Comparative ionomics and growth factors alteration in *Lotus corniculatus* under salt stress

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Abstract

In order to understand the response of *Lotus corniculatus* to salt stress, ions content and some growth factors were analyzed in three varieties of this plant, namely, Ardebil, Karaj, and Jolfa. *Lotus corniculatus* plants were exposed to 0, 50, 100, and 150 mM NaCl in hydroponic condition. The amounts of anions ($Cl^-$and NO_3^-) and cations (K^+ and Na^+) in leaves as well as shoot and root dry weights, stem and root length, leaf area, and number of leaves were analyzed. The amount of Na^+ and Cl^- significantly increased in all varieties. However, with an increase in NaCl concentration, $K^+$and No_3^- content decreased significantly in all varieties ($p<0.05$). Growth factors (root and shoot length and weight, leaf area, and leaf number) declined significantly with increasing NaCl concentration. Considering alterations in growth factors and ions content, it seems that "Jolfa" and "karaj" have a higher capacity to tolerate salt stress particularly in100 and 150 mM NaCl compared to "Ardebil".

Key words: growth factors; ion content; *Lotus corniculatus*; salt stress

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Introduction

Salinity is one of the most important environmental factors limiting crop production often correlated with salt induced osmotic effect, nutrient deficiency or specific ion toxicity (Kouas et al., 2010). Salt stress causes nutrient deficiency because of the reduction of root growth and inhibition of the transporters mediating nutrient acquisition by toxic salts. Chloride is an essential micronutrient that regulates enzyme activities in the cytoplasm. It acts as a counter anion to stabilize membrane potential or as a co-factor in photosynthesis and is involved in turgor and pH regulation. However, Cl^- can be toxic to plants at high concentrations (White and Broadley, 2001). Plant strategies to cope with saline environment include salts exclusion and sequestration, tissue tolerance to accumulate ions, and limitation of K^+ loss, osmotic and molecular responses and changes in growth and development (Tester and Davenport, 2003).

Lotus is a member of *Papilonacea* and is a pasture legume and has a low concentration of tannin so it is suitable and delicious forage for grazers. High quality honey is made by pollens of this plant. Moreover, this plant is used for revitalization and productiveness in low quality soils (Teimouri et al., 2005). Shoots of *Lotus*

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corniculatus have medicinal properties such as anti-spasm, anti-inflammatory and sedative (Mousavi, 2004). The economic importance of postural legumes is related to their capacity to fix atmospheric nitrogen, thereby reducing agricultural cost through a reduction of fertilizer inputs and decreasing environmental contamination and on the other hand their interest as an important source of protein for animal diets (Khan et al., 1992). The aim of this study was investigation and evaluation of three *Lotus corniculatus* varieties' responses under salt stress and analyzing their abilities for salt tolerance and/or resistance.

Materials and Methods

Plant material

The intact seeds with the same size of three varieties of *Lotus* corniculatus, namely, 'Karaj', 'Ardabil', and 'Jolfa' with high viability of 80% to 90% germination were provided from gene bank of Iran Research Institute of Forest and Rangelands (RIFR). The seeds (150 from each variety) were put on wet filter paper in petri dish under 27 °C room temperature. Seed germination started three days after sowing. On the fourth day 72 seedlings of the same size from each variety were selected and transferred to plastic pots with the aerated Hoagland solution (1/8 strength). The Hoagland solutions of the plants were replaced every four days. After fifteen days the plants were transferred to new Hoagland solution of (1/4 strength).

Salinity treatment

Two-month-old plants were treated with NaCl (0, 50,100, and 150 mM) in Hoagland solution for 2 weeks. Three replicates for each treatment and 6 plants for each replicate were taken into account. In order to avoid high salt shock, NaCl was added to the nutrient solution by incrementally until the final concentrations were reached.

Growth parameters

After 2 weeks, plants were harvested and growth parameters including root and shoot dry weights and lengths as well as the number of leaves were determined. Also, total leaf surface for each plant was measured using Flachenberchung-einer-sw-Grafik (1995) software.

