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EVALUATION AND CHARACTERIZATION OF GENETIC VARIATION IN MAIZE (*Zea mays* L.*)* FOR SALINITY TOLERANCE

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Salinity is an important agricultural problem that reduces crop production around the world. Maize (*Zea mays* L.) is an important cereal crop and is not considered tolerant to salinity. The present study was conducted to study the response of different maize genotypes to salinity and to explore the mechanisms of their differential response to salinity. This genotypic variation for salt tolerance in maize was examined in solution culture. Seven days old seedlings of six maize genotypes were transplanted in half strength Hoagland nutrient solution with two treatments (control, 75 mM NaCl) and three replications in completely randomized statistical arrangement. After 30 days of salinity development, the plants were harvested and data for shoot fresh weight, root fresh weight, shoot dry weight root dry weight, shoot length and root length were recorded. Na^+ and K^+ contents were determined from tissue sap and K^+: Na^+ ratio was computed. All the growth parameters of different maize genotypes were significantly reduced due to salinity. The genotypic variation for salt tolerance was also observed among the maize genotypes used in this study. The genotype EV-78 was ranked as salt tolerant genotype because it produced the maximum shoot biomass (6.33 g) and maintained higher K^+: Na^+ ratio (1.5) where as KS-64 was ranked as salt sensitive genotype as it produced the minimum shoot biomass (3.36 g) and could maintain the lowest K^+: Na^+ (0.86) ratio under salinity.
Keywords: maize, salinity, genetic variation, NaCl, *Zea mays*

INTRODUCTION

Salinity is a major stress among the abiotic stresses limiting crop production mainly in semi arid and arid regions of the world (Ashraf *et al*., 2008; Murtaza *et al*., 2011). Approximately 800 m ha land is affected by salinity throughout the world (Munns, 2005). Climate change is also creating serious threats for Agriculture in arid regions (Shakoor *et al*., 2011). Geographically, Pakistan is situated in the arid/semiarid zone of the world. About 6.67 m ha land is affected by salinity in Pakistan which is almost one third of the total cultivated area of Pakistan (Khan, 1998).
High salinity induces specific changes at cell, tissue and organ levels. These changes are physiological, morphological and anatomical in nature (Isla *et al*., 1998). Salt stress consists of three main components: osmotic stress component due to lowered osmotic potential, ion toxicity component due to presence of toxic ions like Na^+ and Cl^- (Liu and Vanstaden, 2001; Khakwani *et al*., 2011), and nutritional imbalance (Gratten and Grieve, 1999). Plants cannot tolerate toxic amount of solutes in their cytoplasm and therefore under saline conditions, they either restrict the large amount of solutes in their vacuole or compartmentalize these toxic ions in tissues to support their normal metabolic activities (Munns and Tester, 2008). High K^+/ Na^+ ratio in plants under salt stress is considered an important selection criterion for salinity tolerance (Saqib *et al*. 2004, 2005). The mechanism to maintain sufficient amount of K^+ in plant tissues under saline environment depends upon selective uptake of K^+ and selective cellular K^+ and Na^+ compartmentation in vacuole and distribution in the shoots (Munns and Tester, 2008). Though plant growth is ultimately reduced under saline conditions yet plant species and genotypes differ in their sensitivity or tolerance to salts (Munns and Termaat, 1986; Saqib *et al*., 2005, 2006). Better nutrient supply improves plant growth (Nadim *et al*., 2011) and stress tolerance as K nutrition protects plants from drought stress (Asgharipour *et al*., 2011). However, selection and introduction of crop species/cultivars which can tolerate high concentration of toxic salts and can produce good economic yield is a practicable approach for the salt affected lands.
Maize (*Zea mays* L.) is an important cereal for human and animal consumption and is grown under different climatic conditions in different parts of the world. Maize grain has high nutritional value and corn oil is used for cooking purpose whereas its green fodder contains high percentage of protein (Dowswell *et al*., 1996). It is a cross pollinated crop and may have great variability for salinity tolerance. In Pakistan due to increasing importance of maize, improvement in its agronomic characteristic has received considerable attention (Mehdi *et al*., 2000; Ahmad *et al*, 2010); however, extensive work is needed to identify maize genotypes for salt affected soils. Maize is C4 plant and

