

Original Research Article

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Impact of Seed Priming on Proline Content and Antioxidant Enzymes to Mitigate Drought Stress in Rice Genotype

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ABSTRACT

Keywords

Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD), Nagina-22 (N-22), Pusa sugandh-5 (PS-5), Methyl-jasmonate (MJ), Paclobutrazol (PBZ)

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Drought is one of the major problems of crop production in most of the countries, particularly in rice growing areas. Rice (*Oryza sativa* L.), is one of the major food grain cereal crops of the Indian and the world subcontinent. It belongs to the family Graminae (Poaceae) and is a model system for cereal biology because of its smaller genome size of 430 Mb that spans across 12 chromosomes. The rice varieties in this study showed differential responses for proline accumulation and enzymatic activities measured. The scavenging system in drought tolerant variety nagina-22 exhibited higher CAT, POD and SOD activities, than in the drought susceptible variety (pusa sugandh-5) drought-susceptible variety, PS-5 was markedly affected even at the lowest drought level used. The activity of antioxidant enzymes CAT, POD and SOD in the drought tolerant and drought susceptible varieties increased markedly during drought stress. Drought tolerance of the rice varieties associated with build up of antioxidant enzymes and proline. Among the biotic elicitors, MJ was found to be the most effective priming reagent, followed by PBZ. Present findings could be explored further to mitigate drought stress in order to improve rice yield in dry land areas.

Introduction

Drought is one of the major abiotic stresses that severely affect and reduce the yield and production of crops up to 65% (Thakur *et al.*, 2010; Akram *et al.*, 2013). Priming seeds with most favourable concentration of phytohormone have shown to be beneficial to growth and yield of some crops by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Dara, 1971). Diminish the harmful effects of stresses by the PGR (Datta *et al.*, 1998). The generation of ROS is limited or scavenged by an antioxidant system including

antioxidant compounds (ascorbate, SA, GSH, Vit-k) and antioxidant enzymes like SOD, APX and CAT (Foyer and Noctor, 2003). The retort of plants to drought stress is complex and involves changes in their morphology, physiology and metabolism. Decrease of plant growth is the most typical symptom of drought stress (Sairam and Srivastava, 2001). Drought stress leads to accumulation of ROS in chloroplast and mitochondria, causing oxidative burst. ROS molecules are singlet oxygen, superoxide anion radicals, hydroxyl radicals and hydrogen peroxide. Plants under

drought stress display some defense mechanisms to protect themselves from the damaging effect of oxidative stress. Plants with high induced antioxidant levels have better tolerance and resistance to oxidative damage (Parida and Das, 2005). The ROS scavenging mechanism is among the common defence responses against abiotic stresses (Vranová *et al.*, 2002). To detoxify ROS, plants can intrinsically develop different types of antioxidants reducing oxidative damage and conferring drought tolerance. The ROS scavengers are antioxidant enzymes containing SOD, APX and CAT (Demiral and Turkan, 2005; Khan and Panda, 2008).

The aim of this work was to study the comparative effects of different concentrations of elicitors on proline accumulation and antioxidant enzyme activities (e.g., CAT, POD and SOD) of two rice varieties.

Materials and Methods

Seed material and priming

Seed material of two contrasting rice genotypes- Nagina-22 (N-22) and Pusa-Sugandh-5 (PS-5). Seeds of the contrasting rice genotypes were sterilized with 0.1 % mercuric chloride (HgCl₂) solution for 4 min and thoroughly washed with distilled water. Three sets of seeds in triplicate were primed with concentration (100µM) of biotic elicitors /priming reagent according to the method given by Afzal *et al.*, (2006) for methyl jasmonate (MJ), and Pill and Gunter (2001) for Paclobutrazol (PBZ). Primed and control seeds (three in each 4/4 inches pot size) were grown in phytotron under controlled conditions. Irrigation of the seedlings with the half strength Hoagland solution was carried forwarded for 7 weeks. Drought stress was induced on 7 weeks-old seedlings by withholding water for 6 days. The shoot samples from the two biological and three technical

replicates were harvested and stored for biochemical studies.

Proline determination

Proline was extracted from 100 mg of leaf sample by using ninhydrin reagent in 3% (w/v) aqueous sulfosalicylic acid (Bates *et al.*, 1973). The organic toluene phase was separated and absorbance of red colour developed was read at 520 nm. Concentration of Proline (mg g⁻¹ FW) was determined using calibration curve.

