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Salinity-Induced Stomatal Resistance, Proline, Chlorophyll and Ion Concentrations of Bean

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Abstract: Salinity-induced effects on the growth and stomatal resistance and proline, total chlorophyll, Na, Cl and K concentrations of bean (*Phaseolus vulganis* L. cv: Strike) were investigated. In the greenhouse experiment, salt was applied to the soil which was taken from Aridisol soil group at the concentrations of 0 and 100 mM as NaCl. NaCl treatment inhibited the growth and significantly decreased the dry mass of bean plants. While stomatal resistance, proline, Na and Cl concentrations of plants were increased by high salinity, total chlorophyll and K concentrations of the bean were decreased.

Key words: Bean, growth, salinity, stomatal resistance, proline, chlorophyll

INTRODUCTION

The extension of irrigated agriculture and the intensive use of water resources combined with high evaporation rates, have drawn attention to the problems of salinity in the soil and in ground water (Lambers, 2003). Not only uncontrolled irrigation but also poor-quality water may cause salinity problems (Tanji, 1990; Maas and Grattan, 1999).

Salinity and salinization threaten agricultural land in the world, especially in arid and semiarid regions (De Pascale and Barbieri, 1997). It is the major environmental factor limiting plant growth and productivity (Allakhverdiev *et al*., 2000). The detrimental effects of high salinity, mainly excessive accumulation of Na and Cl ions, on plants can be observed at the whole-plant level as limited plant growth, the death of plants and/or decreases in productivity. Excessive exchangeable sodium ions decrease the soil permeability and infiltration capacity of the soils, leading to a decrease in growth and yield of plants (Mass and Hoffman, 1977).

Plants adaptation to salinity is associated with osmoregulation adjustment by using some osmotic regulators (such as: Include potassium, soluble sugar, proline and betaine) that are important physiological indicators for evaluating osmotic adjustment ability (Yordanov *et al*., 2003; De Escalada Pla *et al*., 2005; Munns, 2005; Hong-Bo *et al*., 2006).

Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected. The earliest response is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stres intensifies. Growth resumes when the stres is relieved. Carbohydrates, which among other substrates are needed for cell growth, are supplied mainly through the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Parida and Das, 2005).

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The objective of this research is to examine the influence of soil salinity on the growth and stomatal resistance and proline, total chlorophyll and mineral element concentrations of bean plants.

MATERIALS AND METHODS

The experimental soil taken from Aridisol great soil group is calcareous (252 g kg^{-1}), clay loam in texture (clay 31.5%, silt 40.8% and sand 27.7%), slightly alkaline (pH: 8.14, EC: 0.102 dS cm^{-1}; both in 1:2.5 water extract). The soil sample has 40.9 mg kg^{-1} exchangeable Na and 5.53 mg kg^{-1} water extractable Cl.

In the greenhouse experiment, the soil (2000 g) was placed into the pots and soil was salinized with NaCl at the rates of 0 and 100 mM NaCl. On the other hand, for basal fertilizers, 100 mg N kg^{-1} as ammonium nitrate, 60 mg P kg^{-1} as triple super phosphate and 75 mg K kg^{-1} as potassium sulfate were applied to the all pots.

Six seeds of bean (*Phaseolus vulgaris* L. cv: Strike) were planted in each pot, thinned to three per pot after germination. Eight weeks from germination, the vegetative growth was harvested. After weighting the fresh material, plants were washed and sampled taking 2.0 g of fresh material for total chlorophyll and proline determinations. The remaining plant samples were dried at 65°C in order to determine dry mass and the concentrations of Na, Cl and K.

Sodium and potassium were determined by flame photometer (Eppendorf Elex 6361 model). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

Proline was determined 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 mLof 3 % sulfosalicylic acid and filtered through Whatman No 2 filter papers and determined in Shimadzu UV-1201 model spectrophotometer, using pure proline (Merck, L-Proline, Art.7434) as a standard.

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).

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 each crop, situated at the different position of the canopy. Air temperatures (27°C), relative air humidity (65%) and light intensity (340-400 μmol m^{-2} s^{-1}) were also recorded during stomatal resistance measurements.