classified as moderately sensitive to salinity. There is significant reduction in the growth and yield for maize when grown under salt stress condition (Ouda *et al*., 2008).
Under field conditions abiotic stresses (salinity, drought and waterlogging etc.) are unpredictable and variable. Therefore, screening of genotypes in field is not very efficient in addition to being more labor intensive, time consuming and expensive. So screening trials conducted in nutrient solution by adding different amounts of salts to develop the desired salinity levels are good for the selection of genotypes/cultivars for stress environment. This procedure is relatively easy, inexpensive, less time consuming and gives reliable results (Qureshi *et al*., 1990). The present study was conducted to study the genetic variation among different maize genotypes in solution culture on the basis of their growth performance and leaf ionic composition. The selected genotypes can be recommended for cultivation in saline soils.

MATERIALS AND METHODS

Healthy seeds of six maize genotypes namely EV-20, EV-78, KS-64, R-2303, R-2310 and R-2315 were sown in polythene coated iron trays 60 cm × 45 cm × 5 cm containing 5 cm layer of acid washed gravels and irrigated with distilled water daily. At 2-3 leaf stage the seedlings of uniform size were transplanted in foam plugged holes of thermopole sheets floating on 100 L capacity iron tubs. These iron tubs were lined with polythene sheet and were containing ½ strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Proper aeration was provided by bubbling air through nutrient solution for 8 hours a day by aeration pump. The nutrient solution was changed weekly. The experiment was laid out in CRD with factorial arrangement and three replications. After one week of transplantation salinity level of 75 mM NaCl was developed by adding NaCl in three installments (one installment on each day) in the salinity treatment pots where as no salt was added in the "control treatment" pots. The pH was monitored daily and maintained at 5.5-6.5 throughout the experiment by adding either NaOH or HCl. After 30 days of salinity development, the plants were harvested manually and data for shoot fresh weight, root fresh weight, shoot dry weight root dry weight, shoot length and root length were recorded. The leaf samples from the youngest fully expanded leaves were collected, washed with distilled water and frozen. The frozen samples were crushed and tissue sap was collected. Na^+ and K^+ contents were determined by flame photometer (Jenway 480) from the tissue sap and K^+: Na^+ ratio was computed. The data obtained were analyzed statistically and the means were compared by standard error (Steel *et al*., 1997). To evaluate the salt tolerance of the studied genotypes a scoring system has been used from 1-6 for each parameter. The genotype with the best performance for a parameter was awarded 6 score and the genotype with the poorest performance for a parameter was awarded 1 score. On the basis of this scoring the genotype with the maximum scores was called as a salt tolerant and the genotype with the minimum scores was called as a salt-sensitive genotype.

RESULTS

The shoot fresh and dry weights of all the maize genotypes were reduced significantly by salinity and the maize genotypes also differed significantly for these growth parameters (Table 1). In saline conditions (75 mM NaCl), the maximum shoot fresh and dry weights were exhibited by EV-78 (32.8g, 6.33g, respectively) and this genotype did not differ significantly from R-2303. The minimum shoot fresh weight in the saline treatment was noted in R-2315 (22.0g) followed by KS-64 (22.2g) and the minimum shoot dry weight was noted in KS-64 (3.36g) followed by R-2315 (2.39g). The root fresh and dry weights of all the maize genotypes have also been reduced significantly by salinity (75 mM NaCl) (Table 2). Under salinity, the maximum root fresh weight wwas produced by EV-78 (17.9g) and it differed significantly from all the other maize genotypes. It was followed by R-2310 and R-2315 where as the minimum root fresh weight was observed for KS-64 (4.6 g). The maximum root dry weight in saline treatment was produced by EV-78 and it was statistically similar to R-2303, R-2310 and R-2315. The minimum root dry weight in this treatment was produced by KS-64 which did not differ significantly from EV-20. Shoot and root lengths of the maize genotypes under control and applied salinity indicated that salinity (75 mM NaCl) significantly reduced the shoot length of the maize genotypes (Table 3). The decline in shoot length was the maximum in KS-64 (93 cm) and the minimum in EV-78 (113.6 cm) due to 75 mM NaCl salinity treatment. At 75 mM NaCl, the maximum root length was gained by EV-20 (29.6 cm) and it differed significantly from R-2315 (21 cm) which attained the minimum root length.

