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RESEARCH ARTICLE

ENZYMATIC AMELIORATION OF DROUGHT STRESS IN RICE THROUGH THE APPLICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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ABSTRACT

Recently, the role of plant growth promoting rhizobacterial (PGPR) on plant growth promotion and maintenance of plant homeostasis under abiotic stress is gaining importance. In present study, plant growth promoting rhizobacterial (PGPR) strains *Pseudomonas fluorescence strain* P2 and P16, *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, *Bacillus cereus 5507*(1B), *Bacillus cereus BSB 38* (14B) were tested for their role in enhancing plant growth and induction of stress related enzymes in swarna and swarna sub1 varieties of rice (*Oryza sativa* L.). Two PGPRs, *Pseudomonas jessenii,* R62, *Pseudomonas synxantha,* R81 were used as consortia. Most of the PGPR inoculated plants showed enhanced growth parameter as compared to uninoculated plants under drought stress. Quantitative analyses of stress related enzymes indicated that most of the plants inoculated with PGPRs showed higher activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) as compared to uninoculated plants. Among all the treatments, consortia of *Pseudomonas jessenii* R62 and *Pseudomonas synxantha* R81 treated plants showed better improvements in most of the growth parameter as well as stress related enzymatic activities. The greater induction of stress related enzymes in plants may be the mechanism through which these PGPRs help plants to tolerate the consequences of drought stress and maintenance of plant homeostasis under severe drought.

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INTRODUCTION

Rice (*Oryza sativa*, L.) is the leading food grain crop used as a human food for the last almost 5000 years (IRRI, 1997). It is estimated that more than 90% of rice is grown and consumed in Asia where 60% of the people on earth live, to satisfy the growing demand of food we must produce 40% more rice by 2025 without adversely affecting the resource base (Rodrigues *et al*., 2008). In Asia alone at least 20% of total rice area is estimated to be drought-prone (Pandey *et al*., 2006). Environmental stress including water deficit altering the normal homeostasis of plants cells and cause an increased production of Reactive Oxygen Species (ROS) such as the super oxide radical, hydrogen peroxide and hydroxyl radical (Miller and Wood 1996). Oxidative stress caused by accumulation of reactive oxygen species (ROS) results in perturbation of the overall cellular metabolism (Mittler, 2002). In order to protect from deleterious effects of ROS, plants have evolved an antioxidant defense system that includes non-enzymatic compounds such as ascorbate and glutathione and enzymes such as superoxide dismutase, peroxidase, catalase etc. (Agarwal and Pandey, 2004). Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates (Curl and Truelove, 1986). Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as plant growth-promoting rhizobacteria (PGPR) (Herman *et al*., 2008). Besides their growth promotion activity, recent studies indicate that microorganisms can also help crops cope with abiotic stresses (Venkateswarlu *et al*., 2008, Kohler *et al*., 2008). The aim of present study was to evaluate the effect of plant growth promoting rhizobacteria (PGPR) on the growth enhancement, biomass production and antioxidant activity of two genotypes Swarna and Swarna sub-1 of rice, which are considered as high yielding varieties of rice (STRASA news 2010), in which Swarna is widely grown in drought-prone environments due to its high yield and other desirable traits (Mackill *et al.*, 2010).

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MATERIALS AND METHODS

For the study plant growth promoting bacterial strains *Pseudomonas jessenii* (R62), *Pseudomonas synxantha* (R81) (Mader *et al*., 2011), *Bacillus cereus BSB 38* (14B), *Bacillus cereus 5507*(1B), *Pseudomonas fluorescence* strain P2 and *Pseudomonas fluorescence* strain P16 were obtained from Rhizosphere biology lab of department of Biological Sciences

of G. B. Pant university of Agriculture and Technology Pantnagar, and two rice genotype Swarna and Swarna sub1 was obtained from the IRRI Office, NASC Complex, Pusa New Delhi, India.

Preparation of inoculants

For the preparation of bacterial inocula a loop of fresh cultured bacteria were inoculated in 150 ml of nutrient broth media and kept on shaker for overnight at 28°c and colony forming unit (cfu) counted by dilution plate method. In the study two bacterial strains R62 and R81 were used as consortia.

