Bulg. J. Plant Physiol., 2002, 28(1–2), 66–74

THE EFFECT OF SALINITY ON SOME PHYSIOLOGICAL PARAMETERS IN TWO MAIZE CULTIVARS

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Received January 22, 2002

Summary. In this study, effect of salinity with different osmotic potential on shoot length, total fresh and dry weight, amounts of organic (proline) and inorganic (K^+ and Na^+) substance of leaf tissue, the Na^+/K^+ ratio, and leaf area, relative water content (RWC) and leaf osmolality in two maize (*Zea mays* L., var. *intendata*, C.6127 and DK.623) cultivars which are grown as a second yield in the Southeastern Anatolia Region (SAR) of Turkey, were investigated. Plants were grown for 30 days in the controlled growth room. Salinized culture solutions at different osmotic potential (0, -0.1, -0.3 and -0.5 MPa) prepared by adding varying amounts of NaCl and $CaCl_2$ to the main culture solution were applied to plants from the beginning of the germination. As a result the shoot length, total fresh and dry weight and the leaf area decreased, amounts of proline Na^+, Na^+/K^+ ratio and the leaf osmolality increased, but amounts of K^+ did not change significantly with increasing stress, and salt stress caused a similar decrease in leaf relative water content in both maize cultivars.

Key Words: Ions, leaf osmolality, maize, proline, RWC, salinity

Abbreviations: RWC – relative water content; SAR –Southeastern Anatolia Region

Introduction

Salinity is one of the serious environmental problem that cause osmotic stress and reduction in plant growth and crop productivity in irrigated areas of arid and semiarid regions. After finishing Southeastern Anatolia Project (SAP) which is the most impor-

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tant irrigation project in Turkey, 150 000 ha of this region can be irrigated. If efficient methods of irrigation and drainage are not applied, it is thought that these agricultural areas will be affected by salt. Salinity is a significant limiting factor in the agricultural productivity (Hasegawa et al., 1986). The agricultural areas affected by salt need amendment and determination of the most suitable plant species able to grow in these areas.

Tolerances to environmental stresses as salinity of plants can be determined by using different parameters. Plants need to have special mechanisms for adjusting internal osmotic conditions and changing of osmotic pressure in the root environment. Stressed plants diminish osmotic potential by accumulating free amino acids, ions and dissolvable substances. In this way, osmotic adjustment is ensured (Salama et al., 1994; Weimberg, 1986, 1987). Measurement of proline accumulation is also an important criterion for determination of plant tolerance to salt stress (Palfi and Juhasz, 1971). In salt stressed plants osmotic potential of vacuole decreased by proline accumulation (Yoshiba et al., 1997). It was thought that accumulated proline under environmental stress do not inhibit biochemical reactions and plays a role as an osmoprotectant during osmotic stress (Yoshiba et al., 1997). In addition, several possible roles have been attributed to supraoptimal levels of proline; osmoregulation under drought and salinity conditions, stabilization of proteins, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period (Aloni and Rosenshtein, 1984). It is suggested that the low osmotic potential may cause proline accumulation in tissues (Buhl and Stewart, 1983; Sing et al., 1973).

In present study, effect of salinity at different osmotic potential on shoot length, total fresh and dry weight, amounts of proline, K^+ and Na^+ in leaf tissue, Na^+/K^+ ratio, leaf area, relative water content and leaf osmolality in two maize cultivars grown as a second yield in the SAP, were investigated.

Material and methods

In this study, the registered maize (*Zea mays* L. var. *indentata*, C.6127 and DK.623) cultivars which are grown as a second yield in the Southeastern Anatolia Region (SAP) (Ülger et al., 1992) obtained from the Sapeksa and Dako Company were used. The salt levels and germination of cultivars were investigated in preliminary experimental work.

