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Effect of Seed Biopriming on Selected Antagonistic Bacterial Isolates on Growth and Seed Yield of Paddy (*Oryza sativa* L.)

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Abstract

A field experiment was conducted at Zonal Agricultural Research Station, VC Farm, Mandya during *Kharif* 2015 to know the effect of seed biopriming on selected antagonistic bacterial isolates on growth and seed yield of paddy. Results indicated that seed bioprimed with *Bacillus amyloliquifascens* recorded significantly higher number of tillers (17.69), number of panicles per hill (14.55), seed yield (85.45 q ha^{-1}) and 1000 seed weight (19.93 g) as compared to untreated control (7.11, 4.45, 47.79 q ha^{-1} and 13.23g, respectively). Higher number of tillers (16.59), number of panicles per hill (13.21) and seed yield (70.22 q ha^{-1}) were recorded in talc with $MgSO_4$ as compared to carboxymethyl cellulose (CMC) alone (11.20, 7.91 and 59.70 q ha^{-1}, respectively).

Rice (*Oryza sativa* L.) is a staple food crop of the world. In India, rice is grown over an area of 43.97 million hectares with an annual production of 104.32 million tonnes and productivity of 2177 kg h^{-1} (Anon., 2014). Karnataka, occupies a prominent place in rice map of India accounting for nearly 15.40 lakh hectares with total production of 41.88 lakh tonnes with the average productivity of 2719 kg h^{-1} (Anon., 2011). Rice crop is attacked by many pathogens causing substantial yield losses in many countries. Among these, blast disease has become severe recently due to intensive crop production practices. Blast disease alone causes yield losses between 1 to 30 % (Savary *et al*., 1998). Growing resistant varieties and use of fungicides is the main strategies used to manage these diseases in rice (Ghazanfar *et al*., 2009). The use of resistant cultivars is known to be the most effective strategy but, some pathogens develop new races rapidly which breaks down the resistance quickly. The management of diseases with chemicals is effective, but excessive and continuous use of chemical fertilizers coupled with pesticides and fungicides have damaged the soil health, causing deleterious effects on crop cultivation and productivity. Instead of chemicals, eco-friendly micro-organisms can be used to control plant diseases which has dual role as a biocontrol agent and plant growth promoter (Gerhardson, 2002).

A number of such microbes are reported as effective biological control agents (BCA) against plant pathogens *viz*., *Bacillus*, *Penicillium*, *Pseudomonas*, *Trichoderma* etc (Fravel, 1988). One option is the use of bio-control agents applied to seed, which may promote plant growth or provide disease control through a variety of mechanisms, including antibiotic; induced systemic resistance. The concept of bio-priming is a newly developed technology in which the seeds are hydrated using biological compounds to have enhanced host plant resistance to pathogen attack in many host-pathogen interactions (Mathre *et al*., 1999) and inoculation of seeds with biological agents in combination with priming has, in several cases, been reported to enhance and stabilize the efficacy of biological agents (Callan *et al*., 1990). Hence, the present study was formulated to screen different bio control agents along with formulated products to ascertain the transport of bacterial isolates inside the seed by biopriming.

Seeds of rice cultivar Jaya which is popular in Karnataka state was used. The seeds were collected mainly from ZARS, VC Farm, Mandya and six bacterial isolates which offered the highest levels of protection against paddy blast and enhancement of seedling growth under *in vitro* and greenhouse were selected and used for further field studies.

Seed biopriming: Each bacterial isolates with 10^9 CFUs have diluted in water to 6 per cent concentration of different formulations (60 g / kg / 500 ml of water) and the solutions were used for biopriming treatment and soaked the seeds for 12h. Hydro primed, non-primed dry seed and tricyclazole 75 WP was treated @ 3 g /kg of seeds act as controls.

Field evaluations: A field experiment was conducted at Zonal Agricultural Research Station, VC Farm, Mandya, for one season during *Kharif*, 2015. The experiment was laid out in Randomized Complete Block Design with three replications. The experiment consisted of 34 treatment combinations *viz.*, four formulations (F_1: CMC alone, F_2 : Bentonite paste, F_3 : Talc with $MgSO_4$ and F_4: Polyethylene glycol (PEG)-paste) and nine treatments (T_1: *Pseudomonas fluorescens*- RPf-1, T_2: *Bacillus subtillis*-RBs-1, T_3: *Pseudomonas fluorescens*-UASR2, T_4: *Bacillus pumilis*-UASR8, T_5: *Bacillus amyloliquifascens*-UASR9, T_6: *Bacillus subtillis*-UASP17, T_7: Tricyclazol 75 WP @3 g / kg seeds, T_8: formulations without bacterial isolates (untreated control) and T_9: Dry seed. The seedlings of individual treatments were raised in plastic trays. The seedlings were transplanted when they were 28 days old and planting was done with a spacing of 10 cm between plants and 20cm between rows and a single seedling was planted per hill. The recommended fertilizer dose of 40:20:20 N, P_20_5, K_20 kg $acre^{-1}$ was applied. Out of this, 50 per cent N and entire dose of P_20_5 and K_20 were applied at the time of planting and the remaining 50 per cent N was applied in two equal split doses, one at tillering and another at panicle initiation stage.

