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Endophytic movemnet and phylloplane survival of *P. fluorescens* in rice

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Abstract
Fluorescent Pseudomonads belong to plant Growth Promoting Rhizobacteria (PGPR), the important group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. Many strains of *Pseudomonas fluorescens* are known to enhance plant growth promotion and reduce severity of various diseases. The aim of this investigation was to assess the systemic movement and phylloplane survival of *P. fluorescens* (PF_9) in rice crop after treated with PF_9 as seed treatment and foliar spray @ 10ml/kg and 0.5% concentration respectively under green house condition. The results clearly revealed that systemic movement of bacterium (PF_9) could be recovered in roots up to 75 days (3.8×10^3) the maximum period tested, where as in stem and leaves the population declined with increase in the sampling period and the bacterium could be recovered only up to 60 days after sowing the seeds treated with bacterium. Maximum population of the test bacterium could be detected in root (6.8×10^3), stem (5.6×10^3) and leaves (4.1×10^3) at 15 days after sowing indicated the necessity for subsequent application of the antagonist. Also, the population of bacterium in the phylloplane and leaf tissues after foliar spray showed a declining trend with increase in the sampling period. The maximum population (18.2×10^3) on phylloplane and population in leaf tissue (4.9×10^3) was recorded on the first day after foliar spray and then the population showed a gradual decline and reached the minimum population in the phylloplane (0.6×10^2) and in the leaf tissues (0.3×10^3) at 12 days after spraying.

Keywords: Endophytic movement, phylloplane survival, *P. fluorescens*, rice

Introduction
P. 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 plant diseases (Rabindran and Vidhyasekaran, 1996) [12]. Their applicability as bio control agents has drawn wide attention because of the production of secondary metabolites such as side rophores, antibiotics, volatile compounds, HCN, enzymes and phyto hormones (Gupta *et al.*, 2001) [6]. They can be utilized in low input sustainable agricultural applications, such as bio control, 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 (Raaijimakers *et al.*, 2002) [11]. These secondary metabolites include 2,4- diacetylphloroglucinol (DAPG), phenazine (Phz), pyrrolnitrin, oomycin A, viscosinamide, pyoluteorin and hydrogen cyanide (HCN).One of the prerequisites for the efficiency of bio control agents in controlling the plant disease is their capacity to survive in the target sites. Accordingly, the survival of *P. fluorescence* the phyllosphere (Austin *et al.*, 1977) [2] may explain the effectiveness of foliar sprays in controlling the foliar diseases. Beattie and Lindow (1999) [3] suggested that *P. fluorescence* may survive on leaf surfaces by occupying particular surface sites.The antagonists preoccupy the infection site and deprive the same to the pathogen (Gnanamanickam and Mew, 1992) [5] and that the bacterial multiplication and colonization depend on modification of leaf habitat, ingression and egression (Battie and Lindow, 1999) [3].
Several studies were reported that systemic movement and phylloplane survival of *P. fluorescence* after the plant treated with bacterium. Also, the bacterium could be detected 15 to 40 days after the plant treated with *P. fluorescence* was move systemically within the plant and the survive well on the surface of leaf after foliar application (Rabindran and Vidhyasekaran, 1996; Krishnamoorthy and Gnanamanickam, 1998; Vimala and Suriyachandraselvan, 2008; Anand *et al.*, 2010; Meena, 2011; Surjit and Krishnendu, 2013) [12, 8, 16, 1, 9, 15]. Hence, the present study was conducted to assess the systemic movement and phylloplane survival of native isolate of *P. fluorescens*.

Materials and methods

In my previous study an intensive field survey was conducted in rice fields of Cauvery delta districts of Tamilnadu, India, meanwhile 30 number of native isolates of *P. flurescens* (PF_1 to PF_{30}) were collected which was undergoes various *in-vitro* (cultural character, biochemical characterization and antagonistic activity against *S. oryzae*) and pot culture experiment for the management of rice sheath rot disease. All the above experiments conclude that native isolate PF_9 was significantly inhibits the growth of *S. oryzae* and reduced the incidence of sheath rot disease than other isolates. Hence, the isolate was further takeover to investigate their systemic movement and phylloplane survival in rice crop in separate pot culture experiment.

