Effects of Salinity on Ion Exchanges in *Halocnemum strobilaceum* and *Halostachys caspica*

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Abstract. Salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide Approximately 10% of the total land surface is salt affected and about 10 million hectare of agricultural land is lost annually due to salinization and water logging. This study was conducted to determine the ion exchanges in *Halocnemum strobilaceum* and *Halostachys caspica* in saline conditions. The seeds of plants were sown in the pots in a greenhouse in Research Institute of Forests and Rangelands, Iran. After 5 months, plants were exposed to different salinity levels including 0, 100, 200, 300, 400 and 500 mM of NaCl and Na_2SO_4 for 45 days and the amounts of Na^+, k^+, Mg^{2+} and Ca^{2+} were measured in stems and roots. Results showed that ion contents were affected by NaCl and Na_2SO_4 in both species. The minimum and maximum values of Na^+ (568 and 1613mg kg^{-1} DM) were found in the root of *H. caspica* and shoot of *H. strobilaceum,* respectively. Ion content was increased with the increase of salinity up to 100 and 200-300 mM in *H. strobilaceum* and *H. caspica*, respectively. Also, the ion exchanges were higher in *H. strobilaceum* than *H. caspica.* In general, this investigation showed the ion uptake of both species at low salinity but they changed the tolerance mechanism at high salinity. So, Na+ and K+ were translocated from shoot to root while Ca^{2+} translocation from root to shoot was increased by salinity.

Key words: Halophytic plants, NaCl, Na_2SO_4, Cations

Introduction

World population is increasing at an alarming rate and is supposed to reach nine billion by 2050, but our food production is limited (Varshney *et al.,* 2011). Approximately 10% of the total land surface is affected by salt and about 10 million hectare of agricultural land is annually lost due to salinization and water logging (Monirifard and Barghi, 2002). Majority of crop species belongs to the glycophytes category. Thus, salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide (Gupta and Huang, 2014). As the reclamation of salt-affected soils is not completely feasible and always cost-effective, the researchers are searching for biosaline agriculture and thus, it is obvious to explore a better understanding of how naturally adapted plants (halophytes) handle salts. Study of halophytes can be useful from this perspective (Hasanuzzaman *et al.,* 2014). The distribution, exploitation and physiology of salt tolerance of halophytes are intensively studied (Roy *et al.,* 2014).

The initial effect of salt stress is the osmotic stress caused by the presence of ions in rhizosphere which restricts the extraction of water by roots and results in the reduced plant growth. The secondary effects of salt stress are caused by ionic disequilibrium resulting in the inactivation of enzymes, nutrient starvation, ionic toxicity in tissues and oxidative stress (Turan *et al.*, 2012).

As NaCl is the most soluble and widespread salt, it is not surprising that all the plants have evolved some mechanisms to regulate and select its accumulation against it in favor of other nutrients which are commonly present in low concentrations such as K^+ and NO_3^- (Munns and Tester, 2008). Since ionic toxicity caused by Na^+ and Cl^- is the main concern of salt stress in plants, most studies have concentrated on Na^+ exclusion and the control of Na^+ transport within the plant (Hasegawa, 2013). Almost all micro- and macronutrient contents change in the roots and shoots with the increase of salt concentration in the growth medium. Shoot growth was more strongly influenced by salinity than the root growth which resulted in an increase of root/shoot length ratio of the salinity exposed plants (Rasouli and Amiri, 2011; Amiri *et al.,* 2011). But most researches on the effects of salinity on plants have investigated the changes occurring in the leaves whereas the roots are in direct contact with the saline solution. Although there are opportunities to control salt entering leaves at various points along the transpiration stream, the roots must perform a crucial function in the management of input and throughput (Batistice and Kudla, 2009).

In spite of the significant progress in the understanding of plant stress responses, there is still a large gap in our knowledge of trans-membrane ion transport, sensor and receptor in the signaling transduction, molecules in long distance signaling and metabolites in energy supply (Cabello *et al.,* 2014). Thus, the main objective of the present study was to examine the effects of different salt stress types on *Halocnemum strobilaceum* and *Halostachys caspica* and ion imbalance between the roots and shoots.

