

GENETIC DIVERSITY OF MAIZE (*Zea mays* L.) INBREDS UNDER SALINITY STRESS

M. M. ROHMAN¹, B. R. BANIK², A. BISWAS³ AND M. S. RAHMAN⁴

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 twenty five inbred lines formed five clusters at 8 dS level of salinity. The highest intra-cluster distance was recorded in cluster III containing eight genotypes and the lowest was in cluster II having one genotype. The highest inter cluster distance was observed between clusters II & V and lowest was between I & III. Cluster II had the highest cluster means for plant height, cob height, above ground dry mass, cob per plant, cob length, and grain yield per plant. Considering cluster distance, inter-genotypic distance and other agronomic performances the genotypes CZ12, CZ19, CZ26, CZ29, CZ31, CZ32, CZ33 & CML470 from cluster III and CZ27, CZ37, CML251 and CML456 from cluster V may be considered as better parents for future hybridization programs to obtain desirable segregate 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.) plays a significant role in human and livestock nutrition worldwide. It is the world's most widely grown cereal and is the primary staple food in many developing countries (Morris *et al.* 1999). Maize is becoming an important crop in the rice based cropping system in Bangladesh. It is the third leading important cereal crop after rice and wheat. In recent years, maize is gaining popularity among the farmers mainly due to its high yield, more economic return and versatile uses. The area and production of maize is increasing day by day in Bangladesh and it continues to expand rapidly at an average rate of 20% per year (Anonymous, 2008). Plants in saline areas are often exposed to multiple abiotic stresses. High salinity is one of the most important abiotic stress factors limiting plant growth and productivity of a wide variety of crops (Flowers, 2004; Athar *et al.*, 2008). Thus, increased soil salinity has become an increasingly important topic (Flowers, 2004). Over 400 Mha across

¹Senior scientific officer, Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, ²Director, Training and Communication, BARI, ³Scientific officer, Plant Breeding Division, Regional Agricultural Research Station, BARI, Jessore, ⁴Senior scientific officer, Irrigation Division, BARI, Gazipur, Bangladesh.

the world is affected by salinity that is about 25 % of the world's total area (including Bangladesh) (Ghassemi *et al.*, 1995). The response of plants to excess salinity is complex and involves changes in their morphology, physiology and metabolism. Morphologically the most typical symptom of saline injury to plant is reduction of growth (Azooz *et al.*, 2004), which is a consequence of several physiological reasons. Therefore, management and use of morphological variation under salinity condition might give a possibility in selecting inbred lines to develop salinity resistant maize. The genotype of extra polar salinity might offer good genetic combination of better homeostasis.

In southern belt of Bangladesh about 1.2 million hectare (Anonymous, 2010) of land remains fallow every year due to salinity hazard. To use this fallow land it needs to develop variety with high adaptability under salinity stress. This study will not only offer suitable parent for breeding program but also provide opportunity of developing base population for molecular study.

Materials and Method

Twenty five genotypes of maize, locally developed through recycling by plant breeding division, BARI, Gazipur 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 rabi season of 2011-2012. Seeds of each inbred were sown uniformly into the soil of plastic pots by hand. 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 8 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, K, 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, above ground dry mass, cob per plant, cob height, cob length and cob diameter. 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 twenty five genotypes at 8 dS level of salinity was analyzed using GENSTAT 5.13 software program (copyright 1987, Lawes Agricultural Trust, Rothamsted 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 36.51% of the total variation among the genotypes, while seven of these with eigen values accounted for 100%. The first three axes of seven eigen values above the unity accounted for 76.86% 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 7 component characters in 25 maize inbred lines.

Principal component axis	Eigen values	Percentage (%) of total variation	Cumulative percent of variation
Plant height (cm)	2.556	36.51	36.51
Cob height (cm)	1.545	22.08	58.59
Above ground dry biomass (g)	1.279	18.27	76.86
Cob /plant	0.602	8.60	85.46
Cob length (cm)	0.422	6.03	91.49
Cob diameter (cm)	0.321	4.58	96.07
Grain yield /plant (g)	0.275	3.93	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.

