



## Growth promotion of rice seedlings by fungal and bacterial bioagents effective against bacterial leaf blight of rice

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**Abstract:** In the present investigation, fungal and bacterial bioagents which were effective against pathogen of bacterial leaf blight disease of rice (*Xanthomonas oryzae* pv. *oryzae*) was tested for their effect on germination of rice seeds and growth of nursery seedlings under laboratory and glasshouse conditions. Two isolates of fluorescent pseudomonas isolated from rice phylloplane (Pf 83 and FLP 88), *Trichoderma* species were isolated from soil of rice field (isolate 40) and *Trichoderma harzianum* isolated from rice phylloplane. Two commercial formulations (PBA-1 and PBA-2) were tested in the present investigation and compared with chemical treatment (0.76 g Emison + 0.18 g Streptocycline / kg seeds) and untreated check. Under laboratory conditions, chemical treatment was found most effective in increasing seed germination (43.90%) followed by Pf 83 and PBA-1 (34.15 %). Bioagent formulations were significantly effective in increase in root and shoot length as compared to check and chemical treatment. Under glasshouse conditions, maximum increase (29.42%) in seedlings emergence was exhibited by chemical treatment. Maximum increase in root length (100.15%) and shoot length (50.06%) was recorded with FLP 88 and *T. harzianum*, respectively. Bioagent formulations also increased fresh and dry root weight over check and chemical treatment. All bioagents formulations were effective over chemical treatment and check in increasing fresh shoot weight. Results of the present study revealed that in the absence of pathogen of bacterial leaf blight of rice, fungal and bacterial bioagents significantly enhanced germination of rice seeds and growth of nursery seedlings.

**Keywords:** Bacterial leaf blight of rice, Growth promotion, *Pseudomonas* spp., Rice, *Trichoderma harzianum*

### INTRODUCTION

Fungal and bacterial bioagents are reported to induce the growth of various crops. These responses may be due to suppression of deleterious root micro-flora including those not causing obvious disease, production of growth stimulating factors (hormones or growth factors) and /or increased nutrient uptake through solubilization and sequestration of nutrients and/or enhanced root growth (Harman, 2000; Mishra and Sinha, 2000; Bailey and Lumsden, 1998). Enhanced root development is also helpful in tolerating the biotic and abiotic stresses by the plants. Spore/cell suspension as well as dry powder has been used to coat the seeds with potential antagonists (Harman *et al.*, 1980; Chao *et al.*, 1986; Mukhopadhyay *et al.*, 1992). Inoculation of seeds, corms, bulbs, tuber, etc. with bioagents prevents seed/corm decay and seedling blight. This also takes care of seed borne inoculum, particularly externally seed borne and diseases affecting above ground plant parts. For commercial purpose, dry powders of antagonists are used @ 3 to 8 g powder per kg seed, based on seed size and formulations of antagonists (Mukhopadhyay *et al.*, 1992; Mukherjee and Mukhopadhyay, 1995; Vidhyasekaran and Muthamilan, 1995). A large number of seed, seedling, root,

stem, foliar and panicle diseases have been suppressed by seed treatment with antagonists (Singh *et al.*, 2005). Mukhopadhyay (1996) demonstrated increased growth of several crop plants following seed treatment with *T. harzianum* and *T. virens*.

Efficacy of fungal and bacterial bioagents against pathogen of bacterial leaf blight disease in rice (*Xanthomonas oryzae* pv. *oryzae*) was tested under laboratory (Gangwar and Sinha, 2010a,b), glasshouse (Gangwar and Sinha, 2012a,b) and field conditions (Gangwar, 2012 and Gangwar 2013). In the present study, fungal and bacterial bioagents which were effective against pathogen of bacterial leaf blight disease of rice (*Xanthomonas oryzae* pv. *oryzae*) was tested for their effect on germination of rice seeds and growth of nursery seedlings under laboratory and glasshouse conditions.