Materials and Methods

Mature dry seeds of *Halocnemum strobilaceum* and *Halostachys caspica* were collected from Orumieh Lake's marginal lands, Iran. The seeds were surface sterilized in 70% ethanol and 5% sodium hypochlorite (Clorox). Then, they were rinsed 5 times in the sterilized distilled water. Seeds were grown in a greenhouse at 28/21°C day/night temperatures and 65-85% relative humidity of air and planted in plastic pods with the Silica sand bed and were nitrified with Hoagland's nutrient solution for 6 months (Hoagland and Arnon, 1950) in Research Institute of Forests and Rangelands, Karaj, Iran. After 5 months, the salinity stress (deleted) was generated by applying NaCl and Na_2SO_4 concentrations of 0 (control), 100, 200, 300, 400 and 500 mM separately for 6 weeks. Then, the plants were harvested for the analysis of some ion contents in roots and stems. Root and shoot tissues were separately harvested. Na^+, k^+, Mg^{2+} and Ca^{2+} were determined in the extract by an inductively coupled plasma atomic emission spectrometer (ICP) (Navarro *et al.,* 2006). The experiments were conducted in three replicates as a factorial experiment according to the completely randomized design. Data records were analyzed statistically using SPSS17 analysis of variance technique and Duncan's Multiple Range Test at 5%

probability level in order to compare the differences among treatment means.

Results

The results of analysis of variance for the effects of salt stress on ion accumulation in different parts of *H. strobilaceum* and *H. caspica* are shown in Table 1. Ion content was affected by both NaCl and Na_2SO_4 in the root and stem of *H. strobilaceum* and *H. caspica* with the exception of Mg^{2+} and Ca^{2+} in *H. caspica* and *H. strobilaceum* (Table 1). The ions significantly changed as the concentration of salt level increased in the growth medium.

Table 1. Analysis of Variances of ion changes in *H. strobilaceum* and *H. caspica*

Source	*H. caspica*				*H. strobilaceum*			
	Ca^{2+}	Mg^{2+}	K^+	Na+	Ca^{2+}	Mg^{2+}	K^+	Na+
Salt Type (S)	355788**	0.021ns	75375**	646043**	11748ns	675**	63718**	342015**
Concentration (C)	60867**	281**	16744**	536999**	8531*	483**	40677**	768732**
Plant Organ (O)	697183**	858**	16266**	5715355**	268202**	2780**	310275**	1123582**
S x C	44602**	83*	5156*	269954*	5799ns	73ns	5593ns	242694**
S x O	458288**	1.1ns	1922ns	401467**	2866ns	127ns	33260*	1464387**
O x C	70634**	194**	19763**	327966**	12428**	131ns	9399ns	66458ns
O x C x S	39220**	57ns	6554*	258156**	3408ns	41.5ns	10374ns	255831ns
Error	4161.2	28.5	1913.1	50354.9	3490.8	67.7	4959.5	42614.9
CV	13.96	6.8	12.7	18.1	10.4	7.9	14.3	17.3

* and ** = significant at 0.05 and 0.01 probability levels, respectively, ns=non significant

It was observed that Ca^{2+} content was higher in the root and Na_2SO_4 whereas Mg^{2+}, K^+ and Na^+ values were higher in the stem and NaCl in both species (Table 2). Na^+ was of the minimum value (568 mg kg^{-1} DM) in the root of *H. caspica* and the maximum value (1613.3 mg kg^{-1} DM) in the stem of *H. strobilaceum* (Table 2).

