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*Full Length Research Paper*

# Evaluation of bean (*Phaseolus vulgaris)* seeds' inoculation with *Rhizobium phaseoli* and plant growth promoting *Rhizobacteria* (PGPR) on yield and yield components

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**Growing evidence indicates that soil beneficial bacteria can positively affect symbiotic performance of rhizobia. 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 two consecutive years under field condition. 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 *P. fluorescens* P-93 gave the highest seed yield, number of pods per plant, weight of 100 seed, seed protein yield, number of seed per pod, and seed protein yield.**

**Key words:** Bean seeds, *Rhizobacteria*, yield component, plant growth.

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important legume for human nutrition and a major protein and calorie source in the world (Broughton et al., 2003). *P. vulgaris* can grow by assimilation of mineral N or molecular N fixation. A broad range of *Rhizobium* species are able nodule and fix $N_2$ with beans including *Rhizobium leguminosarum* biovar *phaseoli*, *R. tropic* and *Rhizobium etli.* In most Iran soils that are N deficient, $N_2$ 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 (Hardarson et al., 1993). However, its promising potential to fix nitrogen has been shown in several studies (Bliss, 1993; Epping et al., 1994; Asadi et al., 2005; Mnasri et al., 2007). Poor nodulation and variable response to inoculation is mainly attributed to intrinsic characteristics of the host plant, particularly the nodulation promiscuit Michiels et al. (1998)

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**Abbreviations: PGPR,** plant growth-promoting rhizobacteria; **N,** nitrogen; **IAA,** indole acetic acid; **BNF,** biological nitrogen fixation.

(1998) 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 (Graham, 1981; Giller and Cadisch, 1995). 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.

Biological nitrogen fixation reduces costs of production. The use of inoculants as alternatives to N fertilizer avoids problems of contamination of water resources from leaching and runoff of excess fertilizer. Utilizing biological nitrogen fixation (BNF) is part of responsible natural resource management. Legume inoculants do not require high levels of energy for their production or distribution. Application on the seed is simple compared to spreading fertilizer on the field. Inoculants increase legume crop yields in many areas. BNF often improves the quality of dietary protein of legume seed even when yield increases are not detected. Through practices such as green manuring, crop rotations and alley cropping, N fixing legumes can increase soil fertility, permeability and organic matter to benefit non legume crops. Using BNF is part of the wise management of agricultural systems. The economic, environmental and agronomic advantages of BNF make it a cornerstone of sustainable agricultural systems. Legumes comprise the most important plant families in agriculture. Many soils do not have sufficient numbers of appropriate rhizobia for maximum BNF. Rhizobial inoculants and legume crops must be properly matched. There are several methods of inoculating legumes. Inoculants require some special care to maintain their viability.

Although BNF is a natural process, many soils do not have sufficient numbers of appropriate rhizobia for effective symbiosis. Inoculating legume crops with compatible rhizobia ensures maximal BNF. Inoculation is especially important when introducing new legumes to an area (Silva and Uchida, 2000; Dobbelaere et al., 2003; Zhang et al., 1997). Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Glick, 1995; Cleyet-Marcel et al., 2001; Braneix et al., 2005), production of plant growth regulators (De Freitas, 2000) and increasing plant water and nutrient uptake (Okon and Labandera-Gonzalez, 1994; Jacoud et al., 1999). PGPR can also inhibit soil-borne plant pathogens through antifungal activity (Lifshitz et al., 1987) and siderophore production (Suneja et al., 1996). Greenhouse and field studies with PGPR strains have demonstrated enhanced nodulation and nitrogen fixation in soybean, lentil, pea, chickpea and common bean (Dashti et al., 1997, 1998; Chanway et al., 1989; Chanway et al., 1989; Dileep Kumar et al., 2001; Parmar and Dadarwal, 1999; Grimes and Mount, 1984). The effects of native PGPR strains on different crop plants in Iran have been studied earlier (Khalilian, 2006; Mahour, 2005). However, when highly effective *Rhizobium/Phaseolus* combination has been selected, they provide bean yield of 60 - 70% of those obtained with N fertilizer control under field conditions (Santa Maria et al., 1997).

Determination of $N_2$ fixation effectiveness in the process of strain selection is normally a multiple step procedure involving an initial selection under greenhouse conditions and a final testing in field trails (Navarro et al., 1999). In the process of strain selection, variation in the efficiency of the strain bean cultivars association was detected for parameters like total N, yield, plant growth, nodule number and weight and N fixed (Santa Maria et al., 1997).

