ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(4):973-977

ORIGINAL RESEARCH ARTICLE

Studies on the Influence of *Pseudomonas fluorescens* and Chemicals on the Biocontrol Sheath Blight Incidence in Rice

R.Sivakamasundari* and G. Usharani

Department of Microbiology, Annamalai University, Chidambaram-608 002, Tamil Nadu, India

Received 04 May 2012; Revised 03 Aug 2012; Accepted 10 Aug 2012

ABSTRACT

The plant microbes interactions augmented the biocontrol of many Phytopathogens through siderophore mediated induced systemic resistance (ISR). As a prodigious colonizer of plants, *Pseudomonas fluorescens* occurred both in Phyllosphere rhizosphere at rice crop. As the epiphytic *Pseudomonas fluorescens* exhibited hypobiosis, their positive role in augmented biocontrol efficiency at rhizosphere level of rice needs to be elucidated and development and use at these microbes will yield added advantages in the biotechnological application at the microbe to harness maximum benefits. A detailed survey on the occurrence at *Pseudomonas fluorescens* in the Phyllosphere and rhizosphere at rice grown at Cuddalore district revealed the ubiquitous occurrence of the microbes in both phyllosphere and rhizosphere of rice. A total number of 10 strains has been isolated and characterized as per LOPAT test based on the biocontrol characterized, these isolates were screened and the efficiently two isolates were selected for further study. Studies on the combined effect of *Pseudomonas fluorescens* and salicylic acid application during the challenge inoculation of *Rhizoctonia solani* revealed the application of *Pseudomonas fluorescens* at seed and soil level together with application at salicylic acid on 30thay augmented the plant defense enzyme system to a maximum level. The improved ability of Phyllosphere *Pseudomonas fluorescens* for ISR mediated biocontrol ability due to their altered metabolic state.

Key words: Rice, ISR mediated biocontrol, *Pseudomonas fluorescens* and *Rhizoctonia solani.*

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food for over two billion people in Asia and for hundreds of millions in Africa and Latin America. To feed the ever increasing population of these regions the world's annual rice production must be increased from the present 560 to 750 million tones by 2020 [1]. The future increase in rice production has to come from the same or even reduced land area and the productivity yield (per ha) must be greatly enhanced by providing additional nutrient input and through effective control at phytopathogens.

Sheath blight disease of rice was first described from Japan in 1910 and now known to occur in most countries in Asia. For the first time in India, the disease was reported by Paracer and Chaha, 1963 [2]. Kanniyan and Prasad [3] reported the intensity and severity of sheath blight disease in Tamil Nadu. The disease is mainly confined to lower sheath and is known to be soil borne. Kannaiyan and Prasad [4] have reported for the first time the seed borne nature of the disease. Ou [5] reported that when the disease reaches the flag leaves, loss is estimated to be 25 per cent.

Fluorescent *Pseudomonas* have emerged as the largest and potentially most promising group of PGPR, Possessing traits also involved in the biocontrol of phytopathogens. As a prodigious colonizer of plants, *Pseudomonas fluorescens* occurred on the leaf surface [6] on the roots, the soil immediately adjacent to root where microbial activity is influenced by the plants and also within the root. Phyllosphere inhabiting *Pseudomonas fluorescence* represents the important component of leaf community and the distribution pattern of this epiphytic population is seriously affected by climate and cropping pattern of that location [7]. When compared to rhizosphere population the epiphytic population of *Pseudomonas fluorescens* exhibited a state of reduced metabolism or hypobiosis in order to enhance the survivability during physical and chemical stresses of the

***Corresponding Author:** R.Sivakamasundari**, Email:** sivakamikarnan@gmail.com

environment that would be fatal to the cells of rhizosphere population having a high metabolic activity [8].

Induced systemic resistance (ISR) by *Pseudomonas fluorescence* is one of the important mechanisms for the biological suppression of rice phytopathogens including sheath blight disease. The occurrence and association of *Pseudomonas fluorescens* in the rice rhizosphere and the induction of systemic resistance (ISR) during rice *Pseudomonas fluorescens* association have been reported by many authors [9].