Table 2. Mean comparisons of ion content in different salt types and plant parts in *H. strobilaceum* and *H. caspica*

Treatments	*H. caspica*				*H. strobilaceum*			
salt type	Ca^{2+}	Mg^{2+}	K^+	Na^+	Ca^{2+}	Mg^{2+}	K^+	Na^+
NaCl	117.48 b	33.30 a	331.28 a	944.48 a	184.52 a	40.34 a	408.74 a	1435.11 a
Na_2SO_4	258.07 a	33.26 a	266.57 b	755.03 b	158.97 a	34.22 b	349.25 b	999.21 b
Plant organ								
stem	89.4 b	36.7 a	314.0 a	1131.5 a	110.7 b	43.5 a	444.6 a	1613.3 a
Root	286.2 a	29.8 b	283.9 b	568.0 b	232.8 a	31.1 b	313.4 b	821.1 b

Means of each column followed by same letters is not significant based on DMRT method (P<0.05)

Results of data analysis on ion content indicated that they were increased with salinity increasing up to 100 and 200-300 mM in *H. strobilaceum* and *H. caspica* (Fig. 1). But a further increase in salinity decreased ions in both plants. Of course, ion content had the minimum value at the control treatments in *H. caspica* and 500 mM in *H. strobilaceum.* A comparison of ion contents with T-test analysis illustrated that all the studied ions except for Ca^{2+} were higher in *H. strobilaceum* than *H. caspica* (Table 3).

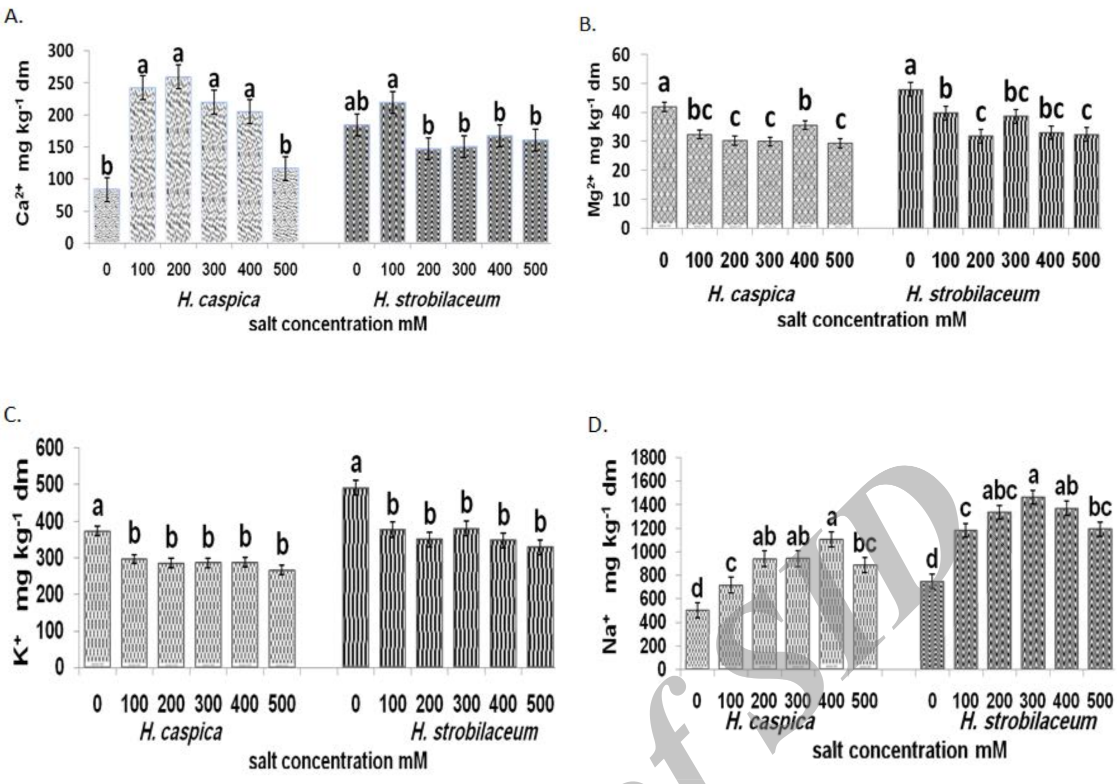

Fig. 1. Means comparisons of the ions content in different salinity concentrations in *H. strobilaceum* and *H. caspica* (1-A: Ca^{2+}, 1-B: Mg^{2+}, 1-C: K^{+}, 1-D: Na^{+}). Bars represent mean ± standard error. Different letters represent a significant difference ($P < 0.05$) between treatments

