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Screening of Fluorescent Pseudomonad Isolates Against *Sclerotium Rolfsii* Sacc., of Soybean (*Glycine Max*)

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ABSTRACT

An attempt was made to isolate and characterize Fluorescent pseudomonads from the rhizosphere soil samples and further screening of the isolates for their antagonistic properties against *Sclerotium rolfsii* Sacc., of soybean. The isolates were subjected for morphological, biochemical and functional characterization. All the isolates exhibited fluorescence under UV light. Cells were rods and gram negative. All the isolates produced clear zone of P- solubilization (TCP) (18.80-21.71mm diameter) on Pikovskaya's agar medium. The fluorescent pseudomonads produced significantly varying quantities of IAA (19.97 µg to 28.89 µg IAA/25 ml of broth) and GA (18.52 µg per 25 ml broth). All the isolates showed antagonistic activity against *Sclerotium rolfsii*. The per cent inhibition ranged from 36.85 to 70.37. Under pot culture experiment, lowest PDI was observed in DFP48 followed by DFP47, among the FP isolates. These isolates also showed maximum peroxidase activity and also plant growth promotional activities.

Key words: Biocontrol, PGPR, Pseudomonas, *Sclerotium rolfsii.*

INTRODUCTION

Diseases play a major role in yield reduction in soybean. More than 100 pathogens are known to affect soybean of which 66 fungi, 6 bacteria, 8 viruses and 7 nematodes are involved (Sinclair, 1978). The world loss of more than seven million tonnes per annum was reported only due to diseases alone (Sinclair, 1988).

Hence production of this miracle crop is great deal, as it attracts many economically important phytopathogens. These diseases result in heavy losses to the farmers. Synthetic chemical fungicides have long been used to reduce the incidence of plant diseases. However they are costly and can have negative effects on the environment. They may also alter biological balance in the soil by disseminating the non-target and beneficial microorganisms and may induce pathogen resistance to chemical fungicide (Bharathi *et al*., 2004).

The excessive use of agrochemicals on the other hand has caused serious environmental and health problems. The high intensity of chemical pesticide application has become serious cause of concern in recent years. Although the use of pesticides is comparatively small in India, the damage caused by them to the environment and health is already evident (Alam, 2000). Furthermore, the current trends suggest that the use of chemical fungicides is likely to continue to increase in the near future. Under these circumstances, there is a growing need to promote the use of alternative methods of crop protection. 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).

Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR) (Ashrafuzzaman *et al*., 2009). PGPR promote plant growth by various factors like ability to produce plant growth regulators, asymbiotic N_2 fixation, antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide, solubilization of mineral phosphates and other nutrients (Sarvanakumar *et al*., 2007).

PGPR may use more than one of above mechanisms to enhance plant growth as experimental evidence suggests that the plant growth stimulation is the net result of multiple mechanisms that may be activated simultaneously (Martinez *et al.,* 2010). Pseudomonas is a diverse genus that occupies many different niches and exhibits versatile metabolic capacity (Clarke, 1982).

Fluorescent pseudomonads (FP's) a group of PGPR, have frequently been considered as effective biological control agents against soil-borne plant pathogens due to their rapid and aggressive colonization of plant roots. They produce secondary metabolites with antibiotic properties such as phytohormones, volatile compounds, hydrogen cyanide (HCN) and siderophores. Plant growth-promoting ability of these bacteria is mainly due to the production of

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indole-3-acetic acid (IAA), siderophores and antibiotics (Lautenberg *et al.,* 2001). Such strains can protect plants from various soil borne pathogens and/or stimulate plant growth (Haas and Defago, 2005). The abundance of literature on genus *Pseudomonas* is due to their elevated metabolic versatility capable of utilizing a wide range of simple and complex organic compounds and holding an important position in biosphere ecology (Scarpellini *et al*., 2004).

Antagonistic activity of *Pseudomonas fluorescens* and *P. putida* in the rhizosphere has been recognized as major factor in the suppression of many phytopathogens. Bacteria of the genus *Pseudomonas* comprise a large group of the active biocontrol strains as a result of their general ability to produce a diverse array of potent antifungal metabolites. Strains of *Pseudomonas fluorescens* are known to show biological control activity against certain soil-borne phytopathogenic fungi and have the potential to produce known secondary metabolites such as siderophore, antifungal antibiotics, HCN and protease which showed antagonistic activity against *Macrophomina phaseolina, Rhizoctonia solani, Phytophthora nicotianae var. parasitica*, *Pythium* sp. and *Fusarium* sp. (Anand *et al*., 2010).

