



Differential growth and antioxidant response to salinity stress in two Indian rice cultivars

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ABSTRACT

The present study investigated the changes in the activity of antioxidant enzymes viz., catalase, peroxidase, superoxide dismutase and glutathione reductase as well as morphological parameters such as root length, shoot length and fresh weight in plants of two rice cultivars (Pusa Sugandha- 5 and Parmal) under salt treatment (150 mM) for different time intervals (0, 6, 18, 24, 30 and 48 h). Results were analyzed in a factorial design based on randomized complete blocks with three replicates. Morphological parameters viz., root length, shoot length and fresh weight showed a decreasing trend with increased duration of stress as compared to the control plants. Increased Catalase activity was found in both the cultivars as compared to the control plants. Peroxidase showed higher activity in Parmal than Pusa sugandha-5. SOD and GR were initially higher up to 24 h of stress and thereafter a declining trend was observed. The plants of cultivar Parmal exhibited higher adaptive potential under salinity stress as evident by the changes in growth parameters and antioxidant defence mechanism as compared to another cultivar, Pusa sugandha-5. These results indicate that, Parmal is salt tolerant with respect to Pusa sugandha-5. The outcome of the present work provides collective information of salinity tolerance in these two Indian rice cultivars. This data would be helpful to researchers engaged in transgenic as well as agricultural institutes. It also helps in a better understanding of physiological and biochemical aspects of salinity adaptation which would help in improvement of agronomically important rice crop.

Keywords: Antioxidant enzymes- SOD, Catalase, GR, Peroxidase, Pusa sugandha-5, Parmal

INTRODUCTION

Salinity is a serious concern globally as it is a major factor limiting agricultural productivity. Increased levels of salt in the soil affect the plant's growth and development, thereby, retarding vital physiological processes like photosynthesis, respiration, Nitrogen fixation and carbohydrate metabolism. Salinity stress triggers a wide range of plant responses affecting cellular structures and metabolism. These responses include cellular dehydration causing osmotic stress, accumulation of low molecular compounds (osmolytes) like glycine betaine, proline, sugar, alcohols, increased abscisic acid levels, increased expression of genes, excessive generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide (H₂O₂) and hydroxyl radical (10) (41). These cytotoxic ROS destroy the normal metabolic processes through oxidative damage of lipids, proteins and nucleic acids (20). However, plants have their own complex antioxidant system in both enzymatic and non-enzymatic response mechanisms as a defence strategy to combat and repair the damage caused by ROS.

Oryza sativa L. is one of the most important food crops globally and is considered to be the

primary staple food for half of world's population. Extensive research is being carried out worldwide for further improvement of rice cultivars to adapt according to the environment with high yield. Rice plant is known to be a glycophyte and is hence susceptible to salt stress. Exploring the physiological and biochemical mechanism of salinity could be helpful in the selection of rice cultivar for the agriculturists as well as breeders. Variation in terms of salt tolerance is a common phenomenon in Rice. There are few reports where salt tolerance mechanism in rice cultivars has been studied. Varieties like Pokkali and Nonabokra are considered as salt tolerant and IR-64, IR-72, IR-29 are considered as salt sensitive. (34)

There is not much information about Parmal and Pusa Sugandha-5 in terms of salt tolerance. So the present study was under taken with the aim to study the role played by various antioxidant components in the salt tolerance mechanism of two India rice cultivars, Parmal and Pusa Sugandha-5 which are known to be agronomically important cultivars.

METHODOLOGY

Seeds of Rice (*Oryza sativa L*) Pusa sugandha-5 and Parmal were obtained from IARI, Pusa, New Delhi. All the reagents and chemicals of analytical grade were procured from SRL, Thomas Baker and Hi Media, India. Glassware used was of Borosilicate, Mumbai, India. Seeds were sterilized with 5% sodium hypochlorite for 5 minutes and then rinsed in sterile distilled water three times. For the first two days the rice seeds were kept in distilled water under dark conditions and then the seedlings were shifted to Yoshida media (44) and light conditions. Seedlings were hydroponically grown in half strength Yoshida solution for seven days at $26\pm 2^\circ$ with 16 h light and 8 h dark photo-cycle in the plant growth chamber. Salt treatments (half strength Yoshida solution containing 150mM NaCl) were given to ten days old plants for different time intervals of 6 hours, 18 hours, 24 hours, 36 hours and 48 hours in a temperature and humidity controlled plant growth chamber.

