Effect of salinity stress on biochemical parameters and growth of borage (*Borago officinalis* L.)

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Abstract

Salinity is one of the most important limiting factors for growth and product of plants around the world. Identification of salt tolerant varieties and improvement of plants tolerance are the most effective way to increase its performance. Experiments were conducted in a hydroponic system in order to study the reaction of borage (*Borago officinalis L.*) to salinity in terms of developmental changes and pigment content. Salinity was applied by 100 mM Sodium Chloride and Sodium Sulfate salts by the ratio of two to one on borage at the 4-leaf stage. Wet and dry weights of shoots and roots, chlorophyll, protein, proline, anthocyanins, soluble carbohydrate, and reducing carbohydrates were determined. Decrease in fresh and dry weights of shoots and roots was observed in the salinity condition and the fresh weight loss in shoots was significant ($p<0.05$). Chlorophyll content also decreased but this was not significant. Salinity stress decreased the amount of protein in shoots and roots compared to control. Proline contents in leaves and roots increased significantly under salt stress compared to control. On the average, in comparison to control, salinity stress decreased the amount of soluble carbohydrates in roots and shoots but this reduction was not significant.The amount of reducing sugars under salinity stress increased in shoots in comparison with control but they decreased in roots. Moreover, the amount of anthocyanin increased under salinity stress in comparison with control plants. It was concluded that borage was able to resist against stress due to the changes in the contents of these compounds under salinity.

Keywords: salinity; biochemical parameters; growth; borage (Borago officinalis *L.*)

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Introduction

Environmental stresses, as the most important factors limiting the plant products, have made human deal with these stresses through various management practices. Use of chemicals against the biological stresses and some corrective actions to deal with these stresses such as drought and salinity have adverse consequences in long run such as environmental pollution and genetic segregation besides decreased stability of farming systems. Soil salinity is caused by irrigation with saline water and most important, improper drainage and accumulation of high levels of salt in the soil. Two strategies recommended are correcting soil and using tolerant plants to solve the salinity problem (Epstein**,** 1985). However, within the last decades, researchers emphasized

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the second strategy as a biological approach. In nature, difference in salt tolerance between species and varieties of a species occurs over time via evolution. Inter- and intra-species genetic variation can be used to select and improve salt tolerance in important crop plants (Ashraf and McNeilly, 2004). Plant breeding for salinity tolerance through the usual methods is difficult because the trait is quantitative (multi-gene). Response of plants to environmental stresses varies and plants ability to adapt to the intensity and duration of stress also depends on the species and stage of stress (Munns and Tester, 2008).

High concentrations of salts in the rhizosphere along with decrease in soil water potential and physiological drought stress and also ion toxicity and ion imbalance caused by salt stress will damage the plant (Munns**,** 2002). Changes in synthesis and stability of photosynthetic and non-photosynthetic pigments under salt stress affect photosynthesis and protective systems depending on some photosynthetic pigments such as flavonoids (Bertrand and Schoefs, 1999). borage *(Borago officinalis*) species is a dicotyledonous herbaceous and annual plant with simple leaves covered with tough hairs. Its leaves and flowered top branch trimmings have medical use. This plant is resistant to salt stress. It has several medicinal, industrial, and forage properties. High salt tolerance and absorption of minerals by this plant has been mentioned in some studies.

Regarding tolerance to salinity, inter- and intra- species differences are observed in the genus cabbage (Ashraf and Ali, 2008). There are differences between Canola varieties in response to various physiological markers under salinity stress (Siddiqui et al., 2008). Although salinity generally decreases pigment content, increasing effects are also observed depending on the plant species (Parida and Das 2005). There are also reports of increasing (Jamil et al., 2007) and decreasing (Shah, 2007) effect of salinity on some photosynthetic pigments in different species of the genus cabbage. In general, the responses of plants to maintain homeostasis are detoxification of harmful substances and return to growth (Hajheidari et al., 2005). So plants to counter or mitigate the effects of salinity may change the expression pattern of genes or content of proteins in their tissues (kanlaya et al., 2005). In the past few years, changes in salinity stress proteins in order to identify and understand the role of proteins in salt stress tolerance have been of considerable interest. However, still the role of majority of proteins in this regard is unknown. Several studies have identified a number of proteins that are induced by salt stress, and this reflects the complexity of biochemical and physiological responses of plants to salinity. From the results of these studies several proteins have been identified that are involved in regulation of transport of sodium or potassium (Maathuis and Ammtmann, 1999). Within the plant cells, proline acts as a substance to maintain osmotic balance between the cytoplasm and vacuole (Matysik and Mohanty, 2002). In addition, proline plays the role of assimilator as a reservoir of carbon and nitrogen. Proline also protects plants from damage by free radicals (Stewart, 19972). Accumulation of organic compounds including carbohydrates and amino acids in the cytoplasm plays an important role in osmoregulation in plants (Sanchez, et al., 1998). Anthocyanins are the most common flavonoids which are responsible for most colors such as red, pink, purple, and blue in different parts of the plant. Non-enzymatic defense system of plants includes antioxidant compounds such as anthocyanins, carotenoids, tocopherols, ascorbic acid, and phenolic compounds. Anthocyanin flavonoids are the most important antioxidant compounds. These compounds not only destroy free radicals, but also prevent their production in the plant. Anthocyanins likely facilitate the entry of salt into the cell vacuoles and its isolation from other parts (Taiz and Zeiger 2008). In this study, we aimed to evaluate the effect of salinity stress on biochemical parameters and growth of *Borago officinalis L.*

