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Effects of Different Salt Sources on Growth, Inorganic Ions and Proline Accumulation in Corn (*Zea mays* L.)

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Summary

The effect of salinity on some agro-physiological properties, such as dry matter, nutrient contents, chlorophyll contents, and ionic balance of corn plants, were investigated. Plants were treated with six salt sources (NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$) and four concentrations (0, 40, 80, and 120 mM doses) for 30 days in a growth media. Salt type and doses affected soil pH and electrical conductivity. Soil salinity affected the parameters considered and changed the nutrient balance of plants. High salt concentration caused substantial reduction in plant growth. Different salt concentrations negatively affected plant dry weight. The highest decrease rates of plant dry weight was obtained for NaCl application followed by Na_2SO_4, $CaCl_2$, $MgCl_2$, $CaSO_4$ and $MgSO_4$. Total chlorophyll and nitrate contents of plants decreased with increasing salt doses, and the lowest value was obtained for NaCl application. Proline contents of plant were increased with increasing salt doses, and the highest value was obtained for 120 mM NaCl application. The effects of salt concentrations on N, K and P content of plants were significant. The highest decrease rate was determined with NaCl application, but the lowest with $MgSO_4$. Iron, Mn and Cu content of plants showed similar variation, as the highest for NaCl, and Na_2SO_4, and the lowest for $CaCl_2$ and $CaSO_4$. The highest decrease in Zn content occurred with $MgSO_4$ application, but the lowest with NaCl.

Zusammenfassung

Wirkung unterschiedlicher Salzquellen auf Wachstum, anorganische Ionen und Prolinanreicherung bei Mais (*Zea mays* L.). Es wurde die Wirkung des Salzgehalts auf einige pflanzenphysiologische Eigenschaften wie Trockensubstanz, Nährstoff- und Chlorophyllgehalt sowie Ionenbalance von Maispflanzen untersucht. Die Pflanzen wurden 30 Tage lang in Substraten kultiviert, die die Salze NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$ in vier Konzentrationen (0, 40, 80 und 120 mM) enthielten. Der Salztyp und die Dosis beeinflusste den Substrat-pH und die elektrische Leitfähigkeit. Der Salzgehalt des Substrats beeinflusste die untersuchten Parameter und veränderte die Nährstoffbalance der Pflanzen. Hohe Salzkonzentrationen führten zu einer deutlichen Reduktion des Pflanzenwachstums. Verschiedene Salzkonzentrationen beeinflussten das Trockengewicht negativ. Die stärkste Minderung des Trockengewichts verursachte die NaCl-Gabe gefolgt von Na_2SO_4, $CaCl_2$, $MgCl_2$, $CaSO_4$ und $MgSO_4$. Der Chlorophyll- und Nitrat- Gesamtgehalt der Pflanzen fiel mit steigender Salzdosis, wobei die NaCl-Gabe den geringsten Wert verursachte. Der Prolin-Gehalt der Pflanzen stieg mit steigender Salzdosis, die Gabe von 120 mM NaCl führte zum höchsten Wert. Der Einfluss der Salzkonzentrationen auf die N-, K- und P-Gehalte war statistisch signifikant. Die höchste Reduktion wurde von NaCl-Gaben und der absolut geringste Wert durch $MgSO_4$ verursacht. Eisen-, Mn- und Cu-Gehalte zeigten ähnliche Variationen, die stärksten bei NaCl und Na_2SO_4 und die geringsten bei $CaCl_2$ und $CaSO_4$. Der höchste Rückgang beim Zn-Gehalt trat bei der $MgSO_4$-Gabe und der geringste bei NaCl auf.

Key words. *Zea mays* – salinity – proline – inorganic ions – chlorophyll

Introduction

Soil salinity increasingly limits plant production in arid and semi-arid regions around the world. Worldwide, 100 million ha or 5 % of the arable land is adversely affected by high salt concentration which reduces crop growth and yield (GHASSEMI et al. 1995). The major cations of concern in saline soils and waters are Na^+, Ca^{++}, Mg^{++}, and K^+ and primary anions are Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^-. Major problem with saline soils is the presence of soluble salts, primarily Cl^-, SO_4^{2-}, and sometimes NO_3^- (SPARKS 1995). The reasons for excessive ion accumulation in the soil solution are inappropriate irrigation, and use of saline irrigation water. In many irrigated areas of the world, farmers are forced to use saline water to irrigate their crops due to inadequate supplies of fresh water. Saline water or some other irrigation waters contain high amounts of NaCl, which contributes to specific ion effects of Cl^-, Na^+ or both, and to antagonistic effects on nutrient el-

ements (AL-RAWAY et al. 1992). Nutrient imbalances may result from the effect of salinity on nutrient availability, uptake, and partitioning within the plant or may be caused by physiological inactivation of a given nutrient, raising the internal requirement of the plant for that essential element (VILLORA et al. 1997).

