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Full Length Research Paper

The effects of salt stress on the growth, biochemical parameter and mineral element content of some maize (*Zea mays* L.) cultivars

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Six cultivars of maize (*Zea mays* L.) (Ada-523, Bora, C-955, PR 3394, Progen-1550 and Trebbia) were subjected to 0 and 100 mM NaCl and their response to salt stress were determined by growths related to relative shoot growth weight (RSGR), shoot and root dry weight and stress tolerance index by biochemical parameters associated with total chlorophyll and proline contents and by mineral element contents such as Na^+ and K^+ contents and K^+\$Na^+$ ratio. Cultivars were grown in greenhouse in perlit supplied with a complete nutrient solution and salt treatment started 14 days after planting. The results indicate that salinity decreased RSGR, shoot and dry weight, stress tolerance index, total chlorophyll and K^+ contents and K^+\$Na^+$ ratio, but increased proline and Na^+ accumulations. Especially, proline accumulation appears to react to salt stress damage rather than a plant response associated with salt tolerance. Another striking point is that the rates of increase in Na^+ content were higher in shoots than in roots. According to the results, salt tolerance index, Na^+ and K^+ contents are reliable criteria for preliminary selection in early growth stage of maize.

Key words: Maize, salt stress, relative shoot growth rate, total chlorophyll, proline, Na^+.

INTRODUCTION

Salinity is one of the most important problems in irrigated soils of the arid and semi-arid areas in the world. Currently, there is about 275 million hectars of irrigated land of which about 20% is salt affected (Ghassemi et al., 1995). On the other hand, the ever growing world population causes great pressure on marginal lands to be brought into cultivation in the developing and under developing countries, which were previously not cropped due to their high degree of natural salinity (Flowers and Yeo, 1995). In Turkey, there are approximately 2 - 2.5 million hectars of arable land suffering from salinity problems (Kaya et al., 2003).

Salinity has three potential effects on plants: Lowering of water potential, specific ion toxicity (sodium and chloride) and interference with the uptake of essential nutrients. The latter may not be considered because it has no immediate effect due to mobile reserve nutrients present in plants (Flowers and Flowers, 2005). Two of the above reasons are important and have part in reduction of plant growth under salt stress. The first one is lowering of external water potential due to salt present outside the root. The second is the senescence of leaves due to the accumilation of ion in the older leaves; there is a true difference in salt tolerance appearance (Akram et al., 2007). Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and progressive death of plant (Munns, 2002). Ion inbalances due to ion accumilation caused by salt stress show their negative effects by reducing shoot and root growths and increasing some amino acids including proline. Proline is a very important indicator because it is osmotically very active and regulates the accumulation of useable nitrogen (N), contributes to membrane salinity and mitigates the effect of NaCl on cell membrane distruption (Ashraf et al., 2004). However, its role in salt tolerance of plants is not so clear. Some findings indicate that there is no healing effect of proline on salt stress (Lacerda et al., 2003) but others indicate clearly that proline enhances plants aganist salt stress (Ahmad et al., 1981; Chowdhury et al., 1993; Petrusa and Winicov, 1997;

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Taban et al., 1999). Beside these, there are findings indicating negative relation between proline and salt tolerant characters of plants (Lutts et al., 1996; Aziz et al., 1998; Lutts et al., 1999).

The mechanism of plant adaptation required to survive in saline conditions is the same in all the plants. However, adaptations are at their extreme in halophytes, but can be found at different degress in glycophytes (Flowers and Flowers, 2005). Variation in salt tolerance of glycophytes occurs between and within species and has been quantified for many crops (Mass and Hoffman, 1976; Francois and Mass, 1994; Flowers and Yeo, 1981). Quick screening procedure has been adopted by many researchers for different crops in their early growth phase (Ashraf et al., 2002; Eker et al., 2006; Khan et al., 2006).

After wheat and rice, maize (*Zea mays* L.) is the third most important cereal crop grown all over the world in a wide range of climatic condition. Maize, being highly cross pollinated, has become highly polymorphic through the course of natural and domesticated evolution and thus contains enormous variability (Paternian, 1990) in which salinity tolerance may exist.

