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Research article

Effect of salinity on osmolytes and relative water content of selected rice genotypes

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Abstract: Reduction in relative water content (RWC) and photosynthesis (dry matter) is a common effect of salinity stress. In the present study, 7 days old rice plants were exposed to 0 (control) and 100 mM NaCl salinity for 7 days to determine the osmolytes accumulation and relative water content (RWC) in leaves. We observed that shoot dry matter, relative water content and K^+ content decreased significantly with the increasing of salinity. In contrast, Na^+ and proline content excessively increased in the leaves of salinity stressed plant. The results revealed that both the organic (proline) and inorganic (K^+) osmolytes accumulation may be responsible for stress alleviation by retaining water in cell.
Keywords: Salinity - Relative water content - Osmolytes - Osmoregulation.

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INTRODUCTION

Salinity is one of the most brutal environmental stresses that adversely influenced the capability of plant to uptake water, and this quickly causes reduction in growth rate, along with a suite of metabolic changes (Munns 2002, Vaidyanathan *et al*. 2003). It seriously limits agricultural productivity with significant crop loss through worldwide (Munns & Tester 2008). Now more than 800 million hectares of land throughout the world are salt affected which is 6% of the world's total land area (FAO 2008) and this area is increasing day by day because of global warming with consequent rise in sea level and increase in tidal surges, particularly in coastal areas in the globe (Wassmann *et al*. 2004). In Bangladesh, about 1.5 million hectares out of 2.85 million hectares of coastal and off-shores land is affected by different degrees of salinity (Murshed 2008).

Salinity exerts its detrimental effect on rice into two phases. Firstly, high concentration of salt in the root zone makes it harder for root to uptake water (osmotic stress). This is caused due to abscisic acid (ABA) mediated root signal that limits the availability of water to plant cell, which leads to reduce relative water content, slower plant growth and finally reduces dry mater accumulation (Fricke *et al*. 2004, Davies *et al*. 2005, Zhang *et al*. 2006). Secondly, high concentration of salt within the plant commences ionic toxicity (hyperosmotic stress) that leads to cell death, which is mitigated by either biosynthesis of organic osmolytes by plant itself or sequestrated role of inorganic osmolyte (K^+). Under various environmental stresses, plant cells have experienced biosynthesis of some organic osmolytes such as sucrose (Hu *et al*. 2000), glycinebetain (Chen & Murata 2002), mannitol (Abebe *et al*. 2003), trehalose (Garg 2002) and proline (Matysik *et al*. 2002, Misra & Gupta 2005) that contribute in osmogerulation as well as maintaining cell turgor. Among these compatible osmolytes, proline is one of the most frequently reported organic osmolyte which involved in osmoregulation in rice (Matysik *et al*. 2002). A large number of data indicate a positive correlation between proline accumulation and adaptation to salt stress in rice (Blum & Ebercon 1976, Hanson *et al*. 1977, Chandler & Thorpe 1987) whilst some recent studies reveal that, inorganic osmolyte exhibits a greater role than organic osmolytes in relation to osmoregulation and maintaining cell turgor in monocot plants (Shabala 2003, Rahman *et al*. 2007) because proline biosynthesis is not always rapid in salt stressed plants (Hanson *et al*. 1979). Plants that contain high

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K^+/Na^+ ratio can tolerate more salinity over those that contain low K^+/Na^+ ratio even though they accumulate proline in several folds higher which is revealed in this experiment. Na^+ and Cl^- which are cytotoxic to cell sequestrated in the vacuole of the cell in relation to K^+ accumulation in the cytoplasm to balance osmotic pressure of the ions in the vacuole (Munns 2002) while proline biosynthesis is not always rapid and occurred when plant espoused to excessive salinity which might damage them fatally. So the present research was designed to measure the magnitude of RWC (%), osmolytes accumulation and their physiological role in stress mitigation under salt stress in selected rice genotypes.

MATERIALS AND METHODS

Study material

Seeds of seven rice (*Oryza sativa* L.) genotypes were collected from Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU) and Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA). The experimental design was completely randomized design (CRD) with three replications.

