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Effects of Salt Stress on Ion Balance at Vegetative Stage in Rice (*Oryza sativa* L.)

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Abstract: Rice (*Oryza sativa* L.) plants were subjected to NaCl stress within 35 days. The shoot dry weight, root dry weight, total survival percentage and contents of inorganic ions in dry samples of stressed plants were measured to identify the physiological adaptive mechanisms by which rice plants at vegetative stage tolerate salt stresses. Laboratory testing of the 10 rice cultivars showed that the salt concentration for the treatment is especially significant for assessment of the relationship between ion accumulation and salt tolerance. Salt treatment strongly stimulated accumulations of Na^+, Na^+/K^+ ratio, Cl^- in shoot and root and reduced K^+, NO_3^- contents in both organs under 1.2% NaCl stress condition, but not always under 0.6% NaCl concentration. It was shown significantly negative correlation ($p<0.5$) between the Na^+ content and dry weight of shoot and root under 1.2% NaCl stress condition. Also, was founded significantly negative impact ($p<0.5$) of Cl^- ions on biomass growth and survival rice plants and positive relationship ($p<0.5$) among the root dry weight and K^+, NO_3^- content under the high NaCl concentration (1.2% NaCl). So, in addition to Na^+, the contributions of Cl^- to abiotic stresses of rice should not be ignored.

Keywords: Salt Stress, Ion Balance, Dry Weight, Adaptation, Rice

Introduction

Rice is important crops used as the staple food of over half of the world's population and the increase rice grain production is the actual goal for today. One of the severe limitations to increase rice production is considered to soil salinity (Ali *et al*., 2014). So, current FAO assessment (2015) specifies that globally the area of soil salinity was more than 400 million ha.

As is known, the adaptations plants to extreme environmental factors are manifested at different levels of organization of the organism (Negrao *et al*., 2011; Usatov *et al*., 2015). The study of morpho-physiological response to salinity is important with the standpoint of understanding the mechanisms of plant adaptation to stressful influences. The pivot to plant survival under salinity is preserving an ion balance in the cells (Das *et al*., 2015). For plants, potassium (K^+) and nitrate (NO_3^-) ions are essential for the adjustment of osmotic pressure, sustentation of membrane potential, activation of enzymes, stomatal movement, turgor pressure and etc., whereas sodium ions (Na^+) are harmful (Mekawy *et al*., 2011; Platten *et al*., 2013). There are some debates about the detrimental effects of chlorine ions (Cl^-) on plant metabolism (Mekawy *et al*., 2011).

The most studies have focused on the evaluating of rice salt tolerance at germination or seedling stage, because the initial plant resistance influences the final yield (Mardani *et al*., 2014; Negrao *et al*., 2011; Hu *et al*., 2012; Ali *et al*., 2014). However, assessment of salt tolerance at the germination or seedling stage does not always correlate with that in the later stages of growth (Mass and Grieve 1994; Ferdose *et al*., 2009; Zeng *et al*., 2002). In this study, we studied the effects of salt stress on ion balance at vegetative stage of rice.

Materials and Methods

Plant Materials

In the present work were used ten rice (*Oryza sativa* L.) cultivars of the Russian breeding. These genotypes were selected based on their agronomic performance and varying of salt tolerance. All the germplasm samples were to subspecies *japonica*.

Evaluation of Salinity Tolerance

Rice seeds were surface sterilized by 5.2% NaOCl solution for 20 min and were then rinsed with distilled water. The seeds were soaked in water for 12 h and placed in special trays with soil (Thomson *et al*., 2010; Usatov *et al*., 2015; Xiong and Choong, 2014).

Artificial salinization of the soil, at the rate of 0.6% and 1.2% on its dry weight, was performed by adding an appropriate quantity of NaCl. Rice was grown in a growth chamber (Binder, GmbH) under the next operated environmental conditions: 72% relative humidity, 26±1°C and a 14 h photoperiod. After 35 days, Root Dry Weight (RDW), Shoot Dry Weight (SDW) and total survival percentage of plants were measured. For dry weight measure, rice shoots and roots were desiccated at 75°C for three days previous to being weighed (Kumar and Kumar, 2014; Usatov *et al*., 2015).

