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Study the Efficacy of *Pseudomonas fluorescens* against Sheath Blight in Rice by *Rhizoctonia solani*

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ABSTRACT

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Rice is an important staple food for more than half of the world's population and is subject to diseases that often place major biological constraints on production. Sheath blight of rice is caused by a soil borne fungus and biological control of the disease is an efficient substitute to replace chemical methods. In the present study thirty native fluorescent Pseudomonads, an efficient biocontrol agent have been isolated and confirmed by cultural, morphological, biochemical characterization and visualization under UV light. The isolates were screened *in vitro* for antagonistic activity against *Rhizoctonia solani*, three strains among them *i.e.*, DMP1 (53.43%), DBP (51.53%) and PVP2 (48.26%) were found to inhibit mycelial growth significantly. The antagonistic activity of the three isolates was further tested by applying them as seed treatment, root dipping and foliar spray in the rice crop under greenhouse conditions. Among all the treatments when compared by inoculating with *Rhizoctonia solani* at the maximum tillering stage, the T₅ treatment (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) not only enhanced the plant growth but also showed the reduction in disease severity of 41.12% when compared with the control.

Introduction

Rice (*Oryza sativa*) is India's pre-eminent crop, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Moreover, the country has the biggest area under rice cultivation, as it is one of the principal food crops. India is one of the leading producers of this crop, with a production, productivity of 105.48 million tons and 2391 kg per hectare respectively (Indiastat, 2014-15).

Sheath blight caused by a soil borne fungus (*Rhizoctonia solani*), is considered to be the

most economically significant fungal rice disease in the world (Groth *et al.*, 1988). Both local and high yielding varieties are susceptible to this disease (Naidu, 1989). The pathogen attacks the rice plant at maximum tillering stage by sclerotium, the primary source of inoculum that over winter in soil and plant debris (Ou, 1985). Yield loss can occur at any stage but is higher when infection occurs at panicle initiation, booting and flowering (Sharma *et al.*, 1990). Sheath blight also interferes with grain filling (Marchetti, 1983) and can reduce rice yield by

39%, but that loss can increase to 50% in terms of kg/ha of milled whole grain rice because grains can be weakened and subsequently break during milling. A possibility of 46% yield loss in milled rice has been estimated if sheath blight lesions reach 90% of the plant height (Ahn and Mew, 1986).

The disease is alarming due to its intensive cultivation of modern high yielding varieties with high doses of nitrogenous fertilizers. Crop with a high plant density and close canopy favours disease build up from panicle initiation onwards. Poor weed management practices and increase in frequency of irrigation have aggravated, incidence of the disease due to modified micro climatic conditions (Srinivas *et al.*, 2013).

The use of chemical fertilizers and pesticides caused an incredible harm to the environment. These agents are both hazardous and may persist and accumulate in natural ecosystems an answer to this problem is replacing chemicals with biological approaches, which are considered more environment friendly in the long term. One of the emerging research area for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which are capable of suppressing or preventing the phytopathogen damage (Nihorembere *et al.*, 2011).

Beneficial rhizobacteria that stimulate plant growth are usually referred as plant growth promoting rhizobacteria (PGPR). PGPR are heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots. They can improve the extent or quality of plant growth by direct or indirect methods. In last few decades, a large array of bacteria have been isolated and reported to enhance plant growth (Glick, 1995). The contributions of PGPR were

emphasized clearly for safe and sustainable agriculture development (Singh *et al.*, 2011).

Pseudomonas fluorescens encompasses a group of common, non-pathogenic saprophytes that colonize soil, water and plant surface environments. Several strains of *P. fluorescens* have been successfully used for the biological control of rice sheath blight (Rabindran and Vidhyasekaran, 1996). Their applicability as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Gupta *et al.*, 2001). They can be utilized in low input sustainable agricultural applications, such as biocontrol, on account of their ability to synthesize secondary metabolites with antibiotic properties and many of such antibiotics produced have a broad spectrum activity but strain to strain variations do exist (Raaijmakers *et al.*, 2002). These secondary metabolites include 2,4-diacetylphloroglucinol (DAPG), phenazine (Phz), pyrrolnitrin, oomycin A, viscosinamide, pyoluteorin and hydrogen cyanide (HCN).

In the present study keeping in view the importance of *Pseudomonas fluorescens* as biocontrol agents they have been successfully isolated and used as seed treatment, foliar spray and root dipping for the control of Sheath blight in rice.

