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Salsolaarbuscula Responses to Salt Stress

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Abstract- ***Salt stress is a world-wide problem and soil salinity is common in arid and semi-arid regions. This study was undertaken to investigate salt tolerance in Salsolaarbuscula in laboratory and natural conditions and recognize the mechanisms that allow it to tolerate these conditions. This study had two parts of greenhouse and natural habitats. The treatment solutions for salinity tests were different concentrations of NaCl and Na2SO4 (0, 100, 200, 300, 400, and 500 mM) with three replicates and growth parameters and proline and soluble sugar were determined in vegetative growth stage in greenhouse. Soil (two depths of 0-10 cm and 10-45 cm) and plant (root and shoot) samples have been harvested from three 200 meter transects in three provinces of Esfahan, Semnan and Markazi.Proline and soluble sugar andsoil texture and EC were measured in laboratory. Collected data were analyzed using a factorial experiment and means were compared by DMRT method by SPSS software. Results indicated that proline and soluble sugar were significantly affected by salinity levels and increased with salinity increase. The rate of growth parameters increased with an increase in salinity up to 400 mM while salinity levels more than 400 mMNaCl caused all growth characteristics decline. Data obtained from the laboratory experiment confirmed the findings noted during the field study. Results also indicated high salt excretion capability in S. arbusculawhich is possible by leaves fall. It has to be mentioned that nature isunpredictable and observing unexpected trends under specific conditions is not impossible.***

Key words: NaCl, Na2SO4, Salsola arbuscula, salt tolerance.

I. INTRODUCTION

Salt stress is a world-wide problem and soil salinityis common in arid and semi-arid regions.Salinity is a scourge for agriculture, forestry, pasture development and other similar practices. Understanding the responses of plants to salinity is ofgreat practical significance.High concentrations ofsalts have detrimental effectson plant growth [4].The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors [11]. All of these cause adverse effects on plant growth and development at physiological and biochemical levels[31].

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Osmotic adjustments require ion accumulation up to a concentration equal to or greater than that of the surrounding root solution in order to achieve an osmotic gradient for the uptake of soil water [9, 10, 37].

So salinity affects water availability due to the limitation of water uptake by plants. In addition, excessive uptake of Na^+ and Cl^- may result in a limited assimilation, transport and distribution of mineral nutrients, as well as nutrient imbalances within the plants [10, 11], while the exclusion or maintenance of low concentrations of toxic Na^+ and Cl^- ions especially in meristematic tissues and reproductive organs is regarded as an essential phyto-physiological mechanism for salinity tolerance [31]. Elevated NaCl levels in the root medium reduce the nutrient assimilation, especially of K^+ and Ca^{2+}, resulting in ion imbalances of K^+, Ca^{2+}, and Mg^{2+} compared to Na^+ [26, 27], aswell as in negative effects on enzymes and membranes.

The salt stress results in the development of leaf necroses and accelerated leaf senescence, thus reducing photosynthetic capability of the plants. In consequence, assimilation of carbohydrates available for fruit production is reduced [3]. Controlled inorganic ion uptake must be accompanied by ion compartmentation in the vacuoles and by the synthesis and accumulation of compatible solutes [10, 32, 37].

This study was undertaken to investigate salt tolerance in *Salsolaarbuscula*in laboratoryand natural conditions and recognize the mechanisms that allow it to tolerate these conditions.

II. METHODS

This study had two stages: laboratory (greenhouse) and natural habitats.

A. Study Area and Plant Species Choice

As best result of salt tolerance screening can be expected from the species that grow naturally in saline environments [8], the native and palatable species of *S. arbuscula* (Xerohalophyte [17] and Psammophyte [39]) has been selected in its natural habitats. The Irano-Turanian species of Chenopodiaceae family which is mostly characteristic for arid to semiarid and/or saline habitats has great importance in livestock grazing and also in salty and dry range improvement [8]. The species of Chenopodiaceae are, however, taxonomically not well-investigated due to the limitation of practical taxonomical characters, the fleshy nature of many species, late flowering and fruiting time, and the fact that they are aesthetically not attractive for most collectors and botanists [12].