The greenhouse experiment was arranged in a completely randomized design with three replicates, including four salts treatments.

The data obtained from different analysis and measurements were analyzed statistically by using Minitab package program (Minitab Release 10.51) and treatment means were compared using the MSTAT package program (Version 3.00) for Duncan's Multiple Range Test.

RESULTS

NaCl application inhibited significantly ($p<0.001$) the growth of bean plant and it was unable to complete its life cycle under high salt concentrations (Table 1). The dry weights were significantly decreased. The decreases was calculated as -57.66% at 100 mM NaCl application (Table 1).

Stomatal resistance of bean plants was influenced by salinity and the augmentation was significant ($p<0.001$, Table 2).

Total chlorophyll (chlorophyll a+b) and proline concentrations of bean plants were affected significantly ($p<0.001$) by salinity (Table 2). NaCl treatment had a negative correlation with total chlorophyll concentration, however, increased the proline contents of bean plants.

Table 1: Salinity induced effects on dry weight

NaCl (mM)	Dry weight (g pot^{-1})	Change (%)
0	4.63±0.31a	-
100	1.96±0.08b	-57.66
Treatments	***	***

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

Table 2: Salinity induced effects on stomatal resistance, proline and total chlorophyll (chlorophyll a+b) concentrations and of bean plants

NaCl (mM)	Stomatal resistance (s cm^{-1})	Proline, (μmol g^{-1} fresh weight)	Total chlorophyll (mg g^{-1} fresh weight)
0	0.92±0.02 a	0.91±0.06a	6.86±1.72a
100	2.68±0.09b	5.11±0.89b	2.13±0.61b
Treatments	***	***	***

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

Table 3: Salinity induced effects on Na, Cl, K concentrations and K/Na ratio of bean

NaCl (mM)	Na (g kg^{-1})	Cl (g kg^{-1})	K (g kg^{-1})	K/Na
0	1.3±0.2a	6.3±0.6a	34.0±3.7a	26.15±4.1a
100	12.5±0.4b	35.62±6.8b	13.8±2.1b	1.104±2.6b
Treatments	***	***	***	***

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

NaCl salinity effected ion contents of bean plants (Table 3). Salt treatments increased significantly ($p<0.001$) Na and Cl concentrations, whereas decreased the potassium concentration and K/Na ratio of plants (Table 3).

DISCUSSION

The proportion of dry mass decreased with salinity, mentioned by Koyro (2006). High concentrations of NaCl inhibited growth of bean plants. This might be due to the toxic effect of NaCl or increased crucial osmotic pressure at which the plant under stress would not be able to uptake the required water (Cusido *et al*., 1987; Haeidari-Sharifabad and Mirzaie-Nodoushan, 2006).

In the present study with bean plants, 100 mM NaCl treatment reduced plant growth. Lauchli (1984) emphasized that increased Na and Cl absorption not only resulted in deficiency in nutrient uptake but also consequent insufficient plant growth.

Stomata are small pores at the surface of leaves which aperture is controlled by 2 guard cells. When they are open, the stomata allow gas exchange, mainly CO_2 for photosynthesis and H_2O, between the leaf and the atmosphere. During a drought stress, mainly due to salinity or limited irrigation, the plant has to close their stomata to limit water loss (Chatrath *et al*., 2000). Measurement of stomatal resistance provides effectual comparison for determining the degree of stress in plants.

It was found that in bean plants salinity increased the stomatal resistance, which could be explained by inhibition of plant growth due to water stress (Chatrath *et al*., 2000). Stomatal resistance and net photosynthesis ratio (P_N) have a strong negative correlation, which indicates that as salinity increases stomatal factors have a more signifcant effect on photosynthesis. This was also reported in spinach (Downtown *et al*., 1985) and rice (Wang *et al*., 1987). Plant photosynthesis might be limited by lower water availability and/or possible the toxic effect caused by salt accumulation becomes visible at higher Na and/or Cl concentration, as proposed for the limitation of salt-stressed plant growth (Neumann, 1997).

