASI	TH	NTB	CPH	NKR	NK	CL	CB	SP	HSW	SPY
PH	1													
DFT	-0.201	1												
DFS	0.268*	-0.253*	1											
ASI	-0.502*	0.227	-0.289*	1										
TH	0.227	-0.488*	0.334*	-0.287*	1									
NTB	-0.036	0.275*	-0.451*	0.342*	-0.277*	1								
CPH	0.228	0.061	0.322*	-0.496*	0.276*	-0.248*	1							
NKR	-0.3006*	0.186	-0.027	0.278*	-0.489*	0.290*	-0.293*	1						
NK	-0.301*	-0.263*	0.128	-0.049	0.282*	-0.452*	0.292*	-0.292*	1					
CL	0.154	-0.290*	-0.341*	0.139	-0.045	0.331*	-0.476*	0.268*	-0.263*	1				
CB	0.044	0.139	-0.232*	-0.316	0.138	-0.037	0.292*	-0.448*	0.258*	-0.234*	1			
SP	0.325*	0.053	0.059	-0.262*	-0.341*	0.204	0.033	0.275*	-0.465*	0.293*	-0.226	1		
HSW	-0.428	0.266*	-0.044	0.131	-0.295*	-0.342*	0.067	0.0067	0.319*	-0.439*	0.260*	-0.311*	1	
SPY	0.315*	-0.414*	0.241*	-0.039	0.102	-0.296*	-0.268*	0.249*	-0.455*	0.271*	-0.418*	0.317*	-0.217*	1

*Significance at 5% level

PH-Plant height, DFT- Days to fifty per cent tasselling, DFS- Days to fifty per cent silking, ASI- Anthesis – silking interval, TH- Tassel height, NTB- Number of tassel branches, CPH-Cob placement height, NKR- Number of kernel rows per cob, NK- Number of kernels per row, CL- Cob length, CB- Cob breadth, SH%- Shelling percentage, HSW- 100 Seed weight, SPY- Single plant yield

was negatively correlated with single plant yield. Similar outcomes were observed in other studies conducted by Tejaswini *et al.* (2022). The anthesis silking interval had positively and significantly correlated with days to 50 percent tasseling. Similar outcomes were pointed out by Prakash *et al.* (2019). Thus, these correlated traits can be used in developing high yielding lines.

As per path analysis studies, it revealed that shelling percentage and plant height exhibited the high direct and positive direct effect whereas cob placement height and number of tassel branches showed high but negative effect on single plant yield. Moreover, the direct effect on single plant yield was moderate and positive for the traits *viz.*, number of kernels per row, tassel height, anthesis silking interval and cob breadth showed moderate negative effect on single plant yield. Furthermore, negligible but positive direct effect of number of kernel rows per cob on grain yield per plant was observed. Similar results were found in the studies done by Kumari *et al.* (2018) and Rajwade *et al.* (2018). This demonstrates that one of the key determining variables for maize production enhancement might be the number of kernels per row. For the purpose of increasing yield attributable traits, the indirect impacts of traits provide us with a set of parameters to be concerned. In this study it was observed that plant height exhibited moderate but negative indirect effect on single plant yield via anthesis silking interval. Plant height exhibited low but positive indirect effect on single plant yield via shelling percentage, days to fifty per cent silking, cob placement height and tassel height. (Table 4). Similarly, the tassel height and number of kernels per row showed a positive effect for grain yield indirectly via anthesis silking interval.

Hence the attributes *viz.*, number of kernels per row, number of kernel rows per cob, tassel height, days to 50% tasselling, plant height and cob placement height are important to be considered for maize improvement.

Cluster analysis based on similarity coefficients between pair of genotypes results grouping of 65 maize genotypes into five groups with subclusters and it is accessible in Table 4 and fig. 1. In the grouping, maximum of 28 maize genotypes were grouped as single cluster followed by 15 genotypes in cluster II and minimum of three in the cluster III. These results indicated that the genotypes utilized in this study having wide diverse in nature. The pairs of maize genotypes *viz.*, UMI 1113 and UMI1075-4-1-1 in cluster I, UMI1153 and UMI504 in cluster II, UMI9188-2-2 and UMI653-2-3 in cluster III, UMI9119-1-1 and UMI1131-5 in cluster IV and, UMI1076-5-4-2 and UMI9233-1 in cluster V were almost genetically similar with a similarity level of 99% between the individuals making up the pair but were distinct from each other. The genotypes were grouped into three clusters, and observed the best-performing genotypes in cluster 3 (Ali *et al.*, 2015). Maize genotypes were categorized into four clusters, and found that cluster 2 contained the largest number of genotypes (Tanavar *et al.*, 2014). Also, the findings were on par with the results of Subramanian and Subbaraman, (2010). The selection of parents having contrasting characters from two different clusters with maximum of dissimilarity is highly helpful to develop superior heterotic hybrids for some useful economic traits and with this view the genotypes UMI1113, UMI1153, UMI 653-2-3, UMI1131-5 and UMI1076-5-4-2 may be utilized in the hybridization programme as they are more diverse.

