

Genotypic Variability in Salinity Tolerance of Maize Pipe Line Hybrids at Seedling Stage

Ratikanta Maiti^{1*}, Humberto González Rodríguez², D. Rajkumar¹, Sanjay Koushik¹ and P. Vidyasagar¹

¹Vibha Seeds, Vibha Agrotech Ltd., Inspire, Plot#21, Sector 1, Huda Techno Enclave, High Tech City Road, Madhapur, Hyderabad, Andhra Pradesh (500 081), India

²Universidad Autónoma de Nuevo León, Facultad de Ciencias Forestales, Carr. Nac., No. 85, km 145, Linares, NL, Mexico

Article History

Manuscript No. 370b

Received in 9th October, 2012

Received in revised form 9th November, 2012

Accepted in final form 5th December, 2012

Correspondence to

*E-mail: ratikanta.maiti@gmail.com

Keywords

Maize, salinity tolerance, genetic variability, emergence, shoot, root

Abstract

In this study using a simple and novel semi-hydroponic technique, 14 maize pipe line hybrids with high yield potentials were evaluated for salinity tolerance at different levels of stress [control, 0.15 (EC=13.6 dSm⁻¹), 0.2 M NaCl (EC=18 dSm⁻¹)] at seedling stage. This technique is proved to be highly effective and consistent to distinguish genotypes for salinity tolerance. Increasing salinity decreases emergence percentage, shoot and root length and number of adventitious roots in susceptible lines, on the contrary the tolerant lines showed increased number of lateral roots with increasing salinity as a function of osmotic adjustment. Two genotypes have even at 0.2 M NaCl showed high emergence percentage starting from 70% to 77% emergence and more number of adventitious roots, viz. revealing these genotypes have high salinity tolerance. Seven genotypes have at 0.15 M NaCl showed highest emergence starting from 70% to 96.7%. The results revealed that seedling emergence and number of adventitious roots may be considered as selection criteria for salinity tolerance in maize. These genotypes have great potential under saline prone areas.

1. Introduction

Maize (*Zea mays* L.) is grown in diverse climatic conditions in various countries of the world as an important basic food grain mainly for the majority of people in Latin American countries. It occupies an indispensable part of the human diet for its high nutritional quality of the grain, but the productivity of the crop is affected by several abiotic factors such as cold temperature, drought and salinity (Maiti et al., 2004). Therefore, there is a great necessity in the development of an efficient technique for the evaluation and selection of maize cultivars for tolerance to salinity as well as other abiotic stresses. Significant progress has been achieved on salinity tolerance in maize.

Salt stress affects the growth of a crop at different stages. Genotypic variability exists for resistance to salinity (Nordquist et al., 1992). Maiti et al. (1996) reported high genetic variability in maize cultivars (*Zea mays*) for resistance to drought and salinity at the seedling stage and diverse genotypes have been selected for resistance to drought and salinity stress. Increasing salinity reduces germination rate (Jan et al., 1995), but addition of CaCl₂ improves seedling growth in terms of shoot and root dry weight (Alberico and Cramer, 1993). Salt shock inhibits root elongation, but it is followed by a gradual recovery

(Rodriguez et al., 1997).

In recent studies, considerable variations were observed in the root, shoot length and biomass of different hybrids at different salinity levels (Muhammad et al., 2010). NaCl concentrations at germinating stage had adverse effect on maize than later stages of growth (Tayyaba et al., 2010).

Salinity causes several biochemical changes in various crops which have been documented in the literature. The salt tolerance of maize at the germination stage was found to be associated with high transport of Na⁺ and Cl⁻ to the growing shoot axis and time course decrease in K⁺ and Ca²⁺ concentration in the embryonic tissues. The adverse effect of salt ion on seed germination is partly due to the damage caused by breakdown of seed lipids in order to supply soluble sugars in the respiratory metabolism of the growing embryo (Ashraf and Wahid, 2000).