Pot Experiment

Rice growth promotion by these bacterial strains under drought stress was performed in net house conditions, during October to December. Rice seeds were surface disinfected by immersion in 70% ethanol and 3 %(v/v) sodium hypochlorite for 1 min and 5 min. Seeds were washed thoroughly three times with sterile distilled water then germinated on sterilized Petri dish. The soil for experiment was obtained from the agriculture field of the G.B. Pant University of Agriculture and Technology Pantnagar. The pH of soil is 8.31 and the soil has 1.2% organic carbon, 186.7kg/h nitrogen, 34.91kg/h of phosphorus, and 145.6kg/h potassium. Before filling the pot the soil were autoclaved at 121psi for 40 minute thrice, every alternate day. The pots were filled with 300 gram of soil and watered to field capacity before sowing the seeds. After two days the equally germinated seeds were selected for sowing. The bacterial inocula were given to 1ml / pot having 10^7-10^8 cfu level. Two seedlings per pot were maintained. After 30 days of sowing 10 ml of Phosphorus free nutrient solution (Hoagland and Arnon, 1950) were given, weekly to the each pot. The experimental design used for the study was complete randomized design. There were six replicate of each isolates. After 55 days of sowing, the pots were irrigated up to water holding capacity of soil and left for drought stress by withholding the irrigation. First harvesting was done after 10 days of drought with three randomly selected replicate for the measurement of growth promoting trait (plant height, shoot fresh and dry weight of plants). Dry weight of sample was determined by placing the root and shoot samples separately into paper bags and drying them in an oven at 60^0C for 48 h. Second harvesting was done after 12^{th} days of drought for the measurement of antioxidant status of plants. Soil water content (SWC) was determined from the pot of second harvesting by the traditional gravimetric method. At the time of harvesting soil was sampled from the middle part of pots. After wet weight determination, soil was dried at 80°C for 48 h or till the complete drying of soil. The SWC were calculated as:

Anti-oxidative enzyme analysis from plant sample

For evaluation of antioxidant status, drought plants were harvested, fresh weight were taken immediately and then placed in -20^0 C for further antioxidant activity. For assays of Superoxide dismutase, Catalase and Guiacol peroxidase, 0.5 g leaf samples (fresh weight) was homogenized with a pestle in an ice-cold mortar in 5ml cold buffer containing: 50 mM potassium phosphate buffer (PH 7.0), 1 mM ethylene diamine tetra acetic acid (EDTA) and 1% (w/v) polyvinylpyrolidone (PVP). Whole extraction procedure was carried out at 4°C. The homogenate was centrifuged at 10,000xg for 30 min at 4°C and the supernatant collected was used to assay enzyme activity. Protein concentration in the enzyme extract was determined by the method of (Bradford, 1976) using bovine serum albumin as a standard. SOD, POD and CAT activity were determined as described by (Zhang and Kirkham, 1996) with few modifications. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS) software, and treatment means were compared in Tukey HSD, at 5% level of significance.

RESULTS

After giving water stress, plants showed droopy appearance and leaves starting turning inwards from the outside edges. Plants were harvested after 10^{th} and 12^{th} days of drought. The moisture content of soil in pots was calculated as 45.43% ± 3.91% after 12^{th} days of drought. After first harvesting at 10^{th} days of drought most of the treated plants showed the higher shoot length as compare to control, however the effect was nonsignificant. Similar to shoot length, the treated plants also showed the non significant effect on root length. In Swarna only *Pseudomonas* strain P2 with 76.85% and 71.09% increase showed significant effect on root and shoot dry weight while in Swarna sub1 *Pseudomonas* consortia R62+R81 and Bacillus strain 14B with 110.37% and 27.77% increase showed significant effect on root dry weight, however in shoot dry weight only *Pseudomonas* consortia R62+R81showed significant (63.04%) effect over control (Table 1). After 12^{th} days of drought none of the treatment showed significant effect on shoot length over control in both the varieties of rice, however in root length only *Pseudomonas* strain P2 showed significant effect in Swarna variety of rice. In Swarna *Pseudomonas* strain P2, consortia of R62+R81 and *Bacillus* strain 14B, while in Swarna sub1 all the treatments except *Pseudomonas* strain P16 significantly increase the shoot fresh weight over control (Table 2). The present study demonstrated that, after 12^{th} days of severe drought, most of the bacterial strains showed the greater activity of SOD, CAT and POD as compare to control. In Swarna consortia of R62+R81 and *Pseudomonas* strain P2 with 1.43 and 1.36 fold, while in Swarna sub1 all the treatments except *Bacillus* strain 1B significantly enhance the SOD activity over control. A consortium of R62+R81 and *Bacillus* strain 14B significantly increased catalase activity (1.38 and 1.62 fold) in Swarna and Swarna sub1 (1.55 and 1.57 fold) verities of rice. Similar to catalase activity a consortium of R62+R81 and Bacillus strain 14B with 1.48 and 1.50 fold significantly enhanced the POD activity in Swarna, however in Swarna sub1 only R62+R81 treated plants showed significant effect over control (Figure1).