## 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 work.

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 et al., 2005). PGPR strains *Pseudomonas fluorescens* P-93 and *Azospirillum lipoferum* S-21 with known positive effects on wheat (Mahour, 2005; Khalilian, 2006) were also used in our study. 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 per litre and mixed thoroughly.

### Experimental conditions

Field trails were established in 2006 and 2007 at Shahrekord (50°51′N 32°17′E) South western in Iran. The soil physical and chemical properties are shown in Table 1. 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. Common bean cultivars formed the subunits and there were a total of three units within each main plot.

Plants were harvested at physiological maturity and seed yield, number of pods per plant, number of seeds per pod, weight of 100 seed, weight of seeds per plant, seed protein yield and total dry matter in $R_6$ were measured from two central rows 4 m long. The micro Kjeldahl method was used to determine the seed nitrogen content which was multiplied by 6.25 to determine seed protein content (El Hadi and Elsheikh, 1999).

Results were analysed statistically by analysis of variance using the SAS computer package (SAS, 9) and treatments meanseparated using Duncan’s multiple range test at $p < 0.05$ level.

**Table 1.** Some physical and chemical properties of soil for field experiment (0 -30 cm).

| Year | Texture | pH | EC | O.C | $N_{total}$ | P | K | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (dS $m^{-1}$) | (%) | | (mg $kg^{-1}$) | | | | | |
| 1 | Loam | 8.3 | 0.47 | 0.79 | 0.07 | 8.1 | 245 | 1.1 | 3.4 | 3.8 | 1.1 |
| 2 | Loam | 7.8 | 0.44 | 0.70 | 0.06 | 7.8 | 235 | 1.1 | 3.1 | 3.5 | 1.0 |

## RESULTS

The effects of bacterial inoculation on plant growth and symbiotic characteristics are given in Tables 3 - 5. 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 of seed per pod, number of 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 R6 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 *P. fluorescens* P-93.

Similar result was obtained for shoot dry matter of plants in R6 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 (4693.29 kg/ha) which showed a 240% increase over control (1381.18 kg/ha) plants.

Plants inoculated with *Rhizobium* alone or co-inoculated with PGPR had greater seed yield than non-inoculated control. The most promising effect for seed yield production was obtained from co-inoculation of *Rhizobium* with *P. fluorescens* P-93 and resulted in a significant ($p < 0.01$) increase over *Rhizobium* alone for cultivar Akhtar than other cultivars. Similar pattern was also demonstrated for seed protein yield. Plants co-inoculated with *Rhizobium* and PGPR showed greater seed protein-content compared to control plants. The highest seed protein-content was achieved by Rb-133+ *P. fluorescens* p-93 in all cultivars.

The amount of seed yield was affected by co-inoculation of *Rhizobium* with PGPR strains in cultivars used in this study (Tables 3 - 5). *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.

## DISCUSSION

This work 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 to 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 (Epping et al., 1994; Mostasso et al., 2002; Asadi et al., 2005; Mnasri et al., 2007). All plant factors 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 - 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, number of seeds per pod, and 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 other researchers(Graham, 1981; Rennie and Kemp, 1983; Lalande et al., 1990; Popescu, 1998; Mostasso et al., 2002). Co-inoculation of the

**Table 2.** Some characteristics of PGPR strains used in the study.

| Strains | P-solubilization ($\mu$g P ml$^{-1}$ week$^{-1}$) | Auxin production ($\mu$g 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 | 12 | – | – |

**Table 3.** Number of 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 R6 (TDM), seed yield, and seed protein yield by bean plants in the field experiment in the first year.

| Cultivar | Treatment | 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 (kgha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| **Sayad** | Rb136 | 10.5 ± 2.7 | 3.9 ± 0.2 | 19 ± 0.4 | 8.1 ± 2.1 | 13 ± 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 | 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 ± 0.3 | 12.9 ± 0.5 | 26.4 ± 1.9 | 1563.7 ± 61 | 325.4 ± 16 |
| | Rb133 | 11.4 ± 1.7 | 3.6 ± 0.1 | 23 ± 1.8 | 10.8 ± 2 | 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 | 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 | 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 ± 0.8 | 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 ± 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 ± 0.1 | 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 | 2395.6 ± 49 | 495 ± 59 |
| | N-fertilized | 14.1 ± 0.2 | 3.3 ± 0.2 | 32 ± 0.1 | 18.3 ± 1.5 | 24.4 ± 1.9 | 32 ± 3 | 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 ± 2.5 | 4 ± 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 | 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 ± 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 4.** Number of 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 R6 (TDM), seed yield, and seed protein yield by bean plants in the field experiment in the second year.