Keeping the above points in view, the present study was undertaken.

MATERIALS AND METHODS

Isolation of fluorescent pseudomonads

Rhizosphere soil samples were collected from the major soybean growing areas of Dharwad and Belagavi districts of Karnataka. Isolation of fluorescent pseudomonads was carried out on King's B medium using serial dilution and spread plate technique.

Characterization of FP isolates

The colonies showing greenish to yellowish pigmentation on King's B medium were picked up and observed for fluorescence under UV light. The isolates were also studied for their colony morphology, cell shape and gram reaction as per the standard procedure given by Barthalomew and Mittewar (1950) and Anonymous (1957). The isolates were subjected to biochemical characterization by employing the standard procedures given by Cappuccino and Sherman (1992). Different biochemical tests performed were Denitrification, Starch hydrolysis, Arginine hydrolysis, Gelatin liquification and Oxidase test.

Functional characterization of FP isolates

The isolates were also tested for their ability to solubilise P, HCN production, Siderophore production and ability to produce plant growth promoting substances like IAA and GA.

In vitro screening of fluorescent pseudomonad isolates against *Sclerotium rolfsii* Sacc., of soybean

The FP isolates were also studied for their antagonistic activity against *Sclerotium rolfsii* Sacc., causing collar or root rot (soil borne) in soybean plant under *in vitro* condition. The pure culture of *Sclerotium rolfsii* Sacc., were obtained from the Department of Plant Pathology, University of Agricultural Sciences, Dharwad.

The dual inoculation technique of Sakthivel and Gnanamanickam (1987) was followed to study the antagonistic activity of the fluorescent pseudomonads against soil borne plant pathogens.

Evaluation of efficient isolates of fluorescent pseudomonads for their antagonistic activity against *S. rolfsii* of soybean under pot culture condition

Pot experiment was conducted with challenge inoculation of *S. rolfsii* along with appropriate control taking soybean as test crop.

This experiment comprised eight treatments and six replications with a view to record wilt incidence and also for destructive sampling at 30 and 60 days after sowing. The treatment details and the different FP isolates used for the experiments are outlined below.

Treatments

T_1: BFP22 + *S. rolfsii*
T_2: BFP38 + *S.rolfsii*
T_3: DFP48 + *S. rolfsii*
T_4: DFP47 + *S. rolfsii*
T_5: DFP62 + *S. rolfsii*
T_6: (Carboxin 37.5 % +Thiram 37.5%) + *S. rolfsii*
T_7: *S. rolfsii* alone
T_8: Absolute control

The seeds of soybean variety, JS 335 were used. The medium black, sandy loam soil collected from experimental field was autoclaved to kill the initial microbial inoculum for making it pathogen free. The soil was mixed with well decomposed FYM @ 10 t/ha. It was also mixed with sand in 9:1 (soil: sand) proportion to improve porosity of soil. Earthen pots of 30 cm top diameter were filled with 10 kg soil. Before sowing, the soil in each pot was mixed with recommended dose of fertilizers.

Challenge inoculation of *Sclerotium rolfsii*

The fungus, *S. rolfsii* causing collar or root rot disease in soybean was multiplied as a mixed inoculum in maize powder and sand (1:4) mixture, which is commonly called as jaint culture. For pot application, top 200 gm of soil was inoculated with 5% of pathogen inoculum (jaint culture) one day before sowing.

Preparation and inoculation of selected fluorescent pseudomonads

The fluorescent pseudomonad isolates were multiplied in King's B broth for 96 h at 30°C under shaking conditions (175 rpm). These broth cultures were diluted to maintain the population of 10^8-10^9 cfu/ml and applied @ 10 ml per pot just one day after sowing. Soybean seeds were treated with respective isolates at the rate of 10 gm per kg of seed, 10-15 min prior to sowing and for the chemical control

treatment, the seeds were treated with Carboxin 37.5% + Thiram 37.5% at the rate of 4 g/kg of seeds.

Observations regarding, per cent seedling emergence, plant growth parameters and yield parameters were recorded.

Disease scoring

Observations on wilt incidence caused by *S. rolfsii* were recorded at 15, 30 and 60 DAS and per cent disease incidence was calculated by using the formula given by Mayee and Datar, 1986.

