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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (15): 1935-1939, 2008
ISSN 1028-8880

Evaluation of Bean (*Phaseolus vulgaris*) Seeds Inoculation with *Rhizobium phaseoli* and Plant Growth Promoting Rhizobacteria on Yield and Yield Components

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Abstract: To study the effect of co-inoculation with plant growth-promoting rhizobacteria (PGPR) and *Rhizobium*, on yield and yield components of common bean (*Phaseolus vulgaris* L.) cultivars was investigated in 2 consecutive years under field condition of plant growing evidence indicates that soil beneficial bacteria can positively affect symbiotic performance of rhizobia. PGPR strains *Pseudomonas fluorescens* P-93 and *Azospirillum lipoferum* S-21 as well as two highly effective *Rhizobium* strains were used in this study. Common bean seeds of three cultivars were inoculated with *Rhizobium* singly or in a combination with PGPR to evaluate their effect on growth characters. A significant variation of plant growth in response to inoculation with *Rhizobium* strains was observed. Treatment with PGPR significantly increased pod per plant, number of seeds per pod, weight of 100 seed, weight of seeds per plant, weight of pods per plant, total dry matter in R_6 as well as seed yield and protein content. Co-inoculation with *Rhizobium* and PGPR demonstrated a significant increase in the yield and yield components. The results showed that all treatments of bacteria increased yield; however, strains Rb-133 with *Pseudomonas fluorescens* P-93 gave the highest seed yield, number of pods per plant, weight of 100 seed, seed protein yield, number seed per pod, seed protein yield.

Key words: Bean (*Phaseolus vulgaris* L.), plant growth promoting rhizobacteria (PGPR), yield

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important legume for human nutrition and a major protein and calorie source in the world (Sharon, 2003). *Phaseolus vulgaris* can grow by assimilation on mineral nitrogen or molecular N fixation. A board range of *Rhizobium* species are able nodule and fix N_2 with beans including *R. leguminosarum* biovar *phaseoli*, *R. tropic* and *R. etli*. The most Iran soils N deficiency, N_2 fixation *Rhizobium* bacteria could increase yield at a low cost and preserve water resources from pollution by nitrates. It occupies more than 125,000 ha in Iran but yield remains low to moderate due to the scarce nodulation, high inputs of chemical fertilizers and low technologies applied. Common bean is usually considered a poor nitrogen-fixing legume however; its promising potential to fix nitrogen has been shown in several studies (Peix *et al*., 2001; Garcia *et al*., 2004; Remans *et al*., 2008). Poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant, particularly the nodulation promiscuity as well as the great sensitivity to other nodulation-limiting factors, such as high rates of N fertilizer used in intensive agriculture, high temperatures and soil dryness (Bais *et al*., 2006; Egamberdiyeva *et al*., 2007). Genotypic variation in beans as well as compatibility of *Rhizobium*-plant cultivars can also greatly affect the efficiency of symbiosis established. This variability often limits the nitrogen-fixing performance of soil native rhizobia or use of commercially available inocula. As a result, application of high amounts of inorganic nitrogen fertilizers is becoming a common practice which has detrimental environmental consequences.

MATERIALS AND METHODS

Plant material and bacterial strains: Three kidney bean cultivars Sayad, Akhtar and Goli were obtained from Seeds and Plant Improvement Institute (SPII) housed at Karaj, Iran and used throughout this research. Two *Rhizobium* strains Rb-133 and Rb-136 with high nitrogen-fixing effectiveness were used in this study. These strains were selected during our previous screening program and demonstrated a good potential to nodulate beans and increase plant growth and yield in greenhouse and field experiments (Asadi Rahmani *et al*., 2005). PGPR strains *Pseudomonas fluorescens* P-93 and *Azospirillum*

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Table 1: Some characteristics of PGPR strains used in this study

Strains	P-solubilization (μg P mL^{-1} $week^{-1}$)	Auxin production (μg auxin mL^{-1})	Nitrogenase activity (10^4 nmol C_2H_4 h^{-1})	HCN production	Siderophore production
P. fluorescens P-93	68	63.7	–	+	+
A. lipoferum S-21	41	32.0	12	–	–

lipoferum S-21 positive effects in this study (Table 1). For preparation of inoculants, bacteria were grown in appropriate growth media for three days and 150 mL of each strain suspension was added to a polypropylene plastic bag containing 50 g of sterile powdered perlite and mixed thoroughly.

