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Full Length Research Paper

The effect of salinity on the growth, morphology and physiology of *Echium amoenum* Fisch. & Mey.

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The salinity of water and soil decreases the growth and yield of agricultural products. Salinity affects many physiological and morphological processes of plant by influencing soil solution osmotic potential and ion absorption and accumulation of minerals. To evaluate the effect of salinity on some physiological and morphological characteristics of the medicinal plant of *Echium amoenum*, an experiment was carried out with completely random design in four replications. In this study, the effect of different levels of salinity, including control (non-saline water), 3, 6, 9 and 12 dS m^{-1} from natural saline water was examined on root length, leaf area, dry weight of roots and shoots, also on the amount of absorption of salts Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+} and the ratio of Na^+/K^+ and Ca^{2+}/Na^+ in root and shoot of plants and proline and total soluble sugars of leaf in the vegetative growth stage in the greenhouses. Results indicated that the application of saline water reduces significantly all morphological traits under study. Also, as the salinity increased, the density of K^+, Ca^{2+} and Mg^{2+} and the ratio of Na^+/K^+ and Ca^{2+}/Na^+ in root and shoot of *E. amoenum* declined. In contrast, by increasing salinity, Na^+ and Cl^- concentration in roots and shoots significantly increased. As the salinity increased, proline concentration and leaf total soluble sugars also increased significantly compared with the control. The results showed that the accumulation of proline and soluble sugars are good indicators of salinity tolerance. Results also suggest that the plant resists against salinity through osmotic adjustment and ion absorption and sharing within its cells. This process is essential for the survival of plants in saline conditions.

Key words: Salinity, *Echium amoenum*, vegetative growth, ion composition, proline, soluble sugars.

INTRODUCTION

Salinity is one of the important issues in world's farmlands. Annually, millions of tons of salt enter the farmlands through irrigation (Kingsbury et al., 1983). On the other hand, by increasing population, lack of food makes pressure on the human in such a way that the demand for more food makes the men to use the saline waters and soils for agriculture and food production in not so far a future (Babaiyan and Ziatabar, 2002). Overall, over 800 million hectares of land are salt-affected throughout the world (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 including 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 an oxidative stress linked to the production of toxic reactive oxygen intermediates (Bajji et al., 1998). Salt tolerant plants have the ability to minimize these detrimental effects by producing a series of anatomical, morphological and physiological adaptations (Hameed and Ashraf, 2008).

Salinity damages plants mainly through the osmotic effect, the effect of specific ion toxicity and subsequently through nutritional stress (Song et al., 2009). In order to fight with the stress effects, the plants make materials which adjust osmotic pressure and accumulate them. These materials include amino acids, sugars and hormones. Proline is among the amino acids which plays

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a vital role in osmotic adjustment of plant's cells. Also, by attracting the ions, the plants save their water potential in a lower level. This helps the increase of water content in plants (Zhao and Harris, 1992). All these processes need energy and result in a decrease in plant production and performance (Hanson et al., 1999). 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, but commonly proposed mechanisms include compartment of ions in vacuoles, accumulation of compatible solutes in the cytoplasm, as well as genetic salt resistance (Girija et al., 2002).

Meloni et al. (2008) reported that resistance to salt is correlated negatively with Na^+ concentration and positively with K^+ concentration. Also, high K^+/Na^+ in plant tissues, has been considered as an important physiological solution for salinity tolerance in some plants (Meloni et al., 2008; Song et al., 2009). Since different plants react to salinity in different ways, therefore, the study of salinity tolerance mechanisms in plants is necessary. It is an important condition for permanent cultivation of salinity tolerant plants. Therefore, aligned with corrective measures for reviving the saline soils, introducing salinity tolerant varieties and plants breeding may be a useful and economical way for solving salinity problem (Levitte, 1980).

Echium genus has 4 species in Iran (Ghassemi et al., 2003; Mehrabani et al., 2005) and only *Echium amoenum* has medicinal uses. *E. amoenum* which belongs to Boraginaceae is a biennial or perennial herb to narrow zone of northern part of Iran and Caucasus, where it grows at an altitude ranging from 60 to 2200 m (Mehrabani et al., 2005; Sadizadeh et al., 2009). Petals of *E. amoenum* has long been used as a tonic, tranquillizer, diaphoretic and as a remedy for cough, sore throat and pneumonia is known in traditional medicine of Iran as Gol-e-Gavzaban (Ghassemi et al., 2003; Mehrabani et al., 2005). New research also shows that this plant has antidepressant effects (Saiiah et al., 2003), analgesic (Heidari et al., 2006) and is stimulating the immune system (Amirghofran et al., 2000).

In order to meet the ever-increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil salinity and other forms of pollutions pose serious threats to plant production (Abdul Jaleel et al., 2007).

Many investigations have already been carried out on the medicinal importance of this plant, but the salinity effects on this medicinal plant attracted a little attention. Since the identification of salinity-tolerant products and varieties and their performance with saline water is of critical importance, the present research investigates the effect of saline water on morphological and physiological specifications of *E. amoenum*.