Five plants were randomly selected in each plot area for taking observations on plant growth, percent disease incidence and yield parameters. The crop in each net plot was harvested as per the treatment and the values were expressed in kg per hectare. The data was statistically analyzed for interpretation of results.

The growth and yield data as influenced by different formulations and bacterial isolates are presented in Tables I and II. The results revealed that combination of formulations had marked effect on growth and yield components of paddy. Among the different formulations significantly higher plant height (108.95 cm) was recorded in F_3 followed by F_4 (105.39 cm) compared to seed treated with CMC alone (98.49 cm) (Table II). Since farmers are advised to use fresh formulations (Jayaraj *et al.*, 2005), this system should overcome any problems related to shelf-life of the formulations. Use of composted organic fertilizers has several benefits such as better soil structure; buildup of antagonistic organisms, and supply of plant nutrients (Siddiqui, 2004).

Higher plant height was recorded in T_5 (108.95 cm). However, it was on par with T_3 (108.88 cm) followed by T_1 (107.89 cm) compared to chemical treated T_7 (101.35 cm) and untreated control T_9 (94.70 cm). Ramamoorthy *et al.* (2001) also reported that production of plant growth regulators increased the availability of minerals and other ions, extensive rooting which facilitates water and nutrient uptake. Yield parameters of paddy were significantly influenced by different formulations. Significantly higher number of tillers (16.59), the number of panicles per hill (13.21) and Seed yield per hectare (70.22 q ha^{-1}) were recorded in F_3 as compared to F_1 (11.20, 7.91 and 59.70 q ha^{-1}, respectively) (Tables I and II).

Among different treatments seed bioprimed with *Bacillus amyloliquifascens* (T_5) recorded higher number of tillers (17.69), number of panicles per hill (14.55) seed yield (85.45 q ha^{-1}) and 1000 seed weight (19.93 g) as compared to T_9 (7.11, 4.45, 47.79 q ha^{-1} and 13.23g, respectively) (Tables I and II). Wu *et al.* (2005) reported that free-living plant growth promoting rhizobacteria (PGPR) trigger plant growth through production of phytohormones. Even though minimum portion (2–5%) of rhizobacteria are occupied by PGPR, they actively colonize around plant roots and increase plant growth and yield. During biopriming process, with the imbibition of bio-agent solution, bacteria may get transported into the seed. Kokila and Bhaskaran (2014) also reported that during biopriming with the imbibition of water, bacteria moved into the seeds of paddy. The bacteria are well protected against adverse biotic and abiotic factors inside the seeds and prolongation of the priming process cannot increase cell density in seeds. So, the establishment of bacteria inside the seed provides better stability and shelf life and the bacterial cells are protected against fluctuating environmental conditions. Carrot seeds artificially inoculated with conidia of *A. radicina* and treated with

TABLE I

Effect of seed bio priming on growth parameters at harvest of paddy cv. Jaya

Treatments (T)	Plant height (cm) Formulations (F)				Mean	No. of tillers (No. hill^{-1}) Formulations (F)				Mean	No. of panicles (No. hill^{-1}) Formulations (F)				Mean
	F_1	F_2	F_3	F_4		F_1	F_2	F_3	F_4		F_1	F_2	F_3	F_4	
T_1	99.43	104.68	117.58	109.88	107.89	12.80	15.83	20.29	18.26	16.79	10.00	13.00	14.30	15.00	13.08
T_2	98.66	102.98	114.58	108.58	106.20	11.10	14.83	19.19	18.06	15.79	7.00	12.00	17.30	14.50	12.70
T_3	100.28	105.68	119.48	110.08	108.88	13.30	16.33	20.29	18.96	17.22	10.00	14.00	18.00	16.20	14.55
T_4	98.77	99.88	114.58	108.58	105.45	11.00	14.83	19.09	16.76	15.42	6.00	11.00	16.20	13.00	11.55
T_5	100.58	104.58	120.18	110.48	108.95	13.80	16.29	21.12	19.56	17.69	11.50	14.20	16.00	16.50	14.55
T_6	98.98	102.58	117.28	107.58	106.61	11.30	15.33	18.99	18.16	15.94	8.50	13.50	17.60	15.00	13.65
T_7	101.05	101.09	101.40	101.87	101.35	11.00	11.13	11.49	11.16	11.19	8.80	11.00	9.00	8.90	9.43
T_8	94.24	95.38	96.58	96.02	95.56	9.90	10.03	11.29	10.26	10.37	5.00	8.80	6.50	4.00	6.08
T_9	94.40	94.69	94.30	95.39	94.70	6.66	7.13	7.59	7.06	7.11	4.40	5.40	4.00	4.00	4.45
Mean	98.49	101.28	110.66	105.39		11.20	13.52	16.59	15.36		7.91	11.43	13.21	11.90	
		S.Em±	CD (P=0.05)	CV (%)			S.Em±	CD (P=0.05)	CV (%)			S.Em±	CD (P=0.05)	CV (%)	
	T	0.937	2.644	3.124		T	0.34	0.97	8.39		T	0.44	1.23	13.60	
	F	0.625	1.763			F	0.23	0.64			F	0.29	0.82		
	T × F	1.875	5.288			T × F	0.69	1.93			T × F	0.87	2.46		

