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Diversity of morphological and chemical traits and assessment of salinity tolerance in medicinal *Portulaca oleracea* L. genotypes

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Abstract

Portulaca oleracea L. is an annual halophyte that can resist salinity as vegetable, forage or medicinal plant. In order to study the effects of different salinity levels on some morphological and chemical properties and salinity tolerance of *Portulaca oleracea* L. a factorial experiment was carried out using randomized complete block design with different salinity levels (0, 7, and 25 deci-Siemens/m) on *Portulaca oleracea* genotypes. Three replications were used and the experiment was conducted in 2013 in the research greenhouse of Islamic Azad University, Isfahan (Khorasgan) Branch. Research results revealed that values of plant height, dry weight of shoot, root wet weight, shoot sodium content, root sodium content, root potassium, ratio of potassium to sodium in shoot, the ratio of potassium to sodium in root were significantly different from the values obtained for the control group. On the other hand, values of root wet weight and shoot dry weight did not differ significantly to a salinity level of 7 deci-Siemens. However, at the 25 deci-Siemens salinity level a significant reduction was observed in the aforementioned values. Examination results indicated that this plant is highly resistant to salinity while genotypes obtained from Ahwaz, Mashhad, and Miandoab also demonstrated a high level of resistance to a salinity of 25 deci-Siemens. Therefore, this plant can be used as a medicinal plant in areas under salinity stress.

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Introduction

Portulaca oleracea L. has numerous medicinal properties and therefore is introduced as a relieving drug for all pains with numerous medicinal uses by the World Health Organization. Some of its properties include its ability to stop pus, relief walking weakness, mitigate gravel, treat helminth, treat headache, and strengthen the stomach (Rahdari *et al.*, 2011). Moreover, this plant is also useful for the treatment of gastrointestinal conditions. It has antifungal and antimicrobial properties and is enriched with vitamins A, C, E, beta-carotene, and minerals. It is also capable of neutralizing the effect of free radicals and preventing the development of cardiovascular diseases, cancer, and infectious diseases (Liu *et al.*, 2000). Every 100 grams of the braches and leaves of *Portulaca oleracea* L contains 92 g water, 1.7 g protein, 4 g fat, 2.5 g carbohydrate, 103 mg calcium, 3 mg phosphorous, 0.3 mg tiamina, 1 mg riboflavin, 5 mg niacin, and 25 g vitamin. The stem of this plant is enriched with the following fatty acids and thus can be effectively used to reduce cholesterol and blood pressure: omega 3, alpha-tocopherol, acid ascorbic, beta-carotene, glutathione. Therefore, this plant is considered among the precious anticancer and anti-blood pressure plants. This plant also contains a considerable amount of vitamin C which is an anti-cancer antioxidant (Simopolous *et al.*, 1992). The active ingredients of this plant include oxalic acid, cinnamic acid, caffeic acid, maleic acid, citric acid, coumarin, flavoinoid, alanine, tannins, alpha-linolenic acid, and menotropin glycosides. It is found out that alkaloids are among the most important chemical compounds of this plant (Mizutan, 1998). Considering the highly important and useful properties of *Portulaca oleracea* L., cultivation and spread of this plant can be very productive for the Iranian pharmaceutical industries. On the other hand, considering the salinity problem in Iran, it is important to find salinity tolerant genotypes.

Salinity is one of the most important factors contributing to the growth and function of plants. Salinity stress and its elimination has been one of human's challenges since thousand years ago. In Iran, more than 15 million hectares of land have salinity problems (Abd Meyshani and Nejat Bushehri, 1995). Amendment of crops and improvement of their resistance to salinity stress forms one of the pillars of the desired mixed method for overcoming this problem (Ashraf, 1994).

