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Induced Biochemical Changes Due to Seed Treatment by Biocontrol Agents for Controlling Sheath Blight of Rice

Sujata Singh, Roopali Sharma, Archana Negi

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Abstract Rice is an important food grain and is a staple food for majority of the world's population. Over the next 20 years it is expected that demand for rice will grow by 2.5% per year. Rice sheath blight (ShB), caused by *Rhizoctonia solani* Kuhn Teleomorph: *Thanatephorus cucumeris* (Frank Donk), is a destructive disease that causes significant yield loss and quality degradation. The classical control method of ShB is the application of fungicides. Biological control offer better opportunity for control of pathogen along with environment protection. *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus* spp. have been shown promising results in controlling many plant diseases of food crops. Under present study, Induction of defence enzymes (PAL (phenylalanine ammonia lyase), PPO (polyphenol oxidase) and PO (peroxidase)) in crop plants treated with bio-control agents and challenged with the pathogen (*Rhizoctonia solani*) was studied. A total of eight biocontrol agents, one standard chemical check along with one control were analyzed for PAL, PPO and PO activity. Analysis of variance study of the increase in spectrophotometric absorbance (minute^{-1} g^{-1}) showed significant differences among treatments for PAL, PPO and PO activity after 0, 2, 4, 6, 8 days of inoculation. Highest PAL and PPO activity was observed with TCMS 43 treatment and PO activity was observed with TCMS 36 treatment. Control treatment (no control measure applied) showed lowest activity of PAL, PPO and PO. Treatment with application of carbendazime fungicide showed significantly higher but overall moderate activity of PAL, PPO and PO. Results suggest probable role of biocontrol agents in inducing higher PAL, PPO and PO activity which ultimately culminate in reduced disease incidence of sheath blight in rice.

Keywords *Rhizoctonia solani*, Phenylalanine ammonia lyase, Polyphenol oxidase, Peroxidase.

S. Singh, R. Sharma*, A. Negi
Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, U. S. Nagar, Uttarakhand, India
e-mail: roopalibiocontrol@gmail.com
*Correspondence

Introduction

Rice is the most widely cultivated food crop in the world. The majority of the rice (90%) is being produced in Asian countries with China and India being the major producers. Rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot and bacterial blight are the important ones. Sheath blight (ShB) in rice is an important soil-borne fungal disease (*Rhizoctonia solani* Kuhn) causing up to 25% of yield losses. Induced resistance may provide an alternative approach to plant protection especially for problems not satisfactory controlled by various fungicides. Induced resistance is defined as an enhancement of the plant defensive capacity against a broad spectrum of pathogens and pests that is acquired after appropriate stimu-

lation. The resulting elevated resistance due to an inducing agent upon infection by a pathogen is called ISR or SAR. Plant has endogenous defense mechanisms that can be induced in response to attack by insects and pathogens. Defense action occurs due to the accumulation of PAL, PPO, and PO. Raj et al. [1] studied the induction of defence enzymes in crop plants treated with bio-control agents and challenged with the *Rhizoctonia solani*. They concluded that defense reaction occurs due to accumulation of peroxidase, phenylalanine ammonia lyase and PR-protein like β-1, 3-glucanase. Induction of systemic resistance in rice by *Pseudomonas fluorescens* against rice root knot nematode *Meloidogyne graminicola* was studied [2]. The objective of the present study is tounravel the induction of various defense related genes encoding proteins implicated in strengthening of plant cell walls by biocontrol agents treatments in response to infection by *Rhizoctonia solani*.

Materials and Methods

Source of *R. solani* and bioagents

R. solani isolated from infected sheath blight plants by the basic isolation technique. The observations taken under compound microscope for morphological identification of *R. solani* and eight bioagents (TCMS 43, TCMS 36, TCMS 9, TH 14, PBAT 3 [TH 14 + Psf 173], Psf 173, Psf 2 and *Bacillus* N 18) were procured from the biocontrol lab, department of plant pathology, GBPUA and T, Pantnagar.

Greenhouse experiment

Rice seeds treated with the nine treatments overnight in moist chamber. Seeds were sown in the pots containing sterilized soil at the rate of 6 seed per pot and grown under greenhouse conditions. Then 30 days old plants raised from the seeds were inoculated with the pathogen *R. solani.* Each treatment was replicated thrice. This inoculated sheath samples were collected after 0, 2, 4, 6, 8 days starting from first day after inoculation. These sheath samples were used for crude enzyme preparation.