Several studies have been undertaken to understand the mechanism of tolerance to salinity in maize and other crops (Naseer, 2001; Mansour et al., 2005; Orak and Ates, 2005; Saboora and Kiarostami, 2006; Giaveno et al., 2007).

Salinity affects plant growth through osmotic ion specific effects and oxidative stress (Pitman and Läuchli, 2002). Osmotic adjustment of both halophytes and glycophytes increased

through accumulation of organic and inorganic solutes (Yeo, 1998). Therefore, greater decrease in cell solute potential than external salt concentration may indicate an osmotic adjustment. This is due to the accumulation of organic solutes in the cytosol to balance the solute potential of the vacuole which is dominated by ions (Flowers et al., 1977, 2004; Greenway and Munns, 1980).

A large number of plant species accumulate glycinebetaine and proline in response to salinity stress and their accumulation may play a role in alleviating salinity stress (Hanson and Grumet, 1985; Ashraf, 1994; Mansour, 2000; Ashraf and Harris, 2004). A positive correlation between osmolyte accumulation and the adaptation to stress has been well documented (McCue and Hanson, 1990; Ashraf, 1994; Lutts et al., 1996; Rodriguez et al., 1997; Mansour, 2000; Ashraf and Harris, 2004).

Molecular mechanism of salt tolerance has been well acknowledged by different authors. Salt-responsive miRNAs are involved in the regulation of metabolic, morphological and physiological adaptations of maize seedlings at the post-transcriptional level. The miRNA genotype-specific expression model might explain the distinct salt sensitivities between maize lines (Dong et al., 2009). Membrane stability appears as a promising trait in the identification of salinity tolerance in maize seedlings (Collado et al., 2010).

Traits associated with seedling vigor, such as seedling weight and growth rate, and photochemical efficiency under stress conditions can be used as selection criteria for salt-tolerant maize in breeding programs (Carlos et al., 2007). According to the above mentioned, the objective of the present study was to evaluate the effect of NaCl stress at the seedling stage in maize hybrids and identify potential tolerant genotypes to be used in crop improvement programs.

2. Materials and Methods

2.1. Plant material and experimental conditions

The present study was conducted at the Seed Physiology Laboratory, Vibha Agrotech Ltd., (VAL), Hyderabad, India (August-September, 2012) using 42 maize genotypes developed by VAL for tolerance to NaCl-salinity at the seedling stage. Initially mass scale screening was conducted at 0.15 M NaCl (EC 13) for 42 genotypes and selected few (14). Selected lines (14) were screened at 0.15 and 0.2 M NaCl (EC 18).

Fourteen maize genotypes developed at VAL were grown in plastic pots using coco peat (coir peat) in room temperature and artificial light was provided to maintain light up to 12 days. Room temperature was about 27°C. A novel technique has been developed for this purpose. The technique consists of sowing the seeds at a depth of 2 cm in a plastic pot (length 7.5 cm, diameter 5 cm) filled with coco peat (neutral delignified coir

fibers) and then applying water or required saline concentration up to two thirds of the pot (about 70 mm). Twenty seeds were sown in each pot in the upper coco peat layer at 2 cm depth which receive water/solution by capillarity. We apply the solution only one time, say water, or saline solution up to the termination of the experiment (10 days after emergence). To protect seeds from fungal attack, seeds were treated with thiram solution (5%, v/v) for 5 minutes before sowing. Seeds were sown in each pot under control (distilled water) along with 0.15 M NaCl or at higher salinity level up to 0.20 M NaCl. Each of the treatments was replicated thrice for all the genotypes. This technique simulates a semi-hydroponic system where the upper layers of coco peat medium receive water/or saline solution only by capillary movement, while the roots are immersed in saturated lower coco peat medium. During capillary movement there is free flow of oxygen owing to constant evapo-transpiration. Observations were taken by taking 12 days old seedlings. Data were taken on average emergence percentage, speed of emergence, shoot length (cm), root length (cm), and number of lateral roots of five seedlings on 12th day after emergence. In the experiment, along to control and each NaCl concentration, sufficient nutrient solution (Knop's solution) was added to the treatments to supply plant nutrients for growing medium.