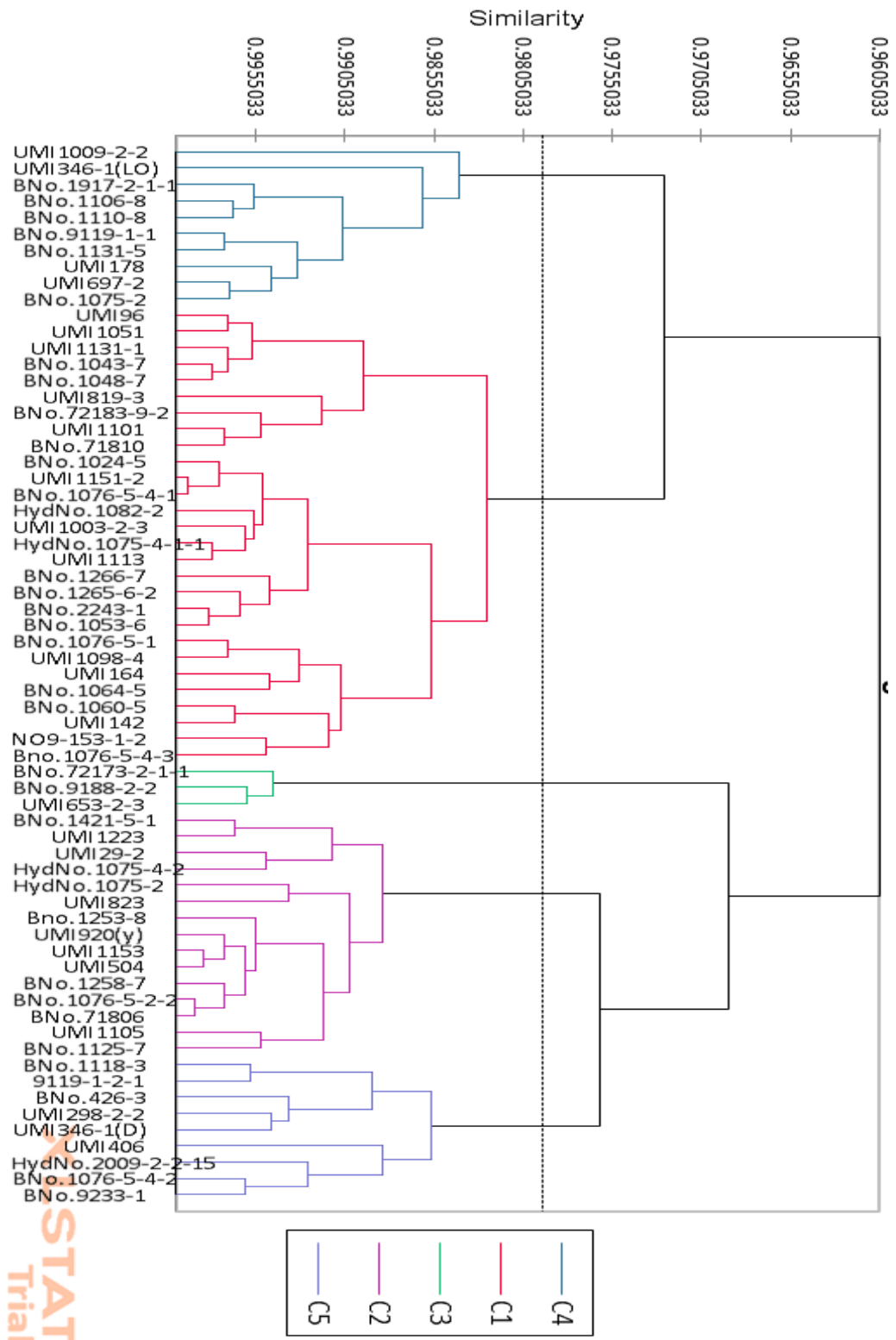


Fig. 1. Dendrogram comprising 65 genotypes was constructed based on 14 quantitative characters using UPGMA cluster analysis

