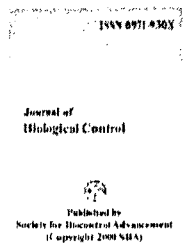
Journal of Biological Control, 25 (2): 156–159, 2011



Research Note

Consortium of fluorescent pseudomonads for the management of rice sheath blight disease

M. SURENDRAN[1]*, G. S. KANNAN[2], KAMALA NAYAR[3] and S. LEENAKUMARY[1]

[1]*Rice Research Station, Moncompu 688 503, Thekkekara P.O., Alleppey District, Kerala, India.*

[2]*Department of Plant Protection, Faculty of Agriculture and Animal Husbandry, Gandhigram Rural University, Gandhigram 624 302, Dindigul, Tamil Nadu, India.*

[3]*Instructional Farm, College of Agriculture, Vellayani 695 522, Kerala, India.*

**Corresponding author E-mail*: surenpath@yahoo.co.in

ABSTRACT: Pure cultures of bacterial antagonist, *Pseudomonas fluorescens* were isolated from different locations in Kuttanad for screening against rice sheath blight disease. Three effective strains, *viz.*, PF43, PF46 and PF47 were tested individually and also in combination against sheath blight under field conditions. Combined application of PF43, PF46 and PF47 was found to be effective for sheath blight disease management during *rabi* 2009-10, *kharif* 2010 and *rabi* 2010-11.

KEY WORDS: Rice, sheath blight, fluorescent pseudomonads, biocontrol agent

(Article chronicle: Received: 21.03.2011; Sent for revision: 06.04.2011; Accepted: 05.05.2011)

Sheath blight of rice (*Oryza sativa* L.) caused by *Rhizoctonia solani* (Kuhn) is a serious disease in Kuttanad, the rice bowl of Kerala. Kuttanad lies about 0-3 m below MSL and enjoys a warm humid climate with fairly uniform temperature throughout the year ranging from 21°C to 36°C. Humidity in general is very high all through the year, which is highly favourable for sheath blight occurrence. The disease can cause yield loss of 5.2 – 50 per cent depending on environmental conditions and crop stages at which the disease appears (Rajan 1987; Sharma and Teng, 1996). Overuse of nitrogen fertilizers (Roy, 1978), closer planting (Kannaiyan and Prasad, 1983) and high relative humidity (Dath, 1990) favour the disease development. The disease can effectively be controlled by use of systemic fungicides, but these cause environmental pollution and human health hazards. In this context, fluorescent pseudomonads can be considered as an ecofriendly strategy for managing *R. solani* infection on rice. Many fluorescent *Pseudomonas* spp. have been reported to induce systemic resistance (Pieterse *et al.*, 1996), and many workers have used antagonistic bacteria against sheath blight disease (Mew and Rosales, 1986; Gnanmanickam *et al.*, 1992; Krishnamoorthy and Gnanamanickam, 1997). The present study was undertaken to test the efficacy of different fluorescent pseudomonads either alone or in combination to manage sheath blight disease in Kuttanad.

Rhizoctonia solani was isolated from rice leaf sheath (cultivar MO 16) showing typical sheath blight symptoms. The pure culture of the pathogen was obtained by the single hyphal tip method (Rangaswami, 1972) and maintained on potato dextrose agar slants at 4°C. Antagonistic bacteria comprising fluorescent pseudomonads were isolated from rhizosphere soil of healthy rice plants from different locations in Kuttanad. The isolated colonies were purified and observed under UV light for fluorescence confirmation. Antagonistic potential of the native *Pseudomonas* isolates to *R. solani* was detected by dual culture technique (Dennis and Webster, 1971) on King's B agar plates with three replications for each isolate using appropriate control. A mycelial disc of about 5mm diameter was placed at one end of a sterilized Petri plate and *Pseudomonas* isolate was streaked opposite to it at a distance of 7 cm. In the case of mixed isolates, streaks were made one over the other at the same time. Observations were recorded following six days of incubation at 25°C by measuring the diameter of mycelial growth of the fungal pathogen. The percentage of inhibition of mycelial growth over control was calculated

by using the formula C-T/C x100 where C – mycelial growth on Control; T – Mycelial growth on treatment. Talc based formulations were prepared for PF43, PF46 and PF47 and PF43+PF46+PF47 mixed strains following the method described by Nandakumar *et al.* (2001) and used for field experiments.

Field experiments were conducted consecutively for three seasons during *rabi* 2009-10, *kharif* 2010 and *rabi* 2010-11 at Rice Research Station, Moncompu, using individual and mixed strains of PF43, PF46 and PF47. Each treatment included seed treatment (10g kg^{-1} of seed), soil application (1kg $acre^{-1}$ at 35 DAS) and foliar application (2% at 55 DAS) of the particular strains. P1 culture received from College of Agriculture, Vellayani and systemic fungicide hexaconazole (0.2%) were used as standard check. The experiment was laid out with ten treatments replicated four times in a randomized complete block design (RBD) using MO 16 (Uma) as the test variety. Pre-germinated seeds were used for direct sowing in plots of 5 x 4 m^2. Fertilizers were applied @ 90: 45: 45 NPK kg ha^{-1}. The pathogen was multiplied on autoclaved paddy straw and artificially applied at the base of the crop at tillering stage. Observations on sheath blight incidence and severity were recorded 25 days after foliar application. Percentage of disease incidence was calculated on 25 plants per sampling unit, by counting the number of infected tillers. Degree of severity was graded (0-9 scale) based on height of the plant portions affected by the disease as per IRRI (1996). Grain yield of each plot was recorded and converted in kg ha^{-1} for analysis. Data on percentages were transformed to arcsine and analysis of variance was performed with transformed values. Significance among mean treatments was determined according to Duncan's multiple range test (Gomez and Gomez, 1984).

