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Evalution of Indian bean (*Lablab purpureus* L. var. typicus) seeds' inoculation with *Rhizobium phaseoli* and *Pseudomonas fluorescens* on growth, yield and nutritional quality

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Abstract
The field experiment was conducted at Horticulture Farm, S.K.N. College of Agriculture, Jobner (Jaipur) during *kharif* season 2016-2017. The experiment consisting four plant growth promoting bacteria like *viz.* P_0 (control), P_1 *(Rhizobium phaseoli*), P_2 (*Pseudomonas fluorescens*) *and* P_3 (*Rhizobium phaseoli* + *Pseudomonas fluorescens*) with three replications. The seeds of Indian bean inoculated with *Rhizobium phaseoli* + *Pseudomonas fluorescens* significantly increased the plant height (cm), number of branches per plant, dry matter accumulation, CGR at 45-60 and 60-75 days after sowing, chlorophyll content in leaves, leaf area, number of green pods per plant, average pod weight (g), green pod length (cm), number of pickings per plant, green pod yield per plant, green pod yield per plot, green pod yield (74.28 q/ha), protein content, net returns (₹ 67875/ha) and B:C ratio (2.56) than control (no inoculation) as compared to control. Whereas, crude fibre content was recorded minimum in treatment inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*.

Keywords: Growth, Indian bean, *Pseudomonas fluorescens*, quality, *Rhizobium phaseoli*, and yield

Introduction

Indian bean or Dolichos bean (*Lablab purpureus* L. var. typicus) belongs to the family fabaceae (2n=22). It is a multipurpose crop grown for pulse, vegetable and forage. There are two type of cultivated species of Indian bean *viz*, *Lablab purpureus* var. typicus which is vegetable type, cultivated for its soft and edible pods and *Lablab purpureus* var. lignosus is the field bean, cultivated for dry seeds as pulse.

The pods of Indian bean are important source of protein, minerals and dietary fibre. Its mature dark coloured seeds contains trypsin inhibitor, which break down into water soluble cyanogenic. During cooking the purple coloured pods have a strong flavour, which disappears after cooking. The nutritional composition of edible green pods contain 86 percent moisture, 2 percent fibre, 4 percent protein, 7.10 percent carbohydrate, 48 Kcal energy, 68mg phosphorus, 1mg iron, 210mg Ca, 668 IU vitamin-A, 0.08mg thiamine, 0.11mg riboflavin, 0.75mg niacin and 9.3mg vitamin C (Gopalan *et al.*, 2004) [14].

PGPB were included as key factors to increase the fertilizer use efficiency as well as to promote/modify the physiological responses in the plants.

Use of Plant Growth Promoting Bacteria (PGPB) can have a greater importance for increasing fertilizer use efficiency and crop productivity. Reduced application rates of chemical fertilizers through inoculation with plant growth-promoting rhizobacteria were supplementing 75 % of the recommended fertilizer rate with inoculants produced plant growth, yield and nutrient (nitrogen and phosphorus) uptake that were statistically equivalent to the full fertilizer rate without inoculants (Adesemoye *et al*., 2009) [3].

Plant Growth Promoting Bacteria (PGPB) enhance plant growth and productivity. *Rhizobium* is well known biological N fixer and *Pseudomonas* has known for its activity of biological control. Co-inoculation of *Pseudomonas spp*. and *Rhizobium spp*. have been shown to increase the degree of colonization of the legume rhizobia resulting in enhanced plant nodulation. This tripartite association composed of legume plant and two soil bacteria *i.e*. *Rhizobium spp.* and

Pseudomonas spp. have been reported to increase root and shoot weight, plant vigour, N fixation and grain yield in legumes. *Pseudomonas fluorescens* is considered most significant phosphate solubilizing bacteria, which not only provide phosphorus to the plants, but also produce siderophore, antibiotic and phytoharmones such as indole-acetic acid (Leinhos and Nacek, 1994) [19].

