

# The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings

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## ABSTRACT

In order to investigate the solutes accumulation associated with salt tolerance of rice (*Oryza sativa* L.), two rice genotypes including IR651 (salt-tolerant) and IR29 (salt-sensitive) were grown hydroponically in the Youshida nutrient solution. Salinity treatment was imposed 3 weeks after sowing using NaCl in two levels 0 and 100 mmol. Samples were separately collected from the youngest (sixth) leaves, leaf sheaths and roots at 72 and 240 h after salinization; then Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, P, Mn<sup>2+</sup>, Cl<sup>-</sup> and total soluble sugars concentration and Na<sup>+</sup>/K<sup>+</sup> ratio were determined. Total dry weight of both genotypes decreased with the application of NaCl. Salinity caused higher accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the sixth leaf and leaf sheath of IR29 than in IR651 while their concentration in root of IR651 was higher. K<sup>+</sup> concentration was decreased in the sixth leaf and leaf sheath of IR29 under NaCl stress. Reduction in Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations were observed in sixth leaves of both genotypes. P concentration was increased in leaf sheath and root of IR29 under saline conditions while it showed no changes in IR651. Our results indicated that the tolerant genotype had mechanisms to prevent high Na<sup>+</sup> and Cl<sup>-</sup> accumulation in the sixth leaf. High total soluble sugars concentration in shoot of IR651 is probably for adjusting osmotic potential and better water uptake under salinity. These mechanisms help plant to avoid tissue death and enable to continue its growth and development under saline conditions.

**Keywords:** ions; rice; salt stress; soluble sugars

Salinity is a common environmental stress factor seriously affecting crop production in different regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectares of land in the world are affected by both salinity and sodicity (Munns 2005). The response of rice to salinity varies with growth stage. In the most commonly cultivated rice cultivars, young seedlings were sensitive to salinity (Lutts et al. 1995). Salinity tolerance at the rice seedling and reproductive stages is only weakly associated; hence, pyramiding of contributing traits at both stages is needed for developing resilient salt-tolerant genotypes.

Salinity has several effects on plant growth via an osmotic effect on plant water uptake, and specific ion toxicities (Munns et al. 2006). Thus, physiological traits used for screening germplasm for salinity tolerance included Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> exclusion (Carden et al. 2003), and Na<sup>+</sup>/K<sup>+</sup> or Ca<sup>2+</sup>/Na<sup>+</sup> discrimination (Yamaguchi and Blumwald 2005). By decreasing the osmotic potential of the soil solution in saline conditions, plant access to water uptake will be reduced. As the soil dries, the concentration of salt in the soil solution will be increased. Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed

by reactive oxygen species (Azevedo-Neto et al. 2006). However, for many plants (such as graminaceous crops),  $\text{Na}^+$  is the primary cause of ion specific damage. Shannon et al. (1998) found no relation between ion concentration in shoot and salt tolerance in rice. Aslam et al. (2003) reported that salt stress in some cultivars of rice is related with  $\text{K}^+/\text{Na}^+$  ratio. Maintaining a better nutrition with  $\text{K}^+$  and  $\text{Ca}^{2+}$ , while limiting sodium uptake, is a highly important trait contributing to high salt stress tolerance in plants. In recent years increasing numbers of salt-tolerant transgenic plants were generated with overexpression of vacuolar  $\text{Na}^+/\text{H}^+$  antiporter proteins mediating lower concentrations of  $\text{Na}^+$  and higher ratios of  $\text{K}^+/\text{Na}^+$  (Yamaguchi and Blumwald 2005). Carden et al. (2003) reported that the cytosolic  $\text{Na}^+/\text{K}^+$  ratio rather than the absolute  $\text{Na}^+$  concentration, may be critical for NaCl tolerance. High levels of  $\text{Ca}^{2+}$  in rice root environment are essential for the maintenance of high root uptake and shoot accumulation of  $\text{Ca}^{2+}$  and  $\text{K}^+$  in saline soils and thus for avoiding salinity damage in plants as shown in rice plants (Song et al. 2006). Earlier studies report on carbohydrate accumulation during various abiotic stresses in plants where long term carbohydrate storage occurs during reproductive development (Colmer et al. 1995).

