International Scholars Journals

African Journal of Agronomy ISSN 2375-1177 Vol. 8 (3), pp. 001-005, March, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant

Murat Ali Turan[1]*, Abdelkarim Hassan Awad Elkarim[2], Nilgun Taban[3] and Suleyman Taban[4]

[1]Department of Soil Science, Faculty of Agriculture, Uludag University, 16059 Bursa, Turkey.
[2]Department of Soil Science, Faculty of Agriculture, Khartoum University, 13314 Shambat-Khartoum, Sudan.
[3]Agricultural Engineer, Kastamonu, Turkey.
[4]Faculty of Arts and Sciences, Kastamonu University, 37100 Kastamonu, Turkey.

Accepted 03 January, 2020

In this study, effect of applied NaCl on growth and stomatal resistance, proline and total chlorophyll concentrations of maize plant (*Zea mays* L. cv: RX 947) was investigated. The experiment was arranged in a completely randomized design with four replications under the greenhouse condition. The experimental soil was salinized with NaCl at the rates of 0 and 100 mM NaCl. Growth of the maize plants was inhibited by salinity. Applied NaCl significantly decreased dry mass of maize plants. Stomatal resistance and proline concentrations were increased by high salinity, while total chlorophyll concentration was decreased. NaCl caused to increase Na and Cl concentrations of plant.

Key words: *Zea mays*, salt stress, stomatal resistance, proline, sodium chloride.

INTRODUCTION

Salinity, due to over-accumulation of NaCl, is usually of great concern and the most injurious factor in arid and semi arid regions. Saline soils are widespread on Earth. More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area (FAO, 2008) . Their genesis may be natural or accelerated by the extension of irrigated agriculture, the intensive use of water combined with high evaporation rates and human activity (Lambers, 2003).

Despite the essentiality of chloride as a micronutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some C_4 species, salt accumulation may convert agricultural areas in unfavorable environments, reduce local biodiversity, limit growth and reproduction of plants, and may lead to toxicity in non-salt-tolerant plants, known as glycophytes. Most of the cultivated plants are sensitive to salt-stress, in which NaCl-salinity causes reduction in carbohydrates that are needed for cell growth. Carbohydrates are supplied mainly through the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Ashraf and Harris, 2004; Parida and Das, 2005), and this would furthermore lead to restriction in water availability and imbalance in nutrient uptake by plants (Pessarakli and Tucker, 1988; Katerji et al., 2004; Arzani, 2008) with inhibition in seed germination due to ionic disturbance, osmotic and toxic effects.

Plants have improved complex mechanisms systems for adaptation to osmotic and ionic stress caused by high salinity, under the salt stress. The adaptation is generally associated with osmoregulation adjustment by using some osmotic regulators, such as potassium, soluble sugar, proline and betaine (Munns, 2005; Hong-Bo et al., 2006). One of the mechanisms is proline accumulation into cell. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Kavi et al., 2005; Ashraf and Foolad, 2007).However, the signi-ficance of proline accumulation in osmotic adjustment is still debated and varies according to the species (Lutts et al., 1996; Rodriguez et al., 1997).

The present study investigated the response of maize plant to a high NaCl treatment (e.g., 100 mM) at dry matter, stomatal resistance, proline, total chlorophyll and

*Corresponding author. E-mail: maturan@uludag.edu.tr.

Table 1. Effect of NaCl on the dry weights (g pot^{-1}) of the maize plants.

NaCl, mM	Dry weight	Range, (%)
0	25.36 ± 2.15 a	-
100	13.83 ± 0.98 b	45.46
Treatments	***	

*** Significant at P < 0.001 level. Means followed by the same letter in column are not significantly different (Duncan's Multiple Range test, P < 0.01).

concentrations of Na, Cl and K.

MATERIALS AND METHODS

Soil material

The experimental soil taken from Aridisol great soil group was non-calcareous (0.58 % $CaCO_3$), clay in texture, slightly alkaline (pH: 7.42 and EC: 0.148 dS m^{-1}; both in 1:2.5 water extract). The soil sample had 82.9 mg kg^{-1} exchangeable Na and water extractable Cl was 9.37 mg kg^{-1}.

