

## RESEARCH ARTICLE

Mohammad M. Ibne Hoque<sup>1</sup>  
Zheng Jun<sup>2</sup>  
Wang Guoying<sup>2</sup>

## Evaluation of salinity tolerance in maize (*Zea mays* L.) genotypes at seedling stage

### Authors' addresses:

<sup>1</sup> Graduate School of Chinese Academy of Agricultural Sciences (GSCAAS), Beijing 100081, P. R. China.

<sup>2</sup> Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, P. R. China.

### Correspondence:

Mohammad Muhebbullah Ibne Hoque  
Graduate School of Chinese Academy of Agricultural Sciences (GSCAAS),  
12 Zhongguancun South Street,  
Haidian District, Beijing 100081,  
P. R. China.

Tel.: +8801717320599

e-mail: muhibleebnehoque@gmail.com

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### ABSTRACT

Reproducible and reliable evaluation methods are the basis of any successful breeding programme especially for abiotic breeding. In this study, solution-culture and vermiculite-culture methods were employed to evaluate the salinity tolerance of nine maize genotypes. Ten seedling traits (shoot length, root length, leaf number, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, Na<sup>+</sup> content, K<sup>+</sup> content and K<sup>+</sup>/Na<sup>+</sup> ratio) were measured under 0 mM, 100 mM and 200 mM NaCl salinity stress and similar results were observed in both systems. Among the genotypes under investigation CZ-7 expressed as the tolerant and B73 appeared to be more sensitive. Biomass especially shoot fresh weight has shown a major growth reduction compare to the control which would be very useful trait in salinity tolerance improvement programme. The newly developed vermiculite-culture method was found an effective, easy and affordable technique for assessment of salt tolerance and which will be useful for plant breeders.

**Key words:** maize, salinity tolerance, biomass, vermiculite-culture method

## Introduction

Soil salinity is one of the major environmental abiotic stresses that limit agricultural productivity and food supply worldwide. The total global area of salt-affected soils has recently been estimated to be approximately 830 million hectares (Martinez-Beltran & Manzur, 2005), of which about 20% salt affected are those of irrigated lands (Pitman & Läuchli, 2002). With the steady increase in population, especially in the under-developed countries of the world and the concomitant decline in new agriculture lands, the need to tackle such soil stresses is urgent (Ali *et al.*, 2002).

Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Läuchli & Epstein, 1990). It is not only affects the morphology, but also modifies the metabolisms of plants by limiting their growth. Salinity affects both vegetative and reproductive development, which has profound implications depending on whether the harvest organ is a stem, leaf, root, shoot, fruit, fiber or grain. Salinity often reduces shoot growth more than root growth (Läuchli & Epstein, 1990).

The extent by which one mechanism affects the plant over the others depends on many factors including the species, genotype, plant age, ionic strength and composition of the salinizing solution and the organ in question. Plants undergo characteristic changes from the time salinity stress is imposed until they reach maturity (Munns, 2002). Nevertheless, a plant affected by salinity can not be productive and will exhibit wilting and droughtiness, even in the wet and moist soil. Above the salinity threshold, a plant performance will be deteriorates and the yield has been affected. Population growth on the one hand and land degradation by salinization on the other have led plant scientists to the concept of developing salt-tolerant crops by genetic approaches (Cuartero *et al.*, 2006; Munns *et al.*, 2006; Yamaguchi & Blumwald, 2005). Therefore, reliable and repeatable screening techniques are the basis of any successful breeding programme specifically for biotic or abiotic stress breeding.

Maize is one of the most salt-sensitive field crops, showing obvious signs of stress, including wilting even when there is adequate soil moisture, dull leaves, and gray leaf tips according to the "Maize Doctor"

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(<http://www.maizedoctor.cimmyt.org>). Maize grown under salinity was showed reduction in growth characteristic and yield production at all (Ouda *et al.*, 2008). In contrast, some variability of maize which belongs to the plants with C4 metabolism is observed as moderately sensitive to soil salinity (Mass & Hoffman, 1977; Ouda *et al.*, 2008). It is also reported that, genetic variability can exist for salt tolerance maize crop (Maiti *et al.*, 1996) like other plant species such as alfalfa (McKimmie & Dorbrenz, 1991), *Trifolium* (Ashraf *et al.*, 1987) and sunflower (Francois, 1996). Keeping this in view, this study was conducted to evaluate the performance of maize genotypes under the different levels of salinity (NaCl) and to endeavor a relatively simple screening method for screening populations of breeding material or a large number of genotypes.

**Materials and Methods****Materials**

These studies were undertaken to develop a suitable method for screening of maize plants at early stage of growth for salinity tolerance. Two methods already known as solution-culture and vermiculite-culture were compared for evaluating 9 maize genotypes. Among these 9 genotypes; eight (CZ-1, CZ-7, CZ-10, BM-7, Bornali, Shuvra, Mohor and Khoi Bhutta) collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Bangladesh and one (B73) originated in USA obtained from Chinese Academy of Agricultural Sciences (CAAS), Beijing, China. Of them, CZ-1, CZ-7, CZ-10 and B73 were inbred lines and others were open pollinated cultivars.