Determining Ions Concentrations

The Na^+, K^+, Cl^- and NO_3^- concentrations in roots and shoots were quantified utilization a capillary electrophoresis system Capel-105M (Lumex, Russia). Dried specimens of plant substance were powdered and treated with 15 mL deionized water at 100°C for 1.5 h and the extract utilized to set the concentrations of free inorganic ions.

Data Analysis

The collected data were processed to t-test. Differences were considered statistically significant when $p<0.05$. All data were presented by an average of the four replicates and the standard deviations.

Results

Evaluation of Salinity Tolerance

Analysis of the total survival percentage of rice breeding material under salt stress showed different decline in this value depending on the genotype (Table 1). The most negative effect of stress factors on the rice plants survival showed under 1.2% NaCl conditions. The highest survival decrease was found in the variety Yuzhanin, compared to the control. The most tolerance lines in this value were K2420-Ostap and K2495-Ostap - 89.5 and 100%, respectively. Also, high tolerance lines were K-7256 and Bacchus-Khazar-deviation from control less than 20%.

Under 0.6% NaCl stress conditions, the Dry Weight (DW) was not significantly changed in all cultivars. The largest decline root and shoot dry weights were revealed in the variety Boyarin. Under 1.2% NaCl stress conditions, the biomass growth was strongly suppressed. The lowest suppression of Shoot Dry Weight (SDW) was shown in the variety Aiceberg, while the largest decline SDW under salt stress (1.2% NaCl) was found in the variety Yuzhanin. The lowest Root Dry Weight (RDW) decrease was shown in the variety Aiceberg and K2495-Ostap, the largest decrease RDW was revealed in the variety Boyarin.

Ion Accumulation

The ion contents in shoot and root of the investigated rice cultivars are illustrated on Fig. 1 and 2. From these figures it is seen that with the rising salinity, ion content indexes are able to distinguish genotypes under investigation to greater extent. It was shown that between rice genotypes at vegetative stage there are significant variations of Na^+, K^+ and NO_3^- contents, but not Cl^- content under control condition. The low salinity treatment (0.6%) influenced on the ion balance of rice plants to a lesser degree than high salinity treatments (1.2%). Under 1.2% NaCl stress conditions, the highest increase of Na^+ content and especially Cl^- content were found in shoot of the cultivar Yuzhanin. For this cultivar has also been shown the lowest survival percentage, compared to the control.

Generally, salt treatment strongly stimulated accumulations of Na^+, Na^+/K^+ ratio, Cl^- in shoot and root and salt stress reduced K^+, NO_3^- contents in both organs under 1.2% NaCl stress condition, but not always under 0.6% NaCl concentration.

Table 1. The Total Survival percentage of plants (TS), Shoot Dry Weight (SDW) and Root Dry Weight (RDW) of rice under control and salt stress conditions