| Cultivar | Treatment | PP (plant$^{-1}$) | SP(Pod$^{-1}$) | WS (g) | WSP (g plant$^{-}$) | WPP (g plant$^{-1}$) | TDM (g plant$^{-1}$) | Seed yield (kg ha$^{-1}$) | Seed proteinyield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Sayad | Rb136 | 8.7 ± 1.4 | 4 ± 0.1 | 21.7 ± 0.4 | 10.7 ±1.9 | 13.4 ± 1.4 | 31.4 ± 1.8 | 1536 ± 74 | 327.9 ± 66 |
| | Rb136+*Pseudomonas* | 11 ± 1 | 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 | 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 ± 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 ± 0.6 | 3.9 ± 0.5 | 27.8 ± 1 | 23.7 ± 1.2 | 27.1 ± 1.8 | 57.6 ± 1.7 | 2867.3 ± 39 | 436.7 ± 50 |
| Akhtar | Rb136 | 10.3 ± 2 | 3.9 ± 0.1 | 35.1 ± 0.5 | 20.2 ± 2.5 | 25.3 ± 2.4 | 35.6 ± 2.5 | 2829.4 ± 84 | 573 ± 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 | 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 | 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 | 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 | 33.1 ± 2.5 | 2678.2 ± 33 | 496 ± 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 ± 82 | 525.8 ± 81 |
| | Rb133 | 8.8 ± 1.6 | 3.7 ± 0.2 | 25 ± 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 |

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 (Rai, 1983; Dashti et al., 1997; Oliviera et al., 1997; Parmar and Dadarwal, 1999; Singer et al., 2000; Figueiredo et al., 2007). The results 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 for *Rhizobium* alone to 1221 - 4693

**Table 5.** Complex analysis of variance of number of pod per plant, number of seed per pod, weight of 100 seed, seed yield, weight of seed per plant, weight of pod per plant, protein seed yield and total dry matter in R6 in bean plants that are affected by several bacteria treatments.

| Source of variation | Degree of freedom | 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 R6 |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean of square | Mean of square | Mean of square | Mean of square | Mean of square | Mean of square | Mean of square | 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$^{**}$ | 0.2$^{**}$ | 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$^{ns}$ | 5.8$^{ns}$ | 64.8$^{ns}$ |
| 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$^{ns}$ | 136392.4$^{ns}$ | 2.71$^{ns}$ | 6.65$^{ns}$ | 2.886$^{ns}$ | 35.3$^{ns}$ |
| Eb | 64 | 2.061 | 0.036 | 1.158 | 133616.1 | 5.228 | 10.018 | 8621.317 | 27.2 |
| Coefficient of variation | | 12.25 | 5.1 | 3.94 | 14.89 | 14.64 | 14.9 | 18.6 | 14.64 |

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

kg/ha for those co-inoculated with *A. lipoferum* S-21 and *P. fluorescens* P-93, respectively. Our 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 2).

The mechanisms of growth and nitrogen fixation promotion by PGPR are not well understood; however, a wide range of possibilities including both direct and indirect effects have been suggested (Dashti et al., 1997). The regulation of root system development in plants depends on auxin activity. In legume root nodules, IAA which is produced by most of PGPR, activates the enzyme $H^+$-ATPase, which is fundamental for energy production in the nodules (Rosendahl and Jochimsen, 1995). It is also well known that plant root flavonoids are the inducers of nodulation gene (nod genes) expression in *Rhizobium* (Maxwell et al., 1989; Peter and Verma, 1990). Rhizobacteria have also been found to induce phytoalexins (a class of fluorescent compounds, closely related to flavonoids and isoflavonoids) in roots of several crop plants (Vanpeer et al., 1991). These phytoalexins have a direct bearing on plant protection mechanisms against pathogens, which help root development (Parmar and Dadarwal, 1999). Mobilization of insoluble nutrients (especially P) followed by enhancement of uptake by plants (Lifshitz et al., 1987) and production of pathogen-inhibiting substances (Lie and Alexander, 1988) can also positively affect the *Rhizobium*-legume symbiosis.

## 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 *P. fluorescens* P-93 resulted in higher number of seed per pod, weight of 100 seed, weight of seed per plant, weight of pod per plant, protein seed yield and total dry matter in R6, 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 inorganic nitrogen fertilizer in soils with low nitrogen content is needed.

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