**Plant growth conditions**

The experiments were carried out in a controlled environment cabinet operating at 25°/23°C (day/night) temperature with 16 h photoperiod provided by fluorescent lighting giving 450  $\mu\text{molm}^{-2}\text{s}^{-1}$  photons and 65% R.H. The plants were tested at two salinity levels i.e., 100 and 200 mM NaCl and full strength Hoagland Nutrient Solution was used as control. The plants were exposed in treatment until the death of susceptible genotype(s) at maximum level of salinity. A completely randomized design with 3 replications was adopted for each method.

**Solution-culture method**

Seeds of each genotype were germinated and were transferred to plastic pots (2 L size hydro culture containers). At two-leaf stage, seedlings of uniform size were transplanted

in foam-plugged holes of polystyrene sheets floating over ½ strength Hoagland Nutrient Solution (Hoagland & Arnon, 1950) for one week. Then the seedlings were exposed in salt solution supplemented with full strength HNS. Nutrient solution was made with deionized water and was aerated with the help of an air pump throughout the experiment. The solution of the containers was changed every two day to maintain the salt concentration of the solution. The plants of all genotypes were exposed in saline solution till the death of susceptible line. Two weeks after treatment the plants were harvested and shoot and root length, the fresh weight of the shoot and roots were recorded and after drying at 80°C for 48 h the dry weights were taken.

**Vermiculite-culture method**

After germination the seedlings of each genotype were transferred to vermiculite-filled plastic pots and irrigated with deionized water. As the first leaf blade became fully expanded, the seedlings along with pots transferred to ½ strength Hoagland Nutrient Solution (Hoagland & Arnon, 1950) for three days and then to full strength solution supplemented with NaCl. The concentration of salt was maintained on alternate day until the end of the experiment by dipping the base of plastic pots along with seedlings in freshly prepared solution of the required concentration of NaCl or full strength nutrient solution (control). The pots along with the seedlings were exposed in solution for 4weeks. The plants of all genotypes were exposed in saline solution till the death of susceptible line. After that the plants were harvested and data were recorded as mentioned in previous method.

**Statistical analysis**

The experiment was conducted by using randomized complete block design with 3 replications. Data were subjected to statistical analysis using ANOVA, a statistical package available from SPSS 16. Significant differences between treatments were determined using LSD test at the 0.05 level.

**Results**

The morphological traits that were measured showed significant and very significant differences for both source of variation (maize genotypes and salinity) and maize genotypes $\times$ salinity interaction. All seedling traits showed significant differences ( $p\leq 0.001$ ) when the saline and control treatments were compared in solution-culture and

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vermiculite-culture methods (Table 1). All the genotypes showed significant differences ( $p \leq 0.001$ ) in their behaviors when they grew in saline conditions, in comparison with their corresponding controls (Figures 1, 2).

**Effect of salt stress on shoot length**

Shoot length was affected significantly with the increase in the salinity (Table 1, Figure 1A, Figure 2A). The rate of reduction in shoot length at 200 mM NaCl in comparison with the control was detected in CZ-1 with 48.04%, CZ-7

