

Effect of Salicylic Acid and *Pseudomonas fluorescens* on Growth and Yield of Paddy Ir-50

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Abstract: Rice (*Oryza sativa* L.) is the most important staple food for over two billion people in Asia and for hundreds of million 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. However, biocontrol of sheath blight disease management has, so far, proved to be inefficient in bringing down the disease incidence below economic threshold level (ETL). Hence, the application of systemic resistance inducing chemicals along with biocontrol agents would be the suitable alternative strategy to improve the sheath blight disease management in rice. In this present study, the effect of salicylic acid and *Pseudomonas fluorescens* on growth and yield of Paddy IR-50 was investigated. Among the various treatments tested, maximum growth and yield was observed in the treatment T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) and the least parameters were recorded in the Control treatment (T₁).

Key words: Salicylic Acid • *Pseudomonas fluorescens* • Sheath Blight And Paddy

INTRODUCTION

Rice production management strategies mainly focus on the use of synthetic chemical fertilizers and pesticides to enhance per hectare yield of the crop. 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 [1]. Kannaiyan and Prasad [2] reported the intensity and severity of sheath blight disease in Tamil Nadu. The disease was mainly confined to lower sheath and was known to be soil borne. Kannaiyan and Prasad [3] have reported for the first time the seed borne nature of the disease. Ou [4] reported that when the disease reaches the flag leaves, loss was estimated to be 25 per cent.

Sheath blight of paddy occurs throughout the tropical and subtropical countries and is more prominent where paddy is grown under intensive cultivation system. Miyake [5] first reported this disease in Japan and named the causal organism as *Sclerotium irregularare*.

Later, the fungus was similar *Kyponussesakii*, which was described by Shirai [6]. Reinking [7] and Palo [8] from Philippines reported the disease and named the organism as *Rhizoctoniasolani*.

The symptom of the disease are the lesions were first greenish grey, ellipsoidal, 10 mm long and 3 to 4 mm broad, gradually enlarge and become greenish white with a blackish brown margin. Butler [9] and Ryker and Exner [10] reported that under wet conditions, the fungus grows rapidly on entire plant and transverse, irregular, discoloured bands were formed on the leaf blades. On account of this symptom, the disease was also described as "Banded sclerotial disease".

Salicylic acid was also known as a resistance inducing substance in various plants against virus and fungal pathogens [11]. Several chemicals are known to induce resistance in plant against pathogens. These include poly acrylic, acetyl salicylic acid [12], 2-4 dichloroisonicotinic acid; methyl salicylate [13], jasmonic acid and jasmonic methyl ester [14], benzimidazole derivatives, DL-B amino-n-butyric acid

(BABA) and oxalic acid [15]. Salicylic acid is known to induce resistance in rice [16], tomato and sunflower [17]. Acibenzolar is also known to induce resistance in wheat, cucumber [18] and rice [19].

MATERIALS AND METHODS

Details of the Pot Culture Experiment: Rectangular cement pots of size 18"x 12"x 12" were filled with 45 kg of paddy field soil flooded with water for 2 days and brought to fine puddle condition. 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. The cement pots meant for transplanting purpose were prepared as mentioned earlier and applied with the recommended basal dose of fertilizers (15:15:75 as NPK/ac) and treatments as given below:

- T₁-Control (uninoculated)
- T₂-PF -S (*Pseudomonas fluorescens* seed application)
- T₃-SA-1 (Salicylic acid applied on 15th day)
- T₄-SA-2 (Salicylic acid applied on 30th day)
- T₅-PF-S + SA-1
- T₆-PF-S + SA-2

Challenge Inoculation of Rice Plants with *Rhizoctonia solani*: The culture which was seed like in potato dextrose agar (PDA) medium was used for the inoculation purpose. Thick mustard sclerotic was harvested from the media with sterile distilled water from *Rhizoctonia solani* day old culture of PDA medium and strained through double layer muslin cloth so as to get a free suspension of conidia.

The spore, polyethylene sheets to preserve moisture and increase the leaf cells more prone to the pathogen. Then, sheets were removed and spraying of spore suspension was done late in the evening. Control plants were also sprayed with sterile distilled water. After spraying, again the plants were covered with polyethylene bags for about 72 hrs to maintain the humidity.