Pot experiment

The experiment was conducted under greenhouse conditions (humidity 65 - 75%, air temperature 25 - 30°C and neutral light intensity 340 - 450 µmol m^{-2} s^{-1}). The soil (3000 g) was placed into pots and was salinised with NaCl at the rates of 0 and 100 mM NaCl. For basal fertilizers, 100 mg N kg^{-1} as ammonium nitrate and 80 mg P kg^{-1} as triple super phosphate were applied to the pots. Five maize (*Zea mays* L. cv: RX947) seeds were sown into each pot which were thinned to three after emergence. Six weeks after germination, the vegetative growth was harvested.

Chemical analysis

After weighting and washing the fresh shoot material, plants were sampled taking 5.0 g of fresh plant material for total chlorophyll and total proline determinations. The remaining plant samples were dried at 65°C and were digested with HNO_3:$HClO_4$ acid mixture (4:1) in order to determine dry matter and the concentrations of Na.

Stomatal resistance was determined during the span of time (14h00 and 15h00) before harvest by use of a "steady-state" porometer (EA 540 - 026 AP4 model) attached to the abaxial side of leaves. The readings were taken on six fully-expanded leaves, situated at the different position of the canopy. Proline was determined by the ninhydrin method described by Bates et al. (1973) . In this method, proline was extracted from 0.5 g of fresh leaf tissue into 10 ml of 3% sulfosalicylic acid and filtered through Whatman No. 42 filter papers and determined in Shimadzu UV-1201 model spectrophotometer. Total chlorophyll (chlorophyll a + b) was extracted in 80% (v/v) aqueous acetone and absorption measured in a Shimadzu UV-1201 model spectrophotometer at 645 and 663 nm (Arnon, 1949).

Sodium and potassium concentration were determined by using Eppendorf Elex 6361 model flame photometry described by Miller (1998). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

Statistical analysis

The pot experiment was arranged in a completely randomized design with two salt concentrations and four replicates. Analysis of variance of data for all variables was computed using MINITAB computer package (Minitab Release 10.51). MSTAT package program (Version 3.00) was used to compare treatment means by Duncan's Multiple Range test.

RESULTS

Dry weights of maize plants

The effects of salinity on the growth of maize were summarized in Table 1. Dry weight results indicated that growth was negatively correlated to the substrate concentration of NaCl (p < 0.001). Maize plants grown at the low levels of NaCl (0 mM) reached relatively higher dry weights and did not imply toxicity symptoms, however, the plant dry weight was significantly reduced at higher levels of salinity (100 mM) indicating the symptoms of salt toxicity as growth depression. The concentration of NaCl that significantly reduced dry weight was 100 mM by 45.46% in comparison to the control.

Stomatal resistance, proline and total chlorophyll concentrations of maize plants

Stomatal resistance, proline and total chlorophyll (chlorophyll a + b) concentration of maize plants was significantly influenced by salinity (P < 0.001, Table 2). While NaCl treatment decreased total chlorophyll concentration, increased stomatal resistance and proline concentration of maize plants.

Ion distribution and accumulation

The concentrations of Na and Cl ions significantly increased in parallel to amount of NaCl (p < 0.01, Table 3). NaCl treatments caused to decrease K concentrations and K/Na ratio of maize plants (Table 3).

DISCUSSION

Dry weights of maize plants

The results of biomass indicated that applied NaCl inhibited the growth of maize plant, and led to a decrease in biomass. This may be related to the effect of salt-stress which resulted in the limitation of water absorption and biochemical processes (Cusido et al., 1987; Parida and Das, 2005). In addition, a decline in the rates of net photosynthesis occurs, due to adverse affect on CO_2 assimilation, which leads to a decrease in nutrient uptake and finally growth of plants (Seeman and Sharkey, 1986;

Table 2. Effects of NaCl on stomatal resistance, proline and total chlorophyll (chlorophyll a + b) concentration of the maize plants.

