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Enhance activity of stress related enzymes in rice (*Oryza sativa* L.) induced by plant growth promoting fungi under drought stress

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Water scarcity is one of the main consequences of changing climate which adversely affects the plant growth and productivity. The present study aimed to investigate the effect of plant growth promoting fungi (PGPF), *Trichoderma harzianum* strain-35 (T-35) and newly discovered *Fusarium pallidoroseum* strain-10 (FP-10) on total biomass production and activities of the stress related enzymes [superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD)] in Swarna and Swarna sub-1 genotypes of rice under drought stress. PGPFs inoculated plants showed enhance shoot and root dry weight as compared to uninoculated plants under water stress. Quantitative analyses of antioxidant enzymes indicated that plants inoculated with PGPFs showed higher activity of SOD, CAT and POD enzymes as compared to uninoculated plants under severe drought condition (41.23% pot moisture content). Higher biomass and greater induction of antioxidant enzymes in plants may be the mechanism through which these PGPFs help plants to alleviate the consequences of drought stress and maintenance of plant homeostasis under severe drought.

Key words: *Trichoderma harzianum*, *Fusarium pallidoroseum,* rice, drought stress, antioxidant.

INTRODUCTION

Rice (*Oryza sativa* L.), is the leading food grain crop. Worldwide, more than 3.5 billion people depend on rice for more than 20% of their daily calorie intake (IRRI, AfricaRice and CIAT, 2010). More than 90% of rice is grown and consumed in Asia where 60% of the people on earth live (Rodrigues et al., 2008). The predominantly rice-growing areas in Asia are often threatened by severe abiotic stresses, of which the most common is drought (Wade et al., 1999). In many Asian rice areas, irrigation water is not available and rice relies almost completely on rainfall during growth under both lowland and upland conditions (Farooq et al., 2009). Among cereals, rice is the most drought-sensitive crop. Severe yield losses can occur in even a mild drought stress during reproductive stage (Verulkar et al., 2010). Water stress induces a number of physiological, biochemical and molecular manipulation within plants which governs growth and productivity (Daie et al., 1988). One of such biochemical

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mechanism includes antioxidant enzymatic system (viz. superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) etc.), which protect plant cells against the detrimental effects of reactive oxygen species (ROS) generated under variety of environmental stresses (Noctor and Foyer, 1998).

Microbial communities can develop a range of activities that may be helpful in improving plant growth. Soil form an interface with the plant roots where symbiotic fungi can interact. Such interaction can manipulate the metabolic activity of plants which may help plants to tolerate the environmental stresses (Marulanda et al., 2007). *Trichoderma* beneficially interact with plant roots and can induced disease resistance, plant growth promotion and tolerance to abiotic stresses including drought (Harman et al., 2004). In limited water availability root size and architecture are the factor which determined yield performance of plants (Price et al., 2000). *Trichoderma* enhanced the growth of roots by colonizing it, thereby increasing plant productivity and the yields of reproductive organs (Bae et al., 2009). A study on *Trichoderma*-plant interaction characterized the possibility of *Trichoderma* species inducing tolerance to abiotic stress, possibly including drought, in cacao (Bailey et al., 2006).

The aim of this present study was to evaluate the effect of two plant growth promoting fungi (PGPF) *Trichoderma harzianum* strain-35 (T-35) and *Fusarium pallidoroseum* strain-10 (FP-10) on the growth enhancement, biomass production and antioxidant activity of two genotype, Swarna and Swarna sub-1 of rice.

MATERIALS AND METHODS

For the study, PGPF *F. pallidoroseum* strain-10 (Srivastava et al., 2011) and *T. harzianum* strain-35 (T-35) were obtained from Rhizosphere Biology Laboratory of Department of Biological Sciences of G. B. Pant university of Agriculture and Technology Pantnagar, and two rice genotype Swarna and Swarna sub-1 was obtained from the IRRI Office, NASC Complex, Pusa New Delhi, India.

Preparation of inoculants

For the preparation of fungal inocula 3-5 disk of fresh cultured fungus were inoculated in 100 ml of potato dextrose broth media and kept on shaker for 5 days at 28°C and colony forming unit (cfu) counted by dilution plate method.