Induced resistance by *Pseudomonas fluorescens* has broad spectrum activity against many fungal, bacterial, viral and pathogenic species [10,11]. *Pseudomonas fluorescens* treatment has increased the plant vigour, height and grain yield of wheat. Mew and Rosales [12] reported increased that the height of rice plant located whit *Pseudomonas fluorescens* then the control plants. Dileep Kumar and Dube [13] reported significant increased in the emergence of tomato seedling when seeds were treated with *Pseudomonas fluorescens* appositive increase in the length of sunflower seedling by seed treatment with *Pseudomonas fluorescens* in 14 strains.

2. MATERIALS AND METHODS

2.1. Isolation and identification of *Pseudomonas fluorescens* from phyllosphere and rhizosphere of rice

The *Pseudomonas fluorescens* was isolated from phyllosphere and rhizosphere of rice by Pour plate technique using King's B medium. The isolated *Pseudomonas fluorescens* was identified by Gram staining technique, motility test, platting on selective medium and biochemical tests.

2.2. Screening of *Pseudomonas fluorescens* isolates for zone of inhibition of *Rhizoctonia Solani*

A loopful culture of each *Pseudomonas fluorescens* isolates was transferred aseptically to the centre of PDA plates which have been pre inoculated with *Rhizoctonia solani*. The plates were incubated at 28±2ºC for 72 hrs. After the incubation period the diameter of inhibition zone was measured. Three replications were maintained for each isolate.

2.3. Studies on the influence of *Pseudomonas fluorescens* growth phases on the sheath blight incidence in Rice

Rice (*Oryza sativa* L.) cv IR 50 seeds were surface sterilized, germinated and transferred to a steel wire mesh (3 mm in dm) in a growth chamber filled with 200 ml of weaver's medium. The *Pseudomonas fluorescens* isolates *viz.,* PFP-5 and PFR-5 were harvested at lag, log and stationary phases, separately. The 10 ml of cultures at each of the three phases was inoculated @ $1x10^{-7}$cfu ml^{-1} into Weaver's medium, separately. Inoculum of *Rhizoctonia solani* was prepared using sterilized maize stem bits and spore suspension was prepared in two per cent sucrose solution containing a drop of Tween-20. The suspension containing about 50,000 spores/ml was used for challenge inoculation. Plants were inoculated by spraying the spore suspension of *Rhizoctonia solani* on 20th DAS with an atomizer and the control plant was sprayed with distilled water only. High humidity was created by sprinkling the water frequently in the polyhouse. The growth chamber was maintained as 30 ±2°C with 10 hrs light and 14 hrs darkness for 20 days. Six replications were maintained for each treatment. After one week of challenge inoculation, the blast disease incidence was estimated by score chart method [14].

2.4. Evaluation of certain resistance inducing chemical against *Rhizoctonia solani*

Rectangular cement pots of size 18" x 12"x12" were filled with 45kg of paddy field soil flooded with water for 2 days and brought to fine puddle conditions. Seeds of the rice variety IR-50 were loosely packed separately in small gunny bag and soaked in water for 12 hrs. Then the bags were subsequently kept in dark place after covering with wet gunny bags to ensure optimum condition for germination. The seeds germinated within about 24 hrs. after soaking. The pre-germinated seeds of IR-50 rice were sown in rows in pots separately. On the 5th day of sowing the seedlings were thinned to get 50 numbers per pot. The seedlings were raised under wet conditions and the age was counted from the time of sowing. After the sowing of rice seeds, four resistance inducing chemicals, namely, Acibenzolar, Naphthaline acetic acid, Jasmonic acid and salicylic acid (Central Drug House) at 0.075 and 0.1 per cent level, were sprayed individually on 15th DAS one day prior to the challenge inoculation of *Rhizoctonia solani* to rice plant. The observations were recorded one week after the challenge inoculation of *Rhizoctonia solani* and expressed as percentage disease incidence as detailed elsewhere in the text.