Mineral elements' measurement

Ion content

One hundred mg of the powdered dry matter of the shoot and root was mixed with 10 ml distilled water and boiled At 100 °C for an hour. After cooling in the room temperature the samples were centrifuged at 400x. The extracts were used to measure Na^+ and k^+ contents using a flame photometer (Fater electronic 405, Iran). Half a millilitre aliquots were analyzed to measure tissue Cl^- concentration using chloride analyzer (926 Sherwood Scientific, UK). Nitrate content was measured using the salicylic sulfuric acid method (Cataldo et al., 1975). Briefly, 0.8 ml of 5% salicylic acid in sulfuric acid was added to the 0.5 ml of the solution. Then, 19 ml of NaOH (2N) was added to the solution. The absorption 410 nm was read by a spectrophotometer.

Statistical analysis

All statistical analysese were done using the Statistical Package for Social Science (SPSS) for windows (Version 16.0). The mean values of three replicates and Standard Error of means were calculated. One-way ANOVA was used to determine the significance of the differences between treatments and the tukey's multiple range tests ($p<0.05$) were performed.

Results

The results obtained from the data analysis showed that different concentrations of NaCl had a significant effect on the stem length. Plant growth decreased significantly ($p<0.05$) in all varieties. The minimum stem length was observed in NaCl (150 mM) and the maximum length was recorded in 'Karaj' and 'Jolfa' varieties (Fig I. A). Also, salinity had a significant impact on the root length which decreased in all of the varieties. The minimum root growth was observed in NaCl (150 mM) and the maximum root growth was recorded

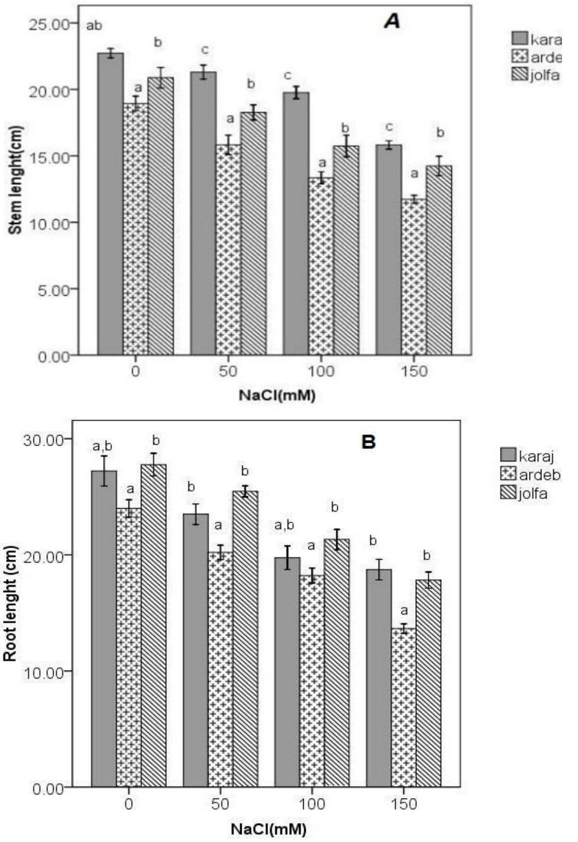

Fig. I. Stem (A) and Root (B) length of the plants treated with different concentrations of NaCl for 2 weeks; Bars are ± SE of the means (n = 3) p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