	Control			0.6% NaCl			1.2% NaCl		
Cultivars	TS, (%)	SDW, (g)	RDW,(g)	TS, (%)	SDW, (g)	RDW, (g)	TS, (%)	SDW, (g)	RDW, (g)
Boyarin	99.0±0.5	0.34±0.04	0.17±0.02	95.0± 3.5	0.18±0.02*	0.06±0.001*	60.0±2.0	0.05±0.007*	0.02±0.003*
Yuzhanin	98.0± 1.0	0.11±0.01	0.03±0.002	45.0± 4.0	0.12±0.02	0.02±0.003*	35.0±3.5	0.02±0.003*	0.01±0.003*
Kuboyar	100.0± 0	0.17±0.01	0.12±0.004	50.0±2.3	0.16±0.003	0.07±0.004*	60.0±2.2	0.04±0.003*	0.02±0.004*
K-7256	90.0±2.0	0.11±0.02	0.08±0.002	72.2±1.5	0.10±0.03	0.05±0.002*	83.3±4.1	0.05±0.001*	0.02±0.003*
Aiceberg	100.0±0.0	0.10±0.01	0.06±0.003	75.0±2.4	0.15±0.01*	0.05±0.004	70±3.0	0.07±0.002*	0.03±0.006*
Bacchus- Donchak	85.0±3.0	0.11±0.02	0.06±0.003	58.8±3.3	0.12±0.02	0.04±0.001*	64.7± 2.8	0.04±0.002*	0.01±0.002*
Bacchus-Khazar	95.0±1.0	0.08±0.01	0.04±0.003	73.7±4.4	0.06±0.01	0.03±0.005	84.2±1.5	0.03±0.005*	0.02±0.002*
Bacchus-Khazar- 9403	100.0±0.0	0.10±0.01	0.05±0.002	85.0±2.5	0.09±0.009	0.03±0.002*	60.0± 4.5	0.03±0.001*	0.02±0.001*
K-2420-Ostap	95.0±1.5	0.16±0.03	0.07±0.004	84.2±3.0	0.15±0.03	0.05±0.005*	89.5± 2.4	0.07±0.001*	0.03±0.006*
K-2495-Ostap	98.0±1.0	0.11±0.01	0.06±0.002	99.0±0.5	0.11±0.02	0.05±0.005	98.0± 0.5	0.04±0.002*	0.03±0.004*

* Display significant dissimilarity from control at $p<0.05$

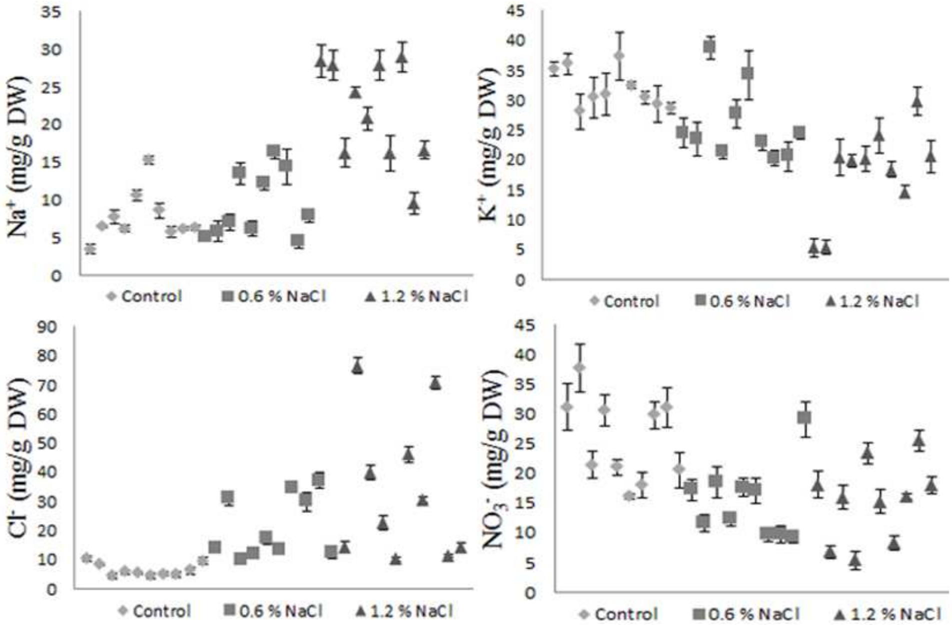

Fig. 1. Ion accumulation in shoots of rice under control, 0.6 and 1.2% NaCl conditions