Preparation of extract

For enzyme assays frozen leaf samples (0.5 g) were ground to a fine powder with liquid nitrogen using pestle and mortar and extracted with ice cold phosphate buffer (pH 7.0) (1 ml) The extracts were centrifuged at 4°C for 15 minutes at 10000 rpm and resulting supernatant was used as the cell free extract. Enzyme assays were standardized by following the standard methodology (25), (26).

Morphological parameters

To study the effect of salinity stress (150mM NaCl) on seedling growth after 6, 12, 18, 24, 30 and 48 hours of salt treatment, various growth parameters such as root length, shoot length and fresh weight of the seedling were measured by comparing with the unstressed control seedlings. Since the cultivars (Pusa and Parmal) had different rates of growth under stressed conditions, these cultivars were compared on the basis of the relative percentage change. Twenty five seedlings were taken at every step of experiment and were repeated at least for three times. Standard error was calculated for each treatment.

Antioxidant enzyme assays/ profile

Catalase

To 100 μl of the diluted enzyme extract, 1mL of 50 mM potassium phosphate buffer (ph 7.0) was added. Then 100 μl of Hydrogen peroxide (100 mM) was added to the above mixture to initiate the reaction. The change in absorbance was then recorded using the spectrophotometer at 240 nm with the interval of 15 seconds up to 2 minutes (1).

Glutathione reductase

To 100 μ L diluted enzyme extract, 1mL of 0.1M phosphate buffer (ph 7.6) containing 1mm of EDTA was added along with 100 μ L oxidized glutathione (0.15mm). Reaction was initiated using 100 μ L NADPH (0.63mm) and change in absorbance was taken at 340 nm at every 30 second interval up to 2 minutes (39) .

Peroxidase

To 100 μ L dilute enzyme extract, 0.9 ml of 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA was added along with 0.1mL of 10 mM guaiacol, 100 μ L hydrogen peroxide was added to initiate the reaction. Absorbance values were recorded using spectrophotometer at 470 nm after every 15 seconds interval upto 2 minutes. (17), (33).

Superoxide dismutase

To 100 μ L diluted enzyme extract, 3 mL of reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13mM methionine, 75 μ M NBT and 100mM EDTA was added. The reaction was initiated by adding 2 μ M of riboflavin. The test tubes were covered with foil and placed under 15W fluorescent lamp for 15 mins. The absorbance values were then recorded using spectrophotometer at 560nm (9).

Estimation of protein

Protein concentration was measured by the method of Bradford (5). Bovine serum albumin was used as a standard.

RESULTS AND DISCUSSION

Morphological Parameters

Studies on growth parameters for two rice cultivars Pusa sugandha-5 and Parmal in response to salt stress (150 mM NaCl) for different time durations, showed significant differences. A decrease in relative growth (root length and shoot length) was observed for both the varieties as has also been reported in Chick pea (14). The percent reduction of root length (Figure I) and shoot length (Figure-II) was higher in Pusa sugandha-5 as compared to the Parmal cultivar. There was a significant decrease in growth (66%) in root length of Pusa sugandha-5 as compared to (50%) in Parmal.

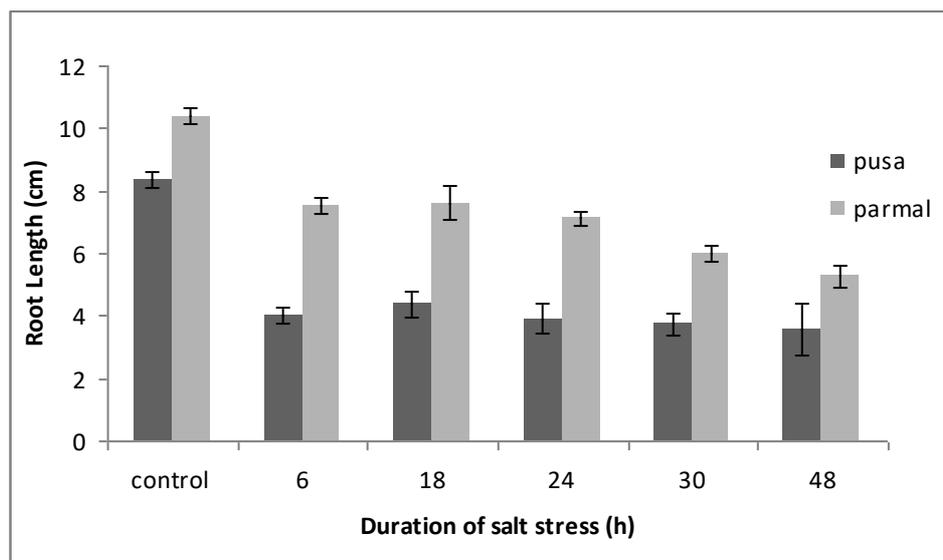


Figure –I Effect of salt stress on relative growth rate in terms of root length of rice varieties Pusa and Parmal (Values are mean from three replicates and bars indicate standard error).