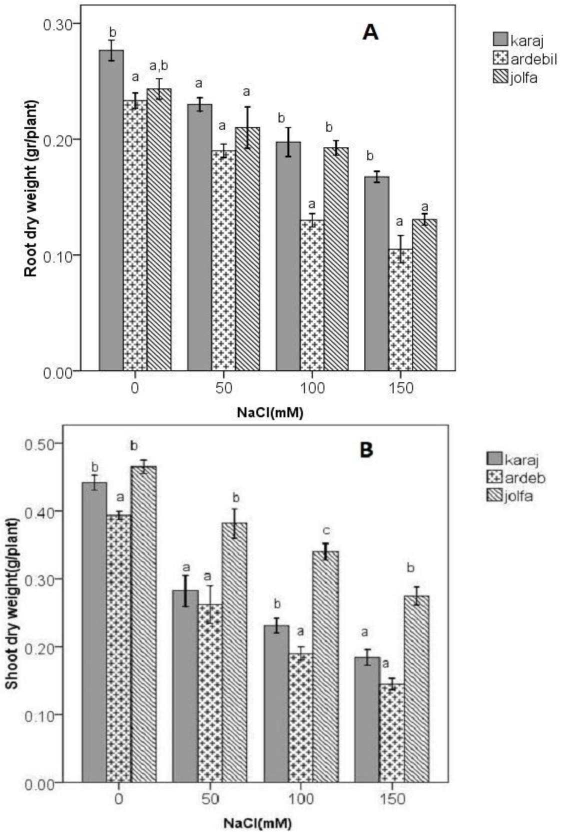

Fig. II. Root (A) and shoot (B) dry weight of the plants treated with different concentrations of NaCl for 2 weeks; Bars are ± SE of the means (n = 3) p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

in control. The maximum root growth under different salinity concentrations was observed in 'Karaj' (Fig I. B).

Increasing the NaCl concentration had a significant effect ($p<0.05$) on shoot dry weight in all of the varieties, in a way that the maximum shoot dry weight was in control and minimum was observed in 150 mM (Fig.II. B). Also, among the varieties the maximum shoot dry weight was observed in 'Jolfa'. Increasing NaCl caused a significant decrease ($p<0.05$) in root dry weight in a way that the 150 mM salinity treatment showed the minimum root dry weight (Fig. II. A).

Results showed that salinity had a significant effect ($p<0.05$) on the leaves decreasing their number in all concentrations. The maximum number of the leaves was observed in control plants. Also, the difference between three verities except for control treatment was significant ($p<0.05$) (Fig. III. A). Data analysis showed that with increasing the salinity the leaf surface was also decreased. A significant difference ($p<0.05$) was observed between control and all treatments except for 50 mM-NaCl. No significant difference was observed in any treatment between 'Jolfa' and 'Karaj' (Fig. III. B).

With an increase in salinity, shoot sodium content increased significantly ($p<0.05$). The minimum level was observed in control. 'Ardabil' variety showed the maximum shoot sodium content. Also, a significant difference ($p<0.05$) between the varieties was observed only in 150 Mm NaCl (Fig. III. A). With increasing salinity, sodium content of root increased significantly ($p<0.05$). Maximum sodium content of the root was also observed in 150 mM NaCl. 'Ardabil' showed the maximum and 'Jolfa' showed the minimum sodium content (Fig. IV. B). Salinity significantly ($p<0.05$) decreased shoot potassium content in a way that the maximum and minimum amount of potassium were observed in control and 150 mM NaCl,' respectively. All the varieties showed a significant difference ($p<0.05$) in all of the treatments except in 100 mM concentration. The minimum decrease was observed in 'Jolfa' and 'Karaj' (Fig. IV. A). Salinity had also a significant effect ($p<0.05$) on decreasing the root potassium content, in a way that the minimum potassium content was observed in the highest salinity concentration (150 mM). Among varieties a significant difference ($p<0.05$) was observed at 100 and 150 mM concentrations (Fig. IV. B).

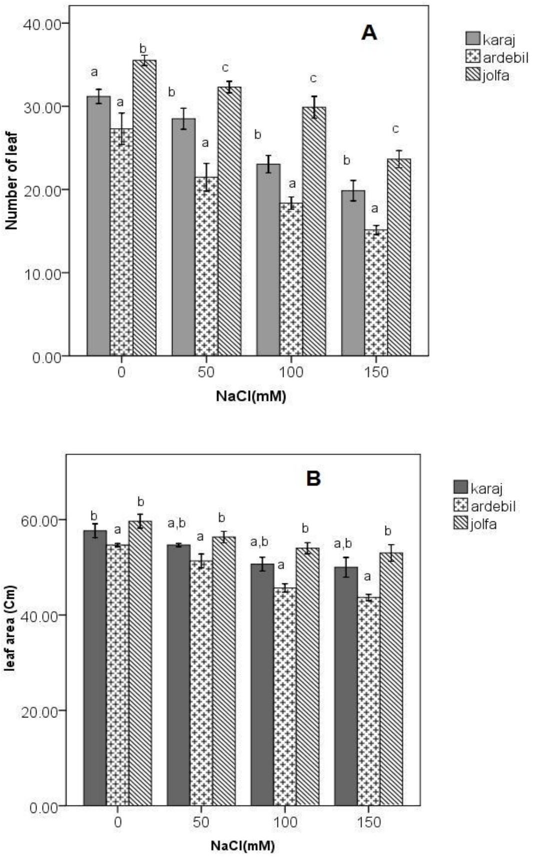

Fig. III. Number of leaf (A) and leaf area (B) of the plants treated with different concentrations of NaCl for 2 weeks; Bars are ± SE of the means (n = 3) p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

