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Induction of systemic resistance (ISR) against sheath blight of rice caused by *Rhizoctonia solani* Kuhn using biological seed treatment with *Trichoderma*

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Abstract: Sheath blight caused by *Rhizoctonia solani* Kuhn has recently noticed one of the most important diseases of rice on almost all the high yielding varieties in major rice growing area. In our study morphologically and molecularly characterized thirty rhizospheric isolates of *Trichoderma* (*T. harzianum* and *T. virens*) from different locations of Uttarakhand were evaluated for their mycoparasitic ability, disease (sheath blight) suppressing potential and inducing systemic resistance against *Rhizoctonia solani*. Maximum inhibition in hyphal growth (58.9 %) against *R. solani* was recorded with isolate PB 2 followed by PB 3 (53.4 %) in confrontation assay. Under glass house condition, minimum disease severity (13.6%) was recorded in the treatment where seeds were treated with PB 22 and this treatment also exhibited highest total phenol content (394 µl/g) 168 hours after pathogen inoculation. Total phenol content was also increased maximally (466 µl/g) before pathogen inoculation in the treatment where seeds were treated with PB 22. Moreover, high quality ISR activity was recorded with isolates PB 21, 3, 1, 4, 23, 2 and 16 as they reduced more than 34 percent disease and total phenol contents 456 µl/g, 449 µl/g, 442 µl/g, 440 µl/g and 440 µl/g, 438 µl/g and 431 µl/g were recorded for respective isolates indicated induction of resistance in paddy against sheath blight disease caused by *R. solani*.

Keywords: ISR, Mycoparasitism, Rice, Sheath blight, Total phenol content, *Trichoderma*

INTRODUCTION

Sheath blight disease caused by the fungus *Rhizoctonia solani* Kuhn is a very destructive disease of all the major high yielding rice varieties under favourable weather conditions causing substantial yield loss (Pal *et al*., 2015). *Trichoderma* is gaining worldwide importance and acceptance, because of its adaptability and variety of mechanisms involved in disease control (VanWees *et al*., 2008, Vinale *et al*., 2014). The capability of *Trichoderma* sp. to antagonize pathogenic microorganisms is fairly well known and understood globally, however we are not able enough to unveil many questions about the process of systemic resistance induced in plants by these fungi till date (Kotasthane *et al*., 2015). Induced systemic resistance is believed to be one of the most important mechanisms of biocontrol effects of *Trichoderma* (Harman, 2006, Segarra, *et al*., 2009). In one of the first comprehensive studies on induction of resistance in cucumber plants by *T. harzianum* demonstrated by Yedidia *et al.* (1999). ISR activity in paddy using *Aspergillus niger* was demonstrated by Sen (2000) when plants raised from strain AN-27 treated seeds exhibited 30 per cent less sheath blight disease as compared to control. Hanson (2000) studied the ability to induce resistance in cotton plants against *Verticillium* wilt when seeds were treated with dried preparations of two strains of *T. virens* and observed significant reduction in disease severity with both the strains. Keeping this in view, present study was undertaken to find out potential indigenous isolates of the *Trichoderma* from different locations of Uttarakhand which can either suppress the pathogen i.e. *R. solani* or the sheath blight disease, in order to find biocontrol isolates for application in the field. To fulfill this objective all rhizospheric isolates of *Trichoderma* were screened in confrontation assay followed by pot experiment under glass house condition and estimation of total phenol content for testing their ability to inhibit the growth of *R. solani* or induce systemic resistance against sheath blight of rice.

MATERIALS AND METHODS

Experimental materials: *Trichoderma* strains were isolated from rhizospheric soils of different crops and locations of Uttarakhand (Table 1) and seeds of paddy (cv. Pant Dhan-4) were obtained from SPC, Pantnagar. The tested phytopathogen used in the present study was isolated from agricultural field of district Udham Singh Nagar (Uttarakhand).