Materials and Methods

Soil sampling

Soil samples were collected from twenty two villages of Rangareddy district for the isolation of *Pseudomonas fluorescens* strains. The samples were collected from rice rhizosphere. Crop plants were selected randomly in the field and the intact root

system was dug out, carefully taken in plastic bags, labelled well and stored at 4⁰C.

Isolation

King's B selective medium was used for the isolation of *P. fluorescens* (King's *et al.*, 1954).

Preparation of inoculum of *Rhizoctonia solani*

Three hundred grams of sterilized barley seed was placed in Erlenmeyer flask, then each flask was inoculated with 5 mm mycelial disc from a 4 days old culture of *Rhizoctonia solani* grown on PDA was placed in the flasks and they were incubated at room temperature (26 ± 2⁰C) for 3 weeks (Kazempour, 2004).

Inoculation of *Rhizoctonia solani* in the rice crop

At the maximum tillering stage the pathogen was inoculated into the crop by placing the barley grains with mycelia at the centre of the hill above the ground level (Singh and Sinha, 2005).

Percent germination

Seed germination of the *Pseudomonas fluorescens* treated seeds was determined by the standard roll towel method (International Seed Testing Association, 1996).

Methods of application of *Pseudomonas fluorescens*

Seed treatment

Rice seeds were surface sterilized with 2% Sodium hypochlorite solution and soaked in a double volume of bacterial suspension (9x10⁸cfu ml⁻¹). After 24 h, the bacterial suspension was drained and the seeds were dried under shade for 30 min. The seeds were

allowed to sprout for another 24 h before sowing (Vidhyasekaran *et al.*, 1997). After 25 days, the seedlings were pulled out from the pots and transplanted at the rate of four seedlings per hill and two hills per pot (30 cm diameter containing 10 kg of clay soil).

Root dipping

Rice seedling in bundles (approximately 200 seedlings) were dipped in 250 ml of bacterial suspension (9x10⁸ cfu ml⁻¹) for 2 h, ensuring that roots alone were immersed in the inoculum and planted in pots.

Foliar application

25 ml of bacterial suspension (9x10⁸ cfu ml⁻¹) per pot was sprayed 30 days after transplanting (Vidhyasekaran *et al.*, 1997).

Growth characteristics

Plant height

The plant height was recorded by measuring the total height from the base of the plant to the tip of the plant at 30, 60 and 90 DAT and is expressed in centimeters.

Total number of tillers hill⁻¹

In each pot the number of tillers were counted on each plant at 30, 60 and 90 DAT and expressed as total number of tillers hill⁻¹.

Number of productive tillers hill⁻¹

The ear bearing tillers, which produced healthy panicles were counted and expressed as number of productive tillers hill⁻¹.

Measurement of chlorophyll content (SCMR Values)

The SPAD-502 (Soil plant analytical development) was used for measuring the

relative chlorophyll content of leaves at 30, 60 and 90 DAT.

The chlorophyll content was measured for recent fully expanded leaves. Mean of the five values from each hill was obtained. This meter enables obtaining instant readings without destroying the plant tissue.

Reduction in disease severity

Development of symptoms was observed and recorded 7 days after inoculation as grades 0±9 (IRRI, 2002). Based on the grades, the disease index was calculated using the following formula

Disease index = Total grade / No. of sheath observed x 100 / maximum grade

Statistical analysis

The data obtained in the experiment was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran (1967) and Panse and Sukhatme (1985).

Data on different characters *viz.*, growth components, PDI and reduction in disease severity, were subjected to analysis of variance procedures as outlined for CRD.

Statistical significance was tested by F-value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

Results and Discussion

The experiment was conducted during *Rabi*, 2014-15 with Completely Randomized Design, in the green house, College of Agriculture, Rajendranagar, Hyderabad and the results are presented and discussed here under various headings.

Screening and selection of *Pseudomonas fluorescens* isolates

Thirty *Pseudomonas fluorescens* isolates were isolated from the soil samples using King's B agar and they were confirmed by cultural, morphological, biochemical characterization and visualization under UV light. The isolates were further studied for the PGPR attributes and found positive for either of them.

In the preliminary screening of 30 *P. fluorescens* isolates for antagonistic activity against *Rhizoctonia solani* under *in vitro* conditions, three strains DMP1 (53.43%), DBP (51.53%) and PVP2 (48.26%) were found efficient to inhibit mycelial growth when compared with other strains and these strains were further used for pot culture study.

