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Full Length Research Paper

Effects of various salt compounds and their combinations on growth and stress indicators in maize (*Zea mays* L.)

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Salinity is an important stress factor for crop plants. In current research work, salt stress was applied to RX 770 hybrid maize plant. The effects of additional supply of Ca, K, and Mg salts to plants under salt stress was evaluated using membrane permeability, relative water content and proline concentrations as stress indicators. Furthermore, effects of the treatments on growth and on macro and micro nutrient concentrations in shoots and roots were determined. It was observed that supplemental Ca, Mg, and K had positive effects on plant performance, by decreasing membrane permeability and enhancing relative water contents (RWC) under salt stress.

Key words: Proline, salt stress, membrane permeability, relative water content.

INTRODUCTION

Maize plant, whose origin and gene center is America, is in the third place among cereal plant groups with its 150,755 million hectars cultivation plant and 76,796 million tons production (FAO, 2006). With the plant-based proteins that maize plant has, it is of great significance in human and animal nutrition.

Soil salinity is part of natural ecosystems under arid and semi-arid conditions (Pathak and Rao, 1998), and an increasing problem in agricultural soils throughout the world (Qadir et al., 2000). In temperate humid climates soil salinity occurs on a smaller scale, mainly in salt marshes, along roads, and on saline waste dumps. Agricultural productivity in arid and semi-arid regions of the world is very low. Soil salinity limits crop production to a great extent (Shannon, 1997; Munns, 2002). The saline growth medium causes many adverse effects on plant growth, which are due to a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors (Ashraf, 1994). All these factors cause adverse effects on plant growth and development at physiological and biochemical levels (Gorham et al., 1985; Munns, 2002; Munns and James, 2003), and at the molecular level (Winicov, 1998; Mansour, 2000; Tester and Devenport 2003). In order to assess the tolerance of plants to Salinity stress, growth or survival of the plant is measured because it integrates the up- or down-regulation of many physiological mechanisms occurring within the plant (Niknam and McComb, 2000).

Katerji et al. (2003) stated that in their study carried out to classify various plants in terms of sensitivity to salt, maize plant is semi- sensitive to salt. When electrical conductivity value of maize plant growth medium is over 5.9 dS m^{-1}, it was pointed out that there was a decrease of approximately 50% in yield. It was stated that there were generally developmental disorders as well as changes in other parameters in maize plant to which NaCl stress was applied. Azevedo Neto et al. (2004) reported that as Na content of leaf and roots in maize increases, content of K decreases. Cicek and Cakirlar (2002) reported that while there was a decrease in total fresh and dry weights and plant height and RWC in maize plant subjected to salt stress, there was an increase in the concentrations rates of prolin, Na, Na/K.

It was reported that Ca and K, macro nutrient elements supplied by adding to nutrition solution and moved to leaf in plants grown in salty medium partly protect plant NaCl and reduce the degree of effect from stress (Cramer, 2002). In plants under the stress of NaCl it was also reported that K is a co-factor for a lot of enzymes and Ca

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decreases the harmful effect of NaCl (Hasegawa and Bressan, 2000).

Since potassium is one of the most important macro-nutrients in plants, understanding the mechanisms of K^+ uptake and transport is essential for revealing the limiting steps of plant growth and for improving crop yields even under unfavorable growth conditions such as salinity. For plants growing on saline soils, it is crucial to maintain the Na:K ratio by favoring the accumulation of potassium over sodium (Sentenac et al., 1992). It was reported that compounds including Ca, K or P applied to plants under salt stress externally decreases intake of Na by competition with Na in leaf and roots of plants and increases the capacity of durability of plant against stress (Kaya et al., 2001).

This study was performed to determine the effects of K, Ca, Mg and Na and different combinations of the salt compounds salts on some stress indicators in maize plants. For this purpose, stem+leaf dry weight, root dry weight, plant height, stem diameter, membrane permeability, relative water content, proline were determined in plant and elements such as N, P, K, Ca, Mg and Na were determined in both leaf and roots. In statistical evaluation of data obtained, SPSS 13 was used.