Note: Treatment details:

Formulations (F)

F_1 : CMC alone

F_2 : Bentonite paste

F_3 : Talc with $MgSO_4$

F_4 : Polyethylene glycol (PEG)-paste

Bacterial isolates (T)

T_1 : RPf-1

T_2 : RBs-1

T_3 : UASR2

T_4 : UASR8

T_5 : UASR9

T_6 : UASP17

T_7 : Tricyclazol

T_8 : Untreated control

T_9 : Dry seed

TABLE II

Effect of seed bio priming on yield parameters of paddy cv. Jaya

Treatments (T)	Seed yield hill^{-1} Formulations (F)				Mean	Seed yield (q ha^{-1}) Formulations (F)				Mean	1000 seed weight (g) Formulations (F)				Mean
	F_1	F_2	F_3	F_4		F_1	F_2	F_3	F_4		F_1	F_2	F_3	F_4	
T_1	29.00	30.60	36.23	33.60	32.36	75.67	75.40	78.00	77.20	76.57	16.20	19.50	23.00	19.50	63.05
T_2	28.40	29.50	34.50	31.50	30.98	63.00	64.00	73.30	63.03	65.83	14.50	18.50	22.40	19.30	18.68
T_3	30.50	31.90	37.50	34.00	33.48	78.00	80.00	81.10	79.23	79.58	16.30	18.30	23.20	20.00	19.45
T_4	28.60	28.50	33.20	30.20	30.13	42.17	51.00	68.17	61.17	55.63	13.50	17.50	21.20	19.00	17.80
T_5	31.00	32.50	37.70	34.20	33.85	79.17	81.30	92.17	89.17	85.45	17.60	17.80	23.80	20.50	19.93
T_6	28.50	30.00	35.60	32.50	31.65	65.27	65.00	75.07	73.00	69.58	15.00	17.00	22.50	19.60	18.53
T_7	28.30	28.50	28.20	28.60	28.40	50.00	51.00	51.07	70.00	55.52	16.30	16.70	16.20	16.40	16.40
T_8	27.00	28.90	29.50	28.60	28.50	43.00	43.00	45.13	44.20	43.83	15.20	15.20	16.80	15.60	15.70
T_9	21.30	21.60	21.20	21.40	21.38	41.00	39.17	68.00	43.00	47.79	13.40	13.20	13.10	13.20	13.23
Mean	28.07	29.11	32.63	30.51		59.70	61.10	70.22	66.67		15.33	36.41	20.24	18.12	
		S.Em±	CD (P=0.05)	CV (%)			S.Em±	CD (P=0.05)	CV (%)			S.Em±	CD (P=0.05)	CV (%)	
	T	0.36	1.02	4.15		T	2.56	7.22	13.77		T	0.53	1.49	8.10	
	F	0.24	0.68			F	1.71	4.82			F	0.35	0.99		
	T × F	0.72	2.3			T × F	5.12	14.45			T × F	1.05	2.97		

Note: Treatment details:

Formulations (F): F_1 : CMC alone; F_2 : Bentonite paste; F_3 : Talc with $MgSO_4$; F_4 : Polyethylene glycol (PEG)-paste

Bacterial isolates (T): T_1 : RPf-1; T_2 : RBs-1; T_5 : UASR9; T_3 : UASR2; T_4 : UASR8; T_6 : UASP17; T_9 : Dry seed; T_7 : Tricyclazol; T_8 : Untreated control isolates of *Pseudomonas cepacia* and *B. amyloliquefaciens*. Hermansen *et al*. (1999), however, found no effect on field emergence.

Bio-priming provides an economically competitive delivery system, because only a relatively small amount of cells are needed and also more stable field efficacy is achieved by biopriming Bennet and Whipps (2008). Ryu *et al*. (2003) demonstrated plant growth promotion in *Arabidopsis thaliana* by *B. subtilis* strain GB03 and *B. amyloliquefaciens strain* IN937a mediated through volatile compounds 2, 3-butanediol and acetoin.

From the present investigation, it can be concluded that bioprimed with *Bacillus amyloliquifascens* combination of talc with $MgSO_4$ (60 g/kg/500 ml of water) soaking for 12 h duration had a dual action of enhancing plant height (120.18 cm) and seed yield (92.17 q ha-1) compared untreated control (97.40 cm and 41 q ha-1, respectively)in Jaya rice cultivar.

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