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Effect of Salinity on Organic Solutes Contents in Barley

F. Khosravinejad, R. Heydari and T. Farboodnia
Department of Biology, Faculty of Science, University of Urmia, Urmia, Iran

Abstract: Salinity (NaCl Stress) was applied with 50, 100, 200, 300 and 400 mM NaCl. The shoot and root water content and organic solutes contents of two barley varieties (*Hordeum vulgare* L. var. Afzal and var. EMB82-12) were determined in various concentrations of NaCl. Soluble sugar and proline contents were increased in two barley varieties in response to increased salt concentration, but this increase in Afzal var. were higher than EMB82-12. Soluble protein content was decreased in two barley varieties in response to different salt regimes and this decrease in Afzal var. was lower than EMB82-12 var. RWC decreased with increasing NaCl concentrations. Decrease of water content in EMB82-12 plants was higher than Afzal plants.

Key words: Barley, proline, RWC, salinity, soluble sugar

INTRODUCTION

Soil salinization is one of the major factors of soil degradation. It has reached 19.5% of the irrigated land and 2.1% of the dry-land agriculture existing on the globe. Salinity effects are more conspicuous in arid and semi-arid areas where 25% of the irrigated land is affected by salts. The increase of salt-affected soils due to poor soil and water management in the irrigated areas, the salinity problem became of great importance for agriculture production in this region. Salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002). Plants resort many adaptive strategies in response to abiotic environmental stresses such as salinity. Among them, the accumulation of compatible solutes according to the metabolic responses has drawn much attention. Adaptation to all these stresses is associated with metabolic adjustments that lead to the accumulation of several organic solutes like sugars, polyols, betaines and proline (Hanson, 2002). Changes in protein expression, accumulation and synthesis have been observed in many plant species as a result of plant exposure to drought stress during growth (Verbruggen and Hermans, 2008). Some of chemical materials (for example ABA) which are induced in response to salt stress might prevent protein synthesis (Rao, 2006). The relative water content decreased significantly with salinity (El-Tayeb, 2005). Furthermore salinity decreased the relative water content in seedling of salt-sensitive cultivars.

In this research, soluble sugar and proline content as well as the relative water content of two barley varieties under different salinity regimes were studied.

The higher relative water content of salt stressed plants which makes plants more tolerant to the salt induced water deficient can be related to the induced accumulation of soluble sugar and proline in the more tolerant plant variety. The result of this study suggests that soluble sugar and proline content of barley varieties might play a crucial role in the moderate tolerance of some barely varieties to the salt stress and drought condition.

MATERIALS AND METHODS

This study was conducted at Biochemistry Laboratory, Department of Biology, Urmia University, Iran, during the winter of 2007. Two genotypes of Barley (*Hordeum vulgare* L.) were used: var. Afzal and var. EMB82-12 which these seeds were obtained from the Agricultural Research Center of Karaj, Iran. Seeds were surface sterilized in 0.5% sodium hypochloride solution for 20 min and grown in pots containing Vermiculite. Plants were watered every second day using half strength of Hoagland nutrient solution in controlled growth room for 4 days, then seedlings were subjected to treatment with 50, 100, 200, 300 and 400 mM NaCl for 3 days. Shoot and root to be used for biochemical determinations were frozen and stored in liquid nitrogen immediately after harvest. Soluble sugars were determined based on the method of phenolsulfuric acid (Dubois *et al*., 1956), 0.5 g fresh weight of roots and shoots was homogenized with deionized water, extract was filtered and the extract treated with 5% phenol and 98% sulfuric acid, mixture remained for 1 h and then absorbance at 485 nm was determined by spectrophotometer (Biochrom S 2100). Contents of soluble sugar were expressed as mg g^{-1} FW. Free proline accumulation was determined using the method of Bates

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Corresponding Author: Fariba Khosravinejad, Department of Biology, Faculty of Science, University of Urmia, Urmia, Iran

(Bates *et al.*, 1973), 0.04 g dry weight of roots and shoots was homogenized with 3% sulfosalicylic acid and after 72 h that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined by UV-visible spectrophotometer (Biochrom S 2100). Contents of proline were expressed as mg g^{-1} DW. Total soluble protein content was determined according to the method of Lowry *et al.* (1951). Relative water content was determined with following equation:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Fresh weight of the plants was measured and after that plants were dried at 105°C until reached constant weight for the determination of dry weight. To determine the turgid weight, samples were soaked in distilled water for 4 h at room temperature (approximately 20°C) and then turgid weight was measured (Fletcher *et al.*, 1988).