A number of strains of *Pseudomonas fluorescens* suppress plant diseases by protecting the seeds and roots from fungal infection (O'Sullivan and O'Gara, 1992) [22]. This effect is the result of production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *Pseudomonas fluorescens* may also be an important factor in disease control. *Pseudomonas fluorescens* induced accumulation of lignin in pea roots was reported by Benhamou *et al*., 1996. *Pseudomonas spp*. can form gluconic acid through the oxidative glucose metabolis (Gyaneshwar *et al*., 2002) [15].

Pseudomonas species have shown to be effective in controlling pathogenic fungi and stimulating plant growth by a variety of mechanisms, including production of siderophores, synthesis of antibiotics, production of phytohormones, enhancement of phosphate uptake by the plant, nitrogen fixation, and synthesis of enzymes that regulate plant ethylene levels (Abdul Jaleel, 2007) [2].

Materials and methods-

The experiment was conducted at Horticulture Farm, S.K.N. College of Agriculture, Jobner (Jaipur) during *Kharif* season 2016-2017. In Rajasthan, this region falls under agro-climatic zone-IIIA (Semi-Arid Eastern Plains). The experiment was laid out in Randomized Block Design with four plant growth promoting bacteria like *viz.* control, *Rhizobium phaseoli*, *Pseudomonas fluorescens and Rhizobium phaseoli* + *Pseudomonas fluorescens* with three replications. The process of inoculation was preceded by seed treatment with fungicide then seed inoculation with *Rhizobium phaseoli* and *Pseudomonas fluorescens* before sowing by putting seeds in 20 % sucrose solution and then inoculated with @ 10 g/kg of seeds by putting the uniform coating of chalk form powder on seeds and were allowed to air dry in shade. The seeds were sown on the same day after inoculation. The seeds of control plot treated with sucrose solution only. Each plot measured 2.8×1.4 m^2 (4.32 m^2) area. The crop geometry was kept at 60 x 30 cm. All the cultural operations were followed which were necessary to raise the good crop. The observations like plant height (cm), number of branches per plant, dry matter accumulation (g/m), CGR at 45-60 and 60-75 (g/m^2/day) days after sowing, chlorophyll content in leaves (mg), leaf area (cm^2), number of green pods per plant, average pod weight (g), green pod length (cm), number of pickings per plant, green pod yield per plant (g), green pod yield per plot (kg), green pod yield per hectare (q), protein content and crude fibre content taken manually. CGR was calculated by Radford, 1967 [26] method. chlorophyll content was deterimented using the method of Arnon (1949) [7] with slight modifications. Nitrogen content in the green pods was estimated by using Nesselar's reagent by spectrophotometer method (Snell and Snell, 1949) [38], protein content in the pods was calculated by multiplying nitrogen concentration (%) by the factor 6.25 (A.O.A.C., 1960) [1]. Crude fibre content in pods was determined by the method suggested by A.O.A.C. (1960) [1].

The data obtained from the trial were subjected to statistical analysis and the results were documented, analysed and presented in tabular form.

Results and discussion

An appraisal of data in table 1 and fig 1 reveals that growth parameters was significantly influenced by the inoculation of plant growth promoting bacteria at sowing time of Indian bean crop. The maximum plant height of (76.70 cm) at final harvesting stage, number of branches per plant **(**10.80) at 60 DAS, dry matter accumulation as 92.17 g, 161.51 g and 218.76 g at 45, 60 and 75 DAS, CGR (25.68g/m^2/day) at 45 to 60 DAS and (21.20 g/m^2/day) at 60 to 75 DAS, total chlorophyll content in leaves (1.931 mg/g) and leaf area (3027 cm^2) at 60 DAS were recorded under treatment P_3 (inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*) and minimum under control and remained statistically at par with treatment P_1 (inoculation with *Rhizobium*) and P_2 (inoculation with *Pseudomonas*) in case of plant height, dry matter accumulation at 60 DAS and CGR at 45 to 60 DAS. However, treatment P_1 (inoculation with *Rhizobium*) remained statistically at par with treatment P_3 in case of leaf area.