Per cent disease index (%) =

$$\frac{\text{Sum of all disease ratings}}{\text{Total leaves observed in a set} \times \text{Max. grade}} \times 100$$

$$\text{PDI} = \frac{\text{No. of infected plants}}{\text{Total number of plants}} \times 100$$

Statistical analysis

The statistical analyzes of the data were carried out by employing completely randomized design (CRD). The critical differences were calculated at P = 0.01 for the pot culture experiments wherever F tests were significant and interpretation of the results was carried out in accordance with Pansey and Sukhatme (1985).

RESULTS AND DISCUSSION

Isolation of fluorescent pseudomonads from rhizosphere soil was carried out by employing serial dilution and plating on King's B selective media. It may be attributed to specific choice of media employed for isolation *viz.,* Nutrient agar and King's B. Several others hence supported to use King's B medium for isolation of fluorescent *Pseudomonad* sp. (King *et al.,* 1954). For the isolation of pseudomonads and fluorescent *Pseudomonad* sp., TSA and King's B agar medium were also used (Raaijmakers and Weller, 1998). Total of eight FP isolates were obtained from the rhizosphere soil samples collected from the major soybean growing areas of Dharwad and Belagavi districts of Karnataka.

All eight isolates were used to study morphological traits. The colony morphology of isolates was found to be round to irregular shape and the irregular shaped colonies were found to be spreading type (Table 1). All the eight isolates exhibited fluorescence under UV light. However variations with respect to intensity of fluorescence and colony morphology were observed. Fluorescence is an important trait for identification, characterization and grouping of fluorescent pseudomonads (Brown and Lowbury, 1968). Among the isolates majority of the isolates produced light green to green pigmented colonies, while some of the isolates appeared to have yellowish orange colonies. It is reported from earlier studies that some pigments such as carotenoids produced by pseudomonad species do not diffuse in to the medium and such colonies were found to have yellowish green colour which could resemble other fluorescing pigments (Indi, 2010).

The cell morphological studies and the gram reaction test revealed that all the 8 isolates were rod shaped and displayed a negative reaction for the gram staining.

The biochemical characterization indicated that out of eight isolates, four were positive for starch hydrolysis and two for arginine hydrolysis, six for oxidase test, five were positive for gelatin liquefaction and seven exhibited the denitrification ability (Table 2). These results are in conformity with Singh *et al.* (2013), who reported that out of seventeen isolates, seven isolates were identified to be *Pseudomonas* sp. on the basis of their cultural, morphological and biochemical characteristics.

All the isolates were also examined for their functional properties like, P-solubilization, production of plant growth promoting substances, HCN production, siderophore

Table 1: Morphological characterization of native fluorescent pseudomonad isolates.

Isolate code	Colony morphology	Pigmentation (Under UV light)	Cell shape	Gram Reaction
BFP22	Round	Yellowish orange	Rod	Gram -ve
BFP38	Irregular	Green	Rod	Gram -ve
BFP42	Round	Dark green	Rod	Gram -ve
DFP47	Round	Green	Rod	Gram -ve
DFP48	Round	Yellowish orange	Rod	Gram -ve
DFP54	Irregular	Green	Rod	Gram -ve
DFP56	Round	Yellowish orange	Rod	Gram -ve
DFP62	Round	Green	Rod	Gram -ve

Table 2: Biochemical characterization of native fluorescent pseudomonads.

Isolates	Denitrification	Starch hydrolysis	Arginine hydrolysis	Gelatin liquification	Oxidase test	Tentaive Identification
BFP22	-	-	-	+	-	*P. fluorescens*
BFP38	+	+	+	+	+	*P. fluorescens*
BFP42	+	+	-	-	+	*P. fluorescens*
DFP47	+	-	-	+	+	*P. fluorescens*
DFP48	+	-	-	-	+	*P. fluorescens*
DFP54	+	-	-	-	-	*P. fluorescens*
DFP56	+	+	-	+	+	*P. fluorescens*
DFP62	+	+	+	+	+	*P. fluorescens*

Note: (+) positive reaction for the test, (-) negative reaction for the test.

production and their biocontrol potential. All the isolates were able to produce clear zone of P-solubilization (TCP) on Pikovskaya's agar medium. These isolates displayed wide variations in the diameter of the zone of solubilization, which varied from 18.80-21.71mm (Table 3). The extent of zone of solubilization may or may not correlate with the amount of P solubilized (Rashid *et al.,* 2004). Isolates of *Pseudomonas fluorescens* species differ in the ability to produce phosphatase enzyme and production of organic acids and hence showed different solubilization efficiency (Priyanka *et al.,* 2017).