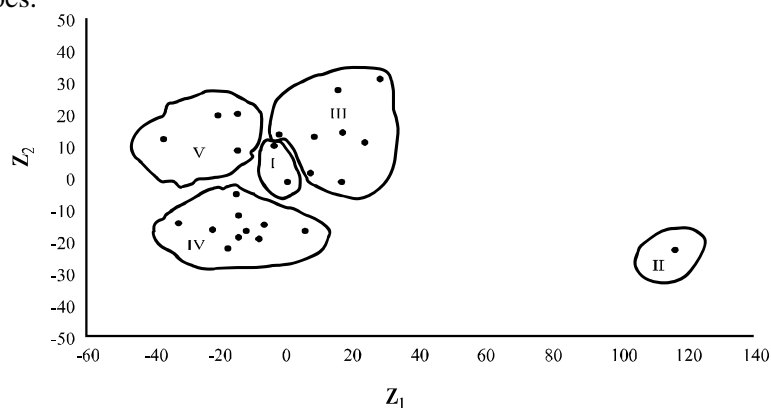


Fig. 1. Scatter distribution of 25 maize inbred lines based on their principal component scores superimposed with clusters.

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 IV composed of the largest number of genotypes (10), 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 5.345. 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 III (5.345) containing eight genotypes followed by cluster V (5.125) containing four genotypes. The lowest intra-cluster distance was observed in cluster II (0.000) having one genotype. The intra-cluster distances of cluster I and IV were 4.440 and 3.560 consisting of 2 and 10 genotypes, respectively. These findings of the present study are in conformity with the findings of Datta and Mukherjee (2004), Singh *et al.* (2005) and Marker and Krupakar (2009).

Table 2. Average inter-cluster and intra-cluster (bold) distance (D^2) for 25 maize inbred lines obtained by canonical variate analysis.

Cluster	I	II	III	IV	V
I	4.440				
II	17.075	0.000			
III	3.724	14.837	5.345		
IV	4.884	15.648	4.703	3.560	
V	4.370	18.392	4.298	4.874	5.125

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 II and V (18.392) followed by I and II (17.075) and II and IV (15.648). The higher inter-cluster distances between these clusters indicated to the wide spectrum of variability in the population. The lowest inter-cluster distance was observed between the clusters I and III (3.724) 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. Debnath (1987) obtained larger inter-cluster distance than the intra-cluster distance in a genetic variability in maize. Similar results were also obtained by Abedin and Hossain (1990) in maize.

With the application of co-variance matrix for non-hierarchical clustering, 25 maize genotypes were grouped into five clusters. Gupta *et al.* (1991) found five clusters; Azam (2012) reported five clusters from 49 maize genotypes. The distribution pattern indicated that maximum 10 inbred lines were included in cluster IV followed by 8 in cluster III. The remainders have been distributed in three clusters. The least number 1 was included in cluster II (Table 3).

These results confirmed the clustering pattern of the genotype according to the principal component analysis. Composition of different clusters with their corresponding genotypes included in each cluster are presented in table 3. Results of the different multivariate techniques were super imposed with the clusters (Fig 1). 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 25 maize inbred lines in different clusters.

Cluster	Total no. of genotypes in the cluster	Genotypes included in different clusters
I	2	CZ36, CML376-1
II	1	CZ35
III	8	CZ12, CZ19, CZ26, CZ29, CZ31, CZ32, CZ33, CML470
IV	10	CZ3, CZ10, CZ24, CZ28, CZ30, CML159, CML206-1, CML216, CML395, CML496
V	4	CZ27, CZ37, CML251, CML456

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 7 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 II followed by those in cluster I and cluster IV. The lowest intra-cluster mean for this trait was observed in cluster V. Cob height had the highest group means in cluster II followed by those in cluster I and cluster IV. It had the lowest mean in cluster V. The intra-cluster mean for above ground dry mass was the highest in cluster II followed by those in cluster IV. The lowest intra-cluster mean for this trait was observed in cluster I. Intra-cluster mean for cob per plant were the highest in cluster II (2.00) and the lowest in cluster I (1.00). Cob length had the highest group mean in cluster II (15.00) followed by those in cluster III (12.13) and cluster I (12.00). The lowest intra-cluster mean for this trait was observed in cluster V (8.06).

