EFFECT OF NACL INDUCED SALT STRESS ON PROLINE, MDA AND ANTIOXIDANT MECHANISM IN RICE (ORYZA SATIVA L.)

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Received: June 12, 2015; Accepted: July 4, 2015

Abstract: The aim of this study was to examine possible alterations in the metabolism of rice plants (Oryza sativa L.) that were exposed to high salt concentrations. NAUR1 seedlings were treated with Hoagland’s solution containing 25, 50, 100 and 200 mM NaCl and maintained for 14 days in these conditions. After 14 days there was a gradual increase in the levels of lipid peroxidation, proline and catalase (CAT); and reduced activity of antioxidant enzymes viz., superoxide dismutase (SOD), and guaiacol peroxidase (GPOX) with increasing salt concentrations in the Hoagland’s solution. Glutathione reductase (GR) activity increased at 25 mM but decreased at 50, 100 and 200 mM salt concentrations. These results suggested that proline may have protective effects against protein degradation. Moreover, although antioxidant enzymes such as SOD and GPOX shown to possess low levels of activity, a large proportion of the hydrogen peroxide that produced is preferentially directed towards lipid peroxidation. High catalase and lower guaiacol peroxidase activities may be regarded as potentially useful biochemical indicators for selection of salt tolerant rice cultivars and targets for improvement through transgenic approaches.

Key words: Salt stress, Oryza sativa

INTRODUCTION

Rice is the most important cereal crop and considered as stable food accounting for 50–80% of daily calorie intake and about 17 % of the protein. More than 90% of the world’s rice is produced and consumed in Asia, where 60% of the people live. In India, rice is grown on about 44.5 M ha and provides food for more than 70% of the population and serves as the principal energy source for most of the people.

Rice is moderately salt sensitive crop species [1]. According to UNEP (United Nation Environment Programme) report, globally 20% of agricultural land under irrigation has become salt affected [2]. Yield losses due to salinity are amounted to 30-50%. Soil salinity in plants imposes ion imbalance or disequilibrium, hyperionic and hypersomotic stress, disrupting the overall metabolic activities and thus, limiting the productivity of crop plants worldwide [3].

Under osmotic stress, proline acts as an osmoregulator and osmoprotectant help to maintain plant growth and development. Overexpression of genes leading to more proline synthesis in transgenic rice resulted in an enhancement of salt tolerance [4]. Free radicals, when produced in excess, may be destructive to cells by reacting with the unsaturated fatty acids of phospholipid membranes, altering their functionalities and promoting lipid peroxidation. Production of ROS, such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide,
is enhanced. Which in turn cause damage to the biomolecules such as membrane lipids, proteins, chloroplast pigments, enzymes, nucleic acids etc. [5]. Many enzymatic activities of plants are adversely affected by high Na\(^+\) concentration [6].

The present study aims to determine the effect of salt on antioxidant enzyme activities, lipid membrane peroxidation and proline content. The comparison of these responses will be useful in identifying the similarities and the differences related to the relative ability of the rice seedlings to cope with different pattern of responses to salinity and useful as potential targets for improvement of rice cultivars by conventional and transgenic approaches.

**MATERIALS AND METHODS**

**Plant growth conditions:** Seeds of rice (O. sativa L. ssp. indica) from cultivar NAUR1 were disinfected with 70% alcohol with tween 20 for 10 min followed by HgCl\(_2\) for 4 min, washed thoroughly and then imbibed in distilled water for one day. After the imbibitions, approximately 15-20 seeds were planted onto bottle containing half-strength Hoagland’s solution with 25, 50, 100 and 200 mM NaCl and maintained for 14 days in these conditions. The inoculated seeds were then incubated at 25 ± 1°C with a 14/10 h (light/dark) period with a photon flux density of 100 µmol photon m\(^{-2}\)s\(^{-1}\). Control seedlings were kept in Hoagland solution without NaCl.

**Malondialdehyde (MDA) determination and proline content:** Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction [7]. Leaves (0.1 g) were homogenized in 5 ml of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 3,500 x g for 20 min and the supernatant was used for the assay [9].