The objectives of this experiment were to compare responses of rice genotypes differing in tolerance to salt stress and to study the association of the physiological traits of rice seedlings such as ion contents and total soluble sugars in leaves, leaf sheaths and roots with the salt tolerance.

## MATERIALS AND METHODS

**Plant cultivation in greenhouse.** Experiments were conducted during 2006 in a greenhouse of Agricultural Biotechnology Research Institute of Iran (ABRII) with a solution culture system and young plants of two rice genotypes. The air temperature ranged 30–20°C (day-night) and 50–75% relative humidity. All the studies were carried out at the seedling stage of rice (*Oryza sativa* L.). Seeds of salt-tolerant (IR651) and sensitive (IR29) rice genotypes were germinated at 30°C for 72 h and pregerminated seeds were transferred to pots containing distilled deionized water for 3 days; then test pots were filled with nutrient solution containing 91.4 g  $\text{NH}_4\text{NO}_3$ , 35.6 g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 117.35 g  $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ , 71.4 g  $\text{K}_2\text{SO}_4$ , 324 g  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 1.5 g  $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ , 0.074 g  $(\text{NH}_4)_6 \text{Mo}_7$ .

$\text{O}_{24} \cdot 4 \text{H}_2\text{O}$ , 0.035 g  $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.934 g  $\text{H}_3\text{BO}_3$ , 0.031 g  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ , 7.7 g  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  and 11.9 g  $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$  per liter (Yushida et al. 1976). The pH was adjusted to 5.5 by 0.1M KOH and HCl. Salinity treatments were imposed 21 days after sowing, when seedlings had six complete leaves, using two salinity levels with 0 and 100 mmol NaCl.

**Plant materials analysis.** All sixth leaves, leaf sheaths and roots were collected for measurements 72 and 240 h after adding NaCl and dried at 70°C for 72 h; the total and each part dry weight were separately determined. Samples digestion was done for  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , P and  $\text{Mn}^{2+}$  determination via wet digestion using sulfuric acid (96%),  $\text{H}_2\text{O}_2$  (30%) and salicylic acid. The concentration of  $\text{Na}^+$  and  $\text{K}^+$  were determined after digestion with a Flame photometer (Corning-410),  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Mn}^{2+}$  were determined with an Atomic Absorption Spectrometer (Perkin Elmer, AAnalyst 300, California, USA). Chloride was measured with Ion-Analyzer using a chloride electrode (ISM146-Cl, Los Angeles, USA) after digestion with distilled deionized water. P concentration in digested samples was estimated after coloring with molybdate-vanadate using spectrophotometer (Varian, Carry 300, California, USA). Soluble carbohydrates were determined using zinc sulfate (5%), barium hydroxide (0.3 N) and phenol (5%) with spectrophotometer (Varian, Carry 300, California, USA). Data were analyzed in a factorial based on completely randomized design with four replications. Means were statistically compared among treatments by the Least Significant Difference (LSD) at the  $P \leq 0.01$  level using the SAS (Ver. 6.12) software.

## RESULTS AND DISCUSSION

**Dry weight.** There was no significant ( $P \leq 0.01$ ) difference between studied genotypes in total dry weight and dry weight of different parts under normal conditions. Both genotypes showed a dry matter reduction under saline condition but observed a decline in dry weight of IR29 higher than in IR651 (Table 1). Salinity caused a decrease of the sixth leaf dry weight of IR651 and IR29 by 12% and 40%, respectively. Analysis of variance showed that all of the treatments and their interactions had significant ( $P \leq 0.01$ ) effects on dry weight of genotypes (Tables 5 and 6).

Results showed that NaCl stress caused a significant ( $P \leq 0.01$ ) total dry weight reduction in both genotypes. Total dry matter reduction in IR29

Table 1. Total dry weight and dry weight of separated parts of plants at 240 h after salinization

	IR29		IR651	
	normal	stress	normal	stress
Total dry weight (g)	451 ± 12.9	243 ± 12.9	468 ± 13.8	385 ± 13.8
Leaf dry weight (g)	39 ± 0.8	24 ± 0.7	33 ± 0.9	29 ± 0.9
Leafsheath dry weight (g)	97 ± 1.9	107 ± 2.3	99 ± 2.1	77 ± 1.8
Root dry weight (g)	122 ± 2.4	89 ± 1.8	121 ± 2.3	101 ± 2.1

Means and standard errors of 4 replications are shown

seedlings was higher than IR651. Munns (2002) reported that biomass production in saline versus control conditions over a long period is related to salt tolerance. A significant reduction in total dry weight under salinity was due to Na<sup>+</sup> and Cl<sup>-</sup> accumulation and osmotic stress. NaCl stress led to a decline of osmotic potential in nutrient solution and plant water uptake and finally plant dry matter production was reduced.