Table 3. Mean comparisons of ions content between *H. strobilaceum* and *H. caspica*

Species Name	Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}
H. caspica	187.8 ^{a}	33.3 ^{b}	298.9 ^{b}	849.8 ^{b}
H. strobilaceum	171.7 ^{a}	37.3 ^{a}	379.0 ^{a}	1217.2 ^{a}
T values	0.7 ^{ns}	3.2 **	7.4 **	8.0 **

** and ns = T values are significant at 0.01 probability level and non significant, respectively
Means of each column followed by same letters is not significant based on T student method

The trends of Ca^{2+} accumulation in exposure to salt stress in the shoots and roots of *H. strobilaceum* and *H. caspica* are shown in Fig. 2. Results indicated that Ca^{2+} was higher in the stem of *H. caspica* in normal conditions. But when it was exposed to salinity, Ca^{2+} translocated to the root, especially in Na_2SO_4. Then, if the salinity increases, this translocation gradually comes back to the primary conditions. Also, (Fig. 2), showed that Ca^{2+} values were increased with the increase of salinity in the stem of *H. strobilaceum* at NaCl. The main difference between two species was high content of Ca^{2+} in the stem of *H. caspica* in the control treatment. Therefore, the Ca^{2+} values were drastically decreased in *H. caspica* with the initiation of salinity at 100 mM, especially in Na_2SO_4 (Fig. 2).

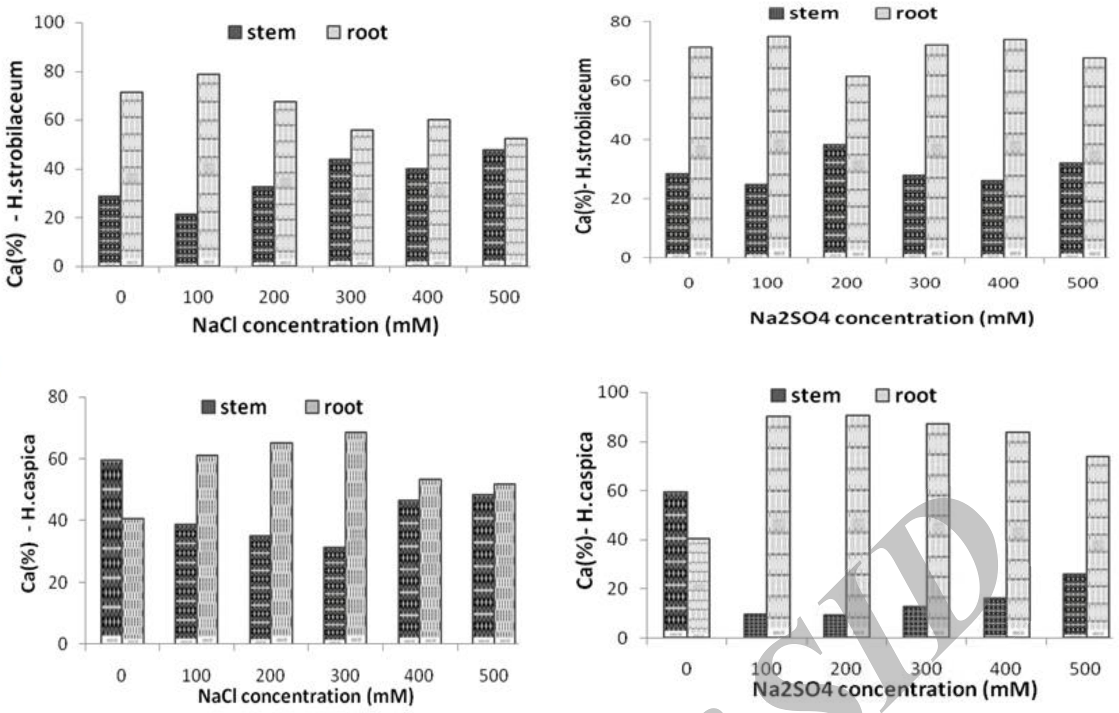

Fig. 2. Changes of Ca^{2+} stem /root of *H. strobilaceum* and *H. caspica* at different salt concentration