Table 4. Path coefficients illustrating the direct and indirect effects of several attributes on the yield of a single plant (SPY)

	PH	DFT	DFS	ASI	TH	NTB	CPH	NKR	NK	CL	CB	SP	HSW
PH	0.4662	0.0302	-0.0367	-0.1156	0.0517	0.0142	-0.0980	-0.0372	-0.0859	-0.0224	-0.0105	0.1641	-0.0050
DFT	-0.0938	-0.1500	0.0347	0.0523	-0.1112	-0.1072	-0.0264	0.0230	-0.0751	0.0419	-0.0333	0.0269	0.0031
DFS	0.1252	0.0381	-0.1366	-0.0665	0.0761	0.1758	-0.1387	-0.0034	0.0366	0.0494	0.0555	0.0302	-0.0005
ASI	-0.2342	-0.0341	0.0395	0.2301	-0.0654	-0.1333	0.2134	0.0345	-0.0140	-0.0202	0.0754	-0.1322	0.0015
TH	0.1060	0.0733	-0.0457	-0.0662	0.2274	0.1081	-0.1190	-0.0606	0.0806	0.0066	-0.0330	-0.1719	-0.0034
NTB	-0.0170	-0.0413	0.0616	0.0787	-0.0631	-0.3897	0.1066	0.0359	-0.1289	-0.0479	0.0090	0.1032	-0.0040
CPH	0.1063	-0.0092	-0.0440	-0.1142	0.0629	0.0966	-0.4300	-0.0364	0.0834	0.0688	-0.0698	0.0166	0.0008
NKR	-0.1401	-0.0279	0.0037	0.0642	-0.1113	-0.1130	0.1264	0.1237	-0.0834	-0.0388	0.1071	0.1388	0.0001
NK	-0.1404	0.0395	-0.0175	-0.0113	0.0643	0.1762	-0.1258	-0.0362	0.2850	0.0381	-0.0617	-0.2343	0.0037
CL	0.0722	0.0435	0.0466	0.0321	-0.0104	-0.1290	0.2047	0.0332	0.0750	-0.1446	0.0560	0.1477	-0.0051
CB	0.0205	-0.0209	0.0317	-0.0727	0.0315	0.0148	-0.1258	-0.0555	0.0737	0.0339	-0.2386	-0.1140	0.0030
SP	0.1518	-0.0080	-0.0082	-0.0604	-0.0776	-0.0798	-0.0142	0.0341	-0.1325	-0.0424	0.0540	0.5039	-0.0036
HSW	-0.1997	-0.0399	0.0061	0.0302	-0.0671	0.1335	-0.0289	0.0008	0.0911	0.0636	-0.0621	-0.1568	0.0116
SPY	0.3151	-0.4149	0.2413	-0.0390	0.1021	-0.2966	-0.2681	0.2493	-0.4552	0.2717	-0.4185	0.3171	-0.2175

Residual effect=0.4005

(High = 0.30-0.99, Moderate = 0.20-0.29, Low = 0.10-0.19)

PH-Plant height, DFT- Days to fifty per cent tasselling, DFS- Days to fifty per cent silking, ASI- Anthesis – silking interval, TH- Tassel height, NTB- Number of tassel branches, CPH-Cob placement height, NKR- Number of kernel rows per cob, NK- Number of kernels per row, CL- Cob length, CB- Cob breadth, SH%- Shelling percentage, HSW- 100 Seed weight, SPY- Single plant yield

Table 5. Classification of genotypes into clusters based on dendrogram using 14 quantitative traits