2.2. Statistical analyses

Data of the seedling traits were analysed statistically from each experiment. Data were statistically analyzed using one-way analysis of variance with a factorial arrangement being genotypes and NaCl concentrations the factors. Where the F-test was significant ($p < 0.05$), differences were validated using the Tukey's honestly significant difference. Assumptions of normality of data were tested using the Kolmogorov-Smirnov test (Steel and Torrie, 1980). For each experiment and NaCl treatment, Pearson's correlation analyses between seedlings traits were also computed (Steel and Torrie, 1980). All applied statistical methods were computed according to the SPSS® (Statistical Package for the Social Sciences) software package (standard released version 13.0 for Windows, SPSS Inc., Chicago, IL).

3. Results and Discussion

With exception of interaction genotypes *NaCl concentration for emergence percentage, studied seedling traits were significantly different between maize genotypes, NaCl concentration and between genotypes within NaCl concentration (Table 1).

Place Table 1 here

Under control conditions, emergence percentage ranged from 50 to 100%; at 0.15 M NaCl from 10 to 96.7%; and at 0.2 M NaCl from 0 to 77% (Figure 1a). With respect to emergence

Table 1: Calculated mean squares (MS) and F values from the statistical analysis corresponding to data of fourteen maize genotypes subjected to 0.15 and 0.2 M NaCl†

Source of variation	Variable									
	Emergence		Emergence index		Shoot length		Root length		No adventitious roots	
	MS	F	MS	F	MS	F	MS	F	MS	F
Genotype (G)	3156.3	2204.0***	328.8	368.3***	21.0	480.3***	60.0	1532.4***	16.4	4655.2***
NaCl	13390.3	9350.5***	6217.1	6963.8***	281.9	6431.7***	691.5	17650.6***	3.8	1077.8***
G*NaCl	273.8	191.2***	75.7	84.7***	1.2	28.4***	8.9	227.3***	1.9	542.1***
Error	1.43		0.89		0.04		0.04		0.004	
Mean	57.4		31.5		7.5		12.9		4.3	
R ²	.998		.999		.996		.998		.997	
CV (%)	2.1		3.0		2.8		1.5		3.3	

^{NS}Not significant, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

†Mean, adjusted coefficient of determination (R²), and coefficient of variation (CV, %) values are provided.

index under control conditions it ranged from 34.5 to 69.6; at 0.15 M NaCl from 9.5 to 34.6; at 0.2 M NaCl from 0 to 33.2 (Figure 1b). With respect to shoot length, under control conditions it varied from 8.6 to 14.2 cm; at 0.15 M NaCl from 3.3 to 9.4 cm; at 0.2 M NaCl from 0 to 6.8 cm; (Figure 1c). Seminal root length under control conditions varied from 14.4 to 22.1; at 0.15 NaCl from 3.5 to 15.7 cm; at 0.2 M NaCl from 0 to 13.6 cm (Figure 1d). Number of adventitious roots control conditions varied from 1 to 7; at 0.15 M NaCl from 1 to 7; at 0.2 M NaCl from 0 to 6 (Figure 1e). Overall correlations, seedling emergence (%) was positively correlated with emergence index ($r=0.834$ at 0.01 level), shoot length ($r=0.794$ at 0.01 level), seminal root length ($r=0.748$ at 0.01 level), and number of adventitious roots ($r=0.647$ at 0.01 level).

