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Salt Tolerance in Rice Cultivars and Changes in Sodium and Potassium Ions

Masomeh Farasat*

Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.

RESEARCH ARTICLE

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ABSTRACT

Salinity is an environmental stress that limits growth and development in plants. Due to high salinity in Khuzestan soils it is necessary to identify cultivars with appropriate yield that are compatible with Khuzestan conditions. This experiment was done to evaluate three rice cultivars for salinity tolerance at seedling stage by measuring the absorption of sodium and potassium ions and the ratio of Na^+/K^+ in the shoots and roots of the rice crop. Experiments were carried out under controlled conditions and in hydroponics culture media. Treatments included three rice cultivars (Amol3, Anbouri and Shahpasand) and five levels of salinity (control: without salt addition, salinity levels of 25, 50, 75 and 100 mM NaCl) according a factorial experiment based on completely randomized design with three replications. Acid digestion method was used to measure sodium and potassium ions in plant tissues. The amount of sodium in the root and also shoot of three cultivars showed a significant difference ($p\leq 0.05$, $p\leq 0.01$). Shahpasand showed the lowest amount of sodium in its shoots compare to the other two varieties. The results showed that Na^+/K^+ ratio was significantly different in three cultivars under salinity conditions. Shahpasand showed the lowest and Anbouri showed the highest ratios. Shahpasand can be considered as a salt tolerant cultivar and also a salt tolerant parent in hybridization and breeding programs for producing new salt tolerant cultivars.

