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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 9 (9): 1738-1743, 2006
ISSN 1028-8880

Effects of Different Strains of *Rhizobium legominosarum* biovar *phaseoli* on Yield and N_2 Fixation Rate of Common Bean (*Phaseolus vulgaris* L.) Iranian Cultivars

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Abstract: In order to evaluate of nitrogen biological fixation ability by different strains of *Rhizobium legominosarum* biovar *phaseoli* in common bean Iranian cultivars, a spilt plot experiment in randomized complete block design was conducted a field in Shahrekord, Iran. The factors were four bacterial strains: L-78, L-47, L-125, l-109, non-inoculated controls including application of nitrogen fertilizer treatment (100 kg N ha^{-1}), without application of nitrogen fertilizer and three cultivars: local cranberry bean, Talash cranberry bean and local red type. The results showed that seed inoculation with strain increased nodule dry weight, N total (shoot) and percentage of fixed N_2 in relation to bean rhizobia population naturalized and +N control. However, an efficient symbiotic was achieved with strain L-109, since total N content of plants inoculated with these strains was similar to that of plants supplied with N-fertilizer treatment.

Key words: Cultivars, nitrogen fixation, *Phaseolus vulgaris*, *Rhizobium legominosarum* biovar *phaseoli*

INTRODUCTION

The increase in the burgeoning population of the world and the shortage of the resources to meet the requirements for food has increased the need for protein sources. The global protein requirement constitutes 70% of the plant sources; about 18.5% of them constitute grain beans for meals. The common bean (*Phaseolus vulgaris* L) is the most important legume for human nutrition and major protein, potassium, calcium, iron and phosphorous source in the world and plays an important role in the enhancement of the level of nourishment in developing countries (Bildirici and Yilmaz, 2005; Graham and Ranallin, 1997). The common bean is now growing extensively in all major continental areas. According to the statistical data obtained by Food and Agriculture Organization (FAO), total 27 million ha of agriculture land for the growing of bean and with a yield of 729 kg ha^{-1}. In Iran, this crop occupies 0.125 million ha of area, the yield is moderate, on average 1470 kg ha^{-1}. The cultivating area of beans in Shahrekord city in Iran is 10000 ha and its yield is 1700 kg ha^{-1}.

The fixation of atmospheric nitrogen represents a major source of nitrogen input in soils. The biological nitrogen fixation (BNF) system can play a significant role in increasing soil fertility. The symbiosis interaction between the Rhizobium and legumes is superior to other nitrogen fixing system due its high potential (El-Deeb and Al-Sheri, 2005). The importance of symbiotic nitrogen fixation is has been overemphasized due to the fact that protein requirement worldwide has been on a rise and serious environmental problems have emerged during the production and use of fertilizers based on minerals and nitrogen. Given requirement for 20.000 kcal energy in order to produce 1 kg of fertilizer containing nitrogen, the importance of biological nitrogen fixation could much more clearly be understood.

Phaseolus vulgaris L. is a legume capable of symbiotically fixing atmospheric nitrogen gas (N_2). Nodules formed on the bean roots contain bacteria that convert N_2 from the air to plant-available form (Giller, 2001). These bacteria can be used as an inoculum that is applied to the seed (Giller and Cadisch, 1995). Board ranges of Rhizobium species are able nodule and fix N_2 with beans including *R. legominosarum* biovar *phaseoli* (Jordan, 1984.) *R. tropic* (Martinez-Romero *et al.*, 1991) and *R. etli* (Segovia *et al.*, 1993).

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Yield response of common bean to inoculation with a specific Rhizobium is often variable and depends on environmental and agronomic (Hardarson, 1993). The lack of response to inoculation can be attributed to intrinsic characteristics of the host plant and bacteria, as well as the great sensitivity of the symbiosis to environmental stress, soil dryness and low soil fertility (Hungria *et al*., 2000). Other important factors to be considered are general limitation to N_2 fixation per se, like the high rate of levels used in intensive agriculture and the residual N remaining in soil (Rodriguez-Navarroet *et al*., 1999). Genotype variation in common bean for traits affecting nodulation and N_2 fixation has been found (Chavera and Graham, 1992). However, when highly effective *Rhizobium/phaseoli* combination have been selected, they provided bean yield of 60-70% of those obtained with N fertilizer control under field conditions (Santa *et al*., 1997).

In Iran, the usual practices of bean cultivation dose not involve inoculation of seeds with a specific rhizobial inoculation and farmers depend on the application of inorganic nitrogen fertilizer to sustain growth to improve yield. In Iran soils, for example high rate of levels of inorganic nitrogen fertilizer used at the rates of 100-300 kg N ha^{-1}. Since chemical N fertilizers are expensive to most farmers. In addition, N fertilizer application had no significant effect on the dry matter yield and N content of *Phaseolus vulgaris* cv. B9 (Shisanya, 2002). However, the most Iranian soils have nitrogen deficiency and N_2 fixation by Rhizobium bacteria can increase the yield at a low cost and preserve water resources from nitrate pollution.

