The effect of salt stress on proline and soluble sugars contents in *borage* under hydroponics condition

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Borage is an important medicinal plant which must be cultivated commercially in order to meet the ever-increasing demand for pharmaceutical industry. This study was done under hydroponics condition. Seeds of Borage were obtained from Neka Research Center (North of Iran). After applying *Benomyl* fungicide, the seeds were rinsed with distilled water and planted in vermiculite. After germination and appearance of two leaves, the plants were nourished by 0.5 Longshtein solutions. At 4-leaf stage, the plants were divided into two groups: control and salinity. Proline and soluble sugars contents were regularly studied under saline condition. While the content of proline increased, soluble sugars experienced a decrease under salinity. It was concluded that Borage was able to resist against stress due to change of contents of these compounds under salinity.

Keywords: salinity; Borage (*Borago officinalis* L.); soluble sugars; proline

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Introduction

Salinity is one of the important problem in world farmlands. Annually, millions of tons of salt penetrate into the farmlands through irrigation (Kingsbury et al., 2012). On the other hand, by increase in the world population, the demand for more food obligates the human to use the saline water and soil for agricultural and food production purposes in near future (Babaiyan and Ziatabar, 2002). Approximately, over 800 million hectares of land are salt-affected throughout the word (Munns, 2002). Salinity induces a wide range of perturbations at the cell and whole plant levels. Salt stress results from a number of detrimental processes involving the toxic action of Na^+ and Cl^- ions, the impairment of mineral nutrition, the modification in the water status of the plant tissues and secondary stresses such as oxidative stress linked to the production of toxic reactive oxygen intermediates (Bajji et al., 2009). Salt tolerant plants have the ability to minimize the detrimental and physiological adaptations (Hameed and Ashraf, 2008).

Primarily, salinity damages plants by the osmotic effect, the effect of specific ion toxicity and subsequently by nutritional stress (Song et al., 2009). In order to fight the stress effects, plants synthesize and accumulate substances which can be adjusted with osmotic pressure. These materials include amino acids, sugars and

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hormones. Proline is an amino acid which plays a vital role in osmotic adjustment of plant cells. Also, by attraction of the ions, the plants can save their water potential at a lower level, which causes the increase of water content in plants (Zhao and Harris, 2001). All these processes require energy and result in a decrease in the productivity and performance of the plant (Hanson et al., 2005). The reduction in plant growth under salinity is a consequence of several physiological responses including modification of water status, photosynthetic efficiency, carbon allocation and utilization (Abdul Jaleel et al., 2007). In general, various mechanisms contribute to salt tolerance in plants. The most common mechanisms consist of compartment of ions in vacuoles, accumulation of compatible solutes in the cytoplasm, as well as genetic salt resistance (Girija et al., 2002).

Borage, an annual herbaceous plant native to Europe, North Africa, and Asia Minor (Beaubaire and Simon, 2005), is a medicinally important plant, which has more than 20% gamma linolenic acid in its seed oil (El Hafid et al., 2002). The leaves of borage are reportedly used as diuretic, demulcent, emollient, and expectorant (Leung and Foster, 2001). In traditional Iranian medicine, the aerial parts of borage are reportedly used for treatment of a variety of ailments (Naghdi et al., 2008). However, borage as an important medicinal plant must be cultivated commercially in order to meet the ever-increasing demand for pharmaceutical industry. Although borage is cultivated in many countries for medicinal uses, no studies were performed concerning the effect of salinity on the phytochemical and production potential of borage during the growth cycle; therefore, it is of great importance to investigate borage for its salt-tolerance capacity in order to exploit the saline lands for its cultivation. The present research investigates the effect of saline water on physiological specifications of Borage.