Pot experiment

Rice growth promotion by these two strains under drought stress was performed in net house conditions. Rice seeds were surface disinfected by immersion in 70% ethanol and 3% (v/v) sodium hypochlorite for 1 and 5 min. Seeds were washed thoroughly three times with sterile distilled water then germinated on sterilized Petri dish. The soil used for experiment has the following: pH 8.31, organic carbon 1.2%, nitrogen 186.7 kg/h, phosphorus 34.91 kg/h, and potassium 145.6 kg/h.

Before filling the pot the soil were autoclaved at 121 psi for 40 min thrice, every alternate day. The pots were filled with 300 g of soil and watered to field capacity before sowing the seeds. After 2 days the equally germinated seeds were selected for sowing. The fungal inocula were given to 1 ml/pot having 10^4 to 10^5 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 each of the 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°C for 48 h. Second harvesting was done after 12 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:

$$SWC\ (\%) = \frac{(\text{Weight of container with soil}) - (\text{Dry weight of soil with container}) \times 100}{(\text{Dry weight of soil with container}) - (\text{Weight of container})}$$

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°C for further antioxidant activity. For assays of SOD, CAT, Guiacol POD, 0.5 g leaf samples (fresh weight) was homogenized with a pestle in an ice-cold mortar in 5 ml 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 10,000 × g 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 Turkey HSD, at 5% level of significance.

RESULTS AND DISCUSSION

Exposure to drought stress caused a droopy appearance of the shoots, and the leaves starting turning inwards from the outside edges. Plants were harvested after 10 and 12 days of drought. The moisture content of soil in pots was calculated as 41.23 ± 2.28% after 12 days of drought. After first harvesting at 10 days of drought both, *F. pallidoroseum* strain FP-10 and *T. harzianum* strain T-35, with 23.59 and 26.54% increase showed significant effect on plant height in Swarna, whereas in Swarna sub-1 only T-35 with 19.48% showed the significant effect as compare to control. Both the strains FP-10 and T-35 efficiently increased the root length in Swarna, while in Swarna sub-1 only T-35 showed increase in root length

Table 1. Rice growth promotion by selected fungal strains after 10 and 12 days of drought stress.

Days of drought	Varieties	Length (cm)			Fresh weight (g/pot)		Dry weight (g/pot)	
		Treatments	Shoot	Root	Shoot	Root	Shoot	Root
10 days	Swarna	Control	24.30^{a}	13.85^{a}	0.97^{a}	0.42^{a}	0.44^{a}	0.22^{a}
		FP-10	30.03^{b}	15.47^{a}	1.51^{a}	0.67^{a}	0.73^{b}	0.37^{b}
		T-35	30.75^{b}	16.58^{a}	1.36^{a}	0.61^{a}	0.62^{ab}	0.29^{ab}
	Swarna sub-1	Control	26.78^{a}	16.73^{a}	0.79^{a}	0.33^{a}	0.50^{a}	0.18^{a}
		FP-10	25.80^{a}	14.72^{a}	1.14^{b}	0.44^{a}	0.56^{ab}	0.23^{a}
		T-35	32.00^{b}	17.40^{a}	1.66^{c}	0.91^{b}	0.74^{b}	0.55^{b}
12 days	Swarna	Control	23.75^{a}	14.43^{a}	0.57^{a}	0.26^{a}		
		FP-10	29.85^{ab}	15.52^{ab}	1.14^{b}	0.50^{b}		
		T-35	32.82^{b}	18.08^{b}	1.19^{b}	0.50^{b}		
	Swarna sub-1	Control	29.33^{a}	18.02^{a}	0.69^{a}	0.28^{a}		
		FP-10	28.2^{a}	14.23^{a}	0.76^{a}	0.40^{b}		
		T-35	30.95^{a}	16.58^{a}	1.11^{b}	0.39^{b}		

Results are means of three replicate. Mean with different letters significantly different from each other ($P < 0.05$).

over control. After 12 days of drought, T-35 showed the significant effect on shoot (38.18%) and root (25.29%) length in Swarna over control, however, both the strains showed non-significant effect on shoot and root length in Swarna sub-1 over control. *F. pallidoroseum* strain-10 showed maximum increase on dry weight of shoot (66.50%) and root (56.05%) in Swarna after 10 days of drought, while in Swarna sub-1, strain T-35 with 47.11 and 205.55% increase showed maximum effect on shoot and root dry weight over control. After 12 days of drought both the strains showed significant effect on shoot and root fresh weight in Swarna, while in Swarna sub-1 only T-35 showed significant effect on both parameter as compare to control (Table 1).

