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MANAGEMENT OF ROOT ROT DISEASE OF WHEAT WITH ENDOPHYTIC PLANT GROWTH PROMOTING *PSEUDOMONAS* ASSOCIATED WITH HEALTHY WHEAT ROOTS

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Abstract

Wheat is the second most important grain crop and is a source of staple food in many countries of the world. Although the production of wheat has increased after green revolution but the attack of various diseases has greatly affected its yield and quality. In this study seven strains of fluorescent *Pseudomonas* isolated from inside of healthy wheat roots were identified as *Pseudomonas aeruginosa.* In dual culture plate assay, one strain of *P. aeruginosa* inhibited the radial growth of all the three test root rotting fungi *Macrophomina phaseolin*a, *Rhizoctonia solani* and *Fusarium solani* by producing the zone of inhibition. One strain also caused lysis of fungal hyphae. In screen house experiment most of the *P. aeruginosa* isolates caused a suppressive effect on *Fusarium culmorum* on two test wheat varieties. However in Blue silver variety of wheat 18.7% control plants showed *R. solani* infection with no infection on *P. aeruginosa* (PS-1, PS-2 and PS-4 isolates) treated plants. Infection of *Macrophomina phaseolina* and *F. solani* was not found on any plants. Application of *Pseudomonas* isolates also showed a positive impact on plant growth of wheat variety Lasani by improving plant height.

KEYWORDS: Wheat, *Pseudomonas*, *Fusarium*, *Rhizoctonia* and Endophytic

INTRODUCTION

The annual wheat (*Triticum aestivum*) production of Pakistan is 23.3 million metric tons, which should be further increased to meet the demand of growing population. Rusts and smut diseases are the major constrains of wheat production (Agrios, 2005). However, introduction of new resistant varieties are minimizing the threat of rusts and smut diseases. Besides rusts and smut, various soilborne fungi can cause root and crown rot disease of wheat. Affected plants may be stunted or less vigorous than healthy plants. Plants may be yellow and wilted and may die prematurely. Root system may be poor with roots and crown tissues discolored and deteriorated (Smiley & Patterson, 1996; Smiley *et al.*, 2005). The pathogens responsible for yield decline in wheat are *Gaeumannomyces graminis* var. *tritici*, causing take-all, *Fusarium culmorum* and *Fusarium pseudograminearum*, causing Fusarium crown root and *Rhizoctonia solani* causing *Rhizoctonia* root rot (Cook, 2001; Paulitz *et al*., 2002). Currently, there are no economically or environmentally acceptable means for controlling these diseases, particularly after wheat crops have been planted.

Endophytic bacteria are those bacteria that live in plant tissues without doing substantive harm or gaining some benefit other than residency (Kado, 1992, Kobayashi & Palumbo, 2000). Considerable evidence has been accumulated in recent years to support and identify the benefits associated with the use of endophytic bacteria in crop protection (Afzal *et al.,* 2013; Siddiqui & Ehteshamul-Haque, 2001; Tariq *et al*., 2009; Dubois *et al.,* 2006). Besides, endophytes also promote plant growth by a number of mechanisms. These include phosphate solubilization activity (Altomare *et al.,* 1999), indole acetic acid production (Brown, 1972) and production of siderophore (Leong, 1986).

Biological control with endophytic bacteria offers an effective strategy for pest management. Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents. Several endophytic bacterial strains have been reported to induce systemic resistance (Dubois *et al.,* 2006; Ramamoorthy *et al.,* 2001). Among the endophytic plant growth promoting bacteria, species of *Pseudomonas* have has been shown to improve plant growth and it is known to synthesize growth-stimulating plant hormones (Tariq *et al.,* 2009; Ehteshamul-Haque *et al.,* 2007a and b). Plant hormones produced by *Pseudomonas* include auxins and cytokinins, as well as volatile signals such as ethylene 2, 3-butanediol and acetonin (Persello-Cartieaux *et al*., 2003), which implicated in stimulation of root growth. The present report describes the biocontrol potential of endophytic fluorescent *Pseudomonas* associated with healthy wheat roots on root rotting fungi of wheat.

MATERIALS AND METHODS

Wheat root samples for the isolation of endophytic fluorescent *Pseudomonas*: For the isolation of fluorescent *Pseudomonas* root samples of healthy wheat plants were collected from agricultural fields of Malir, Karachi and brought to the laboratory and kept at 4^{o}C until isolation was made within 24 hours.