One kg of surface sterilized BPT 5204 rice seeds was taken in a cloth bag soaked in two liters of water for 24 h and the excess water was drained. The seeds were allowed to sprout for 12 h. and *P. fluorescens* (Pf_9) as liquid based formulation (10^8cfu/ml) was added to the sprouted seed @ 10 ml per kg of seed with two per cent CMC and air dried for one h in laminar cabinet and sown in cement pots.The pots were maintained in greenhouse and following standard agronomic practices. There were five pots per replication, six plants/hills per pot, and four replications were maintained for each treatment. Population of *P. fluorescens* (Pf_9) was estimated by pulling out the plants from soil at different days after sowing. Root, stem and leaves (1 gm each) were separated by using scissor were transferred to a sterilized pestle and mortar. They were ground using 10 ml of sterile distilled water and serial dilutions were made. The population of *P. fluorescens* (PF_9) was assessed using King's B medium and recorded (Ragavan, 2003) [13].

Estimation of *P. fluorescens* (PF_9) population on phylloplane of rice plants

To study the survival of *P. fluorescens* in phylloplane of rice plants, the liquid formulation of the Pf_9 (10^8 cfu ml^{-1}) was sprayed @ 0.5% concentration onto 25-days-old BPT 5204 rice plants grown in cement pots. Samples were taken at 1, 3, 6, 9, 12 and15 days after spraying and the phylloplane survival of the test bacterium was assessed by serial dilution technique. One g of leaf bits were taken in 10 ml of sterile dist. water, shaken vigorously and serial dilutions were prepared. The population of *P. fluorescens* (PF_9) was assessed and colonies were counted under the UV (Ragavan, 2003) [13]. From the above experiments, 1g of surface sterilized leaf samples were taken and ground in 10 ml sterile dist. water in a sterile pestle and mortar. From this, serial dilutions were prepared and the population of *P. fluorescens* (PF_9) was assessed and colonies were counted under the UV.

Result

Endophytic survival and systemic movement of *P. fluorescens* (Pf_9) in rice after seed treatment (Pot culture)

The data presented in table 1 revealed that *P. fluorescens* (Pf_9) when treated to the seeds could be recovered in roots up to 75 days (3.8×10^3), where as in stem and leaves the population declined with increase in the sampling period and the bacterium could be recovered only up to 60 days after sowing. Maximum population of the test bacterium could be detected in root (6.8×10^3), stem (5.6×10^3) and leaves (4.1×10^3) at 15 days after sowing. The results indicated the necessity for subsequent application of the antagonist at 60 days after sowing.

Survival of *P. fluorescens* (Pf_9) in the phylloplane and leaf tissues of rice plants as influenced by foliar spray (Pot culture)

The data depicted in table 2 revealed that the population of *P. fluorescens* (Pf_9) in the phylloplane and leaf tissues showed a declining trend with increase in the sampling period. The maximum population (18.2×10^3) on phylloplane and population in leaf tissue (4.9×10^3) was recorded on the first day after foliar spray and then the population showed a gradual decline and reached the minimum population in the phylloplane (0.6×10^2) and in the leaf tissues (0.3×10^3) at 12 days after spraying. The bacterium could not be recovered from the phylloplane or in the leaf tissue at 15 days after foliar spray indicating the necessity of repeated sprays.