Another part of the mixed method is irrigation and drainage management over lands. This task either cannot be accomplished under normal circumstances or is not economic due to the high operational and managerial costs as well as the transient nature of the available methods (Ashraf, 1994). In general, one of the major solutions to this problem is amendment and production of plant genotypes to add to their economic yield under saline conditions. In addition, in field conditions this process is time-consuming and influenced by numerous uncontrollable factors such as soil type, climate and farming operations. Hence, it is necessary to use a greenhouse method under controlled conditions to provide for the quick and relatively precise assessment of plants' response to salinity stress. Salinity has adverse effects such as a reduction in the length and dry weight of radicle and plumule of plants. It also reduces the quality of plants. Potential salinity of water reduces the root zone and leads to a reduction in the water absorption potential of the plant. In addition, with an increase in salinity in the root zone the absorption and transfer of toxic ions to plant tissues increase and finally a reduction is observed in the absorption of essential elements. As a result, the plant loses its ionic balance and the toxicity caused by the accumulation of sodium and chloride ions affects the plant. Many plants demonstrated reduced growth in saline environments. This reduction can be ascribed to the accumulation of toxic ions such as sodium and chloride in plant tissues. Accumulation of toxic ions such as sodium and chloride in plant tissues also leads to a reduction in the enzymatic activities and a change in carbohydrates distribution (Tabatabai Aqdayi, 2000). With the reduction in the extent of

photosynthesis in saline environments, the production of the dry matter in the vegetative organs declines (Gorham, 1996). Salinity leaves a negative effect on the leaf area index (LAI) of plants as well. The reduction in plants' leaf area index, especially in the early stages of growth, is a result of the reduction in water absorption caused by the salinity-induced osmosis. In subsequent phases of growth, accumulation of elements in shoots increases and leads to the early senescence and falling of leaves. Consequently, the leaf area also reduces (Yazdi, 2003). In saline conditions, absorption of potassium by root cells reduces in the presence of sodium and the abundance of sodium ions over the root prevents the absorption of potassium because sodium and potassium have similar chemical properties. Hence, it is necessary to obtain an accurate understanding of salinity tolerance, its mechanisms and its effects on plants as well as the interaction between salt and other environmental factors (Shanon, 1984). This also applies to useful plants such as *Portulaca oleracea* L. which is used as a medicinal plant, vegetable and forage in areas with saline water and soil (Rahimi *et al.*, 2011).

This research was aimed at assessing and comparing the salinity tolerance of different genotypes of *Portulaca oleracea* L. during the vegetative phase. It was also aimed at assessing the effect of different salinity levels on the vegetative characteristics of *Portulaca oleracea* L. genotypes, examining the effect of salinity on the accumulation of potassium, sodium, and the ratio of sodium to potassium in *Portulaca oleracea* L. and investigating the genetic variety of morphologic and chemical properties and salinity tolerance of medicinal *Portulaca oleracea* L.

Materials and methods

Experimental factors

In order to study the effect of salinity stress on the morphological and chemical properties of different cultivars of *Portulaca oleracea* L. a factorial test was carried out using randomized complete block design. Three replications were used in this experiment. The study was conducted in 2013 in the greenhouse of the Islamic Azad University of Isfahan (Khorasgan) branch. The salinity levels applied to the samples were 0, 7 and 25 deci-Siemens. The cultivation medium included 7 liter vases filled with sand, fertilizer, perlite and soil. *Portulaca oleracea* L seeds were obtained from Ahwaz, Shiraz, Mashhad, Kazeron, Mobarakeh, Safashahr, Miandoab, and Isfahan cities.

Research station

A total of 20 seeds were planted in each vase on August 19, 2013. For the purpose of proper drainage and prevention of accumulation of salt, three holes were created at the bottom of each vase. Light expanded clay aggregate was also used to cover the vases' beds. The greenhouse temperature used for the cultivation was 30-35 $^{\circ}C$, which is the optimal temperature for the cultivation of *Portulaca oleracea* L. according to static rules. In order to meet the field capacity, vases were irrigated with tap water depending on the plant's needs until the plants reached the 4-6 leaf stage. Next, in order to prevent osmotic shocks the plants gradually received salinity treatment on September 5, 2013. At the first irrigation phase all vases except for the control one were irrigated using a 7-deci Siemens solution and at higher stages the dosage increased until the required salinity level was obtained after 7 days. On October 2, 2013 the plants blossomed and the desired properties were assessed by harvesting the blossoms.