### MATERIALS AND METHODS

**Bioagents formulations:** The experiment was conducted in the Rice Pathology Laboratory and glasshouse of the Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar. Fungal and bacterial bioagents were tested for their effect on germination of rice seeds and growth of nursery seedlings under laboratory and glasshouse conditions.

Two isolates of fluorescent pseudomonas isolated from rice phylloplane (Pf 83 and FLP 88), *Trichoderma* species isolated from soil of rice field (isolate 40) and *T. harzianum* isolated from rice phylloplane and two commercial formulations viz. PBA-1 (*T. harzianum* PBAT-43) and PBA-2 (*P. fluorescens* PBAP-27) were tested in the present investigation and compared with chemical treatment (0.76 g Emison + 0.18 g Streptocycline / kg seeds) and untreated check.

**Effect of bioagents on germination of rice seeds, under laboratory conditions:** Seeds of susceptible rice cultivar Jaya were soaked in sterilized water for 12 hours. After draining excess water, bioagent formulations (10 g / kg seeds) and chemicals (0.76 g Emison + 0.18 g Streptocycline / kg seeds) were applied as seed treatment. Untreated check was also maintained for comparison. On moist double towel paper 25 seeds were placed by maintaining equal distance between seeds and covered with single towel paper. Then towel papers having seeds inside were rolled by covering one side with butter paper and tied with rubber band. These towel papers were incubated in BOD incubator at  $28 \pm 1$  °C. Data were recorded as germination percent, root length and shoot length at 4, 8 and 12 days after treatment application.

**Effect of bioagents on seedling emergence and growth, under glasshouse conditions:** Bioagent formulations ( $10^6$  cfu/g) @10 g/kg seed and chemical treatment (0.76 g Emison + 0.18 g Streptocycline /Kg seeds) were applied as seed treatment before sowing of rice seeds in nursery. Rice seed amended with different treatment were than soaked separately in sterilized water for 12 hours. Untreated rice seeds soaked in sterilized water were served as check. Plastic pots were filled  $\frac{3}{4}$  by height with natural field soil and fertilized with NPK (@ 120:60:40 kg/ha). Pots were irrigated for puddling. After puddling, 1000 rice seeds from each treatment and check were sown in separate pots. Three replications were maintained for each treatment. Pots were arranged in randomized block design. Regular watering was done by using tap water. Data were recorded as seedling emergence, root and shoot length, fresh and dry root weight and fresh and dry shoot weight after 21 day of treatment application.

**Statistical analysis:** Statistical analysis of the data obtained from the experiment was done using appropriate programme as per the requirement of the experiment. The critical difference (CD) was calculated at 5% level of significance for comparison of difference between the means of different treatments.

## RESULTS AND DISCUSSION

**Effect of bioagents on germination of rice seeds, under laboratory conditions:** It is revealed by the data presented in Table 1, that all bioagents formulations were significantly effective in increasing percent germination

over check, under laboratory conditions. Chemical treatment was most effective which exhibited maximum increase (43.90%) in germination. This was followed by Pf83 and PBA-1, both exhibited increase in germination by 34.15 per cent. All bioagent formulations significantly increased root length as compared to chemical treatment. Maximum increase (73.64%) in root length was obtained by PBA-1 followed by Pf83 (65.76%) and *T. harzianum* (60.81%). All treatments were significantly effective in increasing shoot length over check. FLP 88, Pf83, PBA-1 and *T. harzianum* showed significantly higher shoot length over chemical treatment. Whereas, PBA-2 and isolate 40 were found to be as effective as chemical treatment in increasing shoot length since the values obtained were not statistically different. Chemical treatment and bioagent formulations may inhibited the growth and/or activities of detrimental microorganisms which may resulted in significantly enhanced seed germination, growth and vigour of rice plant, as compared to check. Similarly, *Trichoderma* preparations have been reported to increase emergence and vigour of rice (Mishra and Sinha, 2000). Mukhopadhyay (1996) demonstrated increased growth of several crop plants following seed treatment with *T. harzianum* and *T. virens*.