Maize is considered as moderately salt sensitive (Mass and Hofffman, 1977; Katerji et al., 1994; Ouda et al., 2008; Carpici et al., 2009). Despite its high place among cereals, few findings have been obtained to improve salt tolerance in this crop. Improvement for salt tolerance would be of considerable value for this moderately sensitive crop when it is grown on irrigated areas with salt problems. Thus, the effective and accelarated improvement of salt tolerance cultivars through screening is urgently needed. With this urgency in mind, the present research was conducted to assess the extent of variability in salt tolerance in six maize cultivars and to determine the most tolerant cultivars.

For this purpose, relative shoot growth weight (RSGR), shoot and root dry weights, stress tolerance index, total chlorophyll, Na^+ and K^+ contents, K^+/Na^+ ratio and proline accumulation of six maize cultivars commonly grown as grain and silage were determined in plants grown under normal or salty conditions and these data were used in determining salt tolerance of cultivars.

MATERIALS AND METHODS

Plant materials and salt treatment

Six cultivars of maize (*Zea mays* L.) (Ada-523, Bora, C-955, PR3394, Progen-1550 and Trebbia) were used as plant entries in this study. Cultivars were grown in perlit-filled plastic pots of 5.5 L in the greenhouse of the Uludag University, Turkey, from May to August, 2006.

Seeds were graded and the big and uniform shaped seeds were used and their surface sterilized with 2% sodium hypochlorite for 10 min. After sterilization, seeds were washed with distilled water three times. Six seeds per pot were used. Open surface of pots was covered with aluminum foil to prevent growth of algae. After germination, aluminum foils were removed, the seedlings were thinned to three plant per pot. Pots were irrigated twice a day with nutrient solution. The nurtient solution was prepared according to Maas et al. (1986) and contained 2.5 mM $Ca(NO_3)_2$, 3.0 mM KH_2PO_4, 1.5 mM $MgSO_4$, 0.1 mM KNO_3, 0.1 mM Fe-EDTA, 0.023 mM H_3BO_3, 0.005 mM $CuSO_4$ and 0.01 mM $H_{24}Mo_7N_6O_{24}.4H_2O$.

Salt treatment was started 14 days after planting. Sodium chloride of 100 mM was added to the nutrient solution, and after salt addition, its electrical conductivity was 12.58 dS m^{-1}. The control pots were treated with NaCl-free nutrient solution, with an electrical conductivity of 1.37 dS m^{-1}. To avoid osmotic shock, saline treatment was imposed incrementally, increasing the concentration by 50 mM every 12 h until the final concentration of 100 mM was reached. Day and night temperatures were 35.8 and 19.7°C, respectively in the greenhouse.

RSGR and dry weights of shoot and root

To determine the dry weight of shoots and roots, plants were harvested at 1, 9, 17 and 25 days after salt application. Thus, obtained samples were dried at 78°C for 48 h and then weighed. Hereafter, the shoot samples obtained at 1, 9, 17 and 25 days after salt application were used for calculation of RSGR values. A formula for this calculation was used which was developed by Kingsbury et al. (1984). Samples obtained only 25 days after salt application were used to calculate the shoot and root dry weights.

Measurement of total chlorophyll, proline, Na^+ and K^+

Fully expanded leaves were sampled at 25 days after salt application for total chlorophyll and proline analyses. Fresh samples were used for these analyses. For total chlorophyll content analysis, each sample of 0.1 g was extracted with 10 ml of 80% (v/v) acetone and filtered. Then, absorbancies were determined with a spectrophotometer at 645 and 663 nm. Total chlorophyll content (mg g^{-1} FW) was estimated by the equations of Arnon (1949).