The selected seeds were surface sterilized in 1% sodium hypochlorite solution for 8 min before experimentation (Ashraf and McNeilly, 1990). Then seeds were washed with distilled water three times, and were wetted with culture solutions with different osmotic potential for 12 hours so that they could germinate quickly. In experiment ½ strenght Hoagland's solution was used as a main culture solution. Salinized culture solutions at different osmotic potential were prepared by adding a mixture of NaCl and $CaCl_2$ in a 2:1 molar ratio to the main culture solution (Weimberg, 1987).

Five seeds were planted in each pot containing perlit. A completely randomized block design was used with six replicates. The experiment was carried out in controlled environment room at 25±1°C, with 16 h daylength and with a relative humidity of 60%±5. All conditions were maintained constant during the growth period. Plants were irrigated regularly every two days with culture solutions during the experiment (30 days).

After 30 days, plants were harvested. The data for shoot length and fresh weight were recorded. Samples were then dried at 80°C for 2 days and their dry weight was recorded. To determine free proline in dried leaf powder, extracts were prepared by modifying the method of Weimberg (1987). Chemical method was used to detect proline in the extracts (Bates et al., 1973). For the analysis of K^+ and Na^+ in dried leaf powder, leaves were digested in nitric acid/perchloric acid (4:1), and two cations in the extract were assyed by atomic absorption spectrophotometry (Varian Spectr. AA. 5). Measurement of leaf area was made in the forth leaves according to Zhang and Brandle (1997). Leaf relative water content (RWC) was measured by the method of Conroy et al. (1988). For the determining of leaf osmolality, extracts were prepared from 3rd leaves by using Jones and Turner's (1978) method. The osmolality was determined by using a cryoscopic osmometer (Gonotec Osmomat 030 Cryoscopic Osmometer).

Statistical analyses of data were performed using Statistica Programme. Duncan's Multiple Range Test was used to determine significant differences of means at a 5% level.

Results

Data obtained from preliminary experiments were given on Table 1. Salt treatments did not affect germination significantly in both cultivars. When coleoptile was affected by salinity, the difference between cultivars was not significant. However, the difference of radicle length in both treatment levels and cultivars was significant (Table 1).

Table 1. Effect of salinity on germination of maize. All means followed by the same letter are not significantly different at the 5% probability level.

	Germination (%)		Coleoptile (mm)		Radicle length (mm)	
Cultivars	C.6127	DK.623	C.6127	DK.623	C.6127	DK.623
Osmotic Potential (MPa)						
Control	A* 82.67 a**	A 69.88 b	A 38.53 a	A 39.02 a	A 96.87 a	A 77.93 b
-0.2	A 76.31 a	A 69.57 a	A 33.90 a	A 35.61 a	A 101.82 a	A 73.29 b
-0.5	A 81.53 a	A 65.91 b	B 16.90 a	B 18.35 a	B 75.93 a	B 50.01 b
-0.8	A 76.24 a	A 67.65 a	C 5.42 a	C 2.21 a	C 45.14 a	C 25.46 b

* Values followed by different capital letter in column represent difference of treatment levels within cultivar.

** Values followed by different letter in line represent difference among cultivars.

Effect of salt treatments on shoot length of C.6127 and DK.623 are shown in Fig. 1a. In both maize cultivars, shoot length decreased significantly by increasing salt treatment levels, –0.3 and –0.5 MPa. It seems that salinity affected more significantly the shoot length of C.6127 than that of DK.623, except at 0.5 MPa. Salt stress resulted in a decrease in total fresh and dry weights of 30 day-old plants (Fig. 1b and c). In all culture solutions, was found that DK.623 had more fresh and dry weight than C.6127. Leaf area was reduced by salinity in both maize cultivars (Fig. 1d), but observed decrease was less in DK.623.

