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Measurement of Na^+ Ions in the Root of Rice (*Oryza sativa* L.) under Osmotic Stress Condition at Seedling Stage

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Abstract

Salinity is a major abiotic stress severely threatening the cereal crop production all over worlds. The effect of the osmotic component of salt stress were examined on rice cultivar IR64, Sahbhagidhan and pokkali.Seeds of selected high yielding three rice genotypes (IR64, Sahbhagi Dhan, Pokkali) were germinated in the dark for 3-4 days under normal temperature and pressure.These seedlings were transferred to hydroponic media (Yoshida media) for the culture. The seedlings were allowed to grow for 15 days (days after germination) with continuous media aeration at 25°C illuminated at 400-500 mol $m^{-2}s^{-1}$ using fluorescent lighting (Philips, Kolkata, India) in a day and nightcycle of 48 hours each. The nutrient solution was replaced at 5 days intervaland study biochemical effect. Biochemical studies provided the evidence that Pokkali improved their tolerance by strengthening their antioxidant defensive mechanism. Further studies are required with longer stress duration to achieve a higher Na^+ concentration in plant tissue in several varieties with contrasting tolerance to salt stress.

Key words : *Na^+ ions, osmotic stress condition at seedling stage, ion homeostasis, peroxidase activity, rice.*

Introduction

Rice (*Oryza sativa* L.) is one of the major staple food crops for about 65 % of the world's population and it requires large amount of water to grow as compared to other plants. Rice is one of the important sources of dietary energy and provides a good proportion of proteins (7.3%) and minerals (fat 2.2%, carbohydrate 71.1 %, zinc 2% and iron 3% and dietary fibres). Soil degradation occurred due to Stalinization are considered as major constrainof agricultural productivity. In current scenario approximately 1000 million hectare of land are effected (1). Water is a crucial factor in agricultural and food production. Drought stress is the most prevalent environmental factor limiting crop productivity (2) and global climate change is increasing the frequency of severe drought conditions. Drought and salinity is one of the major abiotic stresses limiting crop production (3). The sheer diversity of plant species grown across climatic regions that include extreme dry conditions suggests that in nature, plants have evolved to endure drought stress with an array of morphological, physiological and biochemical adaptations. The overall rice yield variability due to climate variability over the last three decades has been estimated and it was concluded that approximately 53% of rice harvesting regions experiences the influence of climate variability on yield and approximately 32% of rice yield variability is explained by year-to-year global climate variability (4).

Rice (*Oryza sativa* L.) is highly susceptible to the salinity than other cereals. High sensitivity has been observed at vegetative and reproductive stages in rice. Salt stress has been a serious threat for crop production in irrigated land, as expected salt stressed irrigated land is more than 20% (5) and estimated stressed area will expand to 50% of irrigated land by 2050 (6). High salt level affects seed germination, plant life and crop productivity (7). Rice plant responses to salt stress are complex and depend on duration and type of salt stress, development stage of rice, day length, and other factors (8). Soil salinity, particularly due to NaCl, can be considered as the single most widespread soil toxicity problem that global rice production faces at present (9), and suppressed leaf expansion which ultimately reduces photosynthetic area and dry matter production (10). K^+ in plant tissues evidently decreases when plants are exposed to salt stress, especially rice genotypes.

Effect of osmotic stress on rice : Osmotic stress conditions, such as salinity, drought and low temperature are important factors limiting plant growth and crop productivity. Seed germination is one of the most crucial stage in seedling establishment, determines successful crop yields (11,12). The Salinity and drought creates osmotic stress affects which reduced water uptake or through ionic imbalance due to toxic effects of sodium (Na^+) and chloride (Cl^-) ions and it affect seed germination. At low concentrations, salt suppresses rice plant growth and at higher concentrations can cause death (11) and it