Extraction and determination of proline (µmol g^{-1} FW) was determined spectrophotometrically by an acid ninhydrin procedure (Bates et al., 1973). Leaf samples of 0.5 g were extracted with 3% sulphosalicylic acid. Extracts of 2 ml were held for 1 h in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene of 4 ml was added. Proline content was measured by a spectrophotometer at 520 nm and calculated as µmol g^{-1} FW aganist standard proline.

Shoot and root samples were wet digested by using the HNO_3 + $HClO_4$ (4:1) mixture. Na^+ and K^+ were determined by the flame emission (Horneck and Hanson, 1998). Then, K^+/Na^+ ratio was calculated.

Experimental design and statistical analysis

The experiment was conducted by a completely randomized design with three replications. Analysis of variance was performed by Minitab statistical program. Means were groupped by using the least significant difference (LSD) test at 5% level.

RESULTS AND DISCUSSION

Effect of salinity stress on plant growth

Variance analysis showed that RSGR values of cultivars were affected significantly by salt treatment at every week in which samples were taken ($P < 0.001$). Reduction in RSGR values of genotypes continiously

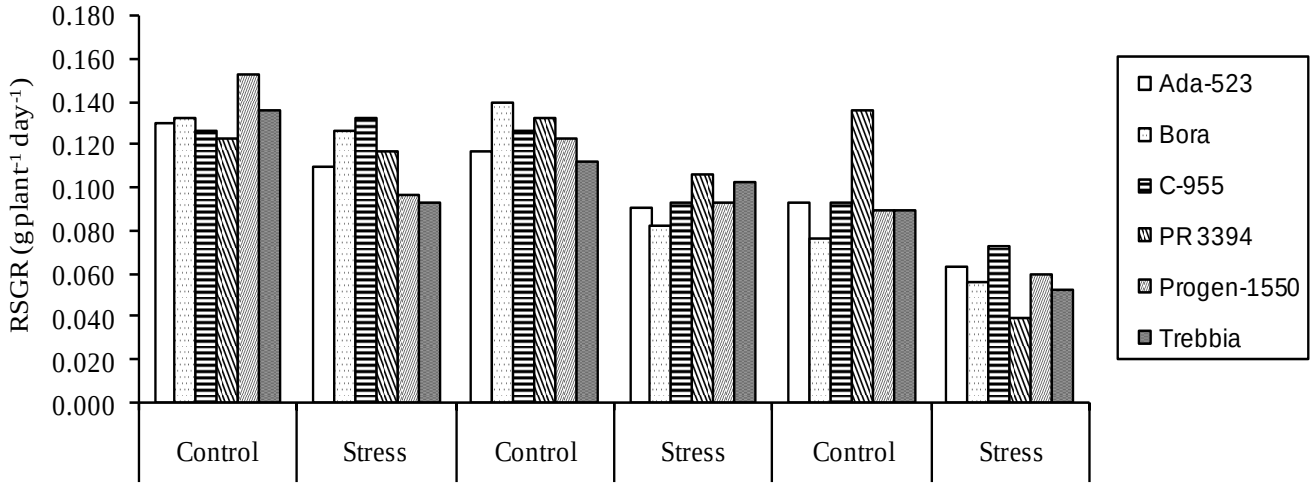

Figure 1. RSGR (g plant^{-1} day^{-1}) of the maize cultivars grown under control and salt stress conditions. RSGR was calculated for days after salt application 1 to 9 (Week 1), 9 to 17 (Week 2) and 17 to 25 (Week 3).

Table 1. Effect of salinity on shoot dry weight (g plant^{-1}), root dry weight (g plant^{-1}), salt tolerance index, total chlorophyll (mg g^{-1} FW) and proline (µmolg^{-1}FW) contents of different maize cultivars.