DISCUSSION

Several environmental stimuli, such as low nutrient or water availability, can reduce or halt cell division or elongation, leading to an arrest of primary-root growth accompanied by a stimulation of lateral-root emergence (Sanchez-Calderon *et al*., 2005). It is well known fact that PGPR play important role in plant growth promotion. IAA and phosphate solubilization properties of PGPR considered as an important properties for the growth promotion of plants (Govindarajan *et al*., 2007).

Table 1. Rice growth promotion by selected bacterial strains after 10 days of drought stress. Results are means of three replicate. Mean with different letters significantly different from each other (P< 0.05)

Variety	Treatments	Length (cm)		Fresh weight (g/pot)		Dry weight (g/pot)	
		Shoot	Root	Shoot	Root	Shoot	Root
Swarna	control	24.30 a	13.85 a	0.97 a	0.42 a	0.44 a	0.22 ab
	P16	27.62 a	16.22 a	0.81 a	0.35 a	0.39 a	0.20 a
	P2	29.85 a	15.53 a	1.67 b	0.75 b	0.75 b	0.39 b
	1B	24.50 a	14.10 a	0.71 a	0.36 a	0.38 a	0.20 a
	14B	30.40 a	18.23 a	0.94 a	0.34 a	0.42 a	0.18 a
	R62+R81	29.87 a	14.43 a	1.09 ab	0.42 a	0.54 ab	0.22 ab
Swarna sub1	control	26.78 a	16.73 a	0.79 a	0.33 ab	0.50 a	0.18 ab
	P16	30.95 a	15.08 a	1.26 b	0.43 bc	0.57 ab	0.21 ab
	P2	31.37 a	13.47 a	1.10 ab	0.38 abc	0.55 a	0.19 ab
	1B	26.50 a	13.40 a	1.03 ab	0.28 a	0.54 a	0.16 a
	14B	26.55 a	13.73 a	1.20 b	0.49 c	0.62 ab	0.23 b
	R62+R81	31.88 a	13.82 a	2.01 c	0.75 d	0.82 b	0.38 c

Table 2. Rice growth promotion by selected bacterial strains after 12days of drought stress. Results are means of three replicate. Mean with different letters significantly different from each other (P< 0.05)

Variety	Treatments	Length (cm)		Fresh weight (g/pot)	
		Shoot	Root	Shoot	Root
Swarna	control	23.75 a	14.43 a	0.57 ab	0.26 a
	P16	26.35 a	15.27 a	0.51 a	0.27 a
	P2	28.57 a	19.32 b	1.21 d	0.49 b
	1B	28.25 a	15.80 ab	0.63 b	0.27 a
	14B	30.46 a	16.97 ab	0.84 c	0.33 a
	R62+R81	26.92 a	15.80 ab	1.10 d	0.46 b
Swarna sub1	control	29.33 a	18.02 ab	0.69 a	0.28 a
	P16	28.45 a	16.38 ab	0.89 ab	0.37 ab
	P2	29.63 a	14.92 a	1.02 bc	0.47 c
	1B	30.38 a	14.92 a	1.21 cd	0.47 c
	14B	30.90 a	16.77 ab	1.15 bcd	0.38 b
	R62+R81	33.10 a	18.65 b	1.44 d	0.54 c