Table 1. Effect of salinity on shoot fresh and dry weights ($g\ plant^{-1}$) of maize genotypes

	Shoot fresh weight		Shoot dry weight	
Genotypes	**Control**	**75 mM NaCl**	**Control**	**75 mM NaCl**
EV-20	52.3 ab	24.9 g	5.22 c	3.84 d
EV-78	55.5 a	32.8 ef	9.50 a	6.33 b
KS-64	39.9 de	22.2 g	4.62 cd	2.09 e
R-2303	48.9 bc	32.3 ef	8.81 a	5.51 bc
R-2310	51.2 ab	26.5 g	8.96 a	4.99 c
R-2315	44.2 cd	22.0 g	4.99 c	2.39 e
Mean	48.6 A	26.8 B	7.02 A	4.19 B

Means of a study parameter sharing the same small letters are statistically similar at $P \leq 0.05$.

Table 2. Effect of salinity on root fresh and dry weights ($g\ plant^{-1}$) of maize genotypes

Genotypes	Root fresh weight		Root dry weight	
	Control	75 mM NaCl	Control	75 mM NaCl
EV-20	19.5 cd	8.5 g	1.59 d-f	1.40 d-g
EV-78	26.0 a	17.9 de	4.72 a	2.00 cd
KS-64	11.2 f	4.6 h	0.91 fg	0.85 g
R-2303	21.9 b	11.5 f	2.48 c	1.67 de
R-2310	20.8 bc	11.2 f	3.21 b	1.60 d-f
R-2315	17.1 de	6.0 h	4.70 a	1.90 cd
Mean	19.4 A	10.0 B	2.93 A	1.57 B

Means of a study parameter sharing the same small letters are statistically similar at $P \leq 0.05$.

Table 3. Effect of salinity on shoot and root lengths (cm) of maize genotypes

Genotypes	Shoot length		Root length	
	Control	75 mM NaCl	Control	75 mM NaCl
EV-20	114.0 c	102.3 e	40.3 a	29.6 de
EV-78	124.3 a	113.6 c	32.0 cd	22.3 f
KS-64	99.6 f	93.0 h	33.0 b-d	25.0 ef
R-2303	118.0 b	106.0 d	38.3 ab	25.6 ef
R-2310	115.0 c	105.3 d	36.0 a-c	28.6 de
R-2315	104.3 de	96.6 g	29.3 de	21.0 f
Mean	112.5 A	102.8 B	34.8 A	25.3 B

Means of a study parameter sharing the same small letters are statistically similar at $P \leq 0.05$.

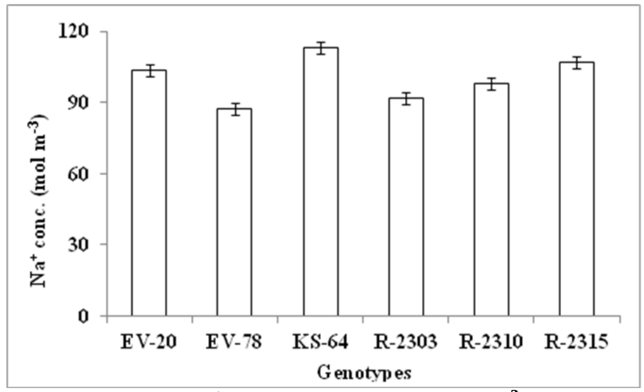

Figure 1. Leaf Na^+ concentration (mol m^{-3}) of maize genotypes grown at 75 mM NaCl salinity