Detail of experimentation

The experiment was conducted in the Plant Physiology laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh from December 2016 to January 2017 To investigate the survivability of seedlings surface sterilized (rinsed with NaOCl) 100 seeds of each rice genotype were placed on water-soaked filter paper (Whatman filter paper-1) in Petridis with three replication at 25^{o}C temperature with 70% relative humidity and 400 µmol m^{-2} s^{-1} light intensity. Day/night ratio was maintained at 14/10 hours. After 3 days of germination the seedlings were treated with 0 (control) and 100 mM NaCl salinity. On the 7th day after Salinization the seedling survivability was recorded. Further, to evaluate the effect of salinity on the seedling stage, seeds of each rice genotypes were surface sterilized with 10% sodium hypochlorite (NaOCl) for 10 minutes. The surface sterilized seeds were then placed on germinating pot for germination containing normal water. After germination healthy and vigorous seedlings with the uniform shoot and root were selected and transferred to perforated cork sheets, each of whole contained 6 seedlings. The sheets were floated on a nutrient solution (Hoagland solution) in 32 liters plastic trays where the seedlings were grown hydroponically at 25^{o}C temperature with 70% relative humidity and a light intensity of about 400 µmol m^{-2} s^{-1}. Day/night ratio was maintained at 14/10 hours. The seedlings were cultured for 14 days at pH 6.5–7.0 in the growth chamber under the control environment. At 15th days of normal growth, salinity was imposed with two concentration of salt (0 and 100 mM of NaCl) for 7 days. On the 7th day after Salinization plants were harvested and necessary data were recorded.

Relative water content

A pre-dawn leaflet sample was taken from 3 plants of each replication in each treatment on 7th day after salinization and its fresh weight was recorded immediately. The leaf sample was then incubated in deionised water for 4 hours as described by Sairam *et al*. (2002) and turgid weight of the leaf sample was recorded. The leaf sample was then filled into a brown paper bag and oven dried at 70^{o}C temperature for 48 hours and the dry weight of the sample was taken. The relative water content (RWC) was estimated as follows:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Growth measurement (Shoot dry matter)

For the measurement of shoot dry weight 6 seedlings of each replication of control and salinity treatments were randomly selected and gently uprooted. The seedlings were separated into roots and shoots. Then the shoots were dried in an aerated oven at 70^{o}C temperature for 72 hours and shoot dry weights were recorded expressed as % dry mater (DM).

Proline determination

Proline was determined according to the method of Bates *et al*., (1973). Briefly, fresh leaf sample (500 mg) was homogenized in 5 ml of 3% sulphosalisylic acid by the means of a mortar and pestle and then centrifuged at 18000 g for 10 minutes to remove cell debris. Then the supernatant (2 ml) was taken in a test tube glacial acetic acid (2 ml) and ninhydrin reagent (2 ml) was added. The reaction mixture boiled in a water bath for an hr. Then the test tube was cooled in ice and toluene (6 ml) was added into the mixture and mixed thoroughly. After that upper toluene phase was separated into a glass cuvette and free proline was estimated from proline standard (0–50 µg ml^{-1}) treated in an identical manner.

Na^+ and K^+ content

About 200 mg dry and ground leaves of each replication in each treatment were placed in a digestion tube and 2.5 ml of digestion mixture (H_2SO_4 + $HClO_4$) was added in each tube. After mixing the tube was allowed to place in a heating block and heated for 5 minutes at 100°C temperature, then for 30 minutes at 180°C temperature. After cooling 1 ml aliquots of 30% H_2O_2 were added and the content of digestion tube was thoroughly mixed. Then the test tube was again heated for 2 hours at 330°C temperature (just below the boiling point of the digestion mixture). The cooled, clear, digested mixture was diluted by deionised water and filtered. Then the filtered was volume up to 100 ml and taken for analysis of Na^+ and K^+ concentration by using flame photometer.

RESULTS

Seedling survivability

Seedling survivability significantly ($P<0.05$) decreased with the increasing salinity (Fig. 1). At 100 mM salt concentration Pokkali (Salt resistant trait) exhibited 10% decrease in seedling survivability while BRRIdhan 29 (salt sensitive trait) exhibited 78.4% decrease over corresponding control. Ashfal, Benapol, Jamainaru, Gunshi and Mohini showed 15, 23, 20, 36.5, 56.6% decrease in seedling survivability respectively over corresponding control.

