



## The effect of salt stress on yield and accumulation of some minerals in two salt-tolerant and susceptible onion cultivars

Zahra Hosseini<sup>1</sup>, Mohammad Reza Zare-Bavani<sup>1,\*</sup>, Ahmad Zare<sup>2</sup>

<sup>1</sup> Department of Horticultural Science, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Khuzestan, Iran

<sup>2</sup> Department of Agronomy and Plant Breeding, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Khuzestan, Iran

Received: 22 February 2021, Revised: 28 September 2021, Accepted: 3 November 2021

© University of Tehran

### Abstract

Salt stress is a major agro-environmental constraint on crop productivity. In this study, the effect of different levels of salinity stress (0, 25, 50, 75, and 100 mM NaCl) on the accumulation of some ions in two salt-tolerant and susceptible onion cultivars (Esfahan and Behbahan cultivars, respectively) was investigated in 2017. The research was done by a factorial experiment based on a completely randomized design with three replications. The results showed that salinity increased Na and Cl contents and also decreased K content in the leaf and root in both cultivars. However, Na and Cl contents in the leaf and root of the susceptible cultivar were significantly higher than those in the tolerant cultivar, while the K value was less. In both cultivars, the Cl content in the leaf and root decreased with increasing salinity. However, the amount of Ca in the tolerant cultivar was higher than that in the leaves and roots of the susceptible cultivar. Salinity only significantly reduced Mg in the leaves. The amount of phosphate decreased at low salinity levels and increased as salinity increased. Yield injury index was in significantly negative correlation with K, Ca, K/Na, and Ca/Na ratios and in significantly positive correlation with Na and Cl in the leaves and roots. The results indicated the salt-tolerant cultivar plays a role in reducing the Na and Cl uptake, as well as increasing the absorption and transfer of K and Ca to the leaves. Therefore, it could cause low yield injury under the salinity conditions compared with the susceptible cultivar.

**Keywords:** Calcium, Chlorine, Correlation, Potassium, Sodium.

### Introduction

Onion (*Allium cepa* L.) is one of the most important vegetables produced in the world, so that its production in 2017 exceeded 97 million tons. Iran annually produces more than 2 million tons of onions; that is about 2% of global onion production, making it the world's fifth largest onion producer (FAO, 2017). Salt stress, one of the most important environmental issues in the world, has a negative effect on the growth and production of crops, especially in arid and semi-arid climates (Karimi *et al.*, 2012). Agricultural soils contain different types of salt ions. However, sodium chloride is usually the dominant and harmful salt, the increase of which reduces vegetative growth and photosynthesis rate (Turan *et al.*, 2007). Among the main adverse effects of salinity on the growth and development of plants, water shortage and ion-induced toxicity of a large amount of chlorine (Cl) and sodium (Na) can be mentioned. These factors often lead to the lack of calcium (Ca), potassium (K), and other nutrients (Marschner, 1995; Munns, 1993). Plants exhibit resistance to salinity stress through various mechanisms such as the uniform distribution of toxic ions in cell vacuoles, accumulation of persistent

\* Corresponding author e-mail: mzarebavani@asnrukh.ac.ir

osmosis in the cytoplasm, Cl and Na uptake decrease by the root, and non-transfer of Cl and Na to the stem (Karimi and Sadeghi-Seresht, 2018). The resistance degree of plants to salinity stress also varies between different species and even varieties of a single species (Loupassaki et al., 2002; Karimi and Sadeghi-Seresht, 2018;). Salinity management through recovery or improving farming techniques is often very expensive and only short-term solutions are available for overcoming salinity problems in arid and semi-arid regions, in which there is severe shortage of irrigation water (Turan et al., 2007).

A permanent solution for minimizing the harmful effects of salinity is to use cultivars that can grow and have economic production under saline conditions (Fooland, 1996; Al-Ashkar et al., 2019; Munns et al., 2006). In this regard, it is important to have a holistic understanding of the whole plant physiology against stress in order to select cultivars and improve salinity tolerance. When selection is made for quantitative characteristics, physiological indices can be more effective than agronomic indices or external evaluation (Ashraf and Harris, 2004). When plants are unable to control Na uptake to K uptake, Na accumulation in plant tissues exceeds the expected levels and causes cell damage. For minimizing the toxic effects of the salt in plant cells, plants should develop mechanisms in order to maintain low concentrations of Na in the cytosol by distributing Na into vacuoles. Several carrier proteins have been detected on the tonoplast membranes, which are responsible for dividing the efficiency of Na into the vacuole (Zhu, 2003). During recent years, several studies have shown that the absolute amounts of elements such as K, Ca, and Na in plants and the amount of equilibrium between the anions in plant organs play an important and determining role in terms of salinity tolerance differences (Ashraf and McNeilly, 2004; Aktas et al., 2006; Akram and Ashraf, 2009). Several research centers have tried to improve salt tolerance in cultivars and plant varieties via the absorbance, transport, and accumulation of nutrients, especially Ca, Na, K, and Cl in plants. Concentration and ratio of these elements are used as screening parameters for tolerating salinity stress in the cultivars (Munns et al., 2006; Colmer et al., 2006).

Most of the current studies on onion salt tolerance have been focused on its growth, yield, shoot and root, and Na and Cl toxicity. According to previous works, some onion cultivars have demonstrated more tolerance to salinity than others (Khodadadi, 2002; Hanci et al., 2016). Studies with different cultivars have found that when the concentration of Na and Cl in onion tissues increase with increasing salinity, the change in the content of nutrient elements varies among different cultivars (Khodadadi and Omid Beigi, 2004; Hanci et al., 2016). Identification of salt-tolerant cultivars is of great importance in onion breeding and production. Investigating the effects of salinity on the amount of nutrient elements in the onion is essential for understanding the effects of salt stress on plant growth and yield as well as identifying the important tolerance mechanisms in the onion. This study is aimed to investigate the relationship between yield injury (YI) and some important nutrients in two onion cultivars with different levels of salinity tolerance.

## Materials and Methods

### *Plant materials*

To investigate the effect of salt stress on the accumulation of some ions in two onion cultivars, a factorial experiment using a completely randomized design with three replications was conducted in Research Greenhouse Complex, Agricultural Sciences and Natural Resources University of Khuzestan, in 2017. The first factor consisted of two onion cultivars (Esfahan [salt-tolerant] and Behbahan [salt-susceptible]) and the second factor was five salinity levels (0, 25, 50, 75, and 100 mM NaCl). First, the seeds were planted in transplant trays (perlite substrate). After the germination and appearance of seedling, those with similar size and

identical vegetative conditions were selected and, after washing the roots with water, they were transferred to the hydroponic media. An aquaculture system was employed for this study. The modified Hoagland nutrient solution (Epstein and Bloom, 2004) was used to feed the plants. After transferring and transplanting them in a hydroponic medium, salinity was applied by adding NaCl to the nutrient solution for each treatment. Moreover, to prevent osmotic shock, the treatments were conducted gradually by adding 12.5 mM NaCl every 12 h (25 mM daily). The greenhouse temperature was controlled between 20 and 25°C. During the growth period, the pH and electrical conductivity of the nutrient solution were adjusted on the daily basis. To maintain the salt and nutrient concentrations, the solutions were changed every two weeks. The nutrient solution was ventilated by the air pump during the day. At the end of the experiment, the plants were removed from the planting medium. Next, different parts of the plant (i.e. root, onion, and leaf) were separated after washing the roots.