Materials and Methods

This research was carried out during 2011-2013 in Hydroponic Research Institute of Isfahan University of Technology and Laboratory of Payam-e-Noor University, Central Tehran Branch. The seeds were provided from Research Center of Neka (North of Iran) and sterilized by Benomyl fungicide 0.25 g/l; then they were washed with distilled water and were transferred to the pots

containing vermiculite for germination and then irrigated. After germination, the plants were fed to the 2-leaf stage with Long Ashton nutrient solution containing 50.6 g/l potassium, 46 g/l calcium and magnesium, 52 g/l phosphorous, 2.5 g/l iron, 0.12 g/l molybdenum, and 5.58 g/l salt. To supply macro- and micronutrients, 1.7 Mn, 0.29 zinc, 0.25 copper, 3.1 boron from Na_2MnO_4, H_3BO_3, $CuSO_4$, $ZnSO_4$, $MnSO_4H_2O$, FeEDTA, $NaHPO_4$, $MgSO_4.7H_2O$, Ca $(NO_3)_2$, and KNO_3 were used. At 4-leaf stage plants were divided into two groups of control and salinity. For salinity treatment, the plants were irrigated twice a week with two volumes of 100 mM Sodium Chloride and one volume of Sodium Sulfate. The average ambient temperature of greenhouse during the test was 21±3° C at nights and 24±3° C in days and relative humidity was 45%. PH of the nutrient solution was adjusted between 6.5 and 7 by H_2SO_4 and KOH. After salinity treatment, the desired parameters were measured.

Measurement of fresh weight (FW) of roots and shoots

Shoot was cut from the root collar. Their individual weights were measured in gram with standard scales with an accuracy of 0.001.

Measurement of dry weight (DW) of roots and shoots

To measure the dry weight, leaves, shoots, and roots were wrapped in aluminum foil and oven-dried at 70° C for 48 h and then weighed by a standard scale with an accuracy of 0.001. Plant chlorophyll content was measured by the chlorophyll metr-digit. (spad)

Statistical Analysis

This research was performed in the form of two-factorial experiment with a completely randomized block design, with minimum three and maximum seven replications. Excel software was used to plot graphs and the obtained data were submitted to one way ANOVA analysis through SPSS software (Version 19). Duncan test was used to compare means ($p \leq 0.05$).

Total protein assay (Lowry et al, 1951)

In this method, in the first step of reaction, protein-copper complex is formed in alkaline solution. Tyrosine, tryptophan, and cysteine residues of this complex, in the next phase reduce yellow Phosphomolybdic- Phosphotungstic reagent (Folin reagent) and strong blue color is caused. Since this reagent is stable only in acidic state while reduction only happens in pH=10, it is necessary to stir the mixture vigorously after adding Folin reagent so that reduction occurs before degradation of Folin reagent in alkaline solution of copper-protein. Standard curve is used to measure protein concentration.

Preparation of plant extract

First 0.02 gram of fresh tissue of leaves was weighed and each sample mixed separately with 4 ml of saline phosphate buffer (pH=7), then it was placed in a porcelain mortar in the ice and grinded and filtered by Whatman paper No. 1.

Preparation of saline phosphate buffer (extraction buffer)

This buffer is used for extraction of tissue proteins without decomposition of their structure. Solution A: 1.78 g of hydrated disodium hydrogen phosphate (Na_2HPO_4) was dissolved in distilled water and volume of the solution was brought to 100 ml.

Solution B: 1.56 g of hydrated sodium dihydrogen phosphate (Na_2HPO_4) was dissolved in distilled water and the solution volume was brought to 100 ml. After preparation of solutions B and A, 60 ml of solution A was mixed with 40 ml of solution B and then 2.925 g of sodium chloride was added. When the salt was well dissolved, saline phosphate buffer was obtained with pH=7.

Preparation of reagents

Reagent A: 2 g of sodium carbonate and 0.4 g of NaOH and 0.02 g of sodium-potassium tartarate were dissolved in distilled water and volume of the solution was brought to 100 ml. Reagent B: 0.5 g of hydrated copper sulfate $CuSO_4$ is dissolved in 100 ml distilled water.