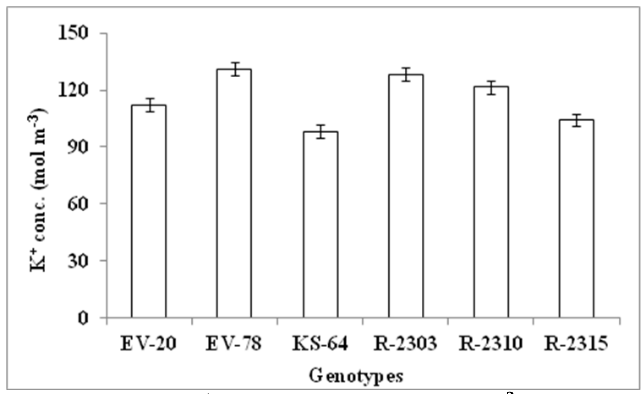

Figure 2. Leaf K^+ concentration (mol m^{-3}) of maize genotypes grown at 75 mM NaCl salinity

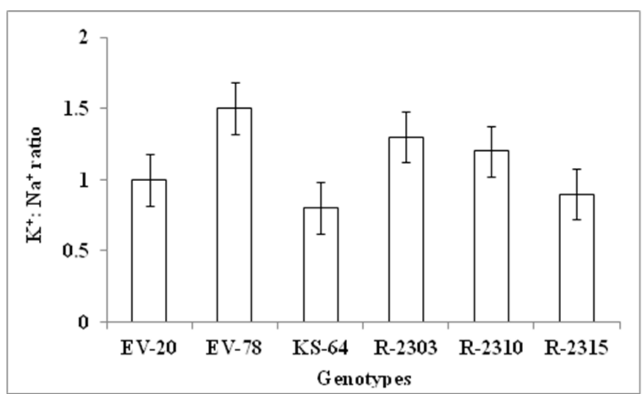

Figure 3. Leaf K^+: Na^+ ratio of maize genotypes grown at 75 mM NaCl salinity

The leaf ionic composition of all the maize genotypes showed a significant increase in Na^+ concentration and decrease in K^+ concentration and K^+: Na^+ due to 75 mM NaCl salinity (Fig. 1, 2, 3). The genotypes also differed significantly for the accumulation of Na^+ and K^+ in their leaf sap. In saline conditions, the minimum leaf Na^+ concentration was found in EV-78 (87.3 mol m^{-3}), whereas KS-64 accumulated the maximum Na^+ concentration (113.3 mol m^{-3}). These genotypes also differed significantly for the accumulation of Na^+ in their leaf sap. The maximum K^+ concentration and K^+: Na^+ ratio was found in the leaves of EV-78 (131 mol m^{-3}, 1.5) and the minimum was noted in KS-64 (98.3 mol m^{-3}, 0.86) with a significant difference between these genotypes.

DISCUSSION

Salinity reduces shoot and root growth of maize. This study reveals the existence of genotypic variation among the maize genotypes for tolerance to salinity. Shoot fresh and dry weights of all the tested maize genotypes were decreased significantly at 75 mM NaCl salinity (Table 1). Reduction was more in KS-64 as compared to EV-78. According to Cicek *et al*. (2002) salinity results in a significant decrease in shoot length, shoot fresh and dry weights and leaf area of maize. Tuna *et al*. (2008) also reported that saline conditions decrease the total dry matter, chlorophyll content and relative water content (RWC) in maize. The reduction in shoot fresh and dry weights may be due to decreased osmotic potential of rooting medium and ion toxicity (Munns *et al.*, 1995). The growth parameters like shoot fresh and dry weights, root fresh weight and dry weight are generally associated with salinity tolerance at early growth stages of crops and could be used as screening/selection criteria for salinity tolerance (Larcher, 1995). According to Maiti (1996) and Bilgin *et al*. (2008) high level of salinity reduces all the growth parameters in maize plants. Plant root is an organ which supplies all essential nutrients from growth medium to growing regions of plant. It has direct contact with medium so root response provides the very useful information for salinity tolerance of plants. In our study root fresh and dry weights were significantly reduced under salinity (Table 2) and the maximum root growth was in EV-78 and the minimum was recorded in KS-64 among the tested genotypes. Our results are in accordance with Akram *et al*. (2007) who observed that root dry weight of the maize hybrids was decreased with salinity. Similar findings were also observed in maize by (Turan *et al*. 2009), they reported that salinity inhibited shoot and root growth of maize plants. Excessive accumulation of sodium in the plant tissue results in nutrient imbalance, osmotic regulation and causes specific ion toxicity (Katerji *et al*., 2004; Arzani, 2008).