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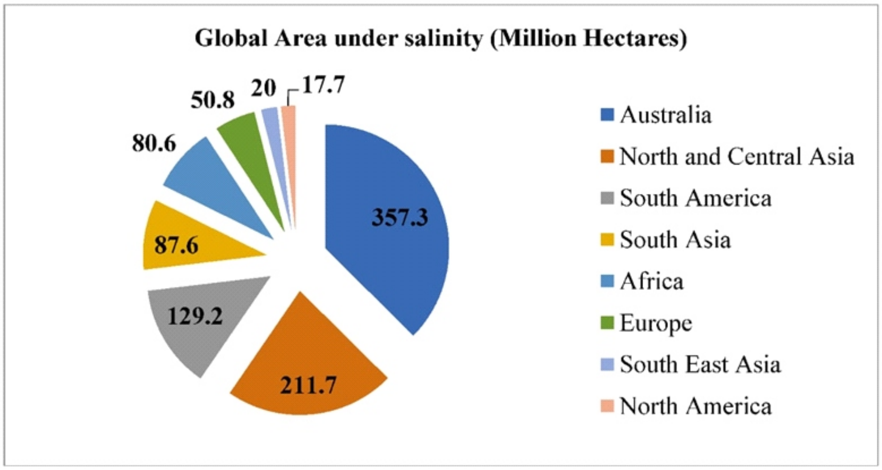

Fig.-1 : Global distribution of salt-affected area.

causes substantial loss in seedling establishment, leaf chlorosis, and final yield reduction are observed in salt-affected rice growing areas (12). Drought has been reported to severely reduce germination and seedling stand.

Crop growth and yield under osmotic stress : Rice is considered as a salinity sensitive crop in the field as compared to other main cereal crops (13). Salt stress strongly influences the morphology traits of rice plants (5). Seedling growth and fresh weight decreased with increased salt stress (14). Root of the plant has direct contact with the biotic and abiotic factors in the soil environment and the rest of the plant. Water and solutes enter plant by the roots with the help of combination of several different pathways such as symplastic, apoplastic and trans-cellular pathway. Transport of water and solutes *via* apoplastic transpiration pathway is very important in rice. Mostly, Na^+ passages in shoots of rice through the apoplastic pathway where Na^+ transports *via* apoplast through solvent drag and Casparian bands. Significant increase in mean root length, mean root numbers per plant, and decrease in shoot length occurred under increased salt stress (5). Thus, root and shoot lengths are two indicators of rice plant response to salt stress.

Ion homeostasis during osmotic stress : The homeostasis of intracellular ion concentrations is fundamental to the physiology of living cells. Proper regulation of ion flux is necessary for cells to keep the concentrations of toxic ions low and to accumulate essential ions. Plant cells employ primary active transport, mediated by H^+-ATPases and secondary transport, mediated by channels and co-transporters to maintain characteristically high concentrations of K^+ and low concentrations of Na^+ in the cytosol.The homeostasis of intracellular ion concentrations is fundamental to the physiology of living cells. Intracellular K^+ and Na^+ homeostasis is important for the activities of many cytosolic enzymes and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation. Under salt stress, the maintenance of K^+ and Na^+ homeostasis becomes even more crucial. Thus, the regulation of ion transport by salt-stress signaling provides a model case for understanding the general regulation of ion homeostasis in plant cells.

Osmotic adjustment : Tolerance or resistance of rice plant to salt stress involves many adaptive responses at molecular, cellular and physiological levels**.** Osmotic adjustment is the most important physiological aspect, as it determines the tolerance limit of rice plant to bear toxic ions concentration. The most favorable strategy to reduce the accumulation of NaCl in the cytoplasm of the plant cell is through compartmentalization in vacuoles.