A positive correlation was determined between proline concentrations and tissue Na-ion concentrations at the during salt stress (Irrigoyen *et al*., 1992; Kundu and Paul, 1997; Bajji *et al*., 2001).

The levels of proline in the plants is mainly designates their ability to tolerate or to adapt to saline conditions. Accumulation of proline in tissues of the plants as a response to drought, salt stress or temperature stress has been attributed to enzyme stabilization and/or osmoregulation system of the plant itself (Madan *et al*., 1994).

The total chlorophyll concentration of bean leaves was reduced by increased level of NaCl treatment. Petolino and Leone (1980) reported that salinity decreased the total chlorophyll concentration of *Phaseolus vulgaris* plant, suggesting a salt-induced weakening of protein-pigment-lipid complex (Strogonov *et al*., 1970) or increasing chlorophyllase (EC: 3.1.1.14) enzyme activity (Stivsev *et al*., 1973).

Under saline conditions, not only in the soil but also in the plant, dominant elements are sodium and chloride, therefore, ionic balance of both soil and plant displays a tendency to accumulate of Na and Cl than the other ions present. For bean plants, Na and Cl concentrations increased in parallel to increased levels of NaCl treatment. These results are in agreement with Alpaslan *et al*. (1999) and Taban *et al*. (1999). Increasing NaCl decreased K concentration of bean plants suggesting an antagonism between Na and K as stated by Siegel *et al*. (1980) and De Pascale *et al*. (2005).

Competition effects between different anions (Bar *et al*., 1997; Kafkafi *et al*., 1982) and different cations (Subbarao *et al*., 1990) are known to occur in saline environment and they may result deleterious for normal plant growth (Grattan and Grieve, 1999). Increasing NaCl decreased K concentration of bean plants suggesting an antagonism between Na and K as stated by Siegel *et al*. (1980)

Overall, soil salinity significantly inhibited growth and decreased dry mass, total chlorophyll and K concentrations of bean plants, however, increased the stomatal resistance, proline and Na and Cl concentrations.

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REFERENCES

Allakhverdiev, S.I., A. Sakamoto, Y. Nishiyama, M. Inaba and N. Murata, 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. Plant Physiol., 123: 1047-1056.

Alpaslan, M., A. Gunes and S. Taban, 1999. Salinity resistance of certain rice (*Oryza sativa* L.) cultivars. Tr. J. Bot., 23: 499-506.

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

Bajji, M., S. Lutts and J.M. Kient, 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.

Bar, Y., A. Apelbaum, U. Kafkafi and R. Goren, 1997. Relationship between chloride and nitrate and its effect on growth and mineral composition of avocado and citrus plants. J. Plant Nutr., 20: 715-731.

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205-207.

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

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

De Escalada Pla, M.F., N.M. Ponce and M.E. Wider, 2005. Chemical and biochemical changes of pumpkin (*Cucumis moschata*, duch) tissue in relation to osmotic stress, J. Sci. Food Agric., 85: 1852-1860.

De Pascale, S. and G. Barbieri, 1997. Effect of soil salinity and top removal on growth and yield of broad bean as green vegetable. Sci. Hortic., 71: 147-165.

De Pascale, S., A. Maggio and G. Barbieri, 2005. Soil salinization affects growth, yield and mineral composition of cauliflower and broccoli. Eur. J. Agron., 23: 254-264.

Downtown, W.J.S., W.J.R. Grant and S.P. Robinson, 1985. Photosynthetic and stomatal response of spinach leaves exposed to salt stress. Plant Physiol., 67: 74-77.

Grattan, S.R. and C.M. Grieve, 1999. Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic., 78: 127-157.

Heidari-Sharifabad, H. and H. Mirzaie-Nodoushan, 2006. Salinity-induced growth and some metabolic changes in three salsola species. J. Arid Environ., 67: 715-720.

Hong-Bo, S., C. Xiao-Yan, C. Li-Ye, Z. Xi-Ning, W. Gangh, Y. Yong-Bing, Z. Chang-Xing and Z. Zan-Min, 2006. Investigation on the Relationship of Proline with Wheat Anti-Drought Under Soil Water Deficits. Colloids and Surfaces Biointerfaces, 53: 113-119.

