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Full Length Research Paper

Evaluating potential of borage (*Borago officinalis* L.) in bioremediation of saline soil

Hassanali Naghdi Badi and Ali Sorooshzadeh*

Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, P.O. Box 14115-336, Tehran, Iran.

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Bioremediation is an efficient, economical and environmentally acceptable strategy used for coping with the salinization of agricultural soils. In this study, borage has been proposed as possible candidate for bioremediation of Na^+ and Cl^-. In this order, the borage (*Borago officinalis* L.) seeds were sown under four levels of salinity (non-saline as control, 5, 10 and 15 dSm^{-1}). Bioremediation and production potentials of borage were evaluated at three growth stages: seedling or early growth, vegetative and flowering stages. This study has been conducted using factorial experiment in randomized complete block design with three replications. The saline ions accumulation in company with changes in growth and chemical composition of borage was studied. The results indicated that the contents of Na^+ and Cl^- in plants increased as salinity levels of growth medium were enhanced. The noticeable contents of Na^+ and Cl^- (9.096 and 5.665%, respectively) were accumulated in borage aerial parts at the highest level of salinity (EC of 15 dSm^{-1}), whereas minimum values, 2.029 and 1.520%, occurred at non-saline condition. Although, borage growth decreased with increasing salinity, its survival/or noticeable growth indicated that this plant could tolerate salinity up to EC of 15 dSm^{-1}. The salinity had a significant effect on the total phenol, alkaloids and tannins and their contents increased with increasing salinity. In contrast, mucilage content and swelling index significantly decreased with increasing salinity. Therefore, borage had noticeable quality and quantity yield up to salinity level of 15 dSm^{-1} and could cumulatively remove considerable amounts of salt from the soil. In addition, if borage can be cultivated as an inter-crop all year round at saline soil with EC up to 15 dSm^{-1}, it can remedy saline soil in respect to Na^+ and Cl^-.

Key words: *Borago officinalis* L., bioremediation, growth stage, salinity, salt tolerance.

INTRODUCTION

Salinity in soil or water is one of the major stresses and can severely limit crop production (Ashraf and Harris, 2004). Under irrigated agriculture, salts are added continuously to the soils with each irrigation event (Qadir et al., 2006). Thus, soil salinity is a major threat to the sustainability of irrigated agriculture (Ghassemi et al., 1995). To combat the salinity problem, there are many solution methods. The recent approach is to use domesticated halophytes (Lieth et al., 1999). In other words, the use of salt accumulating species to remediate aqueous or soil environments has become an option to remove salts from contaminated site (Garnett et al., 2002). Potentials of using some salt-tolerant plants for remediation of saline sodic soil have been reported by several researchers (Chaudhri et al., 1964; Qadir et al., 2006; Abdelly et al., 2007).

Plant species differ greatly in their growth response to salinity (Koyro, 2006). Therefore, the study about mechanisms of salt tolerance in plants is necessarily an important prerequisite for the sustainable use of promising salt tolerant plants with adequate tolerance and yield characteristics (Koyro, 2003). Therefore, identifying such species among the large pool of suitable plants is an effective step to remediation and management of saline soils.

Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism, in which there are much research information about response to salt stress (Parida and Das, 2005). Despite a wealth of published research on

*Corresponding author. E-mail: soroosh@modares.ac.ir.

salinity tolerance of plants (Ashraf and Harris, 2004), the effects of salinity on plants secondary metabolites are not well documented. Thus, the study about changes of secondary metabolites under different environmental conditions such as salinity is necessary. Chemical compounds such as phenolics, alkaloids, steroids and terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance (Bandaranayake, 2002).