The accumulation of toxic ions in plants is often considered to be the main cause of growth reduction under salinity (Muscolo *et al*., 2003). Amzallag (1999) observed that there is negative relationship between the shoot biomass and Na^+ content. Similar relationship has also been found in this study (Fig. 4) where as it has been found in the present study that leaf K^+ concentration (Fig 5) and K^+: Na^+ ratio (Fig. 6) has a positive relationship with shoot growth. The leaf Na^+ concentration was low in EV-78 and performed better as compared to KS-64 which accumulated more Na^+ and showed less growth. K^+: Na^+ ratio was high for EV-78 and low for KS-64. Fortmeier and Schubert (1995) reported that salt tolerant maize genotypes have low shoot Na^+ concentration which shows that Na^+ exclusion is positively correlated with salt tolerance in maize. Toxic concentration of Na^+ and Cl^- in the cells exert many deleterious impacts on vital cellular processes and functions (Serrano *et al*., 1999). If excessive amount of Na^+ and Cl^- cannot be efficiently sequestered in the vacuole, these accumulated ions in the cytosol may result in a significant reduction in photosynthesis, ultra structural and metabolic damage and finally the death of leaves (Yeo, 1985). Under salinity disturbance of K^+ nutrition and a high Na^+: K^+ ratio in the tissues result in salt injury in many plants (Pagter *et al*., 2009). High potassium concentration in leaf facilitates osmotic regulation with relatively less energy expenditure than the accumulation of compatible solutes (Storey and Walker, 1999). More than 50 plant enzymes require K^+ as a co-factor and these enzymes are particularly susceptible to high Na^+ and high Na^+: K^+ ratios (Munns and Tester, 2008).

Plant species and their genotypes differ greatly in coping with the salt stress conditions (Wahid *et al*., 1997). Maize is

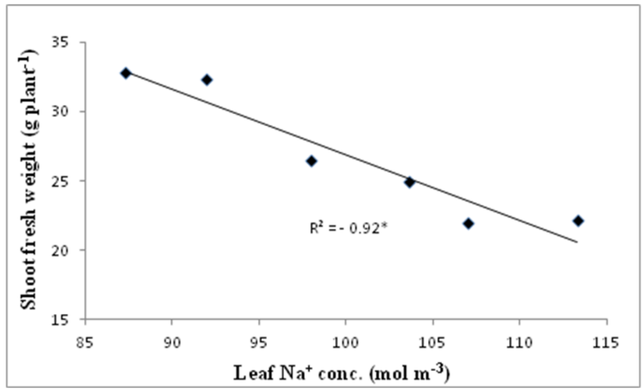

Figure 4. Relationship between shoot fresh weight and leaf Na^+ concentration of maize genotypes grown at 75 mM NaCl salinity

