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Full Length Research Paper

Genetic variation and multivariate analysis of late maturing maize inbred lines using phenotypic and disease related traits

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Abstract

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The determination of diversity among maize inbred lines is important for heterosis breeding. To estimate the genetic diversity amongst the 33 maize inbred lines used, cluster and principal component analyses were carried out for sixteen different morphological and *Aspergillus* ear rot disease related traits. The field experiments were carried out under field artificial infection with *Aspergillus flavus* inoculum following standard procedures. The trials were carried out during the 2020-2021 cropping season at two locations in Cameroon, namely, Bangangte from the Western highlands and Mbalmayo from the Bimodal humid forest zone. In each trial site, the experiment was laid out in an 11 x 3 alpha lattice design with two replications under similar conditions. The PCA identified six principal components (PCs) with Eigen value greater than 1.00 and accounted for 72% of total variation. Cluster analysis based on Ward's minimum variance procedure distributed the maize inbred lines into 7 clusters indicating their broad genetic base of which cluster VII was the largest containing eleven inbred lines and maximum inter-cluster distance was recorded between clusters IV and VI (196.66) suggesting their use in breeding programmes for the exploitation of heterosis for the desirable ear rot and yield traits. Out of all the clusters, cluster I showed highest mean value for grain yield, indicating the importance of this cluster genotypes (87036, 88094, 90156, 90188 and 90301) in maize yield improvement programmes. Similarly, cluster IV showed the lowest mean values for *Aspergillus* ear rot disease incidence (21.28%) and *Aspergillus* ear rot disease severity (6.9%) revealing the importance of this cluster genotype (89291) in maize ear rot disease resistance improvement programmes. The distribution of genotypes in the study revealed that the geographical origin did not have any bearing on clustering pattern. These results showed that the inbred lines having widely divergent clusters can be utilized in hybrid breeding programmes.

Keywords: Genetic divergence, Hierarchical cluster analysis, Maize, Principal Component Analysis.

1. Introduction

Maize (*Zea mays* L.) is the most important cereal food crop in the world after wheat and rice accounting for 9% of the total food grain production (Mounika *et al*., 2018). It occupies a prominent place in Cameroonian agriculture as it is widely grown in the country in varied climatic situations throughout the year suggesting its wider adaptability.

The main objective of any maize breeding programme is to develop hybrids that have higher yields than the existing cultivars as they are popular among the farming community due to the perception that they tend to have yield and disease resistance advantage over the existing varieties, often landraces and open pollinated varieties. To develop high yielding disease tolerant maize hybrids, the development and evaluation of inbred lines contributes a major role in breeding programmes. Hence, inbred lines developed through sib mating and selfing. need to be evaluated for their genetic diversity and performance to plan an effective hybrid breeding programme as genetically diverse parents are known to produce high heterotic effects, while lines closely related can be used in line development.

Multivariate analysis is very useful in quantifying the degree of divergence between inbred lines or any biological population at genotypic level. It is also used in the assessment of relative contribution of different components to the total divergence at both intra and inter-cluster level (Rafique *et al.*, 2018). Evaluation, characterization and classification of inbred lines based on estimates of genetic diversity will facilitate the identification of diverse parental lines which can be exploited in hybrid breeding to develop promising hybrids or varieties. Several methods have been reported (Shrestha, 2016; Mounika *et al*., 2018) to show the pattern and magnitude of variability such as Mahalanobis D^2 analysis, Principal component analysis and hierarchical cluster analysis based on Ward's minimum variance method. PCA and cluster analysis is better utilized for studying the diversity among the genotypes in various crops. In view of the above, 33 inbred lines were investigated to study the nature and magnitude of genetic divergence for *Aspergillus* ear rot, grain yield, and its component characters to provide a basis for the selection of parents in a hybridization program in maize.

2. MATERIALS AND METHODS

2.1 Genetic materials and experimental procedure. The study was carried out during the 2020-2021 cropping season at two locations in Cameroon namely Bangangte from the Western highlands and Mbalmayo from the Bimodal humid forest zone. In each trial site, the experiment was laid out in an 11 x 3 alpha lattice design with two replications under similar conditions using 33 maize inbred lines from The Institute of Agricultural Research for Development (IRAD) and International Institute for Tropical Agriculture (IITA) maize breeding programmes. For all trials and sites, the experimental plot comprised of single rows of 4m long with a planting space of 0.75m between rows and 0.5m within row. Three seeds were planted per stand and was later thinned to two with the aim of achieving a plant population of 53,330 plants per hectare. All agronomic practices were followed from planting to mid-silk stage. At mid silking stage, artificial infection was carried out using the non-wounding technique following standard procedures (Reid *et al*., 1996).