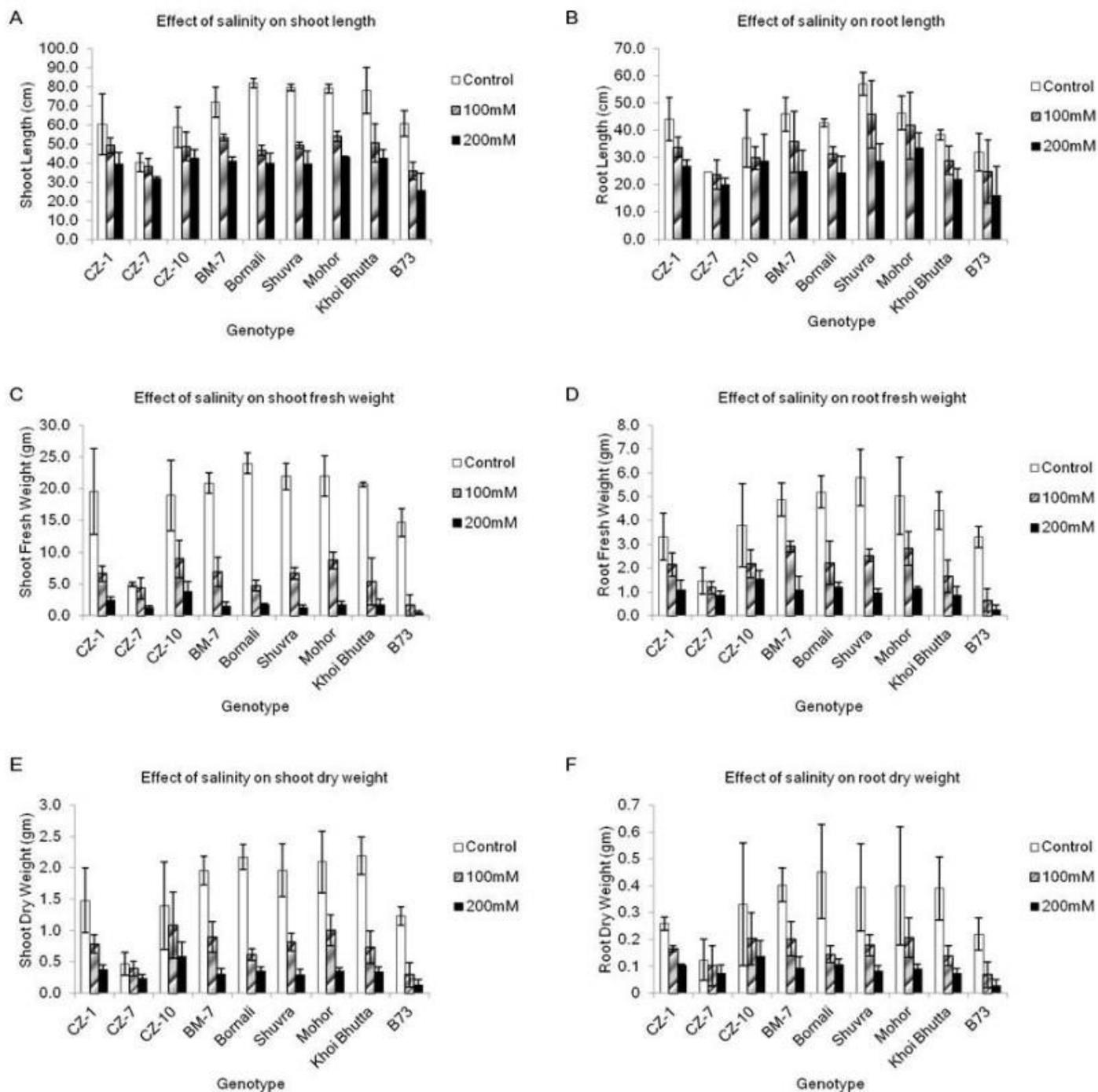
with 41.18%, CZ-10 with 43.48%, BM-7 with 41.80%, Bornali with 45.52%, Shuvra with 52.31%, Mohor with 50.54%, Khoi Bhutta with 52.33% and B73 with 53.29%. According to reduction rate in shoot length from the highest to lowest value the cultivars were arranged as B73>Khoi Bhutta>Shuvra>Mohor>Bornali>CZ-1>CZ-10>BM-7>CZ-7 (Table 2). This result was found in solution-culture method. In vermiculite-culture method maximum reduction rate was also observed in B73 (57.79%) and minimum was in CZ-7 (21.13%) (Table 2).

**Table 1.** *F-values and probability levels of evaluated traits in experiment*

Seedling trait	Sources of variation	Solution-culture method	Vermiculite-culture method
		F value	
Shoot length	Genotypes (G)	5.76***	12.64***
	Salinity (S)	196.93***	144.12***
	G×S	1.77*	3.00***
Root length	Genotypes (G)	20.89***	13.91***
	Salinity (S)	90.94***	55.32***
	G×S	3.58***	1.31 <sup>NS</sup>
Leaf number	Genotypes (G)	13.8***	9.82***
	Salinity (S)	372.79***	365.31***
	G×S	2.08*	4.84***
Shoot fresh weight	Genotypes (G)	7.52***	10.63***
	Salinity (S)	144.37***	386.03***
	G×S	2.10*	5.66***
Root fresh weight	Genotypes (G)	15.25***	9.80***
	Salinity (S)	108.33***	151.71***
	G×S	2.94**	3.12***
Shoot dry weight	Genotypes (G)	10.56***	9.63***
	Salinity (S)	112.48***	185.98***
	G×S	1.89*	4.30***
Root dry weight	Genotypes (G)	14.89***	5.35***
	Salinity (S)	102.89***	87.27***
	G×S	2.71**	1.89**
Na <sup>+</sup> content	Genotypes (G)	135.76***	47.56***
	Salinity (S)	13177.19***	1875.95***
	G×S	50.63***	22.79***
K <sup>+</sup> content	Genotypes (G)	55.59***	32.39***
	Salinity (S)	1427.14***	3876.36***
	G×S	7.96***	30.41***
K <sup>+</sup> /Na <sup>+</sup> ratio	Genotypes (G)	5.16***	28.30***
	Salinity (S)	755.79***	2761.87***
	G×S	5.00***	28.30***

<sup>NS</sup> Not significant, \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$

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**Figure 1.** Effect of salinity on shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E), and root dry weight (F) of different maize genotypes at seedling stage in solution-culture method.