**Effect of bioagents on seedling emergence and growth, under glasshouse conditions:** All bioagent formulations and chemical treatment found statistically effective in increasing percent seed germination over check (Table 2), under glasshouse conditions. Maximum increase (29.42%) in seedling emergence was exhibited by chemical treatment which was followed by *T. harzianum* (22.18%) and PBA-2 (21.73%) and Pf83 (21.73%). All bioagents were significantly effective in increasing root length over check and chemical treatment. Maximum increase (100.15%) in root length was recorded with FLP 88 which was followed by PBA-2 (94.64%) and Pf83 (89.91%). All Bioagent formulations were found significantly effective than chemical treatment and check in increasing shoot length. Maximum increase (50.06%) in shoot length was found with *T. harzianum* followed by Pf 83 (49.05%). Similarly, Dubey (1995) reported a significant increase in root and shoot length, fresh and dry weight and yield of main cultivated crops due to seed bacterization with fluorescent pseudomonads. All bioagent formulations were significantly effective in increasing fresh and dry root weight over check and chemical treatment (Table 2). Maximum increase (53.22 %) in fresh root weight exhibited by Pf83 which was followed by FLP 88 (48.00 %) and PBA-2 (44.22 %). Maximum increase (58.87 %) in dry root weight was showed by PBA-2 which was followed by Pf83 (54.54 %) and FLP 88 (53.67 %). All bioagent formulation was significantly effective over chemical treatment and check in increasing fresh shoot weight. Pf83 was most effective bioagent and resulted in maximum increase (72.85 %) in

**Table 1.** Effect of bioagents formulations (applied as seed treatment) on germination of rice seed, root and shoot length of rice seedling at 4, 8 and 12 days after treatment application, under laboratory conditions.

Treatments	Seed germination (%)			Root length (mm)			Shoot length (mm)		
	After 4 days	After 8 days	After 12 days	After 4 days	After 8 days	After 12 days	After 4 days	After 8 days	After 12 days
Isolate 40	83.33 (66.14)	85.00 (67.40)	85.00 (67.40)	44.00	91.83	117.00	13.00	39.66	70.16
PBA-1	86.66 (68.66)	91.66 (73.40)	91.66 (73.40)	43.53	92.83	128.50	12.33	38.07	74.00
<i>T. harzianum</i>	85.00 (67.40)	83.33 (66.25)	85.00 (67.40)	49.26	89.00	119.00	11.83	33.83	76.66
PBA-2	81.66 (64.69)	83.33 (65.95)	85.00 (67.40)	39.00	79.83	117.50	16.83	32.33	72.16
FLP 88	80.00 (63.92)	83.33 (66.14)	86.66 (68.66)	47.50	89.50	116.66	14.50	39.50	79.50
<i>Pf</i> 83	90.00 (71.95)	91.66 (73.40)	91.66 (73.40)	47.53	86.50	122.66	15.83	36.00	73.66
Chemical treatment	98.33 (85.69)	98.33 (85.69)	98.33 (85.69)	31.16	38.83	84.40	11.50	29.83	60.16
Check	73.33 (58.92)	70.00 (56.83)	68.33 (55.76)	33.00	52.16	74.66	10.50	26.00	47.66
CD at 5 %	9.00	8.09	6.60	7.69	7.68	13.48	ns	6.10	12.05

\*Mean of three replications, ns = non-significant; Values in parenthesis are angular transformed

**Table 2.** Effect of bioagents formulations (applied as seed treatment) on emergence, root length, shoot length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight of rice seedlings at 21 days after application, under glasshouse conditions.