Therefore, it reveals that root length and number of adventitious roots contribute to salinity tolerance in maize. Similarly, under control condition seedling emergence (%) was positively correlated with emergence index ($r=0.515$ at 0.01 level), shoot length ($r=0.459$ at 0.01 level), seminal root length ($r=0.664$ at 0.01 level), and number of adventitious roots ($r=0.695$ at 0.01 level). Under 0.15 M NaCl condition seedling emergence (%) was positively correlated with emergence index ($r=0.814$ at 0.05 level), shoot length ($r=0.640$ at 0.01 level), seminal root length ($r=0.666$ at 0.05 level), and number of adventitious roots ($r=0.834$ at 0.01 level). Under 0.2 M NaCl condition seedling emergence (%) was positively correlated with emergence index ($r=0.866$ at 0.01 level), shoot length ($r=0.812$ at 0.01 level), seminal root length ($r=0.703$ at 0.01 level), and number of adventitious roots ($r=0.752$ at 0.01 level).

Thus the results of the experiment have suggested that there is genetic variability among maize genotypes which may be

utilized for crop improvement for salt tolerance during the early stages of crop growth and development. Results have also indicated that among studied seedling traits, emergence percentage and number of adventitious roots could be selected as salt stress criteria for concentration above 0.15 M NaCl. In addition, among studied maize genotypes, it seems that the most promising genotypes to tolerate concentrations up to 0.2 M NaCl are 1,025 and 1,034, and for emergence above 70%, and emergence index 30 above. In contrast, the most susceptible genotypes for both emergence and emergence index are 1,035 and 1,037. Thus, for massive genetic screening selection, materials could be evaluated up to 0.2 M NaCl in order to identify sources of germplasm tolerant to salt stress.

The results of the present study are in agreement with the results achieved by Giaveno et al. (2007) in screening tropical maize for salt tolerance. Genotypic variability has been reported by some authors (Nordquist et al., 1992; Maiti et al., 1996). Increasing salinity decreased emergence percentage, emergence index, shoot length, root length and number of adventitious roots. These observations coincide with those of several authors (Alberico and Cramer, 1993; Rodriguez et al., 1997). Similar findings to present study, salinity inhibits root growth as reported by Rodriguez et al. (1997). The adverse effect of salt stress is due to the cause of breakdown of lipid in order to supply sugars (Ashraf and Wahid, 2000) and polypeptide pattern (Läuchli et al., 1992).

The adaptation of the embryonic cultures to salinity stress is reported to be associated with the qualitative and quantitative changes in polypeptide pattern (Lusardi et al., 1991). Tolerant lines accumulate glycinebetaine which reduces shoot growth under saline condition and helps in maintaining greater leaf