Experimental conditions: Field trails established in 2006 and 2007 at Shahrekord (50°51'N 32°17'E) south western in Iran. Experiments were arranged in a randomized complete block design using a split plot layout with three replications. Seeds of common bean cultivars Sayad, Akhtar and Goli were planted after they were moistened with a 20% solution of sucrose and then inoculated (7 g inoculant per kg seed) with *Rhizobium* alone or in combination with *P. fluorescens* P-93 or *A. lipoferum* S-21. The experiment also included a non-inoculated control as well as one N-fertilized (100 kg N ha^{-1}) treatments. The main-plots units consisted of 8 treatments (six bacterial combinations, one uninoculated control and one N-fertilized) applied.

RESULTS AND DISCUSSION

We found significant different effects induced by rhizobia on growth parameters of common bean. Plant cultivars also showed different responses due to inoculation with rhizobial isolates.

Inoculation with *Rhizobium* significantly ($p<0.01$) increased number seed per pod, number pod per plant, weight of 100 seed, seed yield, weight of seed per plant, weight of pod per plant, protein seed yield and total dry matter in R_6 in both years. Co-inoculation with PGPR promoted nodulation over *Rhizobium* alone, where the highest values for seed yield and yield components were observed by combination of *Rhizobium* and *Pseudomonas fluorescens* P-93. Similar result was obtained for shoot dry matter of plants in R_6 growth stage. *Rhizobium* inoculation by isolates Rb-133 and Rb-136 increased dry matter production of the shoots. Co-inoculation with *A. lipoferum* S-21 improved dry matter production over *Rhizobium* treatment. This beneficial effect was greater for PGPR strain *P. fluorescens* P-93 and demonstrated the highest dry matter production. Application of PGPR enhanced seed yield compared to either control or *Rhizobium* alone. The highest seed yield was obtained from plants inoculated with Rb-133+ *P. fluorescens* P-93 which showed a 240% increase over control plants.

The amount of seed yield was affected by co-inoculation of *Rhizobium* with PGPR strains in cultivars used in this study (Table 2-4). *P. fluorescens* P-93 resulted in highest amount of seed yield and other characters followed by *A. lipoferum* S-21, when co-inoculated with Rb-133, indicating that *P. fluorescens* P-93 had the most promising effect on enhancement of symbiotic performance of rhizobial strains. Plants inoculated with *Rhizobium* alone showed different values for yield components. Plants inoculated with Rb-133+ *P. fluorescens* P-93 made the most seed yield, which showed over 50% increase compared to *Rhizobium* alone.

This research has shown the effects of co-inoculation with two PGPR strains on the symbiotic performance of common bean nodulating rhizobia in three *P. vulgaris* cultivars. Common bean is believed to be a poor nitrogen fixer due to the genetic characteristics of symbiotic partners as well as soil and environmental conditions. However, selecting rhizobia for increased survival in specific soil types, greater compatibility with crop species or cultivars, superior functioning under diverse climates, improved compatibility and competitiveness with other soil micro organisms and higher nitrogen-fixing efficiency have been shown that can improve growth and yield components of inoculated legumes (Vessey, 2003). Beneficial effects of rhizobia on common bean have been described in several studies with different climatic and soil conditions (Peix *et al*., 2001; Tilak *et al*., 2006; Remans *et al*., 2008). All plant factors we measured in the study were positively affected by inoculation with rhizobia strains Rb-133 and Rb-136. Rhizobia strains were able to increase seed yield, number of pods per plant, number of seeds per pod, weight of 100 seed, weight of seeds per plant, seed protein yield, total dry matter in R_6 and protein content over uninoculated control plants. Amount of seed yield by inoculated plants was ranging from 1221 to 4693 kg ha^{-1} depending on the strain and cultivars used during two years of the study. However, Rb-133 showed a greater symbiotic efficiency than Rb-136. Plant cultivars also had different responses to rhizobial inoculation. Cultivar Akhtar demonstrated highest potential for seed yield, number of pods per plant,

Table 2: Number pod per plant (PP), seed per pod (SP), weight of 100 seed (WS), weight of seed per plant (WSP), weight of pod per plant (WPP), total dry matter in R_6 (TDM) and seed yield, seed protein yield by bean plants in the field experiment in the first year