Phenolic compounds are aromatic compounds with hydroxyl substituents (Bandaranayake, 2002), which have numerous values due to their various biological activities (Bandaranayake, 2002; Kim, 2006; Latha et al., 2007; Kris-Etherton et al., 2002; Bravo, 1998; Oboh, 2006). Only, a few studies have been carried out on the effect of salinity on the total phenol in some medicinal plants. For example, short-term results showed that total phenol concentration increased in the olive oil produced with high NaCl levels in irrigation water (Stefanoudaki, 2004; Weisman et al., 2004), as has also been reported for water stress (Cresti et al., 1994).

Alkaloids are nitrogenous bases (usually heterocyclic), and are structurally the most diverse class of secondary metabolites. Their manifold pharmacological activities have always excited man's interest (Bandaranayake, 2002). However, in order to determine salinity effect on total alkaloids in two medicinal plants (*Datura stramonium* and *Hyoscyamus*), Ahmed et al. (1989) found that the total alkaloid contents as well as the contents of various alkaloids fractions in both plants increased mostly with the rise of salinization level, whatever the organs (leaves, stem and root) analyzed.

Tannins are polyphenolic substances widely distributed among higher plants (Kraus et al., 2003; Ferwerda et al., 2005), which have various uses in industrial products. Recently, evidence has been obtained in support of their potential value as cytotoxic or antineoplastic agents (Bandaranayake, 2002). However, tannin concentrations in plants vary in response to changes in environmental conditions (Chaves and Escuder, 1999). Therefore, it is necessary to evaluate the changes of borage tannins at saline conditions.

The total ash includes both "physiological ash", derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant (WHO, 1998). In addition, total ash content can include the total amount of salts in plant samples (IHP, 2002).

Content of acid-insoluble ash is the amount of silica present, especially as sand and siliceous earth which is present in plant sample (WHO, 1998; IHP, 2002). However, the content of total ash and acid-insoluble ash must not be more than extant mentioned in herbal pharmacopoeias (Naghdi et al., 2008). Khan et al. (2000) found that the ash content of *Suaeda fruticosa* increased from 30% in controls to about 60% in 1000 $molm^{-3}$ NaCl. In addition, the halophytes have high ash contents because of an accumulation of Na^+ and Cl^- (Robinson and Downton, 1985; Naidoo and Raghunanan, 1990).

Borage (*Borago officinalis* L.), an annual herbaceous plant native to Europe, North Africa, and Asia Minor (Beaubaire and Simon, 1987), is a medicinally important plant, which has more than 20% gamma linolenic acid in the seed oil (El Hafid et al., 2002). The leaves of borage are reportedly used as diuretic, demulcent, emollient, expectorant, etc. (Leung and Foster, 1996). In Iranian traditional medicine, the aerial parts of borage are reportedly used for treatment of a variety of ailments (Naghdi et al., 2008). However, borage is an important medicinal plant, which must be cultivated commercially in order to meet the ever-increasing demand for the 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. In this order, it seems important to investigate borage for its salt-tolerance capacity in order to exploit the saline lands for its cultivation. For this reason, the specific objectives of this study were: (a) To determine the effect of salinity on the quality and quantity yields at different growth stages, (b) to quantify levels of sodium and chloride uptake and accumulation in this plant, and (c) to determine the role of borage in remediation of saline soil.

MATERIALS AND METHODS

Plant materials

This study was done in the laboratory and greenhouse of Plant Science Department at Tarbiat Modares University, Iran, from September 2006 to November 2007. Seeds of *B. officinalis* L. were obtained from the Cultivation and Development Department of Medicinal Plants Institute (ACECR). Seeds were selected for uniformity in size, shape, and color. This experiment has been conducted using factorial experiment in a completely randomized design with three replications. In this order, the borage seeds were sown under four levels of salinity (non-saline as control, 5, 10 and 15dSm^{-1}). In addition, the bioremediation and production potentials of borage were evaluated at three growth stages: seedling or early growth stage, vegetative stage and flowering stage. The treatment solutions were made with saline water and distilled water depending on target salinity. Natural saline water was obtained from Hoz-e-Soltan Lake in Qom, Iran. The major ions of saline water were:128 g/l Na^+, 218.7 g/l Cl^-, 1.23 g/l K^+, 19.5 g/l Mg^{2+}, 0.086 g/l Ca^{2+} and 48.8 g/l SO_4^{2-}.