Materials and Methods

This study was done in the laboratory and greenhouse of Plant Science Department of Payam noor University of Iran, from March 2011 to January 2012. Seeds of Borage were obtained from Neka Research Center (North of Iran). After applying *Benomyl* fungicide, the seeds were rinsed with distilled water, planted in vermiculite and irrigated. After germination and appearance of two leaves, the plants were nourished by 0.5 Longshtein solution. An 4-leaf stage, the plants were divided into two groups: control and salt-stressed. To evaluate the effect of salinity on some physiological characteristics of the Borage plants, sodium chloride and sulphate sodium with volume ratio 2:1 and 100 mM were applied at 4-leaf stage. During salinity treatment the content of proline and soluble sugars were measured regularly. Extraction and estimation of proline were conduced according to the procedures described by Bets et al. (1973). Plant material, 0.1 g per sample, was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid; the homogenate was filtered through Whatman No. 2 filter paper. Two millilitres of filtrate was then mixed in a test tube with 2 ml acid ninhydrin solution and 2 ml glacial acetic acid, and the

Table 1
Measured proline and soluble Sugars of aerial parts and root

Number of plants	Proline of aerial parts at control treatment µm/gdw	Proline of aerial parts at salinity treatment µm/gdw	Proline of root at control treatment µm/gdw	Proline of root at salinity treatment µm/gdw	Soluble Sugars of aerial parts at control treatment µm/gdw	soluble Sugars of aerial parts at salinity treatment µm/gdw	soluble Sugars of root at control treatment µm/gdw	soluble Sugars of root at salinity treatment µm/gdw
1	64±3.2	75±3.75	25±1.25	67±3.35	78±3.9	79±3.95	73	51±2.55
2	78±3.9	73±36.5	29±1.45	65±3.25	75±3.75	76±3.5	84±4.2	68±3.4
3	68±3.4	82±4.1	17±.85	64±3.2	92±4.6	84±4.2	73	65±3.25
4	58±2.9	75±3.75	21±1.05	38±1.9	99±4.95	78±3.9	63±3.15	63±3.15
5	35±1.78	71±3.55	28±1.4	56±2.8	71±3.55	80±4	71±3.55	76±3.8
6	43±1.7	72±3.6	36±1.8	38±1.9	76±3.55	77±3.88	71±3.55	48±2.4
7	40±2	69±3.45	25±1.25	39±1.95	99±4.95	85±3.75	70±3.35	41±2.05
Average	55±2.75	74±3.7	26±1.3	52±2.95	84±4.2	80±4	72±3.6	59±2.95

solution was then incubated at 100˚C water bath for 1 h. The reaction was terminated by placing the mixture in an ice bath. It was then extracted with 4 ml toluene and the chromophore phase was aspirated from the aqueous phase. The absorbance rate was read at 520 nm using a spectrophotometer. Concentrations of proline in the leaves were expressed on a DW basis. The total soluble sugars were measured using the phenol-sulphuric acid. To measure total soluble sugars of the leaves, the solution of 5% $ZnSO_4$, 0.3 % $NB(OH)_2$, 5% (v/v) phenol solution and sulphuric acid were used based on Stewart method. Finally, absorption rate was read at 485 nm by spectrophotometry (Stewart, 1989). Concentrations of total soluble sugars in the leaf and root are expressed on a DW basis. Statistical calculations were done by SPSS 16 software. Mean values and significance were determined by Duncans multiple range tests at 5% probability level.

Fig. I. The borage under salinity stress

Results

During salinity treatment, contents of proline and soluble Sugars were measured regularly. Based on the results, the contents of proline in the aerial parts and root increased and soluble sugars decreased significantly after salinity treatment, and the lowest amount of proline was related to the control treatment (Table 1).