2.2 Data Collection

The data were collected following CIMMYT's guideline for maize trial management (CIMMYT, 1985) as described in Table 1.

Grain yield was recorded at harvest on plot basis and adjusted to the 12.5% moisture using the formula:

Grain yield = $\frac{(Field\ ear\ weight\ per\ plot)\ x\ (100-MC)\ x\ 0.8\ x\ 10{,}000}{1000\ x\ (100-12.5)\ x\ Area\ harvested\ per\ plot}$ tonnes ha^{-1}

Where: MC = field moisture content in grains at harvest (%) and 0.8 = shelling coefficient.

During harvest (physiological maturity) in all experimental sites, the primary ears in all plots were harvested and rated for severity of the ear rots, using the 1 – 7 rating scale (Reid *et al*., 1996) as seen in Figure 1.

In order to normalise the data for disease incidence, disease severity, ear declination, husk cover, grain type and ear insect damage scores, angular transformations were carried out and transformed to percentages before analysis. The data were analyzed using principal component analysis (PCA) for dimensional reduction and to determine the importance of different traits in explaining multivariate polymorphism.

2.3 Data analysis

The data were analyzed using Principal Component Analysis (PCA) for dimensional reduction and to determine the importance of different traits in explaining multivariate polymorphism. Hierarchical cluster analysis was performed following the minimum variance method of Ward (1963), based on squared Euclidean distances.

3. RESULTS

In principal component (PC) analysis, the number of variables were reduced to linear functions called canonical vectors which accounted for most of the variation produced by the characters under study. The thirty-three genotypes were grouped into seven clusters using the Ward's minimum variance procedure (Shrestha, 2016) and the distributions of the genotypes into different clusters are shown in Table 2 and Figure 2. Among all the clusters, cluster VII was the largest with eleven genotypes followed by cluster II containing eight genotypes, cluster with five genotypes, cluster V with four genotypes and clusters III and VI with two genotypes each. Cluster IV mono genotypic having only one genotype.

The Eigen values, per cent variance, per cent cumulative variance and factor loading of different characters studied are shown in Table 3. In this experiment, first six principal components (PC) based on 16 quantitative traits showed Eigen values greater than 1. The contribution of these four PCs was 72% in the overall variability among the genotypes. The contribution of PC1 was found to be 19% in the total divergence of the studied population, in which

Table 1. Trait description and measurement.

Trait	Full meaning	Guide
AD	Days to mid pollen	Number of days from planting to when 50% of plants in a plot start shedding pollen.
SD	Days to mid silking	Number of days from planting to when 50% of plants in a plot emerge silks of 2-5cm long.
ASI	Anthesis silking interval	Difference between the number of days to mid silking and number of days to mid pollen shed.
MAT	Days to maturity	Number of days from planting to when 75% of plants in a plot attain physiological maturity.
PH	Plant height (cm)	Measured from the base of the plant at floor level up to the point where the first tassel branch begins.
EH	Ear height (cm)	Measured from the base of the plant at floor level up to the point of attachment of the uppermost ear node.
PLTVIG	Plant vigour	Measured on a scale of 1 to 5, where 1 indicates healthy plants and 5 stands for weak fragile plants.
EarAsp	Ear aspect	Measured on a scale of 1 to 5, where 1 indicates healthy good-looking ears and 5 stands for poor grain filled ears.
LODG	Root and stem lodging	This was rated separately on a plot basis, by counting the number of plants that had inclined more than 45^{o} for root lodging, and those whose stalk had broken below the ear as stem lodging and then multiplied by 100. For the purpose of statistical analysis, these two were combined.
TEX	Grain texture	This was rated on a score scale of 1 - 4, where 1 = flint; 2 = semi flint, more than 50% flint in the kernel row, or slight flint grain; 3 = more than 50% dent in the kernel row or slight dent grain; and 4 = dent.
INSECT	Ear insect damage	The cobs were rated on a scale of 1 - 5, where 1 = clean or no damage and 5 = severe damage with visible holes. The percentage of grains damaged was calculated on per plot basis (Munkvold and Desjardins, 1997; Ajanga and Hillocks, 2000).
Eardec	Ear declination	This was rated on a scale of 1 - 5, where 1 = drooping downwards and 5 = standing upright along the stalk (Betran *et al*., 2002; Rossouw *et al*., 2002).
Moist	Moisture content	Collect a sample of about 200g of shelled grains from each plot and measure the moisture content using a moisture meter.
INC	*Aspergillus* ear rot disease incidence	Measured on plot basis by expressing the percentage of *Aspergillus* ear rot infection ears as a total of the harvested ears.
SEV	*Aspergillus* ear rot disease severity	Measured on a 1 – 7 rating scale By this rating, 1 = Sound, unblemished kernels on the ear (0%); 2 = 1 - 3% of kernels on the ear rotten; 3 = 4 - 10% of kernels on the ear rotten; 4 = 11 -25% of kernels on the ear rotten; 5 = 26 - 50% of kernels on the ear rotten; 6 = 51 - 75% of kernels on the ear rotten; and 7 = 76 - 100% of kernels damaged, covered with fungus or discoloured (Reid *et al*., 1996). See Figure 1.
HUSK	Husk cover	Measured on a scale of 1 to 5, where 1 indicates well covered ear tips and 5 stands for completely exposed ear tips (Kossou *et al*. (1993).
GYD	Grain yield (t/ha)	Yield per hectare at standard 12.5% moisture content.