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**Table 2.** Reduction rate of growth parameters over control due to effect of NaCl

Solution-culture method														
Genotype	% reduction at 100Mm NaCl over control							% reduction at 200Mm NaCl over control						
	SL	RL	LN	SFW	RFW	SDW	RDW	SL	RL	LN	SFW	RFW	SDW	RDW
<b>CZ-1</b>	24.27	6.05	15.04	49.84	23.41	19.75	11.50	48.04	39.56	38.94	93.16	81.60	78.49	81.09
<b>CZ-7</b>	12.81	5.64	1.23	30.28	5.97	11.52	2.45	41.18	15.46	32.10	89.74	63.61	65.13	64.83
<b>CZ-10</b>	20.77	6.63	6.06	34.88	6.94	19.46	4.92	43.48	33.53	37.37	91.31	67.43	77.15	74.24
<b>BM-7</b>	25.06	11.06	12.50	52.24	7.39	42.77	12.43	41.80	26.94	42.31	90.69	71.36	79.96	68.34
<b>Bornali</b>	31.86	14.95	13.64	52.41	19.28	42.81	24.32	45.52	24.78	38.18	89.87	70.90	75.20	71.04
<b>Shuvra</b>	18.00	13.61	8.91	37.30	12.02	10.99	5.79	52.31	46.83	41.58	92.52	81.63	79.04	79.63
<b>Mohor</b>	19.23	34.93	3.92	35.84	19.98	17.76	13.46	50.54	40.87	33.33	92.16	83.21	78.34	83.88
<b>Khoi Bhutta</b>	27.86	22.03	16.67	52.63	41.08	35.79	32.11	52.33	46.27	44.74	93.95	86.07	80.29	86.27
<b>B73</b>	43.25	41.05	25.00	57.88	42.70	44.03	37.53	53.29	46.61	45.00	93.70	87.12	80.55	86.39
Vermiculite-culture method														
<b>CZ-1</b>	18.59	23.09	20.53	66.02	35.19	47.10	36.02	34.41	38.86	39.20	87.47	67.13	75.03	59.95
<b>CZ-7</b>	5.43	3.63	7.69	11.66	19.50	15.17	17.74	21.13	18.54	19.69	73.92	40.95	49.64	39.96
<b>CZ-10</b>	17.04	19.44	15.87	53.01	42.64	22.46	38.53	27.31	22.18	33.86	79.57	59.47	58.09	58.55
<b>BM-7</b>	26.10	21.97	21.14	67.02	40.56	54.13	49.86	42.79	45.90	44.29	92.80	78.16	84.31	76.83
<b>Bornali</b>	42.97	26.70	30.73	80.28	57.34	71.78	68.04	51.25	42.77	43.26	92.64	76.92	83.55	76.65
<b>Shuvra</b>	37.86	19.64	26.25	69.43	56.48	58.25	54.77	50.24	49.54	49.25	94.03	83.65	84.83	79.19
<b>Mohor</b>	31.90	9.92	23.45	60.31	43.67	52.15	48.27	45.69	27.56	46.13	92.11	77.10	83.36	77.17
<b>Khoi Bhutta</b>	35.52	24.52	30.00	73.76	62.60	66.75	64.31	45.48	42.62	46.43	91.60	80.31	84.47	80.67
<b>B73</b>	40.95	22.47	48.95	88.36	80.38	75.79	68.69	57.79	49.97	59.16	97.19	92.77	89.82	87.47

SL: Shoot Length; RL: Root Length; LN: Total Leaf Number; SFW: Shoot Fresh Weight; RFW: Root Fresh Weight; SDW: Shoot Dry Weight; RDW: Root Dry Weight

**Effect of salt stress on root length**

Data regarding to root length is presented in Table 2 and Figure 1B, Figure 2B in all maize genotypes there was a reduction in root length. Maximum reduction in root length was measured in B73 at 200 mM and minimum was observed in CZ-7 in both solution-culture and vermiculite-culture method of screening (Table 2, Figure1B, Figure 2B). Salinity levels means indicate that with the increase in the salinity level the root length decrease significantly (Table 1).

**Fresh biomass reduction due to salt stress**

Due to increase in salinity, biomass accumulation was severely affected consequently; shoot and root fresh weights were reduced. Decrease in shoot fresh weight was parallel with enhancement of NaCl concentration in nutrition

solution. At maximum level of salinization (200 mM) B73 proved to be sensitive and shoot fresh weight was severely reduced up to 93.70% in solution-culture method and 97.19% in vermiculite-culture method (Table 2). The highest shoot fresh weights were obtained in control treatment while the lowest one was observed in 200 mM NaCl treatment (Figure 1C, Figure 2C) in both solution-culture and vermiculite-culture methods. Similar results were achieved when root fresh weights were measured. The highest and the lowest root fresh weights were related to control treatment and 200 mM NaCl treatment, respectively (Figure1D, Figure 2D).