MATERIALS AND METHODS

To study the effect of salinity stress due to natural saline water on some morphological and physiological traits of medicinal plant of *E. amoenum* in the vegetative growth stage, a pilot study with a completely random-base design in four replications was carried out in the Greenhouse of Iranian Academic Center for Education, Culture and Research (ACECR). Treatments included five levels of salinity control (non-saline water), 3, 6, 9 and 12 dS m^{-1}. In order to apply salinity treatment, natural saline water with electrical conductivity of 690 dS m^{-1} was used. Natural saline water was supplied from Huz-e-Sultan Lake in Qom, the chemical characteristics of which is presented in Table 1. The saline water treatments were obtained from diluted natural salt water mentioned earlier.

After preparation of soil and planting seeds, the pots were irrigated by salty water in treatments of salinity and ordinary water in the control treatment up to the six-leaf stage. Irrigation was done up to field capacity in each pot, of course, along with irrigation, to reduce accumulation of salts in the root zone, water leaching was estimated and added to the irrigation water. Six weeks after salt stress, samples of each pot were taken and washed with distilled water. After recording root length, leaf length and width, fresh weights of roots and shoots were oven-dried at 70°C for 48 h and dry weights were recorded.

Ion composition estimation

After harvesting the plant samples, while washing with distilled water for 48 h at 70°C were dry. Then the contents of mineral elements in plant root and shoot were determined. The contents of Na^+ and K^+ in the mineral extract were then determined using a flame photometer. The concentrations of calcium and magnesium were determined by atomic absorption. The ratio of K^+/Na^+ and Ca^{2+}/Na^+ were made to evaluate salt tolerance.

Chloride was determined by titration with 0.1 N $AgNo_3$, with 5% K_2CrO_4 as indicator after samples of oven-dried was extracted in demineralization water for 30 min.

Leaf proline estimation

Extraction and estimation of proline were conducted according to the procedures described by Bates et al. (1973). Plant material, 0.05 g per sample, was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid then; the homogenate was filtered through Whatman No. 2 filter paper. Two milliliters of filtrate was then mixed in a test tube with 2 ml acid ninhydrin solution and 2 ml glacial acetic acid, and incubated in a 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 aspirated from the aqueous phase. The absorbance was read at 520 nm using a spectrophotometer. Concentrations of proline in the leaf are expressed on a DW basis.

Leaf total soluble sugars estimation

The total soluble sugars were measured using the phenol-sulfuric acid. To measure leaves' total soluble sugars from the solution of 5% $ZnSO_4$, 0.3 N $Ba(OH)_2$, 5% (v/v) phenol solution and sulfuric acid was used based on Stewart method. Finally, absorption was read at 485 nm by spectrophotometry (Stewart, 1989).

Table 1. Chemical characteristics of natural saline water.

SAR	K^+(g l^{-1})	Na^+(g l^{-1})	Mg^{2+}(g l^{-1})	Ca^{2+}(g l^{-1})	SO_4^{2-}(g l^{-1})	Cl^-(g l^{-1})	ECW (dS m^{-1})
41.3	1.23	128	19.5	0.086	48.8	218.7	690

Table 2. Mean Squares from analyses of variance of data for different growth and attributes of *E. amoenum.*

Source of variation	df	Root length	Leaf area	Shoot f.wt	Root f.wt	Shoot d.wt
Treatments	4	758.217*	411975.515***	1.227***	0.035***	0.013**
Error	15	247.120	20235.625	0.062	0.003	0.002
		Root d.wt	**Shoot Na^+**	**Shoot Cl^-**	**Shoot K^+**	**Shoot Ca^{2+}**
Treatments	4	0.002***	259.19***	3414.95***	91.26***	11868.04*
Error	15	0.00009	5. 60	191.29	9.42	2594.05
		Shoot Mg^{2+}	**Root Na^+**	**Root Cl^-**	**Root K^+**	**Root Ca^{2+}**
Treatments	4	4.31*	164.86**	848.72***	8.97*	858.44*
Error	15	1.26	26.47	103.25	2.89	251.55
		Root Mg^{2+}	**Shoot K^+/Na^+**	**Shoot Ca^{2+}/Na^+**	**Root K^+/Na^+**	**Root Ca^{2+}/Na^+**
Treatments	4	0.24*	58.30***	24.30***	5.22***	3.58**
Error	15	0.07	3.83	2.08	0.55	0.52
		Proline	**Soluble sugars**			
Treatments	4	1516.357**	43.160***			
Error	15	200.304	5.127			

*, **, *** = Significant at 0.05, 0.01 and 0.001, respectively; d.wt, dry weight; f. wt, fresh weight.

Concentrations of total soluble sugars in the leaf are expressed on a DW basis.

Statistical analysis

Statistical calculations were done by SPSS 16 software. Mean values and significance were determined by Duncan's multiple range tests at 5% probability level.