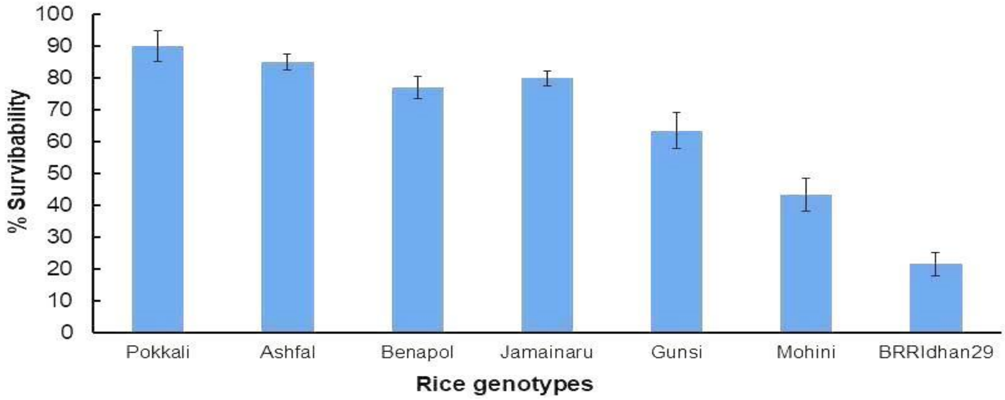

Figure 1. Seedling survivability of some selected rice genotypes under salt stress. [The vertical bars represent the ±SE]

Relative water content

Relative water content significantly ($P<0.05$) decreased with the increasing salinity (Fig. 2). About 6.6% RWC declined in pokkali at 100 mM salt concentration compared to corresponding control. The other varieties Ashfal, Benapol, Jamainaru, Gunshi, Mohini and BRRIdhan 29 with a treatment of 100 mM salinity exhibited a decrease in relative water content at 8.1, 11.5, 12.1, 14.7, 18.3 and 21.7% respectively over corresponding control.

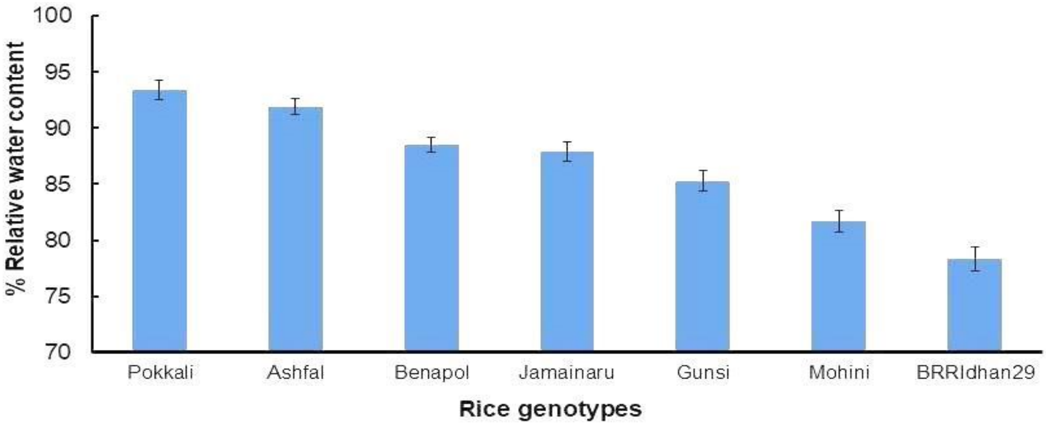

Figure 2. Relative water content (%) of some selected rice genotypes under salt stress. [The vertical bars represent the ±SE]

Growth measurement (Shoot dry matter)

Shoot dry weight significantly ($P<0.05$) decreased with the increasing salinity. Here, pokkali showed maximum dry matter production (1.89 g) at control which gradually decreased with the increase of salinity and at 100 mM salt concentration it showed minimum (1.35 g) dry matter production with 28.57% reduction. The other varieties Ashfal, Benapol, Jamainaru, Gunshi and Mohini with a treatment of 100 mM salinity exhibited a decrease in shoot dry matter at 30, 30, 39.07, 40, 46.09 and 54.76 % respectively over corresponding control.