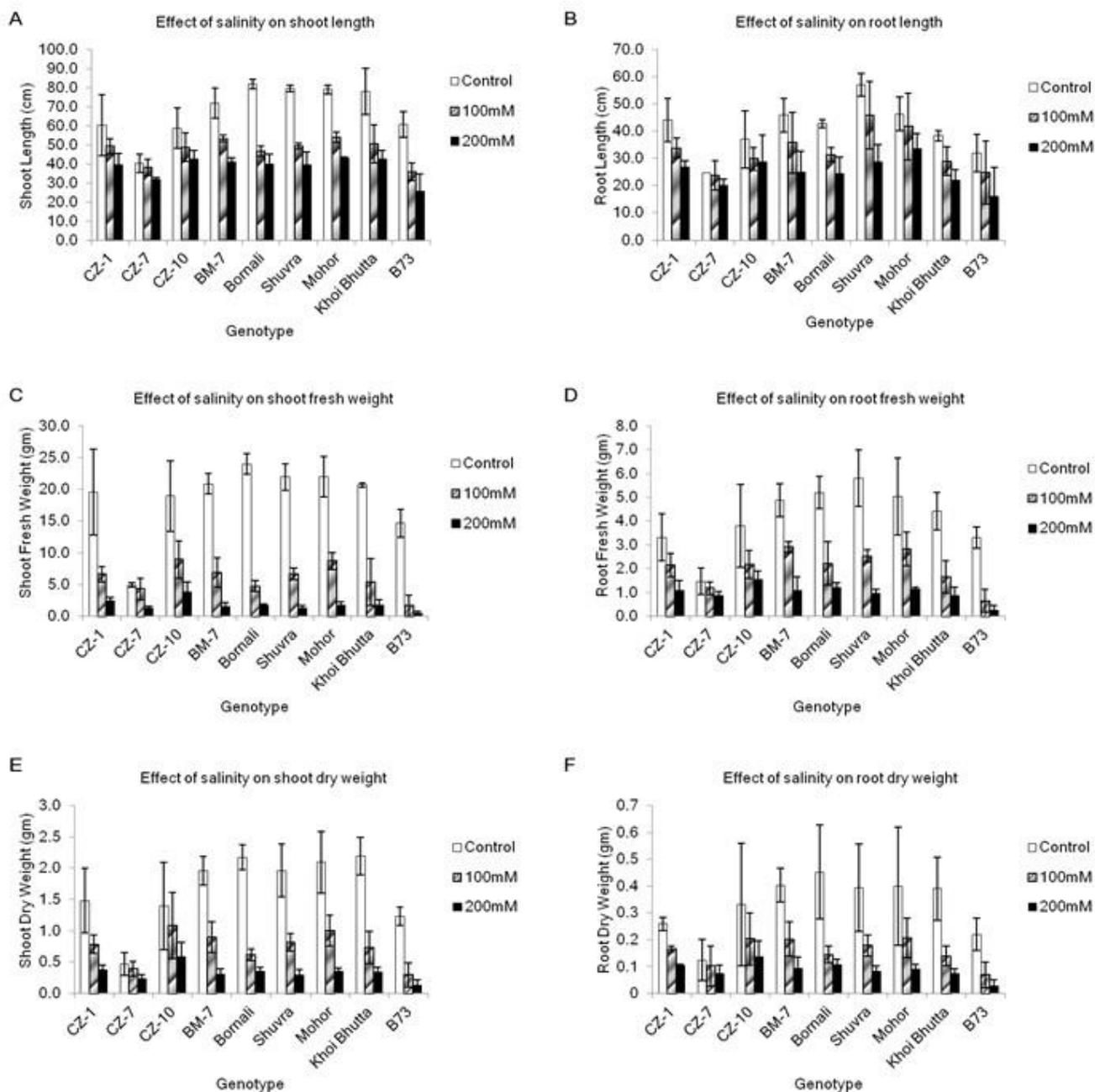
**Dry weights decreased due to salinity**

Salinity stress reduced plant growth and significantly decreased dry weights (Table 1). There was downward decrease in shoot and root dry weights because of deterrent

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effect of salinity on plant growth (Figur 1E, 1F, Figure 2E, 2F). Shoot dry weights of genotypes were negatively affected by increasing salt treatments. The reduction rate in shoot dry weights of genotypes at 200 mM NaCl when compared with the control were detected in CZ-1 with 78.49%, CZ-7 with 65.13%, CZ-10 with 77.15% , BM-7 with 79.96%, Bornali with 75.20%, Shuvra with 79.04%, Mohor with 78.34%,

Khoi Bhutta with 80.29% and B73 with 80.55% in solution-culture method. According to reduction rate in shoot dry weight from the highest to the lowest value the genotypes were arranged as B73>Khoi Bhutta>BM-7>Shuvra>CZ-1>Mohor>CZ-10>Bornali>CZ-7 (Table 2). The maximum decrease in shoot dry weight was also observed in B73 and minimum in CZ-7 in vermiculite-culture method (Table 2).



