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RESEARCH **P**APER

Antipathogenic potentiality of fluorescent pseudomonads for the management of rice sheath blight pathogen, *Rhizoctonia solani*

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Sheath blight (ShB) of rice is an important soil-borne fungal disease caused by *Rhizoctonia solani* (Kuhn) causing up to 40 per cent of yield losses annually. The present investigation was aimed to study the effect of Fluorescent pseudomonads on sheath blight management in rice and impact on plant growth. Fifteen different pseudomonad isolates were evaluated for their antagonistic activity against *Rhizoctonia solani* isolates under *in vitro* condition. Per cent inhibition of mycelial growth of *R. solani* by pseudomonads ranged from 74-100. All the isolates showed antagonism against the pathogen. Five strains, 12, 20, 19, soy2 and soy6 were found potent with 87-100 per cent inhibition of mycelial growth. They were further evaluated in greenhouse as seed treatment, soil application, foliar spraying and combined (seed+soil+foliar application) treatment for sheath blight control. Fluorescent pseudomonad isolate 19 was found potent and promising as it reduced the disease to the maximum extent and stimulated plant and root growth.

Key words : Rice, *Rhizoctonia solani*, Sheath blight, Fluorescent pseudomonads, Biocontrol

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INTRODUCTION

Sheath blight caused by *R. solani* is one of the most widespread diseases of rice and cause serious yield losses up to 50 per cent under favorable environmental conditions (Ou, 1985). The pathogen has very wide host range and the resistance sources in rice against this disease are rare. *R.solani* causing sheath blight in rice belongs to anastomosis group AG1- subgroup IA which mainly exist as vegetative mycelium and sclerotia on rice, although teleomorph have been observed in field. AG1-IA is also known to be the most destructive pathogen on corn and soybean. The pathogen survives as sclorotia in soil or in rice stubbles or on seeds and is disseminated by irrigation water (Premalatha and Dath, 1990). Fungicides for seed treatment (IRRI, 1980), soil application (Chen and Chu,1973) and foliar spray (Dev and Mary, 1986) are being applied to control the disease. However, these treatments are expensive and add pollutants to the environment. Use of biocontrol agents in plant disease management is an ecologically-friendly and cost effective strategy which can be used in integration with other management tactics for sustained crop yields. A successful bioagent should not only be able to control or reduce the disease but also contribute to crop growth promotion and yield. Among different biocontrol agents, plant growth-promoting rhizobacteria (PGPR) are widely used in managing soil borne diseases of several field crops. PGPR group offers an effective means of antagonism against ShB pathogen. Besides, they also contribute to enhanced seedling growth and induced systemic resistance (ISR) against diseases and thereby increase in yield (Pathak *et al*., 2004).

In recent years, fluorescent pseudomonads have drawn attention worldwide because of production of secondary metabolites such as siderophore, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Gupta *et al*, 2001). Fungi from *Trichoderma* genus (Lin, 1994) and

bacteria belonging to *Pseudomonas* and *Bacilllus* genus have been used as biological control agents against *R. solani* (Gasoni *et al.*, 1998). The ideal biocontrol agent for the management of foliar infection by soil borne pathogen may be the one that can survive in both rhizosphere and phyllosphere. Among the various biocontrol agents, fluorescent pseudomonads are known to survive both in rhizosphere (Park *et al.*, 1991) and phyllosphere (Wilson *et al.*, 1992). Considering such qualities of biocontrol agent, the present study was aimed to screen the fluorescent pseudomonads for antagonism under *in vitro* and to evaluate their biocontrol potentiality under glass house condition against *R. solani* in rice.

RESEARCH METHODOLOGY

Plant, pathogen and isolates of fluorescent pseudomonad :

The susceptible rice cv. Samba mahsuri (BPT – 5204) and two isolates of the sheath-blight pathogen, *R. solani viz*., Mandya HRL and VC Farm Mandya were used in this research. The different strains of Fluorescent pseudomonad used here were obtained from Department of Microbiology, University of Agricultural Sciences, Dharwad. This study was undertaken in the Department of Biotechnology, University of Agricultural Sciences, Dharwad, Karnataka, India during 2012-2013.

***In vitro* screening of fluorescent pseudomonad isolates against *R. solani* for antagonism :**

Fluorescent pseudomonad isolates were retrieved from cold storage and grown on King's B medium. The antagonistic potentiality of *F. pseudomonads* against *R.solani* was assessed by dual culture technique. Bioagents were screened by streaking on either side of the plate containing King's B medium and agar plug of *R. solani* was placed at centre of streaked culture plate after 48 hrs of bacterial growth (Kazempour, 2004).