**Solutes concentration.** Root Na<sup>+</sup> concentration was higher than in other parts in both genotypes (Tables 2–4). A significant ( $P \leq 0.01$ ) increase was observed in root Na<sup>+</sup> concentration in both genotypes under salinity (Table 4). Root Na<sup>+</sup> concentration in IR651 seedlings was higher than IR29 at the second harvest while no significant ( $P \leq 0.01$ ) difference was observed between genotypes until the first sampling. Table 2 shows a significant ( $P \leq 0.01$ ) effect of NaCl on Na<sup>+</sup> concentration in the sixth leaves of both genotypes

at the first harvest. More Na<sup>+</sup> was accumulated in the sixth leaf of stressed IR29 seedlings than in IR651 at the second harvest. Application of NaCl caused a significant ( $P \leq 0.01$ ) rise in leaf sheaths Na<sup>+</sup> concentration in seedlings of both genotypes (Table 3). Na<sup>+</sup> concentration showed an increasing change during the experiment conductance. Differences between leaf sheath and root Na<sup>+</sup> concentrations in stressed IR29 seedlings were not significant ( $P \leq 0.01$ ) at the second harvest. Generally, Na<sup>+</sup> in IR29 was accumulated mainly in shoot while in IR651 it was in root. Pierson correlation coefficients showed a negative correlation between Na<sup>+</sup> with K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Mn<sup>2+</sup> but a positive with total soluble sugars (Table 7).

Results showed that plant growth suppression in studied genotypes was related to the rate of Na<sup>+</sup> accumulation in leaves and leaf sheaths. Salt tolerant genotype was able to prevent high Na<sup>+</sup> accumulation in the youngest leaf and thereby de-

Table 2. Solutes concentration in leaf sheaths of IR651 (salt tolerant) and IR29 (salt sensitive) under normal and saline conditions

			Solutes concentration (mmol/kg DW)								
			Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	total soluble sugars	P	Mn <sup>2+</sup>	
After 72 h	IR651	normal	120.3 ± 3.2	935.5 ± 37.5	494.1 ± 17.1	349.4 ± 30.1	212 ± 12.4	442.5 ± 31.6	215.5 ± 8.6	8.9 ± 0.3	
		stress	129.6 ± 4	903 ± 5.7	565.5 ± 7.1	354 ± 20.5	182.4 ± 13.2	722.7 ± 22	193.7 ± 7.9	5 ± 0.2	
	IR29	normal	155.4 ± 6	923.4 ± 19.9	349.7 ± 10.7	333.4 ± 26.4	194.9 ± 5.9	435.8 ± 46.8	211.4 ± 8.6	7.1 ± 0.4	
		stress	159.4 ± 11.5	914.3 ± 30.1	788.4 ± 42.4	227.3 ± 12.7	162.4 ± 7.4	602.3 ± 29.1	205 ± 9.2	5.5 ± 0.3	
	After 240 h	IR651	normal	72.3 ± 3.9	885.6 ± 5.7	501.9 ± 8.5	388.6 ± 16.5	185.2 ± 4.7	401.5 ± 36.1	168.7 ± 17.5	9.3 ± 0.7
			stress	96.5 ± 10	852.7 ± 18.2	559.6 ± 18.8	256 ± 18.2	170.4 ± 7.9	919 ± 17.9	149.3 ± 10.6	7.9 ± 0.4
IR29		normal	122.9 ± 4.8	940.5 ± 58.9	412.7 ± 14.2	318.4 ± 19.9	196.2 ± 7.4	385.6 ± 39.7	152.6 ± 23.3	9.5 ± 0.8	
		stress	525.8 ± 43	818.5 ± 4.8	923.8 ± 28	211.5 ± 6.8	157.8 ± 5.1	857.4 ± 19.7	184 ± 6.8	9.3 ± 0.4	