The experiment at early growth stage was done in Plant Science Laboratory at Tarbiat Modares University, Iran. In this experiment, each replicate contained 1.0 L plastic container in which 50 seeds were sown with dry washed sand. Then, one concentration of the treatment solutions was added to the medium up to the soil field capacity (sufficient water to initiate drainage). The salinity levels were kept constant throughout the experiment period by using containers sealed with plastic bags to avoid evapotranspiration. The growth room conditions were maintained at 24.5 ± 0.5°C, with relative humidity of 35 ± 5% and photoperiod of 12 h. The fifteen-day-old seedlings were then used to determine the growth and chemical compositions. Seedling growth (fresh and dry weight) was determined using ten seedlings from each salinity levels in triplicate. The seedlings were oven dried at 70°C for 72 h (until there was no decrease in weight).

The experiment at vegetative and flowering stages was conducted in a greenhouse of Plant Science Department at Tarbiat Modares University, Iran. In this experiment, the perforated plastic pots of 30 cm diameter were filled with 14.0 kg of soil. Fifty seeds were sown in each pot. After 14 days of germination, plants were thinned to seven plants per pot. This soil is a noncalcareous sandy loam containing 54% sand, 30% silt and 16% clay. The available soil water between wilting coefficient and field capacity ranges from 6.0 to 14.7%, respectively. The total organic carbon content was 0.3% and pH of soil was 7.3. Soil fertility was poor with respect to nitrogen (0.025%) and phosphorus (8 mg/kg) contents.

To simulate non-saline and saline soils before sowing pots, each pot was irrigated at several time with fresh water (0.3 dS/m) or saline water (5, 10 and 15 dS/m) of natural saline water until the effluent salinity at bottom of the pots was equal to that of the supply water. The salinity level was monitored by measuring ECw twice a week at 25°C with a CyberScan Con10/20 conductivity meter.

To determine the growth (fresh and dry weight), fresh weight of plant aerial part was directly recorded and samples for dry weight determination were oven dried at 70°C for 72 h (until there was no decrease in weight).

Biochemical analysis

For the measurement of Na^+ content, the oven dried plant materials were completely digested in concentrated nitric acid at 250°C (Wahid et al., 1998). The extract was evaluated in a Perkin-Elmer atomic absorption spectrophotometer model 560 and Na was determined.

Chloride was measured by titration against silver nitrate (Sato et al, 2006). For the measurement of chloride ion content, 0.1 g of the plant material from each treatment was used. The samples were homogenized and centrifuged at 9200 × g for 20 min. Five milliliters of supernatant was mixed with same amount of purified water and a few drops of 8% potassium chromate (K_2CrO_4), and then subjected for the titration by 0.05N silver nitrate ($AgNO_3$). During titration, the following reaction takes place:

$$Cl^- + AgNO_3 \longrightarrow AgCl + NO_3^-$$

Silver chloride is an insoluble solid. When all chloride ions have reacted with silver ions, the remaining silver ions react with the chromate in the solution and silver chromate (Ag_2CrO_4), which is a pink-orange precipitate indicating the end of the reaction above. The chloride content in sample solution can be calculated from the amount of titrant given (APHA, 1995).

Total phenolic contents were determined using chlorogenic acid as a standard molecule with the Folin-Dennis method, as described by Iranian Herbal Pharmacopeia (2002). The results were expressed as the chlorogenic acid equivalent for the total polyphenols.

Total alkaloids were quantified from the dried shoots tissues of the plants in base of kelidonin as described by Iranian Herbal Pharmacopeia (2002).

Tannins were quantified using tannic acid as a standard molecule with the Folin-Dennis method, as described by Iranian Herbal Pharmacopeia (2002).

