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Effect of PGPR on enzymatic activities of rice (*Oryza sativa* L.) under salt stress

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ABSTRACT

Salt stress makes the agricultural lands unproductive and is an obstacle towards obtaining higher crop yield. To investigate the physiological and biochemical basis of salt stress adaptation in rice plants due to inoculation with plant growth promoting bacteria, two rice genotypes namely ADT43 and IR50 treated with Plant Growth Promoting Rhizobacteria (PGPR Pseudomonas strains PF1 and TDK1) were subjected to 100mM sodium chloride (NaCl), following International Rice Research Institute (IRRI) method in NLC Laboratory of Department of Crop Physiology of Tamil Nadu Agricultural University. Results of our study indicate that enzymatic activities (peroxidase, catalase and nitrate reductase) required to alleviate salt stress were much higher in the plants treated with plant growth promoting rhizobacteria strains PF1 and TDK1 compared to the plants with no external treatment. Among all the treatments ADT43 treated with TDK1 showed the best performance with high enzymatic activities. Phytoremediation through root treatment with PGPR in rice seedlings could lessen the adverse impact of salt stress at later stage of the crop growth and hence increase crop yield.

Key words: Catalase, nitrate reductase, peroxidase, rice, salt stress.

INTRODUCTION

Many crop species do not tolerate salt stress due to the accumulation of salts especially sodium chloride (NaCl) which compete with other nutrients and cause specific toxicity [18]. Salt stress affects adversely growth and development of a non-halophytic crop like rice. Salinity produces oxidative stress by the enhanced occurrence of damaging toxic molecule i.e. reactive oxygen species (ROS) [20]. Evidence suggests that membranes are the primary sites of salinity injury to cells and organelles [2] because ROS can react with unsaturated fatty acids to cause peroxidation of essential membrane lipids in plasmalemma or intracellular organelles [8]. Plant cells produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), and non-enzymatic antioxidants such as ascorbate, glutathione, and α-tocopherol to protect the cells against oxidative stress [4]. Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots. PGPR synthesize hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, which can lyse pathogenic fungal cells [12]. Jha and Subramanian (2013) [7] found that inoculation of plant material with PGPR can protect paddy plants against salt stress through an increase in plant growth parameters and the regulation of ion concentration and antioxidant enzymes. The objective of our present study was to study effect of PGPR treatments on enzymatic traits under salt stress condition and to study effect of PGPR on improvement rice tolerance to salt stress

MATERIALS AND METHODS

Experiment was conducted in the NLC Laboratory, Department of Crop Physiology, Tamil Nadu Agricultural University (TNAU), Coimbatore using Completely Randomized Design (CRD) as the design of experiment. Seeds of two rice genotypes (ADT43 and IR50) were divided into four sets: first set was soaked in water, second set in $CaCl_2$ solution at 50 mM [1], third set in PF1 (*Pseudomonas* strain) solution at 2g per 10 litres of water and fourth set in TDK1 (*Pseudomonas* strain) solution at 2g per 10 litres of water. After 12 hours the seeds were transferred in sand bed and grown for 7 days. The most uniform seedlings were transferred in Yoshida nutrient solution [19] following modified IRRI method. At 28 days after seed germination (7 days after 100Mm NaCl treatment), the plant samples were harvested and rinsed with distilled water and kept in refrigerator for physiological and biochemical analysis. The shoots were dried at 105°C for 2 hours and then extracted in triple acid (Nitric acid, sulphuric acid and perchloric acid in the ratio of 9:2:1 respectively).

Peroxidase activity

Peroxidase activity was assayed according to Perur (1962) [14] and expressed as △ OD 403 nm min^{-1} g^{-1}.

Catalase Activity

Catalase activity was determined from the change in the absorbance at 240 nm over time of the enzyme extract in 3ml of H_2O_2 -PO_4 buffer [10] and expressed as µg of H_2O_2 min^{-1} g^{-1}.

Nitrate Reductase activity (NRase)

Nitrate Reductase (NRase) activity in the leaves was determined by adopting the method of Nicholas *et al*. (1976) [13] and the enzyme activity was expressed as µg of NO_2 hr^{-1} g^{-1}.

Statistical analysis of physiological traits

Using AGRES software, significance of the observed values was determined

RESULTS AND DISCUSSION

Peroxidase and Catalase activity

The peroxidase activity followed an increasing trend except for the genotype ADT43 (no external treatment) which showed comparatively less value of peroxidase activity (0.0394 g^{-1} min^{-1}). ADT43 with $CaCl_2$ and *Pseudomonas* strain PF1 seed treatment had highest peroxidase activity (0.0474 g^{-1} min^{-1}), while the genotype ADT43 with no external seed treatment recorded the lowest peroxidase activity of 0.0394 g^{-1} min^{-1} under salt stress. Genotypes and treatments significantly varied in peroxidase activity. Interaction between genotype and treatment was also found to be significant 5% probability level.