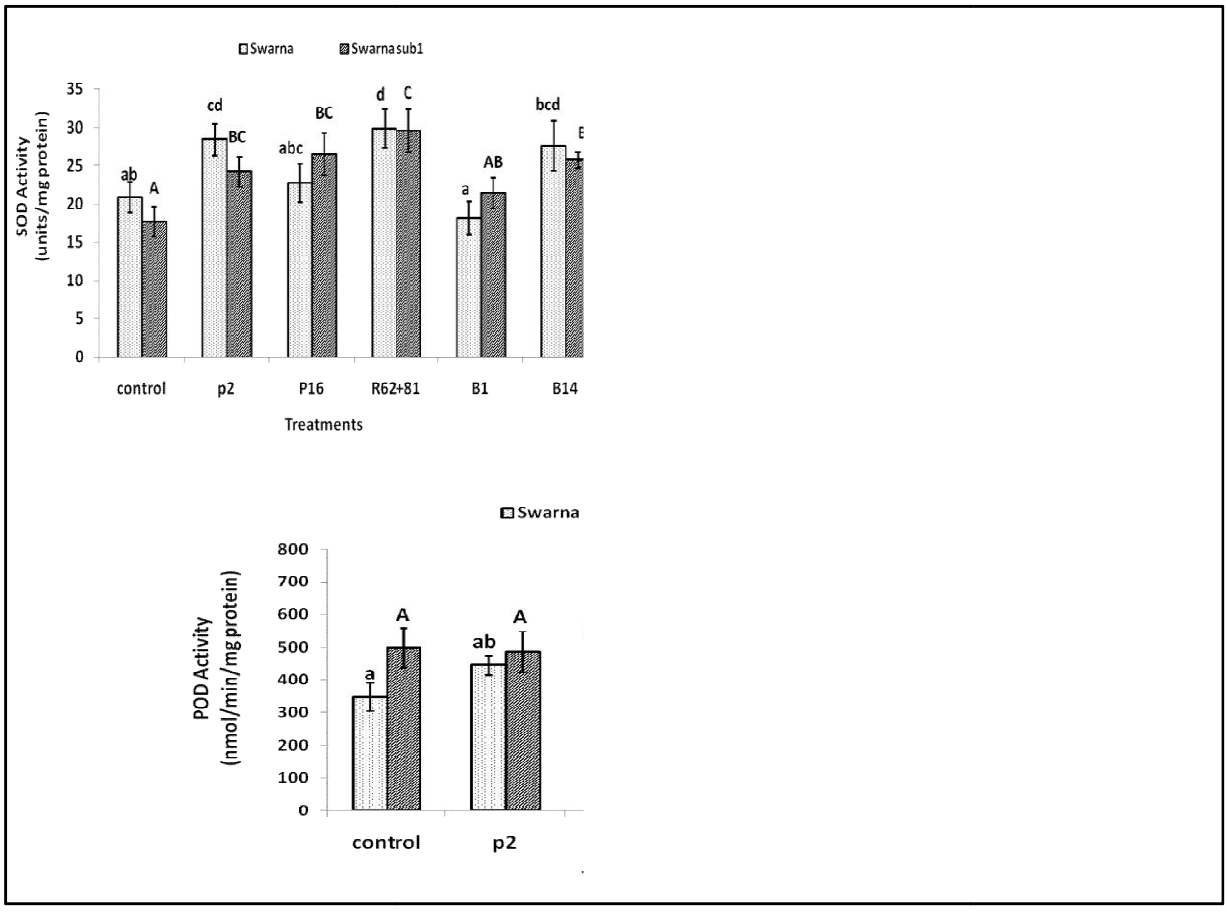

Fig. 1. SOD (a), CAT (b) and POD (c) content of rice after 12 days of drought stress. Different letters denote significant differences (P<0.05) among treatments. Line above bars represents Mean ± standard deviation

Although, it is believed that bacterial growth promotion is a complex phenomenon to which more than one plant growth promoting trait can be attributed (Lifshitz *et al*., 1987). In the present study most of the bacteria showed the positive effect on root and shoot biomass under drought stress. Similar correlation of plant with higher root biomass showing increased stress tolerance due to higher microbial activity was highlighted by Haynes and Francis, 1993. In response to drought, plants respond differently which may involves the new sets of proteins whose function is largely unknown. Under water stress reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide and hydroxyl radicals adversely affect the membranes and DNA of cells (Sharma and Dubey, 2005). A previous study demonstrated that mechanisms that reduce oxidative stress indirectly play an important role in drought tolerance (Bowler *et al*., 1992). SOD and CAT are considered key players in the antioxidant response system as they regulate the cellular concentration of Superoxide radical and hydrogen peroxide (Van Breusegem *et al*., 2001). SOD convert superoxide radical to hydrogen peroxide (H_2O_2) and oxygen, CAT enzyme convert toxic H_2O_2 to water and oxygen (Gill and Tuteja, 2010). In present study most of the treatments increased the antioxidant enzymes in both the genotype of rice as compare to control plants, however the R62+R81, a consortium treated plants showed enhanced activity of most of the growth parameter and antioxidant enzymes. The higher activities of such antioxidant enzymes in treated plants can lower H_2O_2 concentration in treated plants, and thus may protect the rice plants against oxidative stress damage induced by drought. Similar results have been reported by many researchers in other studies under abiotic stresses (Kohler *et al*., 2008, Saravanakumar *et al*., 2011). The results of the current study serve as base for the mediation of PGPR in enhancing water stress resistance in rice plants and need the evaluation of selected bacterial strains in drought prone area.

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