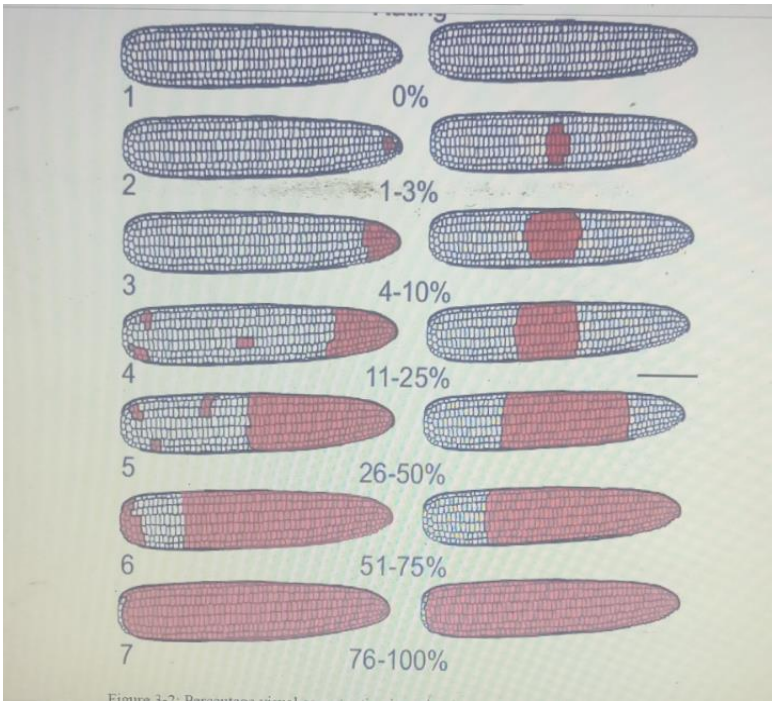

Figure 1 Maize ear rot rating scheme. (Source: Reid *et al*., 1996).

Table 2. Maize inbred lines grouped into seven different clusters.

Cluster	1	2	3	4	5	6	7
	87036	87014 89248	89343	89291	1368	89183	88099
	88094	89320 90183	90323		89243	89311	89193
	90156	90204 90267			89246		8923
	90188	90313			89365		90176
	90301	TZSTR1150					90219
							90251
							90263
							Exp124
							INEW-SR
							M131
							TZI-5-1171

the major contributing traits were days to 50% tasselling, days to 50% silking, grain yield, ear height, husk cover and plant vigour. The second principal component (PC2) was responsible for 14% of the variation and was mainly contributed by anthesis-silking interval, grain texture, plant vigour, husk cover, ear insect damage, ear aspect, grain yield and *Aspergillus* ear rot disease severity. The third principal component (PC3) explained 13% of variation and was associated mainly with *Aspergillus* ear rot disease incidence, *Aspergillus* ear rot disease severity, plant height, ear aspect, days to 50% anthesis, days to 50% silking, grain yield, ear declination, husk cover and anthesis-silking

Table 3. Eigen values, percentage of the total variance represented by first six Principal components, cumulative per cent variance and component loading of different characters in maize.