Johnson, C.M. and A. Ulrich, 1959. Analytical Methods for Use in Plant Analysis. California Agricultural Experiment Station Bulletin, CA, USA.

Irrigoyen, J.J., D.W. Emerrich and M. Sanchez-Diaz, 1992. Water stress induced damages in concentration of proline and total soluble sugars in nodulated alfalfa plant. Physiol. Plant, 84: 55-60.

Kafkafi, U., N. Valoras and J. Letey, 1982. Chloride interaction with nitrate and phosphate nutrition in tomato (*Lycopersicon esculentum* L.). J. Plant Nutr., 5: 1369-1385.

Koyro, H.W. 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environ. Exp. Bot., 56: 136-146.

Kundu, P.B. and N.K. Paul, 1997. Effect of water stress on chlorophyll, proline and sugar accumulation in rape (*Brassica compestris* L.). Bangladesh J.Bot., 26: 83-85.

Lambers, H. 2003. Dryland Salinity: A key environmental İssue İn southern australia. Plant and Soil, 257: 5-7.

Lauchli, A., 1984. Salt exclusion: An adaptation of legume for crops and pastures under saline condition. In salinity tolerance in plants. Strategies for Crop Improvement, Stoples, R.C. and G.H. Toenniessen (Eds.) John Willey and Sons, N.Y.

Maas, E.V. and G.J. Hoffman, 1977. Crop salt tolerance-current assessment. J. Irri. Drain. Division ASCE, 103: 115-134.

Maas, E.V. and S.R. Grattan, 1999. Crop Yields as Affected by Salinity. In Agron. Monogr., Skaggs, R.W. and J. van Schilgaarde (Eds.) No. 38. Am. Soc. Agron., Madison, WI, USA.

Madan, S., H.S. Nainawatee, S. Jain, R.K. Jain, M.S. Malik and J.B. Chowdhury, 1994. Leaf position-dependent changes in proline, proline-5-carboksilate reductase activity and water relations under salt stress in genetically stable salt-tolerant samaclones of *Brassica juncea* L. Plant and Soil, 163: 151-156.

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

Neumann, P., 1997. Salinity resistance and plant growth revisited. Plant Cell Environ., 20: 1193-1198.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A Rev. Ecotoxicol. Environ. Safety, 60: 324-349.

Petolino, J.F. and I.A. Leone, 1980. Saline aerosol. Some effects on the physiology of *Phaseolus vulgaris* (Cultivar Toporop). Phytopathology, 70: 225-232.

Siegel, S.M., B.Z. Siegel, J. Massey, P. Lahne and J. Chen, 1980. Growth of corn in saline waters. Physiol. Plant, 50: 71-73.

Stivsev, M.V., S. Ponnamoreva and E.A. Kuznestova, 1973. Effect of salinization and herbicides on chlorophyllase activity in tomato leaves. Fiziol. Rast., 20: 62-65.

Strogonov, B.P., V.V. Kabanov, N.I. Shevjakova, L.P. Lapina, E.I. Komizerko, B.A. Popov, R.Kh. Dastanova and L.S. Prykhod'ko, 1970. Structure and function of plant cell under salinity. Moscow, Nauka, Russia.

Subbarao, G.V., C. Johansen, M.K. Jana and J.V.D.K. Kumar Rao, 1990. Effects of the sodium/calcium ratio in modifying salinity response of pigeonpea (*Cajanus cajan*). J. Plant Physiol., 136: 439-443.

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

Tanji, K.K. 1990. Nature and extent of agricultural salinity. In agricultural salinity assessment and management. American society of civil engineers., Tanji, K.K. (Ed.) Manual and Report Engineering Practice, 71: 71-92.

Wang, Z., Q. Dai, X. Liu, Z. Want and J. Li, 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.

Yordanov, I., V. Velikova and T. Tsonev, 2003 . Plant responses to drought and stres tolerance. Bulg. J. Plant Physiol., 187-206.