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# GENETIC DIVERGENCE STUDY IN SALINITY STRESS TOLERANT MAIZE (*Zea mays* L.)

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## Abstract

The study was conducted to investigate the genetic diversity of some maize inbreds under salinity stress condition using Mahalanobis's statistic ($D^2$) and principal component analysis. Analysis of variance showed significant difference for all the characters. Results of multivariate analysis revealed that seventeen inbred lines formed five clusters at 12 dS level of salinity. The highest intra-cluster distance was recorded in cluster IV containing three genotypes and the lowest was in cluster V having one genotype. The inter cluster $D^2$ values revealed maximum distance among the clusters. The highest inter cluster distance was observed between clusters IV & III and lowest was between V & I. Cluster IV had the highest cluster means for cob height, tassel length, cob length, SPAD value, number of seeds/cob, 100 seed weight, cob diameter and grain yield per plant. Considering cluster distance, inter-genotypic distance, cluster mean and other agronomic performances the genotypes CZ29, CZ33 and P43 from cluster IV and E135, E158, E169, P29 and P45 from cluster III may be considered as better parents for future hybridization programs to obtain desirable segregates in respect of different yield and yield contributing characters under salinity stress.

Keywords: Maize *(Zea mays* L.), inbred lines, genetic divergence, salinity stress, cluster analysis, grain yield.

## Introduction

Maize (*Zea mays* L.) belonging to the family Graminae is the third most important food grain for human after rice and wheat. It is the world's most widely grown cereal and is the primary staple food in many developing countries (Morris *et al.,* 1999). Maize in Bangladesh is becoming an important crop in the rice-based cropping pattern. In recent years maize is gaining popularity among the farmers mainly due to high yield, more economic return and versatile uses. It is the highest yielding grain crop having multiple uses. The highest average kernel yield is 3.4 t/ha whereas wheat yield is 1.9 t/ha and rice yield is 2.9 t/ha on global basis (Anonymous, 1992; Mallik and Razzak, 1994).

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Among the various environmental stresses, soil salinity has become a critical problem worldwide due to its dramatic effects on plant physiology and performance. Over 400 Mha across the world is affected by salinity that is about 25% of the world's total area (including Bangladesh) (Ghassemi *et al.*, 1995). A major concern of agriculture in Bangladesh could thus be salinity which is a serious threat to the sustainability of crop or seed production in many parts of the country. In the southern belt of Bangladesh about 1.2 million hector of land remains fallow every year due to salinity hazard (Anonymous, 2010). To use this fallow land development of high yielding variety with high adaptability under salinity stress is required. This study was designed with maize inbreds to find out genetically distant parent by observing morphological variation under salinity. This study not only depicts suitable parents for breeding program but also provide opportunity of developing base population for molecular study.

## Materials and Method

Seventeen genotypes of maize collected from CIMMYT, India, were grown in a completely randomized design (CRD) with 3 replications at the research farm of Irrigation Division, coordinated by Plant Breeding Division of BARI, Gazipur, during February to July, 2012. Seeds of each inbred were sown uniformly into the soil of plastic pots by hand on February 11, 2012. The plastic pots were placed according to the FAO standard irrigation system for supplying the saline water. The soil was made wet by normal saline water. The seedlings emerged six to eight days after sowing. The seedlings were thinned to one per pot after ten days of emergence. Irrigation was given at two leaves stage with 12 dS concentration of saline water and repeated at 15 days interval. Fertilizers were applied @ 120, 80, 80, 20, 5 and 1 kg/ha of N, $P_2O_5$, $K_2O$, S, Zn and B respectively. Standard agronomic practices were followed (Quayyum, 1993) and plant protection measures were taken when required. Data were collected on grain yield/plant, plant height, cob height, cob length, cob diameter, tassel length, SPAD value and 100-grain weight. Genetic diversity was estimated using Mahalanabis generalized distance ($D^2$) extended by Rao (1952). Tocher's method was followed to determine the group constellation. Canonical variate analysis was performed as per Rao (1964) to confirm the results of cluster $D^2$ analysis. Mean data for each character was subjected to both univariate and multivariate analysis. Univariate analysis of the individual character (analysis of variance) was done by computer using MSTAT-C software. Genetic diversity of seventeen genotypes at 12 dS level of salinity was analyzed using GENSTAT 5.13 software program (copyright 1987, Lawes Agricultural Trust, Rothamasted Experimental Station, UK).

## Results and Discussion

The maize inbred lines showed significant variation for all the morphological characters. Eigen values of nine principal component axes and percentage of variation of total variation accounting for them obtained from the principal component analysis are presented in table 1. The results revealed that the first axes accounted for 56.37% of the total variation among the genotypes, while nine of these with eigen values accounted for 100%. The first two axes of nine eigen values above the unity accounted for 76.84% of the total variation. Azam (2012) evaluated that days to 50% tasseling, days to 50% silking and plant height together accounted for 71.96% of the total genetic divergence in maize.