Character	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6
AD	0.49	-0.04	0.17	0.20	0.11	0.16
SD	0.49	-0.15	0.15	0.16	0.18	0.13
ASI	-0.08	0.48	0.04	0.13	-0.32	0.08
PH	-0.03	-0.04	0.34	-0.36	-0.38	-0.23
EH	0.13	-0.17	-0.46	-0.18	-0.12	0.38
MAT	-0.18	-0.30	-0.03	0.39	-0.10	-0.11
INC	-0.14	-0.09	0.49	0.19	-0.19	0.28
SEV	-0.33	0.05	0.37	0.02	0.08	0.31
EarAsp	-0.12	0.16	0.30	0.11	0.29	0.27
PLYVIG	0.03	0.36	-0.10	0.28	-0.40	-0.17
HUSK	0.05	0.33	0.06	0.05	0.52	-0.28
LODG	-0.32	-0.08	-0.29	0.17	0.05	0.41
Eardec	-0.29	-0.17	0.07	-0.35	0.26	-0.23
INSECT	-0.01	0.29	-0.03	-0.49	0.06	0.38
TEX	-0.04	0.47	-0.18	0.10	0.16	0.01
GYD	0.36	0.11	0.15	-0.27	-0.18	0.14
Eigen value (Root)	**3.11**	**2.17**	**2.09**	**1.65**	**1.37**	**1.19**
% Var. Exp.	**19**	**14**	**13**	**10**	**9**	**7**
Cumulative % Var. Exp.	**19**	**33**	**46**	**56**	**65**	**72**

interval. The fourth principal component (PC4) explained 10% variation and was contributed by days to 75% physiological maturity, plant vigour, days to 50% anthesis, *Aspergillus* ear rot disease incidence, lodging, days to 50% silking, anthesis-silking interval, ear aspect, grain texture, husk cover and *Aspergillus* ear rot disease severity. The fifth principal component (PC5) contributed to 9% variation and was contributed by husk cover, ear aspect, ear declination, days to 50% silking, grain texture, days to 50% anthesis, *Aspergillus* ear rot disease severity, ear insect damage and lodging. The sixth principal component (PCA6) was responsible for 7% variation and was contributed by lodging, ear height, ear insect damage, *Aspergillus* ear rot disease severity, *Aspergillus* ear rot disease incidence, ear aspect, day to 50%anthesis, grain yield, days to 50% silking, anthesis-silking interval and grain texture.

Cluster analysis based on PCA scores were compared with the results of the principal component analysis on a visual aid in desecrating clusters in the two dimensional other genotypes.

Based on inter-cluster distances and *per se* performance of the genotypes included in the farthest cluster (VII) genotypes 88099, 89193, 8923, 90176, 90219, 90251, 90263, Exp 124, IN WE-SR, M131 and TZI-5-1171 showed maximum inter cluster distance and good *per se* performance (Table 4).

The nearest and farthest cluster for each of the seven clusters are presented in Table 5. All the seven clusters were solitary with intra-cluster distances of zero. The maximum inter-cluster distance was observed between clusters IV and VI (196.66) followed by clusters III and IV (160.22) and clusters II and IV (136.70).

Cluster means were computed for the 16 characters studied by Ward's minimum variance method and are presented in Table 6. Out of all the clusters, cluster I showed