Table 1: Endophytic survival and systemic movement of *P. fluorescens* (Pf_9) in rice after seed treatment (Pot culture)

Method of application	Days after treatment	Population of Pf_9 (cfu $\times10^3$)		
		Root	Stem	Leaves
Seed treatment	15	6.8^a	5.6^a	4.1^a
	30	6.0^a	3.8^b	3.0^b
	45	4.8^b	1.6^c	1.0^c
	60	3.9^c	0.7^d	0.3^d
	75	3.8^c	-	-

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Table 2: Survival of *P. fluorescens* (Pf_9) in the phylloplane and leaf tissues of rice plant as influenced by foliar spray (Pot culture)

Tt. No.	Days after spraying	Phylloplane population of Pf_9 (cfu $\times g^{-1}$)	Population of Pf_9 in leaf tissues (cfu $\times10^3$)
T_1	1	18.2×10^{3a}	4.9×10^{3a}
T_2	3	5.2×10^{3b}	3.0×10^{3b}
T_3	6	3.4×10^{3c}	1.0×10^{3c}
T_4	9	1.1×10^{2d}	0.7×10^{3c}
T_5	12	0.6×10^{2d}	0.3×10^{3d}
T_6	15	-	-

Values in the column followed by same letters not differ significantly by DMRT (p=0.05)

Discussion

The bacteria *P. fluorescens* appeared to move endophytically from seed to roots, stems and leaves. It has been shown in earlier studies that fluorescent pseudomonads could be isolated from aerial parts of plants grown from seeds treated with the bacteria (Colyer and Mount, 1984; Mew and Rosales, 1986) [4, 10]. In case of groundnut *S. marcescens* moved in roots, coleoptiles and the first and second leaves (Kishore *et*

al., 1998). Similarly, the systemic movement of *P. fluorescens* occurred in emerging roots and shoots of rice plants grown from seeds treated with *P. fluorescens* (Ragavan, 2003; Shyamala and Sivakumar, 2012) [13, 14].

In the present study also after seed treatment *P. fluorescens* (Pf_9) could be recovered in roots up to 75 days the maximum period tested, where as in stem and leaves the population declined with increase in the sampling period and the bacterium could be recovered only up to 60 days after sowing. Similar to the present study, Rabindran and Vidhyasekaran, (1996) [12] also obtained decreasing trend in the population of *P. fluorescens* from the roots, stem and leaves after seed treatment with the antagonists. Earlier, fluorescent pseudomonads have been detected on leaf sheaths (Mew and Rosales, 1986) [10] and leaves (Gnanamanickam and Mew, 1992) [5] of rice seedlings grown from seeds treated with the bacteria.

One of the prerequisites for the efficiency of bio control agents in controlling the plant disease is their capacity to survive in the target sites. Accordingly, the survival of *P. fluorescens* in the phyllosphere (Austin *et al.,* 1977) [2] may explain the effectiveness of foliar sprays in controlling the foliar diseases. Beattie and Lindow (1999) [3] suggested that *P. fluorescens* may survive on leaf surfaces by occupying particular surface sites.The antagonists preoccupy the infection site and deprive the same to the pathogen (Gnanamanickam and Mew, 1992) [5] and that the bacterial multiplication and colonization depend on modification of leaf habitat, ingression and egression (Battie and Lindow, 1999) [3]. However, in the present study the population of *P. fluorescens* (Pf_9) in the phylloplane and leaf tissues after foliar spray also showed a declining trend with increase in the sampling period. The bacterium could not be recovered from the phylloplane or in the leaf tissue beyond 12 days after foliar spray indicating the necessity for repeated sprays. Thus, it is quite reasonable to assume that the environmental conditions could have had an impact on the survivability of the antagonist in the phylloplane as the bacterium could not be recovered from the leaf surface after twelve days of spraying. Also this indicated the necessity for repeated sprays at every ten days.

Similar to the present results, Krishnamoorthy and Gnanamanickam (1998) [8] clearly demonstrated that foliar sprays to 30-day-old seedlings resulted with the bacterial number in the leaf up to 35 to 40 days and declined after40 days. Vimala and Suriyachandraselvan (2008) [16] reported that the population of *P. fluorescens* could be detected up to 14 days on foliage of bhendi. Meena (2011) [9] also opined that the foliar application of Pf1 formulation was able to offer protection for groundnut crop from leaf spot disease only up to 15 days. These earlier reports corroborate with the present findings.

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