Increasing salinity level had a significant effect ($p<0.05$) on increasing Cl^- content of the shoot. The minimum and maximum amount of Cl^- in shoots was observed in control and 150 mM concentration, respectively. However, 'Karaj' and 'Jolfa' had the minimum increase in Cl^- content. Also, no significant difference was observed between all of the varieties in 50 and 100 mM NaCl treatments (Fig. V. A). Moreover, salinity had a significant effect ($p<0.05$) on the root Cl^- content. The minimum and maximum root Cl^- content was observed in control and in 150 mM NaCl, respectively. A significant difference ($p<0.05$) between the varieties was observed, so that the minimum increase was in 'Jolfa' and the maximum increase was observed in 'Ardabil' (Fig. V. B). Salinity affects the NO_3^- content in shoots; the maximum amount was observed in control and the minimum amounts were observed in 100 and 150 mM concentrations. The minimum decrease

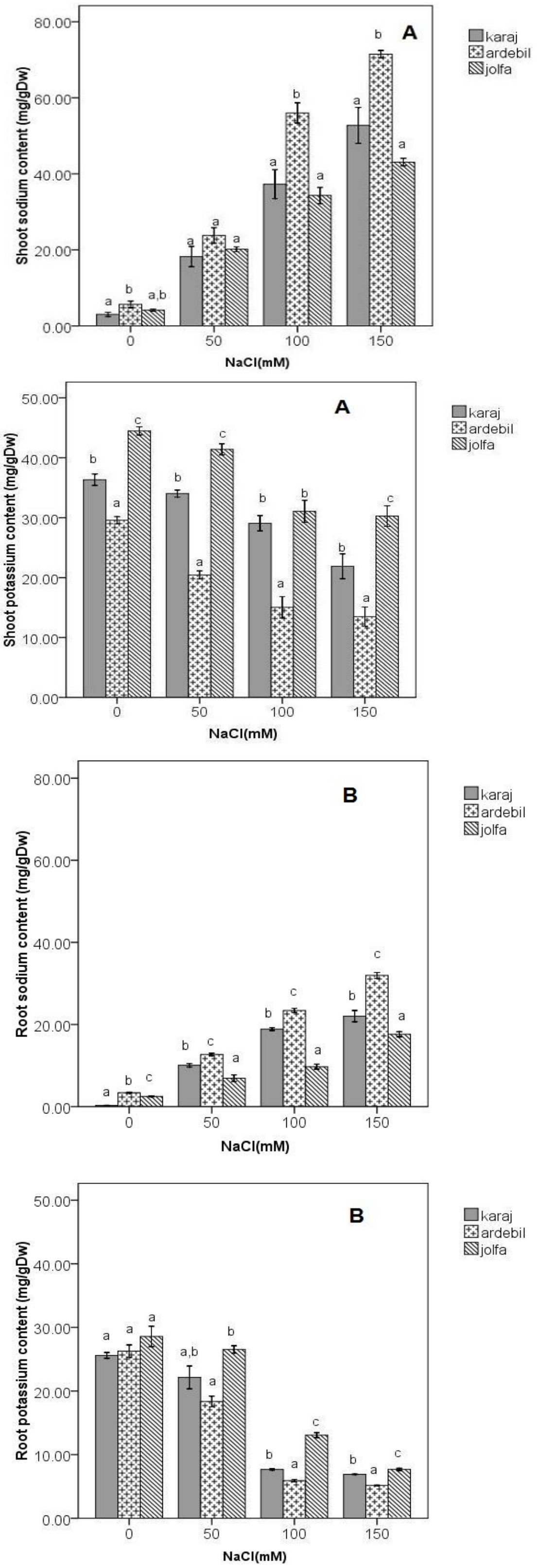

Fig. IV. Shoot (A) and root (B) sodium and potassium of the plants treated with different concentrations of NaCl for 2 weeks; Bars are ± SE of the means (n = 3) p ≤ 0.05. Different letters indicate significant differences between varieties at each salt concentration.

was observed in ‘Karaj’ and ‘Jolfa’ (Fig. V. A). No significant difference ($p<0.05$) was observed between 100 and 150 mM, in a way that control showed the maximum root NO_3^- content (Fig. V. B).