Cultivar	Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		Salt tolerance index (%)		Total chlorophyll (mg g^{-1} FW)		Proline (µmolg^{-1}FW)	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Ada-523	14.663b	7.415d	3.941ab	2.373c	100a	53cd	1.914ab	1.681ab	1.233ab	0.079d
Bora	12.636bc	7.005d	3.896ab	2.144c-e	100a	57c	2.012ab	1.551ab	0.146cd	0.757b-d
C-955	10.982b-d	9.298cd	4.221a	2.139c-e	100a	76b	2.093a	1.933ab	0.423b-d	0.863a-d
PR 3394	21.276a	7.500d	3.649b	1.622e	100a	38d	1.632ab	1.632ab	0.306cd	0.940a-d
Progen-1550	12.900bc	7.319d	4.005ab	1.776de	100a	54c	1.996ab	1.402b	0.317cd	1.706a
Trebbia	12.082bc	7.307d	3.768ab	2.227cd	100a	60c	1.535ab	1.553ab	0.202cd	1.012a-c
Mean (Salt)	14.090A	7.641B	3.913A	2.047B	100A	56B	1.863A	1.625B	0.438B	0.893A

Means followed by the same small letter and by the same capital letter for each components are not statistically different by LSD at 0.05 level.

increased with time. For instance, while at Week 1, the reduction of RSGR value was 15.67%, it was 24.60 and 40.21% at Week 2 and 3, respectively. Interaction effect of salt stress x cultivar was of significance ($P < 0.05$) only in Week 3. The significant interaction effect determined arose mostly from the different response of C-955, Bora and PR 3394 to salt stress. Indeed, the reduction in RSGR value of PR 3394 was very great and reached as high as 70.80%, while RSGR values of C-955 and Bora were rather small (Figure 1). These results indicate that the negative effect of salt stress on RSGR values increased as plants became older and varied with genotypes. Similar results were reported by Netondo et al. (2004).

Shoot development of each genotype was prevented by salt stress. However, prevention degree of shoot development due to salt stress changed with genotypes. The lost of shoot dry weight in C-955 was lower than those of other genotypes. Indeed, shoot dry weight of C-955 decreased only about 15.33%, while reduction percentages of other five genotypes were very high and ranged from 39.52 to 64.74% (Table 1). From these results, it may be expressed that the cultivar C-955 is more salt stress tolerant than the other cultivars. Similar results obtained from researches on maize were reported by other researchers (Hoffman et al., 1983; Zalba and Pienemann, 1998; Cicek and Cakirlar, 2002; Ashrafuzzaman et al., 2003; Neto et al., 2004).

Interaction of salt stress x cultivar was found insignificant, although the effect of salt stress on root growth was found significant (Table 1). However, the roots of all the cultivars were less affected by salinity than their shoots. Most researches on this subject yielded similar results (Lacerda et al., 2001; Neto et al., 2004; Eker et al., 2006; Akram et al., 2007). Under the light of these findings, it may be said that the use of the data of shoots as selection criteria obtained from plant breeding studies would be more realistic than the use of root data. Roots

Table 2. Effect of salinity on Na^+ (%), K^+ (%) and K^+/Na^+ ratio of shoot and root of different maize cultivars.

Cultivar	Shoot						Root					
	Na^+ (%)		K^+ (%)		K^+/Na^+		Na^+ (%)		K^+ (%)		K^+/Na^+	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Ada-523	0.043f	1.117a	2.707	2.517	66.37c	2.28d	0.557d	3.603b	1.220b	0.417d	2.20c	0.12e
Bora	0.043f	0.530e	2.220	2.027	53.91c	3.83d	0.533d	3.257c	1.680a	0.520d	3.15a	0.16e
C-955	0.033f	0.583de	2.730	2.500	85.82b	4.37d	0.507d	3.330bc	0.710c	0.367de	1.41d	0.11e
PR 3394	0.040f	0.977b	2.500	2.133	64.90c	2.18d	0.517d	3.970a	1.130b	0.487d	2.19c	0.12e
Progen-1550	0.020f	0.747c	2.510	2.130	125.78a	2.86d	0.477d	3.633b	1.223b	0.237e	2.59b	0.07e
Trebbia	0.020f	0.660cd	2.263	1.960	113.16a	2.97d	0.493d	3.406bc	1.077b	0.523d	2.16c	0.15e
Mean (Salt)	0.033B	0.769A	2.211B	2.488A	84.99A	3.08B	0.514B	3.533A	1.173A	0.425B	2.29A	0.12B

Means followed by the same small letter and by the same capital letter for each components are not statistically different by LSD at 0.05 level.

absorb ions including Na^+ and Cl^- and transfer them to tops of plants with less harms in root functions. Whereas, salt causes serious demages in biochemical functions of top of plant.