All isolates were screened for their ability to produce IAA and GA. All the fluorescent pseudomonads in the present study produced significantly varying quantities of IAA (19.97 µg to 28.89 µg IAA/25 ml of broth). Among the isolates, DFP48 recorded highest IAA production of 28.89 µg/25ml. Isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992) and IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al*., 2001). The amount of GA production by different FP isolates ranged from 12.19 to 18.52 µg/25 ml of broth. The isolate DFP54 was found to produce maximum amount of GA (18.52 µg per 25 ml broth).

Fluorescent pseudomonads offer an interesting biological system with their ability to promote plant growth directly through production of plant growth promoting substances (IAA and GA) and indirectly through control of plant pathogens and deleterious organisms or both (Bakthavatchalu *et al*., 2012). Seed bacterization with such organisms has emerged as a powerful technology to enhance plant growth and yield, besides providing protection against diseases.

The fluorescent pseudomonads have been the most widely studied group of PGPR with respect to biocontrol of soil borne plant pathogens. The increased interest in the fluorescent *Pseudomonad* sp. in worldwide as biocontrol agents gained momentum after the initial studies conducted at the University of California, Berkeley, during the late 1970s (Weller *et al.,* 1988).

In addition to all the beneficial traits as discussed earlier, the isolates were used to study their antagonistic potential against fungal pathogen *Sclerotium rolfsii* of soybean under *in vitro* condition using dual plate technique (Vincent, 1947).

All the isolates showed antagonistic activity against *Sclerotium rolfsii*. The per cent inhibition of 36.85 to 70.37 was recorded by the isolates. The maximum per cent inhibition of 70.37 was observed in the isolate BFP22. The isolates BFP38 and DFP48 were next highest with per cent inhibition of 60.93 and 58.89 respectively.

These observations were in line with the earlier reports on fluorescent pseudomonads against plant pathogenic fungi like *Fusarium, Rhizoctonia, Macrophomina, Pyricularia, Alternaria, Sclerotium, Colletotrichum, Pythium* and *Phytophthora* (Ahmadzadeh *et al.,* 2006; Kandoliya and Vakharia, 2013; Rakh, 2011; Vishwanath *et al*., 2012; Manivannan *et al*., 2012 and Prasad *et al.,* 2013).

Some rhizobacteria promote plant growth by production of siderophores. The role of sidrophore production in plant growth promotion is described by two mechanisms: one is direct supply of iron to plant; the other is indirect, in depriving fungal pathogens to iron. In the present study, all the isolates were found positive for siderophore production; however the zone of clearance on CAS agar ranged from 21.97-36.37 mm. The isolate DFP54 showed maximum zone of clearance of 36.37 mm on CAS agar media. The next highest zone of clearance (29.23 mm) was recorded by the isolate DFP48 followed by DFP62 (26.70 mm). Similarly, all the isolates were found to be strong (+++) HCN producers.

Biocontrol potential and plant growth promotional ability of selected fluorescent pseudomonads on soybean challenge inoculated with *S. rolfsii* under pot culture condition

Five FP isolates were selected for pot culture studies based on their ability to produce IAA, GA, HCN and siderophore production and dual plate assay against *S. rolfsii* causing collar or root rot disease in soybean. It was also focused to assess the ability of antagonistic isolates for biocontrol potential as well as plant growth promotion.

Per cent seedling emergence

All the treatments had a considerable influence on the seedling emergence (Table 4). Among the treatments, T_5 (DFP62) recorded highest per cent seedling emergence (90.11) followed by the treatment T_4 (DFP47) (89.87) which

Table 3: Functional properties of FP isolates.