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some macro- and micronutrients by crop plants. The uptake of Fe, Mn, Zn, and Cu generally was increased in crop plants under salinity stress (ALAM 1994).

Limited soil water content restricts plant growth under drought and salinity conditions. Plants overcome this difficulty by increasing concentration of compounds in cells. Various studies were made, whether proline accumulation could be used as an indicator in salt tolerance of plants (HANSON et al. 1977; SINGH and RAI 1981; BAL et al. 1984; CHEN et al. 2001; GHOULAM 2002; GIRIJA et al. 2002).

It was pointed out that the more proline is accumulated, the longer plants tend to live, and this accumulation could be used as an indicator for drought tolerance (BARTNETT and NAYLOR 1966; PALFI and JUHASZ 1971). On the other hand, studies related to tomato (ALONI and ROSENSTEIN 1984), chick pea (SINGH and RAI 1981; PANDEY and GANAPATHY 1985), potato (LEVY 1983), and barley (HANSON et al. 1979) showed that resistant cultivars accumulated lower proline than more susceptible ones.

Under salt stress conditions, osmotic adjustment is obtained by the production and accumulation of compatible organic solutes such as proline, glycerol, sugar and glycine betaine in the cytoplasm, while mineral ions are localized in the vacuole (YEO 1998; BOHNERT et al. 1999; SUN et al. 2002). With osmotic adjustment mechanisms, plants may have lower water potential of the cells, and thus the osmotic inhibition effect of salinity could be negated. In addition, proline can inhibit toxic effects of NaCl by stabilizing proteins and enzymes in the cell (AHMAD et al. 1982). However, ZHANG and ARCHBOLD (1993) found that amounts of proline were not significantly changed under water deficit conditions in *Fragaria*. Although numerous studies have been conducted on the impact of salt stress on proline accumulation in different plants (RHODES and HANSON 1993; LUTTS et al. 1996; ZHU et al. 1998; AZIZ et al. 1999; ALIAN et al. 2000), no attempts have to date been made to study proline accumulation in corn plant under salinity conditions.

The objective of this study was to determine the effects of salinity on the growth, proline, and ion accumulation in corn plant.

Materials and Methods

Crop conditions and plant sampling

Corn plants (*Zea mays* L.) were grown under the following controlled greenhouse conditions with 25–30 °C, 30–40 % relative humidity, and 10 °C night temperatures in Erzurum (Turkey). Day length was roughly 14 h during the experimental period. Seeds were sown in plastic pots containing 3 kg soil. The soil had loamy texture (38.6 % sand, 35.4 % silt, and 26.0 % clay), 1.10 % $CaCO_3$, 325.2 mmol kg^{-1} P_2O_5, 455.3 mmol kg^{-1} K_2O, pH (H_2O) = 7.0 and 1.29 dS m^{-1} electrical conductivity. The number of plants per pot was adjusted to three, 15 days after germination. Solution of basal fertilizers including 100 mg kg^{-1} N, 30 mg kg^{-1} P, 130 mg kg^{-1} K was given once a week at 10 days after sowing time. Salt concentrations were established 15 days after sowing time. Six salt sources (NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$) and four concentrations (To=0, T_1=40, T_2=80 and T_3=120 mM) were applied to soil. Plants were regularly irrigated with pure water. Soil water content was carefully controlled. When 70 % of available water in the soil had consumed, pure water was applied to the soil and leakage from the pots was not allowed. Salt sources were dissolved in water and the four concentration levels then applied to the soil. To avoid osmotic shock, salt concentrations were increased gradually by 20 mM every 2 days until the desired concentration was reached. After 30 days (exclusive of the addi-

Table 1. pH and EC of the soil medium at different salt doses and source application.