Salt tolerance ındex

The effect of salinity on salt tolerance indices of cultivars was significant (Table 1). The ranges of the salt tolerance indices of cultivars were very wide. Variations were between 38 and 76% in relation with salt stress. The salt tolerance index of C-955 was the highest and that of PR 3394 was the lowest. These striking results indicate that the salt tolerance index is a reliable criteria for preliminary selection in early growth stage of maize. Similar findings were reported by Bagci et al. (2003).

Salt stress-total chlorophyll relationship

Salinity caused decreases in total chlorophyll of all cultivars except PR 3394 and Trebbia (Table 1). The decrease is more appearent in sensitive genotypes than in tolerant ones. The reduction in total chlorophyll content is to be expected under stress conditions. Its stability depends on membrane stability, which under saline condition seldom remain intact (Khan et al., 2009). Similar results were also reported by Iqbal et al. (2006), Ashraf et al. (2005), Khan et al. (2009), Oncel and Keles (2002), Lacerda et al. (2003) and Almodares et al. (2008). Total chlorophyll of plants may be considered as an indicator in improwing new genotypes for salt stress depending on the present or other findings.

Salt stress-proline content relation

Salt stress in this study, increased proline content in all cultivars except that of Ada-523. However, there were great differences in the increased proline contents of cultivars. Great increases in proline contents were found as 418.49% in Bora, 438.17% in Progen-1550 and 400.99% in Trebbia. Less increases recorded in C-955 and PR 3394 were 104.01 and 207.18%, respectively (Table 1). Proline accumulation in response to environmental stresses has been considered by a number of authors as an adaptive trait concerned with stress tolerance, and it is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Larher et al., 1993). Its accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation. It is believed that the accumulation of proline, a compatible solute, may help to maintain the relatively high water content necessary for growth and cellular function. Further more, it was shown that the capability of a number of crop plants to accumulate proline in response to salt or other stresses was highly variable between or within species (Ashraf et al., 2004; Naqvi et al., 1994; Lutts et al., 1996; Aziz et al., 1998; Lutts et al., 1999).

Na^+ and K^+ concentration and K^+/Na^+ ratio

The earlier mentioned values were affected by salinity and genotypes. While Na^+ content increased, the content of K^+ and K^+/Na^+ ratio decreased by salinity (Table 2). The same studies were conducted and similar results were found by some other authors (Hu and Schmidhalter, 1997; Sagi et al., 1997; Bagcı et al., 2003; Beck et al., 2004; Netondo et al., 2004; Akram et al., 2007). Another striking point is that the rates of increase in Na^+ content were higher in shoots than in roots. However, in contrast to Na^+, K^+ content had a different response to salinity in shoot and root. On the other hand, there were no differences in K^+/Na^+ values of shoots and roots under salt stress. The response of cultivars to salinity were different in respect to shoot and root growth. In saline conditions, the increase of Na^+ uptake in shoots of Bora and C-955 were lower than in shoots of the other

cultivars. The lowest decreases of K^+ content in shoots of Ada-523, Bora and C-955 were recorded. Na^+ contents of roots in Bora, Ada-523 and C-955 indicated less increases than the other cultivars. C-955 lost less K^+ under salt stress than the other cultivars (Table 2). According to the results, the value of K^+, Na^+ and K^+/Na^+ ratio may be more reliable as a selection criteria when they are determined in shoots than in roots.

In conclusion, cultivars with lower reductions in RSGR, total chlorophyll, K^+ content and K^+/Na^+ ratio are resistant to salt stress. In this study, the cultivar C-955 which has these specifications was more tolerant to salt stress than the other cultivars.

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