In each treatment per cent inhibition was calculated by using formula as, I=C-T/C×100 where, I-inhibition of mycelial growth (%), C-growth of pathogen in control (mm), T- growth of pathogen in treatment (mm) (Vincent, 1947). The treatments were replicated thrice and the experiment was repeated twice. The results were statistically analyzed by following Duncan multiple range test (DMRT).

Mass multiplication of potent strains of fluorescent pseudomonad :

A loopful of different PGPR strains was inoculated to nutrient broth separately and incubated in a rotary shaker at 150 rpm for 48 hrs at room temperature (28±2ºC). After 48 hrs of incubation, the broth containing 9×10^8 cfu/ml was used for the preparation of talc based formulation. To 400 ml of bacterial suspension, one kg of the purified talc powder (sterilized at 105ºC for 12 hrs) 15 g calcium carbonate (to adjust the pH to neutral) and 10 g of carboxy methyl cellulose (CMC) as an adhesive were mixed under aseptic conditions following the method described by Vidhyasekaran and Muthamilan (1999). The product was shade dried to reduce the moisture content below 20 per cent and then packed in polythene bags and sealed. At the time of application, the population of the bacteria in talc formulation was checked to 2.5 to 3×10^8 cfu/g.

Evaluation of fluorescent pseudomonad against *R. solani* under glasshouse condition :

The experiment was conducted in pot culture under greenhouse condition with 3 replications following the Completely Randomized Block Design (CRBD). The plastic pots having 4 kg capacity were filled with autoclaved sandy loamy soil and fertilizer was applied @ 100: 50: 50 NPK/ha. The seeds of susceptible rice variety, BPT-5204 (Samba mahsuri) were surface sterilized and sown in pots. The pathogen was soil inoculated @ 4 per cent and later inoculated to sheath of 4 week old plants in the form of agar plug of 4 days old culture covered with wet cotton which was properly bound with aluminium foil.

The treatments were as follows : T_1: seed treatment of bioagent, T_2: soil application of bioagent, T_3: spraying of bioagent on leaves, T_4: combined (seed treatment + soil application + foliar spraying of bioagent) treatment, T_5: only pathogen, T_6: only bioagent, T_7: untreated control.

Seed bacterization was done by treating the seeds with PGPR culture having a population of10^6 cfu/ml in suspension culture. It was treated @ 1g of seed per 10 ml suspension along with 0.2 per cent carboxy methyl cellulose. Seeds were air dried to avoid clumping. After air drying, the treated seeds were sown in pots. Soil application was done by mixing the talc based culture with autoclaved soil @ 2 per cent per kg soil. Foliar spraying made at 45 days after sowing @2 per cent culture suspension having spore load of 10^6 cfu/ml. The disease parameters like lesion length and number of dried leaves due to disease were recorded. In addition growth parameters like shoot length, number of tillers, root length, dry weight of root of rice.

RESEARCH FINDINGS AND ANALYSIS

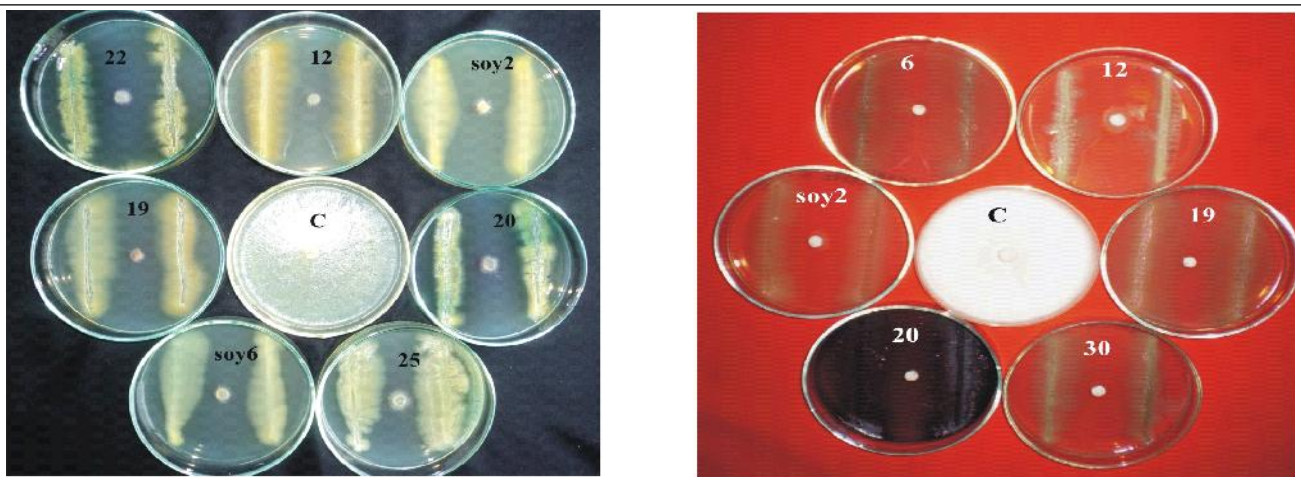
The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