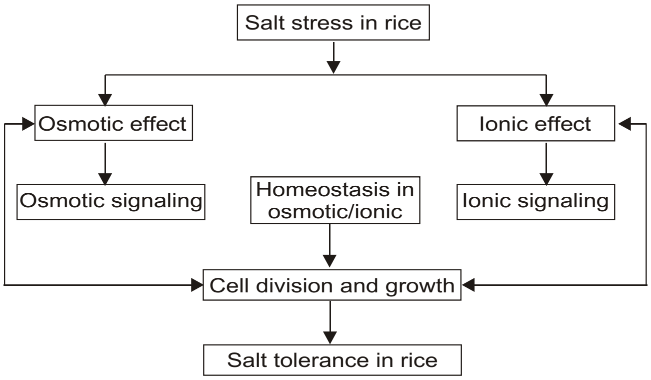

Fig.-2 : Salt stress response in rice plant.

Materials and Methods

Plant material and experimental design : Seeds of selected high yielding three rice genotypes (IR64, Sahbhagi Dhan, Pokkali) were germinated in the dark for 3-4 days under normal temperature and pressure .These seedlings were transferred to hydroponic media (Yoshida media) for the culture. The seedlings were allowed to grow for 15 days (days after germination) with continuous media aeration at 25°C illuminated at 400-500 mol m^{-2} s^{-1} using fluorescent lighting (Philips, Kolkata, India) in a day and nightcycle of 48 hours each. The nutrient solution was replaced at 5 days interval.

Stresprotocol : 15 days old plants grown seedling in Yoshidamedia, were divided into two groups. The first group was kept untreated (control) and the second group was treated with 200mM NaCl (salt) for 48 hours. Morphological and biochemical studies were performed at the seedling stage.

Morphological studies : Root length of plant was studied for all three genotypes under control and stress conditions. Relative change of root length of plant for each genotype was calculated.

Protein extraction : 15 days seedlings after stress treatment for 48 hours, roots are harvested. Harvested roots were washed 2-3 times with distilled water, crushed in liquid nitrogen properly and diluted it in potassium phosphate buffer for 2ml, centrifuged the crushed sample at 1g at 4°C.

Quantification of total protein : Total protein was quantified according to (7). The absorbance of Coomassie Brilliant Blue after binding with the protein was recorded at 595 nm. Total protein was estimated from the BSA standard curve.

Assay of antioxidant enzyme activities : Lyophilized plant tissue was homogenized with ice-cold phosphate buffer (pH 7.0). The plant extract was used to detect different antioxidant enzyme activities.

Superoxide dismutase (SOD) activity : Superoxide dismutase activity was determined at 560 nm by its ability to reduce the formation of blue colouredformazone by nitro blue tetrazolium and O_2^- radical (16).

Peroxidase (POX) activity : Peroxidase activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol (17). The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm for 1 min.

Elemental studies : Plants exposed to salt stress were harvested and washed extensively with distilled water to remove surface contaminating Na^+. The roots were separated from shoots. Weighted the 0.2 g root tissue ,0.01% HNO_3 added in the test tube and heated up to 85-90°C for 30 minutes; transferred the sample into beaker by filtering it by whatman#40 filter paper. The Na^+ and K^+ levels in the acid-digested sample that represents total Na^+ and K^+ in the tissue sample was estimated in PPM by using a Flame photometer (Systronics Flame Photometer 128).

Results and Discussion

Rice genotypes showed increase in root length in response to drought as compared to their normal condition (fig.-3). Sahbhagi Dhan showed increase in root length by 22.83% after 200 mM NaCl (salt) induced salinity for 48 hour, IR64 also exhibited higher increase in root length 10.11% increase after 48 hours salt stress; whereas, Pokkali showed the minimum increase in root length 3.4% increase after 48 hours salt stress.

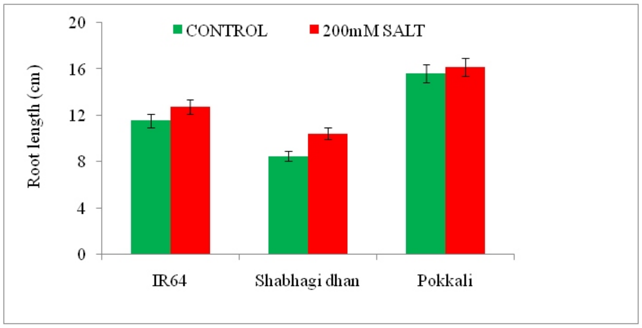

Fig.-3 : 200 mMNaCl induced salinity stress disturbed growth of rice seedlings.