Determination of N_2 fixation effectiveness in the process of strain selection is normally several step procedures involving an initial selection under greenhouse conditions and a final testing in field trails (Bergersen, 1980; Giller, 2001). In the process of strain selection, variation in the efficiency of the strain* cultivars association was detected for parameters like total N, yield, plant growth, nodule number, weight and N fixed (Santa *et al*., 1997).

This investigation was conducted in order to evaluate the effect of four bacterial strains on common bean yield and N_2 fixation rate and to estimate the best bacteria and cultivar's combination response of common bean cultivars to inoculation with different *Rhizobium legominosarum* biovar *phaseoli* strains in Shahrekord environmental condition.

MATERIALS AND METHODS

The experiment was conducted at the field of education and research of Islamic Azad University of Shahrekord (latitude 32°44′ N, 2100 m asl), located at about 100 km of Isfahan and 500 km of the capital town of Iran during May to October 2002. The medial annual rainfall is about 337.2 mm $year^{-1}$. Average annual temperature is 11.9°C. Soil samples were collected to a depth of 0-30 cm before planting. The experiment was performed in soil that had not been cultivated with beans for at least 5 years. C, N, P and K content, electrical conductivity (EC), pH and percentage of sand, silt and clay were determined (Table 1). Total N was measured calorimetrically following Kjeldahl digestion. Also, soil pH and EC were estimated using a glass electrode pH meter and an EC meter in 1:1 soil water suspension. Organic carbon was determined by modified Walkley and Black method (McKeague, 1978.). The rhizobia population evaluated was approximately 10^3-10^4 cells g^{-1} of soil.

The experiment was arranged in a randomized complete block design with a split plot layout and four replications. Four bacterial strains of rhizobia (Table 2) and non-inoculated controls including application of nitrogen fertilizer treatment (40 kg N ha^{-1} as urea at sowing and 60 kg N ha^{-1} at 35 days after sowing) and without application of nitrogen fertilizer were assigned to main plots. Inoculation was added to the seeds with 15% (w/v) source solution to increase adherence. Bacteria strains of *Rhizobium legominosarum* biovar *phaseoli* was kindly donated by Dr. Hadi Asadi Rahmani and Ms Mitra Afshar, Soil Biology Department of Institute of Soil and Water, Karaj, Iran. Three cultivars included local cranberry bean; Talash cranberry and local red Mexican were assigned as the subplots.

Table 1: Physical and chemical characteristics of the soil of experimental site

P (ppm)	N total (%)	OC (%)	K (ppm)	EC (dS m^{-2})	pH	Clay (%)	Silt (%)	Sand (%)
24.4	0.163	1.47	371.4	0.66	7.68	13	40	47

Table 2: Bacterial strains used in this work

Strain	Year and space	Source
L-78	Shahrekord (Chahar Mahal and Bakhtyiari) -2002	Institute of soil and water of soil biology part, Karaj, Iran
L-109	Touysercan (Hamadan)-2002	"
L-47	Feraydonshahr (Isfahan)-2002	"
L-125	Aleshtar (Lorastan)-2002	"

Each block consisted of six main plots spaced 2 m apart. The main plots had three subplots that consisted of five rows spaced 50 cm apart. Phosphate and starter N fertilizer were applied at rates 100 kg P ha^{-1} and 40 kg N ha^{-1} as urea before of planting, respectively. Four seeds of each genotype were planted per hole on 10 June 2002, weeding and thinned 20 days after emergence 135,000 plants ha^{-1} for red bean and 100,000 plants ha^{-1} for cranberry bean (Graham and Ranallin, 1997). Topsoil of the experimental plot area was kept moist throughout the growing season when necessary.

The characteristics under investigation were grain yield, number of pods per plants, number of seeds per pods, number and weight nodule at 40-50% flowering (50 days after emergence) and percentage of nitrogen fixation. Percentage nitrogen fixed was estimated according (Rennie, 1984) with following formula:

$$\text{Percent nitrogen fixed} = \frac{\text{(N content of inoculated plants - total N content of control plants)}}{\text{Total N content of inoculated plants}} * 100$$

Plants were sampled at 25 days after planting, 45-50 days after planting and at physiological maturity (70-80 days after planting). Five plants were randomly samples from each treatment replicated and various parameters assessed. Nodule number and dry weight verified in 5 plants at early (45-50 days after emergence). Plant samples and nodules were dried to constant weight at 75°C at 72 hr in oven. Shoots were dried for dry weight determination and for total nitrogen determination by the Kjeldahl method. Yield and its components were evaluated at final harvest and values were corrected for 13% moisture.