**Figure 2.** Effect of salinity on shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight

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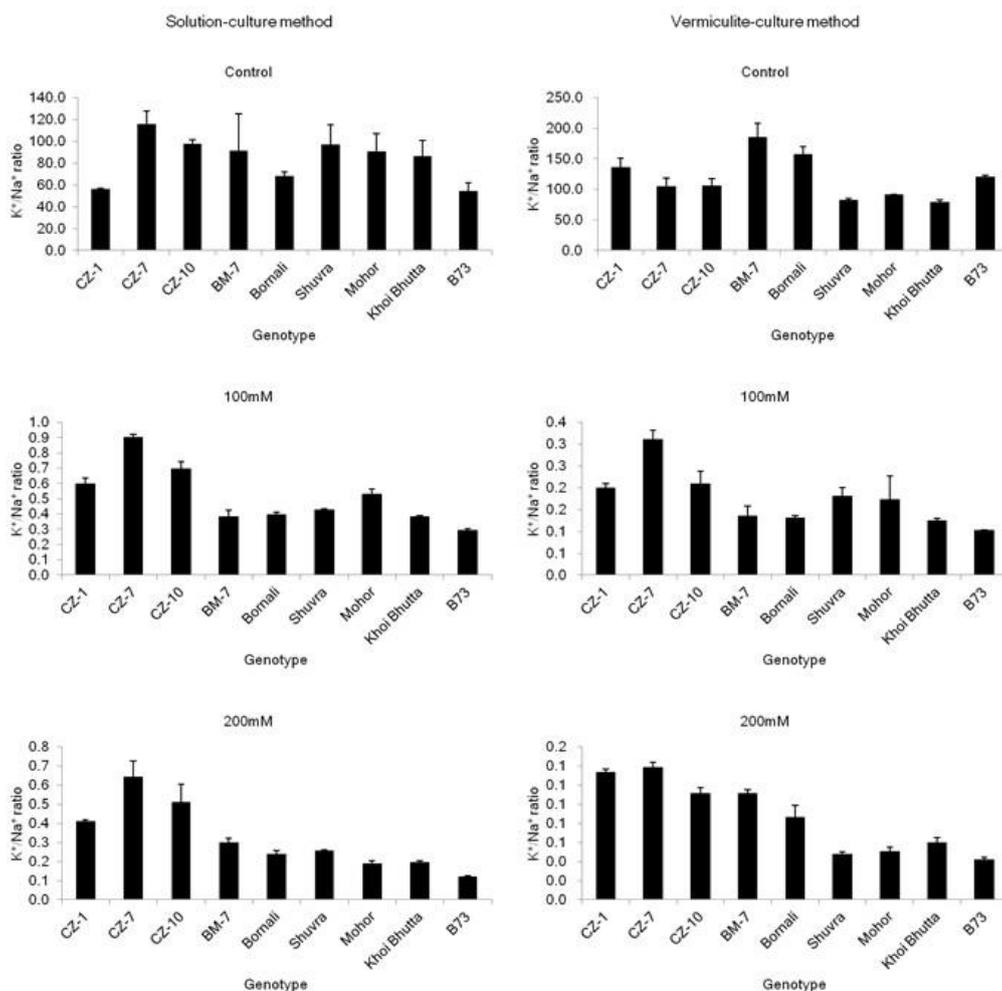
(E), and root dry weight (F) of different maize genotypes at seedling stage in vermiculite-culture method.

Root dry weight of genotypes decreased significantly as the levels of salinity increased from 0 to 200 mM (Table 1). Thus, the highest root dry weight was determined at control and the lowest root dry weight at highest salinity level (Figure 1F, Figure 2F). Among the cultivars, CZ-7 was affected least by salinity. The rate of reduction in root dry weight at 200mM NaCl in comparison with the control was detected in CZ-1 with 81.09%, CZ-7 with 64.83%, CZ-10 with 74.24% , BM-7 with 68.34%, Bornali with 71.04%, Shuvra with 79.63%, Mohor with 83.88%, Khoi Bhutta with 86.27% and B73 with 86.39% in solution-culture method. According to these values, the genotypes were arranged as following: B73>Khoi Bhutta>Mohor>CZ-1>Shuvra>CZ-10>Bornali>BM-7>CZ-7 (Table2). The highest reduction

was also observed in B73 (87.47%) and lowest in CZ-7 (39.96%) at highest salinity level (200 mM) in vermiculite-culture method (Table 2).