MATERIAL AND METHOD

Material

RX 770 is a cultivar of hybrid corn which was improved by Asgrow. The average yield is 1011 – 1234 kg da^{-1}, the average plant height is 260 – 300 cm (Kapar and Oz, 2006; Vartanli and Emeklier, 2007) and the spike/corncob rate is 83.2% (Kapar and Oz, 2006). Being a hybrid cultivar that matured in 110 - 112 days and semi resistible to salinity, it is suggested to be used during the second crop cultivation especially for the Mediterranean Region.

Experimental design

The pot experiment was conducted according to randomised parcel trial design with three replications in greenhouse condition using RX 770 hybrid maize variety. Each pot received 2 kg torf-silt mixture in a ratio of 1:1(w/w). Nutrition of test plants was performed by using formula of Hoagland solution (Hothem et al., 2003). Nitrate ($N0_3^-$) and chloride (Cl^-) salts of Ca, Mg and K in combination with NaCl were added to Hoagland solution in various concentrations. Treatments of the trial were designed as follows:

1. Control
2. 100 mM NaCl + Nutrition Solution
3. 100 mM NaCl + Nutrition Solution + 20 mM $Ca(N0_3)_2$
4. 100 mM NaCl + Nutrition Solution + 10 mM $CaCl_2$
5. 100 mM NaCl + Nutrition Solution + 20 mM $Mg(N0_3)_2$
6. 100 mM NaCl + Nutrition Solution + 10 mM Mg Cl_2
7. 100 mM NaCl + Nutrition Solution + 20 mM KNO_3
8. 100 mM NaCl+ Nutrition Solution + 10 mM KCl

Before trial started, maize seeds were germinated in viols and grown in the medium for 15 days. And then germinated plants were transplanted into 20 cm pots in a way suiting 1 seedling/pot. After this procedure, the plants were watered every 3 days. 200 ml nutrition solution + 200 ml salt solution were added and solutions were increased in three periods lasting 15 days (200 ml nutrition solution + 300 ml salt solution, 200 ml nutrition solution + 400 ml salt solution, 200 ml nutrition solution + 500 ml salt solution). The EC of Hoagland solution was maintained at 2.0 dS m^{-1}. The trial terminated on Day 80. The plants were harvested and their root and stem dry weights were determined. Membrane permeability, RWC and proline contents were analyzed. Plant height and stem diameter were measured and elemental analyses were conducted (N, P, K, Ca, Mg and Na).

Method

Membrane permeability

After harvest, 1 cm diameters disks were removed from leaves of each plant and brought to the laboratory. Disks were washed with pure water and twenty of them were put into brown glass bottles. They were added 10 ml distilled water. Prepared bottles were left in shaker for 24 h and after this procedure solutions in bottles were transferred into tubes and C_1 value was measured in EC meters. Later solutions were again transfered into bottles and were autoclaved in 120°C for 20 min. Afterwards C_2 value was measured in room temperature and its membrane permeability value was calculated in $(C_1/C_2) \times 100$. The analyses were carried out in triplicate. (Lutss et al., 1996).

Relative water content (RWC)

From each group stated in trial design after harvest, 20 discs were removed from plant leaves three times and weighed. They were placed into covered petri plates after determining their fresh weights. Discs were left in petri plates to float in pure water for 4 h so that they could become turgored, after which their turgored weight was determined. After this procedure they were dried in etuves at 70°C for 24 h and their dry weights were determined. RWC (%) value was calculated with the formula stated below (Blum et al., 1999):

$[(FW - DW) / (TW - DW)] \times 100$ (FW: Fresh Weight, DW: Dry Weight, TW: Turgor Weight)

Dry weight

After harvest, plant stem and root samples were kept in etuve at 70℃ for 48 h and then their dry weight was calculated (Kacar, 1972).

Macro element analysis in leaves and roots

One gram of dried plant samples were wet-digested using a HNO_3-$HClO_4$ mixture at volume ratio of 4:1. K and Ca contents were determined by flame emission; Mg by AAS; P by vanadomolybdophophoric method; total N contents was estimated by Kjeldahl digestion procedure (Kacar, 1972).

Measurements of plant height and stem diameter

Plant height was measured from soil level of plant to tasseling. Vernier caliper (over 3rd internodium) was used to measured stem diameter.

Proline analysis

Five hundred mg of fresh leaf sample was grinded pieces with sulfosalicid acid of 3% and filtered. Two ml was taken from filtered

Table 1. Effects of various salt compounds and their combinations on membrane permeability (%), RWC (%) and proline (µmol/g, W/W) in leaves.