Statistical analysis: Mean values were calculated from measurements of four replicates and the SE of the means were determined. One-way ANOVA and Tukey HSDs multiple range test ($p<0.05$) was applied to determine the significance of the result between different treatments. All statistical analysis were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 13.0.0).

RESULTS

Salinity caused an increase in shoot and root soluble sugar and proline contents in both varieties, but the increase in EMB82-12 variety was lower than Afzal var. In 400 mM NaCl, shoot soluble sugar increase was 42.82% in EMB82-12 var. and 43.95% in Afzal var. and root soluble sugar increase was 39.10% in EMB82-12 var. and 60.33% in Afzal var., as compared to control plants (Fig. 1a,b). In highest salinity, shoot proline content increase was 93.33% in EMB82-12 var. and 94.05% in Afzal var. as compared to control plants. Root proline content increase was 92.22% in EMB82-12 var. and 92.73% in Afzal var. as compared to control plants (Fig. 2a,b). The increase of soluble sugar and proline contents in Afzal var. was higher than EMB82-12 plants. Shoot protein content decreased in both varieties and in 400 mM NaCl, reduction of this factor was 58.40% in EMB82-12 var. and 56.01% in Afzal var., root protein content reduction was 22.66% in EMB82-12 var. and 18.07% in Afzal var. as compared to control (Fig. 3a,b). It means that Afzal plants have lower protein content reduction than EMB82-12 var. when salt stressed. Relative water content in root and shoot decreased with increasing NaCl concentrations. In 400 mM NaCl, this decrease was enormous in root and shoot in both varieties. In 400 mM NaCl, RWC reduction was 73.65% in EMB82-12 var. and 37.98% in Afzal var. in root and 61.01% in EMB82-12 var. and 24.39% in Afzal var. in shoot as control plants. RWC reduction in Afzal var. was lower than EMB82-12 plants (Fig. 4a,b).

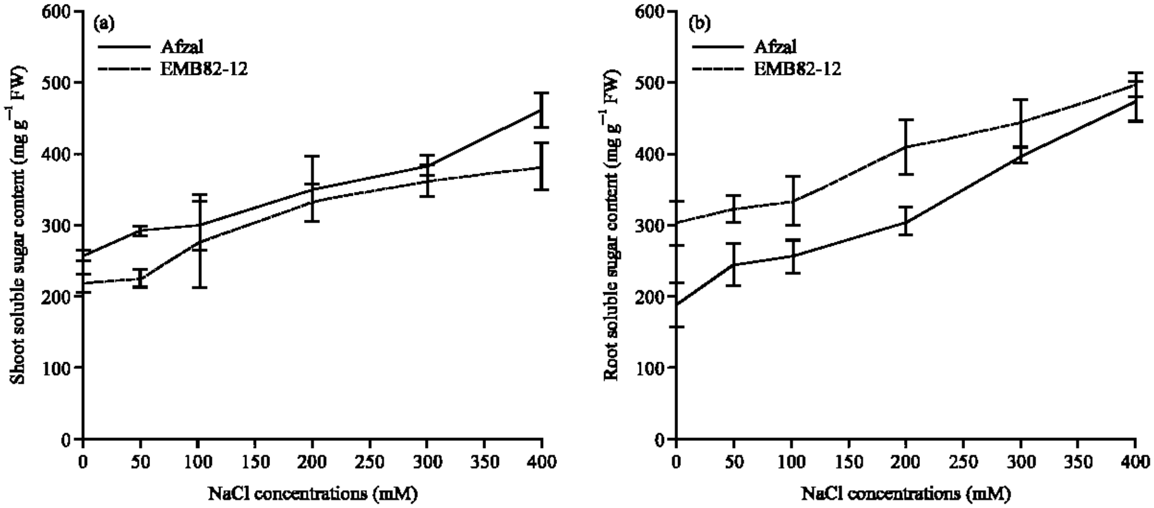

Fig. 1: Effects of different NaCl concentrations on (a) shoot and (b) root soluble sugar content in two barley varieties. Results are shown as Mean±Standard error ($p<0.05$), obtained from four replicates