Reagent C: contains 1 ml of reagent B and 50 ml of reagent A, which should be integrated immediately before use. Reagent D: 10 ml of Folin

phenol solution was added to 100 ml of distilled water and a diluted solution of 10:1 was obtained.

Usage of reagents in protein assay

The prepared plant extracts were centrifuged for 30 min at 5000 g and 1 ml of clear supernatant was removed. Then 4 ml of reagent C was added to the tube containing the extract and incubated for 15 min in vitro. Then 1.5 ml of diluted Folin phenol (reagent D) was added and the resulting solution was stirred vigorously. Tubes were incubated for 45 min in the dark at laboratory temperature. Using a spectrophotometer absorption was measured at the wavelength of 660 nm. Absorption was read in the least possible time by the end of maintenance period, because the color of samples remains stable only 45 minutes to one hour after the end of the maintenance period. Using standard curve, protein concentration was determined and calculated as mg/g. The solution containing distilled water instead of extract and reagents was used as control to set the spectrophotometer.

Drawing standard curve

1.4 g bovine albumin serum was dissolved in one liter of distilled water in order to draw a standard curve and concentrations of 50, 100, 200, 400, and 700 mg/liter were produced from this solution. All stages of protein measurements were done and the absorbance of solutions read at 660 nm using the spectrophotometer. Absorption curve was plotted versus concentration. Equation of a line at the wavelength of 660 nm was calculated as follows.

Y = 0.0017 X + 0.025

Where absorption intensity was applied to the Y and X, that is protein concentration was obtained as milligram per liter.

Proline assay (Bates et al., 1973)

Plant samples were dried and powdered. 0.1 g of the powder was ground in a mortar and 10 cc of Sulfosalicylic acid 3% (W / V) was added. Then, it was filtrated by a filter paper. 0.2 cc of the extract, 2 cc of Ninhydrine reagent, and 2 cc of acetic acid glacial were mixed in a test tube and put in the boiling water bath for one hour. When the tubes got cold, 4 cc of toluene was added to each tube (under a hood) and the tubes were shaken vigorously for 17 to 20 seconds so that two phases were separated (due to its low weight, toluene moves up and color solution moves down.); then, two tubes were fixed so that the two phases were separated where the pink organic phase moved up and transparent colorless aqueous phase moved down). The pink organic phase was used for colorimetery. Blank included 2 cc Sulfosalicylic acid 3% plus 2 cc of Ninhydrine plus 2 cc of acetic acid glacial that was put in hot water bath at 100° C for one hour and after cooling, 4 ml of toluene was added and shaken for 17 to 20 seconds. Then it was fixed so that two phases were separated. First, the spectrophotometer was set on zero with blank at the wavelength of 520 nm and then each color solution related to the sample was poured in the cuvette and its absorption was read.

Ninhydrine reagent: 1.25 g of Ninhydrine powder was poured in 20 ml of acetic acid glacial and 20 cc of 6 M phosphoric acid dissolved for 20 minutes by heating and shaking on a shaker at 60° C. Standard curve of proline was obtained using the following equations:

$$X=\frac{0.0075-y}{0.0029}$$

where y and x are the absorption intensity and the amount of proline in micromole per liter.

$$X^{*}=\frac{A\times B\times C}{DW\times 1000}$$

where a is proline content obtained from the standard curve as micromole per liter, B= 4 cc of the added toluene, C= 10 cc of Sulfosalicylic acid 3%, DW= dry weight of root and stem tissue, 0.1 g, and X^{*}= proline as micromole per gram of dry weight of plant tissue.

Soluble carbohydrate assay

Soluble carbohydrate assay was performed according to Fales method (1951). 0.1 g of the plant sample was ground in 5 cc of 80% ethanol in a mortar, and evened. The extract was

then put in hot water bath at 90 °C for 60 minutes, in two 30-minute phases, and between these two phases the tubes were opened and 80% ethanol was added to compensate the evaporated amount. Then the extract was filtered with a Whatman filter paper No. 1 and alcohol was allowed to evaporate. Resulted deposit was dissolved in 2.5 cc of distilled water and 200 ml of each sample was poured in the test tube and 5 cc of Anthrone reagent was added to each tube. Samples were put in a hot water bath for 17 minutes at 90 °C and after cooling on ice, the sample absorbance was measured by a spectrophotometer at 625 nm wavelength.