#### Measuring the stress index

YI was calculated according to the following formula (1) (Badran, 2015):

$$YI(\%): YR = \frac{Y_p - Y_s}{Y_p} \times 100 \quad (1)$$

Where  $Y_p$  and  $Y_s$  denote yield (bulb fresh weight) in non-stress and stress conditions, respectively, for each cultivar.

#### *Element measurement*

##### *Sample preparation*

First, the leaves were washed with tap water, with 0.1 M hydrochloric acid, and with distilled water, in the order of their appearance. The plant sample was dried in an oven at 70°C for 48 h and, then, milled. The milled sample was passed through a 0.5 mm sieve and used to measure the content of certain minerals (i.e. K, Ca, Mg (magnesium), P (phosphorus), Na, and Cl). The samples of the prepared leaves were extracted for measuring their elements as follows:

##### *Extraction steps*

- 1- Some of the plant samples were digested by wet burning in a volumetric flask with sulfuric acid-salicylic acid-hydrogen peroxide (Wahing *et al.*, 1989).
- 2- Some other plant samples were digested by dry burning in the furnace and the combination was performed with hydrochloric acid (Chapman and Pratt, 1961).

##### *Measuring P by calorimetric technique*

About 5 mL of the extract solution obtained by digestion method 1 was poured into a 25 ml volumetric flask. Next, 5 ml of the ammonium molybdate-vanadate solution was added to it and reached the destination volume. Then, the absorbance value was measured by a spectrophotometer at 470 nm. The amount of P in the dry sample of the plant, in terms of  $\text{mg g}^{-1}$  DW of the sample, was obtained using the following formula (Wahing *et al.*, 1989):

$$P = A \times B \times V / 2000W \times 100 / D.M \quad (2)$$

where A is the concentration of the P sample, B is the concentration of control P, V is the final volume of solution, and W is the weight of the dried plant.

#### *Measuring K and Na by flame photometer method*

The solution obtained by extraction method 2 was diluted with the ratio of 1:9 using the Cesium chloride and the absorbance was measured using a film photometer with the wavelength of 766.5 nm for K and 589 nm for Na (Wahing *et al.*, 1989). K and Na contents in the dry sample of the plant were calculated using the following formula (3) and expressed based on  $\text{mg g}^{-1}\text{DW}$  of the sample:

$$K = A \times B \times V / 1000W \times 100 / D.M \quad (3)$$

#### *Measuring Mg and Ca*

Extracts from dry burning and acid combinations were used to measure the plant Ca and Mg. The procedure was carried out by taking 1 mL of the extract of the samples and the control and diluting it to the ratio of 1:9 with distilled water. Afterwards, 0.25 ml of the diluted extract was transferred to the test tube, to which 4.75 ml of lanthanum nitrate solution containing 1 g/L of lanthanum was added. This extract was used to measure Ca uptake (422.7 nm) and Mg uptake (282.2 nm) by an atomic absorption device (Wahing *et al.*, 1989). The Ca and Mg contents in the dry plant were calculated by the following formula and expressed based on  $\text{mg g}^{-1}\text{DW}$ :

$$\text{Ca or Mg} = (A - B) \times 1 / 500 \times V / W \times 100 / D.M \quad (4)$$

where A is the concentration of Ca or Mg in the extract in mg/L, B is the concentration of control Ca or Mg in mg, V is the volume of the primary extract obtained by digestion in mL, W is sample weight for digestion in g, and D.M is the percentage of plant dry matter.

#### *Measuring Cl in the plant*

The plant sample was ashed in alkaline medium and the Cl from the ash was extracted by hot distilled water and titrated in the presence of standard silver nitrate potassium chromate reagent. Ca oxide was used to alkalize the medium in the ashing phase (Wahing *et al.*, 1989). The amount of Cl in the sample was calculated based on the following formula and expressed in  $\text{mg g}^{-1}\text{DW}$ :

$$\text{Cl}\% = (T + 5 - B) * F * 35.5 / 1000 * 100 / m * 100 / D.M \quad (5)$$

Where T is silver nitrate intake for the sample titers, B is silver nitrate intake for control titers, M is the weight of sample weighed for ashing, DM is the percentage of plant dry substance, and F is standard silver nitrate factor.

#### *Statistical analysis*

The SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis of the generated data. Before analysis of variance, the validity of the normality assumption was checked by Shapiro-Wilk test (Shapiro and Wilk, 1965) and the homogeneity of variance was examined using Bartlett test (Bartlett, 1937). The differences among the means of different treatments were tested using least significant differences (LSD) at probability 5%. To describe

the magnitude of the relationships between YI and some mineral elements, Pearson's correlation coefficients ( $r$ ) were calculated using SPSS (ver. 23). Microsoft Excel (ver. 2013) was used to plot the results.

## Results

Results of analysis of variance (ANOVA) concerning the effect of the interaction of cultivar and different salinity levels on YI, K, Na, Cl, K/Na, and Ca/Na ratio in the root and leaf of the studied cultivars were significant at 1% probability level; but it had no significant effect on Ca, P, and Mg contents (Table 1). The effects of cultivars and salinity on Ca in the leaf and root and the effects of salinity on P and Mg in the leaf and root were significant at 1% probability level (Table 1). The simple effects of salinity and cultivar on the other studied attributes were significant at 1% probability level.

**Table 1.** ANOVA results of the effects of cultivar and salinity treatments on some mineral elements in the leaf and root of two onion cultivars

Sources of variance	d.f.	Mean Squares (MS)								
		YI	Leaf K	Root K	Leaf Na	Root Na	Leaf Ca	Root Ca	Leaf P	Root P
Cultivar	1	1220.450**	0.298**	0.605**	4.461**	51.117**	0.019**	0.023**	0.003 <sup>ns</sup>	0.007 <sup>ns</sup>
Salinity	4	6868.650**	0.812**	1.585**	3.704**	73.447**	0.134**	0.123**	0.091**	0.128**
Cultivar× Salinity	4	135.490**	0.027**	0.047**	0.455**	6.191**	0.003**	0.002 <sup>ns</sup>	0.003 <sup>ns</sup>	0.001 <sup>ns</sup>
Error	20	4.110	0.006	0.006	0.004	0.163	0.002	0.001	0.009	0.004
Coefficient of Variations	of	5.000	4.301	7.398	5.426	11.416	15.167	4.744	9.936	11.641
Cultivar	1	0.001 <sup>ns</sup>	0.002 <sup>ns</sup>	0.736**	0.827**	16.576**	5.738**	3.633**	2.064**	
Salinity	4	0.028**	0.005 <sup>ns</sup>	0.636**	1.708**	327.394**	37.097**	70.095**	10.390**	
Cultivar× Salinity	4	0.004 <sup>ns</sup>	0.002 <sup>ns</sup>	0.088**	0.204**	2.047**	1.339**	0.485**	0.494**	
Error	20	0.007	0.004	0.002	0.005	0.266	0.060	0.028	0.011	
Coefficient of Variations	of	7.178	3.853	5.821	5.921	9.804	14.124	6.680	10.094	