Salt stress resulted in decreased the total protein content in considered genotype. Maximum decrease in protein content was observed in IR64 15.92 % after 48 of stress; whereas, minimum decrease was observed in ShabhagiDhan (5.16 %) and Pokkali (8.97%) after 48 hours of salt stress (fig.-4).

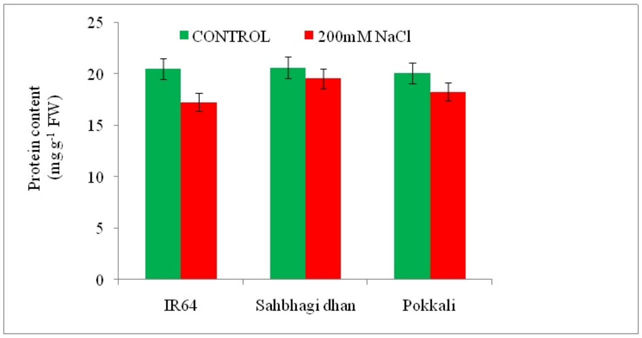

Fig.-4 : 200 mMNaCl induced salinity stress resulted in decreased total protein content.

Osmotic stress increased the antioxidant enzyme activities in all rice genotypes. Successive increase in duration of drought stress increased the antioxidant enzyme activities in all rice genotypes. Superoxide dismutase activity was found to be increased in response to drought as compared to that of the normal condition (Fig.-5). During salt stress, IR64 showed maximum increase in the superoxide dismutase activity; whereas, Sahbhagi Dhan and pokkali showed 69 % and 59% increase, respectively. Pokkalihad the minimum decrease

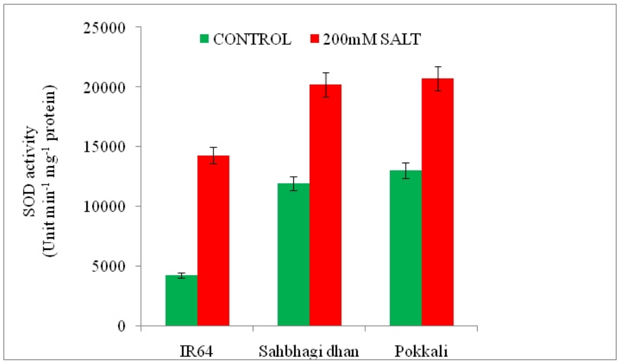

Fig.-5 : Effect of osmotic stress on superoxide dismutase activity of root in different rice genotypes at seedling stage under 200mM NaCl-induced salt stress.

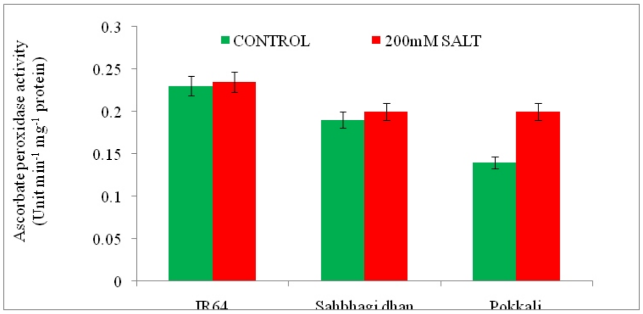

Fig.-6 : Effect of osmotic stress on ascorbateperoxidase activity of root in different rice genotypes at seedling stage under 200mM NaCl-induced salt for 48 hours.