Discussion

Growth is the most basic sign of metabolic activity of a plant. One of the first signs which are created because of the stress is the qualitative and quantitative changes in growth. The longitudinal growth decrease can be the result of high concentration of chloride and sodium ions in nutrition solution. In other words, the toxicity of the Na^+ and Cl^- causes a decrease in longitudinal growth of the aerial organs (Sanches et al., 2011). The plant growth diminishing is the result of salinity stress that decreases photosynthesis area or photosynthesis rate in unit leaf area. High accumulation of salt in the aerial and root organs creates osmosis and ionic effects and thereby, causes growth diminishing (Nedjimi, 2011). In addition, the plant longitudinal growth decrease can be due to changes made by salinity in other metabolic activities such as meiosis, enzyme activities (mostly the protein synthesis is affected), and also decreasing in growth hormones (Amira and Qados., 2011). The results obtained from this research showed that salinity had a significant effect on decreasing the aerial organ and root growth in all of the three varieties. It seems that the salinity has prevented the lengthening of the cells in the target plant. Reviewing on salinity tolerance mechanisms in the plants, Tester and Davenport (2008) reported that the osmosis stress in the first stage of the salinity stress causes the cellular water deficiency and their lengthening will face some problems. Even after recreating the osmosis balance and pressure of the cells, their development and lengthening are slowly progressed. Investigating four model genotypes under salinity stress, Melchiorre et al. (2009) reported that in each of the four target genotypes plant longitudinal growth decrease with increasing the sodium chloride concentration. This decrease has been observed both in long-term (28 days) and short-term (17 days) stress conditions. In saline conditions, with increasing the salt concentrations in nutritious solution, the solution potential