Means and standard errors of 4 replications are shown

Table 3. Solutes concentration in leaf sheaths of IR651 (salt tolerant) and IR29 (salt sensitive) under normal and saline conditions

			Solutes concentration (mmol/kg DW)							
			Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	total soluble sugars	P	Mn <sup>2+</sup>
After 72 h	IR651	normal	191.7 ± 24.4	3400 ± 298.1	212.4 ± 10.2	77.2 ± 7.1	180.4 ± 12.6	358 ± 23.6	265.5 ± 16.4	7.1 ± 0.5
		stress	408.5 ± 14.5	2569.1 ± 94.7	267.3 ± 1.5	115 ± 5.1	157.8 ± 7.6	662.9 ± 32.1	238.9 ± 10.6	5 ± 0.2
	IR29	normal	225.5 ± 12.6	3234.5 ± 80.8	188.6 ± 4.2	182.2 ± 5.6	226.1 ± 10.3	290 ± 23.5	231.6 ± 21.4	7.1 ± 0.4
		stress	618.9 ± 17.6	3160.5 ± 141.1	305.8 ± 16.3	194.6 ± 22.7	156.2 ± 3.9	498.8 ± 53.4	241.3 ± 8.1	5.5 ± 0.3
	IR651	normal	178 ± 4.8	2975.7 ± 46.5	175.7 ± 5.6	98.2 ± 8.2	212.9 ± 7.7	450 ± 49.9	84 ± 9.7	0.3 ± 0.0
		stress	799.9 ± 42.5	2217.9 ± 90.5	215.5 ± 5.5	161.1 ± 13.8	151.8 ± 4.4	1082.7 ± 4.2	190 ± 14.7	0.2 ± 0.0
After 240 h	IR651	normal	188.3 ± 14.1	3014.9 ± 65.1	149.8 ± 4.5	145.3 ± 6.7	255.1 ± 6.3	286.7 ± 6.7	106.6 ± 4.8	0.3 ± 0.0
		stress	2069 ± 85.1	2347.3 ± 149	348.2 ± 11.6	235.1 ± 6	173.3 ± 2.9	459.3 ± 72.1	196.2 ± 6.2	0.3 ± 0.0
	IR29	normal	178 ± 4.8	2975.7 ± 46.5	175.7 ± 5.6	98.2 ± 8.2	212.9 ± 7.7	450 ± 49.9	84 ± 9.7	0.3 ± 0.0
		stress	799.9 ± 42.5	2217.9 ± 90.5	215.5 ± 5.5	161.1 ± 13.8	151.8 ± 4.4	1082.7 ± 4.2	190 ± 14.7	0.2 ± 0.0

Means and standard errors of 4 replications are shown

crease Na<sup>+</sup> damages to active tissues. Na<sup>+</sup> toxicity is strongly linked to the plants' ability to sustain the acquisition and in planta distribution of K<sup>+</sup> (Kader and Lindberg 2005).

There was a significant ( $P \leq 0.01$ ) reduction in root and leaf sheath K<sup>+</sup> concentration in IR651 when adding NaCl to nutrient solution (Tables 3

and 4). Results also showed that there were no significant ( $P \leq 0.01$ ) differences between genotypes in leaves K<sup>+</sup> concentration under control and stress conditions at the first harvest. K<sup>+</sup> concentration was decreased in leaf sheath of IR651 while it was stable in IR29 under stress at 72 h after salinization. Observations indicated that K<sup>+</sup> concentration

Table 4. Solutes concentration in roots of IR651 (salt tolerant) and IR29 (salt sensitive) under normal and saline conditions

