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EFFECT OF NITROGEN FIXING BACTERIA AND NITROGEN RATE ON YIELD AND GROWTH OF COMMON BEAN

Somaye Fallahi[1], Peyman Sharifi[1]

[1] Department of Agronomy and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran

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Abstract

To study the effects of nitrogen (N) rate and co-inoculation of nitrogen-fixing bacteria (*Rhizobium phaseoli)* on common bean, an experiment was performed in Rasht, Iran. Common bean genotype, Guilanian landrace, was planted in a factorial experiment based on a randomized complete block design with three replicates on April, 15th in 2014. The first and second factors were nitrogen rates (0, 30, 60, 90 and 120 kg.ha^{-1} as urea) and *Rhizobium phaseoli* (inoculation and un-inoculation), respectively. Nitrogen had significant effect on seed yield and plant height. The effect of *Rhizobium phaseoli* was significant on seed yield, plant height, pod length, pods per plant, seeds per pod and nitrogen content in leaf. Interaction effects of nitrogen and *Rhizobium phaseoli* were not significant on any of the traits. Application of 60 kg.ha^{-1} nitrogen significantly improved seed yield, plant height and number of pods per plant. Nitrogen supply beyond the 60 kg.ha^{-1} decreased plant height, pods per plant and seed yield. The greatest values of seed yield (1635 kg.ha^{-1}), plant height (30.01 cm), pod length (13.19 cm), number of pods per plant (6.7), number of seeds per pod (4.34) and nitrogen content in leaf (4.12%) was observed in inoculation treatment. The results indicated the inoculation of common bean seeds by *Rhizobium phaseoli* improved the plant growth, productivity and quality. Therefore, the common bean inoculation with *Rhizobium phaseoli* is suitable to achieve the yield potential and reduce the adverse effects of environmental and may be recommended due to its advantages in terms of reduced application of nitrogen fertilizer.

Keywords: *Phaseolus vuigaris*, protected agriculture, *Rhizobium phaseoli*, mineral, yield

INTRODUCTION

Common bean *(Phaseolus vuigaris* L.) are as a perfect food because of its generous amounts of fiber and high protein content, complex carbohydrates and other nutritional needs including folic acid, iron, copper, potassium and zinc. Beans complement other food crops like maize and rice which are primary sources of carbohydrates (Xu and Chang, 2008).

Nitrogen (N) is recognizing a major constraint to crop production and require by plants in comparatively larger amounts than other elements (Habete and Buraka, 2016). Beans use inorganic soil nitrogen or applied fertilizer nitrogen and N_2 fixed by a symbiotic relationship with *Rhizobium phaseoli*. The nitrogen fixing bacteria have ability to improve the growth and yield of plants by fixing the atmospheric nitrogen and play an important role to overcome this problem. Although the association legume plants with *Rhizobium* bacteria provides a part of the nitrogen, but the amount supplied is not adequate for the nitrogen necessary of crop. Bean plants with deficient in nitrogen show lower development and grain yield (Oliveira *et al*., 1996).

Bacteria from *Rhizobium* genera are fixing nitrogen symbiotically in a specific organ called as nodule (Dixon and Kahn, 2004). It is necessary the inorganic and symbiotic nitrogen sources for highest seed yield of legumes; however, as the increases soil nitrogen or fertilizer nitrogen can lead to decreases the fixing of nitrogen by the symbiosis bacteria (Gicharu *et al.*, 2013). *Rhizobium* inoculation increased shoot and root dry weight, yield components and photosynthetic pigments in bean and most of the legumes (Lamptey *et al.*, 2014). Unquestionably, nitrogen fertilizers at different sources with low quantities are important for increasing yield and growth of legumes as well as chlorophyll and carotenoids content in leaves (Daur *et al.,* 2008). A relatively low amount of available N during the initial plant development generally increases nodulation and plant growth (Aslam *et al.*, 2010).

Several studies have tried to discover the effects of *Rhizobia* strains to cope the nitrogen requirements of bean (Stajkovic *et al.,* 2011; Vargas *et al.,* 2000; Habete and Buraka, 2016). Unfortunately, biological nitrogen fixation in common bean field crops has exhibited unstable behavior (Soares *et al.,* 2006). Some of studies indicated beans respond to nitrogen fertilization (Soratto *et al.,* 2010; Salehin and Rahman, 2012; Abdzad Gohari and Amiri, 2010). This indicates that their symbiotic N_2 fixation process generally does not offer sufficient N for most yields. Bozorgi *et al.* (2011) indicated significant effects of nitrogen on seed yield, biological yield, harvest index, plant height, 100 seed weight, pods per plant and seeds per pod in faba bean.