Similarly shoot length in Pusa sugandha-5 also decreased (55%) as compared to Parmal (49%). Earlier reports confirm that percent reduction in shoot length and root length was found to be more in susceptible genotypes (3) (11) (15) Visible signs of stress induced injury were observed during the early growth of seedlings. A change in colour from green to yellow was observed which indicates the adverse effects of stress on photosynthesis as has been reported by Kumar et al., in *Brassica* (24).

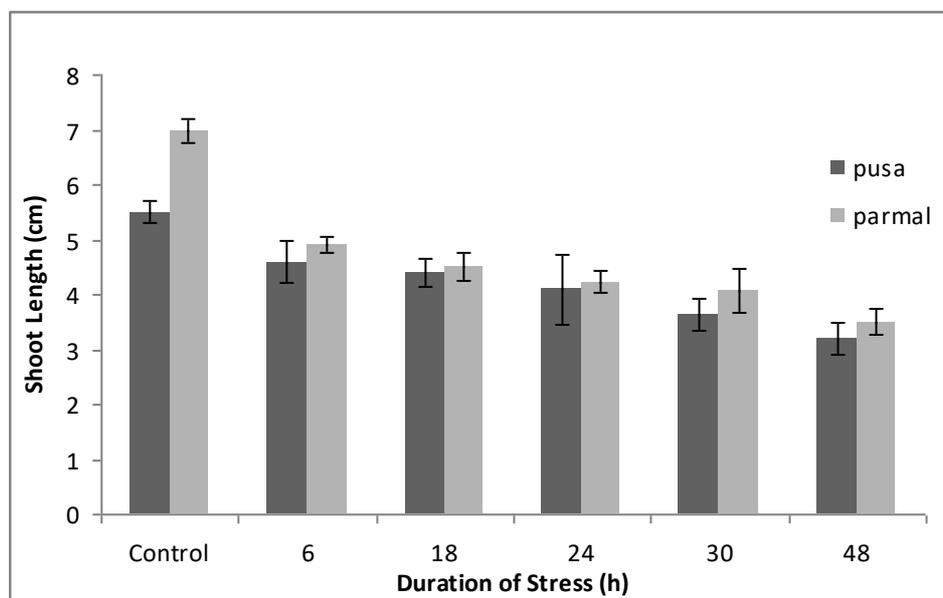


Figure-II. Effect of salt stress on relative growth rate in terms of shoot length of rice varieties Pusa and Parmal (Values are mean from three replicates and bars indicate standard error).

Fresh weight of the rice seedlings was found to be significantly inhibited by higher salinity concentration compared to low and no Salinity (Figure III). Similar results were reported in Rice (10), Wheat (7), Soybean (29) (43) and Chick pea (14).

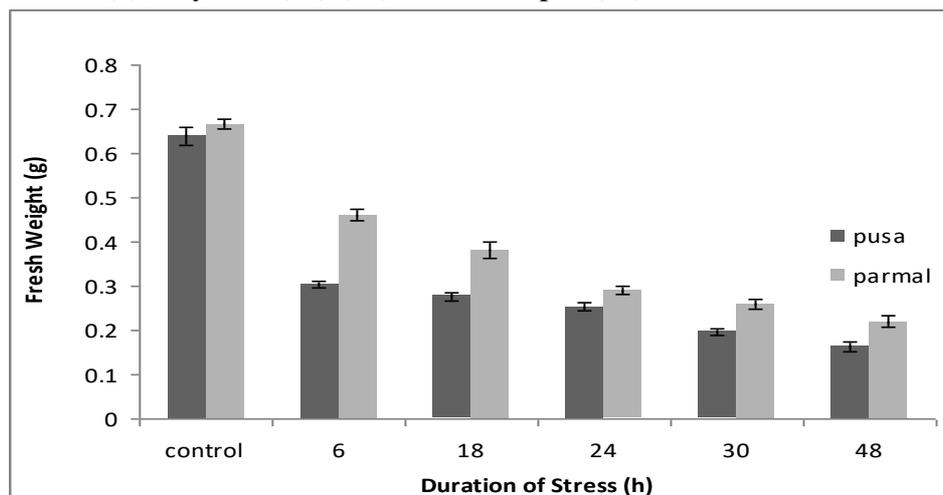


Figure-III. Effect of salt stress on relative growth rate in terms of fresh weight of rice varieties Pusa and Parmal (Values are mean from three replicates and bars indicate standard error).