Table 1: Effect of salt stress on peroxidase (Δ OD 403 nm min^{-1} g^{-1}) in two rice genotypes

Treatments	ADT43	IR50
1. No treatment	0.0394	0.0426
2. $CaCl_2$	0.0474	0.0429
3. PF1	0.0474	0.0446
4. TDK1	0.0470	0.0454
Mean	**0.0453**	**0.0439**

	SEd	CD (P=0.05)
Genotype (G)	0.0002	0.0004
Treatment (T)	0.0003	0.0005
GXT	0.0004	0.0008

Catalase activity exhibited similar pattern of peroxidase activity. The highest enzyme activity of 54.59 µg of H_2O_2 min^{-1} g^{-1} was registered by ADT43 (treated with *Pseudomonas* strain TDK1). Catalase activity was increased in all the genotypes with seed treatment under salt stress compared to the plants without any seed treatment. The lowest value of catalase activity (41.36 µg of H_2O_2 min^{-1} g^{-1}) was observed in ADT43 genotype without any seed treatment. Genotypic variation and treatment effect were significant at 5% probability level.

Figure 1: Effect of salt stress on peroxidase activity in two rice genotypes

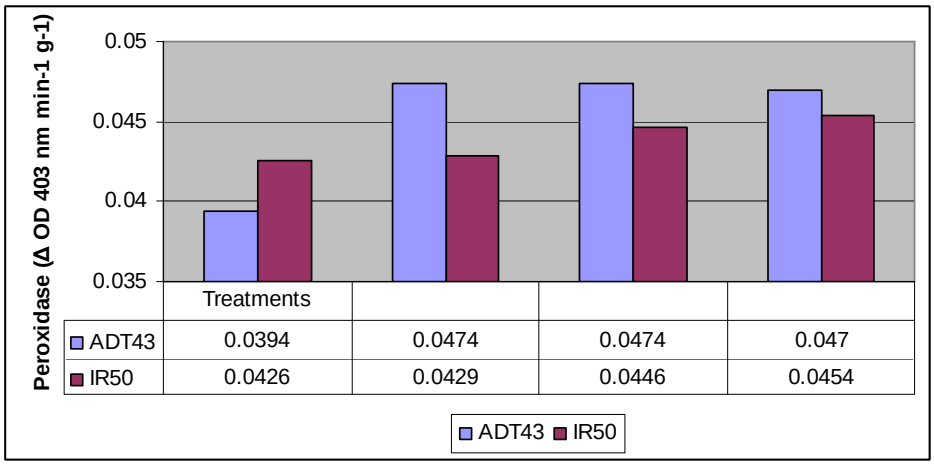
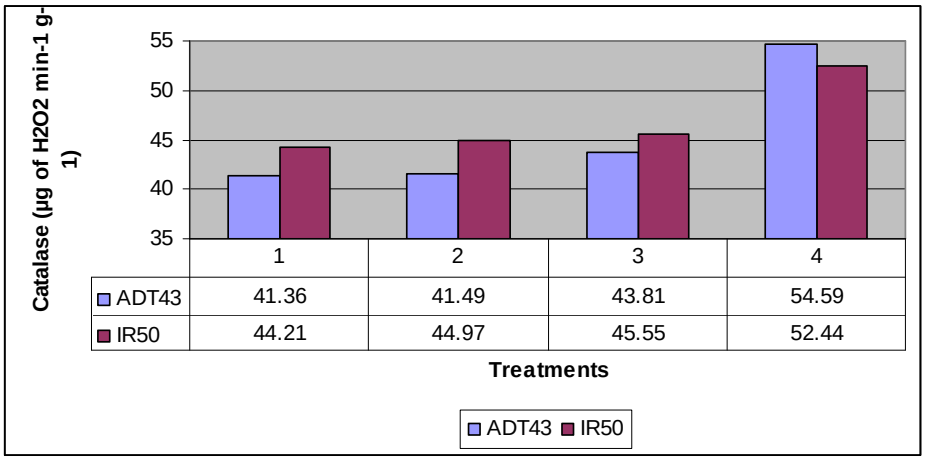


Table 2: Effect of salt stress on catalase (µg of H_2O_2 min^{-1} g^{-1}) in two rice genotypes

Treatments	ADT43	IR50
1. No treatment	41.36	44.21
2. $CaCl_2$	41.49	44.97
3. PF1	43.81	45.55
4. TDK1	54.59	52.44
Mean	**45.31**	**46.79**

	SEd	CD (P=0.05)
Genotype (G)	0.1399	0.2849
Treatment (T)	0.1978	0.4030
GXT	0.2798	0.5699

Figure 2: Effect of salt stress on catalase activity in two rice genotypes



The enzymatic antioxidant system is one of the protective mechanisms including superoxide dismutase, which can be found in various cell compartments and it catalyses the disproportion of two O^{2-} radicals to H_2O_2 and O_2 [16]. H_2O_2 is eliminated by various antioxidant enzymes such as catalases [16] and peroxidases [5] which convert H_2O_2 to water. Peroxidase is an important enzyme involved in morphogenesis and auxin oxidation. This enzyme is sensitive to environmental fluctuation and is considered as the measure of plants' resistance to abiotic stress.

