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Full Length Research Paper

Effects of salt stress levels on five maize (*Zea mays* L.) cultivars at germination stage

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To investigation the effects of salt stress levels (0, 50, 100, 150, 200 and 250 mM NaCl) on five maize (*Zea mays* L.) cultivars at germination stage, this study was performed at Ardabil, Iran in 2011. The results showed that in all cultivars, as the salt concentration increased, both germination percentage and germination index decreased significantly. Increasing salt concentration affected the early seedling growth in all cultivars. There were differences in responses of cultivars to salt stress. For all salt concentrations, Golden west had the highest germination percentage and germination index. Salt concentration decreased shoot and root dry weight. BC678 and Golden west had the lowest reduction of shoot and root dry weight, respectively. OS 499 had the highest reduction of shoot and root dry weight in this study; Golden west showed better results than the other cultivars in respect to salt tolerance index. The results show that Golden west was the cultivar to be recommended for saline soils. OS499 was more sensitive to salinity in this study.

Key words: Corn, germination, salt stress, NaCl, germination index.

INTRODUCTION

Corn (*Zea mays* L.), is one of the most important cereal crops growing in the world. It is used as food for human consumption as well as food grain for animals (Moussa, 2001). The rapid increase in the world population demands an expansion of crop areas to raise food production. In this context, a significant fraction of agricultural crops are cultivated on low quality soils, sometimes affected by salinity (Allen et al., 1983). According to Steppuhn and Wall (1999), salinity could be defined as a water property that indicates the concentration of dissolved solutes. Soil salinity refers to the state in which dissolved constituents concentrate beyond the needs of plant roots. It is well-known that salinity is a common stress factor in agricultural areas as a result of extensive irrigation with saline water and fertilizer application (McKersie and Leshem, 1994).

Seed germination is a major factor limiting the establishment of plants under saline conditions. Salinity may cause significant reductions in the rate and percentage of germination, which in turn may lead to uneven stand establishment and reduced crop yields (Foolad et al., 1999). Salt tolerance at germination stage is an important factor, where soil salinity is mostly dominated at surface layer. High concentration of salts has detrimental effects on germination of seeds (Kayani and Rahman, 1987; Rahman et al., 2000; Sharma et al., 2004; Saboora and Kiarostami, 2006). Plant growth is ultimately reduced by salinity stress but plant species differ in their sensitivity or tolerance to salts (Torech and Thompson, 1993; Munns and Termaat, 1986; Rogers et al., 1995).

Maize, which belongs to the plants with C4 metabolism, is also classified as moderately sensitive to salinity (Mass and Hofffman, 1977; Ouda et al., 2008). For maize grown under salinity, reduction in growth characters and yield were observed (Ouda et al., 2008). As suggested by Souza and Cardoso (2000), a marked increase of germination inhibition is expected at higher NaCl concentrations in the substrate. In general, salt stress is directly related with drought stress due to the capacity of the dissolved solutes to retain water. However, two different mechanisms of salt tolerance enable seeds to germinate at high salt concentrations. Seeds can tolerate the effects of a lower water potential in the substrate (Allen et al., 1983) or they may present specific tolerance to the inhibitory effect of NaCl (Rumbaugh et al., 1993).

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Table 1. Analysis of variance of the traits determined.

SOV	df	MS				
		Germination percentage	**Germination index**	**Shoot dry weight**	**Root dry weight**	**Stress tolerance index**
Salt (S)	4	***	**	**	**	**
Cultivars (C)	5	***	*	**	**	**
C x S	20	***	**	**	*	**
CV (%)	-	12.89	18.62	7.98	11.89	9.75

*, ** and *** significant at the 0.05 and 0.01 and 0.001 levels, respectively.

In some cases, germination inhibition of *Eucaliptus grandis* was higher in NaCl than in PEG 6000 solutions at equal osmotic potential (Souza and Cardoso, 2000). Germination inhibition may be equivalent in species that exhibit a mechanism of Na exclusion or of Na shoot accumulation. In maize, differences in Na shoot accumulation were observed among genotypes and the lack of correlation between this trait and salt tolerance was reported (Wang et al., 2003). According to Amzallag et al. (1993), sorghum plants have the capacity to broaden salt tolerance after previous exposure to sublethal NaCl concentrations. This adaptation period induced the capacity to grow at lethal concentrations.

This study was performed to determine the five maize cultivars tolerance to salt stress which can be grown on cultivated lands having salt problems in Iran.