Treatments	Membrane permeability (%)	RWC (%)	Proline (µmol/g, W/W)
Control	14.1±0.26 e	51.24±0.32 a	2.35±0.32 d
NaCl	64.3±1.98 a	28.43±0.51 d	4.04±0.51 a
NaCl + $Ca(NO_3)_2$	36.1±1.31 cd	44.11±0.63 b	3.24±0.19 b
NaCl + $CaCl_2$	42.5±1.10 b	38.52±0.71 c	2.94±0.43 c
NaCl + $Mg(NO_3)_2$	33.6±1.07 d	42.83±0.40 b	3.56±0.26 b
NaCl + Mg Cl_2	37.5±1.03 c	40.27±0.53 b	3.21±0.41 c
NaCl + KNO_3	24.6±1.21 e	42.51±0.24 b	3.26±0.17 b
NaCl + KCl	28.3±1.17 de	38.56±0.51 c	2.95±0.73 c

The mean of three replication of each characteristic was stated as ± standart error. The difference among values is statistically significant on p<0.05 level.

leaf sample into which 2 ml acetic acid and 2 ml ninhydrin reagent were added. Ninhydrin reagent was prepared using ninhydrin, acetic acid and ortophosforic acid. The samples that were placed in tubes were kept in a water bath (100˚C) for one hour and then in ice. Four ml toluene was added over cooling samples and read in 520 nm by spectrophotometer. Afterwards, calculation was done comparing with proline standarts (Bates et al., 1973)

RESULT AND DISCUSSION

The examined characteristics in plants after harvest were membrane permeability (%), RWC (%) and Proline (µmol g^{-1} w/w). The results of these characteristics and their statistically different groups are given in Table 1.

Membrane permeability has been defined as ion imbalance that develops depending on intracellular and extracelluler osmotic instability especially in plants under salt and water stress. With the examination of this characteristic, it is possible to have information about relative ion content about cell membrane stability (Ghoulam et al., 2002; Munns, 2002). There are some findings that membrane permeability values increase in many plants under NaCl. Ghoulam et al. (2002) reported that under NaCl stress there was an increase in membrane permeability values in sugarbeet (Lutts et al., 1996) and in rice.

Kaya and Higgs (2003) reported that K and Ca had positive effect on membrane permeability of *Capsicum* and C*ucumis sativus*, respectively. As can be seen Table 1, they had significant correlations with membrane permeability levels. Membrane permeability value was lowest in control group but the administration of salt and compounds with added Ca, Mg and K increased membrane permeability 2-4 times compared with control group and had regulatory effect in membrane permeability compared with direct salt supply.

Another characteristic examined in this study is RWC. RWC values decreased depending on reduction of water potential in nutrient solution. Katerji et al. (1997) in sugarbeet and Kaya and Higgs (2003) in *Capsicum* reported that RWC values decreased in plants under NaCl stress and there was improvement in RWC values with additional nutrient elements application. However, RWC produced statistically significant relationships with examined properties (Table 1). While NaCl applied treatments had lowest RWC value, control group had highest RWC value. The compounds with Ca, Mg and K were of regulatory effect compared with RWC values. Results obtained in this study were inconsistent with those found by other researchers (Katerji et al., 1997; Kaya and Higgs, 2003).

Another characteristic examined in the study is proline concentration. Proline amino acid increases significantly in plants under salt and water stress and therefore supports resistant mechanism of plant against stress by stimulating plant defense mechanism (Shannon, 1997). Findings of Bokhari and Trend (1985) showed that there were significant increases in proline ratios in grass under water stress; Ozdemir et al. (2004) also reported the same with rice under NaCl stress.

Proline concentrations produced statistically significant differences among the treatments (Table 1). Highest proline concentration in NaCl application is probably due to resistant of plant to NaCl stress. Moreover, NaCl treatments in combination with other salt compounds decreased proline concentrations significantly and lead to lower salt stress compared with NaCl applied alone. Decrease proline concentrations were inconsistent compared with increases in RWC and decreases in membrane permeability. Under the light of these results, it is concluded that plant develops a tolerance against stress or a protective effect.