Keywords*:* *Na^+/K^+ ratio, Macro Nutrient, Stress, Varieties.*

***Corresponding Author:** Masomeh Farasat ✉ mehfarasat@gmail.com

INTRODUCTION

Rice is major grain crop and carbohydrate source, supplying the necessary daily calories for more than half the world's population. It has been predicted that the demand for rice in the world will increase from 560 million tons to 780 million tons by the year 2020 (Khush, 2005; Far Ebrahimi *et al*., 2012**)**. Rice has generally been introduced as moderately sensitive to saline conditions (Sobahan *et al*., 2012) and is known as the most sensitive plant to salinity among the agronomical important cereals (Hoang *et al*., 2015). Salt-affected soil is one of the serious abiotic stresses that cause reduced plant growth, development and productivity worldwide (Siringam *et al*., 2011). Salinity is one of the important abiotic stresses limiting rice productivity. The capacity to tolerate salinity is a key factor in plant productivity (Momayezi *et al*., 2009). Among abiotic stresses, salinity has been increasing over the time for many reasons like using chemical fertilizers, global warming and rising sea levels. Under salinity stress, the loss of water availability, toxicity of Na^+ and ion imbalance directly reduces carbon fixation and biomass production in plants (Fakhrfeshani *et al*., 2015). In Iran, salinity has already become a major deterrent to crop production, including rice. Addition of salts to water lowers its osmotic potential, resulting in decreased availability of water to root cells. Salt stress thus exposes the plant to secondary osmotic stress, which implies that all the physiological responses, which are invoked by drought stress, can also be observed in salt stress (Sairam *et al*., 2002). The physiological responses of a plant to salinity are often complex. Different species and varieties respond differently when exposed to salinity. The extent of sensitivity varies depending upon the plant growth stage (Zafar *et al*., 2015). salinity reduced tillering, spikelet filling, florets per panicle, 1000 grain weight, grain yield, harvest index, shoot and root dry matter and K^+ uptake and increased leaf and root Na^+ in rice plants (Kranto *et al*., 2016). Adaptation of plants to high levels of sodium salts can be achieved in two very different ways. Plants can either exclude salts from the interior of their cells, or include salts within the leaf cells but at the same time sequester them in the vacuoles. The result of this adaptation process is maintenance of cytoplasmic salt concentration at a relatively low level (Oleary, 2002). Physiological, biochemical and genetically studies have shown that salinity stress in plants is multifactorial, including osmotic stress (Roy *et al*., 2011) and cellular ion toxicity, which inhibits vital enzymes and metabolic processes (Horie *et al*., 2001). Salt tolerance is the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt (Parida and Das, 2005). All plants are sensitive to salts at some concentration. The limiting concentrations change with plant species, variety and stage of development and duration of the salt stress (Eynard *et al*., 2005)._Ion selectivity enables the plant to control uptake of toxic ions like Na^+ and Cl^- and their accumulation in the cytoplasm (Shannon and Grieve, 1999). Plants do this either through strict ion regulation to keep the Na^+ and Cl^- out of the transpiration stream and subsequently the cytoplasm of the aerial parts of the plants (Harvey, 1985), or through ion discrimination, enabling the plant to discriminate between chemically similar ions such as Na^+ and K^+ (Gorham *et al*., 1997). Sodium chloride salts are quickly dissolved in the water and play as ionic effects in higher plant including rice crop (Nishimura *et al*., 2011). Excess Na^+ in plant cells directly damages membrane systems and organelles, resulting in plant growth reduction and abnormal development prior to plant death (Siringam *et al*., 2011). Several factors contribute to the salinity (NaCl) resistance of rice plant, in which limitation of Na^+ uptake at the root is an important factor in determining the resistance to salinity (Yeo and Flowers, 1983). In salt stress, sodium ions cause ionic stress when enter the plant cell. Plants have mechanisms to deal with environmental stress due to their lack of ability to move from their place to another one. Under saline conditions when there is a higher level of Na^+ around the root zone, plant alters its ion uptake and resistant genotypes maintain a lower ratio of Na^+ to Ca^+, Mg^+ and high level of K^+ while sensitive genotypes could not maintain it (Zafar *et al*., 2015). Na^+ and K^+ balance plays a key role in the growth and development of higher plants under saline conditions. Several physiological processes, including the maintenance of membrane potential and turgor, stomatal movement, regulation of osmotic pressure, and tropisms are dependent on the presence of potassium (Rahneshan *et al*., 2018). Under saline conditions, the mineral nutrition of most plants can be expected to be detrimentally affected. The interactions between K and Na may be emphasized under such conditions and ultimately decrease plant growth (Noaman, 2004). Soil salinity decreases crop yield through increasing osmotic stress on the plant. Under saline conditions, nutrient imbalance, reduced nutrient uptake including K^+, and ion toxicity are resulted because of high Na^+ and Cl^- concentrations (Miransari and Smith, 2007). The adverse effect of salinity on crop growth results from disturbed metabolic processes, which are most commonly manifested in stunted plant growth, poor productivity (Jinwoong and Choongsoo, 1998) and distinctly changed concentrations of key biomolecules. Plants grown under saline conditions are stressed and are characterized by increased levels of free proline and carbohydrate content in different tissues as a response to osmotic adjustment (Heuer and Nadler, 1998). High ionic concentration competes with the uptake of other nutrients, especially K^+, leading to K^+ deficiency. Increased treatment of NaCl increases Na^+ and Cl^- and decrease in Ca^{2+}, Mg^{2+} and K^+ levels in number of plant (Khan *et al*., 2003). There is a negative relationship between Na^+ and K^+ concentration in roots and leaves. The selective uptake of K^+ as opposed to Na^+ is considered to be one of the important physiological mechanisms contributing to salt tolerance in many plant species (Ashraf and Khanum, 1997). Thus, under saline and sodic conditions, K fertilization management may need to be modified because of K^+ competition with other cations and especially Na^+ in the plant, and to the effects of salinity on K^+ reactions in soils. Potassium play vital role and stimulates biological process in the plant cell as enzymes activity, respiration, photosynthesis, chlorophyll, creation, carbohydrate formation, water amounts balance in leaves and regulate stomata opining as well as direct effect on the disease resistance (El-Defan *et al*., 1999). Improving plant toward salinity tolerance is one of the most reliable methods that has been studying from many aspects. To improve the ability of crops to grow on saline soil, understanding traits and mechanisms that contribute to salt tolerance in wild and tolerant cultivars are necessary (Kao, 2011). However, mechanisms of plant adaptation to abiotic stresses, particularly for drought (Roy *et al*., 2011) and salinity are complex but understanding the mechanisms of salt tolerance by studying traits that contribute to tolerance separately and identifying natural occurring variation in varieties or wild relatives are scientifically applied methods to confront the complexity and to improve further salt tolerance in crops (Huang *et al.*, 2008). Under salinity stress, the loss of water availability, toxicity of Na^+ and ion imbalance cause growth limitation so plants adopt divert mechanisms to tolerate salinity. It is repeatedly reported that K^+ deficiency and Na^+ toxicity are major restrictions of crop production worldwide (Nieves Cordones *et al.*, 2014; Very *et al.*, 2014). K^+ can counteract Na^+ stresses thus the potential of plants to tolerate salinity is strongly dependent on their potassium nutrition. K^+ composes about 10% of the plants dry weight and is the most abundant mineral cation in the plants. It is involved in many of functions related to enzyme activation, respiration and starch and protein synthesis (Nieves-Cordones *et al.*, 2014; Benito *et al.*, 2014). For instance, about 50mM of K^+ is required for normal starch synthesis and 10-50mM is needed for activation of K^+ dependent enzymes. The optimum concentration of K^+ narrows by increasing the amount of Na^+ for many reasons such as similarity of Na^+ and K^+ in their physicochemical structure. This similarity leads to competition of Na^+ and K^+ at transporters or enzymes binding sites that can result in K^+ deficiency and inhibition of biochemical processes that are dependent on K^+. So the capacity of a cell to maintain a high K^+/Na^+ ratio is assumed to be a critical strategy in salt tolerance. For instance, it is reported that animal cells maintain the K^+/Na^+ ratio around 20 by regulating the K^+ and Na^+ concentration around 100mM and 5Mm respectively. In plants, the optimum concentration of K^+ is reported to be about 100-150mM and the minimum value of K^+/Na^+ is about one. In contrast, the soil K^+ concentration ranges from 1 to 0.1mM and in some cases, the K^+/Na^+ ratio is less than 0.02. So an efficient and controllable potassium supply system should be available for plants (Nieves Cordones *et al.*, 2014; Su *et al.*, 2007; Maathuis and Amtmann, 1999). Khuzestan province has the second place in rice cultivation after Gilan and Mazandaran province. In some parts of the province there are some areas that are affected by the problem of alkalinity, salinity and the lack of suitable drainage, which restricts rice cultivation. So, it is necessary to identify cultivars with appropriate yield that are compatible with Khuzestan conditions. This study was conducted to evaluate three rice cultivars for salinity tolerance at seedling stage by measuring absorption of sodium, potassium ions and ratio of Na^+/K^+ in shoots and roots of plants.