Isolate code	P-solubilization (mm)(Qualitative)	IAA (µg/25ml)	GA (µg/25ml)	Percent inhibition against *S. rolfsii*	HCN production	Siderophore Production (mm)
BFP22	20.00	19.97	12.19	70.37	+++	21.97
BFP38	21.02	25.00	16.29	60.93	+++	24.00
DFP48	21.71	28.89	13.15	58.89	+++	29.73
DFP47	20.96	22.55	12.85	49.63	+++	23.47
DFP62	20.34	26.41	12.99	45.56	+++	26.70
DFP56	18.80	24.81	12.20	36.85	+++	24.00
BFP42	21.11	23.74	13.12	49.63	+++	23.60
DFP54	21.65	28.03	18.52	37.41	+++	36.37

Table 4: Effect of efficient fluorescent pseudomonad isolates on seedling emergence of soybean challenge inoculated with *S. rolfsii.*

Treatments	Per cent seedling emergence
T_1: BFP22 + *S. rolfsii*	79.83 (63.29)*
T_2: BFP38 + *S.rolfsii*	77.69 (61.81)
T_3: DFP48 + *S. rolfsii*	80.00 (63.41)
T_4: DFP47 + *S. rolfsii*	89.87 (71.41)
T_5: DFP62 + *S. rolfsii*	90.11 (71.71)
T_6: (Carboxin 37.5 % +Thiram 37.5%) + *S. rolfsii*	85.90 (67.92)
T_7: *S. rolfsii* alone	67.83 (55.43)
T_8: Absolute control	69.97 (56.75)
S.Em. ±	**0.45**
C.D. @ 1 %	**1.80**

Note: * Arc sine transformed values.

were on par with each other. The next highest per cent seedling emergence of 85.90 was recorded by the treatment T_6 and least was observed in the treatment T_7 (*S. rolfsii* alone) (67.83).

Per cent disease index (PDI) of collar or root rot of *S. rolfsii* in soybean

Per cent disease control was calculated using PDI values at 15, 30 and at 60 DAS (Table 6). At 15 DAS, very less per cent disease incidence of 13.09 was observed in T_3 (DFP48+ *S. rolfsii*), which was on par with T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) (13.59). The treatments T_4 (DFP47+ *S. rolfsii*), T_5 (DFP62+ *S. rolfsii*), T_2 (BFP38+ *S. rolfsii*) and T_1 (BFP22+ *S. rolfsii*) recorded the per cent disease incidence of 15.29, 16.21, 16.93 and 21.22 respectively. The treatment T_7 (*S. rolfsii*) recorded highest per cent disease incidence of 33.73 and no disease was observed in T_8 (absolute control).

At 30 DAS, the per cent disease incidence was increased (PDI) to 47.92 in case of pathogen alone inoculated treatment (T_7) and no disease in T_8 (absolute control). The least per cent disease incidence of 19.89 was observed in T_3 (DFP48+ *S. rolfsii),* which was on par with the treatment T_4 (DFP47+ *S. rolfsii*) (19.96) with PDI of 19.96. The treatments T_5 (DFP62+ *S. rolfsii*), T_1 (BFP22+ *S. rolfsii*), T_2 (BFP38+ *S. rolfsii*) and T6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) recorded the per cent disease incidence of 21.16, 23.78, 24.81 and 27.55 respectively.

At 60 DAS, PDI increased to 56.01 in pathogen alone inoculated treatment (T_7) and no disease in T8 (absolute control). The lowest PDI of 20.98 was observed in T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*). The treatments T_3 (DFP48+ *S. rolfsii*), T_4 (DFP47+ *S. rolfsii*),T_5 (DFP62+ *S. rolfsii*), T_1 (BFP22+ *S. rolfsii*) and T_2 (BFP38+ *S. rolfsii*) recorded the per cent disease incidence of 21.96, 22.71, 24.39, 24.77 and 24.96 respectively.

Prasad *et al.* (2013) reported the antifungal activity of 26 fluorescent pseudomonad isolates from different rhizosphere soil against *Rhizoctonia solani*, *Sclerotium rolfsi* and *Colletotricum truncatum.*

The inhibitory effect of antagonistic bacteria such as *B. subtilis* and *P. fluorescens* against growth reduction of phytopathogenic fungi may be due to the production of hydrolytic enzymes that can degrade cell walls, iron-chelating siderophores and several cyclic lipodepsipeptides (Lim *et al.,* 1991).

Saravanakumar *et al.* (2007a) also showed that inoculation with fluorescent *Pseudomonas* induced a significant increase in root and shoot length over the uninoculated control. *Pseudomonas fluorescens* has been shown to increase seed germination, root and shoot length and seedling vigour in several instances (Ramamoorthy *et al.,* 2002; Khalid *et al.,* 2003; Egamberdieva, 2008). *Pseudomonas* spp. was reported to produce amino acids, salicylic acid and IAA (Sivamani and Gnanamanickam, 1988**;** Osullivan and Ogara, 1992) which might have improved the plant growth and seedling vigour.