Salt doses (mM)	NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$
	pH					
T_0	7.10 ± 0.08 d*	7.12 ± 0.40 d	7.08 ± 0.04 d	7.10 ± 0.03 c	7.14 ± 0.03 d	7.11 ± 0.03 c
T_1	7.60 ± 0.03 c	7.40 ± 0.04 c	7.50 ± 0.01 c	7.60 ± 0.04 b	7.55 ± 0.05 c	7.60 ± 0.07 ab
T_2	7.90 ± 0.06 b	7.70 ± 0.05 b	7.75 ± 0.02 ab	7.85 ± 0.07 ab	7.65 ± 0.03 ab	7.62 ± 0.06 ab
T_3	8.30 ± 0.10 a	7.85 ± 0.05 a	7.80 ± 0.06 a	7.90 ± 0.09 a	7.70 ± 0.03 a	7.68 ± 0.07 a
	EC (dS m^{-1})					
T_0	0.50 ± 0.01 d	0.52 ± 0.02 d	0.52 ± 0.03 d	0.52 ± 0.01 c	0.54 ± 0.01 c	0.50 ± 0.01 c
T_1	6.80 ± 0.02 c	4.15 ± 0.02 c	6.60 ± 0.04 c	3.30 ± 0.01 b	6.75 ± 0.04 b	3.42 ± 0.02 b
T_2	12.00 ± 0.04 b	10.25 ± 0.01 b	9.50 ± 0.03 b	11.60 ± 0.03 a	10.15 ± 0.04 a	9.75 ± 0.06 a
T_3	18.05 ± 0.04 a	16.65 ± 0.03 a	10.42 ± 0.02 a	10.15 ± 0.02 a	12.16 ± 0.08 a	10.10 ± 0.05 a

Values are means ± SE, at 5 % level, of three replications with soil samples.
To=0, T_1=40, T_2=80 and T_3=120 mM

tion ones) of salt treatment, the plants were harvested, measured, and analysed. Soil samples were taken from the plant rhizosphere area from each pot, and then pH and electrical conductivity (EC) were measured. Each pot (three plants) was considered as one replicate with three pots per treatment per salt. The experiment design was randomised block design.

Plant analysis

Total nitrogen was determined using the micro-Kjeldahl method. The tissues sampled of plant were oven-dried at 68 °C for 48 h and ground. K^+, Na^+, Ca^{++}, and Mg^{++} were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-salicylic acid mixture. In the diluted digests, P was measured spectrophotometrically by the indophenol-blue method and after reaction with ascorbic acid. Potassium, Na^+, Ca^{++}, Mg^{++}, Fe, Mn, Zn, and Cu analyses were performed by atomic absorption spectrometry (AOAC 1970).

The nitrate content in the leaves was estimated as described by AGBARIA et al (1996). 100 mg fresh weight of leaf samples were extracted for 60 min in deionized water at 45 °C. After centrifugation at 6000 x g for 15 min, 200 µl of the supernatant were incubated at ambient temperature (around 24 °C) with 0.8 ml 5 % salicylic acid in concentrated sulphuric acid for 20 min. After 12 min, the samples were cooled to ambient temperature and the coloration was measured spectrophotometrically at 410 nm. The nitrate content was determined using a standard curve established with solution of KNO_3.

The proline content was determined using the method of BATES et al. (1973). Proline was extracted from leaf samples of 100 mg fresh weight with 2 ml of 40 % methanol. 1 ml extract was mixed with 1 ml of a mixture of glacial acetic acid and orthophosphoric acid (6M) (3:2 v/v) and 25 ml ninhydrin. After 1 h incubation at 100 °C, the tubes were cooled and 5 ml toluene was added. The absorbance of the upper phase was spectrophotometrically determined at 528 nm. The proline concentration was determined using a standard curve.

For the chlorophyll analysis, 1 cm^2 leaf discs were frozen (–20 °C), ground and the chlorophylls were ex-

Table 2. Sample dry-weight (g pot^{-1}) at different salt doses and source.

Salt doses (mM)	Salt sources					
	NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$
T_0	15.70 ± 1.5 a*	15.07 ± 1.1 a	15.09 ± 1.8 a	15.11 ± 1.9 a	14.90 ± 1.0 a	15.10 ± 1.4 a
T_1	8.74 ± 1.9 b	4.63 ± 1.2 b	10.53 ± 1.7 b	12.83 ± 1.6 b	11.29 ± 1.7 b	12.00 ± 1.6 b
T_2	2.61 ± 0.5 c	3.84 ± 0.9 bc	8.28 ± 1.7 c	9.87 ± 1.5 c	9.01 ± 1.0 c	8.80 ± 1.2 c
T_3	0.56 ± 0.1 d	3.16 ± 1.1 c	4.68 ± 0.9 d	6.53 ± 1.1 d	5.28 ± 0.8 d	6.82 ± 0.8 d

Values are means ± SE, at 5 % level, of three replications with three plants each.