#### Effects of salt stress on $K^+/Na^+$ ratio

The increase in sodium contents demonstrated a decrease in  $K^+/Na^+$  ratio in all the maize genotypes (Figure 3). However, salt tolerant inbred line CZ-7 showed minimum reduction in  $K^+/Na^+$  ratio both in solution-culture and vermiculite-culture method. CZ-7 was successful in maintaining high level of  $K^+$  at all the salinity levels. The highest potassium contents at higher salinity level had resulted in maintaining higher  $K^+/Na^+$  ratio in genotype CZ-7, showed better performance under saline conditions.



**Figure 3.** Effects of different salinity levels on  $K^+/Na^+$  ratio in maize genotypes observed at control, 100 mM and 200 mM NaCl salinity stress.

**RESEARCH ARTICLE****Discussion**

Genetic variability in response to salt stress has been reported in corn (Cramer *et al.*, 1990; Maiti *et al.*, 1996). In the present study, variation in response to salinity was observed among 9 maize genotypes with comparing two screening methods at the seedling stage based on physiological and chemical analysis.

However, some inbred were tolerant or moderately tolerant during the seedling stage. Salinity stress adversely affects plant growth and development and results in significant reduction in yield and quality. It is generally accepted that the germination and seedling stage of plant life cycle is more sensitive to salinity than adult stage (Lianes *et al.*, 2005, Ashraf *et al.*, 1986). Effect of salinity at different growth stages in wheat, sorghum and cowpea was investigated and it was found that the early seedling period was the most sensitive one in all the crops and reduction in growth was observed which decreased with increase in salinity (Shalhevet, 1995). Salinity tolerance is obviously necessary at whole plant level through the whole life cycle to seed production in grain producing species. This has been exploited with success, as a means for selecting enhanced salinity tolerance in maize (Ashraf & McNeilly, 1990), pearl millet (Kebebew & McNeilly, 1994), in several forage grass species (Ashraf *et al.*, 1986), and in lucerne (Al-Khatib *et al.*, 1993).

***Effects of salt stress on physiological traits***

It is observed that significant differences were observed among genotypes and NaCl-concentration with respect to shoot length and root length. No salt (Control: 0 mM NaCl) in culture medium significantly enhanced shoot length. High salinity levels from 100 to 200 mM NaCl caused a decrease in shoot length (Figure 1A). Pessaraki & Kopec (2009) found that shoot length decreased by the increasing salt concentrations. Similar results were reported by Mohammad *et al.* (1998) in tomato and by Gill & Singh (1989) in rice. The reduction in shoot length is due to excessive accumulation of salts in the cell wall elasticity. Further, secondary cell appears sooner and wall becomes rigid as a consequence the turgor pressure efficiency in cell enlargement decreases. Salt stress has considerable effect on root length (Ashraf *et al.*, 2002; Gulzar *et al.*, 2003), but this may vary with plant species or genotype. In the present study, root was severely affected due to salinity (Table 2 and Figure 1B, Figure 2B). It is reported root growth is sensitive to high salt

concentrations in the medium. That is why roots are rapidly reduced or prevented by salinity (Cramer *et al.*, 1988; Ashraf *et al.*, 2005). Similar results were observed in pearl millet by Hussain *et al.* (2008).

The number of leaves was also an important trait to differentiate the genotypes in different treatments of salinization the employed genotypes showed significant differences with controls (Table 1). Due to increase in salinity level total leaf number was highly affected in B73 and less affected in CZ-7 (Table 2). This behavior which seems to indicate a reduction in the appearance of new leaves could be associated with the osmotic stress pointed out by Munns *et al.* (1995, 2002) and Munns & Tester (2008). Leaf number was decreased with increasing in salt concentration in all maize genotypes. The maximum leaf number was recorded under non-saline control and minimum at the highest salinity level.

The obtained results indicated that the increased concentration of NaCl negatively affected the shoot fresh weight (Table 2, Figure 1C, Figure 2C). The highest shoot fresh weight was obtained from control and the lowest shoot fresh weight was found at 200 mM. The increase in plant growth may be due to turgor potential which is decreased by water deficit produced by high concentrations of the salts in the soil (Khatoun *et al.*, 2010). Our result was also supported by the findings of some researches which showed that high NaCl concentration caused an evident suppression of early seedling growth as consequence of the osmotic stress, shoot fresh weight reduced considerably as salt concentration increased (Giaveno *et al.*, 2007). It is observed that significant differences were observed among genotypes and NaCl-concentration with respect to root fresh weight (Table 1, Figure 1D, Figure 2D). Root fresh weight decreased with increasing salinity level. At higher level of salt concentrations, since the root number and root length decreased densely, the fresh root growth also decreased. The findings of our results confirm some researchers' reports which claimed that fresh weight of root was one of the most adversely affected characters by the increasing salt level (Hameed *et al.*, 2008). Hussain *et al.* (2009) concluded that with the increase in salinity levels there was a significant reduction in biomass production including root fresh weight in black seeds.