RESULT

Growth and morphology

In studying the effects of different salinity treatments resulting from natural saline water on physiological specification of *E. amoenum*, it was observed that the effect of different amounts of salinity on all specifications under study was meaningful at the level of 0.01, except for root length ($p \leq 0.05$) (Table 2). By increasing salinity in irrigation water, the root length decreased. The process of changes in the root length resulting from content treatment up to 3 dS m^{-1} salinity had an increasing pattern. However, this increase was not statistically significant and after that showed a decreasing trend (Figure 1). The leaves area experienced decreasing trend by the increase of salinity, of course in 3 dS m^{-1} treatment compared with the control treatment (usual water), the leaf area increased a little. But the increase was statistically non-significant (Figure 1). Dry and wet root's weight and weight of aerial parts decreased like other features by the increase of salinity of water. The least level of the aforementioned specifications was seen in 12 dS m^{-1} treatment. Also, these specifications increased in 3 dS m^{-1} treatment compared with the control group but it showed no statistical significance (Figure 1).

Ion content

The effect of increasing levels of salinity on ions uptake and concentration in root and aerial parts of *E. amoenum* was significant (Table 2).

Increasing salt levels caused a significant increasing effect ($p \leq 0.001$) on Na^+ and Cl^- concentrations of both shoots and roots of *E. amoenum* (Table 2). The concentration of both ions in shoots and roots increased with increasing levels of salt and they were maximal at the

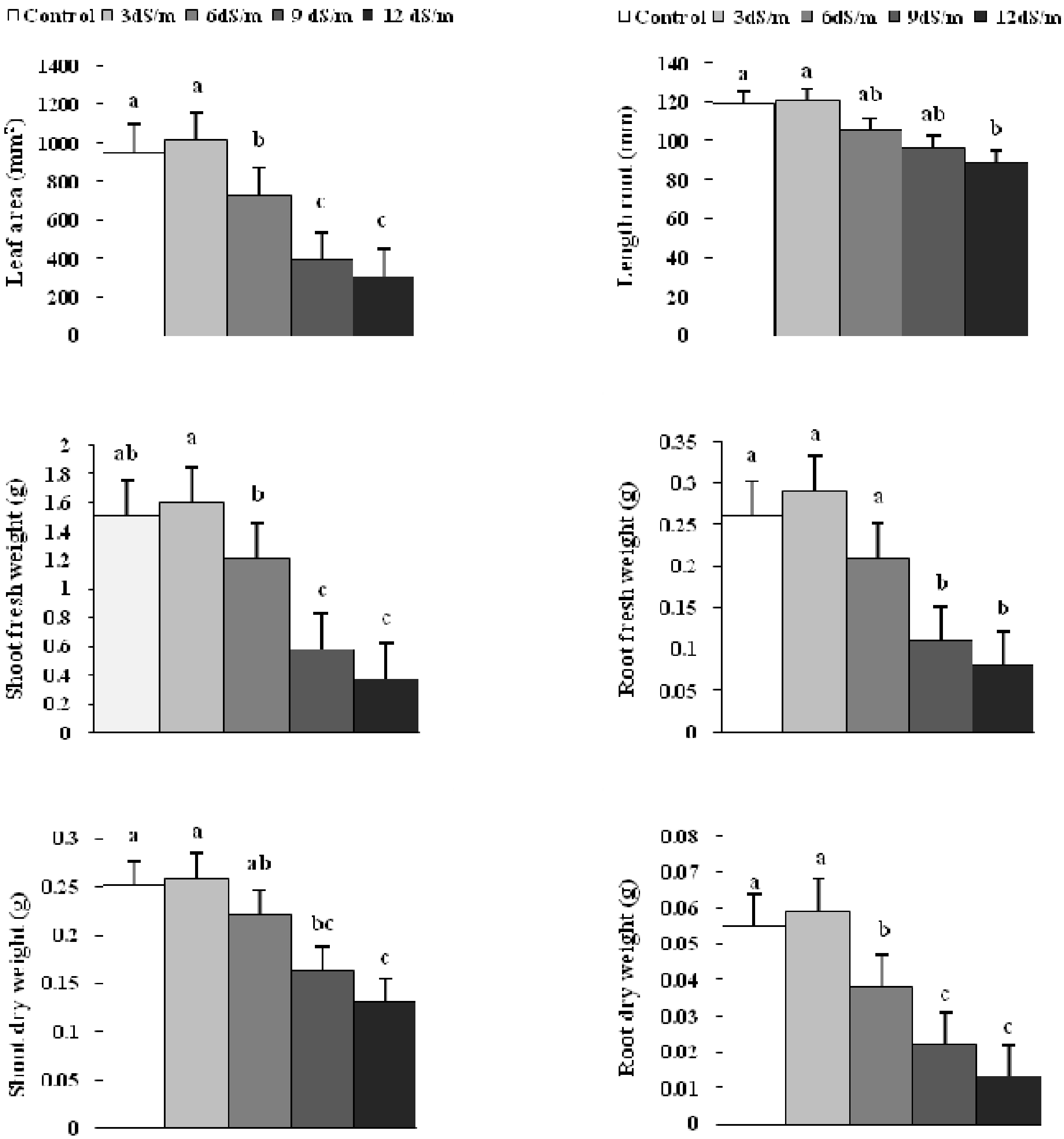

Figure 1. The effect of salt stress on growth attributes of *E. amoenum*.