MATERIALS AND METHODS

Location and Experiment Information

All experiments were carried out at the Ramin University of Agriculture and Natural Resources (at southwest of Iran, Khuzestan province) in an area of 48 square meters. The temperature of the place was maintained using a cooler at a temperature of 30 ±2 ^{0}C a day and 22±2 ^{0}C at night. In addition to the normal daylight, fluorescent lamps were used and the intensity of light was measured 350 $mMol.m^{-1}.s^{-1}$. The duration of the light was 12 hours a day throughout the experiment. This research was conducted under controlled conditions and in hydroponics culture media via factorial experiment based on completely randomized design with three replications. The treatments included three rice cultivars (Amol 3, Anbouri and Shahpasand) and five levels of salinity (Control: without salt addition, salinity levels of 25, 50, 75 and 100 mM NaCl). Table 1 shows the characteristics of rice cultivars tested. 11-day-old seedlings grown under laboratory conditions were transferred to liquid culture containers. To prevent the growth of algae in the culture medium, the containers were covered with aluminum paper. The composition of the culture medium (Yoshida and Coronel, 1976) is shown in Table 2. Sodium chloride salt (NaCl) was added to culture media according to the treatments of experiment. Adjustment of pH was done on a daily basis with the aid of NaOH 1N, and HCl 1N and was adjusted to 5. Replacing of solutions was done every two days with new ones. Fig. 1 shows plants in culture media.

Ion Measurement

Acid digestion method was used to measure Na and potassium ions in tissue (Yoshida *et al*., 1976). In test tube, 10 mg of dry matter from each organ was added to 5 ml HCl 1N solution.