Enzyme extraction

Grinding Leaf sample (400 mg) in liquid nitrogen and finely ground by pestle and motor than the powder was added to 10 mL of phosphate buffer (pH 7.0). Centrifugation of homogenate at 15000 × g for 10 min at 4 oC and supernatant was used as enzyme source for CAT, SOD and POD assays. Assays of Antioxidant Enzyme Activities.

Assay of CAT activity

The assay mixture in total quantity of 3 ml contained 0.5 mL of 0.2 M H₂PO₄⁻ (pH 7.0), 0.3 mL of (v/v) H₂O₂ and 0.1 ml of enzyme and made 3 ml by adding DW. The reaction was started by adding enzyme and vary in optical compactness was measured at 240 nm at 0 min and 3 min on UV-Vis spectrophotometer. The molar extinction coefficient of H₂O₂ at 240 nm was taken as 36 µmol⁻¹ cm⁻¹ and the results were expressed as µmol H₂O₂ min⁻¹ g⁻¹ protein (Luck, 1974; Aebi and Bergmeyer, 1983).

Assay of SOD activity

3 ml of reaction mixture containing 0.1 ml of 1.5 M Na₂CO₃, 0.2 ml of 200 mM methionine, 0.1 ml of 3 mM EDTA, 0.1 ml of 2.25 mM NBT, 1.5 ml of 100 mM potassium phosphate buffer (pH 7.5), 1 ml of distilled

water and 0.05 ml of enzyme samples. One tube was taken as control. Reaction was started by adding 0.1 ml 60 μ M riboflavin and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min than reaction was stopped by switching off the light. Tubes are covered by black cloth. Absorbance was recorded at 560 nm. An illuminated blank without protein gave the maximum reduction of NBT, and therefore, the maximum absorbance at 560 nm. SOD activity is presented as absorbance of blank minus absorbance of sample, giving the total inhibition, calculated per μ g protein. The activity of SOD was expressed as U mg⁻¹ protein. One unit of activity is the amount of protein required to inhibit 50 % initial reduction of NBT under light (Beauchamp and Fridovich, 1971; Dhindsa *et al.*, 1981).

Assay of POD activity

The assay mixture of 3 ml contained 1.5 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared 10 mM guaiacol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H₂O₂. Initial absorbance was read at 436 nm and then increase in the absorbance was noted at the interval of 30 s on UV-Vis spectrophotometer. Activity was calculated using the ϵ 26.6 mM⁻¹ cm⁻¹ for the oxidized tetraguaiacol polymer. Enzyme activity was μ mol guaiacol oxidized min⁻¹ g⁻¹ protein (Putter, 1974; Jebara *et al.*, 2005).

Results and Discussion

Proline content

Proline content was found to increase 27% on drought stress imposition in the drought tolerant (N-22) genotype while it was found that no significant difference in susceptible genotype. It was found that the proline content increases after seed priming and a significant increase in both genotypes after drought imposition (Figure 1).

SOD activity

SOD activity does not show significant differences in control as well as treated sample. SOD activity higher in drought tolerant genotype than the susceptible ones after drought imposition (Figure 2).

CAT activity

Higher CAT activity observed in tolerant genotype than the susceptible ones, maximum activity observed in MJ treated tolerant genotypes after drought. Slightly increased activity found in susceptible ones in MJ and PBZ treated genotype (Figure 3).

POD Activity

Peroxidase activity not observed so much significant in both tolerant and susceptible genotypes after treatment. In both genotypes very low higher activity observed after treatments (Figure 4).

During vegetative growth stage the results obtained from the work confirmed that the rice varieties displayed diverse variation in drought tolerance. We identified the most drought tolerant variety shows higher activity of antioxidant enzymes and shows higher content of proline. The proline content of rice varieties increases in both drought susceptible and drought tolerant under drought condition (Figer 1). Osmotic potential of cell balanced by the proline accumulation in plants under water stress (Pireivatloum *et al.*, 2010). Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates (Athar *et al.*, 2008). To combat the effects of drought induced oxidative stress, plants develop a complex mechanism of antioxidant system. ROS scavenging enzymes have higher activity in tolerant genotypes when compared to susceptible ones.