highest salt level, (12 dS m^{-1}). However, concentrations of Na^+ and Cl^- were considerably higher in the aerial parts than that in the roots; the accumulation of Cl^- in the shoots was markedly higher than that of Na^+ (Figure 2). A progressive decrease in concentrations of K^+, Mg^{2+} and Ca^{2+} in both shoots and roots of *E. amoenum* was found with increase in concentration of salt (Figure 2). However, concentrations of these ions were considerably more in the aerial parts than those in the roots at all external salt levels. Although, a significant decrease in root K^+, Mg^{2+} and Ca^{2+}at external salt levels was found with respect to those at (non-saline) control treatment, the concentrations of the root ions did not vary significantly at all three external salt levels (Figure 2). There was a consistent decrease in K^+/Na^+ and Ca^{2+}/Na^+ ratios in the aerial parts and roots of *E. amoenum* with increase in salt level, *E. amoenum* maintained considerably higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in the aerial parts than those

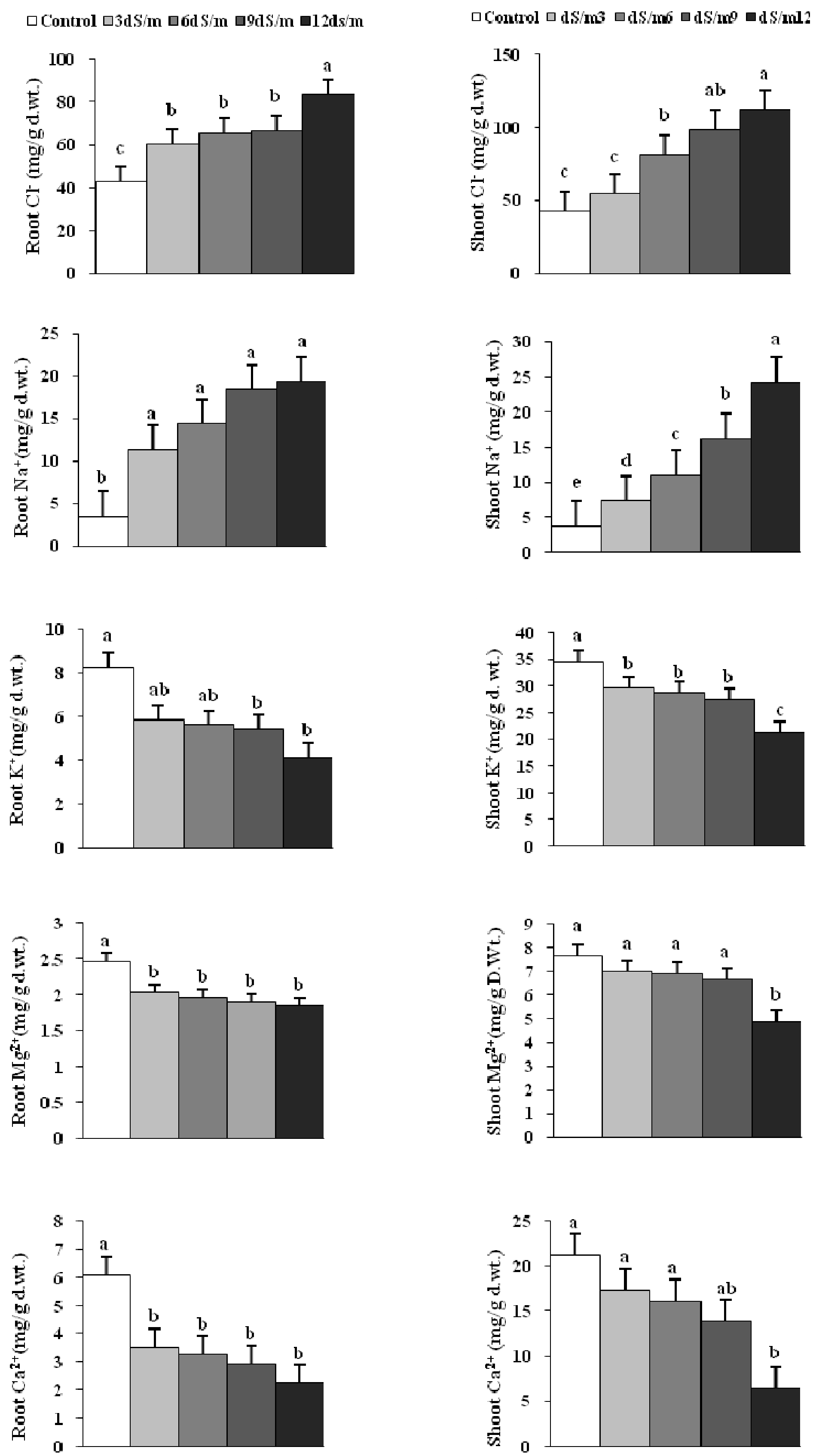

Figure 2. The effect of salt stress on concentration of different ions (mg/g d.wt.) in shoots and roots of *E. amoenum*.