Fig. 1. Rice plants in culture media

Table 1. Characteristics of rice cultivars

Characteristics of rice cultivars	Amol 3	Anbouri	Shahpasand
Original name	Sona	Anbouri	Shahpasand
Original location	India-IRRI	Iraq	Iran-Gilan
Plant height(cm)	84	135	130
Duration of growth(day)	127	129	120
Seed grade	long grain	medium grain	very long grain
Rice class	Sadri	Champa	Sadri
Amylose percentage	24%	21%	20%
Fragrance	none	fragrant	none
Marketability	undesirable	desirable	very desirable
White rice length(mm)	7-7.5	5.5-6	8-9

Table 2. Combination of culture medium and final concentration of elements in culture media

Element	Compound	The amount of compound (g) in 10 L distilled water	Final concentration of the element in the solution (ppm)
N	NH_4NO_3	9.4	40
P	NaH_2PO_4, $2H_2O$	403	10
K	K_2SO_4	714	40
Ca	$CaCl_2$	886	40
Mg	$MgSO_4,7H_2O$	3240	40
Mn	$MnCl_2$, $7H_2O$	15*	0.5
Mo	$(NH_4)6\ Mo_7O_{24},4H_2O$	0.74*	0.05
B	H_3BO_3	9.34*	0.2
Zn	$ZnSO_4,5H_2O$	0.35*	0.01
Cu	$CuSO_4,5H_2O$	0.31*	0.01
Fe	Fe EDTA	**	2.5
Si(SiO_2)	$Na_2SiO_3,5H_2O$	***	5

*First dissolved separately, then combined with 500 ml concentrated sulfuric acid and distilled water added into a volume of 1 liter.

**Iron chelate was made separately and then added to the culture medium.

***The solution was made at a concentration of 50ppm SiO_2 and added to the culture medium.

After 24 hours the solution was centrifuged and 2 ml of supernatant added to 2 ml HCl 1N solution. Standard solutions of sodium and potassium were made at concentrations of 0.20, 40.60, 80 and 100. By using a Flame photometer and plotting the standard curve, sodium and potassium ions were calculated and reported based on the percentage of ions in the dry weight of the plant. Measurements were taken on days 5 and 10 after salt treatment.

Statistical Analysis

Data were analyzed with using one-way ANOVA via SPSS 16.0 software. The differences between treatment means were compared by least significant difference (LSD) test at 1% probability level.

RESULTS AND DISCUSSION

Sodium content in roots and shoots

The cultivars showed significant differences in root sodium content in terms of sodium percentage at 5 percent probability level (Table 3). Shahsavand and Anbouri cultivars had a minimum amount of sodium in their roots 5 and 10 days after the salinity treatment respectively. The effect of salt treatment has also been significant. So that, with increasing salt concentration, the amount of sodium in the root increased (Fig. 2). The effect of the day was significant at 1 percent probability level, meaning that the amount of sodium in the root varied in different days. The interaction effect between cultivars and salinity factors was statistically significant ($p \leq 0.01$) (Table 3). For example, the control plants in the two varieties of Shahpasand and Anbouri have the same sodium values, but with increasing salinity levels, they showed different amounts of sodium at their roots. The amount of sodium in the shoot of three cultivars also showed a significant difference ($p \leq 0.01$) (Table 3). Shahpasand showed the lowest amount of sodium in its shoots compare to the other two cultivars. As the salt increased in the culture medium, the percentage of sodium in the shoot also increased significantly (Fig. 2). The interaction between the day and the cultivar was also significant ($p \leq 0.05$), so that the differences between the cultivars on the 10th day compared to the 5th day were higher in similar treatments (Table 3). Different amounts of sodium in the root can be attributed to a different root selection in three cultivars. Another possibility is that the active transfer of sodium from the base of the plant to the root has been different in three varieties. It has been shown that in rice plant after ion entry into the xylem and reaching the plant base, the bulk of the ion can be transferred back to the roots through the phloem tissue (Matsushita and Matoh, 1991).