			Solutes concentration (mmol/kg DW)							
			Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	total soluble sugars	P	Mn <sup>2+</sup>
After 72 h	IR651	normal	173 ± 7.3	325.3 ± 10	472.1 ± 11.1	99.3 ± 8.7	74.7 ± 2.3	760.6 ± 69	153.3 ± 3.8	0.2 ± 0.0
		stress	1774.6 ± 77.7	280.9 ± 12.4	976.7 ± 25.8	114.7 ± 7.6	54.1 ± 4.3	1112.5 ± 117.1	165.4 ± 4.4	0.1 ± 0.0
	IR29	normal	206.2 ± 11.2	331.5 ± 8.3	283.3 ± 17.1	131.6 ± 9.9	98.1 ± 4.1	582.3 ± 92.5	179.2 ± 11	0.2 ± 0.0
		stress	1651.8 ± 135.8	271.6 ± 17.9	756.3 ± 21.1	141 ± 17.8	77.7 ± 8.2	1200.8 ± 61	200.1 ± 4.8	0.2 ± 0.0
	IR651	normal	153.4 ± 18.6	327 ± 5.5	539 ± 22.4	143.9 ± 15	79.1 ± 2.9	594.4 ± 30.6	84 ± 9.7	0.0 ± 0.0
		stress	2627.4 ± 121.6	205.1 ± 3.5	1094.9 ± 33.2	87.5 ± 11.8	52.2 ± 1.6	1322 ± 119	190 ± 14.7	0.0 ± 0.0
After 240 h	IR651	normal	2187.4 ± 23	332.5 ± 9.5	307.5 ± 8.1	152.7 ± 5.7	101.6 ± 1.3	667.3 ± 99.9	90.4 ± 4.8	0.0 ± 0.0
		stress	2187.4 ± 84.6	234.5 ± 6.2	800.4 ± 38	125.9 ± 4	77 ± 2.4	596.2 ± 51	149.3 ± 12.7	0.0 ± 0.0
	IR29	normal	2187.4 ± 84.6	234.5 ± 6.2	800.4 ± 38	125.9 ± 4	77 ± 2.4	596.2 ± 51	149.3 ± 12.7	0.0 ± 0.0
		stress	2187.4 ± 84.6	234.5 ± 6.2	800.4 ± 38	125.9 ± 4	77 ± 2.4	596.2 ± 51	149.3 ± 12.7	0.0 ± 0.0

Means and standard errors of 4 replications are shown

Table 5. Analysis of variances for determined traits in different parts of plants at 72 h after salinization

Source of variances	df	Mean squares							
		Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	total soluble sugars	P	Mn <sup>2+</sup>
Salinity	1	449180.98**	368511.5**	208.83 <sup>ns</sup>	12667.2**	3340961.12**	1040459.62**	48.9 <sup>ns</sup>	26.8**
Genotype	1	16068.49 <sup>ns</sup>	59163.57 <sup>ns</sup>	3026.2 <sup>ns</sup>	1000.64*	10960.44 <sup>ns</sup>	53318.18*	438.77 <sup>ns</sup>	0.02 <sup>ns</sup>
Salinity × genotype	1	79.38 <sup>ns</sup>	195476.54*	6057.79*	807.53 <sup>ns</sup>	298045.83**	898.47 <sup>ns</sup>	1221.59 <sup>ns</sup>	1.28*
Part	2	5617431.33**	34337726.23**	174339.88**	62382.98**	17316106.79**	691840.93**	19550.62**	218.62**
Salinity × part	2	5166470.3**	232139.05**	6163.41**	651.99 <sup>ns</sup>	356748.86**	58824.06**	1065.87 <sup>ns</sup>	7.83**
Genotype × part	2	55813.59 <sup>ns</sup>	60756.63 <sup>ns</sup>	24751.83**	2297.45**	90250.91**	4679.69 <sup>ns</sup>	2134.85**	2**
Salinity × genotype × part	2	55476.21 <sup>ns</sup>	189786.14**	2978.57*	689.2 <sup>ns</sup>	105165.03**	33188.74 <sup>ns</sup>	205.94 <sup>ns</sup>	16.26**
Error	36	9260.33	32148.48	1105.75	253.34	11050.24	10930.01	354.74	0.28
CV	–	19.8	12.4	16.9	10.7	8.9	17.2	8.9	12.41

\* $P < 0.05$ ; \*\* $P < 0.01$ ; <sup>ns</sup>not significant

was decreased in both genotypes at 240 h after imposing salinity. As shown in Tables 2–4, K<sup>+</sup> concentration in leaf sheaths of both genotypes was higher than in leaves and roots.

