Effect of NaCl Stress on Physiological Parameters of four Rice varieties (*Oryza sativa* L.)

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ABSTRACT

Plants in natural and agricultural conditions are continuously exposed to several biotic and abiotic stresses such as drought and salinity. The objective of this study was to compare the salinity tolerance of four locally cultivable different varieties of rice varieties (Niranjan, Ranjan, Swarnamasuri and Maharaja) to assess their biochemical responses like reducing sugar, proline and malondialdehyde content and morphological effect like shoot and root growth rate. The response against stress showed a gradual reduction in shoot growth in all four varieties of rice was decreased 57.3-80.6% and 69.7-79% in 200mM of NaCl concentration in comparison to control on 10 days and 15 days span respectively. In biochemical estimation the proline accumulation, reducing sugar accumulation and malondialdehyde content there were sharp increase in all varieties of rice with respect to the increasing level of salt stress but the rate decreases as the time of exposure is increased. As in case of Maharaja variety the proline accumulation increase was 4.6 times and slowly reduced to 3.6 times, similarly for malondialdehyde content increase was 1.6 times and became 1.4 and also in reducing sugar accumulation the trend was 1.4 times and reduced to 1.1 times in 200mM of NaCl concentration with respect to control in 10days span and 15days span respectively. This study reveals all the four locally cultivable rice varieties of rice seedlings were trying to cope up as the time of salt exposure is increased.

KEY WORDS: Rice, Salt Stress, Reducing sugar, proline, lipid peroxidation.

1. INTRODUCTION

Salinity is one of the important abiotic stresses that affect growth, physiology, and biochemistry of plants. Soil salinity has lead to the loss of crop productivity of 20% of the cultivated and irrigated area of the entire world (Zheng, 2001). Seedlings when exposed to salinity, have shown to induce various physiological and developmental changes (Islam, 2008). Due to this problem, rice production is facing a problem globally (Hong, 2007). Salinity plays a role in different stages of growth of rice varieties considering both physiological and biochemical aspects (Jamil, 2012). This affects plant growth in various ways like water uptake reduction, toxic accumulation of sodium and chloride, and reduction in nutrient availability. Seedlings have been shown to cope up with this stressed situation in several ways. Among these osmoregulation is one of the best physiological functions**.** Here, the cell osmotic pressure is adjusted by the plant cells using low-molecular-mass compounds, like carbohydrate, proline, glycine-betaine and sugar alcohol (Hasegawa, 2000). Sugar that is produced by photosynthesis in higher plants is a major energy source, plays a critical role in signal transduction in both primary and secondary metabolites. Sugar is the building blocks of macromolecules in the developmental processes of plants (Smeekens, 2000; Price, 2004) which has been shown to play a key role in various salt defense mechanisms, viz, interaction with phospholipid head groups and reactive oxygen species detoxification (Bentsink, 2000; Bohnert, 1996; Roy, 2005). It has been found that, the defense response signals are inside cell are usually regulated in two ways; one is the case when downstream, and the cell consequentially generates secondary messengers like calcium and reactive oxygen species (ROS) (Siringam, 2012) often increased. ROS may lead to metabolic imbalance and cause cell damage by a lipid peroxidation mechanism, resulting in oxidative stress. The objective of this study was to observe the effect of salinity on four local rice varieties to evaluate the biochemical and morphological effect under salinity based on the accumulation of proline and reducing sugars, as well as lipid peroxidation, and growth performance in salt stressed seedlings.

2. MATERIALS AND METHODS

Plantation and morphological studies: Four locally cultivable varieties of rice (*Oryza sativa L.*) (Niranjan (N), Ranjan (R), Swarnamasuri (SM) and Maharaja (M) were chosen for the experiments which were collected from the farmers in Hooghly and Bankura district, West Bengal. The rice seeds of all the varieties were kept in wet soil in cups for germination and seedling growth. The seedlings of all cultivars were then separated into 5 groups based on the saline concentrations as follows: 0 (control), 50, 100, 150, 200mM of NaCl. Rice seedlings were cultured in vitro in a culture room for 15 days, under conditions of 25±2°C temperature, 60±5% relative humidity (RH), 60±5 μmol m^{-2} s^{-1}. Rice seedlings from each concentration were randomly selected after 10 and 15 days intervals for the measurements root and shoot length and for quantification of reducing sugar content, proline content and lipid peroxidation as well.

Reducing sugars extraction and estimation: Estimation of reducing and non-reducing sugar levels was done using Dinitrosalicylic acid (DNSA) reagent by following the method of Miller (1972). Fresh rice seedlings of 0.5g material was sugar analysis was taken into consideration of with or without salt concentrations (0-200 mM NaCl) were homogenized in hot 80% ethanol. The extract was centrifuged at 5000 g for 15 min at room temperature and the supernatant was evaporated by keeping in water bath at 80°C; sugars were dissolved by adding 10 ml distilled water. Reducing sugars were estimated by using DNSA reagent colorimetrically at 530 nm wavelength and calculated from graph plotted using glucose as a standard.