MATERIALS AND METHODS

This study was a laboratory-conducted experiment and carried out in Ardabil region in Iran, 2011. Five cultivars (BC404, BC678, Golden west, Single cross 647 and OS499) of maize (*Zea mays* L.) which were tolerant to drought stress in field were used. The seeds were surface sterilized by dipping the seeds in 1% mercuric chloride solution for 2 min and rinsed thoroughly with sterile distilled water. There were five salinity treatments having osmotic potential: 0 (control), -2, -4, -8 and -10 bars. These treatments were prepared by dissolving separately calculated amounts of NaCl in deionizer water. All the experiments were conducted in 9 cm Petri plate on filter paper beds in growth chambers. 20 seeds were sown in 9 cm diameter Petri plate on filter paper beds, irrigated with 5 ml solution of respective treatment and incubated at 25°C. Each treatment had three replications. The filter paper beds were irrigated daily with 5 ml solution of the respective treatment. The filter beds were changed after 48 h in order to avoid salt accumulation.

Germination percentage

The emergence of plumose was taken as index of germination. Initiation and completion of germination was recorded daily. The germination percentage was recorded daily for 10 days and germination percentage was calculated with the following formula: Germination percentage (%) = Number of germinated seeds / number of total seeds x 100

Germination index

This parameter was calculated using the following formula:

Germination index = (Gt/Tt)

Where, Gt is the number of seeds germinated on tth day and Tt is the number of days up to tth day (Atik et al., 2007).

Seedling growth

After 10 days, the seedlings were harvested and the following observations were made: Root/shoot length; root/shoot biomass.

Salt tolerance

Salt tolerance was calculated using the following formula:

$$\text{Salt tolerance} = \frac{\text{Germination/growth in a particular treatment}}{\text{Germination/ growth in the control}} \times 100$$

The experiment was conducted by using randomized complete block design with four replications. The data of germination percentage and stress tolerant index were transformed using arcsine values prior to statistical analysis. Data were subjected to statistical analysis using ANOVA, a statistical package available from Spss16. Significant differences between treatments were determined using LSD test at the 0.05 level.

RESULTS

The results showed that the measured components of maize cultivars were significantly affected by salt concentrations (Table 1). According to the results, germination percent was significant in 0.001 percentages for salt levels, cultivars and interaction between salt levels and cultivars.

At different salt concentrations, Golden west and Bc678 had the highest and lowest germination percentage as 97.15 and 79.82%, respectively. Single cross 647 had the second highest germination percentage (92.43%). Golden west demonstrated better tolerance to salt stress

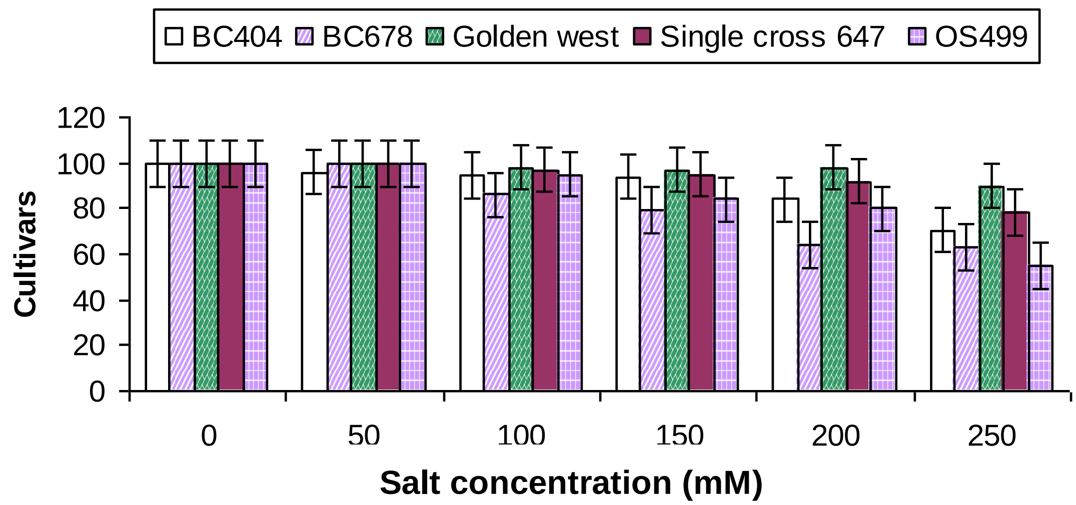

Figure 1. Effects of different salinity levels on germination percentage (%).