Biochemical estimation

Assay of defense related enzymes

Enzymes activity was determined as per the procedure described earlier for PAL, PPO and PO.

Sample collection

Samples were collected from each treatments and kept under –80°C and used for analysis.

Enzyme extract

The leaf sample, collected from treated and pathogen-inoculated rice plants were immediately homogenized with liquid nitrogen. One g of sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. Enzyme extracts prepared from rice tissues were used for estimation of defense enzymes phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) and peroxidase (PO). The supernatants (crude enzyme extract) were immediately used for determination of enzyme activities.

Peroxidase (PO) activity

One g of leaf sample was homogenized in 1 ml of 0.1 M phosphate buffer pH 7.0 in a pre-cooled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to assay activities of PO. 1.5 ml of 0.05 M pyrogallol and 0.1 ml of enzyme extract were taken and added to cuvette. To initiate the reaction 0.5 ml of 1% H_2O_2 was added. The change in absorbance was recorded at 420 nm at 30 sec interval for three min from zero second of incubation at room temperature. The results were expressed as change in absorbance/min/g of fresh tissue.

Polyphenol oxidase (PPO) activity

The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer pH 6.5 with 0.1 ml of enzyme extracts. To this 0.2 ml of 0.01 M catechol was added to initiate the reaction. The change in absorbance

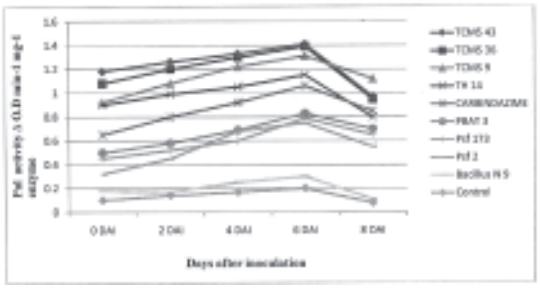

Fig. 1. Phenyl alanine ammonia lyase (PAL) activity of rice leaf sheaths due to seed treatment with biocontrol.

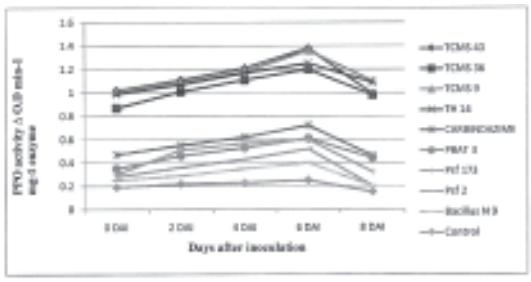

Fig. 2. Polyphenol oxidase (PPO) activity of rice leaf sheaths due to seed treatment with biocontrol.

was recorded at 495 nm and the results were expressed as change in absorbance/min/g of fresh tissue.

Phenylalanine ammonia lyase (PAL) activity

One g of rice leaf was homogenized in 2 ml of ice cold 0.1 M sodium borate buffer, pH 7.0 and centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to assay the enzyme activity. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm. Sample extract of 0.4 ml was incubated with 0.5 ml of 0.1 M borate

Table 1. PAL, polyphenol oxidase and peroxidase activity estimated from rice leaf sheaths. DAI* = Day after inoculation.

	PAL (ΔO.D min^{-1} mg^{-1})					PPO (ΔO.D min^{-1} mg^{-1})				
Treatments	0DA 1*	2DA 1*	4DA 1*	6DA 1*	8DA 1*	0DA 1*	2DA 1*	4DA 1*	6DA 1*	8DA 1*
TCMS 43	1.18	1.26	1.26	1.41	0.97	1.02	1.11	1.21	1.38	0.99
TCMS 36	1.08	1.08	1.30	1.30	0.95	0.87	1.01	1.11	1.20	0.98
TCMS 9	0.92	1.08	1.22	1.31	1.12	1.01	1.09	1.18	1.36	1.10
TH 14	0.90	0.99	1.05	1.15	0.80	0.99	1.07	1.17	1.25	1.09
Carbindazime	0.65	0.80	0.92	1.06	0.85	0.47	0.55	0.62	0.72	0.46
PBAT 3	0.50	0.58	0.69	0.83	0.70	0.35	0.46	0.53	0.61	0.49
Psf 173	0.45	0.52	0.60	0.79	0.65	0.3	0.51	0.57	0.60	0.32
Psf 2	0.32	0.45	0.68	0.75	0.55	0.28	0.36	0.43	0.52	0.20
Bacillus N 9	0.18	0.17	0.25	0.30	0.11	0.25	0.29	0.29	0.40	0.20
Control	0.10	0.14	0.17	0.20	0.08	0.19	0.22	0.23	0.25	0.15
CD (1%)	0.06	0.07	0.05	0.06	0.06	0.06	0.05	0.06	0.07	0.06

Table 1. Continued.