Biometric Observation of Growth and Yield Parameters in Paddy IR-50

Effect of Plant Height: The height of the plants in each treatment was measured at 30th day after transplantation (DAT). The mean value of the plants from 3 replications was recorded.

Total Nitrogen: The plants including root portions were carefully pulled out air dried separately for several days and dried to a constant weight in oven at 60°C, they were powdered sieved and preserved in vials. Their total nitrogen content was determined by Microkjeldhal method [20].

Number of Tillers Clump⁻¹: Number of tillers per clump was recorded at maximum tillering stage by taking average of ten clumps randomly chosen.

Leaf Area Index: Leaf area index at flowering was calculated without removing the leaves by using the formula given by Yoshida *et al.* [21].

Dry Matter Production: Plant samples were taken at three stages of crop growth *viz.*, maximum tillering, flowering and harvest. The plant samples were dried in hot air oven at 80°C for 48 to 72 hours. The oven dry weight was recorded and Dry matter production was calculated.

Panicles Number Hill⁻¹: At harvest number of panicles per hill was recorded by taking average of ten clumps randomly chosen.

Number of Filled Grains Panicle⁻¹: The panicles randomly chosen for recording and the panicle length were used for counting the number of filled grains panicle⁻¹. The differentiation of well-filled and chaffy grains, was made by pressing the grains with fingers and they were counted separately and recorded.

Thousand Grain Weight: The thousand grain weight was taken at 14 per cent moisture level for all the samples and recorded in grams.

Grain and Straw Yield: The matured crop was harvested, hand threshed, winnowed and sun dried. The dried grains from each treatment were weighed and recorded. After threshing, the rice straw was subjected to sun dry and the weight was recorded.

Statistical Analysis: The experimental results were statistically analysed in randomized block design (RBD) as per the procedure described by Gomez [22].

RESULTS AND DISCUSSION

The combined effect of *Pseudomonas fluorescence* and salicylic acid on plant height of paddy var IR-50 was investigated and the results were furnished in Table-1. The plant height of paddy was recorded at 30, 60 and 90 DAT. All the treatments recorded proportionately increased in plant height compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (129.05 cm) showed maximum plant height followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (126.19 cm). Least plant height was recorded in the control pot (97.18 cm).

The combined effect of *Pseudomonas fluorescence* and salicylic acid on total nitrogen content in paddy var IR-50 was studied and the results were presented in Table-2. The total nitrogen content in paddy was recorded at 30, 60 and 90 DAT. All the treatments recorded proportionately increased in total nitrogen content compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (290.42 mg plant⁻¹) showed maximum total nitrogen content followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic

acid applied on 15th day) (285.65 mg plant⁻¹). Least total nitrogen content was recorded in the control pot T₁ (245.13 mg plant⁻¹).

The combined effect of *Pseudomonas fluorescence* and salicylic acid on number of tillers plant⁻¹ in paddy var IR-50 was tested and the results were given in Table-3. The number of tillers plant⁻¹ in paddy was recorded at 30, 60 and 90 DAT. All the treatments recorded proportionately increased in number of tillers plant⁻¹ compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (20.35 tillers plant⁻¹) showed highest number of tillers plant⁻¹ followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (19.56 tillers plant⁻¹). Lowest number of tillers plant⁻¹ was recorded in the control pot T₁ (10.41 tillers plant⁻¹).

The combined effect of *Pseudomonas fluorescence* and salicylic acid on number of leaf area index in paddy var IR-50 was investigated and the results were furnished in Table-4. The leaf area index in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in leaf area index compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (6.75 cm) showed highest leaf area index followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (6.38 cm). Minimum leaf area index was recorded in the control pot T₁ (4.68 cm).

Table 1: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on plant height of paddy

Treatments	Plant Height (cm)					
	30 DAT	% over control	60 DAT	% over control	90 DAT	% over control
T ₁ -Control	52.67	-	65.85	-	97.18	-
T ₂ -PF-S	78.36	48.77	92.98	41.19	123.43	27.01
T ₃ -SA-1	68.56	30.16	81.66	24.0	112.20	15.45
T ₄ -SA-2	71.45	35.65	86.92	31.99	117.35	20.75
T ₅ -PF-S + SA-1	81.27	54.30	95.39	44.85	126.19	29.85
T ₆ -PF-S + SA-2	85.82	62.93	99.56	51.19	129.05	32.79
SE _D	4.81	-	4.96	-	4.77	-
CD (P = 0.05)	9.62	-	9.92	-	9.54	-