Discussion

In this study proline concentration in the leaf and root of Borage was increased with salinity treatment, representing the positive role of proline in the salt tolerance of this crop. Proline, as a signalling/regulatory molecule, can activate multiple responses, which are component of the process of adaptation to abiotic stresses including salt stresses (Ashraf and Orooj, 2006). Increasing of proline under salinity has also been reported in some medicinal plants (Hajar et al., 2011; Munns, 2002; Ashraf and Orooj, 2006; Abdul Jaleel et al., 2007). Soluble sugars of aerial parts and roots were decreased in response to the salinity. Accumulation of soluble sugars in response to environmental stress has been widely reported despite specific reduction in net CO_2 assimilation levels (Chaves et al., 2003; Meloni et al., 2008). Sugars, in addition to the role of regulating osmotic balance, also act as the metabolic signals in the stress conditions (Chaves et al., 2003). It was observed that the osmotic adaptation in plants exposed to salinity can take place by accumulation of high concentration of inorganic ions or solutions with low molecular weight, highly soluble compounds that usually are nontoxic at high cellular concentrations. Generally, they protect plants from stress in different ways, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity and stabilization of enzymes/proteins (Ahmad et al., 2008).

Conclusion

The results suggested that the accumulation of proline and reduction of soluble sugars is a good indicator for salinity tolerance of Borage. Therefore, the plant resists against salt by means of osmotic adjustment and compartment within the cells. This process is essential for plant survival in saline conditions.

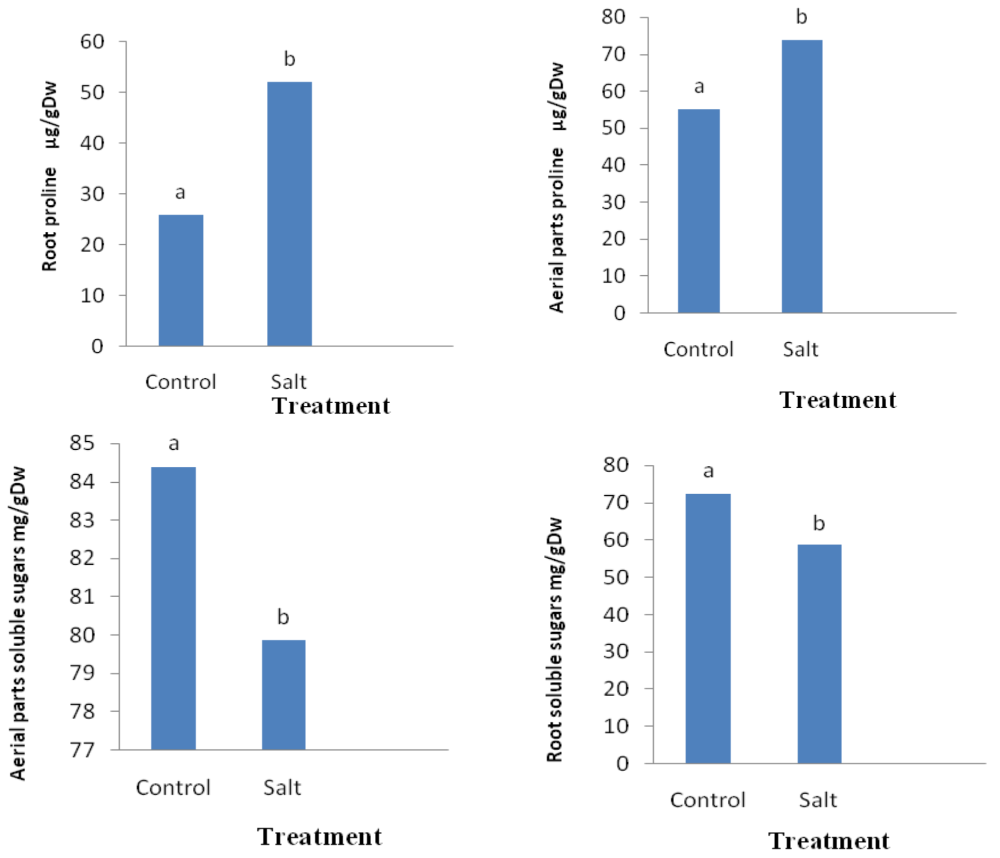

Fig.I. The effect of salt on proline and soluble sugars of Borage

References

Abdul JC, R. Gopi , B. Sankar , P. Manivannan, A. Kishorekumar , R. Sridharan and **R. Panneerselvam** .2007. 'Studies on germination, seeding vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seeding under salt stress'. *J. Bot.* 73 (2):190-195.