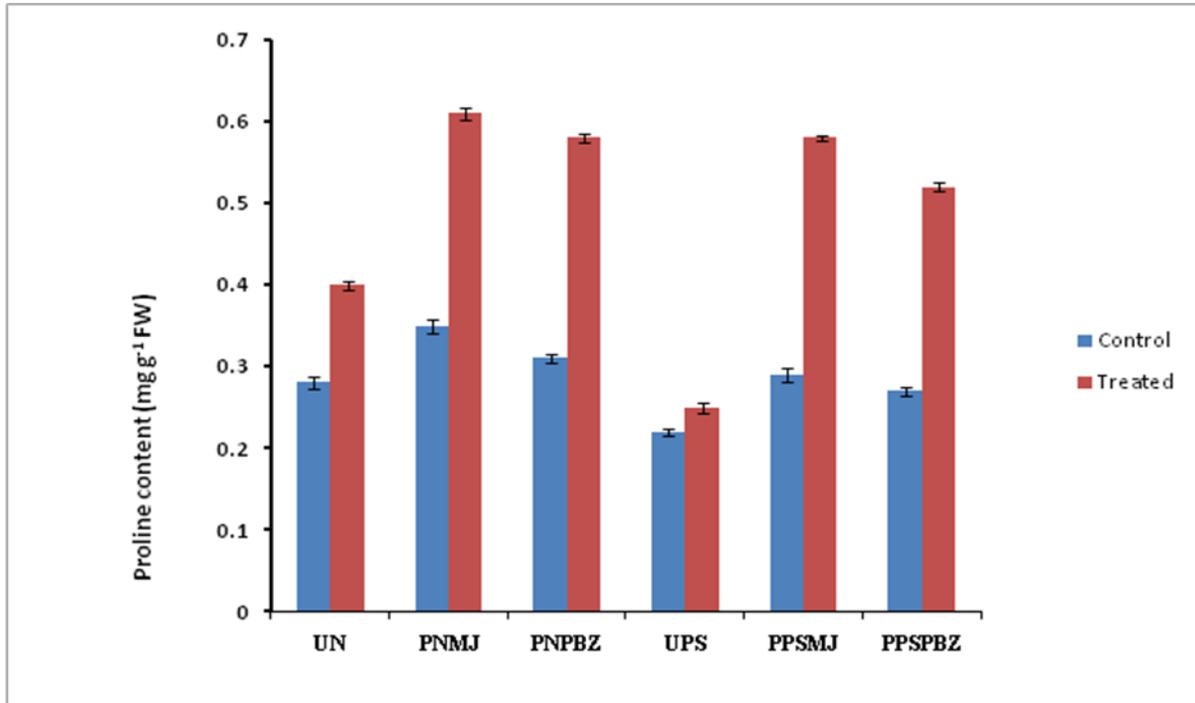


Figure:1 Proline content in shoot region of contrasting rice genotypes-Pusa sugandha-5 (drought-susceptible) and Nagina-22(drought-tolerant) under drought condition against priming with biotic elicitors- methyl jasmonate (MJ) and paclobutrazol (PBZ). Bars represent standard deviations.