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B. Data Collection

One part of experiments was conducted under greenhouse conditions at University of Tehran, Iran. Seeds were sown in pots filled with sand in growth chambers (alternating light periods of 8-h dark and 16-h light and 25°C temperature). After two weeks, the pots were transferred to greenhouse and irrigated with Hoagland's nutrient solution. Seeds were raised from April to October under non-saline conditions. The treatment solutions for salinity tests were different concentrations of NaCl and Na_2SO_4 (0, 100, 200, 300, 400, and 500 mM) which were added to Hoagland's nutrient solution. Three replicates were used for each treatment. Two months after the start treatments, seedlings were harvested and transferred to laboratory and the following parameters were measured at the harvest time. Growth parameters (Root and shoot fresh and dry weight, length and diameter) were determined. Proline and soluble sugar were also considered in vegetative growth stage.

Soil and plant samples have been harvested from three 200 meter transects in three provinces of Esfahan, Semnan and Markazi.Plant samples were collected from root and shoot and proline and soluble sugar were measured. Soil sampling was done in two depths (0-10 cm and 10-45 cm). Soil texture and EC were measured in soil laboratory.

All sampled materials were oven-dried (70°C for 48-h) to obtain the dry weight. Proline was determined by the ninhydrin method described by Bates et al. [19]. In this method, proline was extracted from 0.5 g of fresh leaf tissue into 10 ml of 3% sulfosalicylic acid and filtered through Whatman No. 42 filter papers and determined in Shimadzu UV-1201 model spectrophotometer. In order to measure the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 minutes. The light absorption of the samples was estimated at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard [16]. EC meter and hydrometer method were used to determine Electrical Conductivity and soil texture, respectively.

III. RESULTS

A. Greenhouse

Results indicated that shoot fresh and dry weight significantly ($p<0.01$) increased with salinity increase up to 100mM and 200mM NaCl, respectively. Increasing salinity more than the specified value caused fresh and dry weight significant ($p<0.01$) decrease. Root fresh and dry weight significantly ($p<0.05$) increased when salinity increased up to 500mM NaCl (Table 1, Figure 1).Salinity increase up to 400mM NaCl caused plant height ($p<0.05$) and root length ($p<0.01$) significant increase. Results also showed significant root diameter increase with NaCl increase (Table 1, Figure 2). Salinity increase up to 100mM and 200mM Na_2SO_4 resulted in shoot fresh and dry weight significant increase ($p<0.05$), respectively. Salinity increase up to 500 mM Na_2SO_4 also caused root freshand dry weight significant increase (Table 2, Figure 3). Salinity increase up to 400 mM Na_2SO_4 resulted in plant height ($p<0.01$) and root length ($p<0.05$) significant increase. Increasing salinity up to 500 mM Na_2SO_4, root diameter increased significantly ($p<0.05$) (Table 2, Figure 4).

Table 1- Results of analysis of variance for the growth parameters under NaCl salinity.

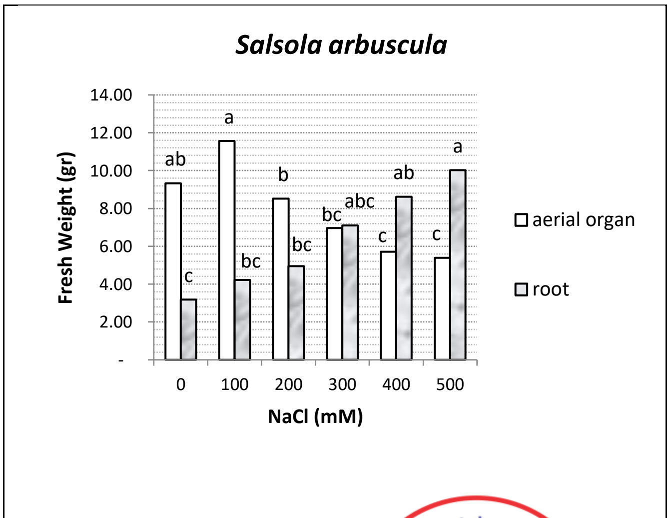
*significantly different at $p<0.05$; ** significantly different at $p<0.01$; ^{ns} no significant difference

source	df	M.S.							
		Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Plant height	Root length	Stem diameter	Root diameter
Salinity	5	16.714**	21.438*	9.508**	9.883*	23.426*	26.521**	0.208^{ns}	1.512*
error	12	2.097	6.722	0.529	2.611	3.536	3.536	0.126	0.356

Table 2- Results of analysis of variance for the growth parameters under Na_2SO_4 salinity.