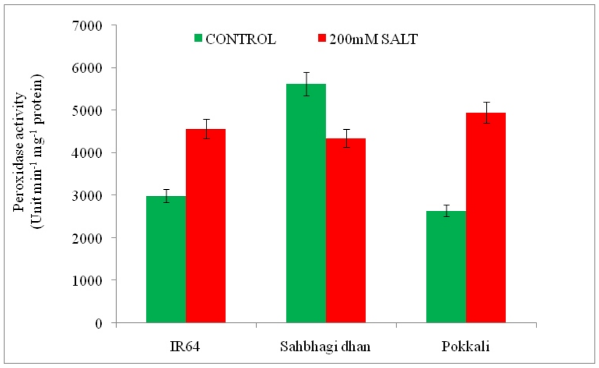

Fig.-7 : Effect of osmotic stress on peroxidase activity of root in different rice genotypes at seedling stage under 200mM NaCl-induced salt stress.

in the superoxide dismutase activity after 48 hours of salt stress.

Ion accumulation during NaCl induced salinity stress : Rice genotypes showed increase in sodium and potassium ion concentration in response to drought and salt stress as compared to their normal condition (Fig.-9). Sahbhagi Dhan showed increase in sodium ion concentration by 3.53% after 200 mM NaCl induced salinity for 48 hours. IR64 also exhibited higher increase in sodium ion concentration by 15.48% after 48 hours salt stress; whereas, Pokkali showed the minimum increase in sodium ion concentration (11 %) after 48 hours salt stress (Fig.-9). Sahbhagi Dhan showed 23.32% increase in potassium ion concentration. IR64 and Pokkali showed 25.65 and 27.59% respective increase in potassium ion concentration after 48 hours of 200m MNaCl stress.

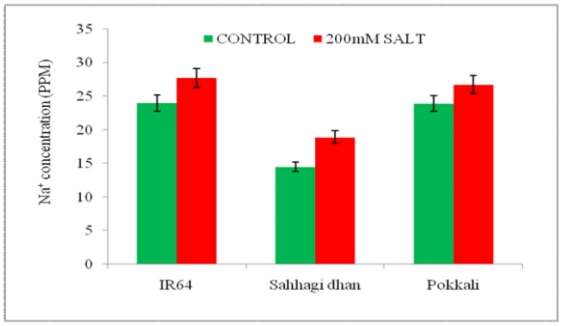

Fig.-9 : Sodium and Potassium ion accumulation inside root of different rice genotypes at seedling stage, 200mM NaCl salt induced and figure, 48 hours respectively.

In the present study, NaCl was used to create osmotic stress in soil solution. Osmotic stress interrupted seed germination of all rice genotypes as described by drought stress increased root growth; whereas, relative root in root length was different depending upon their relative adaptability and tolerance mechanism. Maximum increase in root length was shown by Sahbhagi Dhan and relatively lower increase in root length was shown by IR64 and Pokkali than normal condition during the 200mM NaCl induced salt stress. Osmotic stress is also associated with higher accumulation of ROS (18). Deleterious effect of ROS on cell was determined by the equilibrium between the production and detoxification of ROS through antioxidant enzyme system (19). Reactive oxygen species-induced cell toxicity could be eliminated in Pokkali. Through strong antioxidant defence mechanism against ROS built-up. Furthermore, increased activity of SOD, POX and APX in Pokkali seedlings might result in enhanced ROS scavenging, providing better protection under drought stress (20).

Under the NaCl treatment, rice roots absorbed Na$^+$from the soil solution, subjecting the plants to both ionic and osmotic stresses. The high concentration of Na$^+$ in the plant treated with NaCl suggested that the culture in NaCl solution imposed ionic stress on the plant, in addition to osmotic stress. It can be argued that excess NaCl could lead to the loss of K$^+$due to membrane depolarization and displacement by Na$^+$ ions (21). The two cations also have similar atoms and the K$^+$ transporters are known to be less specific attoxic levels of Na$^+$.

Conclusions

Present study underlined the search for a rice genotype with better tolerance towards drought grounded on morphological, biochemical analyses. In summary, Pokkali exhibited considerably enhanced salt tolerance at seedling. Biochemical studies provided the evidence that Pokkali improved their tolerance by strengthening their antioxidant defensive mechanism. Thus Pokkali may be considered as the potential osmotic tolerant rice genotype.

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