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Effects of seed inoculation by *Rhizobium* strains on yield and yield components in common bean cultivars (*Phaseolus vulgaris* L.)

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Abstract

To study the effect of *Rhizobium* inoculation treatments (inoculation with strains number 133, 116 and their integrated application) and chemical nitrogen fertilizer application (based on soil analysis in form of Urea) on certain agronomic traits of three common bean cultivars (viz. Bahman, Darkhshan and Sayyad) a field experiment was conducted at Agricultural Research Farm of College of Agriculture, University of Tehran (Karaj, Iran) during 2010 and 2011 growing seasons. The treatments arranged as factorial based on a randomized complete block design with three replications. The characteristics such as seed yield, pods number per plant, 100 seed weight, harvest index and biological yield were measured. Inoculated bean cultivars by rhizobium strain 116 produced the highest bean grain yield (5791 kg/ha). Bahman cv. inoculated by rhizobium strains 133+116 produced the highest number of pods per plant (55.97 pods/plant). Sayyad cultivar inoculated by rhizobium strain 116 produced the highest seed yield. Under the environmental conditions of this experiment, application of rhizobium strains proved to have the capability to replace the chemical nitrogen fertilizer in bean production.

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Introduction

Beans are considered as one of the most important gain legumes to provide the calorie and protein sources for human beings. Beans protein content of 20-25% and carbohydrate of 50-56% makes it 2 to 3 times more notorious compared to cereals (Majnoun Hosseini, 2008). The mean bean yield is low (600 kg/ha) across the whole world (Graham and Ranalli, 1997). One of the reasons for this low yield has been reported to be the mismanagement of nitrogen fertilizing system. It has been reported that the average chemical nitrogen application in America and Africa is around 505 kg/ha (Hungria *et al.* 1997). Nitrogen deficiency is considered as one the most important limiting factors in crop production. The mean nitrogen content in different crops varies between 1 to 6 percent. Nitrogen is the main element in bio-molecular structure (Stacey *et al.* 1992). The over application of nitrogen fertilizer in crop production have created adverse effects on soil, under-ground water and the environment.

The environmental friendly pro-biotic bacteria like rhizobium and their role in nitrogen fixation has come to the attention of the researchers in recent years. One of the most efficient symbiotic bacteria with legumes is Rhizobium leguminosarum (Giller, 2001). N2-fixing is important for crop plants as they increase N uptake and play a crucial role as Plant growth promoting rhizobacteria (PGPR) in biofertilization system (Zaidi and Mohammad, 2006). Thus, the application of such microbes as environment friendly biofertilizer may contribute to minimize the use of expensive chemical fertilizers. According to some researcher, nitrogen fixation is a genetically controlled quantitative characteristic and bean cultivars are very diversified in this trait (Anyango *et al.* 1995). Bean shows a good and positive reaction to inoculation by proper bacteria, however, there is a lack of efficient bacteria in most of the agricultural soils (Rodriguez-Navarro *et al.* 2000). Seed inoculation by proper Rhizobium strain caused 78% increase in yield compared to control in wax bean (Asadi Rahmani *et al.* 2005).

Despite of this, bean seed inoculation is still vastly practiced (Barron *et al.* 2000). Tang *et al.* (2001) declared that seed inoculation along with minor amounts of chemical nitrogen and phosphorous fertilizer application at early growth stage (10 day after germination) could stimulate the root nodulation and increase biological nitrogen fixation. Significant interactions have been reported between bean cultivars and different Rhizobium strains in regard to nodulation, biomass production and sap chemical components (Rodriguez-Navarrro, 2000). There are different result about this issue so the objective of this research was to evaluate the seed inoculation of different bean cultivars by different Rhizobium strains to identify the best inoculants in regard to seed yield and component of yield for bean at field condition.

