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Co-inoculated Biopriming with *Trichoderma, Pseudomonas* and *Rhizobium* Improves Crop Growth in *Cicer arietinum* and *Phaseolus vulgaris*

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Abstract

A study was conducted to evaluate the performance of three rhizosphere competent microbial strains, viz., *Pseudomonas fluorescens* OKC, *Trichoderma asperellum* T42 and *Rhizobium* sp. RH4, individually and in combination in bioprimed seeds of chickpea and rajma. Seeds were sown in pots and fields and the results demonstrated that bioprimed seeds showed higher germination percentage, and better plant growth in both the crops compared to non-bioprimed control plants. It was also observed that the combined application of the microbes enhanced seed germination and plant growth better than their individual application. Among the combinations all combinations comprising of *Trichoderma* showed better results compared to the others and the triple microbial combination demonstrated best results in terms of seed germination and seedling growth in both chickpea and rajma.

Highlights

- Germination of chickpea and rajma seeds were higher in combined application of *Trichoderma*, *Pseduomonas* and *Rhizobium*
- Seedling growth enhanced in triple microbe treatment

Keywords: biopriming, chickpea, rajma, *Pseudomonas fluorescens*, *Trichoderma asperellum, Rhizobium* sp.

Plant growth promoting-rhizobacteria (PGPR) are beneficial microbes that have capacity to colonize on root surfaces and promotes plant growth via either direct action or biological control of plant from their pathogens (Kloepper and Schroth,1978). Their association is distributed to many plant species and is commonly present in varied environments. Enhancement in plant growth by root-colonizing species of *Rhizobium* and *Pseudomonas* is well documented. Biofertilizer potential PGPR strains promote plant growth by enhancing nutrient uptake by plants Sahni *et al*., (2008). Phosphate-solubilizing microorganisms (PSM) comprises of a large group that includes bacterial species *Pseudomonas*, *Bacillus*, and *Rhizobium*; actinomycetes and various fungal species such as *Aspergillus*, *Trichoderma* and *Penicillium* (Richardson *et al*., 2009). Application of phosphate solubilizing bacteria alone or in combination with nitrogen fixing bacteria is very beneficial for cotton and wheat fields (Zaidi and Khan 2005). Combined inoculation of Rhizobia and PSM increases the nodulation and availability of phosphorus content into the

soil, which improves the grain yield. Most microbes belong to PGPR group possesses at least two of the three criteria: aggressive colonization, plant growth stimulation and biocontrol (Preston, 2004; Vessey, 2003). Some useful microbes, termed as biofertilizers, have several beneficial roles and possess properties of plant growth promotion (Haas and Défago 2005, Lahan *et al*., 2012).

The genus *Trichoderma* belongs to the Deuteromycetes (Samuels, 1996) class of fungi and has been exploited as biocontrol agents against a range of plant pathogenic fungi because it antagonizes a number of plant pathogens (Chet, 1987). Some strains of *Trichoderma* have been widely used as biological control agents as well as plant growth promoters (Harman, 2000; Rabeendran *et al*., 2000). It is well documented that combinations of biocontrol agents and PGPRs increases disease suppression (Guetsky *et al*., 2002), improves crop yields and facilitates more nutrient uptake by plants (Algawadi and Gaur, 1988) over individual inoculations. Based on the above facts the current study was undertaken to test the hypothesis that combinations of selected *Trichoderma*, *Rhizobium* and *Pseudomonas* strains could improve seedling germination, growth and biomass accumulation in chickpea and rajma. Using selected strains of *Trichoderma asperellum, Pseudomonas fluorescens* and *Rhizobium* sp., the hypothesis was tested under greenhouse conditions as well as in a field to monitor the effect of individual and combined inoculations of these organisms on growth promotion in chickpea and rajma.

Materials and methods

Organisms

Pseudomonas fluorescens OKC (GenBank accession JN128891) was isolated from rhizosphere of a ladyfinger (*Abelmoschus esculentus*) plant whereas *Rhizobium* sp. RH4 was isolated from root nodules of chickpea from the Agricultural Farm of Banaras Hindu University, Varanasi, India. *Trichoderma asperellum* T42 (GenBank accession JN128894) was selected from a pool of *Trichoderma* isolates present in the department of Mycology and Plant Pathology, Banaras Hindu University. All the three strains were selected based on their antagonistic and plant growth promotion activities as well as compatibility with each other. The Pseudomonad strain was identified by sequencing the 16s rDNA region whereas the *Trichoderma* isolate was identified by sequencing the ITS region. The *Rhizobium* strain was confirmed by subjecting to nodulation test in a pot trial conducted with sterilized soil.