*significantly different at $p<0.05$; ** significantly different at $p<0.01$; ^{ns} no significant difference

source	df	M.S.							
		Shoot fresh weight	Root fresh weight	shoot dry weight	Root dry weight	Plant height	Root length	Stem diameter	Root diameter
Salinity	5	15.14*	13.942**	1.571*	9.172**	83.497**	24.119*	0.146^{ns}	1.154*
error	12	4.755	2.724	0.422	1.057	14.124	5.181	0.14	0.241



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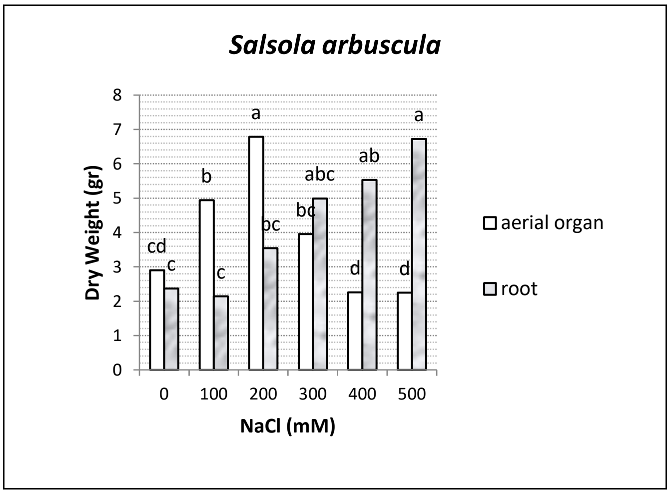


Figure 1- The effect of NaCl on shoot and root dry and fresh weight

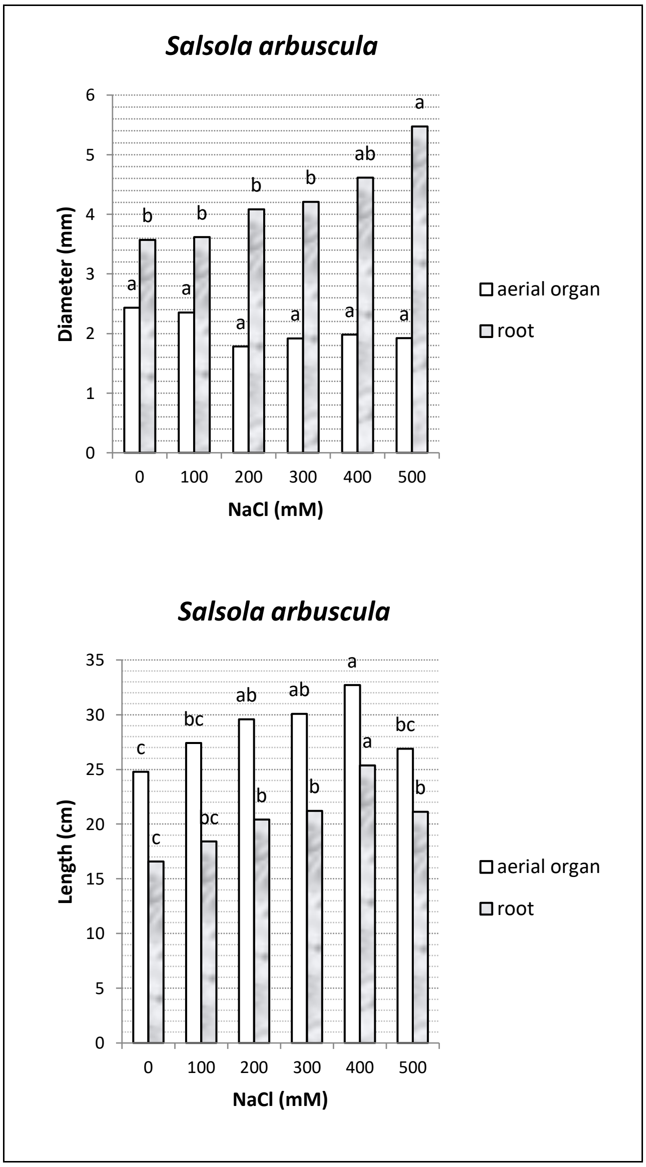
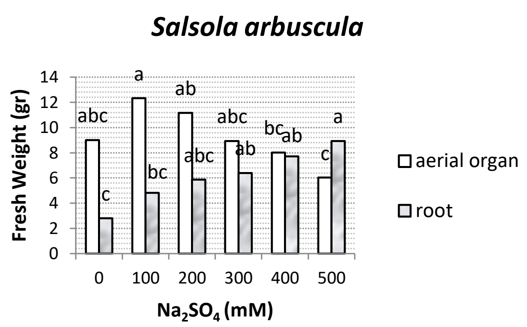


Figure 2- The effect of NaCl on shoot and root diameter and length

Salinity increase more than 300mM NaCl resulted in shoot and root proline ($p<0.01$) and soluble sugar ($p<0.05$) significant increase (Table 3, Figure 5). Results indicated that salinity increase up to 500 mM Na_2SO_4 caused shoot and root soluble sugar ($p<0.01$) and root proline ($p<0.05$) significant increase. The rate of root proline and soluble sugar was mor



e than shoot (Table 4, Figure 6).