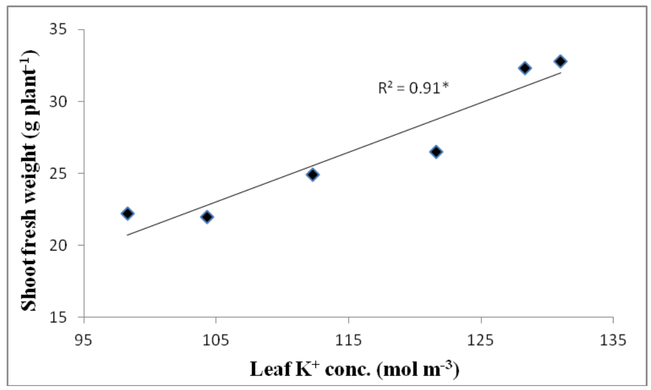

Figure 5. Relationship between shoot fresh weight and leaf K^+ concentration of maize genotypes grown at 75 mM NaCl salinity

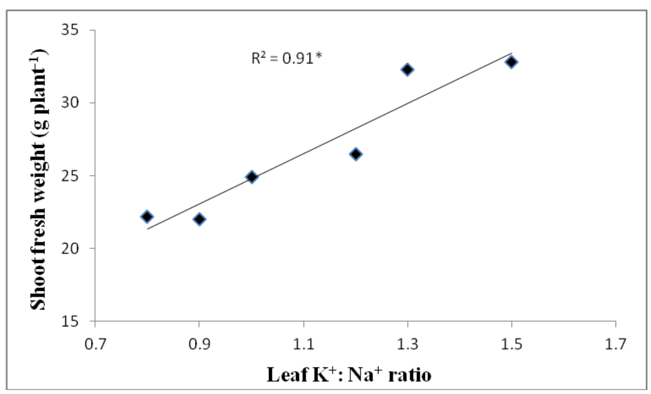

Figure 6. Relationship between shoot fresh weight and leaf K^+: Na^+ ratio of maize genotypes grown at 75 mM NaCl salinity

one of the major cereal crops and is salt sensitive (Mass and Hoffman, 1977; Fortmeier and Schubert, 1995). To evaluate the salt tolerance of the studied genotypes a scoring system has been used and based on their performance each genotype has been awarded a score in each parameter. These scores of

Table 4. The ranking of maize genotypes on the basis of their growth and leaf ionic composition

Genotypes	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Leaf Na^+ conc.	Leaf K^+ conc.	Leaf K^+: Na^+ ratio	Total
EV-20	3	3	3	2	3	3	3	20
EV-78	6	6	6	6	6	6	6	42
KS-64	2	1	1	1	1	1	1	8
R-2303	5	5	5	4	5	5	5	34
R-2310	4	4	4	3	4	4	4	27
R-2315	1	2	2	5	2	2	2	16

the genotypes in different parameters and their cumulative score is shown in Table 4. Based on these scores EV-78 and R-2303 have been declared as tolerant and moderately tolerant genotypes respectively and KS-64 and R-2315 as sensitive and moderately sensitive genotypes, respectively. Genotypic variation has also been observed among the maize genotypes used in this study. The maximum shoot fresh and dry weights were produced by EV-78 (44.19g, 7.91g) whereas the minimum shoot fresh and dry weights (31.09g, 3.35g) were produced by KS-64. KS-64 produced minimum fresh and dry weights due to more accumulation of Na^+ in its leaves and had lower concentration of K^+ and K^+: Na^+ ratio due to which its performance has been poor. Saline environment results in a greater increase in cytoplasmic Na^+ content in the salt sensitive variety than in the resistant variety (Lacerda *et al*., 2003). According to Blanco *et al*. (1998) salt resistant variety contains low Na^+ and high K^+ content as compared to a salt sensitive variety. The selected salt resistant hybrid may be recommended for use on the salt-affected filed.

***Conclusion*:** It is concluded that evaluation at early growth stages of maize genotypes in solution culture is a simple and quick method for determining genotypic differences in response to salinity. The results of solution culture evaluation may however be confirmed in the field. In this study EV-78 has emerged as a salt tolerant maize hybrid as a result of its better management of leaf ionic composition where as KS-64 has been observed as a salt sensitive maize hybrid as it showed lower growth and could manage a poor leaf ionic composition.

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