Reduction carbohydrate assay

Reduction Carbohydrate Assay is based on the method of Somogyi (1952). In this method, 0.02 g of the plant sample is ground with 100 cc of distilled water in a mortar and the mortar content is transferred into test tubes. The tubes are placed in a big beaker containing water and heated on the stove. When the water starts to boil, the tubes are removed from heat about 10-15 minutes later and contents of each test tube are filtered by filter paper. Two cc of the extract is poured into another test tube and 2 cc of copper sulfate solution are added to each tube. The tubes are closed with cotton and kept for 20 minutes at 100 °C in a water bath. In this phase, Cu^{2+} is reduced by aldehyde monosaccharide and turns into Cu_2O and the tubes' bottom turn into brick red. When the tubes are cooled, 2 cc of Phosphomolybdic acid is added to the tubes and a few moments later the blue color appears. Tubes are shaken vigorously until the color is evenly spread. Then absorption intensity of each tube is read at wavelength of 600 nm. The blank is used to set spectrophotometer on Zero.

Blank

Blank containing 2 cc of distilled water and 2 cc of copper sulfate was poured in a test tube and kept in a water bath for 20 minutes at 100 °C and when the tube was cooled, 2 cc of Phosphomolybdic acid solution was added. Standard curve obtained for reduced carbohydrate:

$$Y = 0.012x - 0.014 \quad , X = \frac{Y+0.014}{0.012}$$

In this formula absorptions are applied to the Y and X that is amount of reduction carbohydrate is obtained.

$$X = \frac{A \times B \times C}{DW \times 100}$$

Where A is the same X obtained, i.e., content of reduction carbohydrate as mM per liter in the standard curve; B is precision coefficient, 2.5 (2cc extract of 10 cc ×2 cc copper sulfate /2 cc extract × 2 cc Phosphomolybdic acid /4cc) which is $\frac{10}{2} \times \frac{2}{2} \times \frac{2}{4} = 2.5$ precision coefficient; DW is dry weight of the sample, 0.02 g; X is content of reduction carbohydrate as milligram per gram of dry weight and C is 10 cc water for grinding.

Preparation of copper sulfate

40 g of sodium carbonate was dissolved in distilled water and 7.5 g of tartaric acid and 4.5 g of hydrated copper sulfate were added and using distilled water 1 liter solution was obtained.

Preparation of Phosphomolybdic acid

70 g of molybdic acid and 10 g of sodium tungstate were heated for 40 minutes in 700 cc of 5% sodium hydroxide solution and after being cooled, 250 cc of 85% phosphoric acid was added to it.

Anthocyanins assay

Wagner method (1979) was used to measure the amount of anthocyanin in leaves. Leaf discs were completely ground in porcelain mortar with some amount of acidic methanol (pure methanol and hydrochloric acid to the volume ratio of 1:99) and the extract was poured into test tubes and put in the dark for 24 hours at 25 °C. Then the extract was centrifuged for 10 min at 4000 g and the supernatant absorbance was read using a spectrophotometer at 550 nm wavelength. The anthocyanin content was calculated using the following formula:

$A = \varepsilon bc$

where ε or extinction coefficient equals 3300 mM/cm, A is absorption, b is width of the measurement cell and equals to one centimeter, and c is anthocyanin content as mole per g of the plant fresh weight.

Results

The results in Table 1 show that average value of shoots dry weight of control plants is 5 ± 0.25 mg / g DW and in the plants under salinity it is 4 ± 0.2 mg / g DW. Also average value of roots dry weight in control plants is 2± 0.1 mg / g DW and in salt-treated plants it is 2 ± 0.1 mg / g DW. The average shoots fresh weight in control plants is 56 ± 2.8 mg / g FW and in salt-treated plants it is 14 ± 0.7 mg / g FW. Also average value of roots fresh weight in control plants is 26 ± 1.1 mg / g FW and in salt-treated plants it is 25 ± 1.2 mg / g FW (Fig. I).

Measurement results show that salinity decreased root and shoot dry weight and comparison of fresh weight of root and shoot of control and salt-treated plants shows that salinity decreased fresh weight of roots and shoots and this decrease was significant in the fresh weight of shoots (Fig. I).

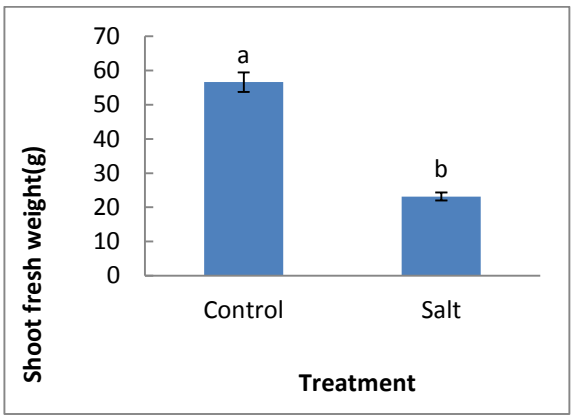
Measurement of chlorophyll index in Table 1 shows that average value of chlorophyll in the leaves of control plants is 51±2.55 SPAD and average chlorophyll content in the leaves of salt-treated plants is 40±2 SPAD.