Statistical analysis

Analysis of variance of the factorial model using randomized complete block design along with the comparison of the mean of main effects of salinity as well as genotypes also interaction effects of them. Mean comparison was carried out using the LSD method at 5% significant level. MSTAT-C and $SPSS_{16}$ softwares were used for statistical calculations while Excel was used to draw charts and tables.

Results

Plant Height

With an increase in salinity a significant difference was observed in the plant heights resulted from different treatments. In the control sample, the plant height was reduced from 30.841 to 13.587 at a salinity level of 25 deci-Siemens (fig. 1). As a result of the interaction between salinity and genotype the highest plant height (33.053) was observed in the control group of Isfahan genotype. The lowest plant height (11.363) was also observed in the Safashahr genotype at the 25 deci-Siemens salinity level (Table 2).

Table 1. Mean squares of properties of the different eight genotypes of *Portulaca oleracea* L.

Sources of variation	Degree of freedom	Plant height	Shoot dry weight	Root wet weight	Shoot sodium content	Root sodium content	Root potassium content	Ratio of potassium to sodium in root	Ratio of potassium to sodium in shoot
Block	2	8.269**	0.634**	0.064**	0.02**	0.012**	0.004**	0.0008**	0.0001**
Salinity	2	1958.72	12.841	0.884	62.37**	48.914*	5.392**	0.131**	0.304**
Genotype		11.468**	0.966*	0.077	2.272*	1.106**	1.052**	0.122	0.081**
Genotype* salinity	14	181.057	2.699	0.201	6.542	4.781	1.639	0.112	0.085
Error	46	8.463	1.362	0.138	0.012	0.007	0.459	0.001	0.0006

*,** : significant at 5 and 1% probability levels, respectively.

Shoot Dry Weight

When salinity was increased up to 7 deci-Siemens no significant difference was observed in the dry weight of shoots receiving different treatments. However, at the salinity level of 25 deci-Siemens/meter a significant reduction was observed. The reduction in the weight of the control sample was from 1.975 to 0.516 (Fig. 2). Moreover, the highest weight (4.249 g) resulting from the interaction between salinity and genotype was seen in the control sample of Isfahan genotype while the lowest weight was seen in Ahwaz and Isfahan genotypes (Table 2).

Table 2. Comparison of the average values of properties for three salinity levels and eight genotypes of *Portulaca oleracea* L.