Peroxidase activity

All the Fluorescent Pseudomonad inoculated treatments exhibited significantly higher peroxidase activity (in terms of D OD/g protein/min) than the pathogen alone and control. Data pertaining to peroxidase activity as influenced by different FP isolates in soybean leaves at different intervals of time *viz.,* 30 DAS and 60 DAS are furnished in Table 6.

At 30 DAS, there was a significant difference in peroxidase activities in all the treatments. Significantly higher peroxidase activity of 12.34 ΔOD/g protein/min was observed in T_3 (DFP48+ *S. rolfsii*). The next highest peroxidase activity (10.97 ΔOD/g protein/min) was recorded in T_4 (DFP47 + *S. rolfsii*), which was on par with T_2 (BFP38+ *S. rolfsii*) (10.26 ΔOD/g protein/min). The treatments T_5 (DFP62+ *S. rolfsii*), T_1 (BFP22+ *S. rolfsii*), T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) and T_8 (absolute control) recorded the peroxidase activity of 10.06, 8.84, 8.15 and 7.87 ΔOD/g protein/min. The least peroxidase activity was recorded in the pathogen alone inoculated treatment (T_7) (7.01 ΔOD/g protein/min).

At 60 DAS, peroxidase activity was further increased in all the treatments. Significantly higher enzyme activity of 17.48 ΔOD/g protein/min was recorded in T_3 (DFP48+ *S. rolfsii*). The next highest peroxidase activity of 15.11 ΔOD/g protein/min was recorded in T_5 (DFP62+ *S. rolfsii*). The other treatments T_2 (BFP38+ *S. rolfsii*), T_4 (DFP47+ *S. rolfsii*), T_1 (BFP22+ *S. rolfsii*) and T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) recorded the peroxidase activity of 14.02, 13.89, 12.89 and 11.30 ΔOD/g protein/min. The least peroxidase activity was recorded in the pathogen alone inoculated treatment (T_7) (10.85 ΔOD/g protein/min) compared to T_8 (absolute control) (11.22 ΔOD/g protein/min).

Effect of efficient fluorescent pseudomonad isolates on growth parameters of soybean challenge inoculated with *S. rolfsii*

Plant height

The results regarding the plant height of soybean is presented in the Table 5. At 90 DAS, maximum plant height of 40.00 cm was recorded by T_3 (DFP48+ *S. rolfsii*) which

was on par with T_2 (BFP38+ *S. rolfsii*) (38.27 cm). Lowest plant height of 18.42 cm was recorded in the treatment T_7 (S. *rolfsii*) which was lesser than the treatment T_8 (absolute control) (33.80 cm).

Number of branches

At 90 DAS, the treatment T_3 (DFP48+ *S. rolfsii*) showed significantly higher number of braches per plant (11.82) over all other treatments. The treatments T_4 (DFP47+ *S. rolfsii*) (11.04), T_5 (DFP62+ *S. rolfsii*) (10.92), T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) (10.89) and T_2 (BFP38+ *S. rolfsii*) (10.73) were on par with each other. Lowest number of branches was observed in T_7 (*S. rolfsii*) (9.96).

Nodule number

At 60 DAS, considerably increase in the number of nodules was observed. The maximum number of nodules of 15.67 was recorded by the treatment T_3 (DFP48 + *S. rolfsii*), which was on par with T_5 (DFP62 + *S. rolfsii*) (14.00), T_2 (BFP38 + *S. rolfsii*) (13.67) and T_1 (BFP22 + *S. rolfsii*) (13.00). (*S. rolfsii*) (T_7) recorded the lowest nodule number of 8.50.

At 90 DAS, significantly higher number of nodules were recorded in T_5 (DFP62+ *S. rolfsii*) (17.67) which was on par with T_3 (DFP48+ *S. rolfsii*) (17.33), T_2 (BFP38+ *S. rolfsii*) (15.50), T_4 (DFP47+ *S. rolfsii*) (15.33) and T_1 (BFP22+ *S. rolfsii*) (15.17). The least number of nodules (9.67) were observed in T_7 (*S. rolfsii*).