**Antioxidant enzymes preparation and assay:**

Extract for determination of antioxidant enzymes activities were prepared from 0.3 g of leaves homogenized with a pre-chilled mortar and pestle under ice cold condition in 3 ml of extraction buffer containing 50 mM sodium phosphate buffer (pH 7.4) with the addition of 1 mM EDTA and 1% (W/V) polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 7378 x g for 20 min and the supernatant was used for the assay [9].

Total SOD (EC 1.15.1.1) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitroblue tetrazolium (NBT). The 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8) 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 0.1 ml enzyme extract, riboflavin was added last [10]. Test tubes were shaken and placed 30 cm below from a light blank consisting of four 15-w fluorescent lamps. The reaction was allowed to run for 10 minutes and stopped by switching the light off. The photoreduction in NBT was measured as increase in absorbance at 560 nm. Blanks and controls were run the same way but without illumination and enzyme, respectively. One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50% in a reaction mixture.

GPOX (EC 1.11.1.7) activity was determined in the homogenates by measuring the increase in absorption at 470 nm due to the formation of tetraguaiacol (ε = 26.6 mM\(^{-1}\) cm\(^{-1}\)) in a reaction mixture containing 50 mM sodium phosphate buffer pH 7.0, 0.1 mM EDTA, 0.05 ml enzyme extract, 10 mM guaiacol and 10 mM H\(_2\)O\(_2\) [9].

Total catalase (EC 1.11.1.6) activity was determined in the homogenates by measuring the decrease in absorption at 240 nm as H\(_2\)O\(_2\) (ε = 39.4 mM\(^{-1}\) cm\(^{-1}\))
Enzyme activity expressed as µmol H$_2$O$_2$ oxidized min$^{-1}$g$^{-1}$ protein. The 3 ml mixture containing 50 mM sodium phosphate buffer (pH 7.0), 10mM H$_2$O$_2$ and 50 µl enzyme extract.

GR (EC 1.6.4.2) activity was assayed by recording the increase in absorbance at 412 nm in the presence of oxidized glutathione (GSSG) and 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) [12]. The reaction mixture contained 1 ml of 0.2 M potassium phosphate buffer (pH=7.5) containing 0.1 mM EDTA, 0.5 ml of 3 mM DTNB in 0.01 M potassium phosphate buffer (pH=7.5), 0.1 ml of 2 mM NADPH, 0.1 ml enzyme extract and distilled water to make up a final volume of 2.9 ml. Reaction initiated by adding 0.1 ml of 2 mM GSSG. Molar extinction coefficient of β-NADPH considered as 6.22 mM$^{-1}$ cm$^{-1}$. Protein content of samples was estimated by Lowry method, bovine serum albumin was used as a standard [13].

Statistical analysis: The mean values were taken from measurements of four replicates and standard error (SE) of the means was calculated. Data obtained by biochemical constituents and enzymes determination were subjected to simple completely randomized design for study in the significance of various data using “F” test [14].

RESULTS AND DISCUSSION

Lipid peroxidation and proline content: Peroxidation of membrane lipids is an indication of membrane damage and leakage under salt stress conditions [15]. The leaves of rice seedling after 14 days exposure to 25, 50, 100 and 200 mM salt showed significant increases of 42.97, 60.67, 75.18 and 80.7 % respectively (Fig. 1A) in the MDA concentration compared with the control plants (6.41µM g$^{-1}$ FW). Similar trend of increase in the concentration of MDA was reported by Hossain et al. [16] where in, increase of 25.88 and 35.01% of MDA was recorded in rice seedlings exposed to 75 and 150 mM of salt concentrations respectively, compared with the control plants (63 nmol g$^{-1}$ FW). Proline content also showed gradual increases of 1.04, 1.8, 1.26 and 1.50 fold respectively on exposure to 25, 50, 100 and 200 mM salt in comparison to unstressed plants (2.23µM g$^{-1}$ FW) (Fig. 1B). Lum et al. [17] also reported increased proline content in drought-tolerant variety, Pulot Wangi. The gradual increase in free proline concentrations that were observed in the rice plants during this study indicated that salinity markedly affects them to produce a rapid osmotic adjustment, suggesting that it is a protective mechanism for salt stress in rice plants of the studied cultivar. These results are in consistent with the findings of studies that have been performed using other cereals. For example, Goudarzi and Pakniyat [18] observed a 2.6-fold increase in the proline content of wheat that was irrigated with salt water over a four-week period.