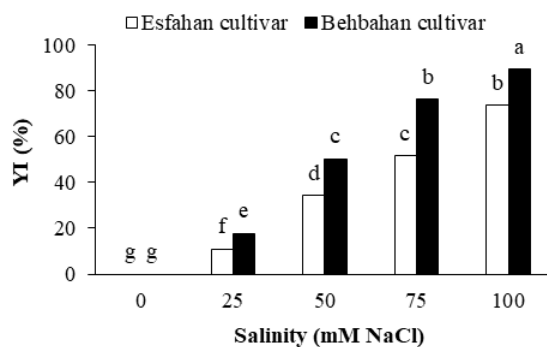
<sup>ns</sup> and \*\*, Not significant and significant at 1% error probability level, respectively. d.f. degree of freedom, YI yield injury, K potassium, Ca calcium, P phosphate

### YI (%)

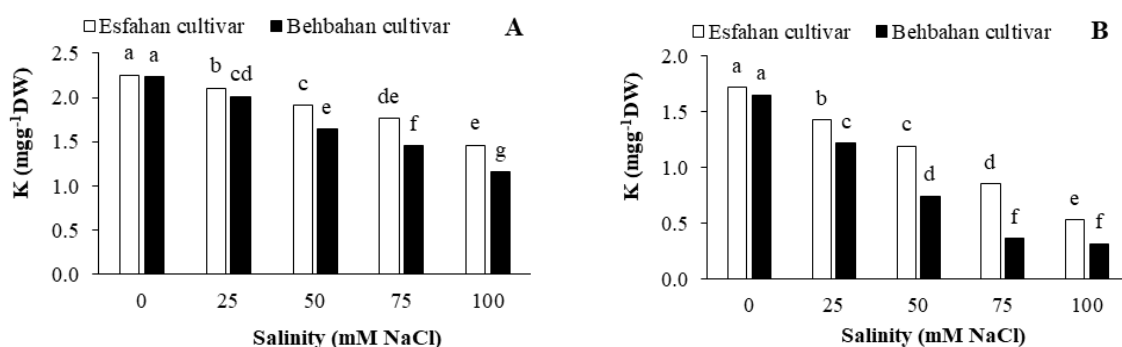
Mean comparison of the interaction of salinity and cultivar on YI of Esfahan and Behbahan cultivars showed that the amount of YI in both cultivars had a significant increase with increasing salinity levels. Here, the highest increase was observed in both cultivars at high salinity levels (100 mM NaCl) (Figure 1). The results also showed that YI of Esfahan cultivar at all salinity levels was lower than that of Behbahan cultivar (Figure 1).

### Effect of salinity on K content in leaf and root

The effects of different salinity levels and cultivars on K content ( $\text{mg g}^{-1}\text{DW}$ ) in the leaf and root are presented in Figure 2. The results showed that the amount of K in both cultivars had a significant decrease with increasing salinity levels and the highest decrease was observed in both cultivars at high salinity levels (100 mM NaCl) (Fig. 2). The results also indicated that the amount of K in the leaf and root of Esfahan cultivar was more than its amount in the leaf and root of Behbahan cultivar at every stress level (Figure 2).



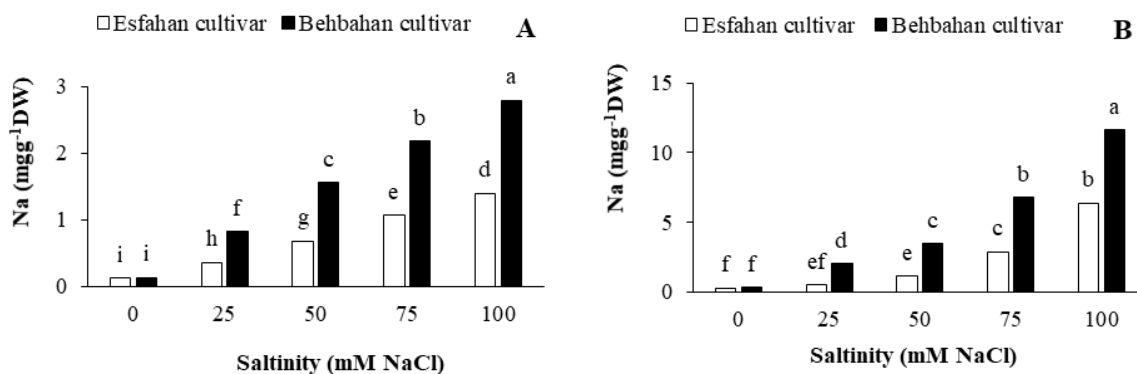
**Figure 1.** Interaction of different levels of salinity and cultivar on YI (%) of two onion cultivars with different salinity tolerance



**Figure 2.** Interaction effects of salinity and cultivar on K content (mg g<sup>-1</sup>DW) in leaf (A) and root (B) of two onion cultivars with different salinity tolerance

*Effect of salinity on Na content in leaf and root*

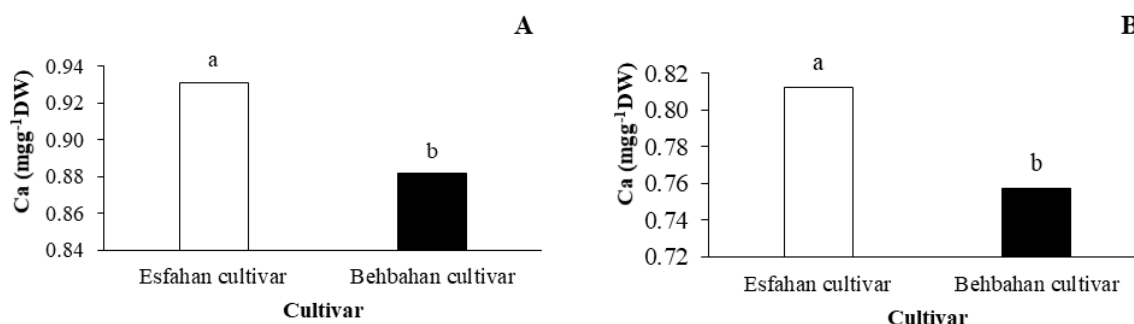
A composition of Na content of leaf and root onions cultivars at different NaCl levels is shown in Figure 3. All salinity levels significantly increased the Na content in the leaf and roots of both cultivars and the highest increase was observed in 100 mM NaCl. Also, the results showed that the amount of Na in Esfahan cultivar in all treatments was less than Na content of Behbahan. Moreover, the Na shortage between tolerant cultivar of Esfahan and susceptible-cultivar of Behbahan created a significant difference (Figure 3).