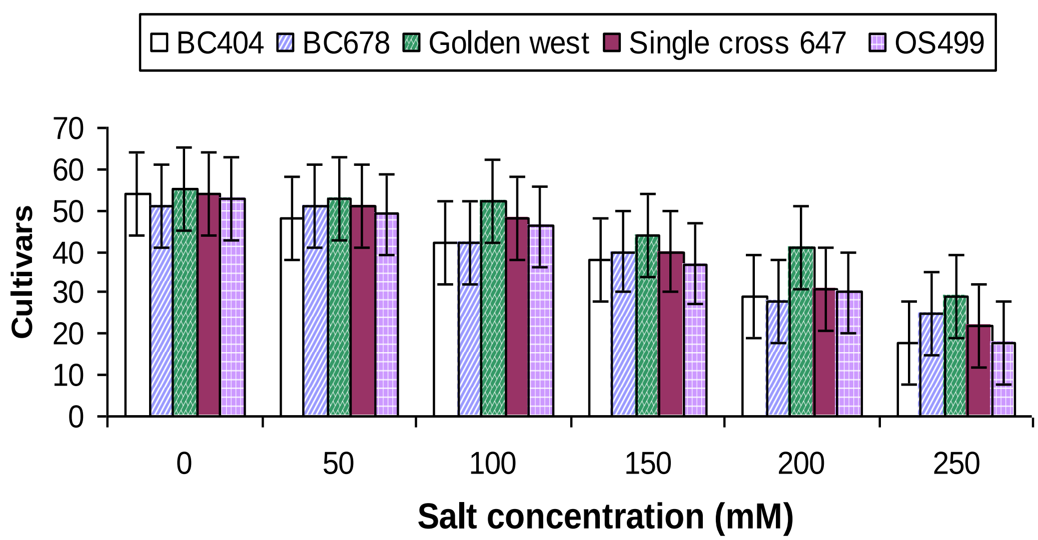

Figure 2. Effects of different salinity levels on germination index.

than other cultivars for germination percentage (Figure 1).

Cultivars had different responses in germination index at different levels of NaCl and cultivar Golden west had the highest germination index (46.89-mean). Germination indices of all the cultivars decreased with increasing salt stress. Mean germination index of cultivars was 53.42 in the control (0 mM NaCl), while it decreased linearly to 21.43 at 250 mM NaCl (Figure 2). Generally, increasing salinity levels decreased germination index in all of the cultivars, but Golden west was less damaged and its germination index was greater than that of the other cultivars at 250 mM NaCl (Figure 2).

Shoot dry weights of cultivars were negatively affected by increasing salt treatments. The average shoot dry weight of cultivars was 24.86 mg $plant^{-1}$ in the control and this value gradually decreased throughout the increasing salt concentrations, and reached 6.87 mg $plant^{-1}$ at 250 mM NaCl. The reduction rate in shoot dry weights of cultivars at 250 mM NaCl when compared with the control were detected in BC404 as 75.43%, BC678 as 41.83%, Golden west as 68.79%, Single cross 647 as 77.82% and OS499 as 94.68% (Figure 3).

Root dry weight of cultivars decreased significantly as the levels of salinity increased from 0 to 250 mM NaCl. Thus, the highest root dry weight was found in the control and the lowest root dry weight at the highest salinity level. Among the cultivars, Golden west and BC678 were affected least by salinity. The rate of reduction in root dry weight at 250 mM NaCl in comparison with the control

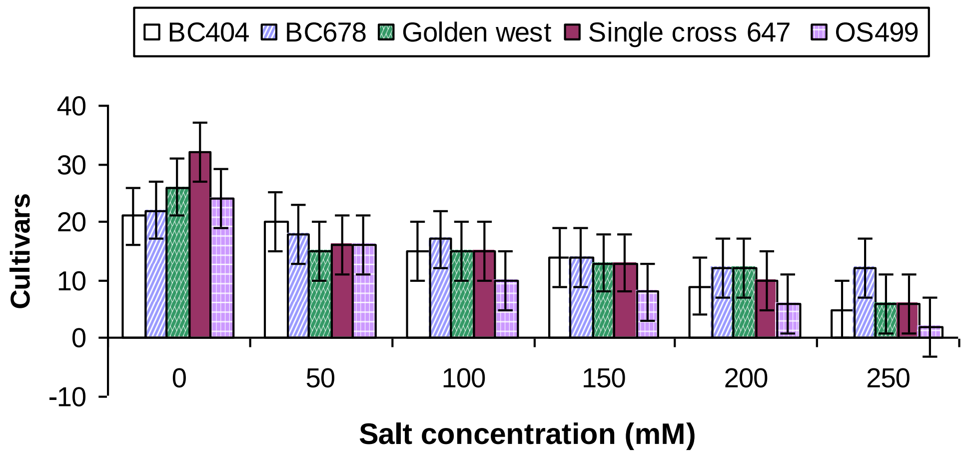

Figure 3. Effects of different salinity levels on shoot dry weight.