Other parameters affected by stress are root and stem dry weights, plant height and stem diameter as presented in Table 2. Because there were significant decreases in the ability of water absorbtion of roots in plants under NaCl stress, some researchers reported that there was recession in root development and stem elongation. Stem diameters of plants decreased as compared with normal plant. NaCl stress negatively affected flowering and fruit growth and there were significant decreases in the weights of dry substance and fresh weights of stem and

Table 2. Effects of various salt compounds and their combinations on stem dry weight, root dry weight, total dry weight (stem + root), plant height and stem diameter.

Treatments	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)	Plant height (cm)	Stem diameter (mm)
Control	63.7±5.35a	20.6±0.78 ha	84.3±6.13 a	133±1.09 b	34.2±3.60 c
NaCl	47.3±3.76c	13.7±1.26 b	61.0±2.92 c	120±3.20 c	22.2±1.04 d
NaCl + $Ca(NO_3)_2$	47.9±3.14c	12.9±1.03 b	60.8±3.60 c	125±1.83 c	28.6±1.62 d
NaCl + $CaCl_2$	53.6±4.10b	11.3±1.62 c	64.9±4.71 b	135±2.62 b	35.4±3.06 c
NaCl + $Mg(NO_3)_2$	49.4±3.45c	13.3±1.28 b	62.7±3.73 b	126±3.01 c	34.8±2.19 c
NaCl + $MgCl_2$	54.1±3.14b	11.9±0.70 c	66.0±3.41 b	144±2.63 a	46.1±1.51 b
NaCl + KNO_3	50.8±5.57b	12.1±0.63bc	62.9±4.80 b	136±3.70 b	54.3±2.36 a
NaCl + KCl	42.7±2.69d	11.6±0.61 c	54.3±3.30 d	124±2.16 c	43.4±1.29 b

The mean of three replication of each characteristic was stated as ± standart error. The difference among values is statistically significant on $p<0.05$ level.

roots (Dasgan et al., 2002; Ghoulam et al., 2002; Irshad et al., 2002).

Stem and root dry weight, total dry weight, plant heights, stem diameters were also given in Table 2.

Plant's water and nutrient element absorption were limited due to osmotic instability in salty soils and decrease in water potential in root. The fact that plant would not absorp the adequate amount of water and nutrient element from soil had stress on plant, which causes important recession in growth, productivity and crop quality. The imbalance of ions themselves especially obtained from roots and accumulation of ions in roots in excessive amounts decreased the growth of stem and caused reduction in height.

Stem and root dry weights treated with NaCl increased compared with control group (Table 2). Two compounds (Ca (NO3)2 and Mg (NO3)2) combined with NaCl showing significant but indifferent values when compared with NaCl. But in KCl, statistically important and lower values were in three compounds (CaCl2, MgCl2 and KNO3), statistically significant values were produced.

As can be seen in Table 2 statistically significant lower values were gotten in root dry weight with NaCl alone and in three compounds ($CaCl_2$, $MgCl_2$ and KCl) combined with NaCl.

While plant height decreased with NaCl application according to control group, higher statistically values were found in $MgCl_2$ supplied with NaCl as compared with both control group and NaCl application. $CaCl_2$ and KNO_3 compounds values increases compared with NaCl.

While decrease in stem diameter was found in NaCl applied treatments, higher and statistically significant increases were produced in three compounds supplied along with NaCl application (KNO_3, $MgCl_2$ and KCl), as compared with both control group and NaCl application; but in two($Mg(NO_3)_2$, $CaCl_2$) compounds, there were higher and statistically significant relationships as compared with NaCl. One of the negative factors causing important instabilities in metabolic function seen in plants grown in salty waters is nutrient element imbalances. As a result of competition taking places depending on Na absorption on root, K, P, N and mainly Ca absorption were negatively affected. This is a good example of antagonism between Na and other elements (Fageria, 2001). It has been known that mainly Ca, K and other elements have important characteristics of the negative effects of salinity on plants. Calcium has a positive effect due to its regulatory effect on Na ion competiting itself in areas to hold the same membrane in plant and has protected cell membrane from toxic effects of salinity (Ehret et al., 1990; Busch, 1995). It was reported in studies conducted with sugarbeet, sorghum and Glycine max that Na content increases in leaf and roots depending on Na concentration increasing root area of plants under salinity stress and the content of cations such as Ca and K decreases (DeLacerda et al., 2002; Essa, 2002; Ghoulam et al., 2002).