Materials and methods

Location and experimental design

This experiment was conducted in Research Farm of College of Agriculture, University of Tehran in Karaj, Iran during 2008 and 2009 growing seasons. The experimental site is located in 35: 56 northern latitude and 50: 58 eastern longitude with 1112.5 m height from the sea level. The treatments arranged as factorial based on a randomized complete block design with three replications. Two Rhizobium strains of 133, 116 *and* their integrated application (133+116), chemical nitrogen fertilizer application (based on soil analysis of 100kg N/ha from Urea source) and control (no inoculation or fertilizing treatment) were considered as the first variable and three common bean cultivars (viz. Bahman, Darakhshan and Sayyad) were the second variable in this experiment. Sayyad and Darkhshan bean cultivars have a semi erect and erect growth habits with Columbia origin, respectively. The Rhizobium strains were provided by Soil and Water Research Institute of Iran.

Soil analysis

Soil analysis of the experimental site is presented in Table 1.

Seed inoculation

Seed inoculation was done according to Subba Rao, (1988) method. Inoculated seeds were dried in shade and instantly planted on May 9th and May 17th in 2008 and 2009, respectively.

Crop management

The experimental plot dimensions were 2.5 x 3 m consisting of 5 rows of cultivated bean 50cm apart. The seeds were planted 15cm apart on each row. A furrow irrigation system was employed during experimental period. All the plots were subjected to hand weeding 25 and 50 days after planting the seeds.

Yield and component yield

After the physiological maturity the experimental plots were harvested and seed yield and seed yield components (100 seed weight, pod/plant, harvest index and ect.) along with biological biomass, and seed protein content were measured.

Statistical analyses

After the homogeneity test for collected data variance, statistical analysis was performed using Mstat-C software and the means were compared at P<0.05% probability level.

Results

Bahman cultivar produced the highest seed yield (5540 kg/ha) among all the evaluated bean cultivars (p<0.05%) (Table 2). The higher seed yield in the first year could be explained by few days delay in planting in the second year (Table 5). Seed inoculation by Rhizobium strain 116 produced more seed yield across all bean cultivars (Table 3). No significant interaction effect of bean cultivar and fertilizing system was observed for seed yield. The difference in seed yield by bean cultivars could be due to the Rhizobium ability to fix and provide nitrogen for bean plants. Also, Bahman cultivar achieved the highest number of pods (49.7) per plant (p<0.05%). Seed inoculation by Rhizobium 116 produced the highest number of pod per plant (43.1) across all bean cultivars (p<0.05%) (Table 3). Bahman cultivar was the best pod producer (66 pods/plant) when it was inoculated by a combination of rhizobium strains (116 + 113) (Table 4).

Table 1. Physical and chemical soil analysis of the experimental site (2008 and 2009)

Year	Texture	Total N mg/kg	P mg/kg	K mg/kg	pH
2008	loam	90	14.2	151	8
2009	loam	97	13.5	180	7.9

Table 2. Analysis of variance on Rhizobium strains effects on different seed yield and seed yield components in bean cultivars

Sources of variance	df	100 seed weight	Seed yield per hectares	Harvest index	Pod number	Biologic yield per hectare
Year	1	0.543	2807736.734**	1.567	45.457	9860422.119
Replication*(year)	4	20.956	806451.721	71.261	25.332	4737008.636
Variety (A)	2	1875.841**	511830.290	207.548	3123.627	28130081.365
Year*variety	2	0.070	25065.430	9.112	8.570	1130836.515
Nitrogen (B)	4	17.445	1247603.664	9.085	132.498	5138002.098**
Year*nitrogen	4	0.446	17610.238	1.305	1.111	87830.016
Variety * Nitogen	8	24.302	427041.303	80.072**	145.514	8010399.231
Year*variety* Nitrogen	8	0.382	35591.158	4.647	2.561	73229.928
Error	56	13.078	364465.035	24.470	22.458	1111462.895
Coefficient of variance (%)	-	12.14	11.18	10.65	11.89	8.98

ns ،*and **: non-significant, significant diferences at 1% and at 5% probability levels

Drakhshan cultivar produced the heaviest seeds (38.8 gr/100 seeds) among the bean cultivars ($p<0.05\%$) (Table 3). The highest HI (49.4) was observed in Sayyad cultivar across all fertilizing treatments ($p<0.05\%$) (Table 3). However, the same cultivar demonstrated the highest HI (52.7) at no fertilizing treatment as an interaction effect (figure 3). This result corresponds with Rahaman-Roy (1992) results working on lentil.

Table 3. Mean comparisons of different agronomic and physiologic traits of bean cultivars.