Inoculation of seed with symbiotic nitrogen fixers might have increased the concentration of an efficient and healthy strain of *Rhizobium* in rhizosphere, which in turn resulted in greater fixation of atmospheric nitrogen in soil for use by the plants and consequently resulting into higher growth (Prasad and Maurya, 1989) [25].

PGPB promote plant growth through production of phytoharmones, siderophores, antibiotics, enzymes and /or fungicidal compounds (Saleim *et al*. 2011, Saharan and Nehra, 2011 and Sharafzadeh *et al*., 2012) [35, 31, 37]. Many PGPB have shown the role of rhizosphere as an ecosystem and has gained importance in the functioning of biosphere (Ahmed and Khan, 2011) [4]. Various bacterial species viz., *Pseudomonas*, *Azospirillium*, *Azotobacter* and *Bacillus etc*. have been reported to enhance plant growth (Wahyudi *et al*., 2011and Kumar *et al*., 2012) [39, 17], germination and seedling vigour in tomato (Rathaur *et al*., 2012), maize (Shasavani *et al*., 2009) [36], rice (Mia *et al*., 2012) [20] and soyabean (Kumar *et al*., 2012) [17]. *Fluorescent pseudomonads*, a group of PGPB are the most studied once. They help in soil health maintenance and are matobolically and functionally most diverse (Lata *et al*., 2000). Presence of *fluorescent pseudomonad* inoculants in combination with microbial fertilizer play an effective role in stimulating plant yield and growth (Kloepper *et al*., 2004, Roesti *et al*., 2006 and Rokhzadi *et al*., 2008) [16, 28, 29]. Another widespread characteristic among the rizosphere bacteria is the ACC deaminase activity, whose regulation is a principal mechanism by which bacteria exert beneficial effects on plants under biotic and abiotic stress (Saleem *et al*., 2007 and Rama *et al*., 2013) [33, 27].

It is evident from data (Table 2) that the inoculation of plant growth promoting bacteria significantly increased the yield and yield attributed. Total number of pods (37.09), average pod weight (3.58 g), pod length (8.16 cm), number of pickings (10) , total green pod yield per plant (133.70g), total green pod yield per plot was recorded maximum (3.209 kg) and pod yield 74.28 q/ha were found maximum under treatment P_3 (inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*) and minimum under control.

The beneficial effects of *Rhizobium* as explained earlier thus might have increased the availability of nitrogen and phosphorus along with other nutrients which in term resulted in to higher production of assimilates and their partitioning to different reproductive structures such as yield attributes and ultimately, green pod yield. Co-inoculation of legumes with *Rhizobium* and PGPR *Pseudomonas* strains, were able to alleviate salt stress of plants, grown on salt affected soils and increased plant growth, yield and controlled the plant diseases of leguminous plants is recorded by Egamberdieva *et al.* (2013) [11].

Application of phosphate solubilizing microbes *i.e.* Pseudomonas, around the roots of plants, in soils and in fertilizers has been sown to release soluble phosphorus, promote plant growth and protect plants from pathogen infection (Biswas *et al.*, 2006, Ouahmane *et al.*, 2007, Rudresh *et al.*, 2005) [10, 23, 30]. The production of phosphate enzyme by phosphate solubilize bacteria and microbial phytases activity was reported by Ponmurugan and Gopi, 2006. The plant growth promoting rhizobacteria colonize in roots of plants and promote plant growth and development through activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion and finally in yield (Glick, 1995 and Lalande *et al*., 1989) [12]. Similar finding have also observed by Anandraj and Leema Rose, 2010 [5], Anitha and Kumudini, 2014 [6], Salehi and Aminpanah, 2015 [34] and Bhadala, 2017 [9].

A perusal of data presented in table 3 and fig 2 revealed that the application of bio-regulators significantly increased the protein content in green pods. The maximum protein content 3.53 per cent was recorded with the application of treatment P_3 (inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*). It is evident from the data (Table 3) that the inoculation of plant growth promoting bacteria significantly reduced the crude fibre content in green pod. The minimum crude fibre content of 1.77 per cent was recorded in treatment P_3 (inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*) while maximum 1.95 per cent under control.