In vitro studies showed that all the tested isolates recorded significant ($P<0.05$) inhibition in mycelial growth of *R. solani* in dual culture studies (Table 1). The highest reduction (58.49%) was achieved by PF43+PF46+PF47 followed by PF43+PF46 and P 1 on King's B agar plates. Mycelial growth reduction recorded in case of PF43, PF46, PF47, PF43+PF47 and PF46+PF47 were statistically on par with each other. Antagonistic effect of *P. fluorescens* against *R. solani* have been reported earlier by Rabindran *et al.* (1996). Growth inhibition caused by antagonists in the present study might be due to production of antibiotics by *P. fluorescens* as reported earlier by O' Sullivan and O'Gara (1992).

In the field trials, treatment PF43+PF46+PF47 (T_7) gave the maximum reduction in disease incidence (6.17%)

Table. 1. Efficacy of *Pseudomonas* cultures on mycelial growth of *Rhizoctonia solani* under *in vitro* condition

Treatments	Mycelial growth (cm)*	(%) reduction in (mycelia growth
PF43	4.70	46.79
PF46	4.73	46.72
PF47	4.97	43.77
PF43+PF46	4.40	50.19
PF43+PF47	4.63	47.55
PF46+PF47	5.43	38.49
PF43+PF46+PF47	3.67	5849
P 1 (Std)	4.60	47.92
Control	8.83	00.00
CD ($P < 0.05$)	0.89	

*After six days of inoculation; data are means of three replications

followed by hexaconazole (8.21%), PF43+PF46 (8.28%), P1 (10.54%) and PF43+PF47 (11.17%). With regard to sheath blight severity, PF43+PF46+PF47 reduced the disease effectively (0.59) when compared with PF43+PF47 (1.11), PF43+PF46 (1.18) and hexaconazole (1.64) and P1 (1.86). Highest yield (5531 kg ha^{-1}) was recorded by the treatment T_7 followed by standard check isolate P1 (5269 kg ha^{-1}), hexaconazole (5210 kg ha^{-1}) and PF43+PF46 (5019 kg ha^{-1}). Even though the standard isolate P1 showed the highest yield during *kharif* 2010, the pooled data of three seasons revealed that the treatment involving the consortium of three isolates (PF43+PF46+PF47) was significantly superior to all other treatments involving single isolates. Fukui *et al.* (1994) reported that a single biocontrol strain may not grow equally well in a variety of environmental conditions. In the current study, the combination treatment PF43+PF46+PF47 showed maximum reduction in sheath blight disease incidence followed by P1, hexaconazole, PF43+PF46 and PF43+PF47 as compared with the application of single isolate (Table 2). The mixed cultures of PF43+PF46 and PF43+PF47 also performed better during all the seasons compared to the individual isolates and they were on par with standard check P1 and hexaconazole in restricting the sheath blight incidence and severity. Van Loon (1998) has emphasized the use of combination of different treatments of biocontrol agents to give better disease suppression. Our results also support the earlier observations that a combination of biocontrol agents with different mechanisms of disease control will have an additive effect and result in enhanced disease

Table 2. Effect of *Pseudomonas fluorescens* cultures on sheath blight disease incidence, severity and grain yield during *rabi* 2009-10, *kharif* 2010 and Rabi 2010-11 at Rice Research Station, Moncompu (Pooled data for 3 seasons)