Comparison of chlorophyll content of the control and salt-treated plants by SPAD shows that salinity decreased chlorophyll content but this decrease was not significant (Fig. II). Salinity stress decreases the amount of protein in the plant shoots and roots compared to control. Fig. (III) shows this decline in the leaf under saline condition although it is not statistically significant. Overall, the highest content of protein is seen in the control plants and the lowest content is seen in the salt-treated plants. Proline contents in leaves and roots significantly increased under salt stress compared to control (Fig. III). Proline

Table I
Content of chlorophyll, fresh and dry weight of aerial parts and root in *Borago officinalis*

Number of plants	Control				Salinity				Measured chlorophyll in leaves (Spad)	
	fresh weight(g)		dry weight(g)		fresh weight(g)		Dry weight(g)		Control	Salinity
	R	Sh	R	Sh	R	Sh	R	Sh		
1	28±1.4	56±2.8	2.4±0.12	4.7±0.23	29±1.4	15.5±0.8	2.2±0.11	4.1±0.2	37±1.8	42±2.1
2	27±1.3	51±2.55	1.9±0.09	4.6±0.23	29±1.4	14.2±0.7	2.2±0.11	4.2±0.2	50±2.5	42±2.1
3	30±1.5	55±2.75	1.8±0.09	4.6±0.23	31±1.5	15.2±0.8	2.4±0.12	5±0.25	57.5±2.9	36±1.8
4	26±1.3	63±3.15	2.2±0.11	5.7±0.18	29±1.4	16.2±0.8	2±0.1	4±0.2	47±2.3	38±1.9
5	29±1.4	53±2.65	2.2±0.11	5.2±0.26	20±1	13.8±0.7	2.2±0.11	3.6±0.2	58±2.9	39±1.95
6	34±1.7	55±2.75	2.8±0.14	5.1±0.25	20±1	10.8±0.5	1.5±0.1	3.4±0.15	50±2.5	37±1.85
7	23±1.1	63±3.15	1.9±0.09	6±0.3	30±1.5	13.5±0.7	2±0.1	4.1±0.2	49±2.45	44±2.2
Average	26±1.1	56±2.8	2±0.1	5±0.25	25±1.2	14±0.7	2±0.1	4±0.2	51±2.55	40±2

Root:R, Shoot: Sh



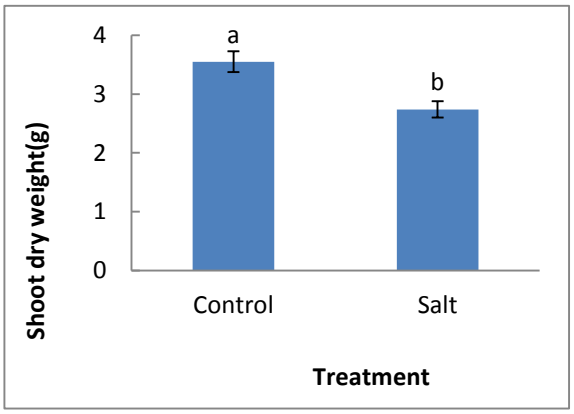
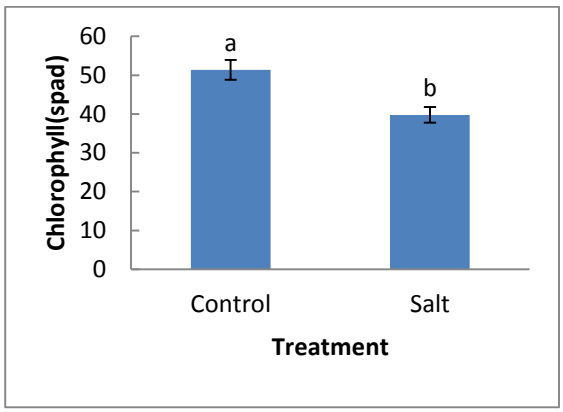


Fig. I. The effect of salt on fresh and dry weight of aerial parts and root



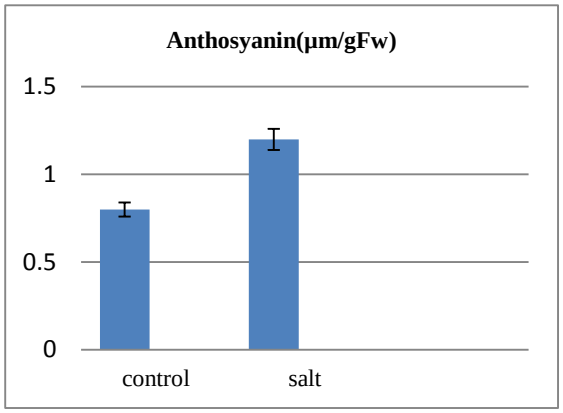


Fig. II. The effect of salinity stress on leaves, chlorophyll, and anthocyanin

contents of the salinity treatment show that in root proline doubled compared to control but this ratio in leaves is less.