Antioxidant Enzyme assays

Salt stress induces a rapid increase in ROS production which stimulates the expression and activities of antioxidant enzymes (22). Bellaire et al (2000) indicated that NaCl- induced increase in antioxidant activity is an early event during acclimation to high salt conditions (4). Manchandia et al.,(1999) suggested that rapid up-regulation of antioxidant activity provides an initial defence against cellular damage from an oxidative outburst (30).

In the present study, a gradual increase in the activity of catalase was observed with increasing stress durations in both Parmal and Pusa cultivars as compared to their respective controls. The activity of catalase was higher in Pusa as compared to Parmal (Figure IV). The activity of catalase in Pusa increases from 0.95 units after 6 hour stress to 2.68 folds after 48 hours of stress where as in Parmal the increase was only up to 1.57 folds. Our results were in accordance with the work of Hattemshabrawi et. al., (17) and Hernandez et. al (19), and Dat etal (6). Shim.,etal, had concluded that catalase activity is a determining factor of salt tolerance in rice (36). However catalase activity in rice leaf is often found to be reduced in response to salt stress (8), (23), (26), (40). Catalase is one of the key enzymes responsible for conversion of the toxic H_2O_2 to H_2O and O_2 .

The accumulation of hydrogen peroxide (H_2O_2) has been reported to function as an intercellular signal (27), and it up regulates a large number of genes and proteins involved in stress responses, such as catalase, peroxidase and alternate oxidase (32, (42).

Peroxidase activity was higher in Parmal as compared to Pusa cultivar. The activity of peroxidase became almost 4 times after 48 hours of stress as compared to control and in Parmal cultivar this activity showed 7 times more increase. The levels of peroxidase showed a more drastic change in its activity in both the varieties (Figure V).

In case of superoxide dismutase, the changes were gradual. The SOD activity in case of Pusa showed an increase at 18 hours after which it began to decrease (Figure VI). However the activity at 24 and 30 hours was still higher as compared to the control. After 48 hours of stress the level of SOD decreased by 4% as compared to the control. A similar trend was observed in Parmal cultivar where the activity showed a gradual increase upto 24 hours after which there was a gradual decrease in activity. Our results were in accordance with Lina et al.,(2011) who did similar experiments on wheat varieties (28). An enhancement in SOD activity under stress was reported in other studies, which indicated that SOD played an important role in plant responses to abiotic stress (22). A relatively high SOD activity was also detected in the shoots of salt tolerant genotypes of both rice and wheat in response to salinity (35).

Initially there was a decrease in the activity of glutathione reductase (GR) upto 18 hours in Pusa after which there was an increase to up to 2.7 folds at the end of 48 hours stress. In Parmal cultivar a similar trend was observed with an increase of up to 2.3 folds at the end of 48 hours of stress (Figure-VII).

Edward et. al., (1994) have also suggested that the increase of total GR activity in oxidative stressed plants of *Pisum sativum* may be due to changes in the composition of GR isoforms (13). However, decreased activity of SOD and GR was observed in the leaves and roots of rice plants (24). Lee et. al., (2001) and Gupta., et. al.,(1993) observed that salt stress in Barely affected the changes in GR activity (26) (16). Our results were similar to the work done by Dong et. al, (2001), in rice (12). It has been shown that salt stress generally enhances the H_2O_2 content as well as OD, APX and peroxidase activity specific to guaiacol in leaves of Rice plant (21). It has been analyzed by various researchers in their studies of antioxidant defense system in various cell fractions that prolonged salt treatment leads to remarkable increase in the activity of enzymes of the ascorbate- glutathione cycle (The Halliwell-Asada pathway) in the soluble fraction in salt-tolerant plants (18). These enzymes often act as an indicator of plant tolerance against stress (2). An increase in the GR activity was observed in the stressed plants as compared to the control which was in agreement with the earlier findings in soybean (2)

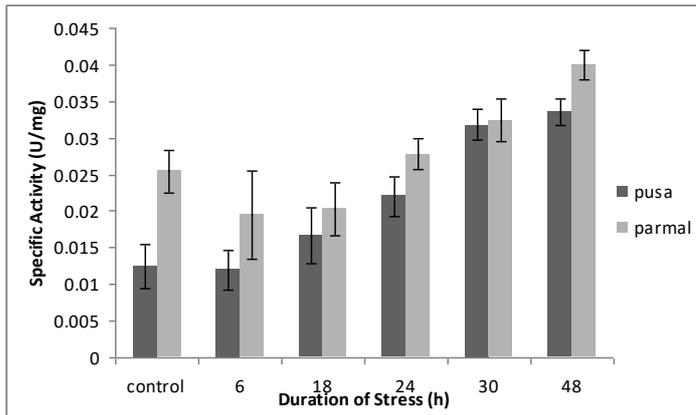


Figure-IV. Effect of salinity (150 mM) on the activity of Catalase in rice cultivars pusa and parmal (Values are mean from three replicates and bars indicate standard error).