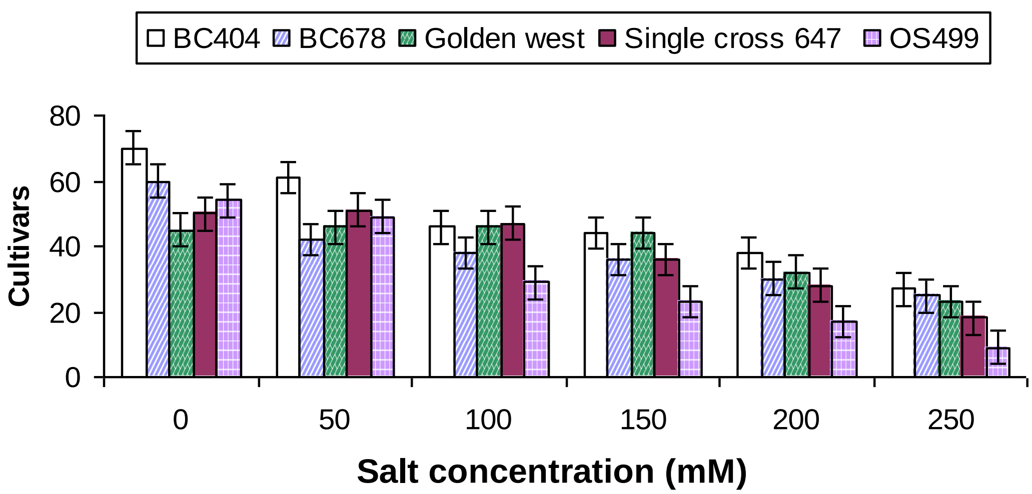

Figure 4. Effects of different salinity levels on root dry weight.

was detected in BC404 as 62.73%, BC678 as 55.32%, Golden west as 45.32%, Single cross 647 as 65.72% and OS499 as 79.82% (Figure 4).

Seedling growth was recorded in terms of shoot/root length and shoot/root biomass at different levels of NaCl salinity. The increase in NaCl concentrations decreased the shoot and root length and biomass of all the corn cultivars. All cultivars responded in same manner to salinity stress. However, the intensity of stress varied with the cultivars. It had been observed that those cultivars that responded poorly at germination stage showed better response at seedling stage. The reduction in shoot growth was greater than that of root growth. The reduction in biomass production was also greater in cultivar having higher germination rates. Maximum decrease in root and shoot length at 250 mM NaCl was recorded in cultivar OS499 which were 75 and 77%, respectively. Salinity reduced the biomass (weight) in the range of 52 to 79% in the root and 61 to 89% in the shoot of different cultivars.

DISCUSSION

Salinity affects germination in two ways: 1) enough salt in the medium may decrease the osmotic potential to such a point which retard or prevent the uptake of water necessary for mobilization of nutrient required for germination (Figure 1) and 2) the salt constituents or ions may be toxic to the embryo.

Our results are in line with the findings of Rahman et al. (2000), in which germination was directly related to the amount of water absorbed and delay in germination due to the salt concentration of the medium. Decrease and

Table 2. Correlation between salt tolerance at germination stage and during later phases of growth.

	Salt tolerance	Germination stage	Later phases of growth
Salt tolerance	1		
Germination stage	0.726**	1	
Later phases of growth	0.568*	0.324	1

delay in germination in saline medium was also reported by Rahman et al. (2000) and Mirza and Mahmood (1986). Rahman et al. (2000) reported that maize cultivars were significantly more tolerant to salt stress at germi-nation than at later stages of growth. Seeds in the control dishes (0 mM NaCl) had the highest germination percentage (100%), and as the salt concentration increased, germination percentage decreased up to 250 mM NaCl concentration (Figure 1). The higher germi-nation percentage of cultivars in the control (0 mM NaCl) was due to lack of salt in the medium. High concentration of NaCl in the salt solution increases its osmotic potential. In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage (Taiz and Zeiger, 2002). In this study, the responses of cultivars to different salt concentrations were found to be significantly different. This condition caused significant interactions between salt treatments and cultivars. This means that there are genetical differences among cultivars with respect to tolerance to salt stress. However, increasing salinity decreased the germination percentage in all cultivars; some of the cultivars were more tolerant than the others. As a result of this fact, the germination percentages of cultivars at 250 mM NaCl were arranged in a gradual decreasing order as: Golden west > Single cross 647 > BC404 > BC678 > OS499, when they were compared with the control (Figure 1). Our results are supported by many researches conducted on this subject (Rahman et al., 2000; Gill et al., 2002; Almodares et al., 2007; Blanco et al., 2007).