**Table 1. Eigen values and percentage of variation for corresponding 9 component characters in 17 maize inbred lines.**

| Principal component axis | Eigen values | Percentage (%) of total variation | Cumulative percent of variation |
|---|---|---|---|
| Plant height (cm) | 5.0796 | 56.37 | 56.37 |
| Cob height (cm) | 1.8425 | 20.47 | 76.84 |
| Tassel length (cm) | 0.7892 | 8.77 | 85.61 |
| Cob length (cm) | 0.6116 | 6.80 | 92.41 |
| SPAD Value | 0.3752 | 4.17 | 96.58 |
| Number of seeds/cob | 0.1549 | 1.72 | 98.30 |
| 100 seeds weight (g) | 0.0793 | 0.88 | 99.18 |
| Cob diameter (cm) | 0.0714 | 0.79 | 99.97 |
| Grain yield /plant (g) | 0.0024 | 0.03 | 100.00 |

Based on the principal component scores I and II obtained from the principal component analysis, a two-dimensional scatter diagram ($Z_1$-$Z_2$) was constructed using component score I ($Z_1$) as X-axis and II ($Z_2$) as Y-axis (Figure 1). The positions of the genotypes in the scatter diagram were apparently distributed into five groups, which indicated that considerable diversity exists among the genotypes.

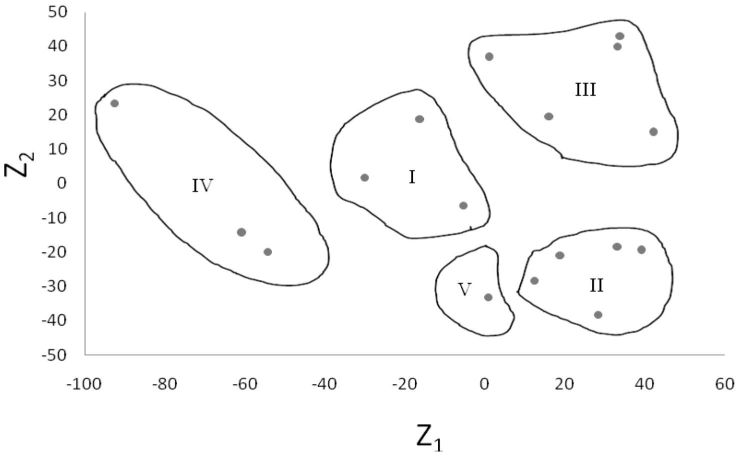

**Figure 1. Scatter distribution of 17 maize inbred lines based on their principal component scores superimposed with clusters.**

The inter genotypic distances were used in computation of intra-cluster distances from distance matrix of PCO according to Singh and Choudhary (2001). The intra-cluster distances were not always proportional to the number of the genotypes in the cluster (Table 2). In the present study the clusters II and III composed of the largest number of genotypes (5), but their intra-cluster distances were not the highest. The statistical distances represent the index of genetic diversity among the clusters. The intra-cluster distances ranged from 0.000 to 3.560. Intra-cluster distances in all the clusters were more or less low which indicated that the genotypes within the same cluster were closely related.

The highest intra-cluster distance was recorded in cluster IV (3.560) containing three genotypes followed by cluster III (3.120) containing five genotypes. The lowest intra-cluster distance was observed in cluster V (0.000) having one genotype. The intra-cluster diversity was the highest in cluster IV *i.e.*, more heterogeneous, while the lowest was in cluster V *i.e.*, comparatively homogenous. The intra-cluster distances of cluster I and II were 2.620 and 2.601 consisting of 3 and 5 genotypes, respectively. Smith (1990) reported 3 groups in a divergence study of 48 maize cultivars. These findings are in conformity with the findings of Datta and Mukherjee (2004), Singh *et al.* (2005), Marker and Krupakar (2009).

**Table 2. Average inter-cluster and intra-cluster (bold) distance ($D^2$) for 17 maize inbred lines obtained by canonical variate analysis.**

| Cluster | I | II | III | IV | V |
|---|---|---|---|---|---|
| **I** | **2.625** | | | | |
| **II** | 4.776 | **2.601** | | | |
| **III** | 8.135 | 7.660 | **3.120** | | |
| **IV** | 10.527 | 12.856 | 18.312 | **3.560** | |
| **V** | 4.605 | 3.430 | 10.510 | 10.084 | **0.000** |

Canonical variate analysis was done to compute the inter-cluster Mahalanobis's $D^2$ values. The intra and inter-cluster distance ($D^2$) values are presented in table 2. Results indicated that the highest inter-cluster distance was between clusters III and IV (18.312) followed by II and IV (12.856) and I and IV (10.527). The higher inter-cluster distances between these clusters indicated to the wide spectrum of variability in the population. However, the highest inter-cluster distance was observed between clusters III and IV which indicated that the genotypes in these clusters were more diverged than those of the others. The lowest inter-cluster distance was observed between the clusters II and V (3.430) suggesting a close relationship among the genotypes within these clusters.