The amount of soluble carbohydrates in roots and shoots of plants were measured. The amount of carbohydrates in salt stress conditions suggests a slight increase in a number of samples while there is a significant decline in others. On the average, in comparison to control, salinity stress decreased the amount of soluble carbohydrates in roots and shoots but this reduction was not significant (Table 2). Results of changes in the content of reduction sugar under salinity treatments compared to control are shown in Figs. III). The amount of reduction sugars under salinity stress increased in shoots in comparison with control but a decrease was observed in the roots. Changes of anthocyanin content in borage leaves under salinity stress and in control are shown in Fig. (II). The amount of anthocyanin increases under salinity stress in comparison with control plants.

Discussion

The results of salinity treatment effect on dry weight of plant organs (Fig.I) and statistical analysis of the results showed that salinity decreases roots and shoots dry weight but this change is not significant. Growth decrease in cells and the whole plant is a general process in dealing with salinity. It seems passing this developmental stage, plants show some degree of resistance against stress. Roots directly face high concentrations of salts and low water potential and cannot be protected against its damages. However, dry weight of shoots undergoes less change under salinity. It can be argued that roots moderate the harmful effects of stress before reaching the shoot. In this experiment, salinity decreased chlorophyll content but this decrease was not significant. Salinity leads to quantitative and qualitative changes in the pigment composition of leaf which in turn depends on the studied plant and salinity level. Decrease in chlorophyll content under salinity has been reported in most studies (Jampeetong and Birx., 2009; Erylmaz., 2006). Few reports are available