NaCl, mM	Stomatal resistance (s cm^{-1})	Proline (μ mol g^{-1}) fresh weight	Total chlorophyll (mg g^{-1}) fresh weight
0	0.95 ± 0.02 a	0.806 ± 0.08 a	6.13 ± 1.72 a
100	3.25 ± 0.10 b	5.812 ± 1.08 b	2.43 ± 0.97 b
Treatments	***	***	***

*** Significant at P < 0.001 level. Means followed by the same letter are not significantly different (Duncan's Multiple Range test, P < 0.01).

Table 3. Effects of NaCl on sodium, chloride and potassium concentrations and K/Na ratio of the maize plants.

NaCl (mM)	Na (g kg^{-1})	Cl (g kg^{-1})	K (g kg^{-1})	K/Na ratio
0	0.32 ± 0.02 a	1.86 ± 0.96 a	27.17 ± 2.62 a	84.91
100	4.46 ± 1.03 b	44.16 ± 5.75 b	17.93 ± 1.35 b	4.02
Treatments	***	***	***	

** Significant at P < 0.001. Means followed by the same letter in column are not significantly different (Duncan's Multiple Range test, P < 0.001).

and finally growth of plants (Seeman and Sharkey, 1986; Cha-Um and Kirdmanee, 2009).

The suppression of plant growth under salt-stress may either be due to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect (Marschner, 1995). There are many reports on osmotic stress and ionic toxicity resulted from salt stress in maize plants (Katerji et al., 2004; Mansour et al., 2005; Eker et al., 2006).

Stomatal resistance, proline and total chlorophyll concentrations of maize plants

During a salt stress, the plant has to close their stomata due to water loss (Chatrath et al., 2000). In general, measurement of stomatal resistance provides effectual comparison for determining the degree of stress in plants. Salinity increased the stomatal resistance, which could be explained by inhibition of plant growth due to water stress (Chatrath et al., 2000). It was found to be a strong negative correlation between stomatal resistance and NaCl (Turan et al., 2007a) . Stomatal factors have also a more significant effect on photosynthesis (Wang et al., 1987).

One of the most important mechanisms by higher plants under salt-stress is the accumulation of compatible solutes such as proline. Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Desingh and Kanagaraj, 2007; Koca et al., 2007; Veeranagamallaiah et al., 2007; Turan et al., 2007a).

On the other hand, a positive correlation was determined between proline and tissue-Na concentrations under salt stress (Bajji et al., 2001). The present study shows that the salt treatments induced an increase in proline concentration in maize plants. Similar result has been reported by Cha-Um and Kirdmanee (2009).

The total chlorophyll concentration of maize leaves was reduced by increased level of NaCl treatment. According to Cha-Um and Kirdmanee (2009), salinity decreased the total chlorophyll concentration of two maize varieties. The reduction in photon yield in the salt stressed seedlings of maize was positively correlated to net photosynthetic rate (P_n); in which the significant drop in P_n of salt stressed seedlings resulted in considerable growth reduction. Similar findings were reported by Petolino and Leone (1980) for *Phaseolus vulgaris* . Many scientists have suggested a positive correlation between decrease in chlorophyll content and salt-induced weakening of protein-pigment-lipid complex (Strogonov et al., 1970) or increased chlorophyllase (EC: 3.1.1.14) enzyme activity (Stivsev et al., 1973).

Ion distribution and accumulation

Increasing levels of NaCl induced a progressive absorption of Na and Cl in plant, agreeing with Chavan and Karadge (1986), Taban et al., (1999) and Turan et al. (2007a, b). Excessive Na concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes toxicity (Bernstein, 1963). Accumulation of Cl in the root tissue is disruptive to membrane uptake mechanisms, and these results in increased translocation of Cl to the shoots (Yousif et al., 1972).

When NaCl was applied to the soil, the levels of K in plant were reduced in accordance with the antagonism between Na and K (Alberico and Cramer, 1993; Azevedo and Tabosa, 2000). Cramer et al. (1985) showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions.