Total dry matter production

At harvesting stage, the treatment T_3 (DFP48+ *S. rolfsii*) achieved significantly highest dry matter production (7.57 g/plant) over all other treatments, which was followed by the treatment T_5 (DFP62 + *S. rolfsii*) (5.75 g/ plant) and T_4 (DFP47+ *S. rolfsii*) (5.58 g/plant). All the treatments with FP inoculation performed very well with respect to total dry matter production compare to the pathogen (*S. rolfsii*) alone inoculated treatment (T_7) (3.02 g/plant) and absolute control (T_8) (4.49 g/plant).

Table 5: Effect of efficient fluorescent pseudomonad isolates on growth parameters of soybean challenge inoculated with *S. rolfsii*.

Treatments	Plant height (cm)	No. of branches /plant	Nodule number /plant		Root dry weight (g/plant) (DAS)	Shoot dry weight (g/plant) (DAS)	Total dry matter production (g/plant) (DAS)
	90 DAS	90 DAS	60 DAS	90 DAS	At harvest	At harvest	At harvest
T_1: BFP22 + *S.rolfsii*	34.62	10.66	13.00	15.17	0.75	3.35	4.10
T_2: BFP38 + *S.rolfsii*	38.27	10.73	13.67	15.50	0.64	3.83	4.47
T_3: DFP48 + *S.rolfsii*	40.00	11.82	15.67	17.33	0.77	6.80	7.57
T_4: DFP47 + *S.rolfsii*	29.65	11.04	11.83	15.33	0.50	5.08	5.58
T_5: DFP62 + *S. rolfsii*	34.55	10.92	14.00	17.67	0.43	5.32	5.75
T_6: (Carboxin 37.5 % + Thiram 37.5%)+ *S. rolfsii*	28.25	10.89	10.83	12.00	0.35	5.05	5.40
T_7: *S. rolfsii* alone	18.42	9.96	8.50	9.67	0.30	2.72	3.02
T_8: Absolute control	33.80	10.08	9.33	10.00	0.44	4.05	4.49
S.Em. ±	**0.60**	**0.10**	**0.81**	**0.80**	**0.03**	**0.28**	**0.01**
C.D. @ 1 %	**2.30**	**0.35**	**3.13**	**3.10**	**0.14**	**1.06**	**0.03**

Table 6: Peroxidase activity of efficient fluorescent pseudomonad isolates and their effect on per cent disease incidence caused by *S. rolfsii*.

Treatments	Per cent disease incidence			Peroxidase activity (DOD/g protein/min)	
	15 DAS	30 DAS	60 DAS	30 DAS	60 DAS
T_1: BFP22 + *S. rolfsii*	21.22 (4.71)*	23.78 (4.98)*	24.77 (5.08)*	8.84	12.89
T_2: BFP38 + *S. rolfsii*	16.93 (4.21)	24.81 (5.08)	24.96 (5.09)	10.26	14.02
T_3: DFP48 + *S. rolfsii*	13.09 (3.75)	19.89 (4.57)	21.96 (4.79)	12.34	17.48
T_4: DFP47 + *S. rolfsii*	15.29 (4.03)	19.96 (4.58)	22.71 (4.87)	10.97	13.89
T_5: DFP62 + *S. rolfsii*	16.21 (4.15)	21.16 (4.71)	24.39 (5.04)	10.06	15.11
T_6: (Carboxin 37.5% + Thiram 37.5%) + *S. rolfsii*	13.59 (3.82)	27.55 (5.34)	20.98 (4.69)	8.15	11.30
T_7: *S. rolfsii* alone	33.73 (5.89)	47.92 (6.99)	56.01 (7.55)	7.01	10.85
T_8: Absolute control	00.00 (1.00)	00.00 (1.00)	00.00 (1.00)	7.87	11.22
S.Em. ±	**0.13**	**0.02**	**0.01**	**0.20**	**0.09**
C.D. @ 1 %	**0.57**	**0.07**	**0.05**	**0.84**	**0.39**

*Öx+1 transformed values.

Table 7: Effect of efficient fluorescent pseudomonad isolate on yield parameters of soybean challenge inoculated with *S. rolfsii*.