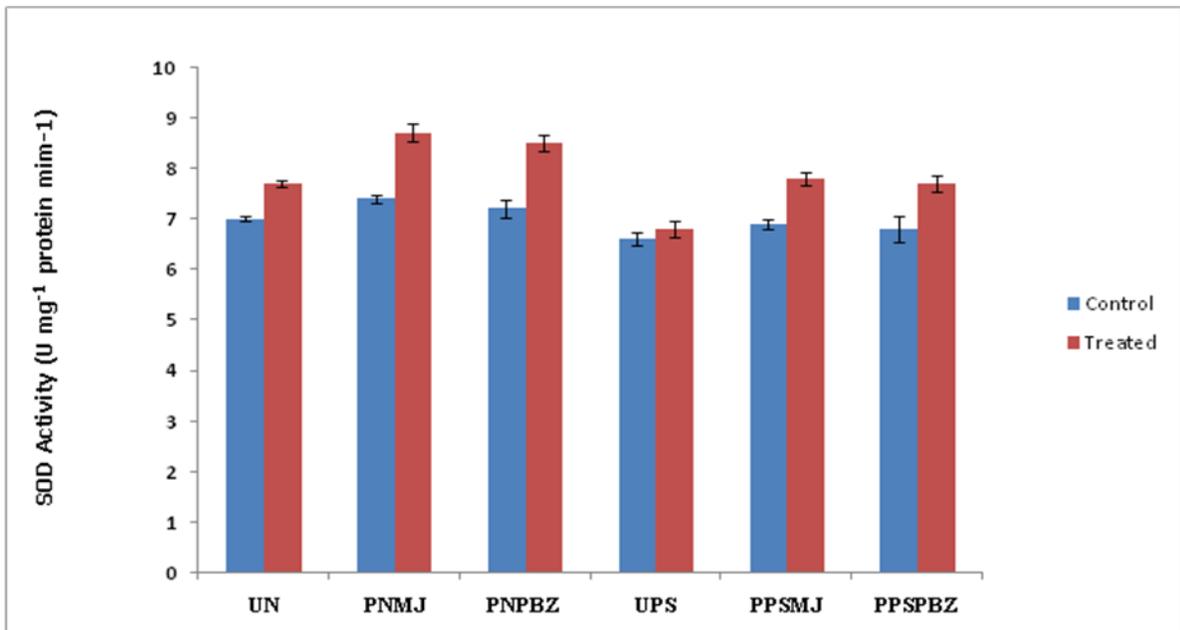


Figure:2 SOD Activity in shoot region of contrasting rice genotypes-Pusa sugandha-5 (drought-susceptible) and Nagina-22(drought-tolerant) under drought condition against priming with biotic elicitors- methyl jasmonate (MJ) and paclobutrazol (PBZ). Bars represent standard deviations.

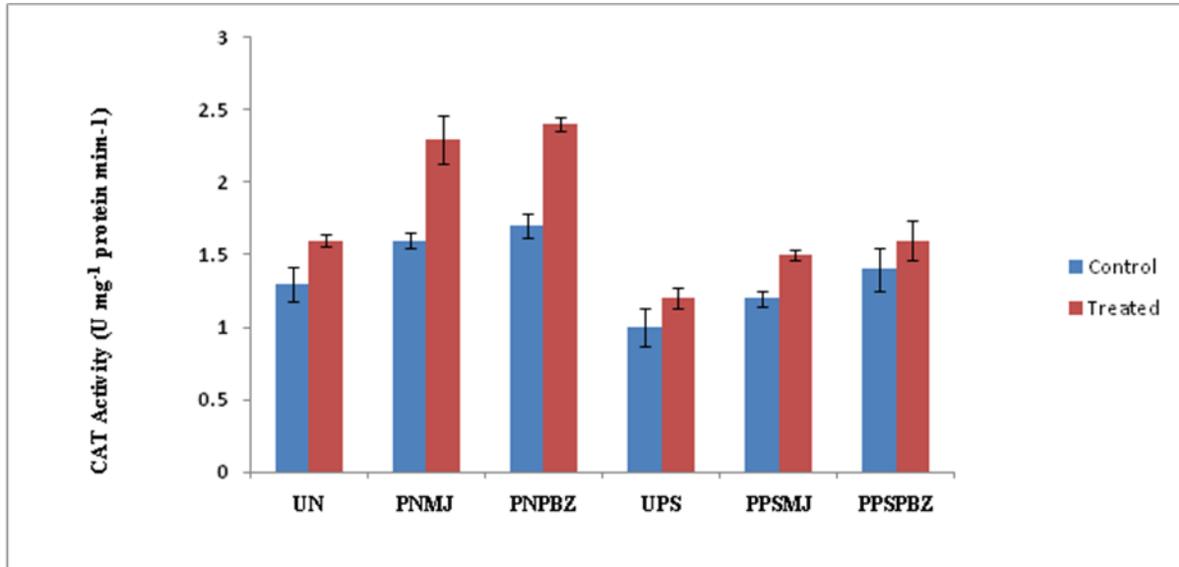


Figure:3 CAT Activity in shoot region of contrasting rice genotypes-Pusa sugandha-5 (drought-susceptible) and Nagina-22(drought-tolerant) under drought condition against priming with biotic elicitors- methyl jasmonate (MJ) and paclobutrazol (PBZ). Bars represent standard deviations.