Screening of fluorescent pseudomonad against *R. solani* under *in vitro* condition

Dual culture studies of fluorescent pseudomonad strains against *R. solani* revealed that the inhibition of mycelial growth of *R. solani* isolates Mandya HRL and VC farm

Mandya ranged from 83-100 per cent and 63-100 per cent, respectively. Among the fifteen strains of fluorescent pseudomonad, strain 19 was found to be highly effective in controlling both the isolates with 100 per cent inhibition. Many strains were found promising with inhibition range of 90-100 per cent against both the isolates, as strain 6, 12, 22, 25, 30 and soy6 (Table 1 and Fig. 1).

Due to the presence of iron chelating ability, a siderophore producing bacterium inhibit harmful microorganisms by competing for iron and thus, reduces the levels of freely available ferric ions (Sayyed *et al.*, 2005). Furthermore, chemically, siderophores are phenolic compounds, which are antimicrobial in nature and may be responsible for antifungal activity of the test pathogen. The mycoparasitic potential of *Pseudomonas* spp. is well documented (Keel and Defago, 1997). Thus, this phenomenon has often been used as means for *in vitro* screening of biocontrol agents (Crowe *et al.*, 2001). Various researchers



C- Control (Mandya HRL) C- Control (VC Farm, Mandya)

Fluorescent pseudomon ad strains- 6, 12, 19, 30, 20, soy2, 22, 25 and Soy6

Fig. 1 : Efficacy of fluorescent pseudomonad against *R. solani* isolates

Table 1 : Evaluation of Fluorescent pseudomonad isolates against *Rhizoctonia solani* isolates

Sr. no.	*F. pseudomonad*	Per cent inhibition : isolate1	Per cent inhibition : isolate-2	Mean
1.	1	83.33 * (65.90) ^{f}	77.78 (61.87) ^{bcd}	80.55
2.	6	84.81 (67.07) ^{ef}	97.04 (84.21) ^{a}	90.92
3.	12	100 (90) ^{a}	96.30 (83.50) ^{a}	98.14
4.	15	87.04 (68.90) ^{de}	70.74 (57.25) ^{cd}	78.89
5.	19	100 (90) ^{a}	100 (90) ^{a}	100
6.	20	88.89 (70.52) ^{cd}	97.04 (84.21) ^{a}	92.96
7.	22	90.74 (72.29) ^{c}	81.11 (64.24) ^{bcd}	85.93
8.	25	92.96 (74.62) ^{b}	78.89 (62.64) ^{bcd}	85.93
9.	30	84.81 (67.06) ^{ef}	91.11 (72.65) ^{b}	87.96
10.	Soy6	94.44 (76.36) ^{b}	81.11 (64.23) ^{bcd}	87.96
11.	Soy2	88.89 (70.52) ^{cd}	87.04 (68.90) ^{bc}	87.78
12.	Wht2	85.56 (67.69) ^{ef}	63.70 (52.99) ^{d}	74.63
13.	Grnrt1	84.07 (66.52) ^{f}	65.56 (54.06) ^{d}	74.81
14.	Safrrt1	88.52 (70.19) ^{d}	79.63 (63.33) ^{bcd}	84.07
15.	IOF3	84.81 (67.07) ^{g}	68.15 (55.66) ^{d}	76.48
16.	Control	0.00	0.00	0.00
	S.E. ±	0.45	2.75	
	C.D. (P=0.05)	1.78	10.68	
	C.V. (%)	1.17	7.49	

*indicates of significance of values at P=0.05, respectively

also demonstrated the role of rhizobacteria in the inhibition of *R. solani* and the mechanisms with which they bring about the beneficial effect. The mechanism behind inhibition of *R. solani* from fluorescent pseudomonad may be attributed to antibiotics and siderophore production (Rini and Sulochana, 2007 and Reddy *et al.,* 2010).

Evaluation of fluorescent pseudomonads against *R. solani* under *in vivo* condition :

Among fifteen fluorescent pseudomonad strains screened against *R. solani* under *in vitro* condition, five strains were found to be potent antagonists. Five strains of fluorescent pseudomonad *viz.*, F. pseudomonad (12) (19) (20) (soy2) and (soy6) were selected for *in vivo* study.