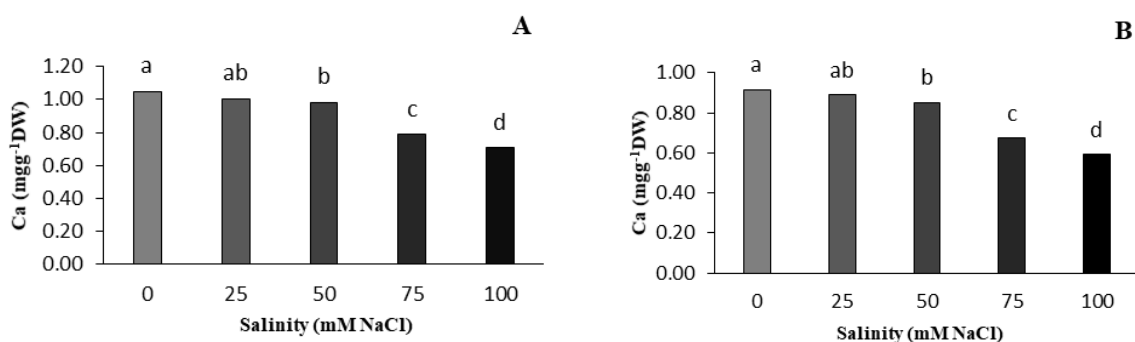
**Figure 3.** Interaction effects of salinity and cultivar on Na content (mg g<sup>-1</sup>DW) in leaf (A) and root (B) of two onion cultivars with different salinity tolerance

### Effect of salinity on Ca content in leaf and root

The mean comparison of the simple effects of cultivar showed that Ca content ( $\text{mgg}^{-1}\text{DW}$ ) in the leaf and root of Esfahan cultivar were significantly higher than that of the Behbahan cultivar (Figure 4). Also, the increase in the salinity levels in the nutrient solution resulted in a significant decrease in Ca content in the leaf and root, irrespective of the cultivar type (Figure 5).



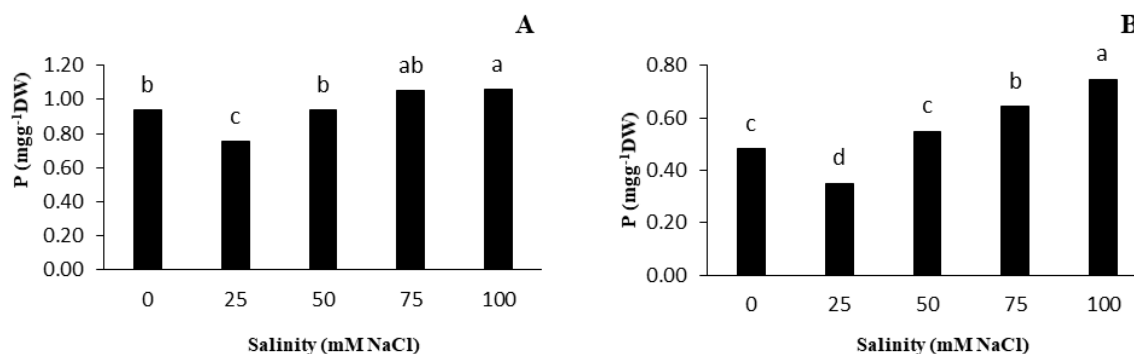
**Figure 4.** Simple effects of cultivars on the leaf (A) and root (B) Ca content ( $\text{mgg}^{-1}\text{DW}$ ) in two onions



**Figure 5.** Simple effects of salinity tension on Ca content ( $\text{mgg}^{-1}\text{DW}$ ) in leaf (A) and root (B) of onions cultivars

### Effect of salinity on P content in leaf and root

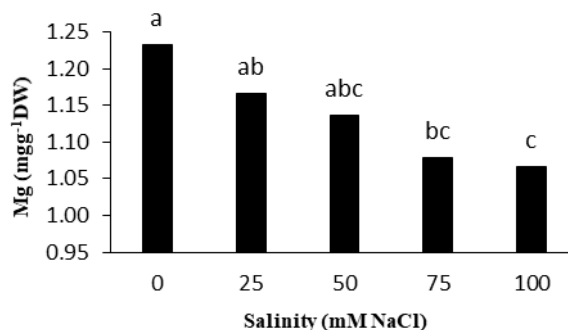
The mean comparison of the simple effects of salinity on P in the leaf and root showed that P concentration had a significant decrease with salinity increase at NaCl level of 25 mM. With an increase in the salinity level, the concentration of P in the leaf and root also increased, so that at 75 and 100 mM NaCl, the amount of P was higher than that of the control (Figure 6).



**Figure 6.** Simple effects of salinity on P content ( $\text{mgg}^{-1}\text{DW}$ ) in leaf (A) and root (B)

### Effect of salinity on Mg content in leaf

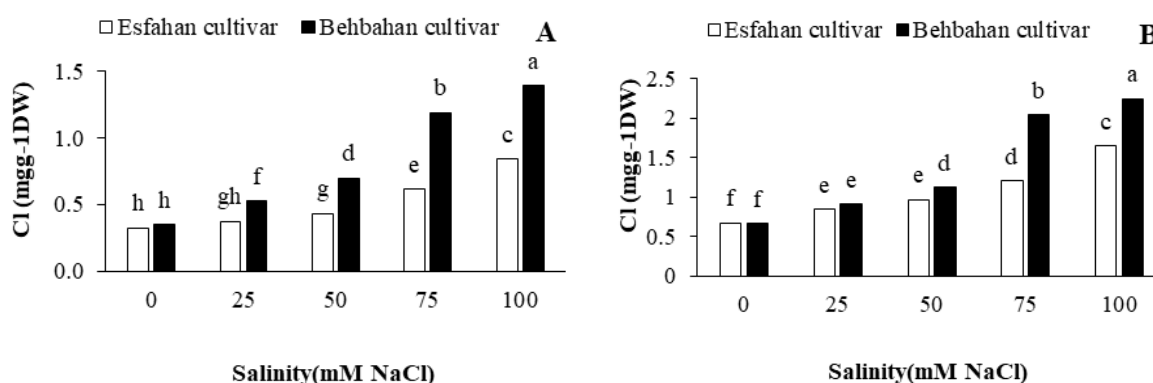
As shown in Figure 7, an increase in the NaCl concentration of the nutrient solution significantly reduced Mg content in the leaf ( $\text{mgg}^{-1}\text{DW}$ ), so that the highest decrease occurred at the high salinity levels (100 mM NaCl).



**Figure 7.** Effect of different salinity levels on leaf Mg content ( $\text{mgg}^{-1}\text{DW}$ )

### Effect of salinity on Cl content in leaf and root

Mean comparison of interaction effects of salinity and cultivar on the amount of Cl ( $\text{mgg}^{-1}\text{DW}$ ) in the leaf and root showed that, with an increase in salinity levels, Cl content had a significant increase and the highest increase in both cultivars was at the highest level of salinity levels (100 mM NaCl) (Figure 8). Also, the results showed that the Cl amount in the Esfahan cultivar at all different levels of salinity was less than that of the Behbahan cultivar (Figure 8).

