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Interaction Between *Pseudomonas fluorescens* FPD-15 and *Bradyrhizobium* spp. in Peanut

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Abstract: In this study the ability of *Pseudomonas fluorescens* FPD-15 to promote plant growth was assessed under greenhouse conditions using JL-24 variety of peanut as a test crop. A pot experiment was carried out in completely randomized factorial design with two main factors to investigate the effect of application of *Pseudomonas fluorescens* FPD-15 with *Bradyrhizobium* strains NC-92 and SSP-24 under preincubated and coinoculated conditions (as a main factor with seven levels) at different time intervals (as the second main factor with three levels) with 3 replicates. Analysis of Variance (ANOVA) on each measured response variable (comprising root and shoot biomass, nodule number and dry weight and nitrogen content) was performed using the GLM procedure of SAS. The inoculation of peanut seeds with FPD-15 significantly increased root and shoot dry weight, nodule number and dry weight and N content in shoot when compared to the control. The interaction between FPD-15 and *Bradyrhizobium* strains NC-92 and SSP-24 were studied under preincubated and coinoculated conditions. The preincubated treatments yielded significantly higher root and shoot dry weight, nodule number, nodule dry weight and percent N content of shoot compared to the coinoculated treatments. Field trials using these strains should be conducted before they can be exploited in a commercial set up.

Key words: Coinoculation, plant growth promoting rhizobacteria, preincubation, *Pseudomonas fluorescens*

INTRODUCTION

The rhizosphere bacteria that aggressively colonize roots were termed as rhizobacteria. The term plant growth Promoting Rhizobacteria (PGPR) was coined by Kloepper *et al.* (1980) to include bacteria inhabiting the root and rhizosphere and having the ability to increase plant growth. These microorganisms have the ability to aggressively colonize plant roots and stimulate growth of plants in addition to suppressing plant pathogens (Kloepper *et al.*, 1999; Weller *et al.*, 2002; Pieterse *et al.*, 2002; Vessey, 2003; Lucy *et al.*, 2004; Preston, 2004). The beneficial effects of PGPR are attributed to improvement of plant growth and health and can be evidenced by an increase in seedling emergence, vigor, root system development and yield. The possible mechanisms by which PGPR increase the yield of crops include biocontrol of phytopathogenic fungi, hormone production and increased uptake of nutrients such as N, P and K (Kapulnik *et al.*, 1985; Lifshitz *et al.*, 1987; Defreitas and Germida, 1990b; Kloepper, 1993). PGPR, such as fluorescent pseudomonads, have been used as seed inoculants to promote plant growth and increase yields (Kloepper *et al.*, 1980; Defreitas and Germida, 1990a). Positive effects of PGPR on diverse hosts such as bean (Anderson and Guera, 1985), cotton (Sakthivel *et al.*, 1986), soybean (Polonenko *et al.*, 1987), peanut (Dey *et al.*, 2004), maize (Shaharoona *et al.*, 2006) and sugarbeet (Cakmakci *et al.*, 2006) are common in literature. A review published recently by Gray and Smith (2005) dealt with the history of PGPR discovery and indicated the progress in understanding each of the PGPR groups. However, PGPR has not yet been fully exploited in Karnataka region and studies on the interaction of *Pseudomonas fluorescens* with *Bradyrhizobium* are meager. The present study deals with the effect of *Pseudomonas fluorescens* FPD-15 alone or in combination with *Bradyrhizobium* sp. on promoting growth of peanut.

MATERIALS AND METHODS

Greenhouse evaluation of bacterial strains: A pot experiment was conducted under greenhouse conditions at the Department of Agricultural Microbiology, UAS,