One of the best-known effects of sodium stress on plant nutrition is suppression of K<sup>+</sup> uptake. K<sup>+</sup> is an essential activator for many enzymes located in the cytosol, and it was shown that Na<sup>+</sup> cannot substitute for this biochemical function (Tester and Davenport 2003). Leaf sheath was the main source for K<sup>+</sup> in both genotypes. Reduction in K<sup>+</sup> concentration under stress is due to Na<sup>+</sup> accumu-

lation because Na<sup>+</sup> engrosses ways for K<sup>+</sup> uptake. Increasing cytosolic Na<sup>+</sup> concentrations with salinity was reported previously (Carden et al. 2003, Halperin and Lynch 2003, Kader and Lindberg 2005). In addition, several studies showed, or inferred, a suppression of the cytosolic K<sup>+</sup> concentration in the presence of Na<sup>+</sup> (Flowers and Hajibagheri 2001, Carden et al. 2003). Many of physiological processes in plants were suppressed with the increase in Na<sup>+</sup> concentration. Jones and Turner (1980) reported that K<sup>+</sup> is an important ion for osmotic adjustment, especially in old leaves, and participates in

Table 6. Analysis of variances for determined traits in different parts of plants at 240 h after salinization

Source of variances	df	Mean squares								
		Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	total soluble sugars	P	Mn <sup>2+</sup>	DW
Salinity	1	15371645.14**	1080213.67**	8889.78**	20435.25**	1116942.82**	1948810**	14017.76**	1.49 <sup>ns</sup>	2465.33**
Genotype	1	576645.89**	16770.28 <sup>ns</sup>	885.04 <sup>ns</sup>	3978.88**	6737.18*	746367.28**	9690.23**	2.99*	108**
Salinity × genotype	1	343311.04**	209.98 <sup>ns</sup>	2073.88 <sup>ns</sup>	588.98*	97787.18**	551923.37**	7043.97**	2.57*	24.08 <sup>ns</sup>
Part	2	3898831.1**	24167102.9**	117968.61**	66694.72**	965056.34**	202125.5**	12685.62**	303.9**	27321.27**
Salinity × part	2	3196845.41**	511900.82**	37210.84**	2741.71**	166050.97**	26768.62 <sup>ns</sup>	2681.82*	0.66 <sup>ns</sup>	461.52**
Genotype × part	2	653236.15**	6643.64 <sup>ns</sup>	14955.58**	1153.24**	174232.8**	141146.03**	2072.12 <sup>ns</sup>	0.65 <sup>ns</sup>	382.68**
Salinity × geno- type × part	2	811591.45**	8218.63 <sup>ns</sup>	3.74 <sup>ns</sup>	200.65 <sup>ns</sup>	64228.59**	137005.88**	217.95 <sup>ns</sup>	0.57 <sup>ns</sup>	682.77**
Error	36	12325.26	13784.72	624.56	103.27	1538.13	13133.36	638.16	0.52	5.5
CV	—	13.6	9.2	6.8	6.7	7.9	17.1	18.8	15.7	2.9

\* $P < 0.05$ ; \*\* $P < 0.01$ ; <sup>ns</sup>not significant



Table 7. Pearson correlation coefficients between determined traits

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Cl <sup>-</sup>	Total soluble sugars	P	Mn <sup>2+</sup>
Na <sup>+</sup>	1	-0.23*	-0.34**	-0.54**	0.05 <sup>ns</sup>	-0.49**	-0.12 <sup>ns</sup>	-0.46**
K <sup>+</sup>		1	-0.16 <sup>ns</sup>	0.66**	0.46**	-0.47**	0.33**	0.45**
Ca <sup>2+</sup>			1	0.48**	-0.24*	-0.28**	0.18 <sup>ns</sup>	0.63**
Mg <sup>2+</sup>				1	0.01 <sup>ns</sup>	-0.65**	0.24*	0.8**
Cl <sup>-</sup>					1	-0.07 <sup>ns</sup>	0.59**	0.14 <sup>ns</sup>
TS						1	-0.32**	-0.45**
P							1	0.4 <sup>ns</sup>
Mn <sup>2+</sup>								1

\* $P < 0.05$ ; \*\* $P < 0.01$ ; <sup>ns</sup>not significant

osmotic adjustment till 30–50%. Reduction in K<sup>+</sup> concentration probably caused a reduction of plant ability for osmotic adjustment and drought stress, which led to plant growth reduction.