Isolation of endophytic fluorescent *Pseudomonas*: For the isolation of endophytic bacteria, 1 g roots from healthy plants were disinfested with 1% $Ca(OCl)_2$ for 3 minutes and rinsed twice with sterile water, submerged for 30 seconds in 15% H_2O_2 and rinsed twice again in sterile water. The roots were macerated in 10 ml of 0.1 M $MgSO_4$ solution with 0.02% Tween-20 and 100 µl aliquots from serial dilution were transferred on S1 medium supplemented with antibiotic trimethoprim (Bashan *et al.,* 1993; Gould *et al.,* 1985; Siddiqui & Ehteshamul-Haque, 2001). Dishes were incubated for three days at 28ºC. Bacterial colonies fluoresced under UV light at 366 nm were purified on King's B agar medium (King *et al.,* 1954) and identified according to Krieg & Holt (1984).

***In vitro* test against root infecting fungi:** Dual culture plate method was used to determine the antifungal activity of bacterial strains (Drapeau *et al*., 1973). The bacterial strains/ isolates were streaked on one side of the Petri dishes containing Czapek's Dox Agar pH 7.2. On the other side of Petri dishes, a 5 mm diam. disc of test fungi *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* (isolated from diseased wheat roots) was inoculated. The dishes were incubated at 28ºC and zone of inhibition (if any) were recorded from 3-7 days (depends upon the growth of test fungus).

Screen house experiment: Biocontrol potential of endophytic fluorescent *Pseudomonas* was evaluated in screen house using two wheat (*Triticum aestivum*) varieties, Blue silver and Lasani as test plants. The experiment was conducted in 12 cm diameter clay pots each containing one Kg soil obtained from the experimental fields of Botany Department. The soil had a natural infestation of 3-6 sclerotia/gm of soil of *Macrophomina phaseolina* as determined by wet sieving and dilution technique (Sheikh & Ghaffar, 1975), 5-10% colonization of *Rhizoctonia solani* on sorghum seeds used as baits (Wilhelm, 1955) and 3000 cfu/gm of soil of mixed population of *Fusarium* spp., (Nash & Snyder, 1962). An aqueous 25 ml cell suspension of five day old culture of *P. aeruginosa* isolates viz., PA-1, PA-2, PA-3, PA-4 and PA-5 were drenched in each pots. Carbendazim (200 ppm), 25 ml per pot served as positive control against root rotting fungi, whereas pots not treated with bacteria or fungicide served as negative control. Six seeds of each wheat variety were sown in each pots. After germination (one week) only four seedling were kept per pot by thinning. Treatments were replicated four times and pots were randomized on a screen house bench in complete randomized block design. To determine the role of endophytic fluorescent *Pseudomonas* on root rotting fungi, plants were uprooted after 6 weeks. Roots were washed with tap water and data on plant growth was recorded.

To determine the incidence of fungal infection, 1-cm long root pieces (five pieces from each plant) were surface disinfested with 1% $Ca(OCl)_2$ and plated onto potato dextrose agar amended with penicillin (100,000 units/litre) and streptomycin (0.2 g/litre). After incubation for 5 days at 28ºC, colonies of *Rhizoctonia solani* and species of *Fusariu*m were recorded. The experiment was conducted twice.

Data analysis: For fungal infection, two way ANOVA was used to compare the means among the treatments and also among different fungal pathogens. The follow up of ANOVA included least significant difference (LSD) at ($p<0.05$) to compare the means. Whereas for plant growth parameters one way ANOVA was used and LSD at ($p<0.05$) was calculated (Gomez and Gomez, 1984).

RESULTS

***In vitro* antifungal activity of fluorescent *Pseudomonas*:** *Pseudomonas aeruginosa* strains isolated from roots of wheat plant were tested for antifungal activity against three pathogenic root rotting fungi *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* isolated from diseased wheat roots. *P. aeruginosa* strain PS-1, PS-5 inhibited all three test fungi by producing zone of inhibition (Table 1). Where as PS4 also caused lysis of mycelium of *Fusarium solani* and inhibited the radial growth of *Rhizoctonia solani.*

Effect of endophytic fluorescent *Pseudomonas* on root rotting fungi of wheat: Infection of *Macrophomina phaseolina* and *Fusarium solani* was not found on any plants. Whereas infection of *F. culmorum* was found on most of the plants. However number of plants grown in bacterized soil was found less infected as compared to control plants. On Blue silver PS-1, PS-2, PS-3 and PS-5 were found effective against root rotting fungi (Table 2). No infection of *F. culmorum* was found on plants treated with PS-3. Plants of wheat variety Lasani showed less infection of *F. culmorum* grown in soil treated with *P.aeruginosa* isolates as compared to untreated control plants (Table 3).

Infection of *Rhizoctonia solani* was found on few plants of Lasani varietiy. However in Blue silver 18.7% control plants showed *R. solani* infection with no infection of *R. solani* on PS-1, PS-2 and PS-4 treated plants. Effect of *P. aeruginosa* was mostly non-significant on plant growth of Blue-Silver, while on Lasani variety PS-1 and PS-2 significantly ($p<0.05$) improved plant height and fresh shoot weight (Table 3).