Influence of *Pseudomonas fluorescens* on growth parameters of rice

Percent germination

The effect of DBP, DMP1 and PVP2 on germination of rice was conducted on standard roll towel. The results showed that highest percent germination was found with the seeds inoculated with PVP2 *i.e.*, 100% followed by DBP (98.35%) when compared with the control (90%) (Table 1).

The isolates obtained in our study when seed treated with the rice grains enhanced germination percentage. *Pseudomonas fluorescens* is one among the PGPR having the ability to produce plant growth regulators such as gibberlins, cytokinins and indole acetic acid etc. which induced higher percentage of germination in the seeds when compared with the untreated control.

Plant height

Plant heights at 30, 60 and 90 DAT are measured and the values are mentioned in

Table 2. Plant height at 30 DAT was found significantly highest in T₅ (Seed treatment with DMP1 +Inoculation of *Rhizoctonia solani*) i.e., 56 cm followed by 52 cm with T₆ (Root dipping with DMP1 +Inoculation of *Rhizoctonia solani*) and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) i.e., 42 cm.

Plant height at 60 DAT was found highest in T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 74 cm on par with 72 cm i.e., T₈ (Seed treatment with DBP + Inoculation of *Rhizoctonia solani*) and T₂ (seed treatment with PVP2 + Inoculation of *Rhizoctonia solani*) and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) i.e., 57.3 cm.

Plant height at 90 DAT was found highest in T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 82.6 cm found on par with T₈ (Seed treatment with DBP + Inoculation of *Rhizoctonia solani*) 82.1 cm and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) i.e., 68.2cm.

Seed treatment, root dipping and foliar application with plant growth promoting rhizobacterial bioformulations significantly enhanced the growth of rice plants compared with the control. Seed treatment had shown effective growth with the organisms DMP1, DBP and PVP2 when compared with root dipping and foliar spray as the inocula to rest of them has been added at later stage.

When the strains are considered, T₅ i.e., Seed treatment with DMP1 had shown best plant growth promotion as it is having the ability to produce siderophores, IAA, Ammonia and HCN followed by T₈ i.e., seed treatment with DBP having IAA, ammonia and HCN producing ability.

Number of tillers per plant

Number of tillers at 30 DAT was found significantly highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) i.e., 13.7 followed by 12.3 with T₆ (Root dipping with DMP1 +Inoculation of *Rhizoctonia solani*), and the least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) i.e., 8.3.

Number of tillers at 60 DAT was found significantly highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) i.e., 17.3 next to it is 16.0 with T₆ (Root dipping with DMP1 +Inoculation of *Rhizoctonia solani*) and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) 11.3.

Number of tillers at 90 DAT was found significantly highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) i.e., 17.3 followed by 14.7 with T₈ (Seed treatment with DBP + Inoculation of *Rhizoctonia solani*) and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) 11.3 (Table 2).

In our study along with the inhibition of *Rhizoctonia solani*, seed treated plants have shown good plant growth promoting traits compared with the root dipped and foliar sprayed plants. Besides plant height, increase in the number of tillers was reported in the rice plants treated with the plant growth promoting rhizobacteria (Salamone *et al.*, 2012).

Number of panicles per plant

Number of panicles at 90 DAT was found highest i.e., 13.7 with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) followed by T₈ (Seed treatment with DBP + Inoculation of

Rhizoctonia solani) 13.3 and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) 8.7 (Table 2).

Chlorophyll content

Chlorophyll content with SPAD meter was measured at 30, 60 and 90 DAT (Table 2). Chlorophyll content at 30 DAT was found highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 39.6 and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) *i.e.*, 32.7. Chlorophyll content at 60 DAT was

found highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 48.17 and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) *i.e.*, 40.60. Chlorophyll content at 90 DAT was found highest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) *i.e.*, 42.13 followed by T₈ (Seed treatment with DBP + Inoculation of *Rhizoctonia solani*) *i.e.*, 40.15 and least was found with control (T₁: Inoculation of *Rhizoctonia solani* alone) *i.e.*, 23.78 (Table 2).