Treatments	Number of pods /Plant	Number of seeds /plant	Seed weight (g/plant)
T_1: BFP22 + *S.rolfsii*	11.40	34.90	5.83
T_2: BFP38 + *S.rolfsii*	16.17	45.87	7.87
T_3: DFP48 + *S.rolfsii*	19.30	55.77	9.23
T_4: DFP47 + *S.rolfsii*	15.80	36.87	6.50
T_5: DFP62 + *S.rolfsii*	11.10	46.17	7.10
T_6: (Carboxin 37.5% + Thiram 37.5%) + *S.rolfsii*	9.40	35.90	6.20
T_7: *S.rolfsii* alone	8.97	28.30	5.00
T_8: Absolute control	10.90	33.47	6.00
S.Em. ±	**0.07**	**0.07**	**0.05**
C.D. @ 1 %	**0.31**	**0.30**	**0.23**

Effect of efficient fluorescent pseudomonad isolate on yield parameters of soybean challenge inoculated with *S. rolfsii*

Number of pods per plant

Data pertaining to the number of pods per plant is presented in Table 7. Significantly highest number of pods per plant were recorded in treatment T_3 (DFP48 + *S. rolfsii*) (19.30) over all other treatments, which was followed by the treatment T_2 (BFP48+ *S. rolfsii*), (16.17) which is on par with T_4 (DFP47+ *S. rolfsii*) (15.80). Lowest pods (8.97) were observed in T_7 treatment compared to T_6 (carboxin 37.5%+Thiram 37.5%+ *S. rolfsii*) 10.90/plant.

At harvest, all the treatments differed significantly with respect to number of pods. Significantly highest numbers of pods were observed in T_3, which was followed by T_2 and T_4.

Number of seeds/plant

The results pertaining to pod weight / plant is presented in Table 7. The treatment T_3 (DFP48+ *S. rolfsii*) recorded higher number of seeds/plant (55.77) and was significantly superior over all other treatments. It was followed by the treatment T_5 (DFP62 + *S. rolfsii*) which recorded 46.17 number of seeds/plant which was on par with T_2 (BFP38+ *S. rolfsii*) (45.87). Pathogen inoculated treatment (T_7) recorded lowest number of seeds/plant (28.30). Whereas T_8 (absolute control) recorded 33.47 number of seeds/plant.

Seed weight (g/plant)

The treatments had a significant influence on the weight of seeds/plant (Table 7). The treatment T_3 (DFP48+ *S. rolfsii*) recorded significantly highest seed weight of 9.23 g/plant over all other treatments, which was followed by the treatment T_2 (BFP38+ *S. rolfsii*) with seed weight of 7.87 g/plant. T_8 (absolute control) recorded the seed weight of 6.00 g/plant which is slightly higher than the pathogen inoculated treatment (T_7) (5.00 g).

The higher plant height, nodule number, dry matter production, number of pods, number of seeds, seed weight compared to control clearly showed the beneficial role of these rhizobacteria, which might be attributed to IAA, production, P-solubilization and many other PGPR activity in favour of plant growth response.

Saravanakumar *et al.* (2007a) also showed that inoculation with fluorescent *Pseudomonas* induced a significant increase in root and shoot length over the uninoculated control. *Pseudomonas fluorescens* has been shown to increase seed germination, root and shoot length and seedling vigour in several instances (Ramamoorthy *et al.,* 2002; Khalid *et al.,* 2003; Egamberdieva, 2008). *Pseudomonas* spp. was reported to produce amino acids, salicylic acid and IAA (Sivamani and Gnanamanickam, 1988; Osullivan and Ogara, 1992) which might have improved the plant growth and seedling vigour.

CONCLUSION

Biological control of plant diseases opened an era of new technology to manage crop diseases and received the attention of researchers throughout the world, which will enhance the sustainability of agricultural production systems and to reduce the use of chemical pesticides. A few of the available biocontrol agents mostly belonging to *Pseudomonas* sp. show broad-spectrum antifungal activity by virtue of volatile and diffusible antibiotics. In this view, the present study aimed at screening efficient fluorescent pseudomonads against *Sclerotium rolfsii* of soybean. Eight isolates obtained from Rhizosphere sample are positive for IAA, GA production, HCN, siderophore production. These isolates were also antagonists to *Sclerotium rolfsii.* Pot culture studies revealed that the treatment T_3 (DFP48 + *S. rolfsii*) recorded highest plant height, nodule number, total dry matter production, no. of pods per plant, number of seeds per plant and seed weight. T_3 (DFP48 + *S. rolfsii*) also showed very less per cent disease incidence compared to all other treatments. These isolates can be further explored for their efficacy as effective PGPR. Application of these multifunctional microbes for diseases management and their practical application require further investigation under field conditions.

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