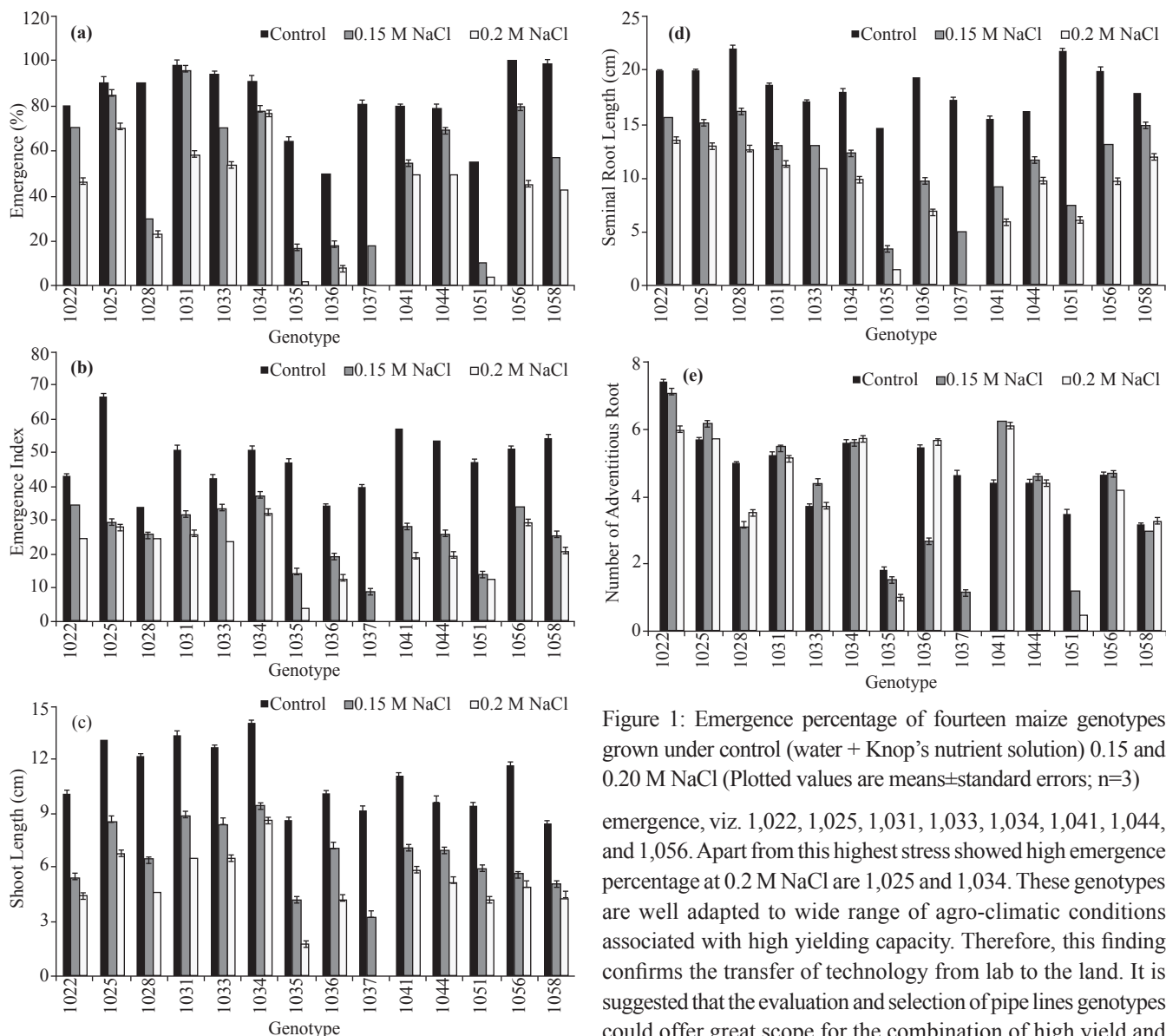


Figure 1: Emergence percentage of fourteen maize genotypes grown under control (water + Knop's nutrient solution) 0.15 and 0.20 M NaCl (Plotted values are means±standard errors; n=3)

emergence, viz. 1,022, 1,025, 1,031, 1,033, 1,034, 1,041, 1,044, and 1,056. Apart from this highest stress showed high emergence percentage at 0.2 M NaCl are 1,025 and 1,034. These genotypes are well adapted to wide range of agro-climatic conditions associated with high yielding capacity. Therefore, this finding confirms the transfer of technology from lab to the land. It is suggested that the evaluation and selection of pipe lines genotypes could offer great scope for the combination of high yield and stress resistance (salinity/drought). This is considered as an effective approach for increasing productivity under salinity stress. Traits associated with seedling vigor, such as seedling weight and growth rate, and photochemical efficiency under stress conditions can be used as selection criteria for salt-tolerant maize in breeding programs (Giaveno et al., 2007). The heritability for relative root growth is moderate suggesting that there is good scope for enhancing salt tolerance in maize through selection and breeding (Khan et al., 2003).

4. Conclusion

Increasing salinity decreases emergence percentage, shoot and root length and number of adventitious roots in susceptible lines, on the contrary the tolerant lines showed increased number of lateral roots with increasing salinity as a function of osmotic adjustment. The results revealed that seedling emergence and number of adventitious

relative water content, a higher rate of carbon assimilation, and a greater turgor potential (Saneoka et al., 1995). The heritability for relative root growth is moderate suggesting that there is good scope for enhancing salt tolerance in maize through selection and breeding (Khan et al., 2003). Salinity affects water transport in maize roots (Azaizeth and Steudle, 1991). The growth and mineral absorption by maize seedling is affected by increasing NaCl (Izzo et al., 1991). Jan (1999) studied salt tolerance of maize cultivars under salt-stressed conditions. In this study, it is observed that though salinity reduced root elongation, but salinity increased the production of adventitious roots in tolerant lines. This may be associated with increase in osmotic adjustment.