Table 3. Analysis of variance of the effect of treatment on measured traits

Treatments	Na^+ (%) Root	Na^+ (%) Shoot	K^+ (%) Root	K^+ (%) Shoot	Na^+/K^+ ratio
Day (D)	**	**	**	ns	ns
Cultivar (C)	*	**	**	**	*
Salt (S)	**	**	**	**	*
C*D	ns	*	**	*	ns
S*D	**	**	ns	ns	ns
C*S	*	ns	ns	ns	**
C*S*D	ns	ns	ns	ns	ns
CV (%)	11.87	16.70	15.02	9.54	8.8

*: significant at 5%; **: significant at 1%; ^{ns}: non significant

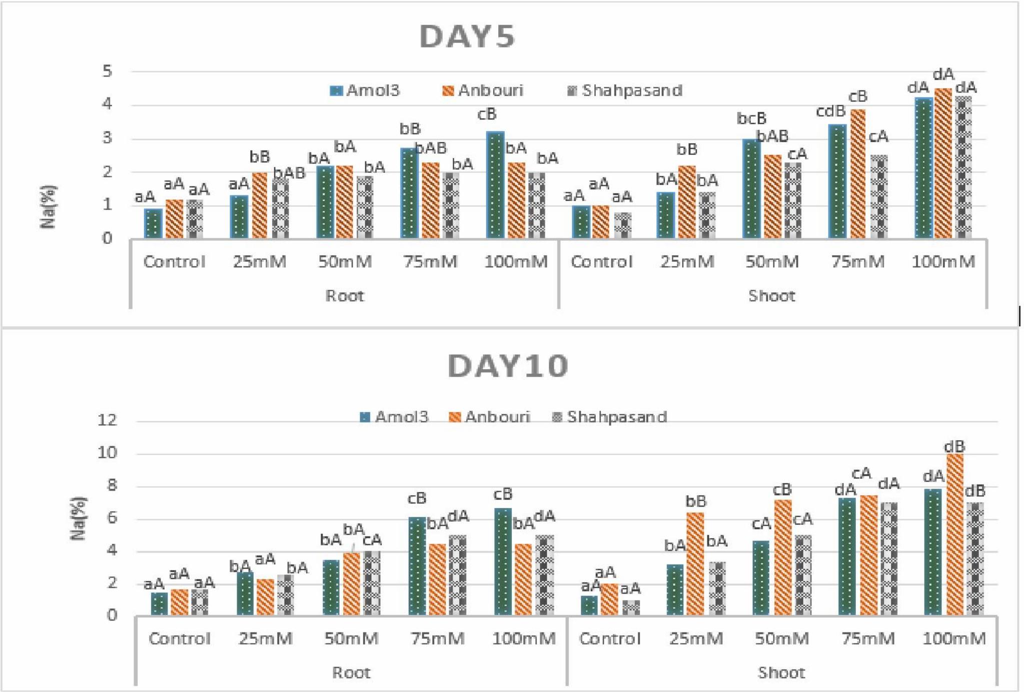

Fig. 2. Interaction effect of different levels of salinity and different cultivars on Na^+ uptake in shoots and roots, 5 and 10 days after treatments.

The lower case indicates a significant difference at 1% level between salinity treatments and capital letters indicating a significant difference at 1% level between cultivars.

There are several transporters involved in salt tolerance in rice. A magnesium transporter is reported to be necessary for salinity resistance which probably regulates the transport activity of a key transporter for the Na^+ removal from the xylem sap at the root (Chen *et al*., 2017). By increasing soil salt concentrations the ability of a plant to take up water decrease and Na^+ are taken up in large amounts by roots. Na^+ can negatively influence on growth of plant. (Deinlein *et al*., 2014). In *Arabidopsis thaliana* a Na^+ transporter has been identified which is involved in Na^+ transport from endodermal cells to stele (Hall *et al*., 2006). It has been suggested that a genotype that collects the lowest sodium in its body in early stages of growth will probably show higher seed yields by increasing salinity levels (Qadar, 1991).