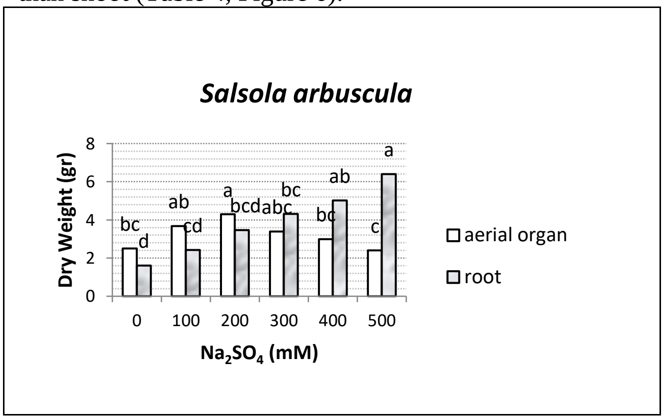


Figure 3- The effect of Na_2SO_4 on shoot and root dry and fresh weight

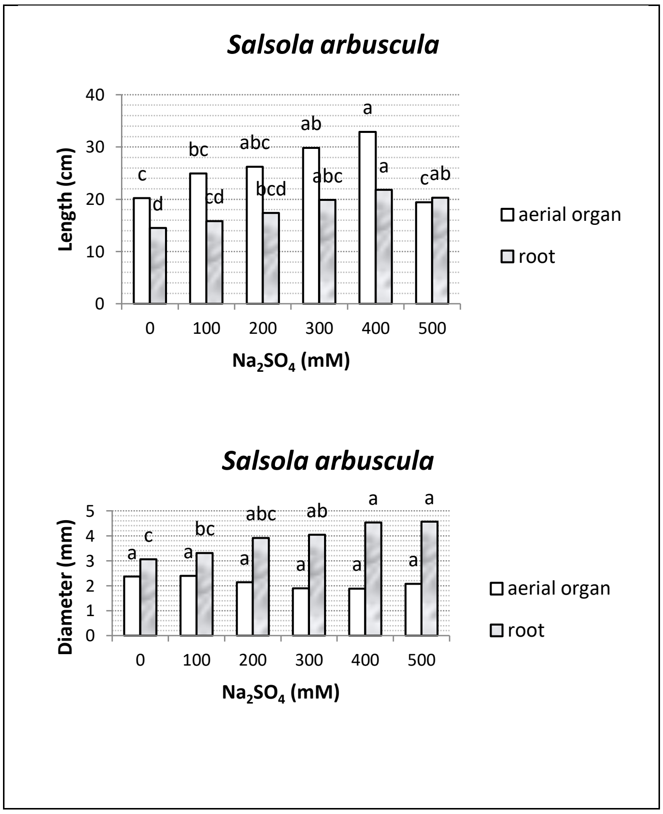


Figure 4- The effect of Na_2SO_4 on shoot and root length and diameter

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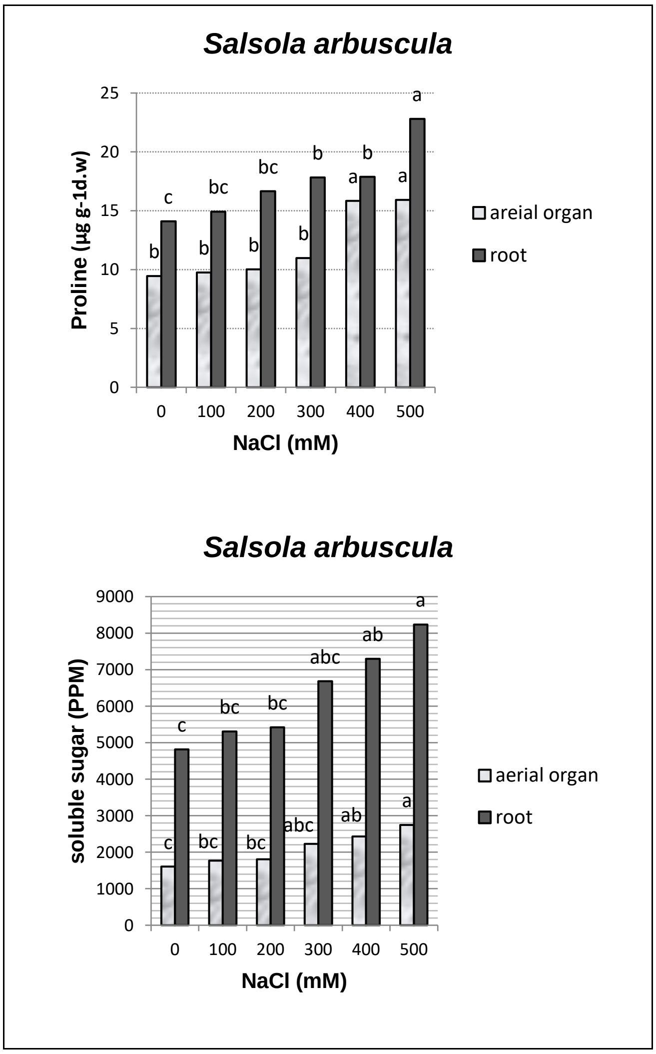