The present study showed that soil salinity inhibited plant growth and caused a decrease in biomass of maize plant. On the other hand, while stomatal resistance and proline concentration of plant were increased by salinity, total chlorophyll concentration was decreased. This suggests the critical importance of need to unravel the cellular mechanisms such as the correlation between chlorophyll content or proline accumulation and sodium intake so as to give a meaning to salt stress studies. Despite a number of studies on salinity effects on plants, neither the metabolic sites at which salt stress damages plants nor the adaptive components of salt tolerance are fully understood. Distinguishing the cellular metabolisms appears to play an important role in the acclimation of crops to salt stress.

REFERENCES

GL, Cramer GR (1993). Is the salt tolerance of maize related to sodium exclusion? I. Preliminary screening of seven cultivars. J. Plant Nutr. 16: 2289-2303.

Arnon DI (1949). Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris.* Plant Physiol. 24: 1-15.

Arzani A (2008). Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro* Cell. Dev. Biol. Plant 44: 373-383.

Ashraf M, Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Ashraf M, Foolad MR (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59: 206-216

Azevedo NAD, Tabosa JN (2000). Salt stress in maize seedlings: II. Distribution of cationic macronutrients and it's relation with sodium. Rev. Bras. Eng. Agric. Amb. 4: 165-171.

Bajji M, Lutts S, Kient JM (2001). Water deficit effects on solute contribution to osmotic adjustment as a function of leaf aging in three durum wheat (*Triticum durum* Defs.) cultivars performing differently in arid conditions. Plant Sci. 160: 669-681.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Bernstein L (1963). Osmotic adjustment of plants to saline media. II. Dynamic phase Am. J. Bot. 48: 909-918.

Cha-um S, Kirdmanee C (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. Pak. J. Bot. 41: 87-98.

Chatrath A, Mandal PK, Anuradha M (2000). Effect of secondary salinization on photosynthesis in fodder oat (*Avena sativa* L.) genotypes. J. Agronomy Crop Sci. 184: 13-16

Chavan PD, Karadge BA (1986). Growth, mineral nutrition, organic constituents and rate of photosynthesis in *Sesbania grandiflora,* L. grown under saline conditions. Plant Soil, 93: 395-404.

Cramer GR, Lauchli A, Polito VS (1985). Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cell. A primary response to salt stress? Plant Physiol. 79: 207-211.

Cusido RM, Palazon J, Altobella T, Morales C (1987). Effect of salinity on soluble protein, free amino acids and nicotine contents in *Nicotiona rustica* L. Plant Soil, 102: 55-60.

Demiral T, Türkan (2005). Comparative lipid peroxidation, antioxidant defence systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53: 247-257.

Desingh R, Kanagaraj G (2007). Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Gen. Appl. Plant Physiol. 33: 221-234.

Eker S, Cömertpay G, Konu kan Ö, Ülger AC, Öztürk L, Çakmak I (2006). Effect of salinity on dry matter production and ion accu-mulation in hybrid maize varieties. Turk. J. Agric. For. 30: 365-373.

Food and Agricultural Organization, FAO (2008). Land and plant nutrition management service. Available online at: http://www.fao.org/ag/agl/agll/spush/ Accessed 25 April.

Hong-Bo S, Xiao-Yan C, Li -Ye C, Xi-Ning Z, Gangh W, Yong-Bing Y, Chang- Xing Z, Zan-Min Z (2006). Investigation on the relationship of proline with wheat anti-drought under soil water deficits. Colloids Surf. B. Biointerfaces 53: 113-119.

Johnson CM, Ulrich A (1959). Analytical methods for use in plant analysis. California Agric. Exp. Stn. Bulletin, 766, pp. 44-45.

Katerji N, Van HJW, Hamdy A, Mastrorilli M (2004). Comparison of corn yield response to plant water stress caused by salinity and by drought. Agric. Water Manage. 65: 95-101.

Kavi KPB, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88: 424-438.