Among several strategies used to improve crop yield under water stress, use of bio-agents such as *Trichoderma* is an effective and easily adaptive strategy (Bailey et al., 2006). In various plants, introduction of *Trichoderma* species is primarily being studied as biocontrol agent (Evans et al., 2003; Holmes et al., 2006), induce plant growth promotion and tolerance to abiotic stresses including drought (Harman et al., 2004). In present study, enhancement of root and shoot length was differ according to genotype of rice as well as the kind of treatments, however *T. harzianum* strain T-35 was found most effective in both the genotype of rice as compare to FP-10. Both the fungal strains markedly increased the root and shoot biomass in both the genotype of rice over control (Table 1). *Trichoderma* effects on plant growth promotion and root architecture are well known (Mastouri et al., 2012; Yedidia et al., 2001). Enhanced rooting system provides increased surface area for absorption of deep seated water and increase plant stand in drought (Malinowski and Belesky, 2000). The ability of *Trichoderma* isolates to enhance plant growth has been characterized in many cropping system, although the mechanisms involved have not been fully explained (Harman et al., 2004). In the present investigation, the enhanced shoot and root biomass of T-35 and FP-10 treated rice plants may be due to enhanced nutrient availability through solubilization and chelation of minerals and thus increased nutrient uptake efficiency, which is the proposed mechanism by which *Trichoderma* induces plants growth (Harman et al., 2004; Altomare et al., 1999; Yedidia et al., 2001). Similar to present study, increased shoot, root fresh and dry weight of *Trichoderma* inoculated plants under drought stress was observed (Bae et al., 2009). *F. pallidoroseum* as a bioinoculant, in previous study, have also been used as biofertilizer for the growth promotion of different crops like wheat, maize etc. (Srivastava et al., 2011).

The present study demonstrated that, after 12 days of severe drought, both fungal strains showed the greater activity of SOD, CAT and POD as compare to control. In Swarna, both the treatments showed higher SOD activity, however, the effect were non-significant, while in Swarna sub-1, both T-35 and FP-10 strains with 1.43 and 1.58-fold showed significant effect over control. Both the fungal strains T-35 and FP-10 significantly increased CAT activity (1.84 and 1.54-fold) in Swarna, while in Swarna sub-1 the effect were non-significant, however, both the strains showed increased CAT activity over control. In both the varieties of rice T-35 and FP-10, treated plants showed greater POD activity over control. In Swarna strain T-35 with 1.33-fold showed maximum POD activity, whereas in Swarna sub-1, strain FP-10