Treatments	100 seed weight (gr)	Seed yield per hectare (kg/ha)	Harvest index (%)	Pod number	Biologic yield per hectare (kg/ha)
Bahman variety	26.44 b	5540 a	44.24 b	49.67 a	12680 a
Derakhshan variety	38.83 a	5376 b	45.71 b	29.30 c	11800 b
Sayyad variety	24.12 c	5282 c	49.35 a	40.60 b	10750 c
Control	28.56a	5132 b	46.20 a	36.89 c	11250 b
100% chemical nitrogen	29.17 a	5291 b	45.51 a	39.50 b	11750 ab
Strain no 133	30.47 a	5248 b	47.05 a	37.66 c	11220 b
Strain no 116	31.02 a	5791 a	47.25 a	43.11 a	12490 a
12000 a	29.75 a	5534 ab	46.15 a	42.11 ab	133*116 strains
ns ،*and **: non-significant, significant diferences at 1% and at 5% probability levels					

The biological yield was significantly ($p<0.05$) affected by bean cultivar, fertilizing treatment and their interactions. Biological dry matter per plant was higher in the first year compared to the second year (Table 5). The highest biologic dry matter (1268 g/plant) was produced by Bahman cultivar and by rhizobium strain 116 inoculation (1249 gr/plant), respectively (Table 3). As a response to interaction effect, Bahman produced the highest biologic dry matter (1368 gr/plant) when it was inoculated by rhizobium strain 116 (Table 4). The findings of Asadi and Saleh (1999) working on beans supports the findings of this experiment.

Table 4. The interaction effects of bean cultivars and fertilizing systems on different agronomic and physiologic traits of bean.

Treatments		100 seed weight (gr)	Seed yield per hectare (kg/ha)	Harvest index (%)	Pod number	Biologic yield (kg/ha)
Bahman variety	Control	24.10 c	5326 g	41.48 d	45.54 bc	12830 abc
	100% chemical nitrogen	24.38 c	5523 d	41.17 d	52.75 a	13420 ab
	Strain no 133	29.15 b	5291 h	50.74 ab	42.44 cd	10440 ef
	Strain no 116	25.42 bc	5640 c	42.47 cd	51.64 a	13680 a
	133*116 strains	29.16 b	5919 b	45.33 bcd	55.97 a	13040 abc
Derakhshan variety	Control	38.43 a	5225 j	44.45 bcd	29.90 fg	11720 cde
	100% chemical nitrogen	38.75 a	5465 e	44.97 bcd	29.93 fg	12160 bcd
	Strain no 133	39.23 a	5192 k	43.46 cd	28.57 g	12000 cd
	Strain no 116	40.52 a	5639 c	50.35 ab	27.23 g	11290 de
	133*116 strains	37.20 a	5362 f	45.35 bcd	30.87 fg	11830 cd
Sayyad variety	Control	23.16 c	4846 m	52.67 a	35.23 ef	9207 f
	100% chemical nitrogen	24.38 c	4887 l	50.41 ab	35.83 ef	9679 f
	Strain no 133	23.04 c	5260 i	46.96 abcd	41.96 cd	11210 de
	Strain no 116	27.13 bc	6093 a	48.92 abc	50.46 ab	12500 abcd
	133*116 strains	22.89 c	5322 g	47.79 abcd	39.50 de	1140 de

Means with the same letter in each column are not significantly different at 5% probability level.

Seed inoculation by Rhizobium strains performed superiority over control (no fertilizer treatment) and chemical nitrogen fertilizer treatment in regard to seed yield, pod number per plant and seed number per pod.

Discussion

Isik *et al.* (1997) reported that varieties with unlimited growth have the ability to produce higher seed yield compared to varieties with limited growth habit. In this study, Bahman cultivar produced the highest seed yield, pod number per plant, biologic dry matter among all the evaluated bean cultivars. Neveen *et al.* (2008) reported dual inoculation increase plant productivity. Ali *et al.* (2008) reported inoculated *Pisum sativum* L. had significantly higher nodule number, nodule weight, root weight, shoot weight, seed yield and foliage yield compared to non-inoculated plants. Our results are supported by findings of other researchers (Shisanya, 2002 and Tamimi, 2002). Abbasi *et al.* (2010) reported that soybean seed yield quadratically increased by Rhizobium inoculation and Phosphorous application.