The main purpose of present study was to assess the effects of nitrogen fertilizer rate and nitrogen fixing bacteria (*Rhizobium phaseoli* L.) on growth and yield of common bean.

MATERIALS AND METHODS

A field experiments carried out during the spring of 2014 at the experimental farm (longitude, 49° 38´ E; latitude, 37° 12´ N and altitude, 7 m below the mean sea level) in Rasht, Guilan, Iran, to study the effect of nitrogen fertilization rate and nitrogen fixing bacteria (*Rhizobium phaseoli* L.) on yield and growth of common bean. The soil chemical properties before the start of the experiment are presented in Tab. I.

The treatment combinations were randomly arranged in a factorial design based on randomized complete block design with three replicates. The first factor was containing five nitrogen rates (0, 30, 60, 90 and 120 $kg.ha^{-1}$ as urea) and the second factor was *Rhizobium* treatment including (inoculation and un-inoculation with *Rhizobium phaseoli*). To inoculate the seeds with N fixing bacteria (*Rhizobium phaseoli*) bacteria, the seeds were first disinfected in 2% sodium hypochlorite solution for three minutes and then rinsed five times with distilled water. For further adhesion, before inoculation, the seeds were impregnated with water and sugar solution and then inoculated at a rate of 7 $ml.kg^{-1}$. They were kept in the shade for two hours and immediately cultured. (Alikhani *et al.*, 2006). The nitrogen rates were applied, using urea, 20 days after seedling emergence between rows.

This experiment was conducted on field and the genotype used was a common landrace of bean (*Phaseolus vuigaris* L.) in Guilan province, which is widely planted in this area of northern Iran. Inoculated and un-inoculated seeds were planted on 15th April. Basal fertilizers were used at the rates of 31 $kg.ha^{-1}$ P and 50 $kg.ha^{-1}$ K, as triple superphosphate and potassium sulfate, respectively. All of the phosphorus and potassium fertilizers were used when of last land preparing stage.

The common bean was planted at the rate of 22 plants m^{-2} (30 × 15 cm spacing distance). All the seeds were sown in plots of 3 × 4 m in dimensions. Weeds were removed by hand and plots were irrigated as required through the growing seasons. Plants were harvested by hand, when pods matured in June. Ten mature plants were randomly sampled from each plot and evaluated for the number of pods per plant, number of seeds per pod, plant height, pod length and 100 seed weight. Seed yield were measured in six meter square area of each plot. The leaves were dried at a temperature of 75 °C for 48 h in an Owen and then total nitrogen content of samples was measured by the Kjeldahl method (Kjeldahl, 1883).

Statistical analysis was performed using SAS, version 9.1 (SAS Institute, Cary, NC). Means were compared using LSD (least significant differences) test at 5% probability level.

RESULTS

The effect of nitrogen rate was significant only on seed yield and plant height. The effect of *Rhizobium phaseoli* was significant on seed yield, plant height, pod length, pods per plant, seeds per pod and leaf N content. Interaction effects of nitrogen and *Rhizobium phaseoli* were not significant on any of traits (Tab. II).

I: *Some of soil characteristics in experimental field*

Sand (%)	Clay (%)	Loam (%)	Acidity or PH	Electrical conductivity ($dS.m^{-1}$)
37.5	23	39.5	6.01	0.41

Absorbable potassium (ppm)	Absorbable phosphorus (ppm)	Nitrogen (%)	Carbon (%)	Tissue
200	7.78	0.08	0.94	Loam

II: *Analysis of variance for yield and yield components*

SOV	df	Mean Square							
		SY	HSW	PH	NPP	NSP	PL	SN	LN
R	2	43093.9	5.83	4.85	0.03	0.26	0.79	0.019	1.12**
N	4	167038.4**	39.58	30.53**	0.58	0.43	0.24	0.077	0.12
Ri	1	448229.6**	40.83	109.44**	1.32**	1.16**	3.67**	0.031	1.03*
N*Ri	4	4990.8	9.58	7.98	0.10	0.06	0.18	0.029	0.10
Error	18	10979.64	16.94	3.05	0.28	0.18	0.37	0.025	0.104
CV		6.92	10.08	6.22	8.19	10.25	4.73	4.99	8.28