Seven genotypes have at moderate stress showed high emergence percentage at 0.15 M NaCl starting from 70 to 96.7%

roots may be considered as selection criteria for salinity tolerance in maize. These genotypes have great potential under saline prone areas.

5. References

- Alberico, G., Cramer, O., 1993. Is salt tolerance of maize related to sodium exclusion? I: Preliminary screening of seven cultivars. *Journal of Plant Nutrition* 16, 2289-2303.
- Ashraf, M., 1994. Breeding for salinity tolerance in plants. *Critical Review of Plant Science* 13, 17-42.
- Ashraf, M., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166, 3-16.
- Ashraf, M., Wahid, S., 2000. Time-course changes in organic metabolites and mineral nutrients in germinating maize seeds under salt (NaCl) stress. *Seed Science Technology* 28, 641-656.
- Azaizeth, H., Steudle, E., 1991. Effects of salinity in water transport of excised maize roots. *Plant Physiology* 97, 1136-1145.
- Collado, M.B., Arturi, M.J., Aulicino, M.B., Molina, M.C., 2010. Identification of salt tolerance in seedling of maize (*Zea mays* L.) with the cell membrane stability trait. *International Research Journal of Plant Science* 1(5), 126-132.
- Dong Ding, Lifang Zhang, Hang Wang, Zhijie Liu, Zuxin Zhang, Yonglian Zheng, 2009. Differential expression of miRNAs in response to salt stress in maize roots. *Annals of Botany* 103, 29-38.
- Flowers, T.J., 2004. Improving crop salt tolerance. *Journal of Experimental Botany* 55, 307-319.
- Flowers, T.J., Troke, P.F., Yeo, A.R., 1977. The mechanism of salt tolerance in halophytes. *Annals of Review of Plant Physiology* 18, 89-121.
- Giaveno, C.D., Ribeiro, R.V., Souza, G.M., de Oliveira, R.F., 2007. Screening of tropical maize for salt stress tolerance. *Crop Breeding and Applied Biotechnology* 7, 304-313.
- Greenway, H., Munns, R., 1980. Mechanism of salt tolerance in nonhalophytes. *Annals of Review of Plant Physiology* 31, 149-190.
- Hanson, A.D., Grumet, R., 1985. Betain accumulation: metabolic pathway and genetics. In: Key, J.L., Kosuge, T., Alan, R. (Eds.), *Cellular and Molecular Biology of Plant Stress*. Liss, New York, 71-92.
- Izzo, R., Navari-Izzo, F., Quartacci, M.F., 1991. Growth and mineral absorption in maize seedlings as affected by increasing NaCl concentration. *Journal of Plant Nutrition* 14, 687-699.
- Jan, N., Khatak, S.G., Khattak, J., 1995. Effect of various levels of salinity on germination of different maize cultivars. *Sarhad Journal of Agriculture* 11, 721-724.
- Jan, N., 1999. Salt tolerance of maize cultivars under salt-stressed conditions. *Sarhad Journal of Agriculture* 15, 205-211.
- Khan, A.A., Rao, S.A., McNeilly, T., 2003. Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.). *Euphytica* 13, 81-89.
- Läuchli, A., 1992. Calcium salinity and the plasma membrane. In: Leonard, R.T., Hepler, P.K., (Eds.), *Calcium in Plant Growth and Development. Current Topics in Plant Physiology Series*, Vol. 4. ASPP, Rockville, MD, 26-35.
- Levitt, J., 1980. Responses of Plants to Environmental Stresses, Water, Radiation, Salt and Other Stresses, Vol. II (2nd Edn.). Academic Press, New York, Page?.
- Lusardi, M.C., Locatelli, C., Stadler, J., Lupotto, E., 1991. *In vitro* characterization of salt-selected maize genotypes. *Journal of Genetics and Breeding* 45, 285-291.
- Lutts, S., Kinet, J.M., Bouharmant, J., 1996. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regulator* 19, 207-218.
- Maiti, R.K., Singh, V.P., Wesche-Ebeling, P., Sánchez-Arreola, E., Hernández-Piñero, J., Aguilar-Nájera, E., 2004. Research advances on cold, drought and salinity tolerance and its mechanism of resistance in maize (*Zea mays* L.): a review. *Crop Research* 27, 1-29.
- Maiti, R.K., Amaya, L.E.D., Cardona, S.I., Dimas, A.M.O., De la Rosa-Ibarra, M., Castillo, H.D.L., 1996. Genotypic variability in maize cultivars (*Zea mays* L.) for resistance to drought and salinity. *Journal of Plant Physiology* 148, 741-744.
- Mansour, M.M.F., Salama, K.H.A., Ali, F.Z.M., AbouHadid, A.F., 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. *Gen. Appl. Plant Physiol.* 1(1), 29-41.
- Mansour, M.M.F., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant.* 43, 491-500.
- McCue, R.F., Hanson, A.D., 1990. Drought and salt tolerance toward understanding and application. *TIBTECH* 8, 358-362.
- Muhammad Akram, Muhammad Yasin Ashraf, Rashid Ahmad, Ejaz Ahmed Waraich, Javed Iqbal, Muhammad Mohsan, 2010. Screening for salt tolerance in maize (*Zea mays* L.) hybrids at an early seedling stage. *Pakistan Journal of Botany* 42(1), 141-154.
- Naseer, S., 2001. Responses of barley (*Hordeum vulgare* L.) at various growth stages to salt stress. *Journal of Biological Sciences* 1, 326-329.
- Nordquist, P.T., Hergert, G.W., Skates, B.A., Compton, W.A., Marwell, J.P., 1992. Phenotypic expression of different maize hybrid genotypes grown in saline-sodic soil. *Journal of Plant Nutrition* 15, 2137-2144.
- Orak, A., Ates, E., 2005. Resistance to salinity stress and available water levels at the seedling stage of the common vetch (*Vicia sativa* L.). *Plant Soil Environment* 51, 51-56.