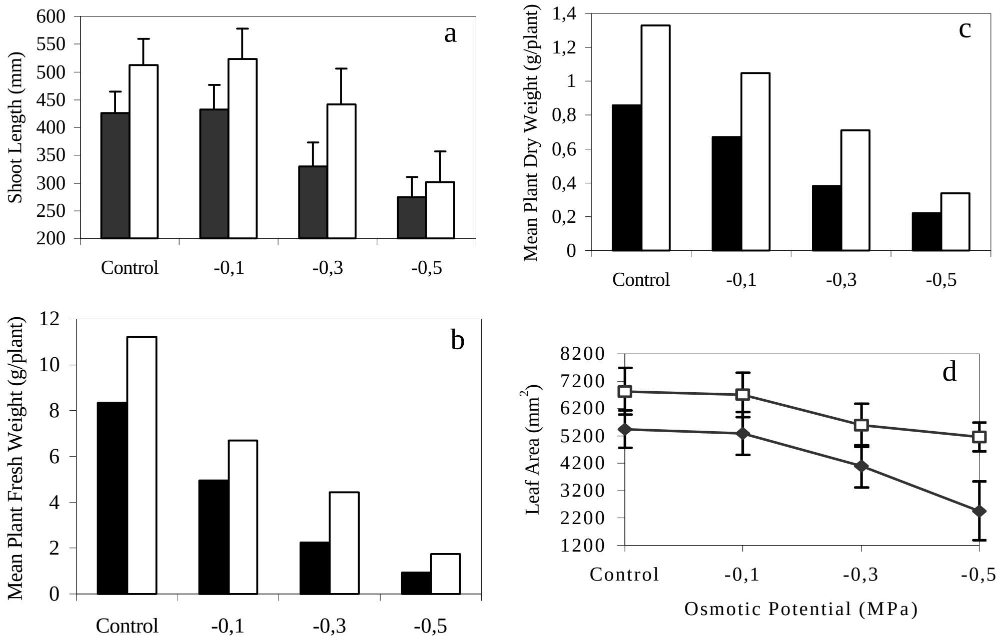


Figure 1. Effects of salinity on the shoot length (a), fresh (b) and dry weight (c), and leaf area (d) of 30-day-old two maize cultivars. (%) C.6127 and (%) DK.623.

Two cultivars showed differences in their amounts of proline in leaf tissue under salt stress (Fig. 2a). In C.6127, proline increased significantly under the increasing levels of salt treatment, whereas in DK.623 it increased only at the –0.5 MPa salt stress.

Salt treatment affected also amounts of K^+, Na^+, and the Na^+/K^+ ratio of both maize cultivars (Fig. 2b, c and d). Differences in amounts of K^+ and Na^+ are significant between both cultivars. There was not a significant difference in K^+ at the all culture solutions, except in DK.623 at the –0.3 MPa (Fig. 2b). However, amount of Na^+ increased markedly in response to increasing stress levels. This increase was more significant in C.6127 than in DK.623, except at the –0.5 MPa (Fig. 2c). Besides increased amounts of Na^+, the Na^+/K^+ ratio rose significantly by increasing stress in both cultivars. This increase was less in DK.623 than in C.6127 (Fig. 2d).