*, ** significant at 5% and 1% probability levels, respectively
N: nitrogen fertilizers, Ri: *Rhizobium phaseoli*, PH: plant height, PL: pod length, NPP: number of pods per plant, NSP: number of seeds per pod, SY: seed yield, HSW: hundred seed weight, SN: Nitrogen content in seed, LN: Nitrogen content in leaf, CV: Coefficient of variation

Effect of *Rhizobium* Inoculation

It is obvious from Tab. III that seed inoculation of common bean with *Rhizobium phaseoli* significantly increased the studied traits. The greatest values of seed yield (1635 kg.ha^{-1}), plant height (30.01 cm), pod length (13.19 cm), pods per plant (6.7), seeds per pod (4.34) and nitrogen content in leaf (4.12%) were recorded when seeds inoculated with nitrogen fixing bacteria (*Rhizobium phaseoli* L.). Seed yield, plant height, pod length, pods per plant, seeds per pod and nitrogen content in leaf were increased by 17.63, 14.60, 11, 5.6 and 11.26%, respectively due to *Rhizobium phaseoli* inoculation.

Effect of Nitrogen Fertilization

For the treatments with 60 kg.ha^{-1} nitrogen, seed yield was higher than in the treatments with no N, even with the application of other amounts of N (Tab. IV). There was not significant difference between rates of 30, 90 and 120 kg.ha^{-1} nitrogen. This emphasizes the importance of nitrogen fertilization in bean crop. With the application of 90 and 120 kg.ha^{-1} nitrogen, plant height was lower than the application of 30 and 60 kg.ha^{-1} N. Nitrogen supplied at the rate of 60 kg.ha^{-1} resulted in higher number of pods per plant. There were not significant differences between 0, 30, 90 and 120 kg.ha^{-1} nitrogen. These results indicated that applying nitrogen at the rate of 60 kg.ha^{-1} might be the optimum rate to cause a desirable increase in plant height and number of pods per plant. Therefore, further increase in nitrogen up to 60 kg.ha^{-1} has a negative effect on plant height and number of pods per plant.

III: *The effect of Rhizobium phaseoli on yield and yield components of bean*

Rhizobium phaseoli	SY (kg.ha^{-1})	PH (cm)	NPP	NSP	PL (cm)	LN(%)
Inoculate	1635^{a}	30.01^{a}	6.7^{a}	4.34^{a}	13.19^{a}	4.12^{a}
UnInoculate	1390^{b}	26.19^{b}	6.3^{b}	3.91^{b}	12.49^{b}	3.67^{b}
LSD (5%)	80.38	1.34	0.41	0.32	0.46	0.32

PH: plant height, PL: pod length, NPP: number of pods per plant, NSP: number of seeds per pod, SY: seed yield, LN: Nitrogen content in leaf, LSD: least significant differences
Common letters in a column indicate no significant difference (P < .05)

IV: *The effect of nitrogen fertilization on yield and yield components of bean*

Nitrogen (kg.ha^{-1})	SY (kg.ha^{-1})	PH (cm)	NPP
0	1285^{c}	27.97^{b}	6.2^{b}
30	1472^{b}	30.33^{a}	6.7^{ab}
60	1750^{a}	30.4^{a}	6.9^{a}
90	1550^{b}	26.33^{bc}	6.4^{ab}
120	1480^{b}	25.43^{c}	6.2^{b}
LSD (5%)	127.1	2.12	0.64

SY: seed yield, PH: plant height, LSD: least significant differences
Common letters in a column indicate no significant difference (P < .05)