The trends of Mg^{2+} accumulation in exposure to salt stress in the shoots and roots of both species are presented in Fig. 3. Results showed that percentage of Mg^{2+} in the root of *H. caspica* was more than the stem in 500 mM regarding different salt types. But it was higher in the stem at NaCl and Na_2SO_4 in both species in the other salt concentrations. Of course, changes of Mg^{2+} did not follow an orderly trend such as the other ions with the increase of salinity.

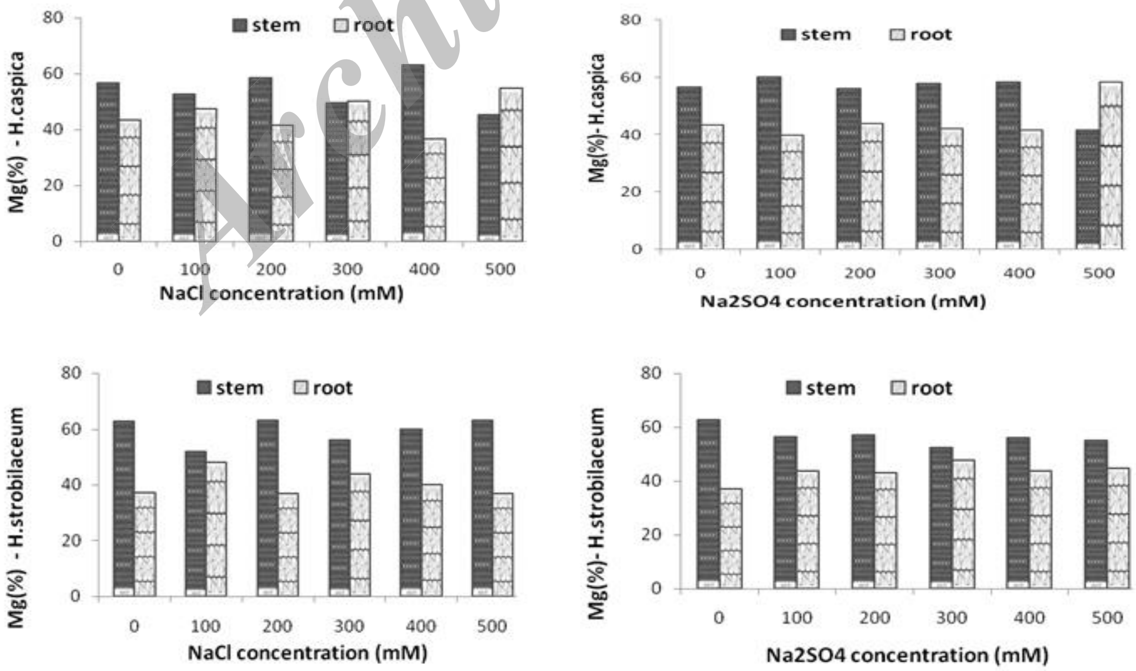

Fig. 3. Changes of Mg^{2++} stem /root of *H. strobilaceum* and *H. caspica* at different salt concentration

According to (Fig. 4), *H. caspica* accumulates higher values of K^+ in the root than shoot at 500 mM salinity in contrast to low salinity. But there were not noticeable changes in K^+ ratio at 0 and 500 mM in *H. strobilaceum*.