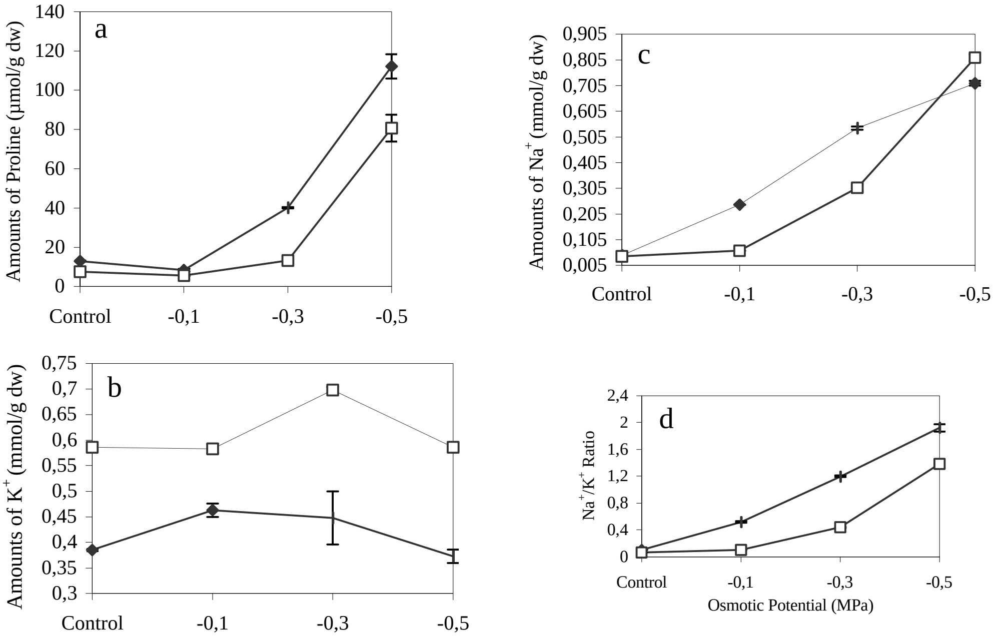


Figure 2. Effects of salinity on the amounts of proline (a), K$^+$ (b), Na$^+$ (c), and Na$^+$/K$^+$ ratio (d). (%) C.6127 and (%) DK.623.

Both cultivars showed decreased RWC after salt stress. But, this reduction was visible only at the –0.5 MPa (Fig. 3). Leaf osmolality was increased by salt stress (Fig. 4). The difference between cultivars was not significant, except at the –0.3 MPa. However, leaf osmolality was higher in C.6127 than in DK.623 at the –0.3 MPa salt stress.

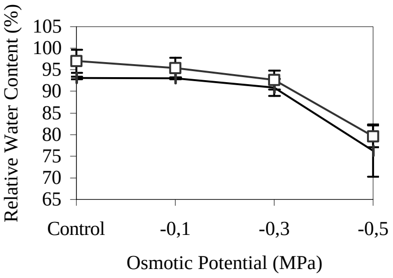
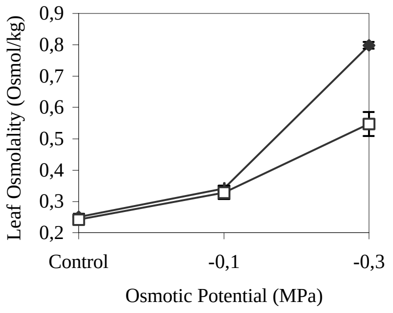


Figure 3. Effect of salinity on RWC. (%) C.6127 and (%) DK.623. n=30.



Figures 4. Effect of salinity on leaf osmolality. (%) C.6127 and (%) DK.623.

Discussion

Salt treatment affects differently early growth stages of plants. In the preliminary experimental work, effect of salinity on germination was of little importance, but effect on development of coleoptile and radicle was considerable, especially on radicle. It is supposed that seeds could germinate at several salt levels, but they could not continue their development. It might be sugested that determining salt tolerant cultivar is insufficient during growth period for 5 days.

Salinity has both osmotic and spesific ion effects on plant growth (Dionisio-Sese and Tobita, 2000). In present study, salt stress caused a significant decrease in shoot length, fresh and dry weights of shoot and leaf area of both cultivars with the increase of stress treatments (Fig. 1a, b, c and d). Reduction in plant growth as a result of salt stress has also been reported in several other plant species (Ashraf and McNeilly, 1990; Mishra et al., 1991; Ashraf and O'leary, 1997). Growth of leaf area is inhibited by salinity (Brungnoli and Lauteri, 1991; Alberico and Cramer, 1993). Richardson and McCree (1985) believe that the greater ability of salinized plants to continue leaf expansion, and carbon gain under water stress can be attributed primarily to a slower development of water stress, which prolonged the osmotic adjustment. But, it was thought that leaf area was not reliable indicators of salt tolerance (Alberico and Cramer, 1993). Probably the negative effect of salinity on plants provoked osmotic potential by salt in the culture medium, so root cells do not obtain required water from medium. Therefore in plants the uptake of some mineral nutrients dissolved in water are also restricted. Thus, growth and development of plants are inhibited due to occurring defect in metabolism. Some investigators thought that because of ion accumulation by changing membrane permeability, metabolism was negatively influenced (Cramer et al., 1985; Grieve and Fujiyama, 1987).