The increase in these values due to inoculation of seed with *Rhizobium* was probably due to more nitrogen fixation of nitrogen resulting in to better utilization of nutrients by plants, which led to more chlorophyll formation and ultimately nitrogen and phosphorus concentration and protein content in green pods. Significant increase in nitrogen and phosphorus concentration of green pod was also observed with *Pseudomonas* inoculation. *Pseudomonas* enhanced the availability of phosphorus to plants, which might have utilized by the crop in greater root development and nodulation that in turn resulted in higher nitrogen fixation in the soil by nodules (Salehi and Aminpanah, 2015 [34] and Bhadala, 2017) [9].

Similar results have been reported by Kumawat and Khangarot (2002) [18], Bahadur *et al.*, (2006) [18], Sajitha *et al.*, (2007) [32] and Nahrawy and Omara, (2017) [21].

It is also indicated that the higher net returns of green pod (₹67,875/ha) obtained under the treatment P_3 (inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens*) and higher B:C ratio (2.56) was obtained under treatment P3(inoculation with *Rhizobium phaseoli* + *Pseudomonas fluorescens).*

Conclusion

On the basis of one year experiment results, it may be concluded that the inoculation of plant growth promoting bacteria as *Rhizobium phaseoli* + *Pseudomonas fluorescens* was found most suitable in terms of comparable green pod yield, net returns and B:C ratio (74.28 q/ha, ₹ 67,875 and 2.56, respectively).

Thus, inoculation of *Rhizobium phaseoli* + *Pseudomonas fluorescens*) to Indian bean crop is recommended.

Table 1: Effect of plant growth promoting bacteria on growth attributes of Indian bean

Treatment combinations	Characters				
	Plant height (cm) at final harvesting stage	Number of branches per plant at 60 DAS	Crop Dry Matter Accumulation (g/m row length) at		
			45 DAS	60 DAS	75 DAS
P_0	73.15	6.00	76.17	137.31	181.56
P_1	75.47	8.80	89.17	157.71	208.76
P_2	74.21	8.60	88.17	156.61	206.76
P_3	76.70	10.80	92.17	161.51	218.76
SEm$\pm$	0.99	0.12	1.05	1.85	2.49
CD at 0.05%	2.84	0.33	3.00	5.31	7.12