Ahmad Ak, **T. KM , Z. KM** and **A. El-Gawad** . 2008. 'Tolerance of seven Faba bean varieties, to drought and salt stresses' .*J. Agric. Biol. Sci*. 4(2): 175-186.

Ashraf M and **A. Orooj** . 2006. 'Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague)'. *J. Arid Environ.* 64: 209-220.

Babaiyan J.N. and **A.M. Ziatabar** .2002. 'Plants growing in saline and arid lands'. (Translation) *Mazandaran Univ. Press*: 407-422.

Bajji M., J.M. Kinet and **S. Lutts** . 2009. 'Salt stress effects on roots and leaves *Atriplex halimus L.* and their corresponding callus cultures'. *Plant Sci.* 137: 131-142.

Beaubaire N.A. and **J.E. Simon** . 2005. 'Production potential of borage (*Borago officinalis* L.)'. *Acta Hort*.208: 101-108.

Chaves M.M., J.P. Maroco and **J.S. Pereira** . 2003. 'Understanding plant response to drought, from genes to the whole plant '.*Functional Plant Biology,*30: 239-264.

El Hafid R.E., S.F. Blade and **Y. Hoyano** . 2002. 'Seeding date and nitrogen fertilization effects on the performance of borage (*Borago officinalis* L.) '. *Industrial Crops and Prod*. 16: 193-199.

Girija C, B. N. Smith and **P. M. Swamy** . 2002. 'Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.)' *Environ. Exp. Bot.* 47 : 1-10.

Hajar A. S., M. A. Zidan and **H. S. Al-Zahrani**. 2011. 'Effect of salinity stress on the germination, growth and some physiological activities of black cumin (*Nigella sativa* L.)'. *Arab Gulf. J. Sci. Res*. 14: 445-454.

Hameed M. and **M. Ashraf** . 2008. 'Physiological and biochemical adaptations of *Cyndon dactylon* L.)' *Perss from the Salt Range (Pakistan) to salinity stress. Flora*, 203 : 68-694.

Hanson B, S. R. Grattan and **A. Fulton** . 2005. 'Agricultural Salinity and Drainage'. *University of California, Davis*. 160pp.

Jampeetong A. and **H. Brix** . 2009. 'Effect of NaCl salinity on growth ,morphology, photosynthesis and proline accumulation on *Salvia natans*'. *Aquat. Bot*. 91: 181-186.

Kingsbury R. W. , E. Epstein and **R. W. Pearcy** . 2012. 'Physiological responses to salinity in selected lines of wheat'. *Plant Physiol*.74: 417-423.

Leung A.Y. and **S. Foster**. 2001. 'Encyclopedia of common natural ingredients-Used in food,drugs and cosmetics'. *2nd ed. A Wiley-Interscience Publication. USA* : 98-99.

Meloni D. A., M. R. Gulotta and **C. A. Martinez** . 2008. 'Salinity tolerance in *Schinopsis quebracho* Colorado: Seed germination, growth, ion relations and metabolic responses'. *J. Arid Environ*. 72: 1785-1792.

Munns, R . 2002 . 'Comparative physiology of salt and water stress'. *Plant Cell. Environ*. 25: 239-250.

Naghdi Badi H , A. Soroshzadeh, SH. Rezazadeh, M. Sharifi, A. Ghalavand and **A. Rezai** . 2008. 'Evaluation of phytochemical and production potential of borage (*Borago officinalis* L.) during the growth cycle'. *J. Med. Plants*, 7(4):37-43

Song J., M. Chen , G. Feng , Y. Jia, B. Wang and **F. Zhang** . 2009 .'Effect of salinity on growth, ion accumulation and the roles of ions in osmotic adjustment of two populations of *Suaeda salsa*'..*J .Plant Soil*, 314: 133-141.

Zhao J. and **K. , Harris** . 2001 . 'Genetic analysis of plant salt tolerance using *Arabidopsis thaliana*'. *Plant Physiol*. 124: 941-948.