Koca H, Bor M, Özdemir F, Türkan I (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ. Exp. Bot. 60: 344-351.

Lambers H (2003). Introduction, dry land salinity: a key environ. issue in Southern Australia. Plant Soil 257: 5-7.

Lewitt J (1980). Salt stresses. In: Responses of plants to environ. stresses. Vol II. Academic Press. New York, USA, pp. 365-454.

Lutts S, Kinet JM, Bouharmont J (1996). Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Plant Growth Reg. 19: 207-218.

Mansour MMF, Salama KHA, Ali FZM, Hadid AFA (2005). Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. Gen. Appl. Plant Physiol. 31: 29-41.

Marschner H (1995). Mineral nutrition of higher plants. Academic Press, London.

Miller OR (1998). Nitric-perchloric acid wet digestion in an open vessel. In Kalra YP (eds) Handbook of reference methods for plant analysis. CRC Press, ISBN, 1-57444-1248.

Misra N, Gupta AK (2005). Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Sci. 169: 331-339.

Munns R (2005). Genes and salt tolerance: Bringing them together. New Phytol. 167: 645-663.

Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: A Rev. Ecotoxicol. Environ. Safety, 60: 324-349.

Pessarakli M, Tucker TC (1988). Dry matter yield and nitrogene^{15} uptake by tomatoes under sodium and chloride stress. Soil Sci. Soc. Am. J. 52: 698-700.

Petolino JF, Leone IA (1980). Saline aerosol. Some effects on the physiology of *Phaseolus vulgaris* (Cultivar Toporop). Phytopathol. 70: 225-232.

Rodriguez HG, Roberts JKM, Jordan WR, Drew MC (1997). Growth, water relations, and accumulation of organic and inorganic solutes in roots of maize seedlings during salt stress. Plant Physiol. 113: 881-893.

Seeman JR, Sharkey TD (1986). Salinity and nitrogen effects on photosynthesis, ribulose-1,5-biphosphate carboxylase and metabolite poll sizes in *Phaseolus vulgaris* L. Plant Physiol. 82: 555-560.

Siegel SM, Siegel BZ, Massey J, Lahne P, Chen J (1980). Growth of corn in saline waters. Physiol. Planta, 50: 71-73.

Stivsev MV, Ponnamoreva S, Kuznestova EA (1973). Effect of salinization and herbicides on cholorophyllase activity in tomato leaves. Fiziol. Rast. 20: 62-65.

Strogonov BP, Kabanov VV, Shevjakova NI, Lapina LP, Komizerko EI, Popov BA, Dastanova RKh, Prykhod'ko LS (1970). Structure and function of plant cell under salinity. Moscow, Nauka, Russia.

Taban S, Ozguven N, Çelik H, Katkat AV (1999). Effect of potassium on macroelements distribution in maize plant grown under salt stress. Dahlia greidinger Int. symposium nutrient management under salinity and water stress, 1-4 March, Hafia- Israel.

Turan MA, Türkmen N, Taban N (2007a). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants J. Agron. 6: 378-381.

Turan MA, Katkat V, Taban S (2007b). Variations in proline, chlorophyll and mineral elements contents of wheat plants grown under salinity stress. J. Agronomy 6: 137-141.

Veeranagamallaiah G, Chandraobulreddy P, Jyothsnakumari G, Sudhakar C (2007). Glutamine synthetase expression and pyrroline-5-carboxylate reductase activity influence proline accumulation in two cultivars of foxtail millet (*Setaria italica* L.) with differential salt sensitivity. Environ. Exp. Bot., 60: 239-244.

Wang Z, Dai Q, Liu X, Want Z, Li J (1987). Some physiological and biochemical differences between salt tolerant and sensitive rice "*Oryza sativa* L" genotypes in response to salinity. Philippine J. Crop Sci. 13: 159-164.

Yousif HY, Bingham FT, Yermason DM (1972). Growth, mineral composition, and seed oil of sesame (*Sesamum indicum* L.) as affected by NaCl. Soil Sci. Soc. Am. Proc. 36: 450-453.