All data were subjected to analysis of variance using the statistical computer package SAS (Release 6.12, Statistical Analysis System Institute., 1996) and treatment means separated using LSD range test at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Seed yield: In the present study, high significant difference ($p \leq 0.01$) in seed yield was observed among seeds inoculated with different strains and non-inoculated controls (N fertilizer treatment and without N fertilizer treatment) (Table 3). Seeds inoculated with L-125 showed higher seed yield than other treatments (Table 4), but similar result were observed in seed inoculated with L-109, L-78 strains and non-inoculated control (N fertilizer treatment). Seeds inoculated with L-125 probably aided the establishment of the most efficient inoculated rhizobia, improving symbiotic performance and could increased nitrogen available for plant. In the Central Regional of Brazil, the Cerrados, where over $0.8 * 10^{6}$ ha are being cultivated with beans and soil N content is low, bean yield is generally increased by inoculation (Hardarson, 1993). Hungria *et al*. (2000) reported that the common bean inoculated with PRF31, PRF55 and PRF81 showing high rates on N_2 fixation and inoculated with PRF81 allowed yield increased of up to 906 kg ha^{-1}, compared with the non-inoculated (control) with a population of native rhizobia in Brazilian soils. Also, the study revealed that tepary bean inoculated with R3254 and non-inoculated (control without inoculated and N fertilizer) the highest and lowest seed yield, respectively (Shisanya, 2002). These results confirm a report also with Iranian cultivar (Asadi Rahmani, 2000) and disagrees with obtained in

Table 3: Analysis of variance for different traits under seeds inoculated with different strains of *Rhizobium legominosarum* bv. *phaseoli*

		Mean square					
SOV	d.f	Shoot N content (%)	Nitrogen fixed rate (%)	Nodule No. $plant^{-1}$	Nodule dry weight (g $plant^{-1}$)	Shoot dry matter (kg m^{-2})	Seed yield (kg m^{-2})
Block	4	13.28	524.68	66.62	0.008	0.005	0.005
Strain	5	1.88*	217.71**	14.58 ^{ns}	0.013**	0.22**	0.016**
Error a	15	0.35	43.58	23.23	0.005	0.051	0.006
Cultivar	2	6.84*	2.54 ^{ns}	84.12 *	0.044**	0.38**	0.053**
Strain*Cultivar	10	0.27	24.89 ^{ns}	6.20 ^{ns}	0.002 ^{ns}	0.042**	0.002^{ns}
Error	36	0.656	43.76	19.00	0.003	0.019	0.003
CV (%)		8.86	35.02	28.10	28.01	22.16	25.78

^{ns} ,* and ** : Not significant, significant at 5 and 1% level of probability, respectively

Table 4: Mean comparison of characteristic in different strains of bean (*Phasseolus vulgaris* L.) using LSD range test at $p \leq 0.05$ level

Strains	Nitrogen fixed rate (%)	Shoot N content N total (%)	Pod dry weight g $plant^{-1}$	Nodule dry weight (g $plant^{-1}$)	Shoot dry weight Kg m^{-2}	Seed yield Kg m^{-2}
Control	0	5.53	11.67	0.055	3740	1441
N 100	5.29	6.21	19.44	0.05	6530	2121
L-47	7.62	5.91	13.51	0.086	3960	1771
L-109	11.9	6.32	14.92	0.126	4280	2103
L-125	10.71	5.98	18.92	0.112	5120	2506
L-78	9.82	6.18	16.53	0.121	5110	2202
$LSD_{(0.05)}$	5.741	0.621	5.171	0.062	0.237	0.067

Table 5: Mean comparison of characteristic in strain *cultivar interaction effects