Cultivar	Treatments	PP (plant^{-1})	SP (Pod^{-1})	WS (g)	WSP (g plant^{-1})	WPP (g plant^{-1})	TDM (g plant^{-1})	Seed yield (kg ha^{-1})	Seed protein yield (kg ha^{-1})
Sayad	Rb136	10.5±2.7	3.9±0.2	19.0±0.4	8.1±2.1	13.0±2.4	26.5±1.4	1579.1±77	327.3±65
	Rb136+*Pseudomonas*	14.7±2.3	4.2±0.1	24.2±1.0	15.1±1.2	24.3±1.2	26.7±0.7	2945.5±68	593.4±72
	Rb136+*Azospirillum*	10.2±1.7	3.3±0.6	23.5±1.2	8.0±0.3	12.9±0.5	26.4±1.9	1563.7±61	325.4±16
	Rb133	11.4±1.7	3.6±0.1	23.0±1.8	10.8±2.0	15.7±1.1	24.3±1.8	1899.9±12	365.5±30
	Rb133+*Pseudomonas*	14.8±3.8	4.1±0.3	24.6±0.5	17.3±1.0	24.5±1.7	25.6±1.9	2970.6±70	641.7±45
	Rb133+*Azospirillum*	12.1±1.5	3.2±0.2	23.4±0.4	11.5±2.1	15.3±2.8	24.9±2.3	1860.9±15	387.9±29
	Control	10.3±2.9	3.7±0.1	22.4±0.8	9.7±1.1	14.1±1.9	21.3±0.6	1714.5±54	335.8±62
	N-fertilized	12.3±2.4	3.5±0.4	24.3±0.9	12.6±2.6	16.8±1.9	25.7±2.0	2034.1±24	439.4±55
Akhtar	Rb136	12.4±2.4	3.4±0.1	30.7±0.5	13.2±2.6	21.7±2.3	29.4±1.8	2590.1±11	571.8±41
	Rb136+*Pseudomonas*	16.8±2.8	3.8±0.5	33.3±0.5	21.5±2.4	34.6±1.8	33.3±1.6	4199.9±47	921.4±56
	Rb136+*Azospirillum*	15.5±0.9	3.3±0.5	32.0±0.8	16.8±3.2	27.1±1.4	27.4±1.7	3279.4±66	703.3±55
	Rb133	12.2±1.7	3.6±0.2	30.1±0.5	15.0±2.5	21.8±1.8	31.1±1.3	2644.4±13	574.5±81
	Rb133+*Pseudomonas*	18.2±3.8	3.8±0.8	32.8±0.1	27.1±1.7	38.7±1.5	32.2±1.1	4693.3±18	1038.2±109
	Rb133+*Azospirillum*	33.1±1.2	3.3±0.3	32.0±0.1	17.1±2.3	22.8±1.4	28.5±1.6	2764.8±19	606.9±26
	Control	12.7±2.8	3.4±0.1	27.5±0.5	13.6±2.6	19.7±1.7	24.6±2.0	2395.6±49	495.0±59
	N-fertilized	14.1±0.2	3.3±0.2	32.0±0.1	18.3±1.5	24.4±1.9	32.0±3.0	2961.1±24	642.4±58
Goli	Rb136	10.7±1.5	3.8±0.2	22.7±2.3	9.8±2.5	15.8±2.2	23.7±1.3	1920.5±15	392.5±21
	Rb136+*Pseudomonas*	14.0±2.5	4.0±0.1	22.4±0.8	12.8±1.9	20.6±3.1	24.6±1.4	2504.5±38	484.9±72
	Rb136+*Azospirillum*	8.3±1.7	3.3±0.5	22.1±2.0	6.2±1.8	10.1±1.1	21.1±1.4	1221.2±37	252.3±80
	Rb133	10.9±1.2	3.3±0.1	21.9±1.7	8.9±1.8	13.0±2.5	21.3±3.2	1575.9±10	340.2±33
	Rb133+*Pseudomonas*	13.9±3.4	4.2±0.3	23.2±0.5	15.9±1.7	22.6±1.8	30.6±1.4	2735.7±71	566.5±61
	Rb133+*Azospirillum*	11.7±1.5	3.2±0.2	22.4±2.1	10.1±2.4	13.5±1.8	21.5±1.4	1631.2±12	330.1±38
	Control	9.8±1.5	3.6±0.1	19.5±0.5	7.8±2.3	11.4±1.7	20.5±0.9	1381.2±52	274.6±57
	N-fertilized	11.3±1.9	3.6±0.1	21.6±1.6	10.6±2.3	14.2±1.1	22.5±1.9	1720.2±38	358.1±67

Table 3: Number pod per plant (PP), seed per pod (SP), weight of 100 seed (WS), weight of seed per plant (WSP), weight of pod per plant (WPP), total dry matter in R_6 (TDM) and seed yield, seed protein yield by bean plants in the field experiment in the second year