The swelling index was the volume in ml taken up by the swelling of 1g of plant material under specified conditions. This index was determined with the method described by WHO (1998).

Mucilage content was quantified as described by Iranian Herbal Pharmacopeia (2002).

The ash remaining (following ignition of medicinal plant materials) was determined by different methods. In this study, the total ash and acid-insoluble ash of the aerial parts were determined according to the method of Iranian Herbal Pharmacopeia (2002).

Statistical analysis

Analysis of variance (ANOVA) was done by SPSS statistical software package version 15 and SAS program (Statistical Analysis System ver. 6.12, SAS Inc., 1995). Mean values and significance were determined by "Duncan's multiple range test".

RESULTS

Plant growth status

Salinity had significant inhibitory effect on the fresh and dry weight of borage (Table 1).The shoot fresh and dry weight decreased constantly with increase in the salinity levels of the growth medium. The minimum fresh and dry weight of shoot was observed at the highest salinity level (Table 2).

In addition, the fresh and dry weight significantly increased with the development of plants, from seedling to flowering stage (Table 3). For this reason, the highest fresh and dry weight of aerial parts was obtained at flowering stage (Table 3).

Na^+ and Cl^- contents

The contents of Na^+ and Cl^- varied with increasing salinity levels (Table 1). The Na^+ and Cl^- contents increased as salinity levels of growth medium are enhanced (Table 2). The maximum Na^+ and Cl^- contents (9.096 and 5.665%, respectively) were recorded in the highest level of salinity (EC of 15 dSm^{-1}), whereas minimum values, 2.029 and 1.520%, occurred at non-saline condition (Table 2).

The contents of Na^+ and Cl^- varied at different growth stages (Table 1) and the maximum (7.047 and 4.081%, respectively) and minimum content (4.373 and 3.501%, respectively) of these ions was observed at vegetative and seedling stage, respectively (Table 3). Therefore, after an initial increase in the Na^+ and Cl^- content at vegetative growth stage, their content declined at the flowering stage.

Phytochemical evaluations

This study indicated that phytochemical compositions of borage were significantly different at different salinity levels. With increasing salinity levels, the content of total phenol, total alkaloids and tannins significantly increased ($P < 0.01$), but the swelling index and mucilage content significantly ($p < 0.05$) decreased (Tables 1 and 2). The highest content of total phenol, total alkaloids and tannins resulted from the highest salinity levels and the lowest ones resulted from the lowest salinity levels (non-saline conditions). In addition, the lowest and highest content of swelling index and mucilage resulted from the highest and lowest salinity levels, respectively.

The salinity had various effects on total ash and acid-

Table 1. Mean squares from analyses of variance of data for measured attributes of *B. officinalis* at different growth stage.

Source of variation	df	Fresh weight	Total alkaloids	Mucilage
Salinity levels (A)	3	126.908***	15.083***	4.708***
Growth stage (B)	2	3802.468***	3.436***	2.619***
AB	6	32.155***	1.307**	$0.364^{n.s}$
Error	24			0.244
		Dry weight	**Tannins**	**Total ash**
Salinity levels (A)	3	2.272***	1.110***	8.446***
Growth stage (B)	2	30.451***	0.168*	8.941***
AB	6	0.631***	0.097*	0.490*
Error	24			
		Total phenol	**Swelling index**	**Acid-insoluble ash acid**
Salinity levels (A)	3	23.971***	25.670***	0.009*
Growth stage (B)	2	$0.317^{n.s}$	1.841**	0.305***
AB	6	$0.188^{n.s}$	1.383***	$0.004^{n.s}$
Error	24		0.253	
		Na^+ content	**Cl^- content**	
Salinity levels (A)	3	84.022***	26.877***	
Growth stage (B)	2	24.613***	1.009***	
AB	6	11.353***	0.471***	
Error	24	0.165	0.047	

n.s: Non-significant; * P > 0.05; ** P > 0.01; *** P > 0.001.

insoluble ash acid content (Tables 1 and 2). Salinity caused a considerable increase in the content of total ash in the plant materials, whereas the content of acid-insoluble ash acid decreased with increasing salinity.