The contents of N, P, K, Ca, Mg and Na determined in leaf and roots after harvest are given in Table 3. As can be seen in Table 3, it was calculated that differences among contents of K, Ca, Mg and Na of leaves are statistically important, differences between N and P contents were not significant. It was observed that the contents of K, Ca, Mg and Na of leaves were highest in NaCl+KNO_3, NaCl+KNO_3, control and NaCl applications respectively. It has been understood that salts containing NO_3^- is the practice which increases the content of plant's nutrient elements, mostly. This condition may be due to an interaction between NO_3 and Cl which has an important effect on growth and develop-ment of NO_3. Consequently, Glass and Siddigi (1985) reported that NO_3 applied excessively decreased considerably the Cl absorption in barley. Although there was no significant difference in N and P contents of leaves depending on applications, it can be seen in tables that applications including NO_3 on N and P concentrations are more effective as in other elements. The effects of salt compounds on roots were examined in Table 3. The interaction between the nutriation element contents of both leaves and roots were statistically calculated in K, Ca, Mg and Na, but it could not

Table 3. Effects of various salt compounds and their combinations on some nutrient element contents of leaf and root.

Treatments	%N	%P	%K	%Ca	%Mg	%Na
Leaf						
Control	1.94±0.03	0.32±0.04	2.63±0.32 c	0.73±0.03 d	0.48±0.01 a	0.24±0.03 b
NaCl	1.44±0.07	0.28±0.01	1.32±0.21d	0.69±0.03 d	0.25±0.01 c	1.50±0.01 a
NaCl + $Ca(NO_3)_2$	2.26±0.10	0.43±0.03	2.23±0.13 c	1.55±0.07 a	0.34±0.02 b	1.49±0.02 a
NaCl + $CaCl_2$	1.74±0.21	0.34±0.02	2.32±0.20 c	1.01±0.04 b	0.23±0.01 c	1.18±0.02 c
NaCl + $Mg(NO_3)_2$	2.20±0.29	0.33±0.03	2.54±0.22 c	0.93±0.04 c	0.42±0.01 a	1.51±0.05 a
NaCl + Mg Cl_2	1.90±0.22	0.28±0.01	2.76±0.30 c	0.72±0.03 d	0.28±0.01 c	1.24±0.03 b
NaCl + KNO_3	2.03±0.13	0.29±0.01	4.55±0.33 a	0.67±0.06 d	0.17±0.02 d	1.29±0.06 b
NaCl + KCl	1.82±0.10	0.33±0.03	3.93±0.35b	0.96±0.07 c	0.20±0.01 c	1.33±0.05 b
Root						
Control	1.41±0.03	0.26±0.04	1.81±0.09 c	0.91±0.05 e	0.38±0.04bc	0.32±0.01 c
NaCl	1.30±0.08	0.24±0.08	1.01±0.05 d	1.14±0.10 e	0.29±0.02cd	3.57±0.13 a
NaCl + $Ca(NO_3)_2$	1.98±0.13	0.29±0.01	1.91±0.10 b	1.87±0.13 a	0.39±0.02 b	3.06±0.15 a
NaCl + $CaCl_2$	1.08±0.16	0.26±0.01	1.95±0.10 b	1.29±0.11 d	0.31±0.03 c	3.40±0.18 a
NaCl + $Mg(NO_3)_2$	1.85±0.14	0.22±0.05	1.85±0.13 c	1.74±0.10 b	0.60±0.02 a	3.10±0.17 a
NaCl + Mg Cl_2	1.34±0.11	0.20±0.01	2.02±0.11 b	1.23±0.13 d	0.47±0.02 b	2.80±0.14 b
NaCl + KNO_3	1.76±0.19	0.18±0.02	3.04±0.15 a	1.38±0.14 c	0.31±0.04 c	3.09±0.12 a
NaCl + KCl	1.39±0.08	0.24±0.01	3.16±0.20 a	1.34±0.12 c	0.28±0.02 d	3.21±0.10 a

The mean of three replication of each characteristic was stated as ± standart error. The difference among values is statistically significant on $p<0.05$ level.

be calculated in N and P. The highest increases in root contents of K, Ca and Mg were in the salts of these elements. Furthermore, it was determined that NO_3 salts are more effective than Cl salts. Other applications to Na contents of roots other than control and KNO_3 are similarly effective.

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