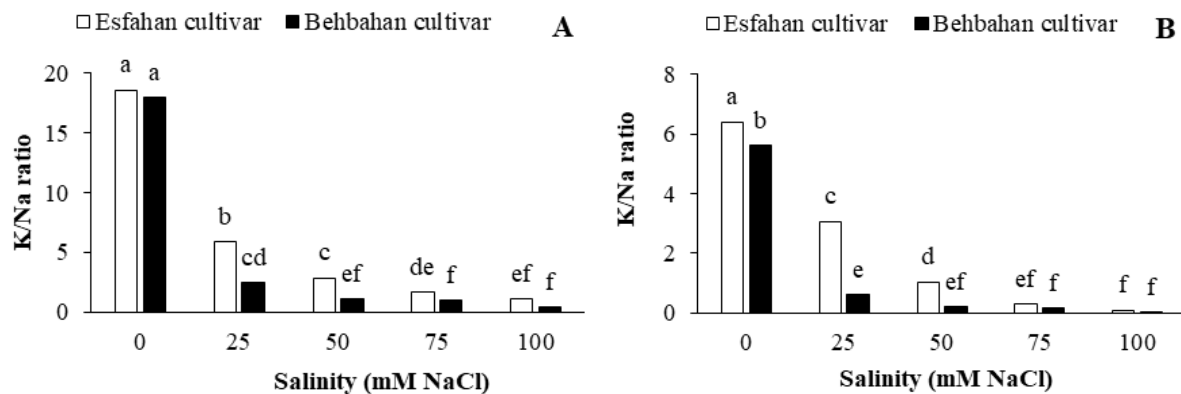


**Figure 8.** Interaction effects of salinity and cultivar on the leaf (A) and root (B) Cl content ( $\text{mgg}^{-1}\text{DW}$ ) of two onion cultivars with different levels of salinity tolerance

### Effect of salinity on K/Na ratio in leaf and root

The effects of different salinity levels and cultivars on K/Na ratio in the leaf and root of Esfahan and Behbahan cultivars showed that this ratio, in both cultivars, had a significant decrease with increasing salinity levels. Here, the highest decrease was observed in both cultivars at high salinity levels. The results also showed that the K/Na ratio in Esfahan at all salinity levels was more than that of Behbahan and there was significant difference between both cultivars (Figure 9).

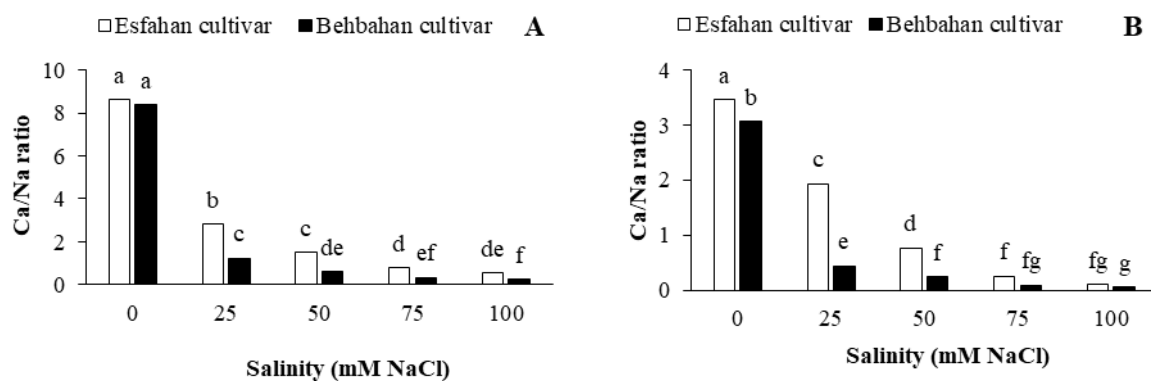




**Figure 9.** Interaction effects of salinity and cultivar on the K/Na ratio of the leaf (A) and root (B) in two onion cultivars with different levels of salinity tolerance

#### *Effect of salinity on Ca to Na ratio in leaf and root*

Mean comparison of the interaction effect of salinity and cultivars on Ca/Na ratio in the leaf of both cultivars showed that this ratio, in both cultivars, had a significant decrease with increasing salinity levels; but, the reduction level was less in the Esfahan cultivar (Figure 10).



**Figure 10.** Interaction effects of salinity and cultivar on Ca/Na ratio in leaf (A) and root (B) of two onion cultivars with different levels of salinity tolerance

#### *Correlation between YI and some minerals*

The correlation between YI and K, Ca, K/Na, and Ca/Na ratio was negative and highly significant. Also, there was a significantly positive relationship between YI and Na and Cl amounts in the leaf and root (Table 3). Correlation between K and Ca contents in the leaf and root was positive and highly significant, while it was negative and highly significant in terms of Na and Cl contents in the leaf and root. K/Na ratio in the leaf had highly significantly positive correlation with K/Na in the root, Ca/Na ratio, and K and Ca in the leaf and root. Moreover, it had highly significantly negative correlation with high Na content in the leaf, and highly significantly negative correlation with Na content in the root and Cl in the leaf and root (Table 2).

## **Discussion**

The results showed that increasing salinity increased the YI of both cultivars. It was also observed that the more tolerant cultivar (i.e., Esfahan) had a significantly lower YI (%) than the susceptible-cultivar (Behbahan) in all the treatments. This is consistent with the findings by

Badran (2015). Hosseini (2019) noted that YI could be a reliable index for selecting high yielding onion cultivars under both with and without salt stress conditions.

**Table 2.** Correlation between YI (stress index) and some mineral elements in leaf and root of two cultivars of edible onions with different degrees of salinity tolerance

	Leaf K	Root K	Leaf Na	Root Na	Leaf Ca	Root Ca	Leaf Cl	Root Cl	Leaf K/Na	Root K/Na	Leaf Ca/Na	Root Ca/Na
Root K	0.95**											
Leaf Na	-0.95**	-0.94**										
Root Na	-0.94**	-0.88**	0.95**									
Leaf Ca	0.89**	0.88**	-0.87**	-0.91**								
Root Ca	0.89**	0.91**	-0.88**	-0.91**	0.96**							
Leaf Cl	-0.92**	-0.91**	0.98**	0.97**	-0.90**	-0.92**						
Root Cl	-0.93**	-0.92**	0.95**	0.96**	-0.93**	-0.94**	0.98**					
Leaf K/Na	0.75**	0.81**	-0.72**	-0.59**	0.61**	0.61**	-0.62**	-0.65**				
Root K/Na	0.78**	0.85**	-0.75**	-0.63**	0.65**	0.65**	-0.65**	-0.68**	0.98**			
Leaf Ca/Na	0.75**	0.82**	-0.72**	-0.60**	0.63**	0.62**	-0.63**	-0.66**	0.99**	0.98**		
Root Ca/Na	0.80**	0.87**	-0.77**	-0.66**	0.68**	0.68**	-0.68**	-0.70**	0.97**	0.99**	0.97**	
YI	-0.98**	-0.98**	0.94**	0.91**	-0.91**	-0.92**	0.91**	0.94**	-0.78**	-0.81**	-0.78**	-0.83**