Salinity levels (ds/m)	Genotypes	Plant height (cm)	Shoot dry weight (gr)	Root wet weight (gr)	Shoot sodium content (%)	Root sodium content (%)	Root potassium content (%)	Ratio of potassium to sodium in shoot	Ratio of potassium in root
Control	Ahwaz	13.77^{a-d}	58.41^{c}	0.335^{c}	3.6^{i}	1.936^{md}	0.588^{n}	0.445^{f}	0.287^{p}
Control	Isfahan	33.053^{a}	4.249^{a}	1.187^{a}	4.363^{i}	2.73k^{l}	0.987^{k}	0.352^{jk}	0.361^{l-n}
Control	Kazeron	30.11^{a-d}	2.085^{bc}	0.636^{a-c}	4.606^{l}	2.993^{j}	2.152^{c}	0.587^{b}	0.718^{b}
Control	Mashhad	28.797 $^{a-e}$	0.897a-e	0.326^{c}	3.306^{m}	2.033^{m}	2.150^{c}	0.496d^{e}	0.06^{a}
Control	Miandoab	30.86^{a-d}	1.668^{a-d}	0.571^{bc}	3.616^{i}	2.064^{m}	0.73^{m}	0.475^{ef}	0.355$^{l.n}$
Control	Mobarakeh	32.175a^{b}	1.653^{bc}	0.474^{c}	4.06^{k}	2.867^{jk}	1.751^{ef}	0.399^{g-i}	0.597^{de}
Control	Safashahr	30.303^{a-a}	1.105^{c}	0.534^{c}	4.42^{j}	2.328^{l}	1.13^{k}	0.366^{h-j}	0.486^{hi}
Control	Shiraz	30.665^{a-d}	3.402^{ab}	1.16^{ab}	2.716^{n}	1.988^{m}	0.896	0.928^{a}	0.468^{ij}
7	Ahwaz	23.02^{f}	1.429^{c}	0.55^{bc}	5.13^{g}	3.832^{ef}	1.639^{f-h}	0.357^{j-k}	0.427^{jk}
7	Isfahan	27.07^{d-f}	0.746^{c}	0.454^{c}	6.0^{ef}	3.951^{e}	0.322^{o}	0.008^{o}	0.08^{q}
7	Kazeron	25.32^{ef}	0.943^{c}	0.544^{c}	5.84^{f}	3.916^{e}	1.35^{j}	0.401^{gh}	0.345^{m-o}
7	Mashhad	27.233^{c-f}	1.77^{bc}	0.579^{a-c}	5.11^{g}	3.734^{fg}	1.459^{ij}	0.462^{ef}	0.389^{k-m}
7	Miandoab	31.833^{a-c}	1.511^{bc}	0.453^{c}	4.626^{i}	3.228^{i}	1.851^{de}	0.373^{h-j}	0.573^{d-f}
7	Mobarakeh	27.183^{c-f}	1.171^{bc}	0.396^{c}	4.533^{h}	3.558^{h}	2.16^{c}	0.55^{bc}	0.608^{cd}
7	Safashahr	28.93^{b-e}	1.919^{bc}	0.753^{a-c}	5.086^{g}	3.64^{gh}	1.125^{k}	0.525^{cd}	0.309^{n-p}
7	Shiraz	25.1^{ef}	1.21^{c}	6281^{c}	4.88^{h}	2.84^{k}	1.559^{g-i}	0.439^{fg}	0.548^{e-g}
25	Ahwaz	11.363^{gh}	0.299^{c}	0.22^{c}	7.716^{a}	2.84^{a}	1.559^{g-i}	0.439^{fg}	0.548^{e-g}
25	Isfahan	16.25^{g}	0.499^{c}	0.274^{c}	7.716^{a}	5.937^{a}	1.734^{ef}	0.232^{h}	0.291^{op}
25	Kazeron	15.868^{g}	0.437^{c}	0.242^{c}	7.83^{a}	5.623^{b}	1.539^{hi}	0.316^{kl}	0.291^{p}
25	Mashhad	14/133^{gh}	0.817^{c}	0.373^{c}	7.365^{b}	4.652^{d}	2.307^{c}	0.316^{kl}	0.495^{g-i}
25	Miandoab	12.873^{gh}	0.468^{c}	0.242^{c}	5.906^{f}	4.834^{c}	1.944^{d}	0.266^{mn}	0.401^{kl}
25	Mobarakeh	13.25^{gh}	0.628^{c}	0.297^{c}	6.096^{e}	4.913^{c}	1.721^{e-g}	0.309^{lm}	0.348^{l-n}
25	Safashahr	11.363^{g}	0.489^{c}	0.26^{c}	6.16^{d}	4.968^{c}	1.71^{e-g}	0.271^{mm}	0.344^{m-o}
25	Shiraz	13.093^{gh}	0.489^{a-b}	0.257^{c}	7.13^{c}	4.964^{c}	2.612^{b}	0.241^{n}	0.526^{f-h}

In each column, means with the same letter(s) haven't significant difference.

Root Wet weight

When salinity increases to 7 deci-Siemens/meter no significant difference was observed in the root wet weight in different groups. However, at 25 deci-Siemens/meter a significant reduction was observed when the weight reduced from 0.652 to 0.27 (Fig. 3). The interaction effect of genotype and salinity on root wet weight indicated that the highest weight (1.187 g) belonged to the control sample of Isfahan genotype while the lowest weight belonged to all genotypes at a salinity level of 25 deci-Siemens (Table 2).