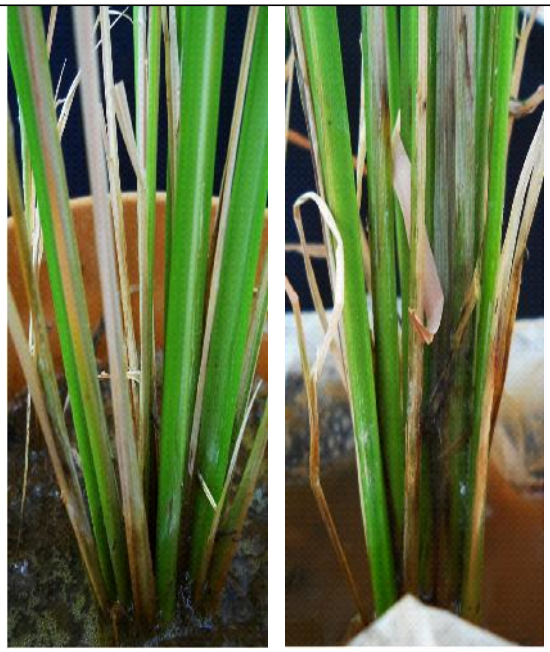
Effect of fluorescent pseudomonads on lesion length and dried leaves due to sheath blight infection :

Five different Fluorescent pseudomonad isolates were applied as seed treatment, soil treatment, foliar application and combination of these treatments. Among the different methods of treatments combined treatment with fluorescent pseudomonad strain 19 was significantly superior with lowest lesion length and lowest number of dried leaves over treated control and other treatments (Table 2 and Fig. 2). Next best treatment was seed treatment with fluorescent pseudomonad strain 19 which showed the lowest mean lesion length of 0.42 cm and mean number of dried leaves of 2.33 leaves/plant, followed by foliar spraying which showed mean lesion length of 0.64 cm and mean number of dried leaves of 3.33 leaves/ plant. Combined treatment with fluorescent pseudomonad strain 19 showed the lowest mean lesion length of 0.22 cm and mean number of dried leaves (1.67 leaves/plant) followed by combined treatment of fluorescent pseudomonad strain 12 with lesion length of mean 0.41.

The combined application of PGPR isolates was most effective method for control of *R.solani* in this study. Possibly both rhizosphere and phyllosphere population of fluorescent pseudomonad helped to control disease. Both

Table 2 : Effect of Fluorescent pseudomonad application on sheath blight and plant growth characters under glasshouse condition

Treatments		Sheath blight disease parameters				Plant growth parameters		
		Lesion length (cm)			No. of dried leaves per plant	Plant height (cm) 60 DAS	Dry wt. of root per plant (g)	Dry wt. of root (g)
		40 DAS	60 DAS	Mean				
T_1	Seed treatment (IABT-A1)	0.30	0.74	0.52	3.33	53.03	0.50	0.50
T_2	Seed treatment (IABT-A2)	0.22	0.62	0.42	2.33	53.29	1.06	1.06
T_3	Seed treatment (IABT-A6)	0.33	0.83	0.58	3.33	45.84	1.02	1.02
T_4	Seed treatment (IABT-A7)	0.53	0.87	0.70	4.33	44.01	0.76	0.76
T_5	Seed treatment (IABT-A8)	0.79	0.94	0.86	3.67	48.41	0.61	0.61
	Mean	0.43	0.8	0.62	3.39	48.91	0.79	0.79
T_6	Soil treatment (IABT-A1)	0.37	0.87	0.62	3.33	48.17	1.08	1.08
T_7	Soil treatment (IABT-A2)	0.32	0.70	0.51	3.00	49.11	1.26	1.26
T_8	Soil treatment (IABT-A6)	0.39	1.03	0.71	3.67	43.52	0.90	0.90
T_9	Soil treatment (IABT-A7)	0.63	1.26	0.94	3.67	42.31	0.53	0.53
T_{10}	Soil treatment (IABT-A8)	0.89	1.01	0.95	3.33	43.36	0.47	0.47
	Mean	0.52	0.97	0.75	3.4	45.29	0.85	0.85
T_{11}	Foliar spray treatment (IABT-A1)	0.47	1.17	0.82	4.33	36.85	0.40	0.40
T_{12}	Foliar spray treatment (IABT-A2)	0.42	0.86	0.64	3.33	45.05	0.70	0.70
T_{13}	Foliar spray treatment (IABT-A6)	0.57	1.27	0.92	3.67	38.36	0.32	0.32
T_{14}	Foliar spray treatment (IABT-A7)	0.87	1.57	1.22	4.00	44.84	0.27	0.27
T_{15}	Foliar spray treatment (IABT-A8)	1.38	1.90	1.64	3.67	48.14	0.20	0.20
	Mean	0.74	1.35	1.048	3.8	42.64	0.38	0.38
T_{16}	Combined treatment (IABT-A1)	0.15	0.67	0.41	2.33	53.47	2.07	2.07
T_{17}	Combined treatment (IABT-A2)	0.11	0.32	0.22	1.67	62.18	2.57	2.57
T_{18}	Combined treatment (IABT-A6)	0.22	0.70	0.46	3.00	57.41	1.66	1.66
T_{19}	Combined treatment (IABT-A7)	0.44	0.92	0.68	3.33	47.37	0.75	0.75
T_{20}	Combined treatment (IABT-A8)	0.64	0.90	0.77	2.33	52.61	0.58	0.58
	Mean	0.54	0.91	0.72	2.53	54.61	1.53	1.53
T_{21}	Pathogen (treated Control)	3.8	4.83	4.31	7.67	37.07	0.44	0.44
T_{22}	Healthy (Untreated control)	0.00	0.00	0.00	0.00	38.26	0.60	0.60
	C.D. (P=0.05)	0.04	0.13	0.39			0.05	
	S.E. ±	0.12	0.39	1.13			0.15	
	C.V. (%)	11.30	20.84	19.76			10.89	