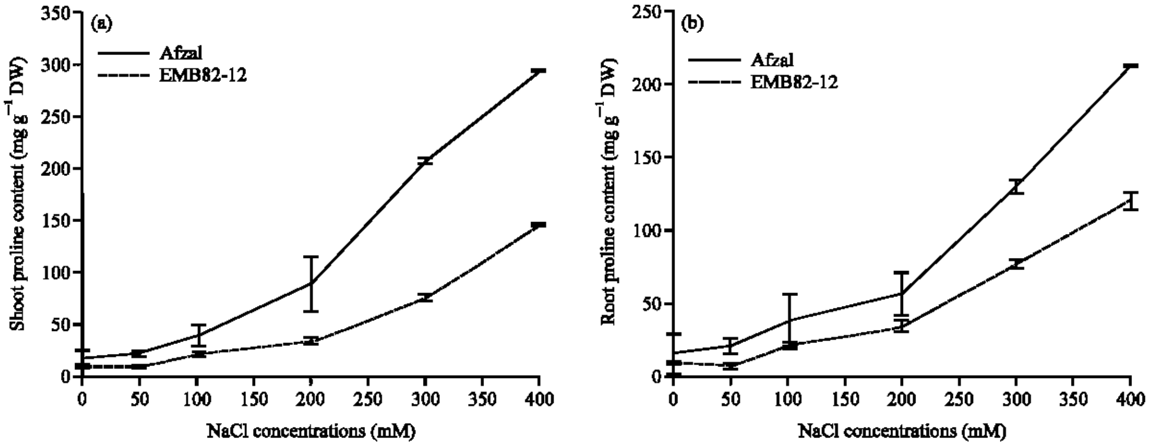

Fig. 2: Effects of different NaCl concentrations on (a) shoot and (b) root proline content in two barley varieties. Results are shown as Mean±Standard error ($p<0.05$), obtained from four replicates

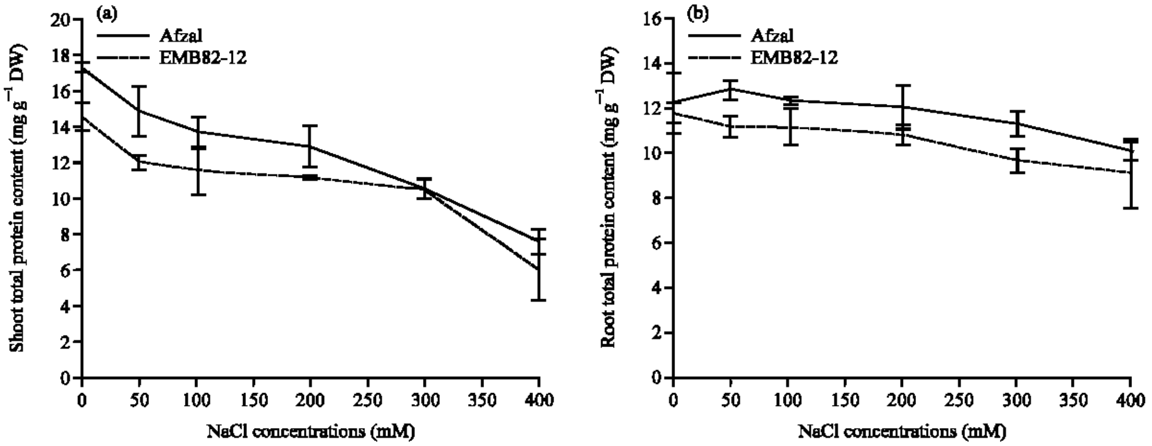

Fig. 3: Effects of different NaCl concentrations on (a) shoot and (b) root protein content in two barley varieties. Results are shown as Mean±Standard error ($p<0.05$),obtained from four replicates