Activities of antioxidant enzymes: The leaves of rice seedling after 14 days exposure to 25, 50, 100 and 200 mM salt showed decrease in SOD and GPOX activity as compared to control. Superoxide dismutase (SOD) is a major scavenger of superoxide (O$_2^-$) and its enzymatic action results in the formation of H$_2$O$_2$ and O$_2$. SOD activity reduced at 35.37, 33.12, 32.36 and 29.54 % than control plant at 25, 50, 100 and 200 mM NaCl respectively (Figure 2A). GPOX activity (Figure 2B) also significantly decreased by 61.26, 51.00, 38.64 and 11.08 % than control (19.41 µM min$^{-1}$g$^{-1}$ protein) in NAUR1. Similarly, Benitez et al. [19] observed a 36% reduction in SOD activity in leaves of rice plant due to salt stress. Kong-ngern et al. [20] also reported that the rice salt-tolerant variety PK showed a slight decrease in GPOX (21.37%) activity than control plants. It is likely to be stated that the lowest SOD activity under salinity might be the key factor for influential membrane damage and increased MDA content and oxidative stress. The SOD activity reduced, it follows by the accretion of O$_2^-$ in leaf cells and as a result there is obstruction of CAT and GPOX activities [21].

Catalase activity of rice var. NAUR1 showed significant increase at 7.14, 13.74, 55.10 and 70.80 % than control plant as the concentration of NaCl increases at 25, 50, 100 and 200 mM NaCl, respectively (Fig. 2C). The action of catalase activity is vital for detoxification of H$_2$O$_2$. The results are in agreement with the previous reports [20,22], who studied the effect of NaCl stress (100-300 mM) on two rice cultivars differing in salt tolerance. They found that the salt-tolerant PK showed higher activity of catalase and lower levels of H$_2$O$_2$ than the salt-sensitive Pusa Basmati 1. Activity of GR increased steadily with NaCl concentra-tration and it nearly doubled (99.94 µM min$^{-1}$g$^{-1}$ protein) in response to 25 mM NaCl. In contrast, there was not much increase
Fig. 1: Effect of salinity on Metabolites constituents. (A) Level of Lipid peroxidation- MDA and (B) Proline in plants of *O. sativa* L., from the cultivar NAUR1, subjected to different concentration of NaCl for 14 days under hydroponics. Column represents the mean of four replicates ± standard errors (SE).

Fig. 2: Effect of salinity on antioxidant enzymes. (A) SOD, (B) GPOX, (C) CAT and (D) GR activity in plants of *O. sativa* L., from the cultivar NAUR1, subjected to different concentration of NaCl for 14 days under hydroponics. Column represents the mean of four replicates ± standard errors (SE).
in the activity of GR at the salt concentrations (50, 100 and 200 mM) compared with the control (Figure 2D). Similar trend was found by Sai Kachout et al. [23], wherein, increase in GR activity of atriplex at 90 mM NaCl for red variety. In contrast, there was not much increase in the activity of GR for green variety at the concentrations 180 and 260 mM compared with the control. Thus, GR is a key enzyme in providing protection against a variety of environmental and abiotic stresses [24]. Activity of GR and hence GSH production is generally elevated in plants upon exposure to xenobiotics and various environmental stresses [25].

**CONCLUSION**

Under salt stress, rice plants especially the cultivar NAUR1 showed that proline may have protective effects against protein degradation. After 14 days of salt exposure, the rice plants showed decreased antioxidant enzymatic activity of (SOD and GPOX) so, the majority of the H$_2$O$_2$ that was produced was preferentially consumed by lipid peroxidation. While higher catalase and lower guaiacol peroxidase activities may be regarded as potential biochemical indicators for selection of salt tolerant rice and targets for improvement through transgenic approaches.

**REFERENCES**