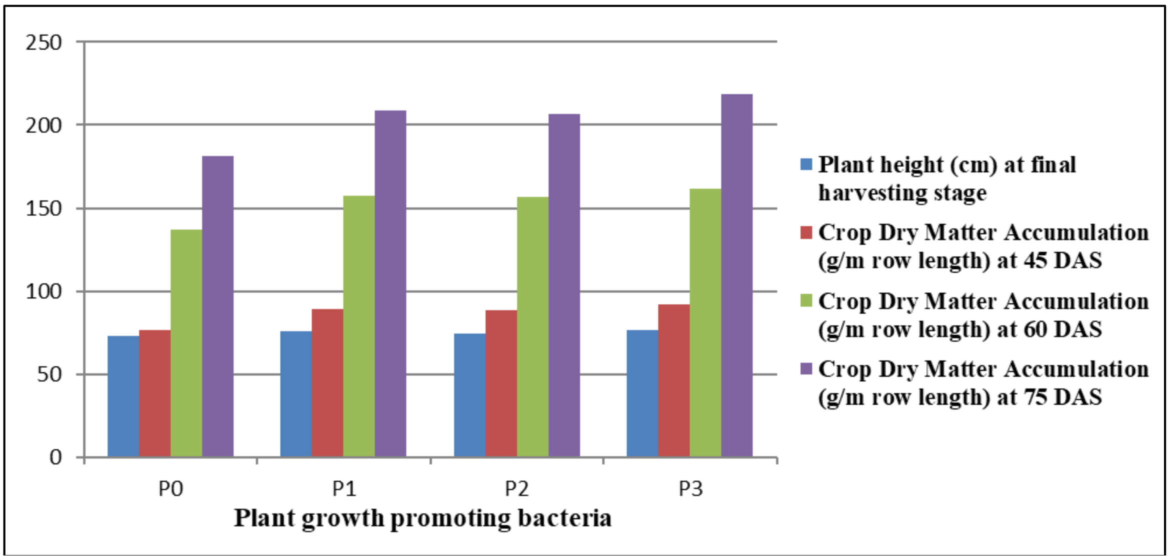

Fig 1: Effect of plant growth promoting bacteria on growth attributes of Indian bean

Table 2: Effect of plant growth promoting bacteria on growth of Indian bean

Treatment combinations	Characters			
	Total Chlorophyll (mg/g)	Leaf area (cm^2)	Crop Growth Rate (g/m^2/day) at	
			45 to 60 DAS	60 to 75 DAS
P_0	1.680	2488	22.65	16.39
P_1	1.820	2974	25.39	18.91
P_2	1.740	2865	25.35	18.57
P_3	1.931	3027	25.68	21.20
SEm±	0.024	33	0.30	0.23
CD at 0.05%	0.070	94	0.86	0.67

Table 3: Effect of plant growth promoting bacteria on yield attributes of Indian bean

Treatment combinations		Characters					
	Number of green pods/ plant	Average pod weight (g)	Green pod length (cm)	Number of pickings	Green Pod Yield		
					g/plant	kg/plot	q/ha
P_0	32.61	3.00	6.94	7.80	98.51	2.364	54.73
P_1	34.32	3.30	7.36	8.80	113.89	2.733	63.27
P_2	33.88	3.20	7.19	8.35	109.14	2.619	60.64
P_3	37.09	3.58	8.16	10.00	133.70	3.209	74.28
SEm±	0.66	0.06	0.06	0.16	2.07	0.050	1.15
CD at 0.05%	1.89	0.18	0.18	0.45	5.91	0.142	3.28

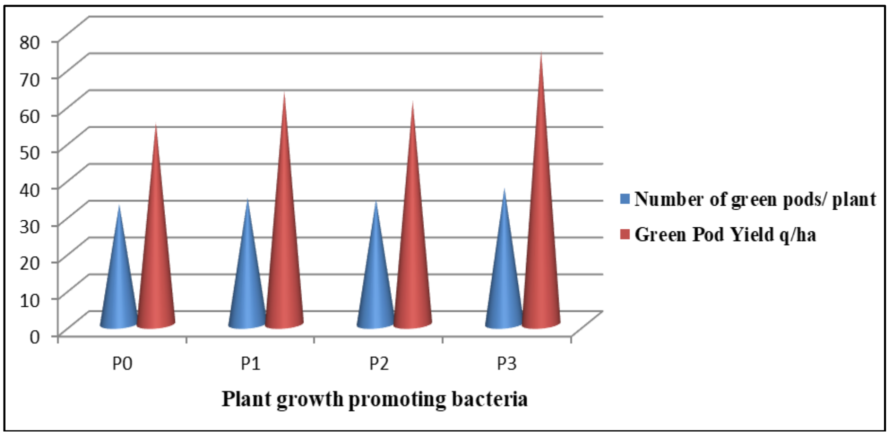

Fig 2: Effect of plant growth promoting bacteria on yield attributes of Indian bean

Table 4: Effect of plant growth promoting bacteria on quality and economic attributes of Indian bean

Treatment Combinations	Characters			
	Protein content (%)	Crude fibre content (%)	Net Returns (₹/ha)	B:C Ratio
P_0	2.35	1.95	38821	1.90
P_1	3.18	1.83	51488	2.19
P_2	2.88	1.80	47524	2.09
P_3	3.53	1.77	67875	2.56
SEm±	0.06	0.03	1672	0.04
CD at 0.05%	0.18	0.10	4786	0.12

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