3. RESULTS AND DISCUSSION

The occurrence and activities of *Pseudomonas fluorescens*, as phyllosphere inhibiting microorganism, have been reported in many field

crops including rice [15]. The cells of *Pseudomonas fluorescens*, inhabiting the phyllosphere, were often exposed to ever change environmental conditions and frequently subjected to various physical, chemical and environmental stresses prevalent therein [16] more over the cells of *Pseudomonas fluorescens* from the phyllosphere were reported to have reduced metabolic state or hypobiosis and thus having added advantages in terms of survival and virulence under stress condition when compared to active metabolic rhizosphere inhabiting cells of the organism [17].

Sheath blight disease of caused by *Rhizoctonia solani* is one of the most destructive phytopathogens of rice and has a yield ubiquitous occurrence in all the rice growing, countries and causing a yield loss upto 90 per cent [18]. Application of *Pseudomonas fluorescens* seed and soil level is known to control *Rhizoctonia solani* through induced systemic resistance (ISR) in rice plant [19]. Moreover, application of resistance including chemical *viz*., salicylic acid along with *Pseudomonas fluorescence* is reported to have augmentation effect on ISR of rice.

After scrutinizing all the above facts, the present investigation was designed in such a way to elucidate the improved functions of stress resistant, phyllosphere *Pseudomonas* cells, upon introduction at rhizosphere level in augmenting the rice – *Pseudomonas fluorescens* association, under lowland rice ecosystem, as regards to plant growth promotions and bio-control against *Rhizoctonia solani*. All the ten isolates exhibited to character of *Pseudomonas fluorescens* according to LOPAT test [20]. Occurrence of *Pseudomonas fluorescens* the phyllosphere and rhizosphere of rice has been reported by Rabindran and Vidhyasekaran [21]. In the present study, all ten isolates were identified characterized as *Pseudomonas fluorescens*

All the ten isolates of *Pseudomonas fluorescens* were screened for its antagonistic activity against *Rhizoctonia solani*. The results are presented in (**Table 1**). The results of the present study revealed the ability of all the ten *Pseudomonas fluorescens* isolates to produce antagonistic activity against *Rhizoctonia Solani* but a variation in their efficiency has been observed among the ten isolates. The phyllosphere isolate of *Pseudomonas fluorescens* namely, PFP-5 recorded a maximum response to biocontrol 11.2 mm as diameter of inhibition zone against *Rhizoctonia solani* followed by other isolates.

Pseudomonas fluorescens isolates, namely, PFP-5 and PFR-5 under different growth phases were tested for their efficiency in sheath blight disease management and the results are presented in (**Table 2**). Among the three different growth phases tested, it was observed that the log phase culture of both *Pseudomonas fluorescens* isolate PFP-5 and PFR-5 showed a reduction in sheath blight disease incidence (18.21 and 22.61% for PFP-5 and PFR-5, respectively) followed by lag and stationary phase cultures. The stationary phase cultures, with less metabolic activity, exhibited lesser degree of sheath blight disease incidence compared to log phases cultures. The isolate PFP-5 performed well in reducing the sheath blight disease incidence when compared to PFR-5.

The influence of *Pseudomonas fluorescens* isolates, namely, PFP-5 and PFP-5 application under different growth phase, namely, lag, log and stationary, was studied on the control of sheath blight disease incidence in IR – 50 rice. The result of the present study indicated the log phase cells of *Pseudomonas fluorescens* isolates effectively controlled the sheath blight disease incidence when compared to lag and stationary phase cells and also point to suggest the active metabolism at the microbes is essential for the induction of systemic in rice plants against *Rhizoctonia solani*. Meyer and Hofte [22] reported a similar observation with *Pseudomonas aeruginosa* against *Botryits cinerea* and in bean.