Many plants accumulate proline as a non-toxic and protective osmolyte under saline or other some stress conditions. 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 (Mattioni et al., 1997). When most plants including halophytes are faced with environmental stresses like drought and salt stress, they accumulate low molecular weights of organic substances as proline (Çakirlar et al., 1986; Tipirdamaz and Çakirlar, 1990; Yoshiba et al., 1997). The accumulation of compatible solutes may help to maintain the relatively high water content necessary for growth and cellular function. We found that salt stress at different osmotic potential increased the amounts of proline of leaf tissues in both maize cultivars (Fig. 2a). But, its accumulation was fast in C.6127. This might indicate that effect of salinity was serious in the same cultivar. Thus, C.6127 responsed to salinity fast, whereas the response in DK.623 gradually increased. At the beginning, salt treatments might not provoke an osmotic adjustment. It was shown that the capability of a number of crop plants to accumulate proline in response to environmental stresses was highly variable from one species to another and even between some varieties of crop plants (Quarrie, 1980).

Most crop plants suffer after exposure to saline conditions and showed decline in growth. The deleterious effect of salinity was suggested as a result of water stress, ion toxicities, ion imbalance, or combination of all these factors (Kurt et al., 1986). In present study, it has been noted that amounts of Na^+ increased in both cultivars, whereas those of K^+ did not change regularly (Fig. 2b and c). According to Weimberg (1987), high levels of Na^+ inhibit the K^+ uptake and as a result of this it causes an increase in the Na^+/K^+ ratio. The increase in the Na^+/K^+ ratio in our study may be attributed to the fact that Na^+ causes a disturbance in the ion balance in plant by an increase in the Na^+ uptake (Fig. 2d). Many of the deleterious effects of Na^+ seem to be related to the structural and functional integrity of membranes (Kurt et al., 1986). It is suggested that the capacity of ion accumulation of plants is related to their tolerance to salt stress. It was found that tolerant species accumulated lower Na^+, and decreasing of K^+ was lower than sensitive species (Weimberg, 1986, 1987; Hagibagheri et al., 1989; Tipirdamaz and Çakirlar, 1989).

We found that salt stress also affected RWC and leaf osmolality (Fig. 3 and 4). Many important physiological and morphological processes, such as leaf enlargement, stomatal opening, and associated leaf photosynthesis are directly affected by the reduction of leaf turgor potential which accompanies the loss of water from leaf tissue (Jones and Turner, 1978). They reported that although RWC was decreased, leaf osmolality increased, the slow development of water deficits resulted not only in osmotic adjustment, but also a decrease in leaf tissue elasticity. Similar trends could be seen in the results of other authors (Tipirdamaz and Çakirlar, 1990; Alberico and Cramer, 1993; Shalaby et al., 1993). Hamada et al. (1992) supposed that an increase of ion concentration in tissues caused an increase in osmolality. Thus, shoot and root cell-sap osmolality increased by increase in salinity concentrations, reflecting enhanced ion concentration in the tissues.

In the light of the findings of this study, it could be said that the DK.623 cultivar is more salt tolerant than the C.6127 cultivar. However, further studies by using new techniques should be carried out to reach at more certain realistic results.

Acknowledgement: This research was supported by The Scientific and Technical Research Council of Turkey (TOGTAG-1741).

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