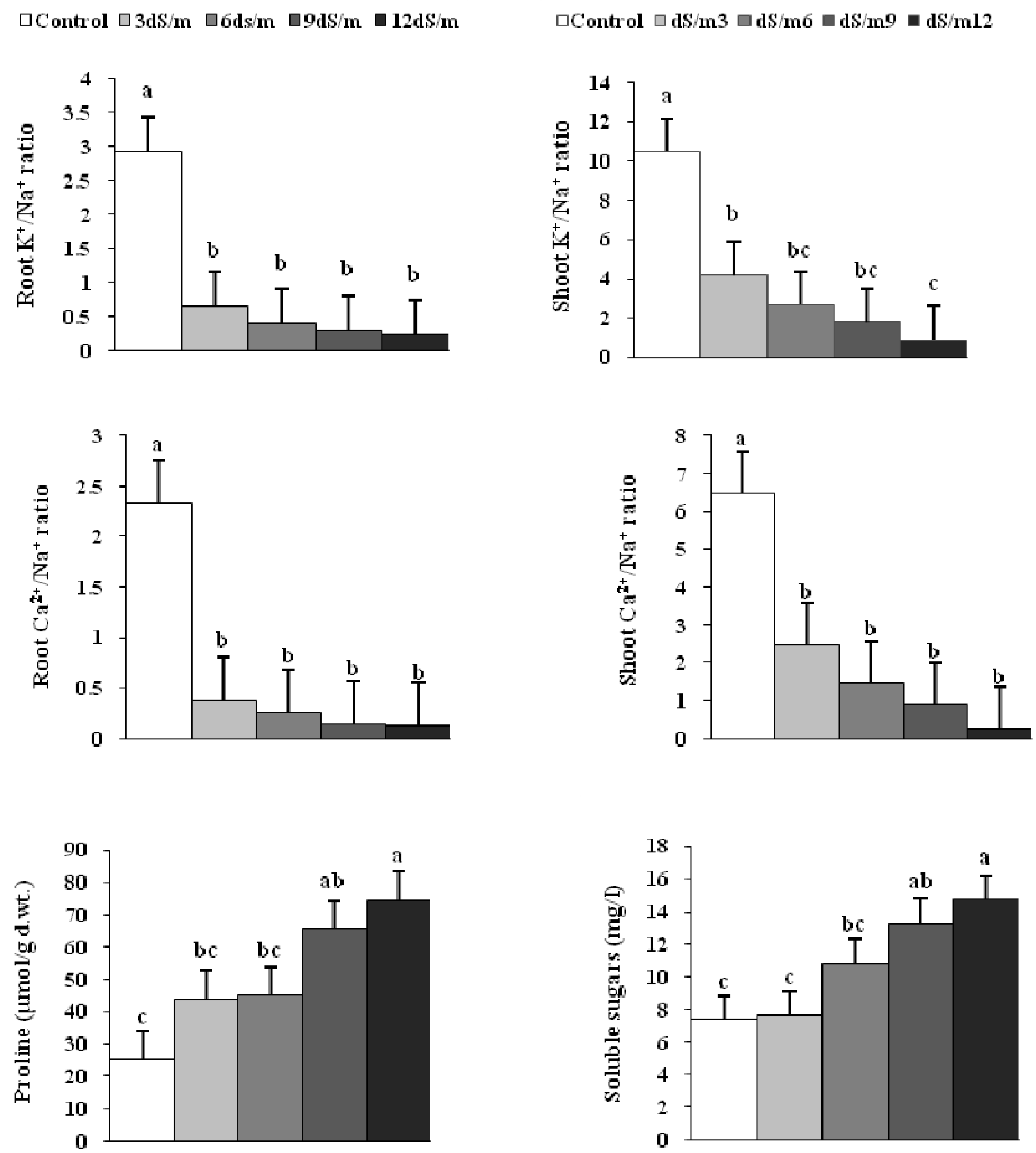

Figure 3. The effect of salt stress on K^+/Na^+ and Ca^{2+}/Na^+ in shoots and roots of *E. amoenum* and concentration of proline and soluble sugars in leaves of *E. amoenum*.

in the roots (Figure 3).

Proline content

Leaf proline content increased significantly with increasing salinity ($p \leq 0.01$) (Table 2), so that the maximum amount in proline was observed at highest level of salinity, that is, 12 dS m^{-1}, by 74.55 µmol g^{-1}. The lowest was related to control by 25.29 µmol g^{-1} (Figure 3).

Total soluble sugars content

Total soluble sugars content of the leaves ($p \leq 0.001$) increased significantly with increasing salinity levels (Table 2). Also, the most content was observed at the highest level of salinity, (12 dS m^{-1}) and the lowest was related to the control treatment (Figure 3).

DISCUSSION

Increasing salinity levels causes a significant decrease in vegetative growth of *E. amoenum,* in which the morphological changes are probably caused by the reductions in turgor pressure within the cells that restricted cell expansion (Jampeetong and Brix, 2009); consequently, the expansion of leaf area is reduced.