\*\*Correlation is significant at the 0.01 level

The results showed that K was significantly decreased in both onion cultivars due to salinity increase. It was also observed that the Esfahan cultivar (the tolerant one) had significantly higher K content than the Behbahan cultivar (the susceptible one) in all the treatments, which is consistent with the findings of Khodadadi (2002), Malik *et al.* (1982), and Arvin and Kazemipour (2003). With increasing salinity, the difference between the amount of K in the leaves and roots of the tolerant cultivar increased compared with the salinity-sensitive cultivar (Figure 2). These results showed that the tolerant cultivar had a greater ability to absorb and transfer K than the susceptible cultivar. The maintenance of K<sup>+</sup> acquisition with the exclusion of Na<sup>+</sup> from photosynthetic leaves was indeed found to be highly correlated with the plant salt tolerance (Hanin *et al.*, 2016).

K plays an important role in many plant processes including ion stability in plant cells, osmotic regulation, opening and closing the stomatal guard cells, and antioxidant systems. As the result of salt stress, an increase occurs in Na ion accumulation in the plant, ion imbalance, nutrient deficiency, and ion toxicity. A decrease in plant growth to high Na concentration has been attributed to the lack of K and Ca uptake (Jouyban, 2012). K is known as a trade-off ion for Na excretion in the cell membrane system because the withdrawal of Na ion in the cortex accompanies the entry of K ions and the presence of large amounts of Na prevents the absorption of nutrients such as K by plant tissues; consequently, the plant is encountered with the lack of this element (Akram and Ashraf, 2009).

Results from other researchers imply that salt-tolerant cultivars have a higher ability in the transport of K into shoots and leaves and, then, maintain the suitable K/Na ratio for normal metabolism (Flowers *et al.*, 2015; Hamdani *et al.*, 2017; Liu *et al.*, 2019).

Salinity increase resulted in an increase in the Na content in the leaf and root of both cultivars, but the tolerant cultivar of Esfahan had significantly lower Na content than the Behbahan susceptible cultivar. In both cultivars, the amount of sodium in the roots was higher than that in the leaves (Fig 3), indicating that onions have an exclusion mechanism. Strong correlation between salt exclusion and salt tolerance does exist in many species. Further removal of sodium from xylem to translocate back into the roots is another way to prevent Na<sup>+</sup> over-accumulation in photosynthetic tissues (Hanin *et al.*, 2016). The results of this study are consistent with the research results of Khodadadi and Omid Beygi (2003) and Arvin and Kazemipour (2003). They have observed that increasing salinity significantly increases Na accumulation in onion cultivars. In the presence of high amounts of Na in the root medium, the

concentration of this element can be increased in plant organs. Numerous studies have shown that NaCl salinity increases the amount of Na in the plant tissue of many crops (Sivritepe *et al.*, 2003; De Pascale *et al.*, 2003; Essa, 2002; Parida and Das, 2005). One mechanism of salt tolerance in plants is the prevention of Na transfer to young organs (Munns *et al.*, 2006, Munns and Tester, 2008). Previous studies have reported that salt-tolerant cultivars have a higher ability for the prevention of Na uptake and transfer of the root to shoots (Flowers *et al.*, 2015; Munns and Tester, 2008). Low Na<sup>+</sup> concentration in the shoots was used as selection criteria to breed salt-tolerant cultivars in some plants (Chen *et al.*, 2017). The lower level of Na amount in the leaf and root of the Esfahan cultivar than the Behbahan cultivar was because of the higher ability of this cultivar to inhibit the absorption and transfer of Na ions, which improved salt tolerance of the Esfahan cultivar compared with the Behbahan susceptible cultivar (Hanin *et al.*, 2016).

Ca content in the root and leaf of both cultivars significantly decreased with increasing salinity. Also, the Esfahan cultivar, which is more tolerant to salinity, had higher Ca content than the Behbahan susceptible cultivar. Previous research has shown that increasing the amount of Na in the root reduces the Ca content in onions (Arvin and Kazemipour, 2001; Khodadadi, 2002), which is consistent with the findings of this study. Significantly negative correlation was found between Na and Ca contents in the leaf and root of onions in this study. According to the findings of some researchers, the major role of Ca in increasing salinity resistance in the plants relates to the effect of its control Na loading in the vascular (Melgar *et al.*, 2006; Husain *et al.*, 2004). Ca is an essential element in plant nutrition that plays an important role in metabolic activities such as membrane stability, transmission of the message as secondary messengers, and controlling the activity of some enzymes. Ca helps reduce the negative effects of salinity by maintaining membrane stability, regulating ion transport, and maintaining selectivity for K/Na and Ca/Na (Nedjimi and Daoud, 2009). Many studies have reported that in the plants under salt stress, Ca contents in the root and shoot decrease; but, this reduction is less in salt-tolerant genotypes than salt-sensitive genotypes (Hadi and Karimi, 2012). Regarding the role of Ca in controlling the effects of salinity stress, higher Ca content in the more tolerant cultivar (Esfahan) may play a key role in its salinity tolerance.

Results showed that at low levels of salinity, the P content decreased in the root and leaf of both cultivars; but, with increasing salinity levels, P content in the leaf and root increased, so that at high levels of salinity in the roots and leaves, P content was higher than the control sample. According to Martinez *et al.* (1996), salinity could increase P uptake through the low-affinity system in the plants under high external P concentrations. However, the results of some other studies have indicated that salinity decreases P uptake because of the possible competition between P and Cl absorption (Martinez and Läuchli, 1994). Some researchers have suggested that there are distinctions between the varieties of a species in terms of P uptake and salinity induction (Grattan and Maas, 1984). Previous studies have reported P content increment in susceptible cultivars and its decrease in salinity-resistant cultivars in the olive (Loupassaki *et al.*, 2002) and rapeseed (Cerdeira *et al.*, 1977). In this study, no differences were found in P of the root and leaf between the two cultivars.

The results showed that increasing salinity decreased Mg in the leaf of both cultivars and there was no difference between the cultivars in this respect. The effects of salt stress caused changes in the uptake, transport, and distribution of the nutrients in different parts of the plant, which affected the nutritional balance in the plant (Karimi *et al.*, 2012). Mg which can be found in chlorophyll molecules plays a key role in photosynthetic reactions. Moreover, it helps in P metabolism, plant respiration, protein synthesis, and activation of several enzymes (Marschner, 1995). In many plants, NaCl salinity decreases the Mg uptake (Sairam and Srivastava, 2002; Francisco *et al.*, 2002; Yildirim *et al.*, 2008).