	PO (Δ O.D min^{-1} mg^{-1})				
Treatments	0DA 1*	2DA 1*	4DA 1*	6DA 1*	8DA 1*
TCMS 43	1.01	1.09	1.18	1.34	1.05
TCMS 36	1.02	1.11	1.21	1.38	0.99
TCMS 9	0.99	1.07	1.17	1.25	1.09
TH 14	0.87	1.01	1.11	1.20	1.98
Carbindazime	0.47	0.55	0.62	0.72	0.46
PBAT 3	0.35	0.46	0.53	0.61	0.44
Psf 173	0.3	0.51	0.57	0.60	0.32
Psf 2	0.28	0.36	0.43	0.52	0.20
Bacillus N 9	0.25	0.29	0.35	0.40	0.20
Control	0.19	0.22	0.23	0.25	0.15
CD (1%)	0.06	0.07	0.07	0.06	0.06

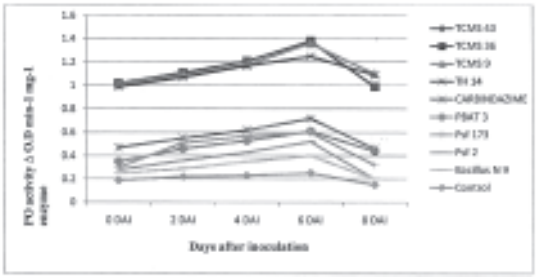

Fig. 3. Peroxidase (PO) activity of rice leaf sheaths due to seed treatment with biocontrol.

buffer, pH 8.8 and 1 ml of 12 mML phenylalanine and incubated for 1 h at 30°C. The reaction initiated by L-phenylalanine was stopped with 0.5 ml of 2 N HCl. A blank was maintained by adding L-phenylalanine after the addition of 2 N HCl. The absorbance was read at 290 nm and the results were expressed as nmol transcinnamic acid/min/g of fresh tissue.

Statistical analysis

The data were analyzed using analysis separately for each experiment and were subjected to analysis of variance (ANOVA) (SPSS, version 16). Significant effects of treatments were determined by the *F* values ($p \leq 0.01$).

Results and Discussion

To evaluate biochemical basis of systemic resistance induced by eight bioagents one fungicide against pathogens *R. solani* the possible role of defense enzymes, viz., PAL, PPO and PO were assessed in rice plant (Table 1). The results revealed that TCMS 43 in PAL and PPO and TCMS 36 in PO induced higher levels of defense enzymes in rice. Similar findings were reported earlier [1]. Plants infested with pathogen alone showed decreased defense enzymes activity. There were a significant increase in PAL, PPO and PO activity in leaf sheaths of plants inoculated with *R. solani* with treatments TCMS 43 and TCMS 36 treated seeds (Fig. 1, Fig. 2 and Fig. 3). The production of enzymes plays an importance role in induced defense reaction. The accumulations of activity of enzymes are known to be associated in biochemical disease resistance. Biocontrol agents increase the enzyme activity and this may induce ISR in plant. Several authors have reported the induction of defense enzymes in crop plants treated with biocontrol agents and challenged with the pathogen [5]. PO is an important enzyme in the synthesis of lignin and it is also known to catalyze the oxidation of lignin of many mono and diphenols and aromatic amines to the highly toxicquinones in the presence of hydrogen peroxide. PO oxidizes phenolics to highly toxic quinones hence has been assigned a role in disease resistance. PAL is the first enzyme of phenyl propanoid metabolishm in higher plants and polyphenol oxidase (PPO) is enzymes which use molecular oxygen to catalyze the oxidation of monophenolic and orthophenolic compounds.

Conclusion

In this study concluded the induction of defense related enzyme activity by biocontrol agents against *R. solani* in rice plant. The application of biocontrol agents as seed treatments could prove to be a beneficial components of integrated pest management. These biocontrol agents are also work as good plant growth promoters and are also able to induce systemic resistance in rice plants, which is use in practical agriculture system.

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