The contents of phytochemical compounds in borage tissues varied during the growing season except content of total phenol, which had no significant differences (Table 1). Although the swelling index and the content of total alkaloids, tannins and mucilage significantly decreased from seedling to flowering stage, the content of total ash and acid-insoluble ash significantly increased with growth and development of plant (Tables 2,3 and 4).

DISCUSSION

This study indicated that the fresh and dry weight of shoot decreased constantly with increasing salinity levels (Tables 2 and 4) and the minimum fresh and dry weight of shoot was observed at the highest salinity level that is, EC of 15 dS m^{-1} (Tables 2 and 4). Although borage had reduction in plant growth with increasing salinity, its survival/ or noticeable growth indicated that this plant could tolerate salinity up to EC of 15 dSm^{-1}. Adverse effects of salt stress on growth, dry matter production and economic yield of a number of cultivated plant species have been subjected to extensive investigations in recent years (Jeschke and Wolf, 1988; Parida and Das, 2005). Salt stress affects all the major processes such as growth, photosynthesis, protein synthesis and energy and lipid metabolism, in which there are much research information about the response to salt stress (Parida and Das, 2005).

Our study indicated that borage exhibited great accumulation of Na^+ and Cl^- in their tissues in company with noticeable quantity yields at saline conditions up to EC of 15 dSm^{-1} (Table 2 and 4). In addition, Na^+ and Cl^- concentrations at EC of 15 dSm^{-1} were 9.096 and 5.665%, respectively. Thus, the borage is able to absorb/remove noticeable amount of Na^+ and Cl^- from soil. Therefore, the borage can be proposed as possible candidates for phytoremediation of Na^+ and Cl^- and this plant can be useful both in managing the highly saline soil from farm.

The salinity had significantly effect on the total phenol and content of total phenol increased with increasing salinity (Tables 1, 2 and 4). As far as the literature is avail-able, no studies were performed concerning the effect of salinity on the total phenol contents of borage. In respect of medicinal plants, a few studies have been carried out on the effect of salinity on the total phenol. For example, short-term results showed that total phenol concentration increased in the olive oil produced with high NaCl levels in irrigation water (Stefanoudaki, 2004; Weisman et al., 2004).

The tannins' content significantly increased with increasing medium salinity (Table 1 and 2). In contrast, it decreased with development of the plant. The highest and lowest of tannin contents were observed in seedling and flowering stages, respectively (Tables 3 and 4).

Table2: Measured attributes of borage aerial parts at different salinity levels.

Salinity levels (dS m^{-1})	Fresh Weight (gr/plant)	Dry Weight (gr/plant)	Total phenol (%)	Total alkaloids (ppm)	Tannins (%)	Swelling index	Mucilage (%)	Total ash (%)	Acid-insoluble ash (%)	Na^+ Content (%)	Cl^- Content (%)
EC0	21.723^{a}±6.104	2.173^{a}±0.634	4.300^{d}±0.132	7.733^{d}±0.141	2.888^{c}±0.051	14.067^{a}±0.264	11.022^{a}±0.210	7.756^{c}±0.266	1.233^{a}±0.042	2.029^{d}±0.247	1.520^{d}±0.189
EC5	18.886^{b}±5.560	1.583^{b}±0.481	4.911^{c}±0.174	8.433^{c}±0.186	3.159^{b}±0.061	12.256^{b}±0.335	10.411^{b}±0.220	8.037^{c}±0.297	1.198^{ab}±0.043	5.390^{c}±0.299	3.651^{c}±0.082
EC10	15.199^{c}±4.689	1.241^{c}±0.393	7.067^{b}±0.168	9.811^{b}±0.300	3.549^{a}±0.091	11.000^{c}±0.173	9.844^{c}±0.272	9.337^{b}±0.327	1.174^{b}±0.057	7.511^{b}±0.691	4.308^{b}±0.152
EC15	13.323^{d}±4.360	1.020^{d}±0.343	7.667^{a}±0.109	10.589^{a}±0.391	3.644^{a}±0.091	10.189^{d}±0.198	9.346^{d}±0.133	9.739^{a}±0.268	1.164^{b}±0.052	9.096^{a}±1.028	5.665^{a}±0.132