The lowest value for cob diameter was found in cluster V (3.17) followed by cluster IV (3.45) and cluster II (3.60), the highest was in cluster I (4.15). Grain yield per plant was the highest in cluster II (163.38) followed by cluster III (70.12) and cluster I (49.52) and the lowest was in cluster V (35.96).

The inter-cluster distance of cluster II with other clusters was higher than the inter-cluster distances between the remaining cluster combinations (Table 2). The

cluster means of this cluster for plant height, cob height, above ground dry mass, cob per plant, cob length and grain yield per plant was also divergent. These indicated that the genotype included in cluster II were very important to contribute to the total divergence among the inbreds for these characters. Cluster I provided the highest cluster means for cob diameter which indicated that the inbred lines within this cluster could be used for increasing cob diameter in maize. Based on cluster means Singh and Chaudhari (2003) also reported wide range of variation for grain yield and its 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.

Table 4. Cluster means for seven different characters of 25 maize inbred lines.

Characters	Clusters				
	I	II	III	IV	V
Plant height (cm)	114.00	135.00 H	95.75	108.05	87.03 L
Cob height (cm)	48.00	65.00 H	40.06	45.65	34.00 L
Above ground dry biomass (g)	45.31 L	86.91 H	56.55	76.83	56.25
Cob /plant	1.00 L	2.00 H	1.19	1.15	1.25
Cob length (cm)	12.00	15.00 H	12.13	11.50	8.06 L
Cob diameter (cm)	4.15 H	3.60	3.99	3.45	3.17 L
Grain yield /plant (g)	49.52	163.38 H	70.12	37.77	35.96 L

Note: H= High, L= Low

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 recycling of different high yielding hybrids were grouped in the same cluster and hybrids were collected from different countries. 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 were calculated to represent the varieties in the graphical form (Rao *et al.*, 1952). 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.

Table 5. Latent vectors for seven principal component characters of 25 maize inbred lines.

Characters	Vector I	Vector II
Plant height (cm)	-0.4484	-0.2755
Cob height (cm)	-0.5076	-0.1601
Above ground dry biomass (g)	-0.2299	-0.5423
Cob /plant	-0.1383	-0.3056
Cob length (cm)	-0.4654	0.3105
Cob diameter (cm)	-0.2635	0.6275
Grain yield /plant (g)	-0.4281	0.1445

In vector I (Z_1) obtained from PCA, no characters had positive values. In vector II (Z_2), the second axis of differentiation, cob length (0.3105), cob diameter (0.6275) and grain yield/plant (0.1445) were important because all these characters had positive values.

Plant height, cob height, above ground dry biomass and cob per plant had negative value in both the vectors, which indicated that they were the less important component characters having lower contribution to the genetic divergence among the materials studied. Among the characters, cob length, cob diameter and grain yield/plant contributed maximum towards the genetic divergence under salinity stress conditions. 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 cob length, cob diameter and grain yield per plant had maximum contribution to the genetic divergence among the genotypes. The cluster means of cluster II for plant height, cob height, above ground dry mass, cob per plant, cob length and grain yield per plant was also divergent. These indicated that the genotype included in cluster II were very important to contribute to the total divergence among the inbreds for these characters. Cluster I provided the highest cluster means for cob diameter which indicated that the inbred lines within this cluster could be used for increasing cob diameter in maize. Inbreds of cluster II and I may be selected for hybridization for obtaining desirable segregants for these traits under salinity stress.