Adding NaCl to nutrient solution had no significant ( $P \leq 0.01$ ) effect on Ca<sup>2+</sup> concentration in roots at the first harvest but caused a significant ( $P \leq 0.01$ ) reduction in Ca<sup>2+</sup> concentration at the second harvest. Table 3 shows there was no change in leaf sheath Ca<sup>2+</sup> concentration in IR29 seedlings at the first harvest while a significant ( $P \leq 0.01$ ) increase was observed in both genotypes at 240 h after salinization.

Observed reduction in leaf Ca<sup>2+</sup> concentration in IR29 was higher than in IR651; this may be due to lower salt tolerance. Ca<sup>2+</sup> functions in plants include alleviation of ionic stress, activation of enzymes, sensing and responding. It was shown in previous studies that increasing Na<sup>+</sup> content in plant environment caused reduction of Ca<sup>2+</sup> content in salt-sensitive plants (Lacerda et al. 2003). Ca<sup>2+</sup> content decreased in maize, chickpea and sorghum under salinity while it increased in bean. In previous studies on soybean and cucumber, an additional supply of Ca<sup>2+</sup> to salt-stressed plants improved the salt tolerance of plants by reducing Na<sup>+</sup> uptake and transport (Dabuxilatu and Ikeda 2005). According to Husain et al. (2004) the major role of Ca<sup>2+</sup> for increasing salt tolerance of plants was related to its inhibitory effect on the xylem loading of Na<sup>+</sup>. Song et al. (2006) reported that high levels of external Ca<sup>2+</sup> are essential for the maintenance of high root uptake and shoot accumulation of Ca<sup>2+</sup> and K<sup>+</sup> on saline soils. It also plays a key role to avoid salinity damage to plants.

Salt stress caused a decrease of Mg<sup>2+</sup> concentration in all plant parts of studied genotypes (Tables 2–4). Determination of Mg<sup>2+</sup> showed that its concentration in the sixth leaf of IR651 was higher than IR29 while in root and leaf sheath it

was lower. Mg<sup>2+</sup> is essential for protein synthesis and chlorophyll structure. It is an activator for many of photosynthetic and respiratory enzymes. Salinity caused Mg<sup>2+</sup> to decrease in plant leaves, leaf sheaths and roots of both genotypes. Contribution of Mg<sup>2+</sup> in osmotic adjustment in leaves of salt-stressed sorghum was higher than in root (Lacerda et al. 2003).

Cl<sup>-</sup> concentration in all parts of stressed IR651 seedlings was significantly ( $P \leq 0.01$ ) higher than IR29. Leaves Cl<sup>-</sup> concentration rose in stressed seedlings of both genotypes. Salinity had a significant effect on Cl<sup>-</sup> concentration in leaf sheaths of genotypes. Cl<sup>-</sup> content in the sixth leaf and leaf sheath of salt-sensitive genotype were about 1.65 and 1.61 times higher than IR651 under stress condition, respectively (Tables 2 and 3). Results showed a significant ( $P \leq 0.01$ ) increase in Cl<sup>-</sup> concentration in roots of both genotypes; but Cl<sup>-</sup> concentration in IR651 was about 1.36 times higher than IR29. Root Cl<sup>-</sup> concentration increasingly changed in IR651 but in IR29 became stable after the primary increase (Table 4). Cl<sup>-</sup> had a positive correlation with K<sup>+</sup> and negative with Ca<sup>2+</sup> (Table 7).

IR651 was able to preserve Cl<sup>-</sup> in root and leaf sheath while a high rate of this ion was observed in the sixth leaf of IR29. Lacerda et al. (2003) said that Cl<sup>-</sup> accumulation in shoot of salt tolerant plant was lower compared to salt-sensitive ones under saline condition. Some halophytes can accumulate high Na<sup>+</sup> content and use it as osmolyte but compartmentalize it in vacuole to decrease toxic effect. The pattern of Cl<sup>-</sup> distribution was similar to Na<sup>+</sup>. It may be associated with salt tolerance in IR651. Na<sup>+</sup> and Cl<sup>-</sup> accumulation in root of IR651 led to suppression of shoot damage.