Seed Biopriming and Sowing

Seeds of chickpea (*Cicer arietinum* L.) and rajma (*Phaseolus vulgaris*) were surface sterilized by using 1% sodium hypochlorite for 3 min. Bacterial strains OKC and RH4 were inoculated in King's B broth and Yeast Extract mannitol (YEM) broth, respectively, and incubated at 28°C in incubator shaker at 100 rpm for 48 hours. *Trichoderma* isolate T42 was inoculated on Potato Dextrose Agar and incubated at 28°C for 4 days. Pellet from the bacterial cultures were obtained by centrifuging at 4 ^{0}C in 10,000x*g* for 2 min. The pellets were washed in sterilized distilled water thrice and the absorbance was measured spectrophotometrically at 600 nm. The optical densities for CFU 10^8 mL^{-1} of RH4 and OKC were determined (OD 0.374 and 0.393, respectively). Similarly, absorbance of the spores of *Trichoderma* strain T42, harvested in sterilized distilled water, was measured at 600 nm for CFU 10^7 mL^{-1} (OD 1.14). Treatments of the seeds with the microbial cultures either singly or in combinations were given by soaking the seeds into the cell suspensions for 2 h and then air dried at room temperature for another 2 h and incubated in moist chamber for 24 h. Equal volume of the spore suspensions were mixed for giving the combined treatments. All the cell suspensions were mixed with 1% Carboxy Methyl Cellulose (CMC) before biopriming the seeds. Seeds treated with only CMC served as control. Pot soil was amended with 20% vermicompost (VC) and soil-vermicompost mixture was sterilized by adding 4% formaldehyde solution. VC used in the experiment was prepared from vegetable peels and leaf litter processed by earthworm *Eisenia fetida* in indoor beds (Sahni *et al*., 2008). The seeds of different treatments (5 seeds per pot) were sown in pots (20 cm diameter) and five replications were made for each treatment. The microbial strains T42, RH4 and OKC were also tested in microplots in the village Bachchaon, Varanasi to evaluate their performance under field conditions for promoting growth and development in chickpea and rajma during the season 2010-11. In the field trial only the individual treatments with the microbes and their triple combinations were tested. Microplots of 3x2 m^2 were prepared and the bioprimed seeds were sown in the microplots in a ramdomized pattern. Five replications were maintained for each treatment. The treatments given are as follows: P = *Pseudomonas fluorescens* OKC, R = *Rhizobium* sp. RH4, T = *Trichoderma asperellum* T42, PT = OKC + T42, RT = RH4 + T42, PR = OKC + RH4, PTR = OKC + T42 + RH4, C= Control. The same combinations of treatments were also sown in agricultural fields.

Statistical analysis

Experiments were performed twice using a completely randomized design. The data are expressed as the mean of five independent experiments ± standard deviations. The one-way variance analysis was performed to test the significance of the observed differences using SPSS version 16. The differences between the parameters were evaluated by means of the Duncan's test and *P* values d"0.05 were considered as statistically significant.

Results and discussion

Biopriming of chickpea and rajma seeds showed increased seed germination and accumulation of biomass in both the crops under both pot and field conditions. However, different treatments had varied effect on the crop growth. In chickpea germination percentage, root length and biomass accumulation was highest in the treatment combination of T42 and OKC. The increase in germination and biomass accumulation of this treatment are marginally high compared to the triple microbe consortium treated plants but the difference was not significant. Treatment of the individual strains T42 and OKC has also showed significant increase in germination percentage and biomass accumulation over the non-bioprimed seeds. Among the three microbes when applied individually most of the characters showed better results when treated with OKC in chickpea (Table 1).

Similarly, the best results were obtained in rajma in the treatment comprising of the triple microbes viz., T42, OKC and RH4 (Figure 1). Although, treatments with the double microbes (T42 and OKC) also showed similar results without significant differences but the biomass accumulation was marginally lower compared to the triple microbe accumulation. However, shoot length was highest in the double microbe treatment comprising of T42 and OKC. The reason is not clearly understood but the effect is very significant. The three microbes when applied individually the strain OKC showed better results in most of the characters (Table 1). The results of field trial also showed better seed germination and plant growth in microbial mixtures compared to control and individual treatments with the microbes. Among the microbes that applied singly the fluorescent *Pseudomonas* strain OKC was best among the three microbial strains in promoting seed germination and plant growth in both the crops chickpea and rajma. The triple consortium bioprimed seeds showed better germination percentage compared to the individual microbes. Similarly, the plant biomass was also high in the plants treated with the triple microbial consortium compared to the individual microbes. However, the individual microbes were also able to induce higher plant biomass compared to the control untreated plants (Table 2).