-
- Pitman, M.G., Läuchli, A., 2002. Global impact of salinity and agricultural ecosystem. In: Läuchli A., Luttge, V. (Eds.), *Salinity: Environment-Plant-Molecules*. Kluwer Academic Publishers, The Netherlands, 3-20.
- Rodriguez, H.G., Roberts, J.K.M., Jordan, W.R., Drew, M.C., 1997. Growth, water relations, and accumulation of organic and inorganic solutes in roots of maize seedlings during salt stress. *Plant Physiology* 113, 881-893.
- Saboora, A., Kiarostami, K., 2006. Salinity (NaCl) tolerance of wheat genotypes at germination and early seedling growth. *Pakistan Journal of Biological Sciences* 9, 2009-2021.
- Saneoka, H., Nagasaka, C., Hahn, D.T., Yang, W.J., Premachandra, G.S., Joly, R.J., Rhodes, D., 1995. Salt tolerance of glycinebetaine-deficient and -containing maize lines. *Plant Physiology* 107, 631-638.
- Tayyaba Khatoon, Khalid Hussain, Abdul Majeed, Khalid Nawaz, M. Farrukh Nisar, 2010. Morphological variations in maize (*Zea mays* L.) under different levels of NaCl at germinating stage. *World Applied Science Journal* 8(10), 1294-1297.
- Yeo, A., 1998. Molecular Biology of salt tolerance in the context of whole plant physiology. *Journal of Experimental Botany* 49, 915-929.