Proline accumulation

Proline accumulation is an exceptional physiological attitude in salinity stressed plant. Table 1 showed a positive correlation between salinity and proline accumulation. With increasing, salinity proline accumulation increased in all rice genotype. The rice genotypes pokkali, Ashfal, Benapol, Jamainaru, Gunshi, Mohini and BRRIdhan 29 at 100 mM salinity showed an increase in proline accumulation about 200%, 209%, 211%, 188%, 283%, 400% and 500% respectively over corresponding control.

Table 1. Effect of salt stress (100 mM) Proline accumulation (μg g^{-1} FW) of selected rice genotypes.

Genotype	Leaf proline content (μg g^{-1} FW)	
	Control	Salt stress
Pokkali	10 [ab]	20 [b]
Ashfal	11 [a]	23 [a]
Benapole	9 [b]	19 [bc]
Jamainaru	9 [b]	17 [c]
Gunshi	6 [c]	17 [c]
Mohini	5 [c]	20 [b]
BRRIdhan 29	4 [cd]	20 [b]

Note: Values marked with the same letter within the columns do not differ significantly @ 5% level of probability.

Na^+ and K^+ content

Na^+ content showed increasing trend while K^+ content showed a decreasing trend in saline stressed plant (Table 2). At 100 mM NaCl stress Pokkali showed 2.91% Na^+ while salt sensitive variety BRRIdhan 29 showed 3.90% Na^+. Others such as, Ashfal, Benapol, Jamainaru, Gunshi, Mohini showed 2.39, 2.49, 2.17, 3.06 and 3.41% Na^+ respectively. In case of K^+ content Pokkali showed 2.20% K^+ while salt sensitive variety BRRIdhan 29 showed 1.48% K^+. Others such as, Ashfal, Benapol, Jamainaru, Gunshi, Mohini showed 2.67, 2.47, 2.68, 2.28 and 1.63% K^+ respectively. Ashfal showed the height K^+/Na^+ ratio (1.117%) followed by Jamainaru (1.101%). The lowest K^+/Na^+ ratio was found in BRRIdhan 29 (0.379%).

DISCUSSION

In the resent study, salinity reduces the seedling survivability (Fig. 1) because the high concentration of salt in the root zone makes it harder for root to uptake water (Munns & Tester 2008). Water is essential for all metabolic processes; lack of water may collapse all the metabolic processes of salt treated seedlings and finally influenced the seedling survivability. Again accumulation of the toxic ions in cellular level may be thought responsible for decreasing seedlings survivability.

Salinity reduces the relative water content (Fig. 2). When plants are subjected to salinity, firstly they face an osmotic challenge that reduces water uptake by roots. Besides ABA mediated stomatal closure affects transpiration pull that lead low/no water uptake by roots, entail low relative water content in the cell (Blatt & Armstrong 1993).

Dry matter production also hampered during salinity (Fig. 3). Salinity disturbs the normal electron flow for carbon reduction in the Calvin cycle by impairing water uptake, which acts as an electron donor. Again ABA mediated stomatal closure limits CO_2 fixation, resulting in decreasing carbon reduction by Calvin cycle (Lawlor & Cornic 2002) that leads to low photosynthesis. As it is proved that dry matter production is associated with photosynthesis, low photosynthesis rate causes low dry matter production.