Statistical distances represent the index of genetic diversity among the clusters. The inter-cluster distances were larger than the intra-cluster distances which indicated wider genetic diversity among the genotypes of different groups. Devnath (1987) obtained larger inter-cluster distance than the intra-cluster distance in a genetic variability in maize. Similar results were obtained by Abedin & Hossain (1990) in maize.

With the application of co-variance matrix for non-hierarchical clustering, 17 maize genotypes were grouped into five clusters. Verma and Sachan (2000) reported 12 clusters; Gupta *et al*. (1991) found five clusters; Azam (2012) reported five clusters in maize. In this study, clusters II and III had maximum of 5 genotypes, genotypes E15, E40, E70, E116 & P35 in cluster II and E135, E158, E169, P29 & P45 in cluster III followed by Cluster I (E32, P37, P40), Cluster IV (CZ29, CZ33, P43) and Cluster V (E6) (Table 3). Azam (2012) studied genetic divergence for 10 traits and had 5 clusters for 49 maize genotypes.

These results confirmed the clustering pattern of the genotype according to the principal component analysis. Composition of different clusters with their corresponding genotypes and collection site included in each cluster are presented in table 3. Results of the different multivariate techniques were superimposed with the clusters. The clustering pattern obtained was coincided with the apparent grouping patterns performed by PCA. For that reason it can be said that the results obtained through PCA were established by non-hierarchical clustering.

**Table 3. Distribution of 17 maize inbred lines in different clusters.**

| Cluster | Total no. of genotypes in the cluster | Genotypes included in different clusters |
|---|---|---|
| **I** | 3 | E32, P37, P40 |
| **II** | 5 | E15, E40, E70, E116, P35 |
| **III** | 5 | E135, E158, E169, P29, P45 |
| **IV** | 3 | CZ29, CZ33, P43 |
| **V** | 1 | E6 |

An attempt was made to characterize the individual genotypes in respect of their mean values for different characters with a view to getting the idea whether the genotypes having similar characteristics could be disseminated. The Intra-cluster mean values for all the 9 characters along with the marking of the highest (H) and the lowest (L) for each of the clusters is presented in table 4. The data revealed that different clusters exhibited different mean values for almost all the characters. Plant height had the highest intra-cluster means in cluster III followed by those in cluster IV and cluster I. The lowest intra-cluster mean for this trait was observed in cluster V. Cob height had the highest group means in cluster IV followed by those in cluster III and cluster I. It had the lowest mean in cluster V. The intra-cluster mean for tassel length was the highest in cluster IV followed by those in cluster I. The lowest intra-cluster mean for this trait was observed in cluster II. Cob length had the highest group mean in cluster IV (9.20) followed by those in cluster III (8.17) and cluster I (7.32). The lowest intra-cluster mean for this trait was observed in cluster V (5.80). Intra-cluster mean for SPAD values were the highest in cluster IV (49.58) and the lowest in cluster II (44.77).

The highest group mean for number of seeds per cob was found in cluster IV (146.33) followed by that in cluster I (96.33) and the lowest mean value was observed in cluster III (49.40). Intra-cluster mean for 100 seed weight was the highest in cluster IV (22.57) and the lowest in cluster II (18.13). The lowest value for cob diameter was found in cluster V (6.63) followed by cluster II (7.11) and cluster I (8.07), the highest was in cluster IV (9.22). Grain yield per plant was the highest in cluster IV (33.08) followed by cluster I (18.91) and cluster V (16.11) and the lowest was in cluster III (9.97).