Total soluble sugars concentration was significantly ( $P \leq 0.01$ ) increased in leaves and leaf sheaths of studied genotypes under salinity. Total

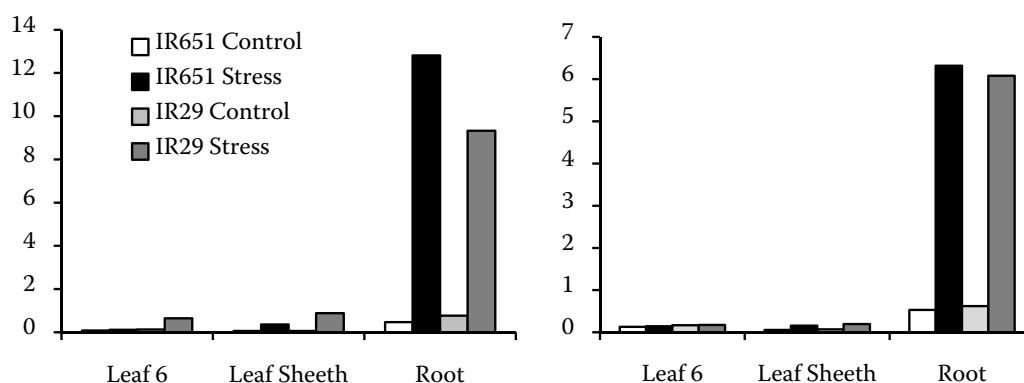


Figure 1. Na<sup>+</sup>/K<sup>+</sup> ratio in studied genotypes of rice at (A) 72 h after salinization and (B) 240 h after salinization

soluble sugars increase in IR651 was continuous till the end of experiment while in IR29 it was stable in leaf sheath and decreased in root. Results showed that total soluble sugars concentration in the sixth leaf and leaf sheath of IR651 were 1.07 and 2.35 times higher, respectively, than IR29 at the second harvest (Table 3).

The first effect of salinity on plants is a drought effect or water deficit. Plants try to reduce their osmotic potential via increasing mineral ions content and compatible solutes synthesis to better water uptake under salinity. Total soluble carbohydrates are important solutes that are synthesized and accumulated in cytosol under salt stress. Total soluble carbohydrates under saline conditions were higher than control in both genotypes. Total soluble carbohydrates increase in IR651 probably caused better osmotic adjustment and maintained turgor for growth under salinity. Kerepsi and Galiba (2000) reported that carbohydrates changes are important because of their relationship with such physiological processes as photosynthesis, translocation and respiration. Some studies reported that plants sugars content rose (Munns and Weir 1981) or remained constant (Morgan 1992) under salinity.

Salinity caused an increase of P concentration in leaf sheath and root of IR29 while it had no significant effect on IR651 at the second harvest. There were no significant ( $P \leq 0.01$ ) changes in P concentration in both genotypes. The effect of salinity was significant ( $P \leq 0.01$ ) on P concentration at the second harvest (Table 6). Changes in P concentration under salinity depended on plant space, salinity level and salt type. The lowest concentration of Mn<sup>2+</sup> was observed in roots of both genotypes. Mn<sup>2+</sup> concentration was decreased in sixth leaf of IR651 while there was no observed change in IR29 under salinity (Table 2). Na<sup>+</sup>/K<sup>+</sup> ratio was increased in all parts with addition of NaCl to nutrient solu-

tion. Roots Na<sup>+</sup>/K<sup>+</sup> ratio increase was higher than in other parts of both genotypes. Na<sup>+</sup>/K<sup>+</sup> ratio in the sixth leaf and leaf sheath of IR29 was about 5.6 and 2.4 times higher than IR651, respectively, while in roots of IR651 it was 1.3 times higher than in IR29 (Figure 1).

Salinity has various effects on Mn<sup>2+</sup> and P content in plants. Mitochondrial SOD (superoxide dismutase) is scavenged with Mn<sup>2+</sup> that can play an important role to remove toxic effects of oxidative stress. Mn<sup>2+</sup> concentration decreasing under salinity lead to a decrease of micro elements solubility in saline and sodic soils. This study has highlighted a relationship between plant ionic status and salinity tolerance in studied rice cultivars. Tolerant genotype was able to accumulate toxic ions in roots better than the sensitive ones, and thereby had better dry matter production. Total soluble sugars that are essential for osmotic adjustment accumulated in shoot of salt-tolerant plants were higher compared to sensitive-ones. Our results showed that IR651 was able to suppress both osmotic and toxic effects of salinity on active leaf using the above mentioned mechanisms, and showed better growth under salt stress.

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