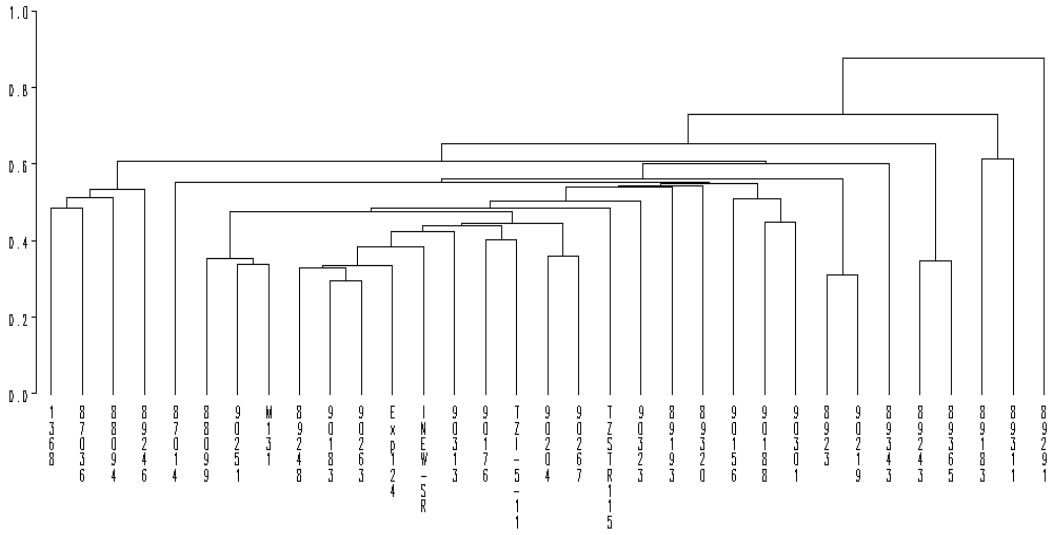

Figure 2. Dendrogram showing relationship of 33 maize (*Zea mays* L.) inbred lines in seven clusters based on Euclidean2 distance.

Table 4. Average intra and inter-cluster Euclidean^2 values among seven clusters in 33 maize inbred lines. Mahalanobis (D^2)

Cluster	1	2	3	4	5	6	7
1	0.00	28.08	48.25	64.27	36.69	75.65*	18.21
2		0.00	34.97	136.70*	73.38**	79.21*	19.20
3			0.00	160.22*	74.47*	71.55	44.78
4				0.00	112.75	196.66*	70.68
5					0.00	43.21	87.50**
6						0.00	112.55**
7							0.00

Note: Diagonal values are intra-cluster distances. Off-diagonal values are inter-cluster distances
*,**= significant at p=0.05 and 0.01 probability levels respectively.

shighest mean value for grain yield, indicating the importance of this cluster genotypes (87036, 88094, 90156, 90188 and 90301) in maize yield improvement programmes. Similarly, cluster IV showed the lowest mean values for *Aspergillu*s ear rot disease incidence (21.28%) and *Aspergillu*s ear rot disease severity (6.9%) revealing the importance of this cluster genotype (89291) in maize ear rot disease resistance improvement programmes.

GYD=grain yield, MAT= days from planting to 75% physiological maturity, EH= ear height, PH= plant height, AD= days from planting to 50% anthesis, SD= days from planting to 50% silking, ASI= anthesis silking interval, LODG= lodging, INC= disease incidence, SEV= disease severity, TEX= grain texture, INSECT= insect damage, Eardec= ear declination, HUSK= husk cover, EarAsp= ear aspect and PLTVIG= plant vigour.

4. DISCUSSION

The estimation of genetic diversity and relationships among germplasm accessions facilitates the selection of parents with diverse genetic background which is very essential for breeding programme (Shrestha, 2016 and Mounika *et al*., 2018). Sokolov and Guzhva (1997) reported significant amount of variability for in maize inbred line populations for their different quantitative traits. In this study considerable morphological variation was found mainly due to genetic factors and also subjected to environmental factors (Table 2, Figure 2). The significant differences ($p< 0.05$, $p< 0.01$) seen among the average intra and interclass cluster Euclidean^2 values (Table 4) suggests wide genetic diversity between these clusters and can be exploited for traits improvement in the breeding

Table 5. The nearest and the farthest cluster from each cluster using Ward's Minimum Variance method in 33 maize inbred lines.

Cluster No	Nearest cluster with D^2 value	Farthest cluster with D^2 value
I	II (28.08)	VI (75.65)
II	I (28.08)	IV (136.70)
III	II (34.97)	IV (160.22)
IV	I (64.27)	VI (196.66)
V	I (36.69)	VII (87.50)
VI	V (43.21)	VII (112.55)
VI	I (18.21)	VII (112.55)

Table 6. Mean values of seven clusters estimated by Ward's minimum variance method from 33 maize inbred line