Potassium content in roots and shoots

Potassium is a macronutrient for plants that is required for physiological processes such as the maintenance of membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stomata movement and tropisms (Golldack *et al*., 2003). Nelson (1978) believed that potassium has a positive role in plant growth under saline conditions, because this element plays an essential role in photosynthesis, osmo regulatory adaptations of plant to water stress. Adequate potassium supply is also desirable for the efficient use of Fe, while higher potassium application results to competition with Fe (Celik *et al*., 2010). The percentage of potassium in the roots were significant at 5 and 10 days after treatment.

The effect of cultivar on root potassium content was significant at 1 percent probability level (Table 3). The highest and lowest potassium content were observed in Shahpasand and Amol3 cultivars respectively. The effect of salt treatment on root potassium was significant ($p \leq 0.01$) (Table 3), so that the concentration of potassium in the root decreased with increasing salt concentration (Fig. 3). The effect of day on potassium content in shoot was not significant and the percentage of potassium in the shoots did not change significantly over time. In contrast, there was a significant difference of varietal effects as well as salinity ($p \leq 0.01$). Shahpasand had the highest percentage of potassium in its shoot. Also, with increasing salt concentration, the percentage of potassium in the shoots of three cultivars decreased. The interaction between the day and salinity was not significant. With increasing salt concentration in two sampling time (5 and 10 days after treatments), the trend of reduction in potassium was relatively similar (Fig. 3). In a study on sensitive and tolerant rice cultivars, sensitive cultivars have accumulated more sodium and less potassium in their organs (Sharma, 1986; Zafar *et al.*, 2015).

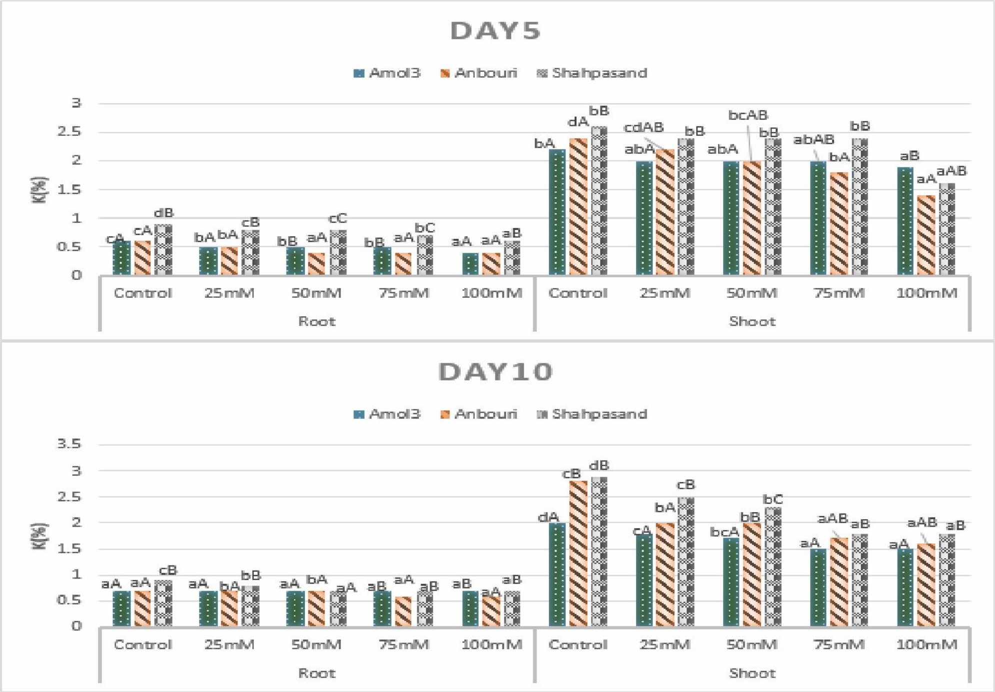

Fig. 3. Interaction effect of different levels of salinity and different cultivars on K^+ uptake in shoots and roots, 5 and 10 days after treatments.

The lower case indicates a significant difference at 1% level between salinity treatments and capital letters indicating a significant difference at 1% level between cultivars.