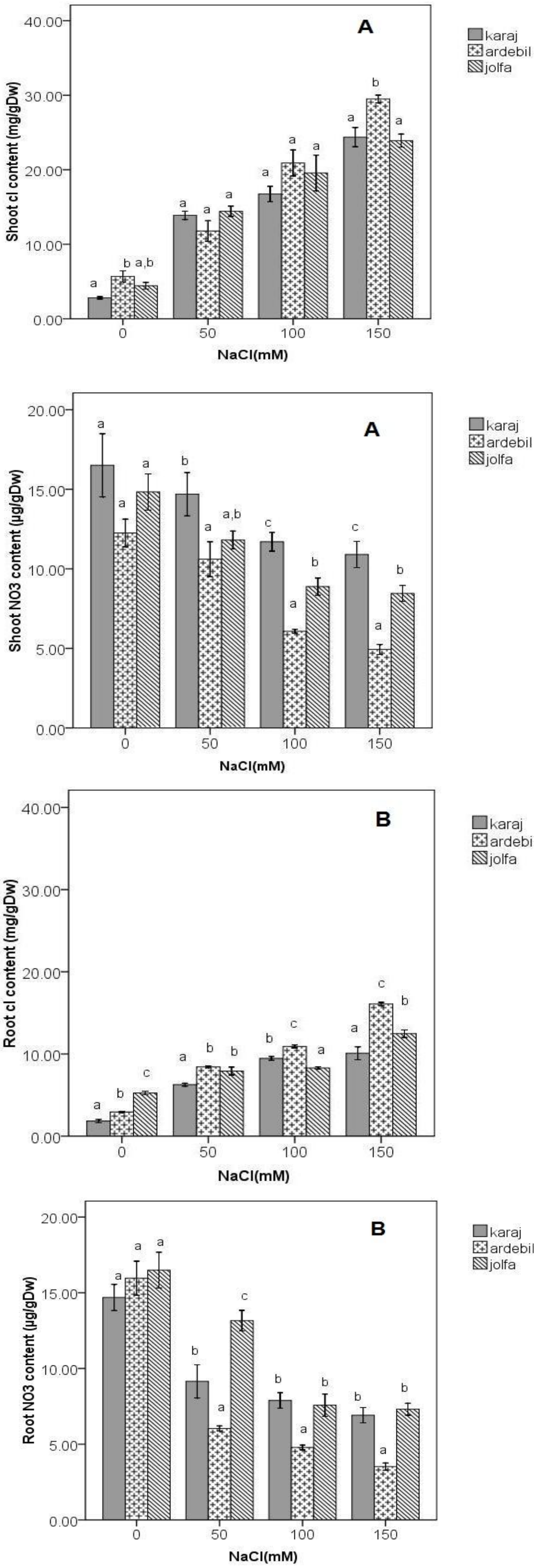

Fig. V. Cl^- and NO_3^- in shoots (A) and roots (B) of the plants treated with different concentrations of NaCl for 2 weeks; Bars are ± SE of the means ($n = 3$) $p \leq 0.05$. Different letters indicate significant differences between varieties at each salt concentration.

decreased and consequently the cells' turgor pressure is decreased too and cells water draining prevents their growth. Also, with shortening and falling, leaf production in plant decreases and as a result, a small amount of material will be absorbed by the cells and this causes a decrease in cell number and dimension, and finally the dry weight of the plant will be diminished (Sibole et al., 1998).

In the present study, salinity has had a significant effect on the dry weight of the aerial organs and the root of the varieties. Decreasing in the amount of stored materials, cells development and plant longitudinal growth, and also decreasing in the leaf production and branching as a result of salinity are the reasons of dry weight loss. Rejili et al. (2007) reported that with increasing the salinity, dry weight of *Lotus certicus* populations were decreased (Rejili et al., 2007). Melchiorre et al. (2009) reported that in each of the genotypes of *Lotus* plant the dry weight in shoot and root decreased significantly in both long-term and short-term treatments. In this study, high salinity caused decrease in leaf number in all of the genotypes. It seems that with decreasing in leaf number, the whole plant area decreased to compensate for the lack of nutrition and water, and to dispel the extra sodium entered into the plant by dropping the older leaves. Since the salinity limits the branching in the plant, the number of leaves also diminishes (Munns, 2003). The results obtained in this study are confirmed by Qados and Amira (2011). These researchers in an investigation on salinity effect on *Vicia faba* reported that salinity causes a decrease in leaf number. Measuring the leaf surface (area) is an appropriate criterion for evaluating the growth which can be done under different stresses including the salinity stress. The remarkable effect of salinity on the leaf surface decrease is due to the sodium chloride increase which causes the photosynthesis decrease and in turn leads to the decrease in plant and leaf growth and chlorophyll content (Parida and Das., 2005).

In the present study, salinity decreased the leaf area in all of the three genotypes. It seems that with lessening the leaf area and net photosynthesis rate, the number of pores (stoma) have been decreased to prevent high transpiration rate. Qados and Amira (2011) and Zhao et al. (2007) reported decrease in the leaf area of *Avena sativa* because of salinity which is consistent with the present findings. Once sodium is increased in the root, its effects may cause changes in osmotic pressure. This factor causes the plasmolysis and decreases the selectivity of ion uptake in root. With increasing the salinity stress, the sodium absorption also increases. Extreme increasing of sodium in cytoplasm causes the extreme depolarization of plasma membrane and prevents the metabolic performances (Bor et al., 2003).