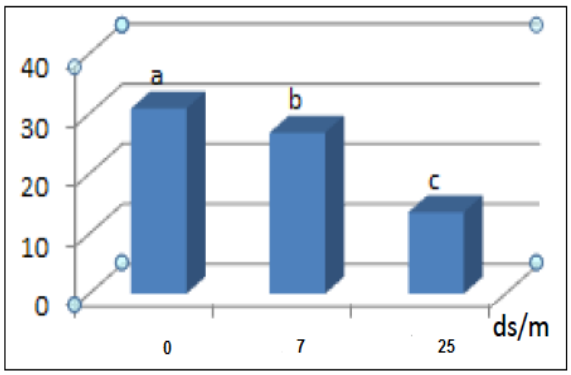

Fig. 1. Mean comparison among salinity levels for plant height.

Shoot Sodium Content

With an increase in salinity a significant difference was observed between the sodium contents of shoots of different treatment groups and the control group. The sodium content increased from 3.836 to 7.047 (Fig. 4). The interaction effect of genotype and salinity on sodium content of shoots indicates that Ahwaz, Isfahan and Kazero genotypes have sodium contents of 7.716, 7.716 and 7.853 at a salinity level of 25 deci-Siemens. The lowest sodium content (2.716) also belonged to Shiraz genotype (Table 2).

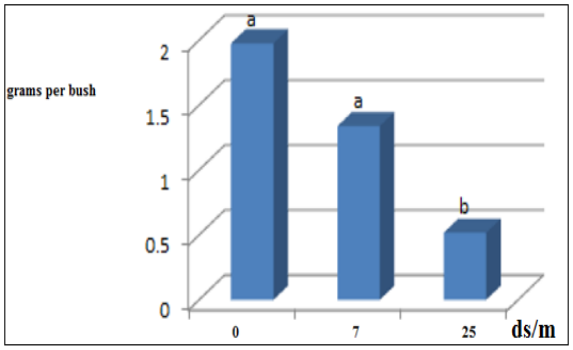

Fig. 2. Mean comparison among salinity levels for shoot dry weight.

Root Sodium Content

When salinity increased the sodium content in the root increased significantly and at a salinity level of 25 deci-Siemens the sodium content reached from 2.367 to 5.213 in the control group (Fig. 5). As a result of the effect of salinity and genotype the highest root sodium content belonged to Ahwaz and Isfahan genotypes at salinity level of 25 deci-Siemens while the lowest content belonged to the control samples of Shiraz and Ahwaz genotypes. The increase in salinity led to a significant increase in root sodium content (Table 2).

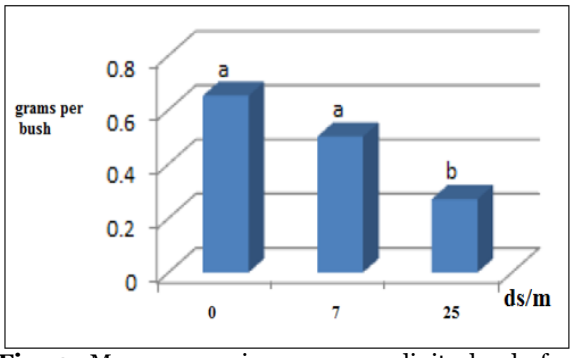

Fig. 3. Mean comparison among salinity levels for root fresh weight.

Root Potassium Content

With an increase in salinity a significant difference was observed in the root potassium contents of all groups. In the control group, the potassium content reached from 1.295 to 2.176 at a salinity level of 25 deci-Siemens/meter (Fig. 6). As a result of the interaction effect of genotype and salinity the highest root potassium content was seen in Ahwaz genotype (3.843) while the lowest was seen in Isfahan genotype (0.322) (table 2).