According to Figure 3, the cultivars were arranged as follows: OS499 > Single cross647 > BC404 > Golden west > BC678. Our results are in agreement with the results of other researchers. For example, Hussein 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^{-1} in the control, while it decreased linearly to 25.26 mg plant^{-1} at 4000 ppm. The same results were also obtained by other researchers (Mansour at al., 2005). Akram et al. (2007) reported that root dry weight of all corn hybrids showed a decline towards increase in salinity level. On the other hand, reduction in plant growth as a result of salt stress has also been reported in several other plant species (Ashraf and O'leary, 1997).

Salinity affects the seedling growth of plants (Tezara et al., 2003) by slow or less mobilization of reserve foods, suspending the cell division, enlargement (Meiri and Poljakoff-Mayber, 1970) and injuring hypocotyls. Other researchers (Francois, 1994), demonstrated that plants exhibit different sensitivities to salinity at different stages of growth. Among the varieties tested, Leucurum cultivar appeared to be more sensitive at the germination stage than others. Although Leucurum cultivar had comparatively low germination at higher salinity levels, it performed quiet satisfactorily at seedling stage. Ayers and Hayward (1948) reported that there may not be a positive correlation between salt tolerance at germination stage and during later phases of growth as observed in this study (Table 2). Many plants are most sensitive to ion stress during germination (Catalan, 1994) or young seedling growth (Carvajal, 1998). Mahmood and Malik (1986) observed greater salt tolerance at growth than germination stage. It is clear from the results that behavior of cultivars varies both at germination and seedling growth stages. This shows that species/varieties can never be selected simply on the basis of higher germination percentage. According to Mass and Grieve (1990), the ability of seed to geminate and emerge in saline soil not only depends upon the concentration of salts, but also upon various other biological factors, that is, viability of seed, seed age, dormancy, seed coat permeability, internal inhibitors and genetic makeup. George and William (1964) have the opinion that greater tolerance to salinity during germination is associated with lower respiration rates and greater reserve of respiratory substances.

The salt tolerance index of cultivars at the germination stage showed a large genotypic variation. Golden west had the highest salt tolerance index, while OS499 had the lowest (Figure 5). Therefore, Golden west demonstrated a better tolerance to salt stress than other cultivars. The effects of different salt concentrations on salt tolerance indices of cultivars were of importance. As the salt concentrations increased, the salt tolerance indices of cultivars decreased. Therefore, the lowest value of salt tolerance index was determined at 250 mM NaCl (Figure 2).

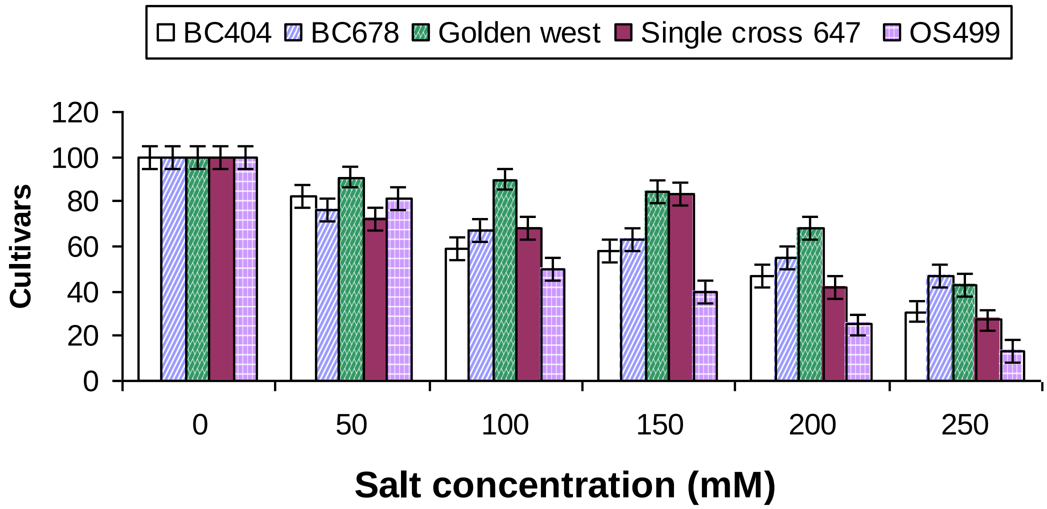

Figure 5. Effects of different salinity levels on salt tolerance index

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