Table 2: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on total nitrogen content in paddy

Treatments	Total Nitrogen (mg plant ⁻¹)					
	30 DAT	% over control	60 DAT	% over control	90 DAT	% over control
T ₁ -Control	37.16	-	105.13	-	245.13	-
T ₂ -PF-S	47.15	26.88	113.75	08.19	275.00	12.18
T ₃ -SA-1	44.00	18.40	122.15	16.18	256.77	04.74
T ₄ -SA-2	40.15	08.04	109.66	04.30	269.06	09.76
T ₅ -PF-S + SA-1	48.30	29.97	132.65	26.17	285.65	16.53
T ₆ -PF-S + SA-2	49.84	34.12	136.94	30.25	290.42	18.47
SE _D	2.02	-	5.22	-	7.02	-
CD (P = 0.05)	4.05	-	10.45	-	14.05	-

Table 3: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on Number of tillers per plant in paddy

Treatments	Number of tillers plant ⁻¹					
	30 DAT	% over control	60 DAT	% over control	90 DAT	% over control
T ₁ -Control	05.60	-	07.92	-	10.41	-
T ₂ -PF-S	11.15	99.10	13.57	71.33	17.85	71.46
T ₃ -SA-1	06.60	17.85	8.56	08.08	13.14	26.22
T ₄ -SA-2	09.64	72.14	12.90	62.87	16.16	55.23
T ₅ -PF-S + SA-1	12.60	125.00	16.45	107.70	19.56	87.89
T ₆ -PF-S + SA-2	13.34	138.21	18.74	136.61	20.35	95.48
SE _D	1.29	-	1.73	-	1.57	-
CD (P = 0.05)	2.61	-	3.46	-	3.16	-

Table 4: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on Leaf area index in paddy

Treatments	Leaf area index (cm)	
	90 DAT	% over control
T ₁ -Control	4.68	-
T ₂ -PF-S	5.98	27.77
T ₃ -SA-1	5.36	14.52
T ₄ -SA-2	5.75	22.86
T ₅ -PF-S + SA-1	6.38	36.32
T ₆ -PF-S + SA-2	6.75	44.23
SE _D	0.30	-
CD (P = 0.05)	0.67	-

Table 5: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on dry matter production in paddy

Treatments	Dry matter production (g plant ⁻¹)					
	30 DAT	% over control	60 DAT	% over control	90 DAT	% over control
T ₁ -Control	1.80	-	3.55	-	4.91	-
T ₂ -PF-S	2.17	20.55	5.69	60.28	7.07	43.99
T ₃ -SA-1	2.14	18.88	4.31	21.40	6.00	22.19
T ₄ -SA-2	2.11	17.22	4.79	34.92	6.80	38.49
T ₅ -PF-S + SA-1	2.25	25.00	5.98	68.45	7.20	46.63
T ₆ -PF-S + SA-2	2.38	32.22	6.57	85.07	8.95	82.28
SE _D	0.07	-	0.46	-	0.54	-
CD (P = 0.05)	0.18	-	0.95	-	1.09	-

The combined effect of *Pseudomonas fluorescens* and salicylic acid on dry matter production in paddy var IR-50 was estimated and the results were given in Table- 5. The dry matter production in paddy was recorded at 30, 60 and 90 DAT. All the treatments recorded proportionately increased in leaf area index compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (8.95 gram plant⁻¹) showed highest dry matter production followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (7.20 gram plant⁻¹).¹ Minimum dry matter production was recorded in the control pot T₁ (4.91 gram plant⁻¹).

The combined effect of *Pseudomonas fluorescens* and salicylic acid on number of panicles per hill in paddy var IR-50 was determined and the results were presented in Table-6. The number of panicles per hill in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in number of panicles per hill compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (6.38 panicles hill⁻¹) showed highest number of panicles per hill followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (5.97 panicles hill⁻¹). Minimum number of panicles per hill was recorded in the control pot T₁ (3.97 panicles hill⁻¹).