Treatments	Mean disease incidence (%)				Mean disease severity (0-9 scale)				Grain yield (kg ha^{-1})			
	Rabi 2009-10	*Kharif* 2010	*Rabi* 2010-11	Mean	*Rabi* 2009-10	*Kharif* 2010	*Rabi* 2010-11	Mean	*Rabi* 2009-10	*Kharif* 2010	*Rabi* 2010-11	Mean
PF43	9.43	24.16	12.99	15.53)	1.8	3.05	1.45	2.1	5420	3545	5016	4660
PF46	(17.85)	(29.47)	(21.33)	(23.18)								
PF47	12.10	24.08	29.37	21.85	1.83	3.65	1.75	2.41	5650	3613	4455	4573
PF43+PF46	(20.36)	(29.40)	(32.83)	(27.90)								
PF43+PF47	16.35	34.17	33.97	28.16	2.43	3.98	1.96	2.79	5590	3225	4463	4426
PF43+PF47	(23.89)	(35.79)	(35.67)	(32.08)								
PF43+PF46+	10.60	6.62	7.63	8.28	1.75	1.03	0.75	1.18	5830	3485	5741	5019
PF47	(19.00)	(14.89)	(16.00)	(16.74)								
P 1 (Std)	13.18	7.18	13.16	11.17	1.32	0.88	1.13	1.11	5570	3870	5418	4953
Hexaconazole	(21.30)	(15.56)	(21.30)	(19.55)								
Control	14.43	20.68	10.32	15.14	2.83	2.98	1.65	2.49	5610	3893	5203	4902
	(22.30)	(27.06)	(18.72)	(22.87)								
	5.01	3.17	10.33	6.17	0.76	0.5	0.5	0.59	6340	3825	6429	5531
	(12.92)	(10.30)	(18.72)	(14.42)								
	9.65	10.51	11.46	10.54	2.03	1.53	2.03	1.86	6300	4240	5268	5269
	(18.15)	(18.91)	(19.82)	(18.91)								
	6.68	7.67	10.28	8.21	1.38	0.95	2.58	1.64	6128	4128	5375	5210
	(15.00)	(16.11)	(18.72)	(16.64)								
	45.70	58.06	55.53	53.10	4.35	7.03	4	5.13	4170	2668	3655	3498
	(42.53)	(49.66)	(48.13)	(46.78)								
CD (0.05)	9.12				1.38				555.25			

Mean of four replications; figures in parentheses are angular transformed values

control compared to their individual application (Guetsky *et al.*, 2002). Nandakumar *et al.* (2001) reported that the mixtures of PGPR strains gave better suppression of sheath blight in rice than when they are applied singly.

The present study also proves the efficacy of three isolates (PF43+PF46+PF47) in reducing the sheath blight disease of rice and thereby increasing the yield. Therefore combined application of the above strains of *P. fluorescens* can be recommended for achieving an ecofriendly management of the disease in Kuttanad, Kerala.

REFERENCES

Dath, A. J. 1990. *Sheath blight disease of rice and its management.* Associate Publishing Company, New Delhi, 152 pp.

Dennis, C. and Webster, J. 1971. Antagonistic properties of species groups of *Trichoderma* I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, **57**: 25–39.

Fukui, R., Schroth, M. N., Hendson, M. and Hancock, J. G. 1994. Interaction between strains of pseudomonads in sugar beet sphermospheres and the relationship to pericarp colonization by *Pythium ultimum* in soil. *Phytopathology*, **84**: 1322–1330.

Gnanamanickam, S. S., Candole, B. L. and Mew, T. W. 1992. Influence of soil factors and cultural practices on biological control of sheath blight of rice with antagonistic bacteria. *Plant and Soil*, **144**: 67–75.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical procedures for agricultural research.* John Wiley & Sons, New York, USA.

Guetsky, R., Stienberg, D., Elad, Y., Fischer, E. and Dinoor, A. 2002. Improving biological control by combining bio-control agents each with several mechanisms of disease suppression. *Phytopathology*, **92**: 976–985.

IRRI. 1996. *Standard evaluation systems for rice.* International Rice Research Institute, Manila, The Philippines.

Kannaiyan, S. and Prasad, N. N. 1983. Effect of spacing on the spread of sheath blight disease of rice. *Madras Agricultural Journal*, **70**: 135–136.

Krishnamurthy, K. and Gnanamanickam, S. S. 1997. Biological control of sheath blight of rice: Induction of systemic resistance in rice by plant-associated *Pseudomonas* spp. *Current Science,* **72**: 331–334.

Mew, T. W. and Rosales, A. M. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. *Phytopathology,* **76**: 1260–1264.

Nandakumar, R., Babu, S., Viswanathan, R., Sheela, J., Raghuchander, T. and Samiyappan, R. 2001. A new bio-formulation containing plant growth promoting rhizo-bacterial mixture for the management of sheath blight disease and enhanced grain yield in rice. *BioControl,* **46**: 493–510.

Pieterse, C. M. J., van Wees, S. C. M., Hoffland, E., van Pelt, J. A. and van Loon, L. C. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell,* **8**: 1225–1237.

O' Sullivan, D. J. and O' Gara, F. 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiological Review*, **56**: 662–676.

Rangaswami, G. 1972. *Diseases of crop plants in India.* Prentice Hall of India Pvt. Ltd., New Delhi, India, 520 pp.

Roy, A. K. 1978. Horizontal spread of sheath blight to rice plants in relation to spacing and nitrogen application. *Current Science,* **47**: 307–308.

Rabindran, R. and Vidhyasekaran, P. 1996. Development of a formulation of *Pseudomonas fluorescens* PfALR2 for management of rice sheath blight. *Crop Protection,* **15**: 715–721.

Rajan, C. P. D. 1987. Estimation of yield loss due to sheath blight disease of rice. *Indian Phytopathology,* **40**: 174–177.

Sharma, N. R. and Teng, P. S. 1996. Rice sheath blight: effect of crop growth stage on disease development and yield. *Bangladesh Journal of Plant Pathology,* **12**: 43–46.

Van Loon, L. C., Bakker, P. A. H. M. and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology,* **36**: 453–83.