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Dharwad using peanut (variety JL-24) as a test crop. The experiment was conducted in pots (15×15 cm) set in a factorial design which was completely randomized. The soil used in the study was medium black clay obtained from E-block, Main Research Station, UAS Dharwad, India. The peanut seeds were sown after bacterizing them with respective treatments containing bacterial cultures. In the beginning two seeds were planted in each pot and after germination only one seedling was retained. All the pots were maintained in the greenhouse up to a period of 49 days. The plants were sampled at 35, 42 and 49 days after inoculation and were used for analysis. Each pot comprised of an experimental unit and the treatments (inoculation×time) were assigned randomly to the pots. The trial consisted of treatments (with 8 levels) and days after inoculation (independent pots with 3 levels of 35, 42 and 49 Days After Inoculation (DAI)) as two main factors with eight treatments replicated three times. The treatment combinations were: 1) Uninoculated control; 2) *Pseudomonas fluorescens* FPD-15 single inoculation; 3) *Bradyrhizobium* sp. NC-92 single inoculation; 4) *Bradyrhizobium* sp. SSP-24 single inoculation; 5) *Pseudomonas fluorescens* FPD-15 + *Bradyrhizobium* sp. NC-92 preincubated; 6) *Pseudomonas fluorescens* FPD- 15 + *Bradyrhizobium* sp. SSP-24 preincubated; 7) *Pseudomonas fluorescens* FPD-15 + *Bradyrhizobium* sp. NC-92 coinoculated; and 8) *Pseudomonas fluorescens* FPD-15 + *Bradyrhizobium* sp. SSP-24 coinoculated.

The population of *Pseudomonas fluorescens* FPD-15, *Bradyrhizobium* sp. NC-92 and *Bradyrhizobium* sp. SSP-24 at the time of sowing was 8.2×10^8, 6.2×10^8 and 6.5×10^8 cfu mL^{-1}, respectively. The interaction between *Pseudomonas fluorescens* FPD-15 and strains of *Bradyrhizobium* sp. were studied in two ways. In one set called preincubation, equal suspensions of both bacteria were mixed and incubated together for five hours on a rotary shaker at 50 rpm and 28°C as per the protocol of Nishijima *et al*. (1988) and then used for inoculation. For coinoculation, the individually grown cultures were mixed in equal proportions just before sowing and were then applied. In both preincubated and coinoculated treatments, the seeds were bacterized with one mL of bacterial suspension. Bacterial cultures used in the present study were obtained from the Department of Agricultural Microbiology, UAS, Dharwad, India. The root and shoot dry weights were estimated after drying in an oven at 65°C till constant weight and N content in shoot was determined by Kjeldahl method (Jackson, 1973).

Statistical analysis: The statistical model was factorial with a completely randomized design. Treatment with 7 levels and Days after Inoculation (DAI) were considered as main effects and their effects on response variables (root and shoot dry weight, nodule number, nodule dry weight and nitrogen content) were analyzed using General Linear Model (GLM) procedure of SAS (SAS Institute, 1999). Data matrix comprised a data set of the differences between each observation and its un-inoculated control. Multiple comparisons of individual means were performed by Duncan's multiple range test and using mean statement in the GLM procedure.

RESULTS

The effect of both main factors (treatment and time) was highly significant ($p<0.001$) on all response variables investigated in this study (Table 1). The highest root dry weights were observed by NC-92 single inoculation and FPD-15 + NC-92 preincubated treatments at 49 DAI. Root dry weights at 49 DAI were also significantly ($p<0.05$) higher than those at 35 and 42 DAI (Fig. 1a). Inoculation with FPD-15 significantly improved the dry matter accumulation in shoot at 49 DAI over others at all stages of growth. Compared to all stages of growth, the preincubated treatments recorded significantly ($p<0.05$) higher shoot dry weight than their coinoculated counterparts and highest shoot dry weight was observed due to inoculation of preincubated treatment with FPD-15 and NC-92. The treatments FPD-15 + NC-92 preincubated, FPD-15 + SSP-24 preincubated and FPD-15 + NC-92 coinoculated at 49 DAI produced the first, second and third significantly ($p<0.05$) highest shoot dry weights, respectively (Fig. 1b).

The treatment involving inoculation with *Pseudomonas fluorescens* FPD-15 recorded significantly ($p<0.05$) higher nodule number and dry weight at 49 DAI than other stages of growth, whereas it had no effect on nitrogen content over time (Fig. 1c-e). The preincubated treatments recorded significantly ($p<0.05$) higher nodule number and dry weights than the coinoculated treatments. Highest nodule number and dry weight was for the preincubated treatment inoculated with FPD-15 and NC-92, followed by FPD-15 and SSP-24 (Fig. 1c and d). At 35, 42 and 49 DAI, the preincubated treatments were statistically significant over coinoculated treatments with regard to the percent of N content in shoot of peanut plants and generally fewer treatments showed significant change in N content over time (Fig. 1e).