Confrontation assay: All the 30 *Trichoderma* isolates were evaluated for their mycoparastic ability against *Rhizoctonia solani* following dual culture technique (Dennis and Webster, 1971) on poured PDA medium plates. The Petri dishes were incubated at 25 ± 2^0C. Per cent reduction in hyphal growth of pathogen

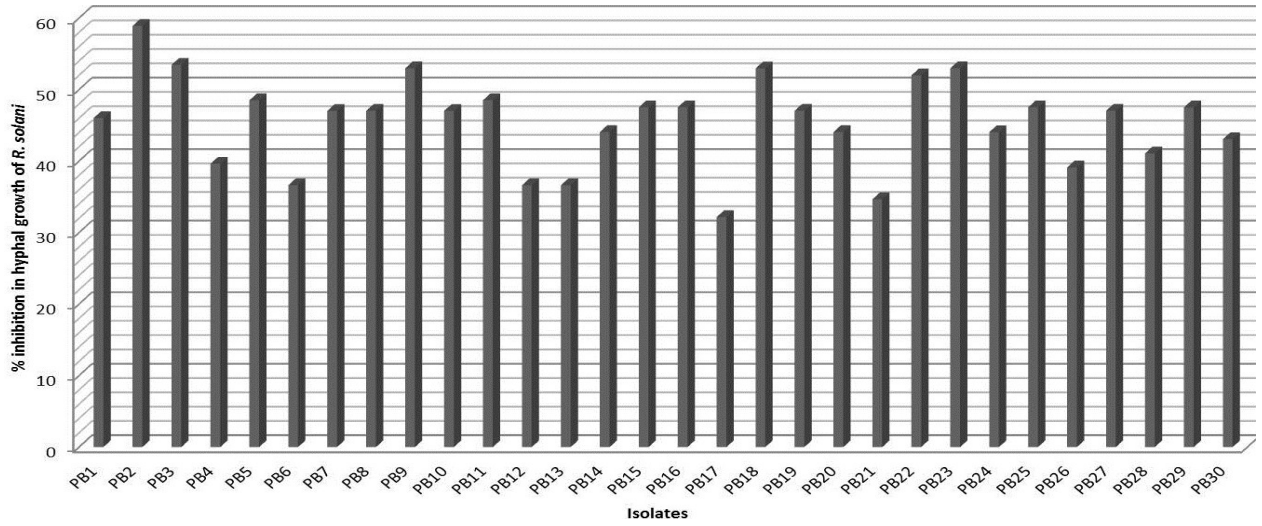

Fig. 1. *Percent reduction in hyphal growth of Rhizoctonia solani.*

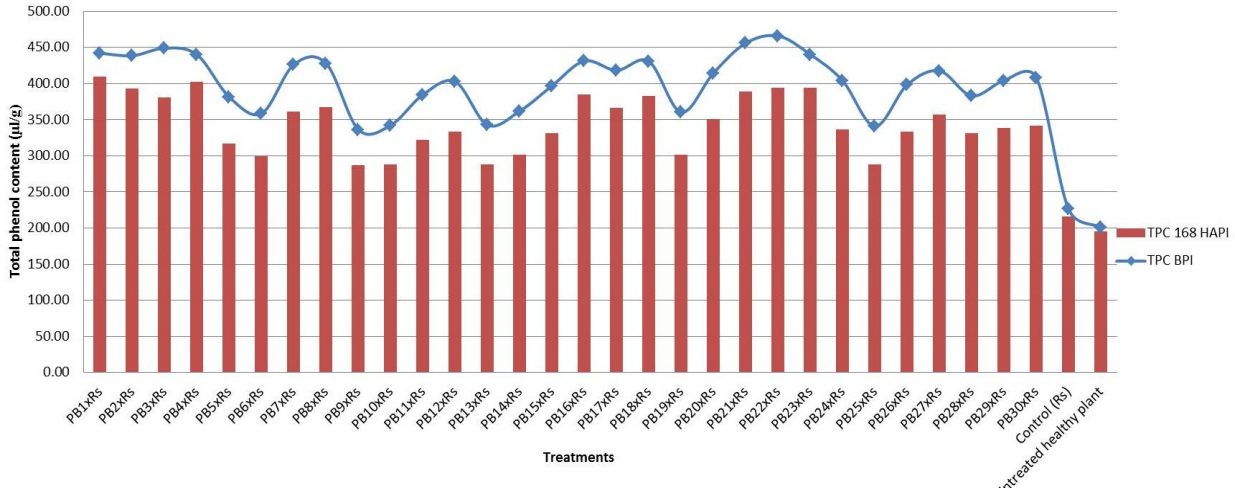

Fig. 2. *Total phenol content in rice leaf before pathogen inoculation and 168 hours after pathogen inoculation.*

was calculated by using following formula:

I = **(C**-T) / C X 100 ……..Eq 1

I = Per cent inhibition in mycelial growth, C = Growth of pathogen in control plate, T = Growth of pathogen in dual culture plate

Pot experiments: All isolates of Trichoderma were then tested for their pathogen and/or disease suppression ability against sheath blight (*Rhizoctonia solani*) of rice via pot experiment under glass house conditions and potential isolates were identified for Induction of systemic resistance. Paddy seeds (surface sterilized) were treated with powdered formations of biocontrol agents (@ 10g/kg seeds; cfu=10^9/g powder) and ten seeds per pot were sown with triplicates in plastic pots (5kg capacity) containing sterilized soil. Five days after germination pots were thinned to three plants per pot and watered regularly to keep the proper soil moisture. After 30 days of sowing, second leaf sheaths (from the top) of paddy seedling were inoculated with 3 day-old immature sclerotia of *R. solani* by following the method described by Singh *et al*. (2000). Observation was recorded on lesion length development after 7th day of inoculation and percent disease severity and percent reduction in disease severity were calculated by using following formulae:

Percent Disease Severity (PDS) = Lesion length / Plant height X 100 ……Eq 2

Extraction of phenols: Phenols were estimated by the procedure described by Sadasivam and Manickam (1997). One gram of leaf tissue was grinded in 5 ml 80% methanol. The extract was agitated at 70ºC for 15 minutes. Afterwards in 1 ml sample of methanolic extract, 5 ml distilled water was added to make the final volume 6 ml. To this 250 µl Folin's reagent was added and the mixture was incubated for 3 min at room temperature. After incubation, 1 ml 20% sodium carbonate and 1 ml distilled water were added and the solution was incubated for 1 hr. at room temperature. Absorbance was recorded at 725 nm. The amount of total phenols was estimated from the standard curve for tannic

Table 1. Soil samples collected from different locations.

Sl. No.	Crop	Location	Isolate code
1	Paddy	Kathgodam-Haldwani	PB1
2	Paddy	Halduchaur-Haldwani	PB2
3	Paddy	Lamachaur-Haldwani	PB3
4	Paddy	Khema-Almora	PB4
5	Paddy	Khema-Almora	PB5
6	Paddy	Khema-Almora	PB6
7	Paddy	Khema-Almora	PB7
8	Paddy	SPC-Pantnagar	PB8
9	Paddy	SPC-Pantnagar	PB9
10	Paddy	Rudrapur-U.S. Nagar	PB10
11	Paddy	Rudrapur-U.S. Nagar	PB11
12	Paddy	Rudrapur-U.S. Nagar	PB12
13	Paddy	Rudrapur-U.S. Nagar	PB13
14	Paddy	Rudrapur-U.S. Nagar	PB14
15	Apple	Mukteshwar-Almora	PB15
16	Broccoli	Mukteshwar-Almora	PB16
17	Pea	Mukteshwar-Almora	PB17
18	Pea	Mukteshwar-Almora	PB18
19	Strawberry	Mukteshwar-Almora	PB19
20	Walnut	Mukteshwar-Almora	PB20
21	Paddy	Premnagar-Dehradun	PB21
22	Mustard	Premnagar-Dehradun	PB22
23	Maize	Dhalwala-Rishikesh	PB23
24	Maize	Bhaniawala-Dehradun	PB24
25	Paddy	Bhaniawala-Dehradun	PB25
26	Paddy	Mazra-Ranipokhri	PB26
27	Maize	Geetanagar Rishikesh	PB27
28	Paddy	Raipur-Dehradun	PB28
29	Paddy	Raiwala-Haridwar	PB29
30	Paddy	Nagani, Tehri Garhwal	PB30

Table 2. ISR activity of *Trichoderma* isolates against sheath blight of paddy.