In the present study, the peroxidase activity followed an increasing trend compared to control genotypes except for the genotype ADT43 grown with no external treatment which showed comparatively less value of peroxidase

activity. PGPR alleviated the oxidative damage produced under water shortage [9] confirmed by our study. Sandhya *et al*. (2010) [15] reported that *Pseudomonas* inoculated maize plants showed increased antioxidant enzymes activity on exposure to drought stress. Inoculation of lettuce *(Lactuca stiva* L.) with PGPR *Pseudomonas mendocina* augmented an antioxidant catalase under severe drought conditions, suggesting that they can be used as inoculants to alleviate the oxidative damage elicited by drought [9].

Nitrate Reductase activity

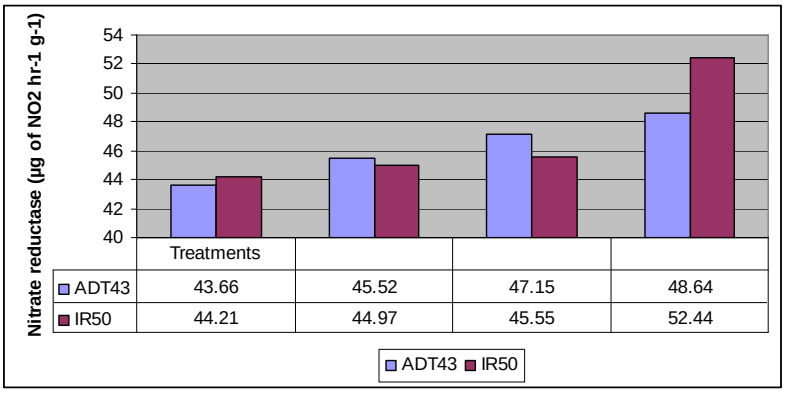
During stress Nitrate reductase (NRase) plays important role in reducing stress effects. It reduces nitrates to nitrite ions. Higher amount of NaCl (600 mmol. L^{-1}) reduces nitrate reductase activity considerably in leaves than in roots [11]. In the present study, among all the treatments used to treat the seeds of two genotypes IR50 genotype treated with *Pseudomonas* strain TDK1 showed highest NRase activity (52.44 µg of NO_2 hr^{-1} g^{-1}), while the genotype ADT43 without any external seed treatment recorded the lowest NRase enzyme activity of 43.66 µg of NO_2 hr^{-1} g^{-1}. Genotypic variation in nitrate reductase activity was significant. Different *Pseudomonas* strain seed treatments improved the enzyme activity significantly. Interaction between genotype and treatment was significant.

Table 3: Effect of salt stress on nitrate reductase (µg of NO_2 hr^{-1} g^{-1}) in two rice genotypes

Treatments	ADT43	IR50
1. No treatment	43.66	44.21
2. $CaCl_2$	45.52	44.97
3. PF1	47.15	45.55
4. TDK1	48.64	52.44
Mean	**46.24**	**46.79**

	SEd	CD (P=0.05)
Genotype (G)	0.1419	0.2890
Treatment (T)	0.2007	0.4088
GXT	0.2838	0.5781

Figure 3: Effect of salt stress on nitrate reductase activity in two rice genotypes



The pathway of NO_3 assimilation is considered as the major route of conversion of inorganic N into a biologically useful organic compound. The primary step in NO_3 assmilation involves reduction of NO_3 to NO_2 catalyzed by the enzyme, nitrate reductase (NRase). Nitrite is subsequently reduced to NH_4^+ by the enzyme nitrite reductase. These two enzymes reduce NO_3 to the end product NH_4^+ which is then incorporated into amino acids. Activity of NRase in plants gives a good estimate of the N status of plants and is very often correlated with growth and yield of crops [17]. Garg and Singla (2005) [6] reported decrease in NRase activity in chickpea leaves and roots at all stages of the plant under salt stress. Similarly Debouba *et al*. (2007) [3] found reduction in NO_3^- content in tomato leaves affected by salinity. In the present study, among all the treatments used to treat the seeds of two genotypes IR50 genotype treated with TDK1 *Pseudomonas* strain showed highest NRase activity, while the genotype ADT43 without any external seed treatment recorded the lowest NRase enzyme activity. Genotypic variation and treatment effect was found to be significant.

CONCLUSION

Plant growth promoting rhizobacteria has long been known to promote plant growth under abiotic stress conditions. Our study confirms the relationship among the antioxidative enzymes and the rhizobacteria. Peroxidase and catalase activities increased followed by *Pseudomonas* treatments. The activities of nitrate reductase were inhibited, as a function of NaCl concentration in the growth medium. The inhibition of NRase activity was comparatively more when the plants were grown under salt stress without any seed treatment. The results show that nitrate reductase is sensitive to NaCl stress. IR50 genotype treated with *Pseudomonas* strain TDK1 showed highest NRase activity. A lot of cultivated lands get salinized every year in India either due to natural or anthropogenic activities. Treating the rice seeds with PGPR prior to sowing would ameliorate the effects of salt stress to large extent.

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