**Table 4. Cluster means for nine different characters of 17 maize inbred lines.**

| Characters | Clusters | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| Plant height (cm) | 78.66 | 41.93 | 92.47 H | 80.00 | 41.67 L |
| Cob height (cm) | 33.22 | 17.93 | 38.00 | 42.11 H | 15.00 L |
| Tassel length (cm) | 32.11 | 28.80 L | 29.67 | 35.56 H | 29.67 |
| Cob length (cm) | 7.32 | 6.00 | 8.17 | 9.10 H | 5.80 L |
| SPAD Value | 47.18 | 44.77 L | 46.33 | 49.58 H | 44.87 |
| Number of seeds/cob | 96.33 | 62.60 | 49.40 L | 146.33 H | 89.00 |
| 100 seed weight (g) | 19.65 | 18.13 L | 19.80 | 22.57 H | 18.17 |
| Cob diameter (cm) | 8.07 | 7.11 | 8.86 | 9.22 H | 6.63 L |
| Grain yield /plant (g) | 18.91 | 11.14 | 9.97 L | 33.08 H | 16.11 |

Note: H= High, L= Low

The inter-cluster distance of cluster IV with other clusters was higher than the inter-cluster distances between the remaining cluster combinations (Table 2). The cluster means of this cluster for cob height, tassel length, SPAD value, number of seeds per cob, 100 seeds weight, cob diameter and grain yield per plant was also divergent. These indicated that the genotype included in cluster IV were very important to contribute to the total divergence among the inbreds for these characters. Cluster V provided the highest cluster means for plant height which indicated that the inbred lines within this cluster could be used for increasing plant height in maize. Based on cluster means Singh and Chaudhari

(2001) also reported wide range of variation for grain yield and it's components in maize. Marker and Krupakar (2009) also have assessed the range of variability of 16 genotypes for 14 traits in maize. The present results are in agreement with those of Tang *et al.* (2002) and Alom *et al.* (2003) who also identified the above mentioned characters as the principal components contributing maximum to the total variation in maize.

Generally genetic diversity is associated with geographical diversity but the former is not necessarily directly related with geographical distribution. In the present study pattern of clustering revealed that genotypes originating from different countries and collected by CIMMYT, India, were grouped in the same cluster (Table 4). This indicates that geographic diversity was not related to genetic diversity, which might be due to continuous exchange of genetic materials among the countries of the world. Verma and Sachan (2000) observed no parallelism between geographic and genetic diversity. Chatterjee and Khare (1991) studied a negative relationship between geographic and genetic diversity. Gupta *et al.* (1991) showed no correlation between geographic and genetic diversity.

Contribution of characters towards the divergence obtained from canonical variate analysis is presented in table 5. In this method vectors was calculated to represent the varieties in the graphical form (Rao *et al.*, 1952).

**Table 5. Latent vectors for nine principal component characters of 17 maize inbred lines.**

| Characters | Vector I | Vector II |
|---|---|---|
| Plant height (cm) | 0.3418 | 0.3913 |
| Cob height (cm) | -0.3581 | 0.3206 |
| Tassel length (cm) | -0.2211 | 0.3622 |
| Cob length (cm) | 0.3901 | -0.2607 |
| SPAD Value | -0.3597 | 0.1557 |
| Number of seeds/cob | 0.2789 | 0.4908 |
| 100 seed weight (g) | 0.3209 | -0.0960 |
| Cob diameter (cm) | -0.3595 | -0.2818 |
| Grain yield /plant (g) | -0.3381 | 0.4372 |

This is helpful in cluster analysis as it facilitates the study of group constellations and also serves as a pictorial representation of the configuration of various groups. The absolute magnitude of the coefficients in the first two canonical vectors also reflects to a great extent, the importance of the characters for primary and secondary differentiation. The character which gives high absolute magnitude for vector II, is considered to be responsible for secondary differentiation. Likewise, the characters which give higher absolute magnitude for vector I is considered to be responsible for primary differentiation. If the same character gives equal magnitude for both the vectors then the character is considered responsible for primary as well as secondary differentiation.

In vector I ($Z_1$) obtained from PCA, the important characters responsible for genetic divergence in the axis of differentiation were cob length (0.3901) and 100 seed weight (0.3209). In vector II ($Z_2$), the second axis of differentiation, cob height (0.3206), tassel length (0.3622), SPAD value (0.1557) and grain yield/plant (0.4372) were important because all these characters had positive values.

Plant height and number of seeds/cob had positive value in both the vectors, which indicated that they were the important component characters having higher contribution to the genetic divergence among the materials studied. Negative values in both vectors for cob diameter had lower contribution towards the divergence. Among the characters, plant height, cob length, number of seeds per cob and 100 seed weight contributed maximum towards the genetic divergence. The current consequences are in concurrence with those of Tang *et al.* (2002), Alom *et al.* (2003) , Marker and Krupakar (2009) who also identified above mentioned characters as the principal components contributing maximum to the total variation in maize.

## Conclusion

The results indicated that the plant height and the number of seeds/cob had maximum contribution to the genetic divergence among the genotypes considering the cluster distance, inter-genotypic distance, cluster mean and other agronomic performances the genotypes CZ29, CZ33 & P43 from cluster I and genotypes E135, E158, E169, P29 & P45 from cluster III may be considered as better parents for future hybridization programs to obtain desirable segregations in respect of different yield and yield contributing characters under salinity stress.

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