Shoot dry weight of all maize genotypes decreased significantly by increasing NaCl concentration in Hoagland's solution in both solution-culture and vermiculite-culture method (Table 1 & Figure 1E, Figure 2E). Our results are in

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agreement with the results of other researchers. For example, Hussain *et al.* (2007) reported that a negative relationship was detected between vegetative growth parameters and increasing salinity. In the same study, shoot dry weight was 52.01 mg plant<sup>-1</sup> in the control, while it decreased linearly to 25.26 mg plant<sup>-1</sup> at 4000 ppm. The same results were also obtained by other researchers (Cramer *et al.*, 1994; Mansour *et al.*, 2005). In this experiment dry weight of root was significantly inhibited by salt (Table 1, Figure 1F, Figure 2F). A consistent decrease in root fresh weight was observed with increase in salt level. Thus the highest root dry weight was determined at control and the lowest root dry weight at the highest salinity level. Akram *et al.* (2007) reported that root dry weight of all corn hybrids showed a decline towards increase in salinity level.

The present study showed high genetic variability in salinity tolerance with respect to different seedling traits (shoot length, root length, fresh and dry weight in different experiments at different levels of salinity). Consequently, these traits would be very useful in salinity tolerance improvement programs, especially shoot fresh weight which has shown a major growth reduction compared to the controls. The of the present study are in agreement with the results achieved by Giaveno *et al.* (2007) in screening tropical maize for salt tolerance. The same were published by Prajuabmon *et al.* (2009) who reported that all three cultivars of rice seedlings grown under high salinity had shoot length, fresh and dry weight of shoot and relative growth rate of shoot decreased. The results are similar to those reported by Ghoulam & Fares (2001) and Salim (1991).

***Effects of salt stress on nutrient uptake***

It is well established fact that Na<sup>+</sup> is a toxic element whose higher concentration disturbs the different metabolic activities (Akram *et al.*, 2007). It is also reported that salt tolerance is associated with K<sup>+</sup> contents (Ashraf & Sarwar, 2002), because of its involvement in osmotic regulation and competition with Na<sup>+</sup> (Ashraf *et al.*, 2005). Regulation of K<sup>+</sup> uptake and prevention of Na<sup>+</sup> entry, efflux of Na<sup>+</sup> from cell are the strategies commonly used by plants to maintain desirable K<sup>+</sup>/Na<sup>+</sup> ratio in the cytosole. In the present study, the tolerant genotypes are expressing the same trend for K<sup>+</sup>/Na<sup>+</sup> ratios. K<sup>+</sup>/Na<sup>+</sup> ratio is the criteria which is established by the scientist and the genetically approved for salt tolerance. So the varieties maintaining higher K<sup>+</sup>/Na<sup>+</sup> ratio are the salt tolerant. Increasing the salt concentration in the root medium in both solution-culture and vermiculite-culture

method genotypes decreased the K<sup>+</sup>/Na<sup>+</sup> ratio (Figure 3). Accumulation of Na<sup>+</sup> and impairment of K<sup>+</sup> nutrition is a major characteristic of salt-stressed plants. Therefore, many reports have alluded to the beneficial effects of high K<sup>+</sup>/Na<sup>+</sup> ratios with regard to salt tolerance (Shabala *et al.*, 2010). Selection or breeding genotypes with high K<sup>+</sup>/Na<sup>+</sup> ratios is an important strategy to minimize growth decreases in saline soils (Santa-Maria & Epstein, 2001).

***Evaluation methods for salt screening***

For practical purposes, a good mass screening method should be efficient, reproducible, reliable and simple and for this purpose selection of a suitable criterion for assessing the relative salt tolerance in various methods is important (Mahmood, 2009). In the present study, two screening methods were tested to determine the consistency in response of various genotypes grown in two salt levels and in control. The genotypes showed similar response to salt in both methods used in the study which indicates that any of the techniques is effective for assessment of salt tolerance. The hydroponic and soil pots have already been used in many studies (Aslam *et al.*, 1993; Munns & James, 2003). However, for the determination of salt tolerance of maize lines the use of vermiculite-culture method is reported first time. As compared to solution-culture method, vermiculite-culture method is easy, simple and economical. Vermiculite-culture method is more comparable with screening in saline soil conditions than that of hydroponic as solid media is provided to roots. Similar results were found by Mahmood (2009) using vermiculite-filled Japanese paper pots for screening wheat plants at early stage of growth for salinity tolerance.

From the results it can be concluded that screening at the early stage of plant growth in vermiculite-culture method is a convenient and fairly reliable technique for determining differences with respect to salt tolerance of maize genotypes. And evaluation of genotypes can be done through laboratory experiments using accumulation of plant biomass under saline conditions. It is suggested that the evaluation and selection of pipe-line genotypes could offer great scope for the combination of high yield and stress resistance (salinity/drought). Our results provide guidelines for the selection of salt tolerant maize genotypes and this information is relevant and very important to breeders and plant physiologists interested in improving salt tolerance of maize.

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