Isolate Code	Lesion length (mm)	Lesion width (mm)	% Disease Severity*	% reduction in Disease Severity*
PB1x*Rs*	21.7	2.7	19.1 (25.9)	38.5 (38.3)
PB2x*Rs*	11.3	3.7	17.7 (24.9)	37.0 (37.5)
PB3x*Rs*	7.7	3.3	16.0 (23.6)	42.5 (40.7)
PB4x*Rs*	12.7	2.0	20.7 (27.0)	37.7 (37.9)
PB5x*Rs*	5.3	4.0	25.4 (30.2)	17.3 (24.5)
PB6x*Rs*	15.3	2.7	27.4 (31.6)	9.6 (18.0)
PB7x*Rs*	13.7	3.7	19.5 (26.2)	29.4 (32.9)
PB8x*Rs*	7.7	3.0	19.2 (26.0)	29.7 (33.0)
PB9x*Rs*	12.3	3.7	26.6 (31.0)	5.9 (13.9)
PB10x*Rs*	15.3	5.7	25.6 (30.4)	6.0 (13.8)
PB11x*Rs*	22.3	4.7	26.8 (31.2)	18.4 (25.4)
PB12x*Rs*	20.7	5.3	23.9 (29.2)	22.3 (28.1)
PB13x*Rs*	27.0	6.0	25.7 (30.5)	6.0 (13.8)
PB14x*Rs*	12.7	4.7	21.7 (27.8)	10.0 (18.3)
PB15x*Rs*	20.0	4.3	20.4 (26.9)	21.2 (27.4)
PB16x*Rs*	10.3	3.7	17.2 (24.5)	34.4 (35.9)
PB17x*Rs*	24.3	3.0	18.9 (25.8)	30.0 (33.2)
PB18x*Rs*	13.0	4.7	16.5 (23.9)	34.0 (35.7)
PB19x*Rs*	17.3	3.3	23.8 (29.2)	10.0 (18.3)
PB20x*Rs*	7.0	2.7	25.6 (30.4)	25.9 (30.6)
PB21x*Rs*	11.7	3.0	14.3 (22.2)	46.6 (43.0)
PB22x*Rs*	7.7	5.7	13.6 (21.6)	50.4 (45.2)
PB23x*Rs*	15.7	4.7	16.5 (24.0)	37.1 (37.5)
PB24x*Rs*	6.3	2.7	22.2 (28.1)	22.4 (28.2)
PB25x*Rs*	13.3	5.7	26.6 (31.0)	6.0 (13.8)
PB26x*Rs*	22.0	5.7	20.5 (26.9)	21.7 (27.8)
PB27x*Rs*	10.7	2.7	21.8 (27.9)	26.2 (30.7)
PB28x*Rs*	14.3	2.0	27.1 (31.4)	18.3 (25.3)
PB29x*Rs*	10.0	2.7	21.9 (27.9)	22.5 (28.3)
PB30x*Rs*	7.3	1.7	25.0 (30.0)	23.8 (29.2)
Control(*Rs*)	31.3	5.7	34.2 (35.8)	-
CD(p= 0.05)	1.19	0.73	0.48	3.81

* Values in bracket are the angular transform values

acid and expressed as µg phenol g-1 fresh leaf weight. Data was recorded as total phenolic content in rice leaves, before pathogen inoculation (BPI) and 168 hours after pathogen inoculation (HAPI).

RESULTS

Results of the mycoparasitism of Trichoderma against *Rhizoctonia solani* are summarized in figure 1. All thirty isolates were effectively suppressed the hyphal growth of *R. solani in-vitro* which was ranged from 37.2 % to 58.9 %. Maximum reduction in hyphal growth was recorded with isolate PB 2 (58.9 %) followed by PB3 (53.4 %) while isolate PB 17 resulted in minimum reduction of hyphal growth. Only six isolates viz. PB 2, 3, 9, 18, 22 and 23 inhibited more than 50 percent hyphal growth of the pathogen *in-vitro*. Isolates PB 3, 9, 18 and 23; PB 5 and 11; PB 15, 16 and 25; PB 8, 10, 19, 27 and 29; PB 14, 20 and 24; PB 4 and 26; PB6, 12 and 13 were at par. Furthermore, all the Trichoderma isolates were evaluated through seed treatment under glass house pot experiments for their disease suppression potential to induce systemic resistance (ISR) in paddy against sheath blight fungus and results are summarized in table 2. Results revealed that all the isolates significantly suppressed sheath blight development 30 days after application as seed treatment. More than 50 percent disease suppression was observed only with PB 22 (50.4 %) which was the highest among all the screened isolates. Among the all treatments PB 18 and 23; PB 14, 27 and 29; PB 24, 27 and 29; PB 5 and 30; PB 5, 10 and 20; PB 10, 13 and 20; PB 11 and 25; PB 11 and 28; PB 6 and 28 were at par. Results revealed that 42.5 %-50.4 % reduction in disease was recorded only with 3 isolates PB 22, PB 21 and PB 3 out of 30 isolates. In all the Trichoderma treatments, total phenolic content in rice leaves was found significantly higher as compare to untreated healthy control before pathogen inoculation (figure 2) which was also higher as compare to those treatments recorded 168 HAPI (figure 2). Maximum phenol content (468 µl/g) was recorded with isolate PB 22 followed by PB 21(456 µl/g) and PB 3 (449 µl/g) which was significantly higher as compare to untreated healthy control (201.33µl/g). Significantly higher total phenolic content of rice leaves was also observed 168 hours after pathogen inoculation over untreated pathogen inoculated control (control *R.s.*). Maximum mean total phenolic content (394 µl/g) in rice leaves was observed with PB 22 as compare to untreated pathogen inoculated control (216 µl/g), which is followed by PB