Table.1 Percent germination of *Pseudomonas fluorescens* strains treated seeds

Organism/strain	Percent germination
Control	90
DBP	98.35
DMP1	96.65
PVP2	100
CD	0.781
SE(±m)	0.236

Treatments

- T₁: Inoculation of *Rhizoctonia solani* alone
- T₂: Seed treatment with PVP2 + Inoculation of *Rhizoctonia solani*
- T₃: Root dipping with PVP2 + Inoculation of *Rhizoctonia solani*
- T₄: Foliar spray with PVP2 + Inoculation of *Rhizoctonia solani*
- T₅: Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*
- T₆: Root dipping with DMP1 + Inoculation of *Rhizoctonia solani*
- T₇: Foliar spray with DMP1 + Inoculation of *Rhizoctonia solani*
- T₈: Seed treatment with DBP + Inoculation of *Rhizoctonia solani*
- T₉: Root dipping with DBP + Inoculation of *Rhizoctonia solani*
- T₁₀: Foliar spray with DBP + Inoculation of *Rhizoctonia solani*

Table.2 Influence of *Pseudomonas fluorescens* on growth parameters of rice, PDI and Percent reduction of the disease

Treatments	Plant height(cm)			Number of tillers plant ⁻¹			No. of panicles	Chlorophyll content			PDI	Reduction in disease severity
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	90 DAT	30 DAT	60 DAT	90 DAT		
T₁	42.00	57.30	68.20	8.3	11.3	11.3	8.7	32.70	40.60	23.78	62.33	0.00
T₂	51.33	72.00	78.30	10.3	14.0	13.7	12.7	36.07	45.70	35.27	44.36	28.84
T₃	47.67	70.00	75.40	9.7	13.3	13.3	10.3	35.40	44.50	29.32	52.74	15.39
T₄	45.67	59.70	73.30	9.3	12.7	12.0	9.7	34.53	42.57	27.39	57.25	8.16
T₅	56.00	74.00	82.60	13.7	17.3	17.3	13.7	39.60	48.17	42.13	36.70	41.12
T₆	52.00	70.70	78.90	12.3	16.0	14.3	12.7	39.40	47.87	37.13	43.34	30.47
T₇	42.33	64.00	75.60	10.3	14.3	13.7	12.0	36.63	47.50	30.79	53.08	14.85
T₈	51.67	72.00	82.10	11.0	15.0	14.7	13.3	37.87	47.43	40.15	43.38	30.41
T₉	48.00	63.00	78.00	10.7	13.7	13.3	11.3	37.13	46.60	33.29	46.57	25.29
T₁₀	45.67	61.00	69.30	10.3	12.7	12.3	9.7	33.33	44.23	25.77	59.71	4.21
CD	3.525	2.236	1.250	0.939	1.129	1.129	0.939	2.596	2.998	2.705	3.128	0.578
SE(±m)	1.187	0.753	0.421	0.316	0.380	0.380	0.316	0.874	1.009	0.508	1.053	0.195

Chlorophyll content increased with increasing crop up to 60 DAT and then decreased. As the crop is inoculated with the *Rhizoctonia solani*, the fungus after inoculating damages the chlorophyll content of the crop and the result was found best with the seed treatment method with the organism DMP1, as it is showing plant growth promotion as well as disease resistance more compared with the other treatments.

Percent Disease Index (PDI)

Percent Disease Index (PDI) was calculated and found significantly highest 62.33% with the control (T₁: Inoculation of *Rhizoctonia solani* alone) followed by 43.34% with T₆ (Root dipping with DMP1 +Inoculation of *Rhizoctonia solani*) and PDI was lowest with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 36.70% (Table 2).

In our study, application of the biocontrol agents as seed treatment reduced the PDI to a maximum extent, as the fungal pathogen *Rhizoctonia solani* is a soil borne and seed treatment inhibited the pathogen from the initial stages of plant growth followed by root dipping and foliar spray. In seed treatment the bacterial bioagent appeared to move from seed to roots, stems and leaves. The fluorescent Pseudomonads could be isolated from aerial parts of plants grown from seeds treated with the bacteria (Colyer and Mount, 1984).

Reduction in disease severity

Reduction in disease severity was calculated and maximum reduction in disease severity was found with the treatment T₅ (Seed treatment with DMP1 + Inoculation of *Rhizoctonia solani*) 41.12% followed by 30.47% in T₆ (Root dipping with DMP1 +Inoculation of *Rhizoctonia solani*) when

compared with the control (T₁: Inoculation of *Rhizoctonia solani* alone) (Table 2). Seed treated plants had shown more resistance towards the disease and the percent reduction of disease severity was also found highest in them.

The maximum reduction in disease severity (67.8%) along with increase in grain yield (31.6%) was recorded when *Rhizoctonia solani* was applied 7 days after application of *Pfr1* followed by *Pfr5* which resulted in 64.5 and 40.7% reduction in disease severity and incidence respectively was reported by Singh and Sinha (2009).

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