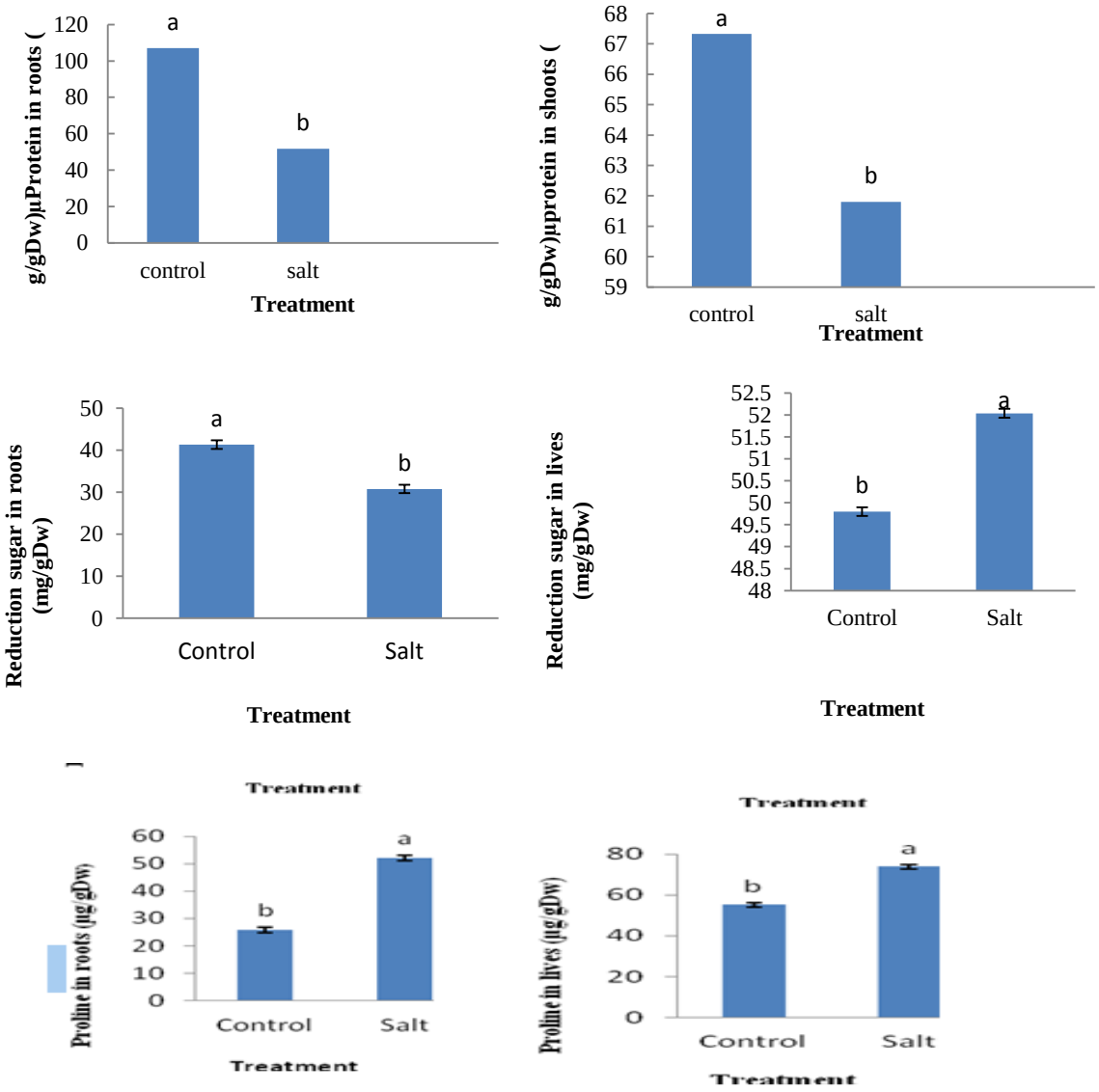


Fig. III. The effect of salt on fresh and dry weight of aerial parts and root

on the effect of salinity on chlorophyll content (Le-Dily et al., 1993). This increase may be due to increase in the number of chloroplasts in the leaves under stress (Jamil et al., 2005).

Difference in the rate of chlorophyll synthesis in various plants under salinity is the result of different synthetic pathways that can be followed by different enzymes and these enzymes show different responses to salinity. Competition for the precursors between chlorophyll and proline synthesis pathways is another issue, in addition to inhibitory effect of salinity on chlorophyll synthesis pathways (Le-Dily et al., 1993). Decrease in chlorophyll is a negative result of salinity on plants but this decline effectively intervenes to prevent light inhibitory damages and reduces the amount of photon received by the leaves (Munns, 2002). Degradation of chloroplast fine structure and instability of pigment-protein complexes, chlorophyll degradation, and changes in the content and composition of carotenoids are also results of salinity. The decrease in chlorophyll content in plants under stress can be related to increase in activity of chlorophyll-degrading enzyme (chlorophyllase) (Bertrand and Schoefs, 1999). It can be said that salinity has different effects on plant. All of these effects work towards growth and production of borage under salinity. It seems that root system is more vulnerable to salinity than shoots. Invulnerability of

Table 2
Content of proline, soluble sugars, and reduction sugars of aerial parts and root in *Borago officinalis*

Number of plants	Proline				Soluble Sugar				Reduction sugars			
	Control		Salinity		Control		Salinity		Control		**Salinity**	
	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh	R	Sh
1	25.86	63.79	66.54	74.82	73	77.8	50.7	79.4	33.77	31.62	37.91	45.2
2	29.28	77.56	65.17	73.44	84.15	75.48	68.23	76.21	47.28	61.87	27.5	47.28
3	16.88	67.9	63.79	81.72	73	92.15	65.05	83.75	50.41	62.91	31.66	50.41
4	21.03	58.27	37.58	74.82	63.43	99.26	63.45	77.78	25.41	59.78	33.75	69.16
5	27.93	34.82	55.51	70.68	71.4	71.24	76.21	79.83	56.66	38.95	30.62	46.25
6	36.2	43.1	37.58	72.06	71.4	76.21	47.21	76.64	31.62	51.45	28.53	53.53
7	25.16	40.34	38.96	69.31	69.81	98.53	41.13	85.34	44.16	42.07	25.41	52.5
Average	26±1.3	55±2.75	52±2.6	73.84±3.7	72±3.6	84±4.2	59±2.95	80±4	41±2.05		31±1.55	52±2.6
									50±2.5			

Root: R, Shoot: Sh

photosynthetic pigments under saline conditions is considered as a very important reason for salt tolerance of borage. After passing critical stages of growth and fixing pigments content and probably photosynthetic capacity, this plant manages good compatibility for dealing with salinity applied later. Considering the results of this study and medicinal importance of the plant and presence of significant level of saline soils in the country, medical borage cultivation will be possible in relatively saline soils or irrigation with relative saline water.

When plants are exposed to salt and drought stress, they respond to the stress throughout the plant, in molecular or cellular levels. Production pattern of many proteins is changed in response to water shortage. These proteins include the ones involved in stress signaling pathways, detoxification proteins for oxidative stress, and proteins that have indirect actions during the stress conditions. Reduction in the amount of proteins can be caused by a decrease in protein synthesis, increased activities of enzymes that hydrolyze the proteins, reduction of the available amino acids or denaturation of enzymes involved in the synthesis of amino acids or protein. It is concluded that reduction in the content of protein in borage is related to the increased resistance to salinity by different mechanisms. The most important mechanisms for resistance to salt stress may be associated with metabolic pathways that can result in an increase in the resistance of cells to cope with stressful situations. Further tests are needed to identify these mechanisms more precisely. According to a study reported by Tayeb (2005), content of soluble proteins in shoots and roots of plants under stress conditions is declined but this reduction is compensated by applying treatments such as salicylic acid, which leads to an increase in antioxidant enzymes in the plants. So reduction in protein or increase in their breakdown can be attributed to decrease in the activity of enzymes of the plants under the stress conditions because increase in the antioxidant activity prevents oxidation of proteins (Sairam, et al., 1998).