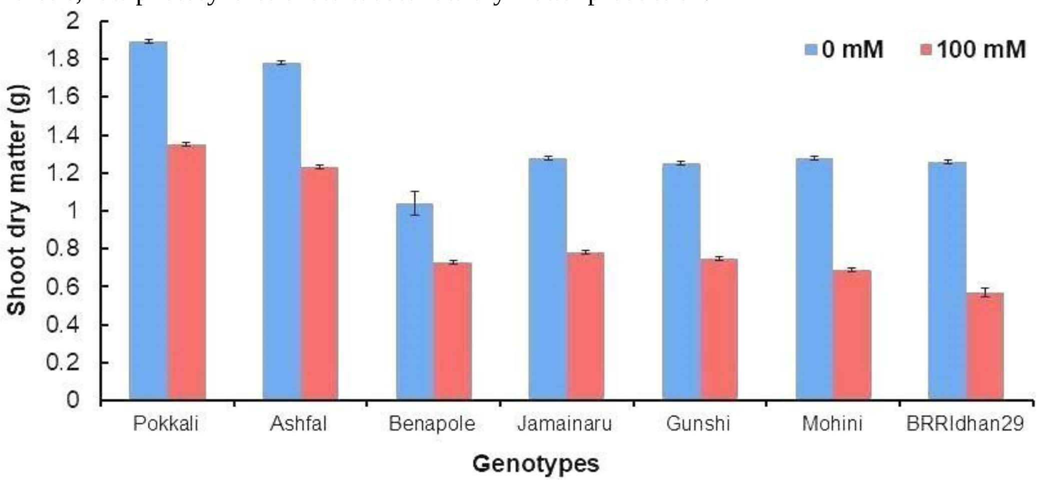

Figure 3. Shoot dry matter (g) of some selected rice genotypes under salt stress. [The vertical bars represent the ±SE]

Proline accumulation is a common metabolic process to be involved in stress tolerance mechanism (Lutts *et al*. 1999, Sivakumar *et al*. 2002). In the present study, a significant increase in proline accumulation was recorded in rice leaf after 7 days of exposure to NaCl salinity (Table 1). The result also revealed that the magnitude of proline accumulation was positively associated with the concentration of NaCl in the culture solution. These results are likely to with the findings of some earlier study (Chen & Murata 2002, Abebe *et al*. 2003). The mechanism that evolved in proline accumulation is, salinity stress causes ABA mediated stomatal closure that limits the fixation of CO_2, resulting in decreasing carbon reduction by Calvin cycle (Lawlor & Cornic 2002) makes $NADP^+$ non available for electrons acceptance during photosynthesis. In this circumstances, Photosynthetic reducing power $NADPH_2$ donate election to glutamate for biosynthesis of proline and regenerate $NADP^+$ for further election acceptance (Wang *et al*. 2007).

Table 2. Effect of salt stress on Na^+ and K^+ content (%) of selected rice genotypes.

Genotype	**Na^+ and K^+ content (%) at leaf blade**		
	Na^+	**K^+**	**K^+/ Na^+**
Pokkali	2.19^e	2.20^c	1.004^b
Ashfal	2.39^d	2.67^a	1.117^a
Benapole	2.49^d	2.47^b	0.991^b
Jamainaru	2.17^e	2.68^a	1.101^a
Gunshi	3.06^c	2.28^c	0.745^c
Mohini	3.41^b	1.63^d	0.478^d
BRRIdhan 29	3.90^a	1.48^e	0.379^e

Note: Values marked with the same letter within the columns do not differ significantly @ 5% level of probability.

Recently some studies revealed that, inorganic osmolyte exhibit a greater role than organic osmolytes in relation to osmoregulation and maintaining cell turgor in rice which is revealed in this experiment in table 2. According to Hanson *et al.* (1977) and Moftah & Miche (1987), Proline biosynthesis is not always rapid in salt-stressed plants, the maximum accumulation of proline might have occurred when plant espoused to excessive salinity which might damage them fatally. In these circumstances, K^+ an inorganic osmoticum other than proline might be involved in retaining leaf water content as well as cell turgidity by maintaining ionic balance (Shabala 2003). The evolved mechanism behind this science is Na^+ and Cl^- are sequestrated in the vacuole of the cell in relation to K^+ accumulation in the cytoplasm and organelles to balance osmotic pressure of the ions in the vacuole (Munns 2002). Again K^+ (known as water buoy) plays an important role in the opening of stomata by regulating guard cells with its water holding capacity. If the K^+ concentration is high in the guard cell it keeps the stomata open for carbon fixation thus help in plant growth and productivity (Murata *et al*. 1994, Shabala 2003). So plant that accumulates more proline over control but content less k^+/Na^+ ratio are less tolerance to stress and vice-versa (Table 2).

CONCLUSION

From this study, it might be concluded that, salinity affects the seedling survivability and also exhibits the reduction in relative water content and dry matter accumulation. Photosynthetic impairment induced by salinity was mitigated by proline biosynthesis whereas its function in maintaining ionic balance and cell turgidity was not so strong over k^+.

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