Combine treatment of psedomonad-19

Pathogen treatment

Fig. 2 : Lesion length due to sheath blight infection

1 - Treated control (pathogen)
2 - Untreated control (healthy)
3 - Combined treatment
4 - Seed treatment
5 - Soil treatment
6 - Foliar spray

Fig. 3 : Effect of pseudomonad strain 19 on root growth

direct inhibition of pathogen and systemically induced resistance in the rice plants could be involved in control (Kazempour, 2004).

Effect of fluorescent pseudomonads on plant growth parameters :

Plant height :

All the isolates tested in this study promoted the plant growth and significantly superior over control. Among them, combined treatment of Fluorescent pseudomonad 19 was significantly superior over other treatments and promoted the plant height to the maximum extent (61.65 cm/plant). Next best was combined treatment with isolate 20 with plant height of 56.83 cm. However, at par results were recorded in seed treatment with isolate 19 (52.77cm) and isolate 12 (52.78 cm) (Table 2 and Fig. 3).

Number of tillers :

Application of biocontrol agents increased the number of tillers/ plant compared to control. In case of fluorescent pseudomonad strains, number of tillers was maximum in the combined treatment method with fluorescent pseudomonad 19 (4 per plant), significantly followed by soil treatment (3.67 per plant) and seed treatment with fluorescent pseudomonad 19 (3.33 per plant). However, significantly at par results was recorded in combined treatment with Fluorescent pseudomonad 12 (3.33 per plant). Least number of tillers was recorded in pathogen inoculated control treatment (1.33 per plant).

Dry weight of root :

Combined treatment of *F. pseudomonad* 19 was significantly superior over other isolates with maximum dry weight of root as 2.57g/plant. Next best isolate treatment and isolate was combine treatment with isolate no.12 with dry weight of root as 2.07 g/plant. Least dry weight of root was recorded in pathogen treated control as 0.44g/plant.

Similar results were recorded on significant increase in rice plant growth and control of sheath blight by application of *Pseudomonas fluorescens, Trichoderma* and salicylic acid (Anitha and Das, 2011). Thilagavathi *et al.* (2007) reported that the combined application of *P. fluorescens* (Pf1) in seed and soil applications was effective in reducing the root rot disease in green gram under greenhouse and field conditions. Significant increases in plant growth parameters in the present study may be attributed to the production of plant growth regulators such as auxins, gibberellins, cytokinins and ethylene (Frankenberger and Arshad, 1995). Indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway. Gupta *et al.* (2002) reported the positive colonization of Pseudomonas GRC2, its ability to increase seedling emergence, and establishment in the rhizosphere of peanuts giving protection against *M. Phaseolina* resulting in enhanced yield.

P. fluorescens isolate 19 used in the present study not

only controlled sheath blight, but also stimulated plant growth which is an additional advantage over the use of chemical fungicides against sheath blight management. Amongst the different methods of treatments, soil application + seed bacterization + foliar spraying of *P. fluorescens* isolate 19 found to be suitable for the management of *R.solani* in greenhouse condition. Field evaluation is underway to determine its efficacy under natural ecosystem.

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