Table 5. Mean comparisons of different agronomic and physiologic traits of bean cultivars in two years of the experiment.

Years	100 seed weight (gr)	Seed yield per hectare (kg/ha)	Harvest index (%)	Pod number	Biologic yield per hectare (kg/ha)
2010	29.718 b	5575.91 a	46.565 a	40.566 a	12075.026 a
2011	29.873 a	5222.65 b	46.301 b	39.144 b	11413.029 b

Upadhyay *et al.* (1999) demonstrated a significant increase in number of seed and pod per plant as well as seed yield (2000 kg/ha) in Mung bean when the seed was inoculated by proper Rhizobium bacteria. In the experiment conducted by Rodriguez-Navaro *et al.* (2000), a significant increase in pod number per plant was observed among different bean cultivars in which Morada cultivar produced the highest number of pods (91 pod/plant) when it was inoculated by Semia-481 Rhizobium strain. These results are also supported by Sharma *et al.* (2000) working on black Mung bean. Mahmoud *et al.* (2010) reported biological fertilizer (Nitrobin) application significantly increase pod number in bean. Some researchers reported that inoculation of bacteria and nitrogen applications did not affect the number of pods per plant (Bozoglu *et al.* 1997). However, Karahan and Şehirali (1999) explained that both inoculation of bacteria and application of nitrogen significantly increased the number of pod per plant. Zhang *et al.* (2002) and Kazemi *et al.* (2005) reported that seed inoculation by Rhizobial bacteria significantly increased the number of pods and seed per plant, thousand grain weights and finally the yield of soybean

This confirms that varieties have different genetic potential in producing seeds. Westermann and Crother (1997) reported that beans with erect growth habit have higher 100 seed weight compared to prostrate varieties. Yield and yield components of Mung bean such as number of pods per plant, number of seeds per plant, 100-seed weight and seed yield crop were significantly affected by both inoculation and nitrogen fertilizer application (Shehzad Anjum *et al.* 2006). However, Bozoglu *et al.* (1997) and Kacar *et al.* (2004) reported that bacteria inoculation and nitrogen application didn't affect the 1000 seed weight.

Giller (2001) indicated that Rhizobium bacteria increase nitrogen absorption from soil and stimulate plant biomass production. Sharma *et al.* (2000) reported the significant effect of seed inoculation on plant height and biomass dry mater compared to control. The influence of Rhizobium bacteria on promoting legumes growth has been documented by many researchers (Rudresh *et al.* 2005; Malik *et al.* 2006). The observed benefits on bean by rhizobium inoculation seem to be due to the supply of N to the crop (Togay *et al.* 2008). Moreover, growth promoting substances

(phytohormones) are produced by these organisms. Rhizobium bacteria synthesize phytohormones like auxin as secondary metabolites in inoculated plants. These results corresponds to the results reported by Gamini Senevirante and Ekanayake, (2000) working with different strains of *Bradyrhizobium japonicum* and soybean.

Table 6. Percent increase in seed yield as affected by inoculation by different Rhizobium strains compared to 100% chemical nitrogen fertilizer and control (no fertilizer treatment) during the two years of experimental period

100% chemical nitrogen	Control	Strain no 133	Strain no 116	133*116 strains	
5291	5122	5248	5791	5534	Mean seed yield in each treatment
3.09	–	2.26	12.84	7.82	Percent increase compared to control (no inoculation)
–	–	-0.81	9.45	4.59	Percent increase compared to 100% chemical nitrogen fertilizer

Conclusions

Seed inoculation by Rhizobium strains performed superiority over control (no fertilizer treatment) and chemical nitrogen fertilizer treatment in regard to seed yield, pod number per plant and seed number per pod. Seed inoculation by Rhizobium strain 116 significantly produced higher seed yield across all bean cultivars (Table 6). Sayyad bean cultivar inoculated by Rhizobium strain 116 had the best yield and yield component performance. The results of this experiment suggest that under the environmental conditions of this experiment, application of rhizobium strains have the capability to replace the chemical nitrogen fertilizer in bean production.

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