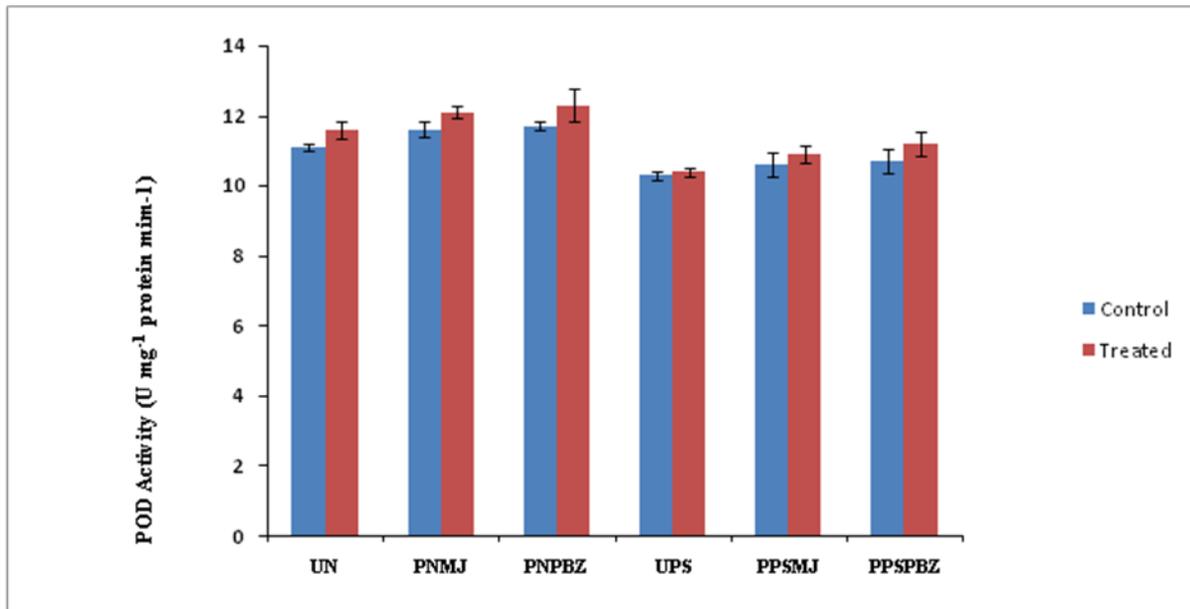


Figure:4 POD Activity in shoot region of contrasting rice genotypes-Pusa sugandha-5 (drought-susceptible) and Nagina-22(drought-tolerant) under drought condition against priming with biotic elicitors- methyl jasmonate (MJ) and paclobutrazol (PBZ). Bars represent standard deviations.

This suggests that the antioxidant system plays an important role in plant tolerance against environmental stress. Rice varieties showed lowest enzymatic activity under normal condition. This indicated plants will produce more CAT, SOD and POD under drought conditions to remove the ROS. In this study, CAT SOD and POD activities increased markedly in the drought tolerant varieties than the sensitive one. Drought-tolerant varieties were efficient hunter of free radicals, which may result in better protection against oxidative stress. The CAT is one of the highest turnover rates for all enzymes with the potential to directly dismutate H₂O₂ into H₂O and O₂ and is indispensable for ROS detoxification in peroxisomes during stress condition (Sairam and Srivastava, 2001). Anion free radicals converted to H₂O₂ by the SOD enzyme and detoxify to less toxic compound and the H₂O₂ can be eliminated by CAT and POD (Hasheminasab *et al.*, 2012). Moreover, POD also involved in various plant processes, including lignification (Hendriks *et al.*, 1991), phenolic compound oxidation (Largrimini, 1991), regulation of cell elongation (Mohammadkhani and Heidari, 2008) and detoxification of toxic compounds such as H₂O₂ (Chaparzadeh *et al.*, 2004). Antioxidant enzymes provide tolerance to rice genotypes to environmental stresses. For exm. drought tolerant species of pigeon pea (*Cajanuscajan*) (Kumar *et al.*, 2011), wheat (*Triticumaestivum*) (Hasheminasab *et al.*, 2012; Omar, 2012) and black gram (*Phaseolus mungo*) (Pratap and Sharma, 2010) had higher activities of SOD, POD and CAT than the drought-sensitive species. Under water stress conditions, the proline content showed highest. Accumulation of proline content under water stress indicates accumulated proline might act as a compatible solute regulating and reducing water loss from the plant cell during water deficit (Yokota *et al.*, 2006) and play

important role in osmosis regulation (Fedina *et al.*, 2002). Proline accumulation provides energy for survival and growth of the plant under oxidative stress (Kumar *et al.*, 2011). Thus, the proline content is a good indicator for screening drought tolerant varieties in water stress condition (Bayoumi *et al.*, 2008; Kumar *et al.*, 2011; Rahdari *et al.*, 2012).

In conclusion, the rice varieties in this study showed differential responses for proline accumulation and enzymatic activities measured. The scavenging system in drought tolerant variety nagina-22 exhibited higher CAT, POD and SOD activities, than in the drought susceptible variety (pusa sugandh-5). Thus, the drought tolerance of these rice varieties seems to be linked to the activities of these antioxidant enzymes. The drought tolerance of rice varieties could induce antioxidative enzyme system more efficiently, resulting in growth suppression and higher proline content under drought stress.

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