Table 1: Factorial analysis of variance of root dry weight, shoot dry weight, nodule number, nodule dry weight and nitrogen content in peanut plants inoculated with *Pseudomonas fluorescens* and strains of *Bradyrhizobium*

Variables	Source	Degrees of freedom	Type III	Mean square	f-value	Pr>F
Root dry weight						
	Treat	6	0.0163	0.003	3.98	0.0031
	DAI	2	0.0481	0.024	35.33	<0.0001
	Treat*DAI	12	0.0210	0.002	02.57	0.0119
Shoot dry weight						
	Treat	6	0.2525	0.0421	53.63	<0.0001
	DAI	2	0.0749	0.0375	47.76	<0.0001
	Treat*DAI	12	0.0954	0.0079	10.13	<0.0001
Nodule No.						
	Treat	6	2846.58	474.43	12.1	<0.0001
	DAI	2	3799.19	1899.60	48.45	<0.0001
	Treat*DAI	12	535.48	44.62	01.14	0.3572
Nodule dry weight						
	Treat	6	29036.28	4839.38	08.18	<0.0001
	DAI	2	57472.00	28736.00	48.59	<0.0001
	Treat*DAI	12	4956.93	413.08	00.70	0.7437
Nitrogen content						
	Treat	6	4.86641	0.81107	25.64	<0.0001
	DAI	2	0.24743	0.12372	03.910	0.0277
	Treat*DAI	12	0.49401	0.04117	01.300	0.2538

Results in terms of significance probabilities (P>F). There were 7 treatments (Treat) and 3 Days after Inoculation (DAI) as main effects and the interaction between the main effects (Treat*DAI) were also tested