Treatments	Seedling emergence		Root length (mm)		Shoot length (mm)		Fresh root weight (g)		Dry root weight (g)		Fresh shoot weight (g)		Dry shoot weight (g)	
	(%)	(mm)	(mm)	(mm)	(mm)	(mm)	(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
Isolate 40	89.33 (71.10)	6.33	22.66	6.33	22.66	5.21	1.08	6.03	1.34	6.03	1.34	6.03	1.34	1.34
PBA -1	88.00 (69.76)	7.56	23.83	7.56	23.83	5.68	1.09	6.22	1.41	6.22	1.41	6.22	1.41	1.41
<i>T. harzianum</i>	90.00 (71.81)	7.83	25.00	7.83	25.00	5.66	1.16	6.53	1.48	6.53	1.48	6.53	1.48	1.48
PBA -2	89.66 (71.93)	8.23	24.00	8.23	24.00	5.77	1.22	6.54	1.47	6.54	1.47	6.54	1.47	1.47
FLP 88	88.00 (70.69)	8.46	24.00	8.46	24.00	5.92	1.18	6.73	1.48	6.73	1.48	6.73	1.48	1.48
<i>Pf</i> 83	89.66 (72.16)	8.03	24.83	8.03	24.83	6.13	1.19	7.17	1.52	7.17	1.52	7.17	1.52	1.52
Chemical treatment	95.33 (77.86)	4.36	20.33	4.36	20.33	4.43	0.84	4.79	1.11	4.79	1.11	4.79	1.11	1.11
Check	73.66 (59.27)	4.23	16.66	4.23	16.66	4.00	0.77	4.15	1.03	4.15	1.03	4.15	1.03	1.03
CD at 5 %	10.17	1.92	2.19	1.92	2.19	0.66	0.19	0.52	0.11	0.52	0.11	0.52	0.11	0.11

\*Mean of three replications; Values in parenthesis are angular transformed

fresh shoot weight which is followed by FLP 88 (62.32%) and PBA-2 (57.75%). Maximum increase (47.89%) in dry shoot weight was obtained by Pf 83 which is followed by FLP 88 (44.33%) and *T. harzianum* (43.68%). Similarly, Sakthival *et al.* (1986) and Mishra and Sinha (1998) also reported enhanced growth of rice seedling with bioagent application. Van Peer and Schippers (1988) documented increases in shoot and root fresh weight for tomato, cucumber, lettuce, and potato as a result of bacterization with *Pseudomonas* strains. Increased fresh and dry weight of shoot, roots and nodules of broad bean was also reported by Yehia *et al.*, 1985 when seeds were coated with *T. viride*. This may be because of production of growth stimulating factors by the bioagents (Windham *et al.*, 1986; Schipper *et al.*, 1987).

The results of the present study depicts that the effect of bioagents on growth and vigour of rice plants was significantly higher than chemical treatment. This exhibits the superiority of bioagents over the chemical treatment. However, in few other cases growth and vigour enhanced by bioagents was significantly higher than check but it was similar or lower than chemical treatments. This may be because of growth promoting effect of bioagents may be ignored because comparatively healthy crops were obtained in conventional rice cultivation. Kloepper *et al.* (1980) reported that *P. fluorescens* and other plant growth promoting rhizobacteria enhance plant growth by showing antagonisms to potentially deleterious rhizoplane fungal and bacterial pathogens. The direct application of microorganism to seed or other plant part gives them a competitive advantage over pathogen that must compete for nutrients and sites for attachment prior to infection. Reports are on hand indicating that bacteria may promote plant growth by suppression of deleterious root micro flora indicating those not causing obvious disease (Baker, 1986), altered root growth because of N production (Baker and Scher, 1987; Voisard *et al.*, 1989), normal effect on plant growth (Imam *et al.*, 1977), hydrogen cyanide (HCN) production (Rangeshwaran and Prasad, 2000), increased root exudation (Prikrýl and Vancura, 1980) and induce host resistance (Kempe and Sequeira, 1983).

### Conclusion

In the present study, bioagents formulations were found to be effective in promoting growth of rice plant under laboratory and glasshouse conditions when applied as seed treatment. These bioagent formulations were also effective against bacterial leaf blight disease of rice in previous studies. On the basis of above facts, it can be concluded that these bioagent formulation can also be used under no disease condition which may result in growth promotion of rice plant. Further studies are considered necessary under field conditions.

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