Table 3. Proline and chlorophyll content of plant at different salt doses and sources.

Salt doses (mM)	NaCl	Na_2SO_4	$CaCl_2$	$CaSO_4$	$MgCl_2$	$MgSO_4$
	Proline (µmol g^{-1} dry weight)					
T_0	0.62 ± 0.11 d*	0.58 ± 0.21 d	0.60 ± 0.14 c	0.55 ± 0.21 c	0.58 ± 0.10 c	0.64 ± 0.13 c
T_1	3.84 ± 0.53 c	4.10 ± 0.35 c	2.54 ± 0.21 bc	2.65 ± 0.24 bc	2.83 ± 0.15 bc	2.67 ± 0.27 bc
T_2	5.65 ± 0.62 b	6.20 ± 0.22 b	4.10 ± 0.42 b	4.25 ± 0.70 b	3.86 ± 0.23 b	4.32 ± 0.36 b
T_3	12.52 ± 1.10 a	10.18 ± 0.81 a	9.15 ± 0.66 a	8.65 ± 0.92 a	8.66 ± 0.31 a	8.14 ± 0.57 a
	Total chlorophyll (mg g^{-1} dry weight)					
T_0	2.22 ± 0.10 a	2.28 ± 0.12 a	2.38 ± 0.12 a	2.35 ± 0.14 a	2.37 ± 0.17 a	2.33 ± 0.13 a
T_1	1.92 ± 0.50 b	1.83 ± 0.07 b	2.02 ± 0.06 b	2.23 ± 0.12a	2.05 ± 0.10ab	2.22 ± 0.10 a
T_2	1.38 ± 0.03 c	1.49 ± 0.09 c	1.60 ± 0.03 c	2.15 ± 0.18 a	1.93 ± 0.09 b	2.02 ± 0.09 a
T_3	1.15 ± 0.02 c	1.26 ± 0.08 c	1.35 ± 0.05 c	2.05 ± 0.07 a	1.78 ± 0.07 b	1.99 ± 0.07 a
	Nitrate content (mg NO_3 g^{-1} dry weight)					
T_0	50 ± 9.8 a	51 ± 2.5 a	50 ± 8.2 a	52 ± 7.8 a	51 ± 7.8 a	52 ± 7.2 a
T_1	41 ± 3.8 ab	48 ± 1.5 a	45 ± 1.2 ab	49 ± 9.2 a	46 ± 9.2 ab	48 ± 7.3 a
T_2	38 ± 2.4 b	47 ± 1.2 a	42 ± 4.6 b	49 ± 1.4 a	43 ± 1.4 b	47 ± 4.1 a
T_3	27 ± 4.4 c	45 ± 1.1 a	34 ± 9.2 c	50 ± 0.8 a	37 ± 0.8 c	46 ± 2.4 a

* Values are means ± SE, at 5 % level, of three replications with three plants each.

tracted in acetone. The pigment density was measured in petrol ether with a spectrophotometer at 642 nm (Chl b) and 660 nm (Chl a).

Statistical analysis

Each pot was considered as a replicate and all of the treatments were repeated three times. A two-way analysis of variance (ANOVA) was performed using a statistical software (SAS 1982) program.

Results and Discussion

Soil pH and EC measured over the sample from the plant rhizosphere raised with increasing salt concentrations. The highest increasing rate in pH and EC were determined from NaCl treatments, while the lowest values were determined from $MgSO_4$ treatments (Table 1). Salt type and doses affected plant dry-matter (Table 2). As the salt dose increased, plant dry-matter decreased for all types of salts. The reduction of dry-matter was higher in NaCl treatment than with the other salt treatments. The percentage of reduction in plant dry-matter was greater at 80 (T_2) and 120 (T_3) mM salt doses for all of the salt types. The highest reduction was obtained in NaCl treatment, where it reached around 96 % at 120 mM NaCl, followed by Na_2SO_4 with 76 % compared to control. KARIM et al. (1993), reported that plant dry-matter declined steadily as salinity concentration was raised. Salt treatments caused a decrease in growing in salt sensitive bean