Reduction in plant growth has also been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and decrease in many metabolic activities (Kumar et al., 2005). It appears that the decreased root and shoot fresh weight is due to the reduced water absorption, which in turn causes a reduction in the amount of water in plant tissue (Prado et al., 1995; Sharma et al., 2004). Moradi and Ismail (2007) found that the cause of decrease of production of dry plant in saline conditions created by irrigation water salinity increased energy costs, reduced carbon capture and photosynthesis per unit leaf area. The results show that all growth characteristics in treatments 3 dS m^{-1} compared with control plants had a slight increase, the increase in plant growth and production of shoot and root indicates the significance and necessity of having a suitable level of absorbing water and minerals in stress conditions, which are accompanied by production lateral roots (Poljakoff and Lerner, 1994).

In this study, increasing salinity led to a significant increase in the concentration of Na^+ and Cl^- and also reduced the concentration of K^+, Ca^{2+} and Mg^{2+} in aerial parts and roots of *E. amoenum*, though, increased ions Na^+ and Cl^- in the aerial parts were more than that in the roots. Accumulation of Na^+ and Cl^- in leaves may be responsible for more negative water potential and osmotic potential values of shoots; this pattern increases with increase in salinity (Hameed and Ashraf, 2008). Chloride ion concentration also was markedly higher than that of sodium ions. Such pattern of accumulation of the toxic ions has earlier been reported in other plant species (Munns, 2002; Grewal, 2010). Also, the results show that the sodium ion concentration in root has increased up to 3 dS m^{-1} by the increase of salinity, but in higher salinity this trend is not significant. This shows the limited capacity of root in keeping and storing sodium, in such a way that by the increase of salinity, the capacity of sodium preservation in root becomes saturated and full (Naeini et al., 2004). Also, it was seen that the accumulation of sodium ion in some salinity levels in root is more than that of aerial parts. Probably, *E. amoenum* resists and tolerates salinity because of limited Na^+ transfer from roots to aerial parts.

Zandstra-Plom et al. (1998) announced that internal sodium recycling mechanisms plays an effective role in Na^+ concentration increase in roots. Also, it was reported that the lower Na^+ transfer from roots to aerial parts in many varieties increases tolerance considerably (Munns et al., 2006). Darvishi et al. (2009) announced that root's cells may help the salinity tolerance through concentrating nutrient elements in their protoplasm. The increase of Na^+ causes the decrease of other cations and it imbalances the cation balance of the plants. This increase causes the decrease of K^+, Ca^{2+} and Mg^{2+} in root and aerial part of *E. amoenum*. Na^+ accumulation causes a meaningful decrease in K^+ concentration. In many studies, the cause of potassium decrease has been reported to be due to the existence of antagonistic relationship between sodium and potassium (Zhu, 2001; Meloni et al., 2008). El-Hendawy et al. (2005) observed that decrease of potassium attraction may be related to the salt tolerance of plant.

However, the rate of reduction of K^+ in roots was far more than that of the aerial parts. These results can be attributed to: (1) transfer of K^+ from roots to leaves; (2) an exchange of K^+ ions with Na^+ ions in root tissues; (3) direct interference of Na^+ with K^+ uptake (Ramoliya et al., 2004). In fact, a few amount of potassium that has entered root is transferred to the aerial part for being used in metabolism and osmotic adjustment process, which causes a decrease in potassium in the roots.

It has been generally observed that plants exposed to saline environment take up high amounts of Na^+, whereas the uptake of K^+, Ca^{2+} and Mg^{2+} is significantly reduced.

Calcium is important during salt stress, for example, in preserving membrane integrity (Rengel, 1992; Ashraf and Orooj, 2006), signalling in osmoregulation (Mansfield et al., 1990) and influencing K^+/Na^+ selectivity (Cramer et al., 1987). It was reported that the decrease of calcium attraction under saline conditions is because of the increase in Na^+/Ca^{2+} ratio, this also limits the root's growth (Orcutt and Nilsen, 2000; Garcia-Sanchez et al., 2002).

In addition to the role of Mg^{2+} in chlorophyll structure and as an enzyme cofactor, another important role of Mg^{2+} in plants is the export of photosynthates, which is impaired and leads to enhanced degradation of chlorophyll in Mg^{2+} deficient source leaves, resulting in increased oxygenase activity of RuBP carboxylase (Ramoliya et al., 2004).

Salinity stress also leads to gradual reduction of magnesium ion concentration, so that changes in the shoot only at the highest level of salinity are significantly different from the control. The findings are consistent with Ramoliya et al. (2004).