Table 3. Measured attributes of borage aerial parts at three stages of growth cycle.

Growth stage	Fresh weight (g/plant)	Dry weight (g/plant)	Total phenol (%)	Total alkaloids (ppm)	Tannins (%)	Swelling index	Mucilage (%)	Total ash (%)	Acid-insoluble ash (%)	Na^+ content	Cl^- content
Seedling	0.123^{c}±0.006	0.009^{c}±0.0002	5.825^{a}±0.487	9.625^{a}±0.449	3.447^{a}±0.148	12.267^{a}±0.382	10.692^{a}±0.281	7.739^{c}±0.247	1.009^{b}±0.020	4.373^{c}±0.272	3.501^{c}±0.504
Vegetative	16.063^{b}±1.513	1.325^{b}±0.161	6.150^{a}±0.391	9.233a±0.462	3.246^{b}±0.086	11.883^{ab}±0.575	9.542^{b}±0.224	9.038^{b}±0.375	1.272^{a}±0.016	7.047^{a}±1.018	4.081^{a}±0.399
Flowering	35.662^{a}±1.593	3.179^{a}±0.241	5.983^{a}±0.445	8.567^{b}±0.194	3.238^{b}±0.077	11.483^{b}±0.465	9.834^{b}±0.197	9.373^{a}±0.199	1.297^{a}±0.014	6.599^{b}±1.159	3.776^{b}±0.477

Table 4. Chemical composition of borage aerial parts at different salinity levels.