Cluster	1	2	3	4	5	6	7
AD	67.90	68.94	65.50	72.50	67.88	70.75	67.82
SD	66.10	66.63	62.50	71.00	65.63	68.75	65.73
ASI	**1.85**	**2.31**	**3.00**	1.50	**2.25**	**2.00**	**2.09**
PH	**133.30**	105.78	**128.23**	**160.90**	**152.64**	**129.88**	**122.03**
EH	46.69	58.04	49.33	65.10	45.70	55.52	64.07
MAT	118.60	**117.78**	119.63	113.50	117.81	121.63	117.73
INC	118.60	47.23	55.77	21.28	64.06	82.48	34.84
SEV	118.60	11.19	15.25	6.90	13.05	13.49	10.44
EarAsp	118.60	18.62	21.69	16.13	18.90	16.90	16.80
PLTVIG	118.60	18.21	17.29	15.28	16.76	15.99	17.65
HUSK	118.60	12.41	12.79	12.08	11.22	14.30	12.00
LODG	118.60	9.76	24.99	**0.00**	2.58	12.30	13.63
Eardec	118.60	9.34	11.83	10.11	9.96	9.74	10.19
INSECT	118.60	10.84	10.72	10.93	10.83	9.08	10.25
TEX	118.60	14.34	14.45	12.90	12.64	12.90	14.74
GYD	118.60	3.11	2.41	4.54	3.34	3.61	3.27

Note: Bold figures indicate minimum and maximum values in each character.

programmes. Ihsan *et al* (2005) have also reported that there was substantial variability for days to anthesis among different maize genotypes. Shah *et al* (2000) have also noticed the different maturity traits among maize populations and variability for different morphological traits in maize. Dijak *et al.,* (1999) observed significant amount of variability among long and short stature maize populations for ear and plant height. In the present study, seven clusters of maize were formed based on quantitative traits (Table 6); these findings agree with those founded by Singh *et al*., (2005). Kamara *et al.,* (2003) used PCA to categorize traits of maize (*Zea mays* L.) that resulted for most of the variance in the data. Shrestha, 2016 reported important contribution of the first PCs in total variability while studying different traits. Greenacre (2010) reported that Eigen values (in PCA) have primary importance for numerical diagnostics to assess variation attributed by number of large variables on the dependent structure and their data matrix in a graphical display.

The principal component scores of genotypes were used as input for cluster analysis using Euclidean[2] distances in order to group the genotypes into various clusters and to confirm the results of principal component analysis. Cluster IV showed the lowest mean values for *Aspergillu*s ear rot disease incidence (21.28%) and *Aspergillu*s ear rot disease severity (6.9%) revealing the importance of this

cluster genotype (89291) in maize ear rot disease resistance improvement programmes. Similar findings have been reported by Mounika *et al*., (2018) in India who realized that a cluster showed highest means for yield and yield related traits in maize. The average intra and inter-cluster Euclidean^2 distance were estimated based on Ward's minimum variance and are presented in the Table 4. Based on inter-cluster distances and *per se* performance of the genotypes included in the farthest cluster (VII) genotypes 88099, 89193, 8923, 90176, 90219, 90251, 90263, Exp 124, IN WE-SR, M131 and TZI-5-1171 showed maximum inter cluster distance and good *per se* performance. Hence, they can be included in maize breeding programmes for generating heterotic hybrids for various yield traits in maize.

Similar results of clustering were reported by Sandeep *et al.,* (2015) and Mounika *et al*., (2018) who depicted that maize genotypes with maximum inter cluster distances showed good performances in grain yield.

CONCLUSION

There was genetic diversity for both grain yield and resistance to *Aspergillus flavus* in the test genotypes assembled for breeding. The presence of high level of diversity among the inbred lines grouped into divergent clusters indicated their suitability for hybridization and various crosses can be made among them in breeding programmes. The thirty-three inbred lines were grouped into seven clusters. Among all the clusters, cluster VII was the largest with eleven genotypes followed by cluster II containing eight genotypes, cluster with five genotypes, cluster V with four genotypes and clusters III and VI with two genotypes each. Cluster IV mono genotypic having only one genotype. Out of all the clusters, cluster I showed highest mean value for grain yield, indicating the importance of this cluster genotypes (87036, 88094, 90156, 90188 and 90301) in maize yield improvement programmes. Similarly, cluster IV showed the lowest mean values for *Aspergillu*s ear rot disease severity (6.9%) revealing the importance of this cluster genotype (89291) in maize ear rot disease resistance improvement programmes. Based on inter-cluster distances and *per se* performance of the genotypes included in the farthest cluster (VII) genotypes 88099, 89193, 8923, 90176, 90219, 90251, 90263, Exp 124, IN WE-SR, M131 and TZI-5-1171 showed maximum inter cluster distance and good *per se* performance. Hence, they can be included in maize breeding programmes for generating heterotic hybrids for various yield traits in maize.

Conflict of interest statement. The authors declare that they have no conflict of interest.

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