Estimation of proline: Proline content was analyzed by the modified procedure of Bates (1973). For the experiment 0.5 gm of fresh of rice seedlings were homogenized in 3% Sulphosalicyclic acid. Homogenate was filtered. 10ml of filtered homogenized extract was reacted with 1ml of freshly prepared acid ninhydrin solution along with 1ml of glacial acetic acid then it was was heated at 100°C for 45 min in a water bath. The reaction of the mixture was then stopped by using an ice bath. The mixtures were then extracted with toluene and measured using an UV-visible spectrophotometer. The proline concentration was determined using standard curve and expressed as µmole proline per g fresh weight of tissue.

Estimation of malondialdehyde: The level of lipid peroxidation in the tissues was determined as 2-thiobarbituric acid (TBA) (a reactive metabolites chiefly to work with malondialdehyde (MDA) accumulation) as described by Heath and Packer (1968). This protocol is slightly modified from the original one where 1gm of rice seedlings were homogenized in 20 ml TBA (0.25%) made in 10% Tri-chloroacetic acid (TCA). Extract was heated at 95ºC for 30 min and then quickly cooled in ice. After centrifugation at 10,000g for 10 min, the absorbance of the supernatant was measured at 532 nm. The level of lipid peroxidation is expressed as µg MDA g-1 F.W.

Statistical Analysis: The experimental design was completely randomized with five replicates per treatment. Statistical analysis was performed using one-way ANOVA and the difference between the mean values was compared using Duncan's Multiple Range Test (DMRT) at the $p<0.05$ level.

3. RESULTS

Morphological result: Salt stress had reducing effect on shoot and root growth of rice seedlings. In all the varieties of rice seedlings the root and shoot length decreased rapidly initially but as the time span increased the rate of decrease was reduced. This trend was observed in all the varieties as the concentration varied viz. root growth decreased 9.8- 46.4% in 50 mM and 60-95.2% in 200mM of NaCl on 10 days span whereas, it was 7.9-16.9% in 50mM and 65.2-70.2% in 200mM of NaCl concentration in comparison to control on 15 days span; similarly the shoot growth decreased 21.9-34.3% in 50 mM and 57.3-80.6% in 200mM of NaCl on 10 days span whereas, it was 14.7-20.1% in 50mM and 69.7-79% in 200mM of NaCl concentration in comparison to control on 15 days span in the four rice varieties. The changes in both the shoot and root length in all the varieties were statistically significant at 5% level of significance (Fig.1A, 1B).

Figure.1. Effect of increased NaCl concentration on shoot length (A), root length (B) [10days and 15days seedlings of Ranjan (R), Swarnamasuri (S), Maharaja (M), Niranjan (N)]

(Values are mean ±SE of five measurements per data point)

Reducing sugar accumulation: The reducing sugar accumulation increased in the four varieties of rice seedlings; In 200mM NaCl the increase rate was 3.5-10.4 times more than 50mM NaCl with respect to control in 10 days and it was 3.2-9.3 times in 15 days span respectively The changes in all the varieties were statistically significant at 5% level of significance except for the Niranjan variety (Fig.2A, 2B**).**

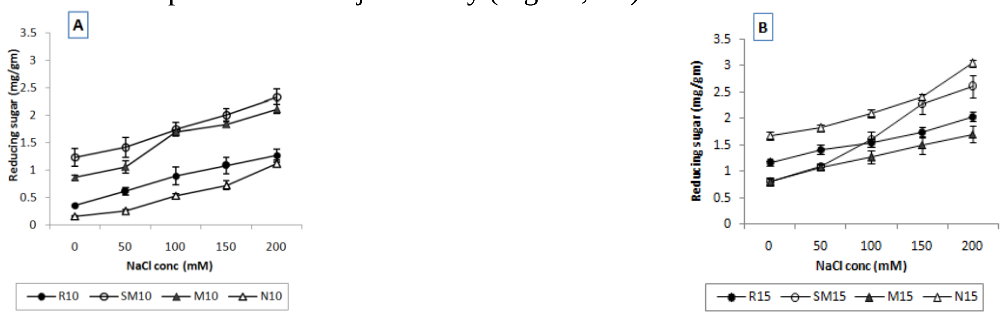

Figure.2. Effect of increased NaCl concentration on reducing sugar accumulation in seedlings of [A]10days, [B] 15days [Ranjan (R), Swarnamasuri (S), Maharaja (M), Niranjan (N)]

(Values are mean ±SE of five measurements per data point)

Proline accumulation: There was a significant positive correlation between proline accumulation and salt concentration. The proline accumulation increased in all the four varieties of rice seedlings at the highest concentration i.e. 200mM NaCl concentration of exposure. Proline accumulation was 3.1-12.7 times more in 200mM NaCl than with 50mM NaCl with respect to the control in 10 days; and it was 4.5-7.6 times in 15 days span, respectively. The changes in proline content in all the varieties were statistically significant at 5% level of significance (Fig.3A, 3B).