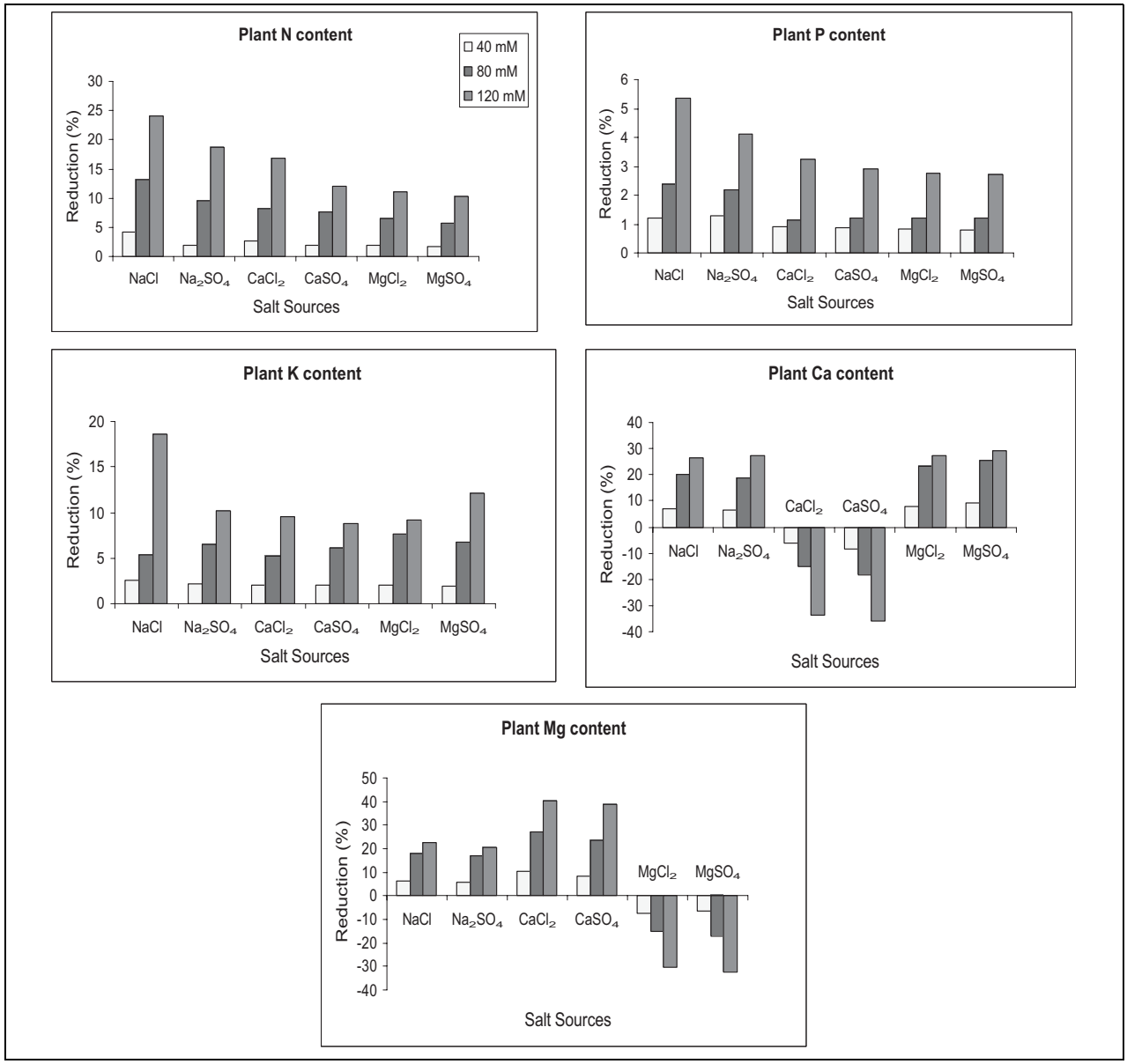


Fig. 1. Effects of different salt concentrations in the rooting medium on plant macro nutrient content. The plants were exposed to salt treatments for 30 days. Results are means of three replicates and expressed as reduction percentage of control.

(*Phaseolus vulgaris*), but did not significantly affect salt tolerant cotton (*Gossypium hirsutum*) (GOUIA et al. 1994).