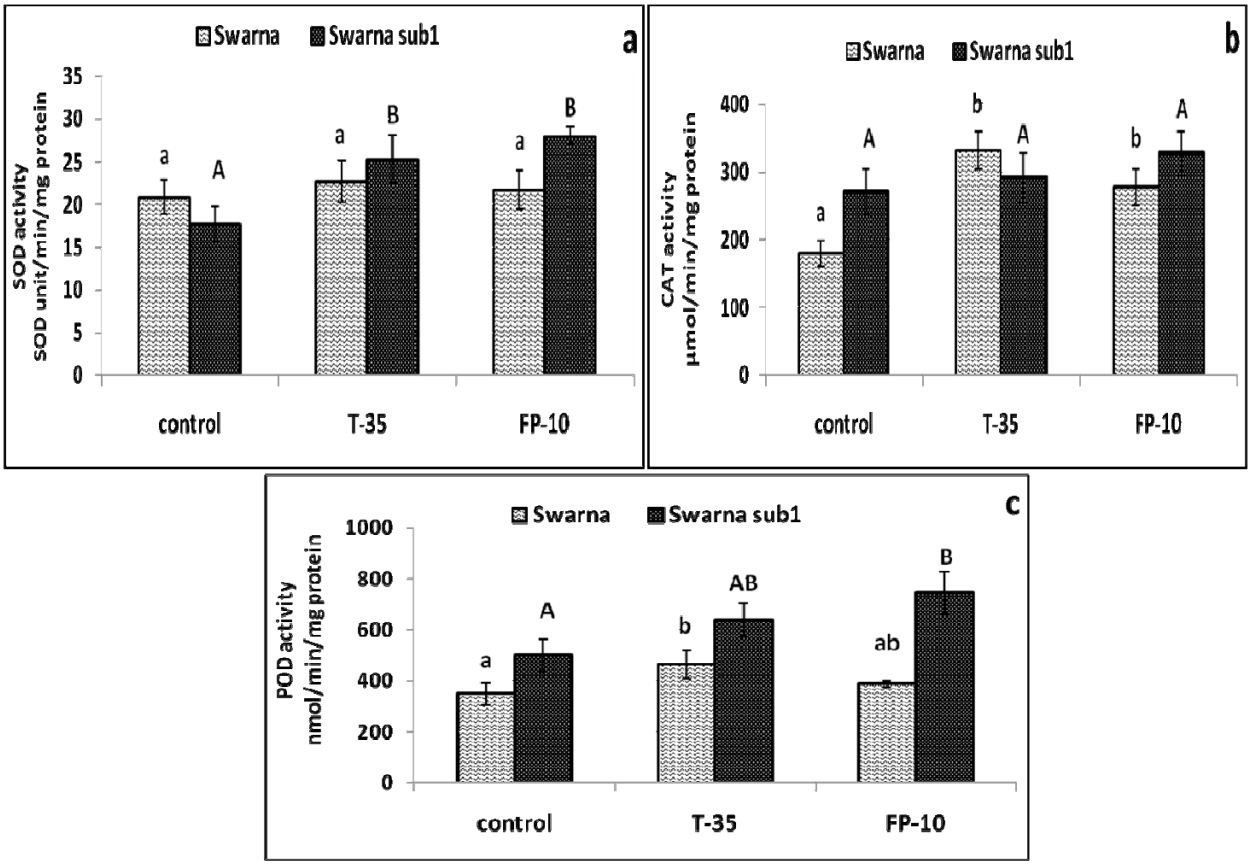

Figure 1. SOD (a), CAT (b) and POD (c) enzymatic activity of rice after 12 days of drought stress. Different letters denote significant differences (P<0.05) among treatments. Line above bars represents Mean ± standard deviation.

with 1.49-fold showed significant effect over control (Figure 1).

Plant response to drought differently that may involve the synthesis of a new set of proteins whose function is largely unknown. Under environmental stress ROS such as superoxide radical, hydrogen peroxide and hydroxyl radicals adversely affect the membranes and DNA of cells (Sharma and Dubey, 2005). Increased SOD activity has been correlated with induced resistance of plants to drought stress (Pastori and Trippi, 1992, 1993; Moran et al., 1994). SOD enzyme convert superoxide radical to hydrogen peroxide (H_2O_2) and oxygen, CAT enzyme convert toxic H_2O_2 to water and oxygen (Blokhina et al., 2003). In this respect under water stress only increased SOD activity cannot protect the plants from toxic effect of oxygen free radical and other antioxidant enzymes (CAT and POD) is necessary to remove toxicity of H_2O_2 (Arora et al., 2002). In present study, both the fungal strains increased the SOD, CAT and POD enzymes in both the genotype of rice as compare to control plants. When we compare both the genotype, irrespective of treatments, Swarna sub-1 showed higher activity of all enzymes as compare to Swarna. The result indicated that these fungal strains may help plants to tolerate stress under severe drought through the reduction of oxidative stress. Similarly, observation of enhanced activity of ROS-scavenging enzymes in *T. harzianum* colonized tomato plants in response to water stress have also been reported (Mastouri et al., 2012).

Conclusion

The result of the present study serve as base for the mediation of PGPF in enhancing water stress resistance in rice plants and need the evaluation of both the strains for growth promotion and productivity of rice under different environmental stress condition.

Conflict of Interests

The authors have not declared any conflict of interests.

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