Table 1. *In vitro* growth inhibition of *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina* by the endophytic isolates of *Pseudomonas aeruginosa,* plant growth promoting rhizobacterium.

***P. aeruginosa* strain**	***F. solani***	***R. solani*** Zone of inhibition(mm)	***M. phaseolina***
PS-1	25	12	26
PS-2	-	-	5.5
PS-3	20	25	23.6
PS-4	21.6*	17.5	-
PS-5	22.5	12.6	26.5
PS-6	20	12.6	-
PS-7	-	21.6	-

* = Fungal hyphae lysed

- = Not tested

Table 2. Effect of endophytic *Pseudomonas aeruginosa* on the infection of root rotting fungi on wheat variety Blue silver and Lasani.

Treatment	**Blue silver**		**Lasani**	
	Fusarium culmorum	***Rhizoctonia solani***	***Fusarium culmorum***	***Rhizoctonia solani***
Control	37.5	0	31.2	0
Carbendazim	18.75	0	18.75	0
P. aeruginosa (PS-1)	6.25	18.75	6.25	6.2
P.aeruginosa (PS-2)	6.25	0	12.5	0
P.aeruginosa (PS-3)	6.25	0	12.5	6.2
P.aeruginosa (PS-4)	12.5	0	6.2	0
P.aeruginosa (PS-5)	31.25	18.75	18.7	12.5
$LSD_{0.05}$	**Treatments=22.7[1]**	**Pathogen=11.6[2]**	**Treatments=21.2[1]**	**Pathogen=11.2[2]**

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$

[2]Mean values in rows showing differences greater than LSD values are significantly different at $p<0.05$

Table 4. Effect of *Pseudomonas aeruginosa* on the growth of wheat variety Blue silver and Lasani.

Treatments	**Blue silver**				**Lasani**			
	Shoot length (cm)	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**	**Shoot length (cm)**	**Shoot weight (g)**	**Root length (cm)**	**Root weight (g)**
Control	24.4	0.46	15.2	0.12	29.1	0.87	9.3	0.36
Carbendazim	21.9	0.60	11.0	0.16	39.0	0.79	8.7	0.10
P.aeruginosa (PS-1)	21.25	0.48	14.5	0.19	40.1	1.347	9.6	0.38
P.aeruginosa (PS-2)	23.3	0.48	7.7	0.17	41.7	1.11	9.6	0.32
P.aeruginosa (PS-3)	21.7	0.45	8.5	0.08	38.2	0.58	8.7	0.25
P.aeruginosa (PS-4)	23.68	0.68	11.7	0.16	39.0	1.09	9.3	0.32
P.aeruginosa (PS-5)	23.5	0.55	8.6	0.15	26.7	0.49	10.8	0.22
$LSD_{0.05}$	ns	ns	ns	ns	11.7[1]	0.46[1]	ns	0.2[1]

[1]Mean values in column showing differences greater than LSD values are significantly different at $p<0.05$

ns = Non-significant

DISCUSSION

Endophytic microbial communities, both bacteria and fungi are known to affect root health. In the present study some strains of endophytic fluorescent *Pseudomonas* isolated from roots of healthy wheat plants were identified as *P. aeruginosa* which showed significant activity against root rotting fungi both *In vitro* and *In vivo.* Several studies have shown that the interaction between plants and some endophytic bacteria was associated with beneficial effects such as

plant growth promotion and biocontrol potential against plant pathogens (Hallmann *et al*., 1995). Of the various rhizospheric bacteria, the bacteria belonging to the fluorescent *Pseudomonas* which colonize roots of a wide range of crop plants are reported to be antagonistic to soil-borne plant pathogens (Siddiqui & Ehteshamul-Haque, 2001; Raaijmaker *et al*., 2002; Weller *et al.,* 2002).

According to Berg (2009), plant growth promoting rhizobacteria (PGPR) are employed for controlling plant pathogens, enhancing efficiency of fertilizers and degrading xenobiotic compounds. The beneficial effects of PGPR on root growth have also been reported in wheat (Levanony & Bashan, 1989). The mechanisms involved in supporting plant growth and health include increasing soil nutrient availability, improving soil structure, inducing the plant defense mechanisms, producing antibiotics, competing pathogens and providing growth stimulating substances or enzymes (Van Loon & Bakker, 2004). Plant hormones produced by *Pseudomonas* include auxins and cytokinins, as well as volatile signals such as ethylene 2, 3-butanediol and acetonin (Lambrecht *et al*., 2000; Persello-Cartieaux *et al*., 2003). Endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents (Berg *et al.*, 2005). The plant growth-promoting endophytes are now being used in the developing areas of forest regeneration and phytoremediation of contaminated soils (Harman *et al*., 2004a and b). Biological control with endophytes offers an effective strategy for pest management.

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