Treatment	Total phenol (%)	Total alkaloids (ppm)	Tannins (%)	Swelling index	Mucilage (%)	Total ash (%)	Acid-insoluble ash (%)	Na^+ content (%)	Cl^- content (%)
E1S1	4.000^{e}±0.153	7.633^{f}±0.219	2.750^{f}±0.104	13.467^{b}±0.260	11.533^{a}±0.371	6.933^{e}±0.176	1.076^{b}±0.038	2.933^{g}±0.072	0.832^{h}±0.022
E1S2	4.600^{de}±0.231	7.667^{f}±0.328	2.970^{ef}±0.035	14.900^{a}±0.321	10.933^{ab}±0.291	7.733^{d}±0.472	1.310^{a}±0.027	1.887^{h}±0.113	2.100^{f}±0.087
E1S3	4.300^{de}±0.208	7.900^{f}±0.252	2.943^{ef}±0.072	13.833^{b}±0.328	10.600^{bcd}±0.289	8.600^{bc}±0.221	1.313^{a}±0.026	1.267^{h}±0.067	1.627^{g}±0.089
E2S1	4.533^{de}±0.260	9.000^{de}±0.115	3.303^{bcd}±0.116	13.400^{b}±0.321	11.067^{ab}±0.348	7.077^{e}±0.126	1.037^{bc}±0.032	4.322^{f}±0.108	3.753^{d}±0.080
E2S2	5.367^{c}±0.273	8.033^{f}±0.260	3.017^{def}±0.060	11.867^{c}±0.353	10.100^{cde}±0.265	8.030^{dc}±0.271	1.293^{a}±0.030	6.262^{d}±0.223	3.767^{de}±0.145
E2S3	4.833^{cd}±0.203	8.267^{ef}±0.285	3.157^{cde}±0.081	11.500^{cd}±0.321	10.067^{de}±0.260	9.003^{b}±0.211	1.263^{a}±0.032	5.587^{de}±0.217	3.433^{e}±0.136
E3S1	7.167^{ab}±0.328	10.533^{ab}±0.467	3.833^{a}±0.120	11.433^{cd}±0.291	10.800^{abcd}±0.265	8.103^{dc}±0.207	0.957^{c}±0.029	5.087^{e}±0.131	4.008^{d}±0.080
E3S2	6.900^{b}±0.379	9.967^{bc}±0.318	3.497^{b}±0.071	10.700^{d}±0.306	9.333^{e}±0.285	9.8873^{a}±0.255	1.253^{a}±0.0318	9.787^{b}±0.346	4.897^{c}±0.067
E3S3	7.133^{ab}±0.260	8.933^{de}±0.318	3.317^{bc}±0.109	10.867^{d}±0.186	9.400^{e}±0.231	10.020^{a}±0.167	1.313^{a}±0.023	7.660^{c}±0.215	4.020^{d}±0.076
E4S1	7.600^{ab}±0.208	11.333^{a}±0.333	3.900^{a}±0.115	10.767^{d}±0.338	9.367^{e}±0.318	8.843^{b}±0.184	0.965^{c}±0.0164	5.150^{e}±0.055	5.412^{b}±0.130
E4S2	7.733^{a}±0.203	11.267^{a}±0.367	3.500^{b}±0.153	10.067^{e}±0.233	9.400^{e}±0.173	10.503^{a}±0.286	1.230^{a}±0.026	10.253^{b}±0.339	5.560^{b}±0.199
E4S3	7.667^{a}±0.233	9.167^{cd}±0.260	3.533^{b}±0.120	9.733^{f}±0.145	9.270^{e}±0.276	9.870^{a}±0.217	1.300^{a}±0.032	11.883^{a}±0.475	6.023^{a}±0.228

Therefore, tannin content differed in response to changes of plant development (growth stages) and environmental conditions such as salinity. It has been previously observed that tannin concentrations in plants vary in response to changes of environmental conditions (Chaves and Escuder, 1999). In addition to varying with environmental conditions, tannin concentrations also vary by genotype, phenology and season (Kraus et al., 2003). Alternatively, plasticity in tannin production may be adaptive and provide such benefits as compensatory defense, UV protection, oxidative prevention, and nutrient uptake (Close and McArthur, 2002; Nitao et al., 2002). Therefore, increasing tannin content at saline conditions and/or early growth stages suggested that there is a defense mechanism to cope with environmental stresses.

The content of total alkaloids significantly increased ($P < 0.01$) with increasing salinity levels (Tables 1, 2 and 4) and the highest and lowest content of total alkaloids resulted from the EC of 15 and 0.3 dS m^{-1}, respectively. Ahmed et al. (1989) work on the effect of salinity on *D. stramonium* and *Hyoscyamus muticus* and found that the total alkaloid contents as well as the contents of various alkaloids fractions in both plants increased mostly with the rise of salinity level, whatever the organs (leaves, stem and root) analyzed.

Although aerial parts of borage had noticeable mucilage amount in different growth stages and salinity levels, mucilage content and thereafter swelling index significantly decreased with increa-sing salinity and promoting growth stages (Tables 1 and 2). The pervious study indicated that borage leaves contain mucilage at different growth stage (Naghdi, 2008). On the other hand, carbo-hydrates such as sugars (glucose, fructose, sucrose, and fructans) accumulate under salt stress (Parida et al., 2002). The decreasing mucilage can be in order to increase soluble carbohydrates. Their major functions are osmoprotection, osmotic adjustment, carbon storage, and radical scavenging (Parida and Das, 2005). This mechanism can be the reason of borage tolerance to salinity.