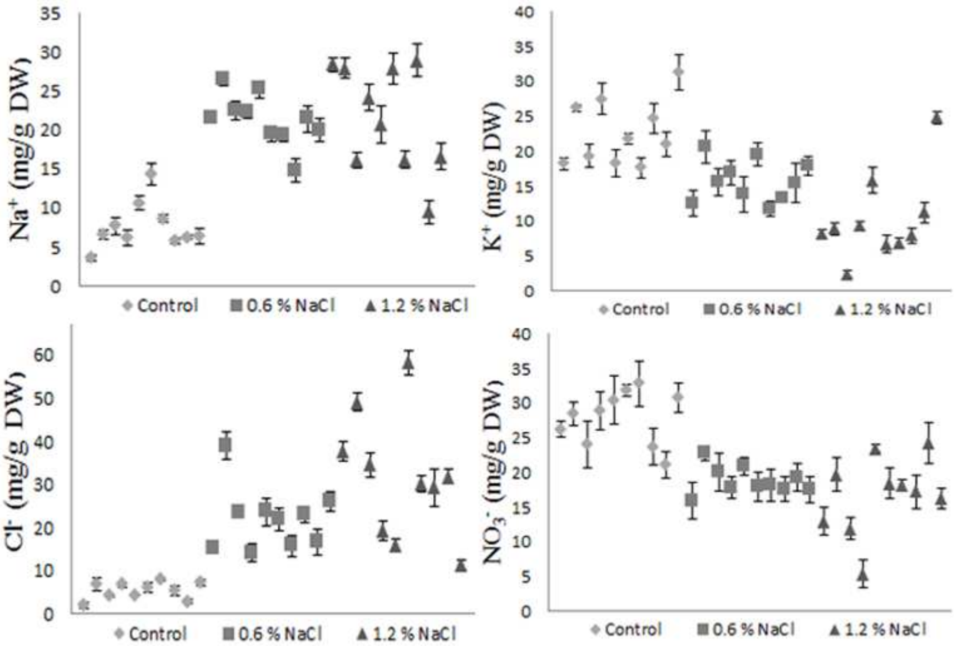

Fig. 2. Ion accumulation in roots of rice under control, 0.6 and 1.2% NaCl conditions

Correlation Analysis

The relationships between the dry weight indices, total survival percentage of plants and ion content at different salinity levels are presented here.

There was a significant negative correlation ($p<0.5$) between the Na^+ content and RDW under 1.2% NaCl condition, but no significant correlation was revealed under 0.6% NaCl and the control conditions (Table 2 and 3). The relationship among SDW and Na^+ was highly significant both under 0.6 and 1.2% NaCl conditions.

The correlations between Cl^- content and RDW, SDW were strong negative only under 1.2% NaCl concentration and not under 0.6% NaCl and the control conditions. The correlations for NO_3^- and K^+ were significantly positive only between ions content and RDW under 1.2% NaCl condition.

Table 2. Correlation matrix between the variables at 0.6% NaCl conditions

Variables	Variables TS (shoot/root ion content)	SDW	RDW
TS	-	-	-
SDW	0.05	-	-
RDW	0.27	0.94*	-
Na^+	-0.42/-0.35	-0.83*	-0.35
K^+	-0.48/-0.51	0.36	-0.32
Cl^-	-0.35/-0.48	-0.35	-0.5
NO_3^-	0.45/-0.54	0.62	0.33

TS- Total Survival percentage of plants; SDW- Shoot Dry Weight; RDW- Root Dry Weight. *Display significant dissimilarity from control at p<0.05

Table 3. Correlation matrix between the variables at 1.2% NaCl conditions

Variables	Variables TS (shoot/root ion content)	SDW	RDW
TS	-	-	-
SDW	0.53	-	-
RDW	0.26	0.15	-
Na^+	-0.01/-0.67*	-0.85*	-0.67*
K^+	0.65*/0.50	0.63	0.71*
Cl^-	-0.72*/-0.68*	-0.83*	-0.69*
NO_3^-	-0.14/0.32	0.61	0.69*

TS- Total Survival percentage of plants; SDW- Shoot Dry Weight; RDW- Root Dry Weight. *Display significant dissimilarity from control at p<0.05

The relationship among the total survival percentage of plants and ion concentrations was also examined and there were a fairly strong negative linear association between survival of rice plants and Cl^- concentrations in root and shoot, under 1.2% NaCl stress condition (Table 3).