21(389 µl/g) and PB 3 (380.67 µl/g).

DISCUSSION

The isolates PB 2, 3 and 22 were found most effective and gave more 50 % inhibition of hyphal growth of *R. solani* in dual culture were also suppressed the sheath blight disease by more than 34 % when the paddy seeds were treated with dry formulation of these isolates before sowing (Figure 1). Isolate PB 2 that gave maximum per cent inhibition (58.9 %) however it was not found the best isolate to induce the systemic resistance against sheath blight fungus. Li *et al.* (2001) also studied antagonistic effect of Trichoderma spp. against *Rhizoctonia solani*. According to Punja and Utkhede (2003) Trichoderma spp. are the most widely studied mycoparasitic fungi. A lot of work has been done with Trichoderma sp. (Elad, 2000, Howell, 2003, Benitez, *et al.*, 2004, Kotasthane *et al*., 2015) which improved our understanding about mycoparasitism. A similar study was conducted by de-França *et al*. (2015) a mixture of four isolates of *T. asperellum* was found efficient in reducing the severity of sheath blight and increasing the rice yield and grain weight in Brazil. In addition to the well-recognized mycoparasitic nature of Trichoderma, induction of resistance against pathogens in plants has also been reported by several workers (Benitez *et al.*, 2004, Harman *et al.*, 2004 and Shoresh, *et al.*, 2005) as indirect mechanism of biocontrol. Saksirirat *et al*. (2009) reported that isolate of *T. harzianum* (T9) induced resistance in tomato plant (cv. Sida cultivar) with reducing 69.32% bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) after 14 days post inoculation. Accumulation of increased level of phenols in plants under pathogen challenge or application of bioagent is the first stage of defense mechanism which slows the growth of the pathogen (Kumawat *et al*., 2008, Gangwar and Sinha, 2014, Vinale *et al*., 2014). One important observation was recorded with isolate PB 9 that gave more than 50 % inhibition of hyphal growth of *R. solani* in dual culture but found least effective against sheath blight with minimum (5.9 %) reduction in disease which was also supported with least phenolic content (286.67 µl/g) in paddy leaf 168 HAPI. However, isolate PB 22 identified most potential isolate for the disease suppression ability which was evidenced with highest TPC (394 µl/g) under pathogen challenge (Fig. 2). Sivakumar and Sharma (2003) recorded an increase in phenolic content in maize leaf sheaths inoculated with *R. solani* or plants raised from *P. fluorescens* treated seeds. Karthikeyan *et al*. (2006) also reported induction of phenolics in coconut roots treated with biocontrol agents (*Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum*) against Ganoderma disease and they reported maximum level of phenolics after 9 days of treatment application.

Conclusion

Isolate PB 22 was identified highly efficient to suppress the disease (50.4 %) and induced resistance in rice against sheath blight pathogen which is evidenced by highest phenolic content (394 µl/g) in leaves of diseased rice plants 168 HAPI. Hence, PB 22 can be utilized under field condition as an alternative of chemical fungicide. In our study, we can also conclude that it is not essential that a good antagonist must be good inducer of systemic resistance as isolate PB 2 was best antagonist against *R. solani* in confrontation assay but weakest to suppress the disease under glass house.

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