Figure 5- The effect of NaCl salinity on soluble sugar and proline in *Salsolaarbuscula*

Table 3- Results of analysis of variance for the impact of NaCl salinity on soluble sugar and proline in *Salsolaarbuscula*

*significantly different at $p<0.05$; ** significantly different at $p<0.01$; ns no significant difference

Source	df	M.S.			
		Root soluble sugar	shoot soluble sugar	Root proline	shoot proline
salinity	5	3479040.046**	869760.011**	48.781*	25.002ns
error	12	348137.469	87034.367	11.353	8.426

Table 4- Results of analysis of variance for the impact of Na_2SO_4 salinity on soluble sugar and proline in *Salsolaarbuscula*

*significantly different at $p<0.05$; ** significantly different at $p<0.01$; ns no significant difference

Source	df	M.S.			
		Root soluble sugar	shoot soluble sugar	Root proline	shoot proline
salinity	5	5307013.176*	589668.131*	28.256**	27.975**
error	12	1616583.728	179620.414	3.652	2.179

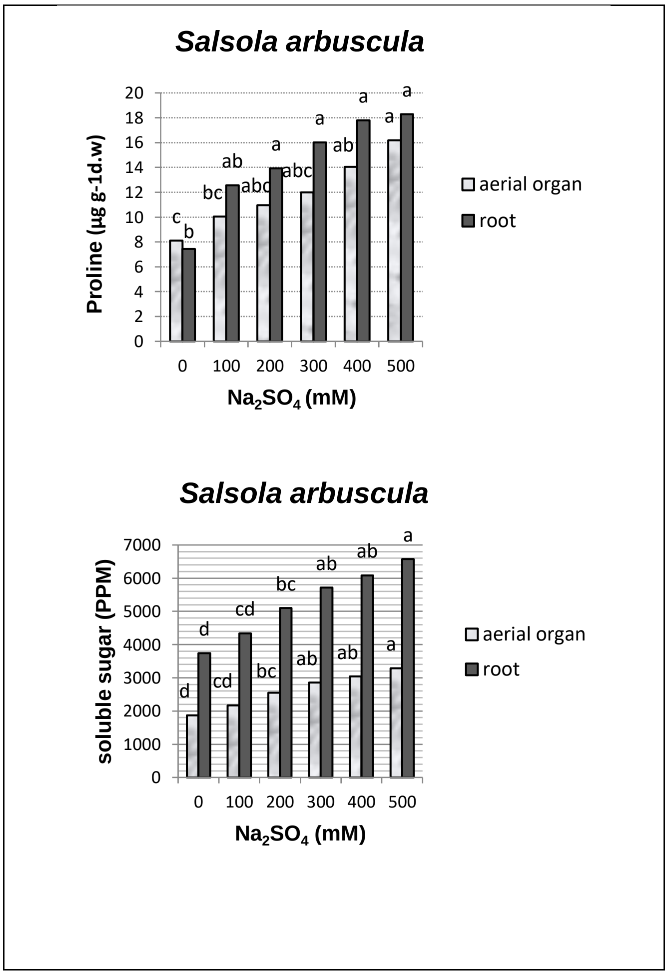

Figure 6- The effect of Na_2SO_4 salinity on soluble sugar and proline in *Salsolaarbuscula*