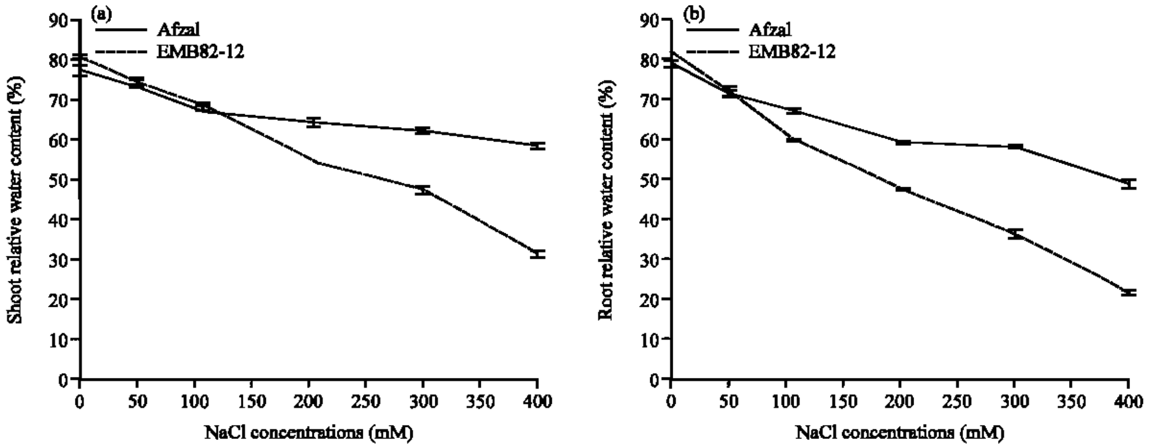

Fig. 4: Effects of different NaCl concentrations on (a) shoot and (b) root water content in two barley varieties. Results are shown as Mean±Standard error ($p<0.05$), obtained from four replicates

DISCUSSION

Earlier reports mentioned that sugars protect the cells during stress. The hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins. Thus, sugars interact with proteins and membranes through hydrogen-bonding, thereby preventing protein denaturation (Sanchez *et al.*, 1995). Accumulation of soluble sugars may be to counter the osmotic stress. The salt tolerance mechanism may be associated with accumulation of osmoprotectants such as proline and soluble sugars (Morgan, 1984). The concentration of soluble sugars increased under salt stress in both varieties in our study but increase in Afzal var. was higher than EMB82-12 var., this showed that Afzal var. have more tolerant than EMB82-12 var. Among amino acids, the accumulation of proline is frequently reported in many plants or tissues in response to a variety of abiotic stresses (Hare and Cress, 1997). Proline (Pro) accumulation is a common physiological response in many plants in response to a wide range of biotic and abiotic stresses. Controversy has surrounded the possible role(s) of proline accumulation. In a review, knowledge on the regulation of Pro metabolism during development and stress, results of genetic manipulation of Pro metabolism and current debate on Pro toxicity in plants are presented (Verbruggen and Hermans, 2008). Whether it is to act as an osmo-regulator (Delauney and Verma, 1993), an osmo-protector (Csonka, 1989), or a regulator of the redox potential of cells (Hare and Cress, 1997) has not been decided. Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes and repressed activity of proline degradation (Delauney and Verma, 1993; Peng *et al.*, 1996). This leads to a proline cycle, the homeostasis of which depends on the physiological state of tissue. Proline catabolism is catalyzed by proline dehydrogenize and P5C dehydrogenize (Peng *et al.*, 1996). Evidence for the transport of proline to the root tip, where is accumulates during stress, has been reported by Verslues and Sharp (1999). The increase of proline in severe salt stress in our experiment was consistent with them. The data indicate that plants may have evolved a mechanism to coordinate synthesis, catabolism and transport activities for the accumulation of proline. During the experiment, we found that Afzal var. had increase of proline content higher than EMB82-12 var. The means differences of proline content in both varieties were significant at the 0.05 level between all treatments. It means that Afzal var. had higher tolerance than EMB82-12 var. in severe salt stress. These results indicate that proline accumulation by repressed catabolic pathway under oxidative stress helps plants to decrease oxidative damage. Usually the magnitude of proline accumulation is relatively dependent on the levels of carbohydrates (Morgan, 1984). Larher mentioned that sucrose was a positive effector of proline accumulation (Larher *et al.*, 1993). In this study, protein reduction in EMB82-12 var. was higher than Afzal var. It seems that the decrease in total soluble proteins during salt stress was due to a severe decrease in photosynthesis. Photosynthesis decreased in salinity (Lee *et al.*, 2004) and materials for protein synthesis weren't provided; therefore, protein synthesis dramatically reduced or even stopped. The decrease in total soluble proteins under drought stress was consistent with the findings of André Dias *et al.* (2004) in sorghum, Garg *et al.* (1997) in cluster bean and Surabhi *et al.* (2008) in mulberry (*Morus alba* L.). These authors reported that salinity resulted in a decrease of some soluble proteins. Soil water potential reduction and plant ability reduction in absorbing water lead to relative water content reduction in salt stress (Munns, 2003). The decrease of water content in EMB82-12 plants was higher than Afzal plants. Afzal plants have higher water content than EMB82-12 plants. This study showed that plants grown at high salinity will be more tolerant to salt stress if they can mange K^+/Na^+ imbalance properly. At salt stress plants more suffer from high accumulation of Na^+. Instead of taking up suitable amount of K^+ which is necessary for plant's normal growth and development they accumulate Na^+ to toxic level.

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