Table 6: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on number of panicles per hill in paddy

Treatments	Number of panicles hill ⁻¹	
	90 DAT	% over control
T ₁ -Control	3.97	-
T ₂ -PF-S	5.47	37.78
T ₃ -SA-1	4.71	18.63
T ₄ -SA-2	5.28	32.99
T ₅ -PF-S + SA-1	5.97	50.37
T ₆ -PF-S + SA-2	6.38	60.70
SE _D	0.35	-
CD (P = 0.05)	0.73	-

Table 7: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on number of filled grains per panicle in paddy

Treatments	Number of filled grains panicles ⁻¹	
	90 DAT	% over control
T ₁ -Control	58.08	-
T ₂ -PF-S	68.85	18.54
T ₃ -SA-1	64.21	10.55
T ₄ -SA-2	67.50	16.21
T ₅ -PF-S + SA-1	72.37	24.60
T ₆ -PF-S + SA-2	74.10	27.58
SE _D	2.37	-
CD (P = 0.05)	4.76	-

Table 8: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on thousand grain weight in paddy

Treatments	Thousand grain weight (g)	
	90 DAT	% over control
T ₁ -Control	20.12	-
T ₂ -PF-S	20.65	2.63
T ₃ -SA-1	20.48	1.78
T ₄ -SA-2	20.58	2.28
T ₅ -PF-S + SA-1	20.71	2.93
T ₆ -PF-S + SA-2	20.78	3.28
SE _D	0.09	-
CD (P = 0.05)	0.21	-

Table 9: Effect of combined application of *Pseudomonas fluorescens* and salicylic acid on grain yield and Straw yield in paddy

Treatments	Grain yield (g pot ⁻¹)		Straw yield (g pot ⁻¹)	
		% over control		% over control
T ₁ -Control	24.28	-	36.20	-
T ₂ -PF-S	41.72	71.82	59.01	63.01
T ₃ -SA-1	38.00	56.50	57.90	59.94
T ₄ -SA-2	39.78	63.83	58.67	62.07
T ₅ -PF-S + SA-1	42.13	73.51	59.92	65.52
T ₆ -PF-S + SA-2	46.91	93.20	63.18	74.53
SE _D	3.15	-	3.99	-
CD (P = 0.05)	6.32	-	8.00	-

The combined effect of *Pseudomonas fluorescens* and salicylic acid on number of filled grains per panicle in paddy var IR-50 was studied and the results were furnished in Table-7. The number of filled grains per panicle in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in number of filled grains per panicle compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (74.10 filled grains panicles⁻¹) showed highest number of filled grains per panicle followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (72.37 filled grains panicles⁻¹). Minimum number of filled grains per panicle was recorded in the control pot T₁ (58.08 filled grains panicles⁻¹).

The combined effect of *Pseudomonas fluorescens* and salicylic acid on thousand grain weight in paddy var IR-50 was tested and the results given in Table-8. The thousand grain weight in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in thousand grain weight compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (20.78 g) showed highest thousand grain weight followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (20.71 g). Minimum thousand grain weight was recorded in the control pot T₁ (20.12 g).

The combined effect of *Pseudomonas fluorescens* and salicylic acid on grain yield in paddy var IR-50 was tested and the results given in Table-9. The grain yield in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in grain yield compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (46.91 g pot⁻¹) showed highest grain yield followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 15th day) (42.13 g pot⁻¹). Minimum grain yield was recorded in the control pot T₁ (24.28 g pot⁻¹).

The combined effect of *Pseudomonas fluorescens* and salicylic acid on straw yield in paddy var IR-50 was tested and the results presented in Table-9. The straw yield in paddy was recorded at 90 DAT. All the treatments recorded proportionately increased in straw yield compared to control. Among the six treatments, T₆ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on 30th day) (63.18 g pot⁻¹) showed highest straw yield followed by the treatment T₅ (*Pseudomonas fluorescens* seed application + Salicylic acid applied on

15th day) (59.92 g pot⁻¹). Minimum straw yield was recorded in the control pot T₁ (36.20 g pot⁻¹). All the results of the present study was in line with the findings of Saranraj *et al.* [23] and Sivasakthi *et al.* [24].

CONCLUSION

From this present study, it was concluded that the Plant growth promoting rhizobacterial isolates *Pseudomonas fluorescens* have the capacity to produce plant growth promoting substances. The treatment containing *Pseudomonas fluorescens* and salicylic acid showed maximum plant height, root and shoot dry weight, chlorophyll content, nitrogen content, grain yield and straw yield. Further work on the enhancement of this strain's antagonistic activity and characterization of the mechanism of action is currently underway.

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