Cultivar	Treatments	PP (plant^{-1})	SP (Pod^{-1})	WS (g)	WSP (g plant^{-1})	WPP (g plant^{-1})	TDM (g plant^{-1})	Seed yield (kg ha^{-1})	Seed protein yield (kg ha^{-1})
Sayad	Rb136	8.7±1.4	4.0±0.1	21.7±0.4	10.7±1.9	13.4±1.4	31.4±1.8	1536.0±74	327.9±66
	Rb136+*Pseudomonas*	11.0±1.0	4.7±0.2	27.7±1.2	20.3±2.6	25.4±3.3	69.5±1.7	2900.6±83	594.5±72
	Rb136+*Azospirillum*	10.2±1.7	3.8±0.6	26.9±1.4	12.5±2.0	17.8±0.7	35.1±1.3	2041.1±80	326.1±15
	Rb133	9.7±3.7	4.1±0.2	26.3±2.1	11.8±2.2	18.3±2.1	33.1±3.8	2089.0±33	366.3±30
	Rb133+*Pseudomonas*	10.7±1.1	4.7±0.3	28.2±0.6	21.4±2.5	24.5±1.8	71.1±1.6	2796.3±66	643.1±45
	Rb133+*Azospirillum*	10.3±1.6	3.6±0.2	26.8±0.4	15.6±2.4	17.7±1.1	42.5±1.7	2030.2±10	388.5±30
	Control	10.3±2.9	3.4±0.4	25.6±0.9	10.2±2.1	15.7±1.7	30.2±1.3	1798.6±48	336.5±12
	N-fertilized	13.0±0.6	3.9±0.5	27.8±1.0	23.7±1.2	27.1±1.8	57.6±1.7	2867.3±39	436.7±50
Akhtar	Rb136	10.3±2.0	3.9±0.1	35.1±0.5	20.2±2.5	25.3±2.4	35.6±2.5	2829.4±84	573.0±42
	Rb136+*Pseudomonas*	12.3±2.3	4.3±0.6	38.2±0.5	28.1±0.9	35.1±1.1	71.1±5.0	4007.4±31	923.3±56
	Rb136+*Azospirillum*	12.1±2.2	3.7±0.6	36.6±1.4	20.6±2.4	29.2±1.0	36.8±1.4	3332.4±89	704.7±55
	Rb133	10.6±1.8	4.1±0.3	34.5±0.5	16.9±2.3	26.3±2.3	38.1±1.8	3000.9±48	575.7±81
	Rb133+*Pseudomonas*	13.7±2.6	4.3±0.9	37.6±0.1	32.5±2.5	37.1±2.8	72.8±5.2	4240.3±29	1040.3±10
	Rb133+*Azospirillum*	11.3±3.0	3.7±0.3	36.6±0.1	23.9±2.4	27.3±1.5	45.4±6.1	3118.8±67	607.8±27
	Control	11.4±0.5	3.7±0.2	31.5±0.5	15.1±1.3	23.4±2.0	33.1±2.5	2678.2±33	496.0±59
	N-fertilized	10.4±0.6	3.7±0.3	36.6±0.1	28.8±1.2	32.9±1.4	62.7±5.7	2847.7±73	643.3±58
Goli	Rb136	7.9±1.6	3.9±0.2	25.9±2.6	11.7±2.3	14.6±1.7	28.8±3.8	1668.5±70	387.3±10
	Rb136+*Pseudomonas*	12.1±0.9	4.5±0.1	25.6±0.9	19.7±0.9	24.7±1.1	54.2±2.1	2817.5±31	485.8±73
	Rb136+*Azospirillum*	8.3±1.7	3.7±0.6	25.3±2.4	9.8±2.5	13.9±1.2	33.5±1.6	1594.0±82	525.8±81
	Rb133	8.8±1.6	3.7±0.2	25.0±1.9	9.4±1.7	14.6±2.8	29.6±2.5	1664.1±70	340.8±33
	Rb133+*Pseudomonas*	12.3±0.5	4.8±0.3	26.5±0.9	24.1±2.3	27.5±3.8	53.4±5.7	3143.4±41	567.7±61
	Rb133+*Azospirillum*	10.5±1.3	3.7±0.2	25.6±0.9	14.9±1.3	17.1±1.1	38.9±1.7	1948.9±97	330.5±39
	Control	9.8±1.5	3.3±0.4	22.3±0.5	8.1±2.3	12.6±1.4	28.1±2.5	1439.4±58	275.2±57
	N-fertilized	12.8±1.9	4.1±0.3	24.7±1.8	21.8±1.3	24.9±1.9	52.5±1.7	2583.6±48	358.6±67