Systemic resistance induced against the pathogen, through exogenous application of the inducers *viz.*, salicylic acid, was studied and the results are presented in (**Table 3**). The results revealed the efficiency of salicylic acid in reducing the sheath blight incidence by 19.10 per cent and 28.122 per cent at 0.1 and 0.075 per cent concentrations, respectively, followed by Acibenzolar, NAA and Jasmonic acid. The studies on the effect of foliar application at certain resistance inducing chemical, namely, Acibenzolar, NAA, salicylic acid and jasmonic acid on the control of sheath blight disease incidence in IR-50 rice indicated the improved efficacy at salicylic acid to other chemical induced systemic resistance to Pathogen by SA-treatment at root or soil level has been reported in tobacco, radish and arubidiopsis [23, 24, 25]. In the present study also, Salicylic acid was found to decrease the sheath blight incidence in IR -50 rice and it might be clue to the SA augmented ISR of rice plant against *Rhizoctonia solani* as suggested by Meyer and Hofte [26].

Table 1: Screening the *Pseudomonas fluorescens* isolates for on biocontrol activity

Isolates	ZOI[a] of *Rhizoctonia solani*	Isolates	ZOI[a] of *Rhizoctonia solani*
PFR-1	8	PFP-1	7
PFR-2	6.7	PFP-2	9
PFR-3	8.2	PFP-3	8
PFR-4	5	PFP-4	6.8
PFR-5	9.1	PFP-5	11.2

a - Zone of Inhibition (mm in dm)

Table 2: Influence of *Pseudomonas fluorescens* growth phases on sheath blight disease Management of rice

Isolate	Growth phase* of *Pseudomonas*	% disease incidence**
Pseudomonas fluorescens PFP-5	Lag	30.12 ± 1.7
	Log	18.21 ± 0.6
	Stationary	28.69 ± 1.3
Pseudomonas fluorescens PFR-5	Lag	35.01 ± 1.9
	Log	22.61 ± 0.8
	Stationary	34.10 ± 1.5

** OD at 420 mm at 1 x 10^7 cfu ml^{-1} of inoculum level*

*** Values are mean of three replications ± SD*

Table 3: Effect of foliar application of certain resistance inducing chemicals on sheath blight incidence of rice

Chemicals	Dose (%)	Disease incidence (%)
Control	-	74.5 (59.67)
Acibenzolar	0.075	36.9 (37.41)
Acibenzolar	0.10	36.3 (37.05)
NAA	0.075	37.5 (37.76)
NAA	0.10	34.9 (36.21)
Salicylic Acid	0.075	28.12 (32.02)
Salicylic Acid	0.10	19.1 (25.91)
Jasmonic Acid	0.075	37.8 (37.94)
Jasmonic Acid	0.10	37.5 (37.76)
CD (p=0.05)	-	5.21