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Table 5- Results of analysis of variance for four soil properties in two depth and 3 locations in *Salsolaarbuscula* *significantly different at p<0.05; ** significantly different at p<0.01; ^{ns} no significant difference

Source	**M.S.**			
	EC	**sand**	**silt**	**clay**
Location	12.895**	340.056**	70.056**	172.056**
Depth	0.004^{ns}	46.772^{ns}	0.5^{ns}	40.5^{ns}
Location*depth	0.558^{ns}	13.389^{ns}	10.167^{ns}	0.167^{ns}
error	0.152	37.889	8.833	24.444

Table 6- Results of analysis of variance for proline and soluble sugarin shoot and root in three locations *significantly different at p<0.05; ** significantly different at p<0.01; ^{ns} no significant difference

Source	**M.S.**	
	Soluble sugar	**proline**
Location	65057.723**	1.031^{ns}
Organ	487074.63**	0.224^{ns}
Location*Organ	115223.626**	0.169^{ns}
Error	4253.106	0.963

B. Field

Statistical analyses showed a significant difference (p<0.01) between the rate of EC, sand, silt and clay in three habitats of *S. arbuscula* (Table 5). The maximum and minimum rate of EC and sand was observed in Esfahan and Semnan locations, respectively. Markazi location had the minimum rate of silt and there was not any significant difference between two depths (Table 5, Figure 7).

Results indicated a significant difference (p<0.01) between the rates of soluble sugar of three locations and also between shoot and root soluble sugar. It has to be mentioned that the maximum rate of soluble sugar was observed in Esfahan (Table 6, Figure 8).

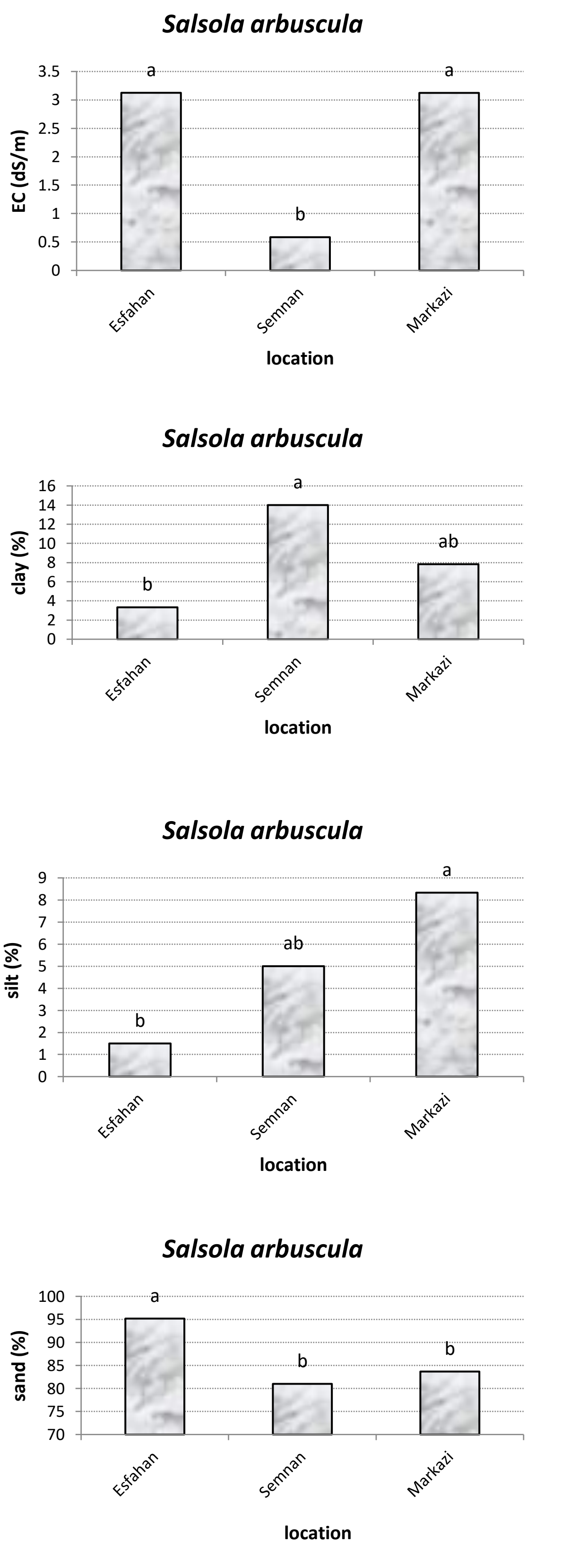

Figure 7- Mean differences of four soil characteristics in three provinces of Iran in *Salsolaarbuscula* habitats.