In the present study, with increasing the salinity rate the sodium concentration in shoot and root increased in the varieties. Sodium highly accumulated in shoots rather than roots and this probably indicates the fact that this plant is not able to prevent the sodium ions to reach to aerial organs. Meanwhile, decreasing in the plant length and weight in the aerial organ was more than the root and it can be resulted from the more sodium accumulation in the shoot. Investigating the relationship between growth rate and ionic content in response to salinity in two forage plants of *Lotus cornicolatus* and *Lotus tenuis,* Teakel et al. (2006) reported that salinity causes the accumulation of sodium in leaves and roots and this increase in *Lotus cornicolatus* was more than *Lotus tenuis* . By comparing the amount of ions and osmolites in cultivars of *Lotus*, Sanches et al. (2011) reported that the sodium content increases with increase in salinity and this increase was more in *Lotus certicus* than others. This shows high resistance in *L. certicus* compared with other species of *L. cornoculatus* and *L. tenuis,* because shoot Cl^- content is the most important criterion of salinity tolerance in this plant. Generally, salinity stress decreases potassium concentration in plant organs through creating disorders in potassium absorption mechanisms by root. Since potassium acts as a co-enzyme in activating more than 40 enzymes, any change in its concentration has a remarkable effect on the plant growth. High sodium concentration under the salinity conditions not only disables the potassium absorption mechanism but also has a negative effect on these enzymes. Sufficient amount of potassium in plant causes the osmosis potential regulation and improves water absorption and in this way, one of the salinity stress effects that is physiological dryness is balanced (Qiv et al., 2004). Usually, the resistant plants have a high proportion of potassium rather than sodium in their different parts (Garg, 1997). In the current study salinity caused a decrease in potassium amount in plant organs in three varieties. This decrease was observed more in aerial organs. It was also observed that the sodium content in roots and shoots increased. It seems that increase in sodium content because of higher concentration of salt was the cause of potassium decrease. Low potassium content in shoots compared to roots can be due to more sodium accumulation in shoots. This finding is consistent with the results obtained in other studies. For example, investigating four genotypes of *Lotus,* Melchiorre et al. (2009), found that with increasing the salinity level, the amount of potassium decreased and this decrease was observed only in (LJNG2) genotype and *L. burtil* (Rejili et al., 2007). Comparative study of ions and osmolites content in *Lotus* species, Sanches et al. (2011) reported that the potassium content decreased with increasing the salinity in plants. Decreasing the nitrogen and phosphorus concentrations under the salinity stress is attributed to chloride. As a result, with increasing the chloride content, the species encounter nitrogen and phosphorus deficit and their growth decreases. Meanwhile, chloride and sodium ions accumulation has always been simultaneous and their toxic effects on plants are complementary. Chloride is an osmotic active solution in vacuoles and acts in turger and osmosis regulation processes. It regulates the key enzyme activities in cytoplasm and is an essential co-factor in photosynthesis. Also, it is the opposite anion for establishing the membrane potential and pH regulation inside the cell. But its increase leads to toxicity which accompanies some signs for example folding the leaf margin, tissue destruction, and the leaves death or fall because of chlorosis and necrosis (Teakel et al., 2010).

In the present study, shoot and the root chloride contents increased with increase in salinity level. This increase was observed in all the three varieties and it was more in shoots than in other plant parts. A significant difference in resistance to salinity and shoot chloride uptake was observed in 100 mM NaCl treatment which indicates the relative tolerance or resistance of this plant in higher salt concentrations. Conducting a comparative study between ions and osmolites contents in *Lotus* species, Sanches et al. (2011) reported that chloride increases with an increase in salinity level. Teakel et al. (2006) suggested that chloride content in plant parts increased because of salinity and this increase in both leaves and roots of *Lotus corniculatus* was more than in the same organs of *lotus tenuis* which is probably the cause of *Lotus tenuis* resistance. Chloride and nitrate absorption is competitive and when the chloride absorption increases the accumulation of nitrate decreases in the plant. Also, during the salinity stress because of the osmotic compounds synthesis such as proline and glycine betaine, the nitrogen content of the plant decreases (Heidari and Messri., 2010). Because of the decrease in nitrogen, the photosynthesis decreases and finally the plant growth decreases.

In the present research, with increasing the salinity, nitrate decreased and this decrease was observed in all three varieties. Increasing the amount of chloride can decrease nitrate absorption in *Lotus corniculatus* plants and more decrease of nitrate in aerial organs is because of the accumulation of more chloride in this organ and reciprocal effect of these two ions. Studying the sensitive and resistant wheat cultivars, Khan et al. (1992) found that salinity decreases the amount of nitrate in both roots and aerial organs. In another experiment on Indian pepper, Grag et al. (1993) reported the same level of nitrate decrease as a result of salinity.

In conclusion, regarding the results obtained in this study, the amount of Cl^- and Na^+ in 'Jolfa' and 'Karaj' varieties was less than in 'Ardabil' and the growth factors as well as K^+ and NO_3^- were more in them. Therefore, it seems that 'Ardabil' showed a lower capacity to tolerate salt stress condition when compared to the other varieties studied in this experiment.

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