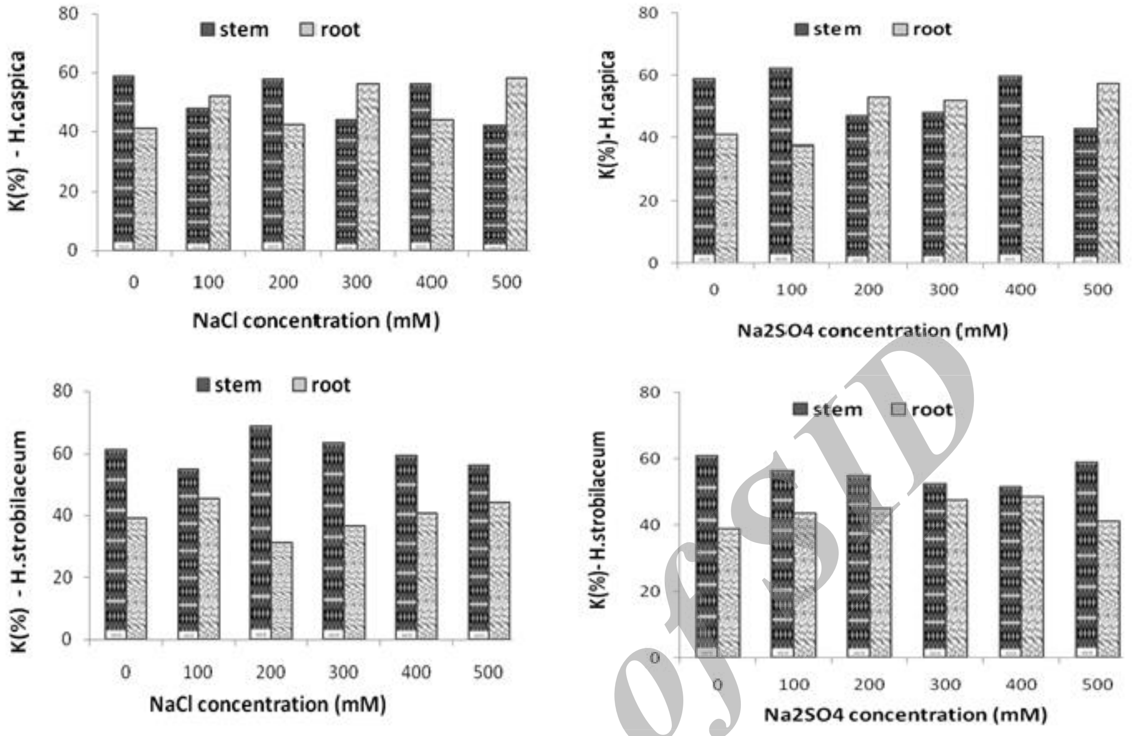

Fig. 4. Changes of K^+ stem /root of *H. strobilaceum* and *H. caspica* at different salt concentration

The trends of Na^+ accumulation in exposure to salt stress in the shoots and roots of both species are presented in Fig. 5. Results showed that both species tend to translocate Na^+ to the root with the increase of salinity. Of course, this trend exceeds at Na_2SO_4 more than NaCl.