Ratio of Na^+/K^+ in shoots and roots

The sodium ion (Na^+) is harmful, whereas the potassium ion (K^+) is an essential ion. The cytosol of plant cells normally contains 100-200 mM of K^+ and 1−10 mM of Na^+ (Taiz and Zeiger 2002); this Na^+/K^+ ratio is optimal for many metabolic functions in cells. Physico-chemically, Na^+ and K^+ are similar cations. Therefore, under the typical NaCl-dominated salt environment in nature, accumulation of high Na^+ in the cytosol, and thus high Na^+/K^+ ratios, disrupts enzymatic functions that are normally activated by K_+ in cells (Tester and Davenport 2003, Munns *et al.* 2006). Therefore, it is very important for cells to maintain a low concentration of cytosolic Na^+ or to maintain a low Na^+/K^+ ratio in the cytosol under NaCl stress (Maathuis and Amtmann 1999). Sodium to potassium ratios in rice cultivars was significantly different at the 5th and 10th days after treatment. Shahpasand cultivar showed the lowest ratio in both days of sampling in root and shoot compared to the other two cultivars (Table 4). In general, sodium ion changes were much more dramatic than changes in potassium ion. It has been shown that salinity can cause a significant reduction in K^+/Na^+ ratio in both susceptible and tolerant rice cultivars. In low salinity conditions (25 mM NaCl stress) the addition of exogenous proline can increase the K^+/Na^+ ratio in rice plants (Bhusan *et al.*, 2016). Resistance to salinity is the result of the presence of genes that limit the entry of salt from the soil to the plant's body and adjust the ionic and osmotic balance of cells in roots and shoots (Munns, 2005). In an experiment on transgenic rice plants, the transgenic plants expressing *AtBAG4, Hsp70* and *p35* genes maintained lower Na^+ levels and a lower Na^+/K^+ ratio following NaCl exposure at the seedling stage (Hoang *et al.*, 2015). It has been reported that Na/K ratio is the key factor for salinity tolerance (Puram *et al.*, 2017). In the present study, Shahpasand variety showed the lowest sodium percentage and the highest percentage of potassium with the lowest Na^+/K^+ ratio, indicating that this variety has more ability to tolerate salinity than the other two varieties and can be considered as a parent for producing and breeding new resistant rice cultivars. Saqib *et al.* (2000) reported a significant reduction in all growth parameters considered and an increased concentration of Na and Cl, decreased concentration K and decreased K^+: Na^+ ratio.

Table 4. Mean comparison interaction effect of cultivars and salinity along time on Na^+/K^+ ratio in the shoots and roots via LSD test at 1% probability level

Treatments		Day 5		Day10	
		Root	**Shoot**	**Root**	**Shoot**
Amol 3	**Control**	1.50*	0.45	2.14	0.66
	25mM Nacl	2.60	0.70	4.00	1.76
	50mM Nacl	4.40	1.50	5.00	2.83
	75mM Nacl	5.40	1.70	8.71	4.77
	100mM Nacl	8.00	2.21	9.57	5.17
Anbouri	**Control**	2.00	0.42	2.43	0.72
	25mM Nacl	4.00	1.00	3.28	3.17
	50mM Nacl	5.50	1.25	5.57	3.70
	75mM Nacl	5.75	2.17	7.50	4.41
	100mM Nacl	5.75	3.21	7.50	6.17
Shahpasand	**Control**	1.33	0.31	1.88	0.33
	25mM Nacl	2.25	0.58	3.25	1.37
	50mM Nacl	2.37	0.96	5.71	2.17
	75mM Nacl	2.86	1.04	7.14	3.89
	100mM Nacl	3.33	2.69	7.14	3.89

CONCLUSION

Response of 11 days old seedlings of three varieties of rice plants to five levels of salinity stress were investigated in this study. Salinity showed a significant effect on uptake of sodium and potassium ions in roots as well as shoots. Na^+/K^+ ratio was significantly different in the 3 varieties under salinity conditions. Shahpasand showed the lowest and Anbouri had the highest Na^+/K^+ ratio. The results showed that, root selectivity against Na^+ is the primary mechanism involved in resistance to the salt. Shahpasand variety showed the lowest sodium percentage and the highest percentage of potassium, indicating that this variety has more ability to tolerate salinity than the other two varieties and can be considered as a parent for producing and breeding new resistant rice cultivars.

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