Cluster	Number of genotypes	Genotype accessions
I	28	BNo.2243-1, BNo.1060-5, BNo.1024-5, NO9-153-1-2, BNo.1053-6, UMI1151-2, HydNo.1075-4-1-1, UMI819-3, BNo.1265-6-2, UMI1101, BNo.1076-5-1, BNo.71810, UMI1003-2-3, BNo.72183-9-2, BNo.1043-7, UMI164, UMI1131-1, BNo.1064-5, BNo.1266-7, BNo.1048-7, UMI1113, UMI196, UMI1051, BNo.1076-5-4-1, HydNo.1082-2, UMI142, Bno.1076-5-4-3, UMI1098-4
II	15	UMI1153, HydNo.1075-2, UMI29-2, UMI504, BNo.1076-5-2-2, BNo.1421-5-1, UMI1105, BNo.1125-7, BNo.1258-7, BNo.71806, HydNo.1075-4-2, UMI1223, Bno.1253-8, UMI920(y), UMI823
III	3	BNo.9188-2-2, BNo.72173-2-1-1, UMI653-2-3
IV	10	BNo.1106-8, BNo.9119-1-1, UMI178, BNo.1131-5, UMI697-2, BNo.1075-2, BNo.1917-2-1-1, UMI1009-2-2, BNo.1110-8, UMI346-1(LO)
V	9	UMI298-2-2, UMI346-1(D), BNo.1118-3, UMI406, BNo.1076-5-4-2, BNo.9233-1, HydNo.2009-2-2-15, BNo.426-3, 9119-1-2

The total variation present among the genotypes were partitioned into 14 principal components (PC) of which first five principal components (PC) had Eigen value >1 and contributes to about 70.26 per cent of total variation. (Table 6 and Fig. 2).

The first principal component (PC 1) contributed about 25.02 per cent of the total variation and had an Eigen value of 3.50. The traits viz., cob breadth, cob length, plant height, tassel height, number of tassel branches and cob placement height contributed maximum towards divergence in PC 1. The second principal component (PC 2) had an Eigen value of 2.36 and accounts for about

16.89 per cent of the total variation. The major contributors in PC 2 are days to 50% tasseling, days to 50% silking, cob placement height and number of kernel rows per cob. The third principal component (PC 3) had an Eigen value of 1.56 and it explains about 11.19 per cent of the total variation. The major contributors in PC 3 are 100 seed weight, shelling percentage, anthesis silking interval with negative loading (-0.559) and number of kernels per row. The fourth principal component (PC 4) contributes about 9.32 per cent of the total variation and had an Eigen value of 1.30. The traits viz., cob placement height with negative loading (-0.521), 100 seed weight and anthesis silking interval in PC 4 (Table 7). Similar findings were

Table 7. Relative contribution of 14 characters towards divergence in extracted principal components in maize

Characters	Principal Components				
	PC1	PC2	PC3	PC4	PC5
Plant height (cm)	0.746	0.202	-0.037	-0.111	0.05
Days to 50% tasseling	-0.074	0.946	-0.023	0.112	-0.034
Days to 50% silking	-0.096	0.923	-0.163	0.247	0.078
Anthesis silking interval	-0.069	-0.036	-0.559	0.49	0.428
Tassel height	0.732	-0.226	-0.141	-0.275	0.238
Number of tassel branches	0.563	-0.177	-0.361	-0.201	-0.15
Cob placement height	0.556	0.435	0.007	-0.521	-0.029
Number of kernel rows per cob	0.342	0.393	-0.341	-0.117	-0.159
Number of kernel rows	0.468	0.251	0.496	0.187	0.296
Cob length	0.747	-0.175	-0.108	0.208	0.249
Cob width	0.767	-0.066	-0.007	0.347	-0.071
Shelling percentage	-0.083	0.189	0.572	-0.358	0.353
100 seed weight	0.392	-0.114	0.586	0.448	-0.029
Single plant yield	0.349	0.052	0.177	0.224	-0.72

for the maize improvement program, priority consideration should be given to these traits. Similar outcomes were pointed out by Azad *et al.* (2012). Additionally, Mustafa *et al.* (2015) emphasised the use of PCA in selecting drought-tolerant maize lines by focusing on factors that significantly contribute to the genetic diversity of maize germplasms. In agglomerative hierarchical clustering, the genotype B.NO.1076-5-4-2, UMI 1003-2-3 & UMI 178 falls in cluster IV and B.NO.9119-1-1 & HYD.NO.2009-2-2-15 in cluster V. So, these genotypes can be used as parents in improvement in maize.

Thus, it may be concluded that the genotypes studied exhibited sufficient amount of variability and selection based on the traits like number of kernel rows per cob, number of kernel rows, cob length, cob breadth, plant height and shelling percentage can bring enhancement in yield attributes in maize. Based on agglomerative hierarchical clustering and principal component analysis, genotypes B.NO.1076-5-4-2, UMI 1003-2-3, UMI 178, B.NO.9119-1-1 & HYD.NO.2009-2-2-15 can be used as parental lines in hybridization programme.

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