number of seeds per pod, weight of 100 seed compared to cultivars Sayad and Goli. Differences among strains of common bean rhizobia and plant cultivars in their nitrogen-fixing performance were previously observed by Vladimir (2001), Bais *et al.* (2006), Ahmad *et al.* (2006) and Remans *et al.* (2008). Co-inoculation of the common bean with *Rhizobium* and PGPR resulted in better nodulation which was translated into higher shoot dry matter and seed yield production. This is in agreement with previous reports demonstrating the beneficial effects of PGPR belonging to *Pseudomonas* spp. and *Azospirillum* spp. on symbiotic efficiency of rhizobia nodulating different legume crops (Preston, 2004; Bashan and Holguin, 2004; Valverde *et al.*, 2006; Figueiredo *et al.*, 2007). The results

Table 4: Complex analysis of variance of number pod per plant, number seed per pod, weight of 100 seed, seed yield, weight of seed per plant, weight of pod per plant, protein seed yield, total dry matter in R_6 in bean plants that affected by several bacteria treatments

Source of variation	Degree of freedom	Pod of plant^{-1}	No. seed pod^{-1}	Weight of 100 seed	Seed yield	Weight of seed plant^{-1}	Weight of pod plant^{-1}	Protein seed yield	Total dry matter in R_6
		(Mean of square)							
Year (Y)	1	113.33^{ns}	5.53**	501.94**	1095394.24^{ns}	807.5**	340.18*	11.53^{ns}	13107.38**
R/Y	4	153.55**	0.1605^{ns}	2.418^{ns}	5697282.977**	183.94**	384.04**	406656.576**	15.66^{ns}
Bacteria (A)	7	38.93^{ns}	2.008**	34.31**	5851868.26**	365.03**	417.05**	325782.421**	9047.76**
Y*A	7	5.19^{ns}	0.22^{ns}	0.25^{ns}	177017.64^{ns}	46.82^{ns}	39.59^{ns}	3.72^{ns}	800.13*
Ea	28	30.53	0.381	2.07	1004158.33	38.9	70.51	69901.871	299.79
Cultivar (B)	2	57.31**	0.20**	1452.38**	21822391.31**	887.96**	1715.1**	1413991.57**	793.08**
Y*B	2	12.88**	0.05^{ns}	6.96**	127748.12^{ns}	5.07^{ns}	0.95^{ns}	5.80^{ns}	64.80^{ns}
A*B	14	4.61*	0.12**	10.33**	325631.66**	8.55^{ns}	16.58	23816.371**	17.04^{ns}
Y*A*B	14	2.46^{ns}	0.03^{ns}	0.06^{ns}	136392.40^{ns}	2.71^{ns}	6.65^{ns}	2.886^{ns}	35.30^{ns}
Eb	64	2.061	0.036	1.158	133616.10	5.228	10.018	8621.317	27.20
Coefficient variation		12.25	5.10	3.94	14.89	14.64	14.9	18.60	14.64

^{ns}, * and **: Non significant, significant at the 5 and 1% levels of probability, respectively

revealed that application of PGPR together with *Rhizobium* improved the growth and seed production by inoculated beans. As a result, gross average of seed yield increased from 1536-3000 kg ha^{-1} for *Rhizobium* alone to 1221-4693 kg ha^{-1} for those co-inoculated with *A. lipoferum* S-21 and *P. fluorescens* P-93, respectively. Present data showed that *P. fluorescens* P-93 had better promoting effect on yield components of rhizobia than *A. lipoferum* S-21. This difference is probably attributable to siderophore production as well as higher ability for auxin production and P-solubilizing activity of *P. fluorescens* P-93 (Table 1).

CONCLUSION

This study showed that plant growth and seed yield potential of *Rhizobium-P. vulgaris* varies with *Rhizobium* strains and plant cultivars. Co-inoculation of the common bean with Rb-133 and *Pseudomonas fluorescens* P-93 resulted in higher number seed per pod, weight of 100 seed, weight of seed per plant, weight of pod per plant, protein seed yield, total dry matter in R_6 and thereby produced greater seed yield. The results indicate that in spite of the fact that Rb-133+ *P. fluorescens* P-93 can increase the proportion of seed per pod and productivity in plants, application of complementary inorgamic nitrogen fertilizer in soils with low nitrogen content is needed.

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