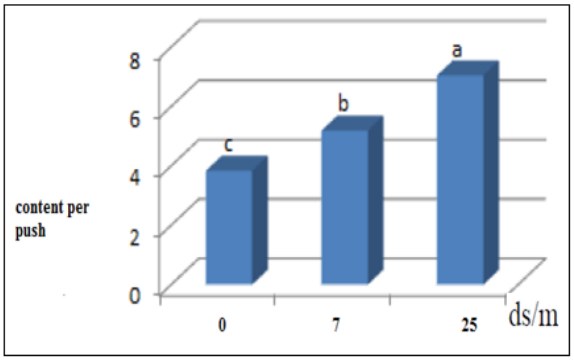

Fig. 4. Mean comparison among salinity levels for sodium content of shoot.

Ratio of Potassium to Sodium in Root

The increase in salinity led to a significant difference between the ratios of potassium to sodium in the roots of different samples. However, no significant difference was resulted between 7 and 25 deci-Siemens/meter. The increase in salinity led to a significant decrease in the ratio of potassium to sodium of the root (Fig. 7). As a result of the interaction effect of genotype and salinity the control sample of the Mashhad genotype demonstrated the highest ratio (1.06) while Isfahan genotype showed the lowest ratio (0.08) (Table 2).

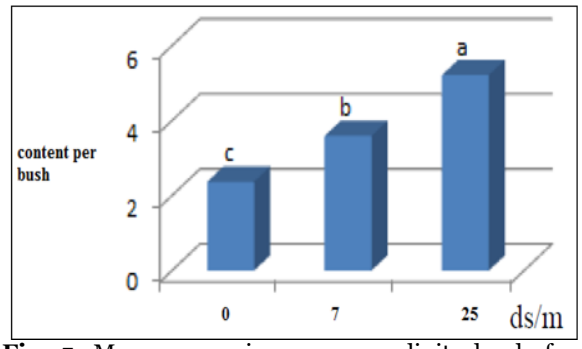

Fig. 5. Mean comparison among salinity levels for sodium content of root.

Ratio of Potassium to Sodium in Shoot

With an increase in salinity a significant difference was observed between the ratios of potassium to sodium in the shoots of different groups. The ratio dropped from 0.506 to 0.281 at a salinity level of 25 deci-Siemens/meter (Fig. 8). As a result of the interaction effect of genotype and salinity, the control sample of the Shiraz genotype demonstrated the highest ratio (0.928) and the Isfahan genotype showed the lowest ratio (0.008) at 7 deci-Siemens/meter (Table 2).

Discussion

Reduction in plant height as a result of the increase in salinity is caused by the adverse effects of salinity on absorption and transfer of nutrients to shoots. In order to explain the reduction in plant length as a result of salinity stress, Mir Samy *et al.* (2005) argue that salinity leads to the formation of epidermal cells containing a large number of vacuoles. Vacuoles in plant cells are responsible for inflammation and elongation which in turn may cause problems in the presence of salt. Early formation of vacuoles may also lead to an increase in the resistance of plants to salinity. This resistance is manifested in the form of inhibition of growth.

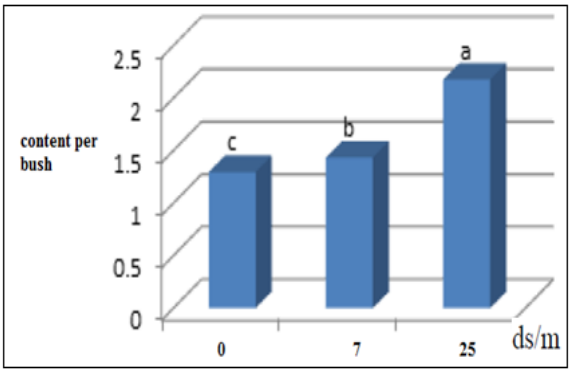

Fig. 6. Mean comparison among salinity levels for potassium content of root.

Moreover, a reduction in plant height by salinity can be ascribed to the reduction in water potential and meiosis. Flowers *et al.* (2007) carried out a study to examine the effect of sodium chloride on mung bean under salinity stress. They found out that salinity reduces plant height.