A two-way ANOVA analysis indicated a significant effect of salinity sources and salt doses and their interaction on the proline content in plants (Table 4). Proline content in plant increased with the increasing salt doses (Table 3). The highest proline amount was observed with NaCl 120 (T_3) mM doses and the lowest the $MgCl_2$ treatments. The proline content of plants increased about 6.2, 9.1, and 20.2 fold compared to control in NaCl salt treatment (40, 80 and 120 mM doses), and 4.2, 6.8, and 12.7 fold in $MgSO_4$ salt treatment (40, 80 and 120 mM doses), respectively. The increasing in proline content was positively correlated to the level of salt but plant inorganic ions such as N, P, K, Ca, Mg, Fe, Mn, and Cu contents of plant was negatively related to salt doses. Similar result were reported for other plant species, such as *Brassica napus* L. (CHANDLER and THORPE 1987), *Lycopersicon esculantum* L. (INAL et al. 1997), and *Cucurbita pepo* L. var. *moschata* (VILLORA et al. 2000).

Leaf total chlorophyll content (LTCC) decreased with increasing salt doses for all of the salt sources. The highest LTCC was observed in the 120 mM NaCl treatment compared to control (Table 3). The lowest LTCC decreasing rate was determined for the $MgSO_4$ treatment. Decreasing in chlorophyll concentration in salinised plants could be attributed to increasing activity of chlorophyll-degrading enzyme chlorophyllase (REDDY and VORA 1986). Ion accumulation in leaves also adversely affected chlorophyll concentration (YEO and FLOWERS 1983).

Plant nitrate content decreased with increasing doses for all the salt treatments. At increased chlorine salt