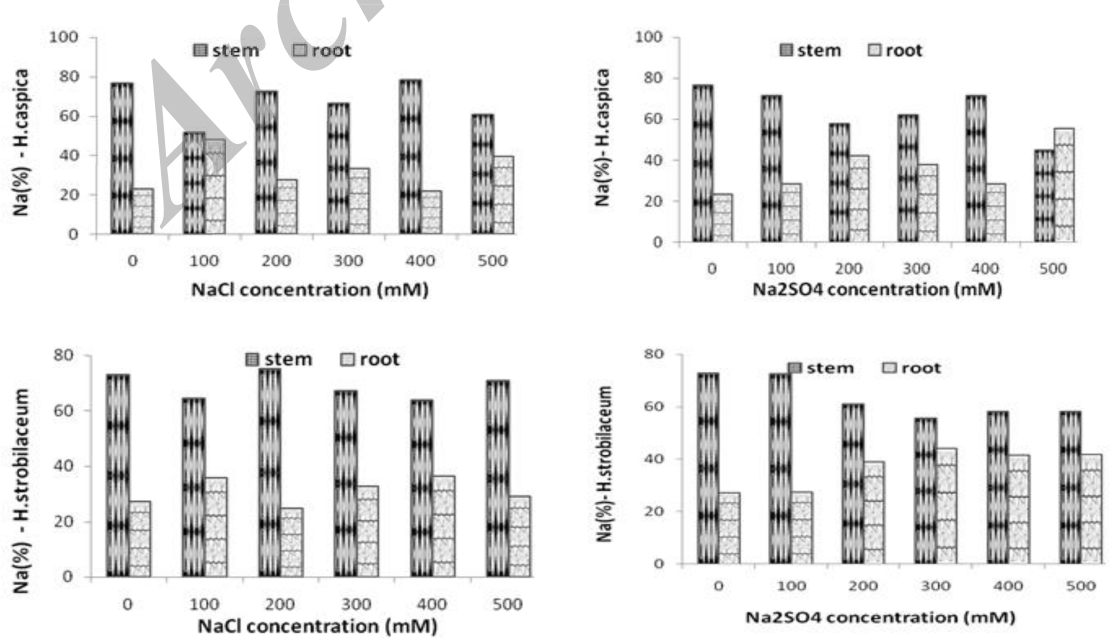

Fig. 5. Changes of Na^+ stem /root of *H. strobilaceum* and *H. caspica* at different salt concentration

Discussion

The results of the current research indicated that both plants including *H. strobilaceum* and *H. caspica* absorb ions at low salinity and excrete them at high concentrations of NaCl and Na_2SO_4. It was accepted that halophytes utilize salts in the osmotic adjustment to low water potentials of their environments (Cicek and Cakırlar, 2008; Duan *et al.,* 2007; Gama *et al.,* 2007). They must accumulate sufficient ions in their leaves for this purpose while avoiding the toxic effects of those ions (Hussain *et al.,* 2011). Many plants have developed an efficient method to keep the ion concentration in the cytoplasm in a low level. Membranes along with their associated components play an integral role in maintaining ion concentration within the cytosol during the period of stress by regulating ion uptake and transport (Zhang and Shi, 2013).

In some species, growth and ion accumulation are balanced (Garbarino and Dupant, 1988) while excess ions are secreted via salt glands in the other species (Ramadan, 2000 and Farkhondeh *et al.,* 2012). It seems that perennial halophytes tend to avoid high salinity through producing compatible soluble because the accumulation of ions needs thick leaves and these leaves have smaller intracellular spaces and more mitochondria. The mitochondria of the salt affected plants might be larger; evidencing that extra energy is needed in these plants for salt compartmentalization and excretion (Apse and Blumwald, 2002; Amiri and Rasouli, 2011).

According to the results, mineral content of shoots was more than roots. The capacity of salt exclusion is directed by several factors like selectivity of uptake by root cells involving preferential loading of K^+ rather than Na^+ into the xylem by the cells of the stele, the removal of salts from the xylem in the upper parts of roots, the stem and leaf sheaths based upon the exchange of K+ for Na+ and loading of the phloem (Hasanuzzaman *et al.,* 2014). Teakle *et al.* (2013) indicated that tolerance to salinity in the halophytic grasses like *Puccinellia* and *Thinopyrum* is facilitated by the development of adventitious roots and a superior ability to maintain negative membrane potential in root cells resulting in greater retention of K^+ in the shoots. Turan *et al.* (2007) stated that salts are located on or depressed into the epidermis and are found in almost every aerial part of the plant but they tend to be concentrated in the leaves. The results of present study are in accordance with the findings of Shi *et al.* (2003), Amiri *et al.* (2010) and Wu *et al.* (2010). Also, most of the studied ions were absorbed at NaCl. It can be attributed to NaCl composition which contains chloride that makes dissolvable composition with Na^+, K^+ and Mg^{2+}. However, Tirmizi *et al.* (1993) and Joshi and Kumar (1993) found that NaCl inhibited the growth of plants more than the other salts and seawater.