Comparison of the effect of salinity on Cl content in two onion cultivars of Esfahan and Behbahan showed that, in both cultivars, Cl content in the leaf and root increased significantly with increasing salinity levels, which is consistent with results by Khodadadi and Omid Beigi (2003) and Hanci *et al.* (2016). Results of the correlation between Cl data in the leaf and root and the salinity tolerance and yield showed the very negative effect of Cl on salinity tolerance and yield. Some researchers suggested that salt tolerance in some species is directly related to Cl ion transport (Munns and Tester, 2008). As observed, the Esfahan salt-tolerant cultivar had a lower amount of Cl in the leaves and roots. Keeping concentrations of Cl at the low level in vital organs may be a good indicator of tolerance to salinity stress.

Results showed that with increasing salinity, K/Na and Ca/Na ratios decreased in both cultivars; but, the reduction in the tolerant cultivar was significantly less than that of the susceptible cultivar. Also, YI showed significantly negative correlation with K/Na and Ca/Na ratios. The results were consistent with the results by Ashraf and McNeill (2004) and Aktas *et al.* (2006), showing that salt-tolerant cultivars have higher K/Na and Ca/Na ratios than their susceptible cultivars in their aerial part. Increasing Na concentration and decreasing K/Na ratio in response to salinity stress have been reported in several studies. However, in the tolerant cultivars, less Na enters the plant tissues and, hence, the K/Na ratio in the plant is used as an indicator for salinity tolerance (Ashraf and McNeill, 2004; Colmer *et al.*, 2006). In large quantities of Na, the absorption of nutrients such as K and Ca in plant tissues is prevented, which results in a reduction in K/Na and Ca/Na ratios (Akram and Ashraf, 2009). Salinity-tolerant cultivars have higher K/Na and Ca/Na ratios than their susceptible cultivars (Ashraf and McNeill, 2004; Aktas *et al.*, 2006) in their aerial organs. K/Na ratio has been proved as a reliable parameter for determining salinity tolerance in some plants such as tomatoes (Dasgan *et al.*, 2002), cotton (Ashraf, 2002), rice (Aslam *et al.*, 2003), and wheat (Poustini and Siosemardeh, 2004; Colmer *et al.*, 2006).

## Conclusion

One of the problems for the plants that is made by salinity stress is the disturbance caused by high amounts of ions such as Na and Cl on the physiological and biochemical processes. The results showed that the Esfahan tolerant cultivar was able to decrease the amount of Na and Cl uptake and increase K and Ca uptake in the roots and leaves. However, in the susceptible cultivar, higher levels of Na and Cl and lower levels of Ca and K increased the loss yield level. Therefore, measuring the concentration of K, Na, and Ca ions and K/Na and Ca/Na ratios in the leaves and roots can be used as an indicator for screening salt-tolerant genotypes in the onion.

## References

- Akram MS, Ashraf M. 2009. Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of potassium nitrate. *Journal of Applied Botany and Food Quality*, 83; 19- 27.
- Aktas H, Abak K, Cakmak I. 2006. Genotypic variation in the response of pepper to salinity. *Scientia Horticulturae*, 110; 260-266. doi.org/10.1016/j.scienta.2006.07.017
- Al-Ashkar I, Alderfasi A, El-Hendawy S, Al-Suhaibani N, El-Kafafi S, Seleiman MF. 2019. Detecting salt tolerance in doubled haploid wheat lines. *Agronomy*, 9(4); 211- 237. doi.org/10.3390/agronomy9040211
- Arvin MJ, Kazemipour N. 2001. Effects of salinity and drought stress on growth and chemical and biochemical composition of four onion cultivars. *Journal of Agricultural Science and Technology*, 5 (4); 51-41.
- Ashraf M. 2002. Salt tolerance of cotton: Some new advances. *Critical Reviews in Plant Sciences*, 21; 1-30. doi.org/10.1080/0735-260291044160

- Ashraf M, Harris P 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166(1); 3- 16. doi.org/10.1016/j.plantsci.2003.10.024
- Ashraf M, McNeilly T. 2004. Salinity tolerance in brassica oilseeds. *Critical Reviews in Plant Sciences*, 23; 157-174. doi.org/10.1080/07352680490433286
- Aslam M, Muhammad N, Qureshi RH, Ahmad Z, Nawaz S, Akhtar J. 2003. Calcium and salt-tolerance of rice. *Communications in Soil Science and Plant Analysis*, 34; 3013-3031. doi.org/10.1081/CSS-120025222
- Badran AE. 2015. Comparative analysis of some garlic varieties under drought stress conditions. *Journal of Agricultural Science*, 7(10); 271- 280. doi:10.5539/jas.v7n10p271
- Bartlett MS. 1937. Properties of sufficiency and statistical tests. *Proc. R. Soc. Lond.* 1670; 268–282.
- Cerda A, Bingham FT, Hofman B. 1977. Interactive effects of salinity and phosphorus on sesame. *Soil Science Society of America Journal*, 41; 915- 918. doi.org/10.1098/rspa.1937.0109
- Chapman HD, Pratt PF. 1961. *Methods of analysis for soils, plants and waters*. 1th ed., University of California, Division of Agricultural Sciences. California. doi.org/10.2136/sssaj1963.03615995002700010004x
- Chen CL, Schoot H, Dehghan S, Alvim Kamei CL, Schwarz KU, Meyer H, Visser R, Linden CG. 2017. Genetic diversity of salt tolerance in *Miscanthus*. *Frontiers in Plant Science*, 8; 187. doi.org/10.3389/fpls.2017.00187
- Colmer TD, Flowers TJ, Munns R. 2006. Use of wild relatives to improve salt tolerance in wheat. *Journal of Experimental Botany*, 57; 1059- 1078. doi: 10.1093/jxb/erj124. Epub 2006 Mar 2.
- Dasgan HY, Aktas H, Abak K, Cakmak I. 2002. Determination of screening techniques to salinity tolerance in tomatoes and investigation of variety responses. *Plant Science*, 163; 695- 703. doi:10.1016/S0168-9452(02)00091-2
- De-Pascale SD, Ruggiero C, Barbieri G, Maggio A. 2003. Physiological responses of pepper to salinity and drought. *Journal of the American Society for Horticultural Science*, 128; 48- 54. doi:10.21273/JASHS.128.1.0048
- Epstein E, Bloom A. 2004 *Mineral nutrition of plants: principle and perspectives*. 2th ed., Oxford university press. London.
- Essa TA. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. *Journal of Agronomy and Crop Science*, 188; 86- 93. doi.org/10.1046/j.1439-037X.2002.00537.x
- FAO. 2017. *Agricultural Statistical Database*. Available from <http://faostat.org>.
- Flowers TJ, Munns R, Colmer TD. 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Annual Botany*, 115; 419. doi: 10.1093/aob/mcu217
- Fooland MR. 1996. Genetic analysis of salt tolerance during vegetative growth in tomato, *Lycopersicon esculentum* Mill. *Plant Breeding*, 115; 245- 250. doi.org/10.1111/j.1439-0523.1996.tb00911.x
- Francisco G, Jhon L, Jifon S, Micacle C, Tames PS. 2002. Gas exchange chlorophyll and nutrient content in relation to Na<sup>+</sup> and Cl<sup>-</sup> accumulation in "sunburst" mandarin grafted on different root stocks. *Plant Science*, 35; 314-320. doi.org/10.1016/S0168-9452(02)00010-9
- Grattan SR, Maas SV. 1984. Interactive effects of saline and substrate phosphate on soybean. *Agronomy Journal*. 76: 668- 676. doi.org/10.2134/agronj1984.00021962007600040038x
- Hadi MR, Karimi N. 2012. The Role of Calcium in Plants Salt Tolerance. *Journal of Plant Nutrition*, 35(13); 2037-2054. doi.org/10.1080/01904167.2012.717158
- Hamdani F, Derridj A, Rogers HJ. 2017. Multiple mechanisms mediate growth and survival in young seedlings of two populations of the halophyte *Atriplex halimus* (L.) subjected to long single-step salinity treatments. *Functional Plant Biology*, 44; 761–773. doi: 10.1071/FP17026.
- Hanci F, Cebeci E, Uysal E, Dasgan HY. 2016. Effects of salt stress on some physiological parameters and mineral element contents of onion (*Allium cepa* L.) plants. *Acta Horticulturae*, 1143; 179- 185. doi:10.17660/ActaHortic.2016.1143.26
- Hanin M, Ebel C, Ngom M, Laplaze L, Masmoudi K. 2016. New insights on plant salt tolerance mechanisms and their potential use for breeding. *Frontiers in Plant Science*, 7; 1787. doi: 10.3389/fpls.2016.01787
- Hosseini Z. 2019. *Evaluation of Salt Tolerance in some Cultivars of Onion (Allium cepa L.)*. M.Sc. Thesis, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