Discussion

Rice is highly susceptible to salinity, but the degree of susceptibility differs with varieties (Negrao *et al*., 2011; Hoang *et al*., 2015). Accurate phenotyping is important for studying impacts of salinity on ion balance (Wang *et al*., 2012). Previous studies showed that the salt tolerance of genotypes could be determined by comparing the production of biomass after a sufficiently long period of growth under salinity (Leland *et al*., 1994). The accumulation of plant biomass is closely related to their productivity and varies considerably depending on the salinity and salt tolerance of variety (Hu *et al*., 2012; Ali *et al*., 2014; Usatov *et al*., 2014). Under 1.2% NaCl condition the lowest suppression of dry weight was shown in the variety Aiceberg whereas under 0.6% salt concentration there was a significant stimulation (50% of control) of shoot biomass growth of this variety. Also high tolerance to salinity was revealed in the samples K-7256, K-2495-Ostap and K-2420-Ostap.

Cellular ion homeostasis is fundamental in the processes of growth and development of higher plants (Negrao *et al*., 2011; Senadheera *et al*., 2009). Salinity stress reasons an unbalance of ion homeostasis in cells, leading to ion toxicity (Ferdose *et al*., 2009; Mekawy *et al*., 2011; Cotsaftis *et al*., 2012). At the initial stages of growth, the ability to maintain a high K^+/Na^+ ratio in roots or shoots is regarded an indicator of probable salt tolerance in rice (Mardani *et al*., 2014; Wang *et al*., 2012). A similar result was observed at vegetative stage in our research. In this study there were significantly negative correlations (p<0.5) between the Na^+ content and DW of root and shoot under 1.2% NaCl condition and under 0.6% NaCl condition a significant negative correlation was identified between the Na^+ content and shoot DW. Also, we revealed a strong negative impact of Cl^- ions on biomass growth and survival rice plants under the high NaCl concentration (1.2% NaCl). Under salt stress condition, plants have K^+ and NO_3^- deficiencies due to the competitive inhibition of their uptake by Na^+ and Cl^- (Mekawy *et al*., 2011; Negrao *et al*., 2011). In our study were revealed significantly positive relationships among the root DW and K^+, NO_3^- content, but not between K^+, NO_3^- and shoot DW or survival of rice plants. In this case the capacity to maintain the transport and stockpiling of K^+ and NO_3^- in root is important salt-tolerant trait at vegetative stage. Thus, salinity stress at vegetative stage of rice leads to serious disruption of ion homeostasis.

Conclusion

Laboratory examination of the rice cultivars showed that shoot dry weight, root dry weight and total survival percentage of plants at vegetative stage declined under salinity. The minimal reduction of growth under salt stress was revealed in the variety Aiceberg and in the lines K7256-Ostap, K2420-Ostap and Bacchus-Khazar, while the largest decline shoot dry weight, root dry weight and total survival percentage of rice plants was found in the varieties Boyarin and Yuzhanin. It was shown that the salt concentration for the treatment is especially significant for efficient assessment of the relationship between ion accumulation and rice salt tolerance. Salt treatment strongly stimulated accumulations of Na^+, Na^+/K^+ ratio, Cl^- in shoot and root and reduced K^+, NO_3^- contents in both organs under 1.2% NaCl stress conditions, but not always under 0.6% NaCl concentration. The studies of ion ratio revealed a valid negative correlation ($p<0.5$) between the Na^+ content and DW of root and shoot under 1.2% NaCl condition. Also, we founded significantly negative impact of Cl^- ions on biomass growth and survival rice plants and positive relationship among the root DW and K^+, NO_3^- content under the high NaCl concentration (1.2% NaCl). Therefore in addition to Na^+, the contributions of Cl^- to abiotic stresses of rice should not be ignored. Thus, salinity stress at vegetative stage of rice initiates significant alterations to many physiological processes of plant cells, including ion homeostasis, which all may cause to yield reduction.

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Author's Contributions

All authors equally contributed in this work.

Ethics

The authors declare that this article is original and corresponds to the ethical norms specified by the OnLine Journal of Biological Sciences.

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