References

- Abedin, J and M. A. Hossain, 1990. Variability in maize composites. Bangladesh . *Agric. J. Res.* **15**(1): 24-31.
- Alom A. K., M. M. Masum, A. S. M. H. Nahar, M. A. Matin and A. K. M. J. Pasha 2003. Genetic divergence in maize (*Zea mays* L.) *Pakistan J. Biol. Sci.* **6**(22): 1910-1911.
- Anonymous. 2008. Achievements of The Bangladesh CIMMYT partnership for agricultural research and development CIMMYT. Bangladesh, Banani, Dhaka. Pp.1-9
- Anonymous. 2010. Annual Report, Plant Breeding Division, BARI
- Athar, H., A. Khan and M. Ashraf, 2008. Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.*, **63**:224-231

- Azam, M.G. 2012. Morphological and molecular diversity analysis in maize inbreds. MS. Thesis, Department of Genetics and Plant Breeding, BSMRAU, Salna, Gazipur : 58-59.
- Azooz, M.M., M.A. Shaddad and A.A. Abdel-Latef, 2004. Leaf growth and K⁺/Na⁺ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. *Acta Agron. Hungarica* **52**: 287-296
- Chatterjee, A. and D. Khare 1991. Multivariate analysis in niger (*Guizotia abyssinica*). Research and Development Reporter **8**(2): 111-114.
- Datta D. and B. K. Mukherjee. 2004. Genetic divergence among maize (*Zea mays* L.) inbreds and restricting traits for group constellation. *Indian J. Genet.* **64**(3): 201-207.
- Debnath, S. C. 1987. Genetic variability in maize (*Zea mays* L.) *Bangladesh J. Agric.* **12**(4): 217-221.
- Flowers, T. J., 2004. Improving crop salt tolerance. *J. Exp. Bot.* **55**: 307-319
- Ghassemi, F., A.J. Jakeman, H.A. Nik, 1995. Salinisation of land and water resources. Human causes, extent, management and case studies, University of New South Wales Press, Sydney, Pp 526
- Gupta, V. P., M. S. Sekhon and D. R. Satiya. 1991. Studies on genetic diversity, heterosis and combining ability in Indian mustard [*Brassica juncea* L. (Czern and Coss)]. *Indian J. Genet. Pl. Breed.* **51**(4): 448-453.
- Mahalanobis, P.C.1936. On the generalized distance. Proc. Nat. Inst. Sci. India **11**(1): 49-55.
- Marker, S. and A. Krupakar. 2009. Genetic Divergence in Exotic Maize Germplasm (*Zea mays* L.) *ARPJ. Agr. Biol. Sci.* **4**: 44-47.
- Morris, M. L., J. Risopoulos and D. Beck. 1999. Genetic change infarmer-recycled maize (*Zea mays* L.) seed; a review of the evidence. CIMMYT Economic Working paper No. 99-07. Mexico, D.F., CMMYT, 1p.
- Quayyum, M. A. 1993. Bhuttar Chash Paddhati (in Bengali). In: Chowdhury, M. K and M. A. Islam (ed.). Bhuttar Utpadan O Babohar. Bangladesh Agricultural Research Institute, Gazipur. Pp 43-48.
- Rao, C. R. 1952. Advanced statistics methods in Biometric Research, Ed. John Wiley and Sons Inc. New York. P. 390.
- Rao, C. R. 1964. Statistical genetic consideration for maintaining germplasm collection. *Theor Appl. Genet.* **86**: 673-678.
- Singh P. K. and Chaudhari L. B. 2003. Genetic divergence in maize (*Zea mays* L.). *J. Res. Birsa Agric. Univ.* **13**(2):193-195.
- Singh, P. K. and L. B. Choudhary. 2001. Genetic divergence in maize (*Zea mays* L.) *J. of Res. Birsa Agricultural University* **13**(2): 193-195.
- Singh, P., S. Dass, V. K. Dwivedi, Y. Kumar and O. Sangwan. 2005. Genetic divergence studies in maize (*Zea mays* L.). *Annals of Agric Bio-Res.* **10**(1): 43-46.
- Smith, M. F. 1990. A multivariate approach to specific problem of grouping maize cultivars. *South African J. Plant and Soil.* **7**(3): 167-171.
- Tang, D. F., Yang A. G., Pan G. T. and Rang T. Z. 2002. Study on combining ability and clustering analysis of CIMMYT maize population and local population in China. *J. Siehuan Agric. Univ.* **20**(4): 317-320.
- Verma, S. K. and J. N. Sachan., 2000. Genetic divergence in Indian mustard [(*Brassica juncea*. L.) Czern and Coss.]. *Crop Res. Hisar.* **19**(2): 271-276.