REFERENCES

1. Kloepper, J.W., S.Tuzun and J.A. Kuz.1992. Proposed definitions related to induced disease resistance. Biocontrol Sci.Techn.,2:349-351
2. Paracer.C.S and Chalal, D.S 1963. Sheath blight of rice caused by *Rhizoctonia solani* kuhn, a new record in India. Curr.Sci,32 :328-329
3. Kanniyan, S and Prasad, N. 1976. Quantitative sugar changes in barley infected with a faculative parasite. Phytopathol.,55 :341-345
4. Kanniyan, S and Prasad, N. 1978. Effect of potassium on the incidence of sheath blight of rice. Indian potash J., 3:23.
5. Ou, S.H. 1985. Rice Disease. Commonwealth Mycological Institute, Kew. Surrey, England, U.K.380pp.
6. Hirano, S.S and C.D Upper. 2000. Bacteria in the Leaf Echosystem with Emphasis on *Pseudomonas* species a Pathogen, ice nucleus and epiphyte. Microbiol. Mol. Rev., 64: 624-653.
7. Van Outryve, M.F., F. Cossle and J.Swings. 1989. The bacterial microflora of wit loof chicory *(Cicborium intybus L.Foliosum aegi)* leaves. Microb. Ecol.,18: 175-186.
8. Knudsen, G.R., and G.W. Hudler. 1987. Use of a Computer simulation model to evaluate a plant disease biocontrol agent. Ecol. Modell., 35:45-62
9. Chen.F.F., K.Lee and W.S.Klu. 2000. Biological and chemical control of *Rhizoctonia solani* by pea seed treatment, memoirs of the college of Agriculture, National Taiwan University. 21:19-28
10. Hoffland,E., J.Hakulinem and J.A Vanpelt. 1996. Comparison of Systemic resistance induced by avirulent and non-pathogenic *Pseudomonas* species, Phytopathology, 86: 757-762.
11. Zehnder, G.N ., J.F.Marphy, E.J. Sikora and J.W. Kloepper. 2001. Application of rhizobacteria for induced resistance. European J.Plant Pathol., 107: 39-50.
12. Mew, T.W and A.M Rosales. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. Phytopathol., 76: 1260-1264.
13. Dillep Kumar, B.S and H.C.Dube. 1991. Plant growth promoting activity of a fluorescent *Pseudomonas* from tomato rhizosphere. Indian J. Exp. Biol., 29: 366-370.
14. Kessmen,H., T.Staub, C.Hofmann, T.Maetzice, J. Herzog, E.Ward, S.Ukness and Ryals. 1994. Induction of systemic acquired disease resistance in plants by chemicals. Annu. Rev. Phytopathol., 32: 439-459.
15. Mew, T.W and A.M Rosales. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. Phytopathol.,76: 1260-1264.
16. Lindow, S.E. and M.T. Brandl. 2003. Microbiology of the phyllosphere. Appl. Environ. Microbiol., 69 :1875-1883.
17. Sen. S.P. 1982. Associative symbiosis: Potentialities and problems (Ed.S.K. Roy) In: Frontiers of Research in Agriculture. Golden Jubilee of Symposium, Indian Statistical Institute, Calcutta, p.204.
18. Mercier,J. and S.E. Lindow. 2000. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. Appl. Environ. Microbiol., 66: 369 - 374.

19. Meyer, G.D and M. Hofte. 1997. Salicylic acid produced by rhizobacterium *Pseudomonas aeruginosa* FNSK induced resistance leaf infection by *Botrytis cinerea* on bean. Phytopathol., 87: 588-593.
20. Lee.F.N and Rush M.C. 1983. Rice sheath blight: A major rice disease. Indian phytopath., 67: 827-832.
21. Rabindran, R and P. Vidhyasekaran. 1996. Development of a formulation of *Pseudomonas fluorescens* PFAL 2 for management of rice sheath blight. Crop protection. 15: 715-721.
22. Meyer, G.D and M. Hofte. 1997. Salicylic acid produced by rhizobacterium *Pseudomonas aeruginosa* FNSK induced resistance leaf infection by *Botrytis cinerea* on bean. Phytopathol., 87: 588-593.
23. Enyedi., A.J., N. Yalpani, P.Silverman and I.Raskin 1992. Localization, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction of mosaic virus Proc. Natl. Acad. Sci. U.S.A., 89: 2480-2484
24. Leeman, ., F.M Damouden, J.A. Vanpelt , F.P.M. Dirkx, H.Steijl, P.A.H.M. Bakker and B.Schippers. 1996. Iron availability affects induction of systemic resistance of *Fusarium* wilt of radish by *Pseudomonas fluorescens*. Phytopathol., 86: 149-155.
25. Van Loon, L.C., P.A.H.M. Baker and C.M.J. Pieterse, 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol., 36: 435-483.
26. Meyer, G.D and M. Hofte. 1997. Salicylic acid produced by rhizobacterium *Pseudomonas aeruginosa* FNSK induced resistance leaf infection by *Botrytis cinerea* on bean. Phytopathol., 87 588-593.