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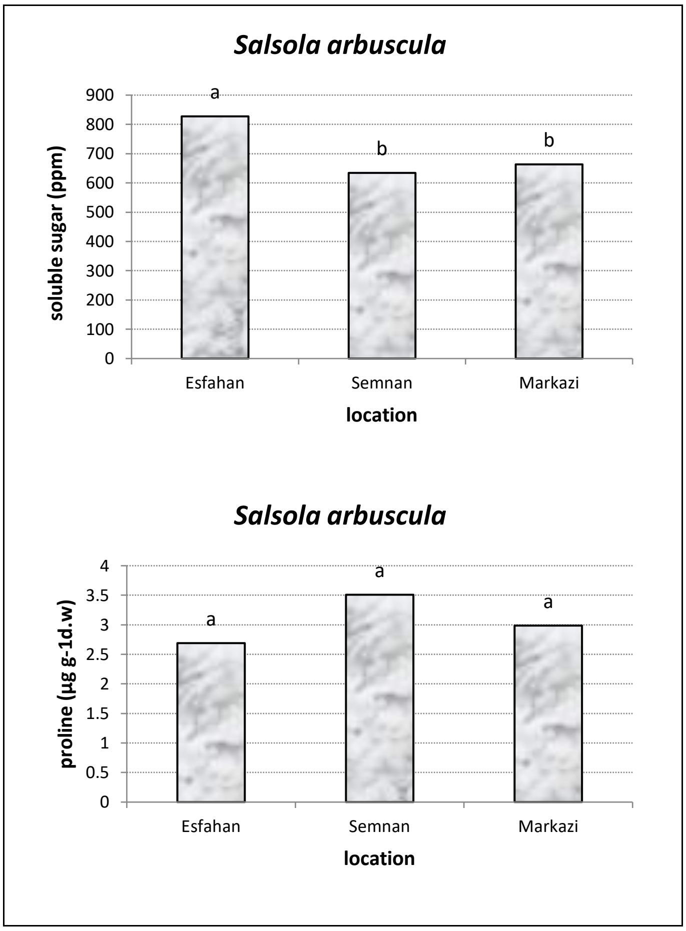

Figure 8- Mean differences of soluble sugar and proline in three provinces of Iran in *Salsolaarbuscula* habitats.

IV. DISCUSSION

Shoot and root length are the most important parameters of salt stress, because root has direct contact with soil and absorbs water from soil and shoot spreads it to the other parts of the plant. So shoot and root length are main indicators of plant responses to salt stress [23].Different results on the effects of salinity on plant growth and sensitivity of root and shoot dry and fresh weight of plant species are presented in various researches. Reduction of plant growth under salt stress has reported in many plant species [13, 36]. Our results also suggest that growth parameters decrease with increasing salinity levels more than the specified levels. Garg and Gupta [4] reported that salinity causes reduction in leaf area as well as in rate of photosynthesis which together result in reduced crop growth and yield. Jamil et al. [22] studying sugar beet, cabbage and amaranthreported that salinity limits plant growth (shoot and root length and fresh weight). In this study, results showed a reduction in growth parameters of *S. arbuscula* with increasing salinity more than 400mM concentration.

In general,salinity can reduce the plant growth or damagethe plants through: (i) osmotic effect (causing waterdeficit), (ii) toxic effects of ions, and (iii) imbalance ofthe uptake of essential nutrients. These modes ofaction may operate on the cellular as well as on higherorganizational levels and influence all the aspects ofplant metabolism [4]. Our results for reduction of shoot fresh and dry weight for salinity increase more than 100mM and 200mM, respectively, are in conformity with the findings of Jennette et al. [34] and Shannon et al.[28], who reported fresh weight significant decrease with salinity increase.

Various researchers have shown that shoot growth is more sensitive than root growth in plants under salt stress. The results of Da Silva et al. [6] show that *Spondiastuberosa*shoot growth is more sensitive than root growth. In the species studied in this research, it is somewhat obvious, in a way that increasing salinity to 500mMNaCl, root dry and fresh weight and diameter significantly increased. Although, shoot diameter decline was observed with increasing salinity more than the specified level. Shoot diameter decrease is also reported for Avocado [30]. According to Munns[33], shoot sensitivity to salt stress is of cation imbalance due to the complex interaction of the xylem transport system but it is related to more rapid osmotic regulation and slower loss through turgor in roots [14]. Morphologic adaptation of halophytes to salinity is a part of their evolution and natural selection in saline environments [39]. Salinity also causes specific structural changes such as fewer and smaller leaves, fewer stomata per leaf area, leaves thicker cuticles andwaxy surfaces, differentiation and development of vascular tissue and premature root lignification. These changes vary depending on the species and the type of salt [1]. In this research, salt excretion by leaf fall was observed that may be the result of shoot weight decrease by salinity increase up to 200mM.