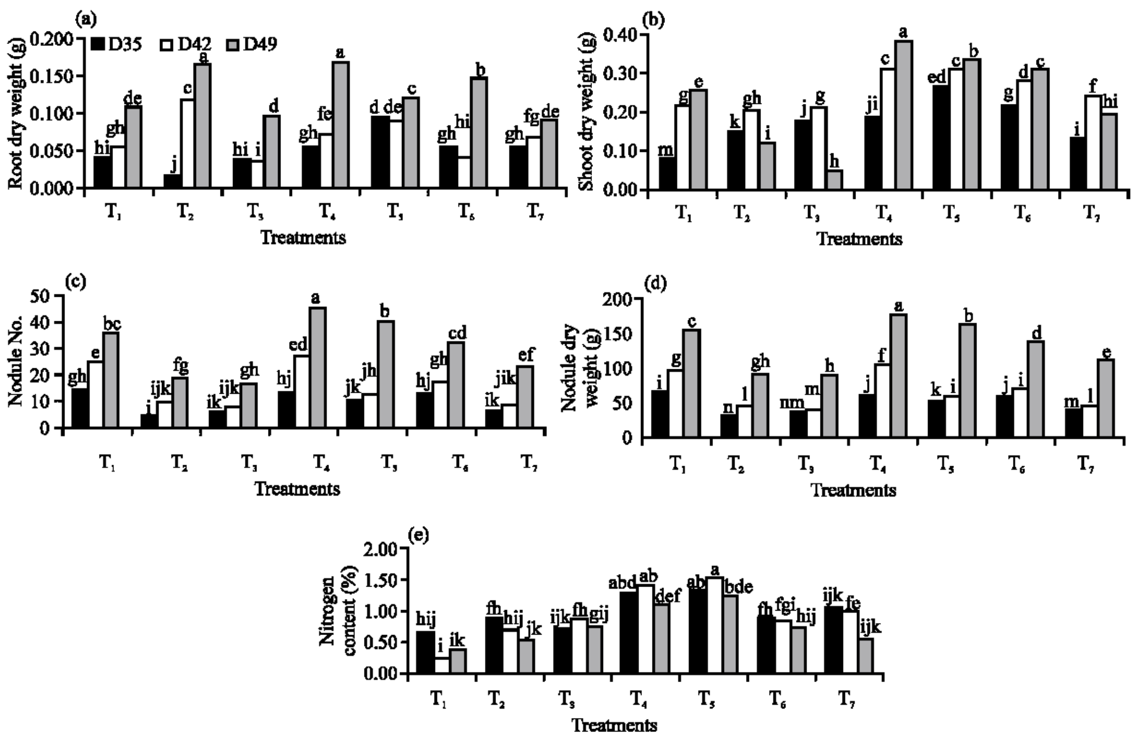

Fig. 1: Mean profiles of a) root dry weight, b) shoot dry weight, c) nodule number, d) nodule dry weight and e) nitrogen content over a period of 14 days after inoculation. The comparison of mean are done using Duncan's Multiple Range test at $p<0.05$ and bars with same letter are not significantly different. Means are deducted from un-inoculated control, T_1 = FPD-15 single inoculation, T_2 = NC- 92 single inoculation, T_3 = SSP-24 single inoculation, T_4 = FPD-15 + NC-92 preincubated, T_5 = FPD-15 + SSP-24 preincubated, T_6 = FPD-15 + NC-92 coinoculated and T_7 = FPD-15 + SSP-24 coinoculated; D35, D42 and D49 = 35, 42 and 49 days after inoculation

DISCUSSION

Plant Growth Promoting Rhizobacteria (PGPR) competitively colonize plant roots, stimulate plant growth and reduce the incidence of plant diseases. Fluorescent pseudomonads are a group of PGPRs which have also been responsible for improving the overall growth of many crops (Wang *et al*., 2000; Dey *et al*., 2004). In the present investigation, the effect of inoculation of FPD-15 to improve root growth, shoot growth, nodulation and N uptake in peanut plants was assessed. The interaction effects of *Pseudomonas fluorescens* FPD-15 and strains of *Bradyrhizobium* were also studied. Inoculation of FPD-15 was found to have a positive effect on improving root biomass, shoot growth, nodulation and shoot N concentration. Increased root and shoot growth could be attributed to the ability of this strain to solubilize phosphate and to release growth promoting substances such as auxins and cytokinins. The inoculation of mineral phosphate solubilizing bacteria increased the total biomass and grain yield in chickpea (Alagawadi and Gaur, 1988) and other leguminous crops (Gaur, 1990). The role of phytohormones such as auxins and cytokinins in enhancing plant cell division and root development is well known (Arshad and Frankenberger Jr., 1993). *Pseudomonas fluorescens* improved plant growth through the production of growth promoting substances such as indole acetic acid (Dey *et al*., 2004) and cytokinins (Neito and Frankenberger, 1989). Plant growth promotion observed in agronomic crops due to inoculation of rhizobacteria could be attributed to the increase in nitrogen fixation, production of growth hormones, solubilization of phosphates, oxidation of sulphur, increase in nitrate availability, extra cellular production of antibiotics, lytic enzymes, hydrocyanic acid, increase in root permeability, competition for available nutrients and root sites and induction of plant systemic resistance (Chanway *et al*., 1991; Kloepper, 1993; Enebak *et al*., 1998). It is also suggested that a combination of a few of the probable mechanisms may be operative for any particular PGPR strain (Chakraborty *et al*., 2006). Thus, the improvement in the characters under study may be attributed to the growth promoting substances produced by between test organism as well as enhanced P availability in the rhizosphere.

Another parameter that influences shoot growth in legumes is N_2 fixation. The inoculation with FPD-15 significantly increased nodule number. Exopolysaccharides are known to influence legume root infection and nodulation (Chen *et al*., 1985; Leigh *et al*., 1988). FPD-15 was capable of producing exopolysaccharides in the form of slimy growth and also solubilized P when grown on hydroxy apatite medium. It is suggested that the release of such exopolysaccharides might have resulted in noticeable increase in nodulation. The shoot N concentration was also significantly higher in FPD-15 inoculated plants. In leguminous oilseeds, increased nodule number results in increased N fixation and N uptake (Joshi *et al*., 1990). Inoculation of FPD-15 with NC-92 and SSP-24 both by addition of preincubated mixture or coinoculation, significantly improved the percentage of N accumulated over the respective NC-92 and SSP-24 single inoculations indicating an overall net positive effect of both strains on N uptake. Positive interactions of *Pseudomonas fluorescens* and *Bradyrhizobium* have been reported in soybean and chickpea (Polonenko *et al*., 1987; Alagawadi and Gaur, 1988). Coinoculation of *Bacillus polymyxa* and *Rhizobium etli* stimulated *Rhizobium etli* populations and nodulation in the rhizosphere of *Phaseolus vulgaris* (Petersen *et al*., 1996).