Increase in the content of proline is because of its role in osmoregulation and osmotic protection. As a result, the more content of this substance is increased, the more tolerance of plants against osmotic stress increases. Increase in proline is the result of protein breakdown under

salinity. Several researchers have studied proline accumulation in plants and its remarkable increase has been reported in plants under stress. Report of content of proline in 29 cultivars of chickpea under water stress shows that it is increased about 4-44 times (Mc cu and Hanson 1999). Even 3-300 times increase in proline in different species and different treatments of osmotic stress have been reported (Duke, 1981). Proline content is increased with salinity stress treatment that is one of the biochemical mechanisms in response to salt stress. Reduction in the intake of proline for protein synthesis during stress may be probable reason of proline accumulation (Guo, et al., 2004; Farzamisepehr, 2015). With the osmotic stress imposed on plant and osmotic imbalance, the plant increases proline and glucose as a mechanism of resistance to salinity in order to survive and osmotic adjustment in saline conditions (Sudhakar, 1993; Tattini, et al., 1996). Effects of salinity in many plant species like sugar beet, tomato, rice, and mulberry have been reported (Tayeb, 2005).

In this study, under salinity stress, proline content increased and that indicates borage tolerance under stress. Several factors affect the increase in soluble carbohydrates under salt stress. Reports indicate that under salinity conditions complex carbohydrates may break down into simple carbohydrates. Also, under stress conditions, increase in the ratio of sucrose to starch and starch degradation and also decrease in sucrose transport out of the leaves result in an increase in content of soluble carbohydrates. Such a process is seen under short-term and long-term deficiency of water which plays an important role in osmoregulation (Morgan, 1984). This increase in the content of soluble carbohydrates plays a crucial role in reducing the osmotic potential and finally, in sufficient gradient in the soil and plant and results in the increase in water absorption because carbohydrates play an important role as osmotic regulators. Xu, et al. (2001) reports that the accumulation of osmolytes like proline and sugars is directly related to the increased resistance of plants to abiotic stresses. The results of this study suggest that accumulation of carbohydrates may have a larger share in osmotic adjustment than proline. Increase in the content of soluble carbohydrates plays an important role in reducing the osmotic potential and ultimately creates a gradient between the soil and plant and increases water absorption. It can be concluded that borage shows adaptive responses to salinity in order to be protected from the stress damages. Increased content of soluble and reducing sugars under salinity, water logging, and cold have been reported. Havani and Johnson (1995) believe that accumulation of reducing sugars in stress conditions may regulate intracellular osmolarity and protect important biomolecules and membranes. Activity of invertase and sucrose synthase, which are two enzymes of sucrose breakdown to non-reducing sugars in cytoplasm and vacuole, decreases the content of reducing sugars in plants and catalyzes sucrose in the cell wall and vacuoles. The activity of this enzyme is reversible and plays an important role in energy metabolism by metabolizing sucrose in the metabolism-related pathways and in the storage and structural functions of the cell. Invertase belongs to a class of enzymes with optimal conditions of pH and special location. This enzyme in the vacuole may be attached to the cell wall and hydrolyze sucrose into glucose and fructose. On the other hand, combination of sugars in the roots decrease compared with the control group and as a result, absorption of water and CO_2 decreases during photosynthesis. This reduction mechanism suggests that salt may be entered into the cell after destruction of cell membrane. Then it comes into play with other compounds in the cell and affects their metabolism and reduces stem growth by increasing the metabolic rate. So we can guess that carbohydrate storage protects stressed plants for basic metabolic processes and maintenance of optimal metabolism. So it is concluded that under stress, borage protects its cells from oxidative damage by increasing carbohydrate and maintains the structure of membrane proteins.

In this study, content of anthocyanins increased under salt treatment. Research shows that anthocyanins can do their act in coordination with protective molecules in plant cells and compensate deficiency of molecules concentration during stress. Anthocyanins enter into action in special locations within the leaves for optimal performance of the plant. Accumulation of anthocyanins is induced by

different environmental stimuli like UV (Ramani, 2009), low temperature (Christie and Walbot, 1994), pathogens (Colmer, and et al., 1995; Hipskind and Nicholson, 1996), and several growth regulators like cytokinins (Deikman, and Hammer, 1995), gibberellins (Mealem- Beno, and et al., 1997), ethylene (Woltering, and Somhors, 1990), and acetylsalicylic acid (Marschner, 1995). Adequate nutrition in most cases can have beneficial effects on plants tolerance to biotic and abiotic stresses. Addition of NaCl to the nutrient solution affected borage growth, yield, and nutrient balance that is probably due to the adverse effects of sodium and chloride, decrease in the osmotic potential of nutrient solution, and decrease in water intake in the medium.

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