Inthisresearch,bothNaCl andNa$_2$SO$_4$salinityincreasesignificantlyincreased the rate of proline. Jipingand Zhu [18] also pointed to proline increase as a symbol of salt stress. Madan et al. [35] reported that the proline biosynthetic enzymes of *Brassica juncea* increased under salt stress, conversely, proline degrading enzymes decreased under salt stress in leaf tissue. Studying *Suaeda salsa*, Song et al.[15] stated that proline is one of organic solutions having influence on salt stress decrease. The exactnature ofprolineagainstsalt stressis not known [24]. Proline increase is probably due to plants capacity in organic and no-organic compounds accumulation in the cytoplasm which plays an important role in water potential decrease and the osmotic gradient change and as a result flowing of water to the plant. However, proline concentration in salt-tolerant plants is more than salt-sensitive plants [24]. Petrusa and Winikov[20] reported that the rate of proline rapidly doubles in salt-tolerant alfalfa roots. In this research, proline accumulation of the roots significantly increased and was more than the shoots. There is a threshold of salt for proline accumulation, in other words, there won't be any proline accumulation unless salt concentration and as a result, univalent cations specifically Na^+ reach to a certain extent [24]. In this study, salinity increase more than 300mMNaCl caused significant shoot proline accumulation increase. The results of this research confirm a direct link between salinity increase and proline content increase which is in conformity with the results obtained by Joshi and Lyengar[2] studying*Suaedanudiflora*,Bajji et al. [21] studying *Amaranthustricolor* and Zandi[5] studying*Suaedavermiculata*and *Atriplexleucoclada* who also pointed to the direct relationship between salinity and proline.

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In saline environments, soluble sugar accumulation may be observed in response to osmotic pressure, but its concentration differs depending on the plant species and organs [24]. In this research, shoot and root significant soluble sugar increase was observed with salinity increase.Irigoyen et al. [16] reported that shoot soluble sugar increase is caused by conversion of starch to soluble sugar, reducing their consumption or transmission in vessels. Bajji et al. [21] reported soluble sugar accumulation in *Atriplexhalimus*.Balibrea et al. [29] stated that root soluble sugar increased under salt stress, but it had no effect on the leaves. Our study also shows more soluble sugar accumulation in roots in comparison with the shoot.

Results showed a significant difference between soil characteristics in different habitats. A significant difference was also observed between the rate of soluble sugar and proline in different locations which was influenced by climatic conditions.Some halophytes use salt accumulation [9] and some other synthesize compatible solutions [7] in order to regulate the osmotic pressure. However, compatible solutions are produced in all three locations.Zandi[5]also pointed to the significant difference between the rate of proline and soluble sugar in different habitats of*Atriplexleucocladaa*nd *Suaedavermiculata*. The high rate of proline in all three habitats can be a result of climatic conditions such as high temperature, rainfall distribution and low annual precipitation.Nature is unpredictable and observing unexpected trends under specific conditions is not impossible and there is the probability of observing various reactions of species in different climates [8]. In general, the results obtained from greenhouse were in conformity with the results of natural habitats. Studying*Salsolatomentosa*, *Salsolaarbuscula*, *Eurotiaceratoides*,*Peganumharmala*,*Zygophylumeurypterum*and*Eurotiaceratoides*, soil and the type of vegetation in Yazd Nadushan rangelands, MousaeiSanjerehei[25] pointed to *S. arbuscula* as a xerohalophyte[17] and psammophyte[39] and an important indicator of soil salinity and finally the high impact of soil properties on the type of plant species.

According to the results of greenhouse and field, it can be concluded that different salinity levels have significant influence on growth parameters and proline and soluble sugar accumulation in *S. arbuscula*. This species can tolerate salinity by salinity regulation and salt excretion through old leaves fall. According to the results obtained, it can be suggested using this species in salt affected areas and also in sand dune stabilization.

At last, it has to be mentioned that scientists have been reluctant to study *S. arbuscula* and similar species with such tiny and fleshy leaves and difficult recognition so these species have been oppressed for centuries. Further studies on different aspects of them will be helpful for science and its progress.

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