We found that increasing the salinity caused the exchange of different ions between soil and plant and translocation of ions in different parts of the plant. Gupta *et al.* (2013) showed that maintaining cellular Na^+/K^+ homeostasis is pivotal for the plant survival in saline environments. Plants maintain a high level of K^+ within the cytosol of about 100 mM which is ideal for cytoplasmic enzyme activities. Also, Ahmad and Prasad (2012) expressed that with the increase in the concentration of Na^+, there is a sharp increase in the intracellular Ca^{2+} level. El-Fauly *et al.* (2002) expressed that salt tolerance requires not only the adaptation of sodium toxicity, but also the acquisition of potassium whose uptake is affected by high external sodium concentration due to the chemical similarity of two ions. Therefore, potassium transport systems involving good selectivity of potassium over sodium can also be considered as an important salt tolerance determinant. Also, Miam *et al.* (2011) suggested that the plant increases salt tolerance by promoting potassium and calcium accumulation and inducing osmoregulation by the accumulation of organic solutes. In fact, plants adopt different mechanisms to resist salinity stress like excluding salts or accumulating ions into different tissue compartments, vacuoles or old leaves (Munns, 2005).

Conclusion

In conclusion, this investigation showed that *H. strobilaceum* and *H. caspica* uptake ions at low salinity but they change the tolerance mechanism at high salinity. Also, the study demonstrates that the plants translocate Na^+ and K^+ from the shoot to root as well as Ca^{2+} translocation from the root to shoot with the increase of salinity. In addition, the results indicate that ion uptakes change with different salt types. In fact, it can be concluded that due to the existence of such wide range of salt tolerance traits in halophytes, it is to be applied differently depending on the individual plant and its surrounding environment.

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تأثير شوری بر تبادلات يونی دو گونه شورپسند ***Halocnemum strobilaceum*** و ***Halostachys caspica***

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چکیده. شوری به عنوان عامل استرس‌زا محیطی، یکی از عوامل بسیار مهم تاثیرگذار بر روی حاصلخیزی غلات در تمام دنیا است. تقریبا ۱۰ درصد کل اراضی دنیا متاثر از شوری می‌باشند که سالانه حدود ۱۰ میلیون هکتار از اراضی کشاورزی بر اثر شوری و آبشویی از حیز انتفاع خارج می‌شوند. این مطالعه برای تعیین تبادلات یونی در *Halocnemum strobilaceum* و *Halostachys caspica* در شرایط شوری انجام شد. بذرهای این گیاهان در شرایط گلخانه‌ای در موسسه تحقیقات جنگلها و مراتع کشت شدند. بعد از گذشت ۵ ماه، تحت تنش شوری با غلظت‌های ۰، ۱۰۰، ۲۰۰، ۳۰۰، ۴۰۰ و ۵۰۰ میلی مولار بوسیله دو نمک NaCl و Na_2SO_4 برای مدت ۴۵ روز قرار گرفتند و مقادیر Na^+,k^+,Mg^{2+} و Ca^{2+} در اندام هوایی و زمینی گیاهان اندازه گیری شد. نتایج نشان داد که محتوای یونی در هر دو گیاه تحت تاثیر مقادیر مختلف هر دو نمک NaCl و Na_2SO_4 قرار گرفت. حداقل مقدار Na^+ به میزان (568 $mg\ kg^{-1}$ DM) در ریشه *H. caspica* و حداکثر مقدار آن (۱۶۱۳ $mg\ kg^{-1}$ DM) در ساقه *H. strobilaceum* مشاهده شد. محتوای یونی با افزایش شوری محیط تا ۱۰۰ mM در گونه *H. strobilaceum* و تا ۲۰۰–۳۰۰ mM در گونه *H. caspica* افزایش یافت. همچنین غلظت اکثر یون های مورد مطالعه در گونه *H. strobilaceum* بیش از گونه *H. caspica* بود. در مجموع، این تحقیق نشان داد که هر دو گونه در شوری های کم محیط، اقدام به جذب یونها می‌نمایند، اما در شوری های بالا مکانیزم مقابله با شوری را تغییر می دهند. در ضمن این گیاهان از طریق تغییر محل تجمع یون های Na^+ و K^+ از ریشه به ساقه و یون Ca^{2+} از ساقه به ریشه به مقابله با شوری می‌پردازند.

کلمات کلیدی: گیاهان هالوفیت، کلرید سدیم، سولفات دی سدیم، کاتیون‌ها