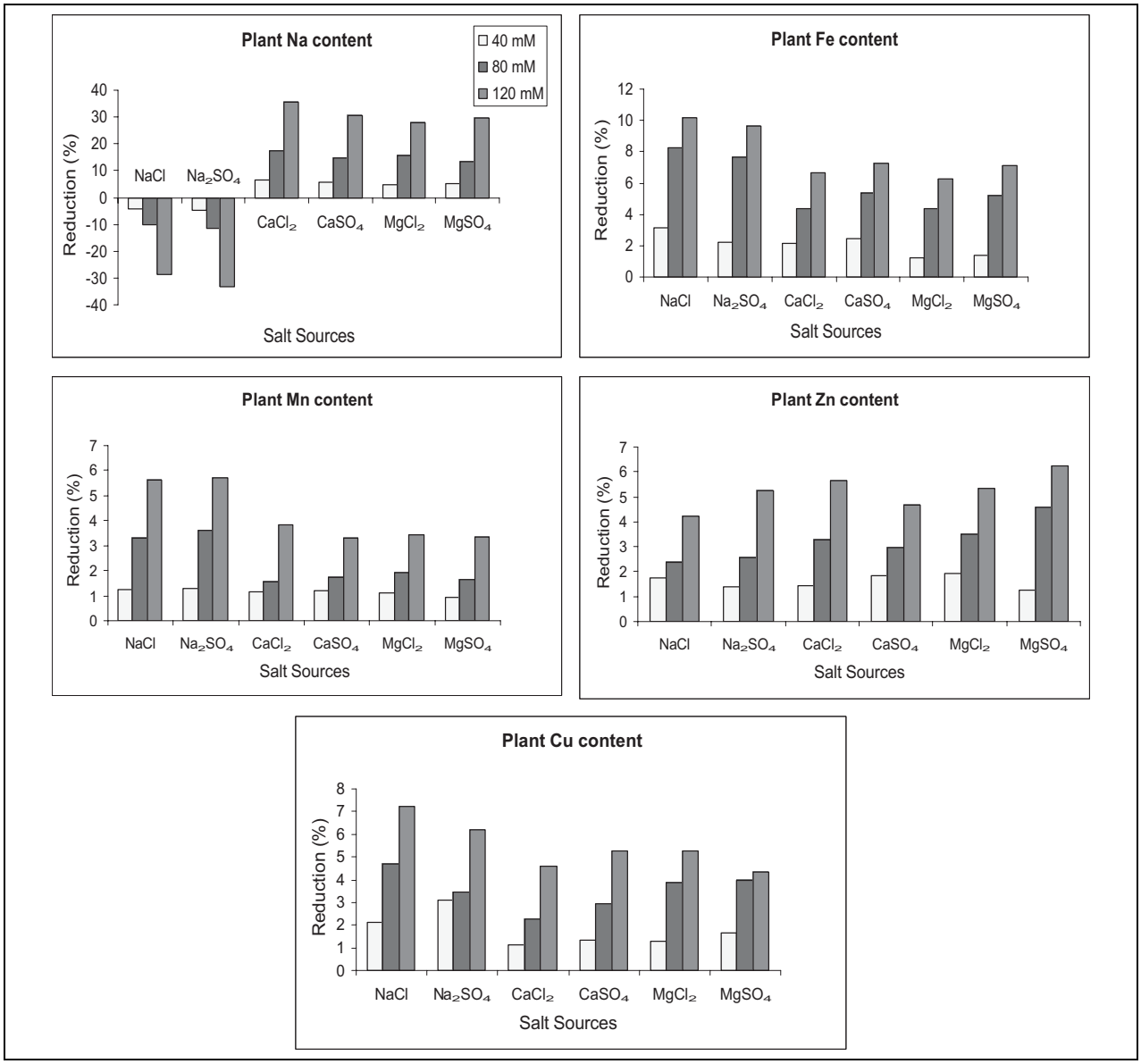


Fig. 2. Effects of different salt concentrations in the rooting medium on plant micro nutrient content. The plants were exposed to salt treatments for 30 days. Results are means of three replicates and expressed as reduction percentage of control

Table 4. Result of two-way analysis of variance (ANOVA) of salt sources (SS) and salinity doses (SD) effects and their interactions (SS x SD) for the parameter consider.

Dependent variable	Independent variable		
	SS	**SD**	**SS x SD**
Plant dry-matter	153.60 **	379.07 **	21.40 **
N	45.32 **	489.15 **	27.29 **
P	46.16 **	706.13 **	27.05 **
K	31.48 **	852.36 **	18.79 **
Ca	76.22 **	25.13 ns	0.18 ns
Mg	63.12 **	7.30 ns	1.15 ns
Na	118.10 **	13.20 ns	0.67 ns
Fe	210.13 **	1018.30 **	23.22**
Mn	185.22**	628.19 *	33.10 **
Zn	380.62 **	965.82 **	28.12 *
Cu	128.10 *	422.17 *	30.31 *
Proline	7.14 **	16.60 **	13.10 *
Nitrate	5.31 **	13.20 **	4.60 **
Total chlorophyll	14.60 *	47.80 **	3.70 *
EC	54.13**	18.24**	11.18**
Soil pH	98.24**	254.21**	24.50**

* $p< 0.05$, $p< 0.01$ Number represent F values at 5 % level. ns not significant

of Na^+, Ca^{++} and Mg^{++}, plant nitrate content drastically decreased especially with 120 mM NaCl treatments. It has been reported that nitrate uptake was reduced by salinity (BOTELLA et al. 1994).

The presence of salt in the growth medium induced an important decrease in the macro and micro nutrients of the plant, such as N, P, K, Fe, Mn, Zn and Cu content, but the Ca^{++}, Mg^{++}, Na^+ content of the plant was increased or decreased depending on the salt sources. The highest N, P, K, Fe, Mn, and Cu reduction in the plant was obtained with the NaCl treatment at 120 (T_3) mM application doses (Fig. 1 and 2).

The highest decreases occurred in N, K, Ca, Mg, and P contents of plants when NaCl was applied (24 %, 18 %, 28 %, 22 % and 5.6 % respectively). After $MgSO_4$ application a decrease in N, K, Ca, and P contents of plants took place (10 %, 13 %, 30 % and 3 %, respectively). Decreases in dry mater content in NaCl and $MgSO_4$ applications occurred at a rate of 40–70 % and 25–36 %, respectively. Responses of plants to chemical variations in soil were so different. Owing to salt and its doses in soil, plants respond to salts by changing nutrients in the cell and accumulating proline. The highest salt stress occurred in the NaCl application and in this case, because of unbalanced nutrient uptake, plants tend to resist stress conditions by accumulating proline and the highest reaction occurred in NaCl application.

Conclusion

The assessment of the effect of salinity on the growth parameters by different salt sources and doses enabled us to conclude that all of the considered parameter are affected by salinity. Ion balance in the soil solution is very important plant growth and tolerance of salinity. Plant inorganic ions, such as N, P, K, Ca, Mg, Fe, Mn, and Cu, were negatively related to salt doses, but proline content of plants was positively correlated to the level of salt doses. Under stress conditions, corn plants have evolved complex mechanisms allowing for adaptation to osmotic and ionic stress caused by high salinity.

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