- Husain S, Caemmerer S, Munns R. 2004. Control of salt transport from roots to shoots of wheat in saline soil. *Functional Plant Biology*, 31; 1115-1126. doi: 10.1071/FP04078.
- Jouyban Z. 2012. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences*, 2; 7- 10. doi.org/10.1590/1678-4324-2020200072
- Karimi E, Abdolzadeh A, Sadeghipour HR. 2012. Increasing salt tolerance in olive, *Olea europaea* L. plants by supplemental potassium nutrition involves changes in ion accumulation and anatomical attributes. *International Journal of Plant Production*, 3 (4); 49- 60.
- Karimi HR, Sadeghi-Seresht E. 2018: Effects of salinity stress on growth indices, physiological parameters and element concentration in Banebaghi (*Pistacia* sp.) as rootstock for pistachio. *Journal of Plant Nutrition*, 41(9); 1094-1103. doi.org/10.1080/01904167.2017.1392572.
- Khodadadi M. 2002. Effect of salinity stress and seed preparation on morphological and physiological characteristics of Iranian onion cultivars. M.Sc. thesis, Tarbiat Modares University, Tehran, Iran.
- Khodadadi M, Omid-Beigi R. 2004. Effect of sodium chloride salinity on some physiological characteristics of two onion cultivars. *Journal of Agricultural Science*, 14(3); 25-11. doi.org/10.17660/ActaHortic.2016.1143.26
- Liu C, Zhao X, Yan J, Yuan Z, Gu M. 2019. Effects of Salt Stress on Growth, Photosynthesis, and Mineral Nutrients of 18 Pomegranate (*Punica granatum*) Cultivars. *Agronomy*, 10(1); 27. doi.org/10.3390/agronomy10010027.
- Loupassaki MH, Chartzoulakis KS, Digalaki NB, Androulakis II. 2002. Effects of salt stress on concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. *Journal of Plant Nutrition*, 25 (11); 2457- 2482. doi.org/10.1081/PLN-120014707
- Malik YS, Singh K, Pandita ML. 1982. Effect of salinity on quality of bulb and chemical composition of leaves in onion (*Allium cepa* L.) varieties. *Haryana Journal of Horticultural Sciences*, 226-230
- Marschner, H. 1995. Saline soil in: mineral nutrition of higher plants, Academic Press, New York, 657-680 pp.
- Martinez CA, Maestri M, Lani EG. 1996. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. *Plant Science*, 116; 177–184. doi.org/10.1016/0168-9452(96)04374-9
- Martinez V, Läuchli A. 1994. Salt-induced inhibition of phosphate uptake in plants of cotton (*Gossypium hirsutum* L.). *New Phytologist*, 97; 118–122. doi.org/10.1111/j.1469-8137.1994.tb02955.x
- Melgar JC, Benlloch M, Fernández-Escobar R. 2006. Calcium increases sodium exclusion in olive plants. *Scientia Horticulturae*, 109(3); 303-305. doi.org/10.1016/j.scienta.2006.04.013
- Munns R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. *Plant, Cell & Environment*, 16: 15-24. doi.org/10.1111/j.1365-3040.1993.tb00840.x
- Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59; 651- 681. doi.org/10.1146/annurev.arplant.59.032607.092911
- Munns R, James RA, Lauchli A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57; 1025-1043. doi: 10.1093/jxb/erj100
- Nedjimi B, Daoud Y. 2009. Ameliorative effect of CaCl<sub>2</sub> on growth, membrane permeability and nutrient uptake in *Atriplex halimus* subsp. *schweinfurthii* grown at high (NaCl) salinity. *Desalination*, 249; 163- 166. doi.org/10.1016/j.desal.2009.01.019
- Parida AK, Das AB. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety*, 60; 324- 349. doi.org/10.1016/j.ecoenv.2004.06.010
- Poustini K, Siosemardeh A. 2004. Ion distribution in wheat cultivars in response to salinity stress. *Field Crops Research*, 85; 125- 133. doi.org/10.1016/S0378-4290(03)00157-6
- Sairam RK, Srivastava GC. 2002. Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes to long term salt stress. *Plant Science*, 162; 897-804. doi.org/10.1016/S0168-9452(02)00037-7
- Shapiro SS, Wilks MB. 1965. An analysis of variance test for normality. *Biometrika*, 52; 591–611. doi.org/10.2307/2333709
- Sivritepe N, Sivritepe HO, Eris A. 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Scientia Horticulturae*, 97; 229- 237. doi.org/10.1016/S0304-4238(02)00198-X

- Turan MA, Katkat V, Taban S. 2007. Variations in proline, chlorophyll and mineral elements contents of wheat plants grown under salinity stress. *Journal of Agronomy*, 6; 137-141. doi: 10.3923/ja.2007.137.141
- Wahing I, Van W, Houba VJG, Lee VD. 1989. Soil and plant analysis, a series of syllabi. Part 7, plant analysis procedure. Wageningen Agriculture University. 179 p.
- Yildirim E, Turan M, Guvenc I. 2008. Effect of foliar salicylic acid applications on growth, chlorophyll and mineral content of cucumber (*Cucumis sativus* L.) grown under salt stress. *Journal of Plant Nutrition*, 31; 593-612. doi.org/10.1080/01904160801895118
- Zhu JK. 2003. Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology*, 6; 441– 445. doi: 10.1016/s1369-5266(03)00085-2.