Figure 1: Growth of *Phaseolus vulgaris* in pot after seeds bioprimed with *Pseudomonas fluorescens* OKC, *Trichoderma asperellum* T42 and *Rhizobium* sp. RH4 after 28 days of sowing.

According to Kleifeld and Chet (1992) *T. harzianum* applications increased (i) germination of pepper seeds, (ii) emergence of seedlings of bean, radish, tomato, pepper, and cucumber, (iii) seedling length and leaf area in pepper, and (iv) dry weight of cucumber plants. Other reports also showed contribution of different *Trichoderma* strains in increasing fresh shoot weight, root weight and/or root length in pea (Naseby *et al*., 2000), shoot dry weight in corn Va´squez *et al*. (2000), plant root area of cucumber by 95%, root length by 75%, dry weight by 80%, shoot length by 45%, and leaf area by 80% when compared to the control (Yedidia *et al*. 2001). Another strain of *Trichoderma atroviride* increased shoot and root dry weight of seedling by three-fold (Gravel *et al*., 2006) and a significant increase in total and/or marketable fruit (Gravel *et al*., 2007) in healthy tomato plants. Similarly, Ahmed *et al*. (2012) showed that combined application of rhizobial strains and plant growth promoting *Pseudomonas* improves

Table 1: Germination of bioprimed seeds and biomass accumulation in chickpea and rajma in pots after 28 days of sowing.

Treatments	% germination		Shoot Length (mm)		Root Length (mm)		Dry Shoot Biomass (g)		Dry Root Biomass (g)		Total Dry Biomass (g)	
	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma
P	87a	84a	122.5a	139a	108a	99a	0.074a	0.316b	0.075ab	0.563a	0.149ab	0.879ab
T	89a	82a	105a	120a	89a	89a	0.066ab	0.274b	0.079ab	0.524a	0.145b	0.799b
R	86a	80a	91.5ab	105b	76b	81ab	0.075a	0.362ab	0.070ab	0.410b	0.146b	0.772b
PT	92a	82a	129.5a	144.5a	110a	101a	0.091a	0.335ab	0.086a	0.492ab	0.172a	0.828ab
RT	89a	68b	120a	138a	94a	95a	0.066ab	0.298b	0.064b	0.557a	0.131b	0.855ab
PR	86a	64b	108.5a	123.5a	76b	97a	0.060ab	0.270b	0.061b	0.517a	0.121bc	0.787b
PTR	89a	92a	109.5a	125a	98a	106a	0.076a	0.547a	0.081a	0.438ab	0.162ab	0.985a
Control	70b	64b	85b	102.5b	70.1b	69b	0.044b	0.250b	0.057b	0.460ab	0.101c	0.710c

Results are expressed as means of ûve replicates and different letters indicate signiûcant differences among treatment results according to Duncan's multiple range test at P d" 0.05.

Table 2: Germination of bioprimed seeds and biomass accumulation in chickpea and rajma in pots after 28 days of sowing.

Treatments	% germination		Shoot Length (mm)		Root Length (mm)		Dry Shoot Biomass (g)		Dry Root Biomass (g)		Total Dry Biomass (g)	
	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma	Chickpea	Rajma
P	76.29ab	55.2b	102.45a	242.37b	83.38a	149.2a	1.1653b	4.66ab	0.845a	3.380a	2.010ab	8.870ab
T	71.85ab	30.55b	99.68a	282.91a	83.5a	156.6a	0.9569b	3.84b	0.747a	2.986a	1.704b	6.826b
R	78.52a	43.75b	93.54b	289.16a	83.02a	143.4a	1.2047b	4.09b	0.851a	3.405a	2.056ab	7.495ab
PTR	80.37a	83.33a	110.24a	291.36a	86.24a	158.4a	1.7514a	5.42a	0.9345a	3.715a	2.686a	9.135a
Control	68.89b	28.47b	95.61a,b	237.5b	74.5b	115.8b	0.4201c	1.97c	0.410b	1.639b	0.830c	3.609c

Results are expressed as means of ûve replicates and different letters indicate signiûcant differences among treatment results according to Duncan's multiple range test at P d" 0.05.

growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. Singh *et al*. (2013) recently also showed that combined application of *Trichoderma*, *Pseudomonas* and rhizobial strains can also protect chickpea from infection by the collar rot pathogen *Sclerotium rolfsii*. From the present study, therefore, it can be concluded that (i) seed biopriming with potential bioagents can improve seed germination and seedling growth in chickpea and rajma; (ii) co-inoculation of biopriming agents has synergistic activity and hence, co-inoculation is recommended.

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