Studies involving preincubation of *Bradyrhizobium japonicum* with *Pseudomonas fluorescens* increased the level of nodulation in soybean (Nishijima *et al*., 1988). Hence, the differences between inoculation of preincubated mixture and coinoculation of *Pseudomonas fluorescens* FPD-15 with *Bradyrhizobium* strains on growth parameters of peanut was assessed in this trial. While comparing both preincubated and coinoculated treatments it was noted that preincubated treatments had a significantly higher impact on the plant biomass, nodule dry weight and N content in shoot over coinoculation. Preincubation of *P. fluorescens* and *Bradyrhizobium* strains significantly increased nodulation over coinoculated treatments. Results of enhanced levels of nodulation were recorded when soybean was treated with *Bradyrhizobium japonicum* and *Pseudomonas fluorescens* (Nishijima *et al*., 1988). They were of the opinion that the enhanced nodulation observed in soybean by *Bradyrhizobium japonicum* in presence of *Pseudomonas fluorescens* could be due to a substance produced by *Pseudomonas fluorescens* SSJ2. Interaction between *Bradyrhizobium* and plant growth promoting rhizobacteria increased nodulation and nitrogen fixation in soybean and *Lupinus albus* (Dashti *et al*., 1998; Garcia *et al*., 2004). *Pseudomonas fluorescens* F113 enhanced nodulation by *Rhizobium leguminosarum* 1112 fourfold in pea plants when they were inoculated after mixing them together (Andrade *et al*., 1998). The nodules obtained were much larger and strongly pigmented compared to single inoculation of *R. leguminosarum* 1112. In chickpea, coinoculation of fluorescent *Pseudomonas* and effective strains of *Rhizobium* resulted in a significant increase in nodule weight, root and shoot biomass and total plant nitrogen in sterilized chillum jars or under pot culture conditions (Parmar and Dadarwal, 1999). Coinoculation of *Bradyrhizobium japonicum* USDA 110 with rhizobacterial strains increased nodule number and dry weight of *B. japonicum* USDA 110 when compared to their single inoculations (Polonenko *et al*., 1987). These rhizobacteria can promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation, or nodule occupancy. The fact that inoculation of FPD-15 alone or in combination with *Bradyrhizobium* spp. increased nodulation and N_2 fixation than individual inoculation of *Bradyrhizobium* in the present study could be attributed to two possible reasons. One reason for this observation may be that FPD-15 produced some growth promoting substances which aided in improved nodulation and N_2 fixation by *Bradyrhizobium*. The second reason may be that FPD-15 strain has the capability to fix nitrogen. In studies which were conducted earlier, *Pseudomonas fluorescens* and *Pseudomonas* sp. have been shown to fix nitrogen (Gowda and Watanabe, 1985; Chan *et al*., 1994). However, there are suggestions that the contribution of bacterially fixed nitrogen to plants is minimal and that enhanced growth by an inoculated plant does not necessarily mean that the bacteria associated with the roots do fix nitrogen or pass the products of nitrogen fixation to the plant (James and Olivares, 1997). There are also reports that although PGPR have the ability to fix atmospheric nitrogen, they are not likely to provide large amounts of this fixed nitrogen to the plants (Mantelin and Touraine, 2004). The possible answers to the above could all be answered if acetylene reduction assay of the strain, *in vitro* nitrogen fixation and refined plant N uptake analysis are conducted. It is suggested that it would be appropriate to test if FPD-15 has the capability to produce growth promoting substances. The ability of *Pseudomonas fluorescens* FPD-15, alone or in combination with *Bradyrhizobium*, to promote plant growth in peanut can be commercially exploited only after conducting suitable field trials.

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