K^+/Na^+ and Ca^{2+}/Na^+ ratios decreased consistently in the aerial parts and roots of *E. amoenum* with increase in salt level, both ratios were markedly higher in aerial parts when compared with those in roots. Reductions in these ratios in plants due to salinity have also been reported in other studies (Ashraf and Orooj, 2006; Safavi and Khajehpour, 2007; Grewal, 2010). Benlloch et al. (1994) stated that the increase of sodium ions in the root environment decreases uptake of potassium ions and lowers K^+/Na^+ ratio. Safavi and Khajehpour (2007) also studied the effect of soil salinity on crop borage and *Echium* and stated that with increasing soil salinity, sodium levels increased in both plants, while K^+ and Ca^{2+} contents and K^+/Na^+ ratio of aerial parts reduced in both plants. These results are consistent with our findings. Grewal (2010) stated that excessive accumulation of Na^+ in plants at high salt levels led to detrimental effect on the ionic balance (K^+/Na^+ and Ca^{2+}/Na^+ ratios) of plants. High K^+/Na^+ selectivity in plants under saline conditions is considered as one of the important selection criteria for salt tolerance (Gorham et al., 1997). As observed, K^+/Na^+ and Ca^{2+}/Na^+ ratios up to the level of salinity 9 dS m^{-1} was greater than 1. This amount was the minimum level suggested for normal functioning of most mesophytes under saline conditions (Wyn Jones, 1981).

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. Compatible solutions are low molecular weight, highly soluble compounds that are usually non-toxic 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).

In this study, proline concentration in the leaf of *E. amoenum* was increased with increasing salinity, particularly at the highest external salt level, thus, showing the positive role of proline in the salt tolerance of this crop. Proline, as a signaling/regulatory molecule, can activate multiple responses, which are component to the process of adaptation to abiotic stresses including salt stress (Ashraf and Orooj, 2006). Increase in proline under salinity has also been reported in some medicinal plants (Hajar et al., 1996; Munns., 2002; Ashraf and Orooj, 2006; Abdul Jaleel et al., 2007). Leaf soluble sugars considerably increased in response to the increase in salinity. Accumulation of soluble carbohydrates 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).

Conclusion

High concentrations of salt on the roots bring down the soil water potential and available water (Fornes et al., 2007; Jampeetong and Brix, 2009). The cumulative effects of reduced osmotic potential of soil solution, ion toxicity (high concentrations of Cl^- and Na^+) in soil/plants and ionic imbalance (reduced K^+/Na^+ and Ca^{2+}/Na^+ ratio) within plant system under salt stress highly contributed to the reduction of water uptake and plant growth (Grewal, 2010).

The results showed that the accumulation of proline and soluble sugars is a good indicator for salinity tolerance of this plant. Also maintaining of high K^+/Na^+ and Ca^{2+}/Na^+ ratios by the *E. amoenum* is an important mechanism for salt tolerance. So this plant through osmotic adjustment and ion uptake and compartment within the cells resists against salt. This process is essential for plant survival in saline conditions.

REFERENCES

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

Ahmad AK, Tawfik KM, Zinab KM, Abd El-Gawad A (2008). Tolerance of seven Faba bean varieties to drought and salt stresses. J. Agric. Biol. Sci. 4(2): 175-186.

Amirghofran Z, Azadbakht M, Keshavarzi F (2000). *Echium amoenum* stimulate of lymphocyte proliferation and inhibit of humoral antibody synthisis. Iran. J. Med. Sci. 25: 119-124.

Ashraf M, Orooj A (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 JN, Ziatabar AM (2002). Plants growing in saline and arid lands, (Translation), Mazandaran Univ. Press. p. 407.

Bajji M, Kinet JM, Lutts S (1998). Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures. Plant Sci. 137: 131-142.

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

Benlloch M, Ojeda MA, Ramos J, Rodriguesnavarro A (1994). Salt sensitivity and low discrimination between potassium and sodium in plant. Plant Soil, 166: 117-123.

Chaves MM, Maroco JP, Pereira JS (2003). Understanding plant response to drought: from genes to the whole plant. Funct. Plant Biol. 30: 239-264.

Cramer GR, Lynch J, Lauchli A, Epstein E (1987). Influx Na^+, K^+ and Ca^{2+} into roots of salt stressed cotton seedling: Effects of supplemental Ca^{2+}. Plant Physiol. 83: 510-519.

Darvishi B, Postini K, Tavakol Afshari R (2009). Study of Pattern of ion distribution in different organs of alfalfa and its relation to performance in conditions of salt stress. J. Crop Sci. 2: 43-31.

El-Hendawy SE, Hu Y, Schmidhalter U (2005). Growth, ion content, gas exchange, and water relation of wheat genotypes differing in salt tolerances. Aust. J. Agric. Res. 56: 123-134.

Fornes F, Marı´a Belda R, Carrio´n C, Noguera V, Garcı´a-Agustı´n P, Abad M (2007). Pre-conditioning ornamental plants to drought by means of saline water irrigation as related to salinity tolerance. J. Hort. Sci. 113: 52-59.

Ghassemi N, Sajjadi SE, Ghannadi AR, Shams-Ardakani MR, mehrabani M (2003). Volatile constituents of a medicinal plant of Iran, *Echium amoenum* Fisch. C.A. Mey. Daru. J. 11(1): 32-33.

Girija C, Smith BN, Swamy PM (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.

Gorham J, Bridges J, Dubcovsky J, Dvorak J, Hollington PA, Luo MC, Khan JA (1997). Genetic analysis and physiology of a trait for enhanced K+/Na+ discrimination in wheat. New Physiologist. 137: 109-116.