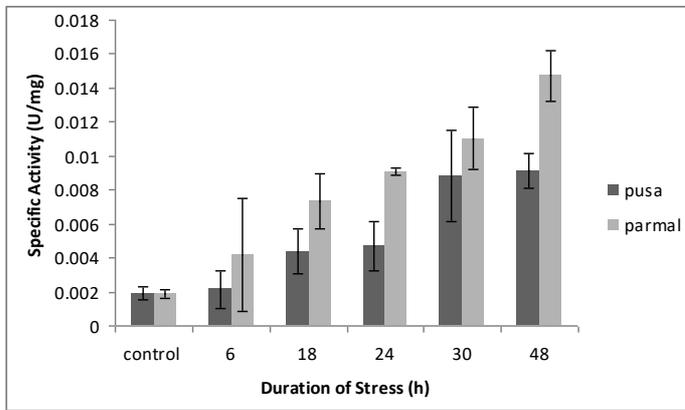


Figure V. Effect of salinity (150 mM) on the activity of Peroxidase in rice cultivars pusa and parmal (Values are mean from three replicates and bars indicate standard error).

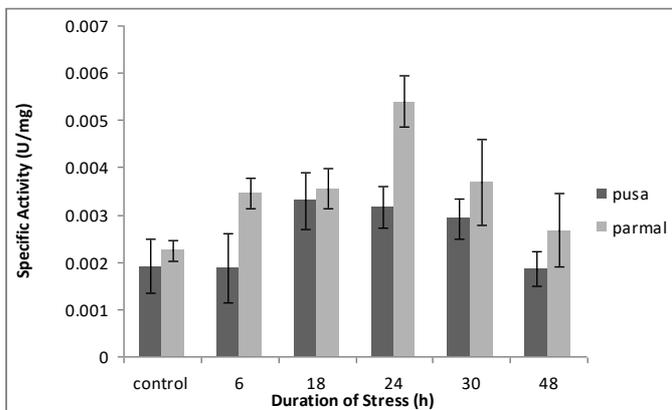


Figure VI. Effect of salinity (150 mM) on the activity of Superoxide dismutase in rice cultivars pusa and parmal (Values are mean from three replicates and bars indicate standard error).

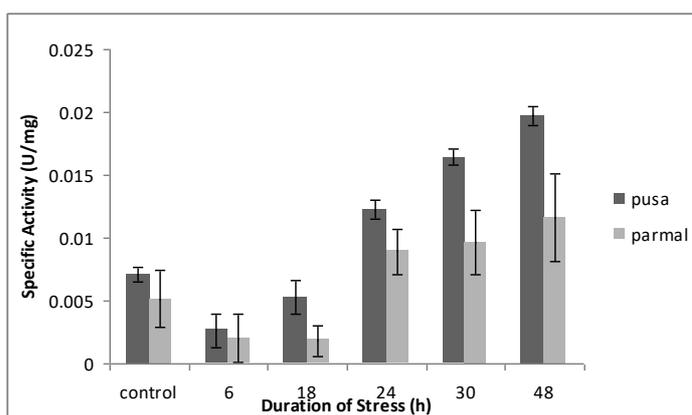


Figure VII. Effect of salinity (150 mM) on the activity of Glutathione Reductase in rice cultivars pusa and parmal (Values are mean from three replicates and bars indicate standard error).

CONCLUSIONS

The present study indicated that salinity stress caused significant changes in morphological parameters including decreased root length, shoot length and fresh weight and activity of antioxidant enzymes in both varieties of *Oryza sativa*.L. Significant enhancement was detected in activities of all four enzymes as compared to control plants. However there was a substantial difference in antioxidant enzyme activities when the two rice varieties were compared. The differences suggest that plants of Parmal showed higher adaptive potential under salinity stress as evident by the changes in growth parameters and antioxidant defence mechanism as compared to another cultivar Pusa sugandha.

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