Figure.3. Effect of increased NaCl concentration on Proline accumulation in seedlings of [A] 10days, [B] 15days [Ranjan (R), Swarnamasuri (S), Maharaja (M), Niranjan (N)]

(Values are mean ±SE of five measurements per data point)

Result of Lipid Peroxidation (MDA): In case of lipid peroxidation**,** there is an increased concentration of MDA in all the four varieties of rice seedlings from 50mM to 200mM during both 10 days and 15 days time-span *i.e.*, in 200mM NaCl, Lipid peroxidation was increased 2.1-6.5 times more than with 50mM NaCl with respect to the control in 10 days; and it was 2.9-5.8 times more in 200mM than with 50mM in 15 days span respectively. The changes in Niranjan and Ranjan varieties were not statistically significant at 5% level of significance (Fig.4A, 4B).

Figure.4. Effect of increased NaCl concentration on Lipid Peroxidation (malondialdehyde content) in seedlings of [A] 10days, [B] 15days [Ranjan (R), Swarnamasuri (S), Maharaja (M), Niranjan (N)]

(Values are mean ±SE of five measurements per data point)

DISCUSSION

It has been observed that the deleterious effects of salinity stress on plants causes osmotic stress. This, in turn, is due to water deficit and the effects of excess sodium ions on critical physiological parameters and biochemical processes. Rice cultivars chosen for this study represented the salt tolerance capacity to compare different patterns of physiological and biochemical responses. It was found from the above study that, maximum rice seedling growth occurred at 50mM salt concentration after the control set. Similar type of results were reported where the length of roots and shoots were reduced with the increasing salt concentrations (Jamil, 2012).

Many plants show an increased accumulation of sugar when exposed to salinity (Gilbert, 1997). Salinity stress in rice induce increase in concentrations of reducing sugars and non reducing sugars as well as elevated activities of sucrose phosphate synthase (Dubey, 1999). The phenomena of sugar accumulation has been associated with drought and salinity tolerant mechanisms in many species. It is observed the accumulation of compatible solutes is accompanied by the influx of water into or at the least a reduced flux from cell which results the increase in cellular osmolarity which provide the turgor pressure for cell expansion (Hare, 1998).

There are several compatible solutes which accumulate in plants in response to a wide variety of environmental stresses and confer stress tolerance by contributing to osmotic adjustment; protecting proteins and membranes; and by quenching reactive oxygen species where Proline has been widely considered to be one of the most compatible solutes in this respect. The present work reveals that all four rice cultivars showed an increase in proline levels in seedlings with increasing salinity levels as compared with the control sample plants on both days of exposure. Similar findings were observed in other plants e.g. in six different citrus cultivars where proline content in leaves was higher and was more useful than that in roots as a determinant of the level of salt stress experienced by plants (Matysik, 2002).

Membrane injury under salt stress is related to increased production of highly toxic reactive oxygen species. There are reports that salinity actually disrupts membrane permeability by peroxidation of the lipid membrane (Hernandez, 2000). Lipid peroxidation measured as the amount of MDA is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals. Lipid peroxidation is ascribed to oxidative damage and is often used as an indicator of increased damage (Hernandez, 2000; Bor, 2003; Meloni, 2003). Similar kind of result found a sharp increase in MDA accumulation in rice during the two first weeks of stress (Lutts, 1996). The present work reveals that all four rice cultivars showed an increase in reducing sugar levels in seedlings with increasing salinity levels as compared with the controlled plants in both days of exposure.

4. CONCLUSION

It was concluded from the study that salinity affects physiological and biochemical characteristics of all the four locally cultivable rice varieties but the response varied depending upon the different days of exposure on seedling growth. It also can be concluded from this study that salinity stress alters carbohydrate metabolism in all the rice varieties. The present data reveals a marked increase in the accumulation of proline as it plays an important role in osmotic adjustment in plants under salt stress which is clear from the present results for all the rice varieties. In this investigation, MDA formation was used as an index of lipid peroxidation and the data here shows that MDA content increased with increasing NaCl concentration. From this it also can be concluded that salinity induces an increase in lipid peroxidation. This study says that all the varieties of rice seedlings were trying to cope up as the time of salt exposure is increased.

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