Grewal HS (2010). Water uptake, water use efficiency, plant growth and ionic balance of wheat, barley, canola and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. Agric. Water Manage. 97: 148-156.

Hajar AS, Zidan MA, Al-Zahrani HS (1996). 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, Ashraf M (2008). Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range (Pakistan) to salinity stress. Flora, 203: 68-694.

Hanson B, Grattan SR, Fulton A (1999). Agricultural Salinity and Drainage, University of California, Davis. 160 pp.

Heidari MR, Moein-Azad E, Mehrabani M (2006). Evaluation of the analgesic effect of *Echium amoenum* Fisch & C.A. Mey. Extract in mice: Possible mechanism involved. J. Ethnopharmacol. 103: 345-

349.

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

Kingsbury RW, Epstein E, Pearcy RW (1983). Physiological responses to salinity in selected lines of wheat. Plant Physiol. 74: 417-423.

Kumar R, Goyal V, Kuhad MS (2005). Influence of fertility-salinity interactions on growth, water status and yield of Indian mustard *(Brassica juncea)*. Indian. J. Plant Physiol. 10: 139-144.

Levitte J (1980). Responses of plants to Environmental stresses. 2nd edition. Academic Press. New York. USA.

Mansfield TA, Hetherington AM, Atkinson CJ (1990). Some aspects of stomatal physiology. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41: 55-75.

Mehrabani M, Ghassemi N, Sajadi E, Ghannadi AR, Shams-Ardakani MR (2005). Main phenolic compound of petals of *Echium amoenum* Fisch. and C. A. Mey., a famous medicinal plant of Iran. DARU. 2(13): 65-69.

Meloni DA, Gulotta MR, Martinez CA (2008). Salinity tolerance in *Schinopsis quebracho colorado*: Seed germination, growth, ion relations and metabolic responses. J. Arid Environ. 72: 1785-1792.

Moradi F, Ismail AB (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot. 99: 1161-1173.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell. Environ. 25: 239-250.

Munns R, James RA, Lauchli A (2006). Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57: 1025-1043.

Naeini MR, Khoshgoftar AH, Lessani H, Mirzapour MH (2004). Effect of NaCl-induced salinity on mineral nutrients and soluble sugar in three commercial cultivars of Pomegranate. J. Soil Water Sci. 18: 97-105.

Orcutt MD, Nilsen ET (2000). The physiology of plants under stress: soil and biotic factors. John Wiley and Sons, New York. p. 683.

Poljakoff MA, Lerner HR (1994). Plants in saline environments. In: M. Pessarakli (ed). Handbook of plant and crop stress. Marcel Dekker. Inc. pp. 65-96.

Prado FE, Gonzales JA, Gallardo M, Moris M, Boero C, Kartsarz A (1995). Changes in soluble carbohydrates and invert as activity in *Chenopodiom quinoci*. Developed for saline stress during germination. Curr. Trop. Phystol. 14: 1-5.

Ramoliya PJ, Patel HM, Pandey AN (2004). Effect of salinazation of soil on growth and macro- and micro-nutrient accumulation in seedlings of *Salvadora persica* (salvadoraceae). For. Ecol. Manage. 202: 181-193.

Rengel Z (1992). The role of calcium in salt toxicity. Plant Cell Environ. 15: 625-632.

Sadizadeh M, Abbassi F, Baghizadeh A, Yazdanpanah A (2009). The effects of salicylic acid and ascorbic acid on some of resistance mechanisms to drought stress in *Echium amoenum*. American-Eurasian J. Agric. Environ. Sci. 6(3): 262-267.

Safavi S, Khajehpour MR (2007). Effects of salinity on Na, K and Ca contents of borage (*Borago Officinalis* L.) and echium (*Echium amoenum* Fisch. & Mey.). RPS. 2: 23-27.

Saiiah Bargard M, Assadi SM, Amini H, Akhondzadeh S (2003). Efficacy of aqueous extract of *Echium amoenum* L. in the treatment of mild to moderate major depressive disorder: A randomized double blind clinical trial. Institue of medicinal plant Iran.

Sharma AD, Thakur M, Rana M, Singh K (2004). Effect of plant growth hormones and abiotic stresses on germination, growth and phosphates activities in *sorghum bicolor* L. Moench seeds. Afr. J. Biotechnol. 3: 308-312.

Song J, Chen M, Feng G, Jia Y, Wang B, Zhang F (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.

Stewart EA (1989). Analysis of vegetation and other organic material. In: Acad. Press, New York. pp. 46-60.

Wyn JRG (1981). Salt tolerance. In: Johnson CB. (Ed.), Physiological Processes Limiting Plant Productivity. Butterworths, London. pp. 271-292.

Zandstra-Plom M, Vogelrang SA, Veen BN (1998). Sodium fluxes in sweet pepper exposed to varying sodium concentrations. J. Exp. Bot. 49: 1863-1868.

Zhu JK (2001). Genetic analysis of plant salt tolerance using *Arabidopsis thaliana*. Plant Physiol. 124: 941-948.