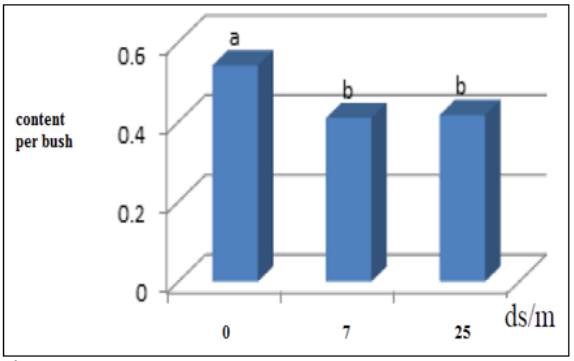

Fig. 7. Mean comparison among salinity levels for potassium to sodium ratio in root.

An increase in salinity led to an increase in the accumulation of sodium in shoots. The accumulation of sodium in shoots can be ascribed to osmoregulation, which plays an important role in the adjustment of the plant to salinity stress. The reason is that osmoregulation increases inflammation and cell volume and consequently leads to the growth of the plant and accumulation of nutrients in shoots (Ghanavati and houshmand, 2006).

Root sodium was significantly influenced by salinity, genotype and the interaction effect of genotype and salinity. With an increase in salinity levels the content of sodium in roots increases. This can be ascribed to the further increase in the growth and length of root and the subsequent increase in the absorption of elements such as sodium. These researchers carried out a study on wheat and indicated that salinity leads to an increase in the content of sodium in shoots and roots. With an increase in salinity the root sodium content increased. This increase can be ascribed to the limited absorption of potassium in root. As a result, the concentration of potassium in the root unit volume grows at higher salinity levels (Gorham, 1990).

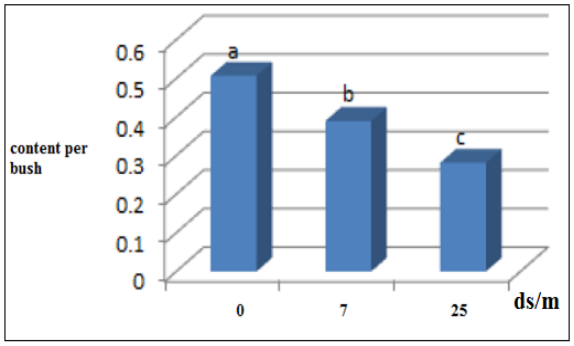

Fig. 8. Mean comparison among salinity levels for potassium to sodium ratio in shoot.

The ratio of potassium to sodium in shoot and root reduced significantly with an increase in salinity. Although sodium can contribute to turgor by increasing pressure, unlike potassium it cannot trigger activities such as enzyme activation and protein synthesis to produce adequate growth. Therefore, the toxicity effects of sodium chloride (caused by the excessive accumulation of salt in plant) may not be directly caused by sodium ion and may rather be caused by the reduction in the concentration of elements such as Manzo Potassium in the plant (Aminpanah and Soroushzadeh, 2005). Salinity leads to ionic imbalance. As a result of this imbalance the ratio of potassium to sodium decreases and the excessive accumulation of sodium leads to the inactivity of enzymes which eventually affects plant's metabolic processes. High concentrations of sodium are usually accompanied by low levels of potassium in plants (Munns, 1980; Bassil and Kaffka, 2001).

According to the results of this study, Ahwaz, Mashhad and Miandoab genotypes demonstrated considerable levels of resistance at a salinity level of 25 deci-Siemens. This plant can be used as a medicinal plant in areas under salinity stress. It is recommended to repeat this research in future to further investigate the stability of different cultivars. It is also recommended to study the treatments used in this research under average salinity levels of 7 to 25 deci-Siemens/meter with higher salinity levels. It is also better to use the glycine amino acid, other anti-stress substances (such as other such as amino acids) and soil amenders (such as humic acid and folic acid) to reduce salinity stress.

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