To explain variations in secondary compounds, there are several different hypotheses. The carbon-nutrient balance hypothesis (CNBH) (Bryant et al., 1983) and the growth-differentiation hypothesis (Herms and Mattson, 1992) are resource-based hypotheses, which explain changes in concentrations of secondary compounds to changes in resource availability. Both of these hypotheses suggest that given an excess of C over other nutrients (primarily N), plants increase allocation to C-rich secondary compounds such as phenolics rather than nutrient-rich compounds such as proteins. In support of these hypotheses, most studies find that total phenolic and tannin concentrations increase in response to lower fertility and greater atmospheric CO_2 levels (Kraus et al., 2003). Therefore, variation in secondary metabolites can be due to changes in resource availability such as C and other nutrients at saline conditions. Therefore, increasing secondary metabolites such as total phenol, total alkaloids and tannins can be due to lower fertility and excess of carbon in borage at salinity.

It is clear that saline soil reclamation was carried out by different methods including using halophytic vegetation, chemical remediation by using organic or mineral amendments and by mechanical remediation by utilizing excavation and removal of the salt-affected soil. Revegetation of a salt-affected land with halophytes is an example of proactive phytoremediation (Yensen et al., 1999). In addition, a recent approach is to use domesticated halophytes to combat the salinity problem (Lieth et al., 1999). It is an important prerequisite for the sustainable use of promising salt tolerant plants with adequate tolerance and yield characteristics (Koyro, 2003). The use of phytoremediation techniques to cleanse saline-polluted substrates offers new options for the reduction of crop damage and loss (Garnett et al., 2002). However, the cost of leaching by chemical and mechanical or by drainage system is higher than bio/or phytoremediation (Ravindran et al., 2007).

Several researchers have evaluated the potential use of some salt-tolerant plants for remediation of saline sodic soil. For example, it was reported that the salt content in leaf and stem fresh tissue of *S. fruticosa* were 9.06 and 4.29%, respectively, and this plant has effective role in remediation of saline soil (Chaudhri et al., 1964). Other studies showed that *Sesuvium portulacastrum* accumulated large amounts of Na^+ and Cl^- (about 6.5 mmol.g^{-1} DW, or 30 – 40% of the biomass) and at the end of the experiment the quantities of Na^+ and Cl^- in soil were significantly reduced (10%) by planting *S. portulacastrum* (Abdelly et al., 2007). Published data indicate that some halophytes can accumulate high levels of Na^+ in the shoot (up to 50% of dry weight) without dying (Tester and Davenport, 2003). However, salts are being added continuously to the soils with each irrigation event under irrigated agriculture. Removal of salts via aerial plant parts under irrigated conditions is an inefficient way of saline soil amelioration (Qadir et al., 2006) which is possible by cultivation of salt-hyperaccumulator plants at crop rotations. This study indicated that borage plant is able to remove large amount of salt from saline soil and further studies must be done for potential evaluation of borage in remediation of saline soils under actual field conditions.

Our results indicated that the content of Na^+ and Cl^- varied at different growth stages. In addition, the maximum and minimum contents of these ions was observed at vegetative and seedling stages, respectively. Therefore, this plant had a mechanism that could control these ions content at seedling and flowering stages. Because seedling and flowering stages are two sensitive and critical stage of plants, this control can be of a valuable and vital process.

However, increasing the content of some valuable compounds such as total phenol, total alkaloids and

tannins at saline conditions is very valuable, which suggests that borage could be used for bioremediation of saline soils in company with noticeable quality yield.

Conclusion

Our result indicated that borage is a salt-accumulating species and can uptake and accumulate high levels of toxic salt ions (Na^+ and Cl^-) with its above ground tissues from the growth medium. This study was the first to suggest that borage could be used to desalinate soil in company with noticeable quality yield.

Since fruit trees are in general salt, in particular, Cl^- sensitive, borage can be suited to intercropping with fruit trees. Because borage growth cycle is short and can be grown in different areas including arid and semiarid conditions throughout the year, it can be used continuously for salt removal in the orchard soils.

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