DISCUSSION

Results indicated seed inoculation of common bean with *Rhizobium phaseoli* significantly increased seed yield, plant height, pod length, pods per plant, seeds per pod and nitrogen content in leaf. In agreement the results of present study, Ronner *et al.* (2016) revealed *Rhizobium* inoculants improve the availability of soybean seed yield. Ahmadi-Rad *et al.* (2016) indicated foliar application of bacteria (*Azotobacter* and *Azospirillum*) significantly increased the yield and growth of canola. Gicharu and Gitonga (2013) were also indicated the inoculation of climbing common bean increased the nodules formation and seed yield in comparison to control. Bacem *et al.* (2007) stated common bean is as an inefficient N-fixation crop as compared with other legumes. However, co-inoculation with *Rhizobium* had higher nitrogenase activity, leghemoglobin concentrations and atmospheric nitrogen fixation efficiency and thereby formed associations of greater symbiotic efficiency. Inoculation with *Rhizobium* stimulated nodulation and N fixation (Figueiredo *et al.,* 2007). Martins *et al.* (2003) stated co-inoculation of *Rhizobium* significantly affected the nitrogenase activity in common bean root nodules. Otieno *et al.* (2009) were also reported the increases of food legumes yield with use of bacteria inoculation. Uyanöz and Karaca (2011) indicated inoculation with *Rhizobium tropici* is important to decrease the soil salinity and to make better the yield of bean. The favorable effects of bacterial inoculation on yield were also reported in the other leguminous crops (Lamptey *et al*., 2014; Tairo and Ndakidemi, 2013; Aslam *et al*., 2010). The positive effects of *Rhizobium phaseoli* inoculation on these traits means that rhizobia bacteria were effective in fixing nitrogen and shows that there is a possibility for mineral nitrogen partially replaced by biological nitrogen. These effects of *Rhizobium phaseoli* on yield and the other traits were related to the symbiotic relationship between bacteria and bean plants, that arises from fixation of N_2 to roots and translocation of amino acids to the shoots, which lead to increases the seed yield (John Ndlovu, 2015). The other reason of *Rhizobium* can be because of the effect of these bacteria on phosphorus availability. *Rhizobium* makes the insoluble form of phosphorus to soluble forms and makes phosphorus available to the plant. These bacteria can live together with legumes and non legumes plants and enhance the uptake of nutrient and improve the structure of soil (Oufdou *et al.*, 2016). Rhizobial inoculation prepared the application P-fertilizer economically interesting unlike the use of P alone and it is an inexpensive way to increase seed yields with low financial risks and reduce the environmental adverse (Ronner *et al.*, 2016).

The results were also indicated application of 60 $kg.ha^{-1}$ nitrogen had significantly improved seed yield, number of pods per plant and plant height. Similar results were reported by Geetha and Varughese (2001). The results indicated that the bean crop requires N for its initial growth (Soratto *et al.,* 2010). The positive and significant effect of nitrogen fertilization on seed yield, seed weight, number of pods per plant and number of seeds per plant were also reported by Abdzad Gohari and Amiri (2010). Soratto *et al.* (2010) indicated even with the application of 60 $kg.ha^{-1}$ nitrogen at planting, common bean yield was increased. This result may be due to the important role of nitrogen in structure of amino acid, protein, enzymes and chlorophyll molecule and its vital importance for plant growth (Otieno *et al.*, 2009). The reason of reduction in number of pods per plant, plant height and seed yield with increase the amount of nitrogen is according to that the excessive available N can result in reduced and delayed yield and reduced dry matter content (Salehin and Rahman, 2012). Oliveira *et al.* (1996) were also indicated lower development and seed yield of bean plants with deficient in nitrogen. Evans *et al.* (2001) stated that N accumulations from legume crops varied from 45–225 $kg.ha^{-1}$ nitrogen. It is necessary the inorganic and symbiotic nitrogen sources for greatest yields of seed legumes (Gicharu *et al.*, 2013). Application of nitrogen fertilizer is useful for yield and growth of bean. Because the first added nitrogen is utilized for nodule growth, early growth of bean is excited by use of mineral nitrogen fertilizer. The use efficiency of nitrogen mineral fertilizer is less than 50%, because of lost through run-off water and leaching and leading to higher production costs substantial environmental contamination. Therefore, the utilization of legumes with high ability to atmospheric nitrogen fixation and improvement the fixation of N_2 by bacteria (Habete and Buraka, 2016) can be resolve the high use of nitrogen mineral fertilizer.

CONCLUSION

Application of 60 $kg.ha^{-1}$ nitrogen had significantly improved seed yield, plant height and the number of pods per plant. Nitrogen supply beyond the 60 $kg.ha^{-1}$ generally resulted in decline plant height, pods per plant and seed yield. It can also concluded from the results of present study that inoculation of seeds with *Rhizobium* significantly increased growth and yield of common bean. The higher yields achieved with *Rhizobium* inoculation revealed this technology is effective in supplying nitrogen to legumes as mineral nitrogen fertilizer and it is a cost-effective for resource-poor farmers.

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Contact information

Peyman Sharifi: sharifi@iaurasht.ac.ir