Cultivar	Strains	Seed yield Kg m^{-2}	Nodule No. ($Plant^{-1}$)	Nodule dry weight (g $plant^{-1}$)	Pod dry weight (g $plant^{-1}$)	Nitrogen fixed rate (%)
	Control	1416.7	5.45	0.050	8.63	0.00
	N 100	2651.6	1.98	0.031	28.40	4.63
Cranberry bean	L-47	1946.7	6.73	0.010	17.40	7.22
Talash cv	L-109	2370.1	4.45	0.121	18.10	11.60
	L-125	2701.7	4.12	0.132	21.83	9.10
	L-78	1718.4	5.20	0.071	11.63	9.41
Mean		2134.2	4.60	0.069	17.67	6.99
	Control	1995.1	6.11	0.081	17.13	0.00
	N 100	2172.2	1.63	0.091	18.45	7.11
Cranberry bean	L-47	2065.3	5.20	0.085	15.24	11.58
Local	L-109	1271.7	5.96	0.052	7.58	12.30
	L-125	2123.3	4.49	0.041	13.68	10.46
	L-78	2188.9	9.75	0.142	18.19	7.49
Mean		1969.42	5.52	0.082	15.04	8.16
	Control	912.8	4.51	0.031	9.23	0.00
	N 100	1538.3	0.81	0.021	11.45	10.12
Mexican red bean	L-47	1301.7	5.73	0.063	7.89	4.04
Local	L-109	2668.2	7.87	0.0191	19.02	11.97
	L-125	2694.4	7.88	0.152	21.25	12.39
	L-78	2698.3	5.13	0.144	19.77	12.74
Mean		1968.95	5.32	0.072	14.77	8.54
ANOVA	Cv.	p<0.01	ns	p<0.01	p<0.01	p<0.01
	St.	p<0.01	p<0.05	p<0.01	p<0.01	ns
Significance of F	Cv. * St	ns	ns	ns	p<0.01	ns
CV %		35.02	28.10	28.01	23.59	25.78

other countries (Olivera and Graham, 1990; Streit *et al.*, 1992). However, the different strains substantially in symbiotic properties, such as, nodulation and N_2 fixation capacity, as well as in the synthesis of nod factors after inoculation.

The results of analysis of variance indicated that there were high significant differences ($p\leq 0.01$) between cultivars in seed yield (Table 3). The interaction between strain * cultivar effect was no significant difference, but the results Table 5 showed the highest and lowest seed yield were associated to Talash * L-125 strain and local red bean * control. However, local red bean * L-125, local red bean * L-78 and local red bean* L-109 similar combinations were observed in seed yield with Talash * L-125 strain. Other authors (Buttery *et al.*, 1998; Shisanya, 2002) found no interaction strain * cultivar effects on plant growth and seed yield. While, a significant interaction Rhizobium strain * common bean cultivar for seed yield, shoot and pods dry matter were found (Rodriguez-Navarro *et al.*, 1999).

Shoot and pods dry matter: Shoot and pods dry matter were influenced ($p\leq 0.01$) by seeds inoculated with strains and non-inoculated controls (Table 3). Seeds inoculated with strains showed lower shoot dry matter than non-inoculated N-fertilizer, but similar pods dry matter was observed in seeds inoculated with strains L-109, L-125 and L-78 and the non-inoculated N-fertilizer (Table 4). There were high significant differences between cultivars and strains in shoot and pods dry matter as well as an interaction strain * cultivar for both parameters (Table 3). Non-inoculated N-fertilizer treatment produced high shoot and pods dry matter in Talash cultivar (Table 5). The difference in vegetative and reproductive (pods) growth between treatments reflected changes in assimilates partitioning which led to significant differences in harvest index (Rodriguez-Navarroet *et al.*, 1999). The results showed that + N control gave highest shoot and pods dry matter (Table 4). The results of study by Tamimi *et al.*, 2002 showed that shoot dry matter was the highest for common bean inoculated with isolate JOV1. Rodriguez-Navarro *et al.*, 1999 working in greenhouse also found that non-inoculated + N control higher shoot growth than the plants inoculated with strains, but similar pods growth was observed in plants inoculated with strains ISP-1 and CIAT 899 and the non-inoculated + N plants. In tepary bean, inoculated with strain R3254 and non-inoculated control (without N-fertilizer) demonstrated the highest and lowest shoot and pods dry matter, respectively (Shisanya, 2002). Also, the other investigation by Rodriguez-Navarro *et al.*, 1999 showed that the highest shoot dry matter was associated to c.v Cnellini * strain CIAT and c.v Cnellini * strain ISP-1 and c.v Bina * non-inoculated + N treatment for pods dry matter.

Nitrogen fixation rate: Table 3 shows that nodule dry weight (at 40-50% flowering), N total (shoot) and percentage of fixed N_2 were influenced by seeds inoculated with strains and non-inoculated controls (N-fertilizer and without N-fertilizer treatments). While, there were no significant differences between cultivars and strains in nodule number at 40-50% flowering. Following, the results revealed that all treatments were capable of nodulation, however, strains L-109 gave highest nodule dry weight, N total (shoot) and percentage of fixed N_2 (Table 4). Furthermore, seed inoculation with strain increased nodule dry weight, N total and percentage of fixed N_2 in relation to bean rhizobia population naturalized and N fertilizer treatment. An efficient symbiotic was achieved with strain L-109, since total N content of plants inoculated with these strains was similar to those plants supplied with

N-fertilizer treatment: The results of analysis of variance indicated that there were high significant differences between cultivars in nodule dry weight and N total (Table 3). While, there were no significant differences between cultivars in nodule number and percentage of N_2 fixed. Also, no significant interaction Rhizobium strain *common bean cultivar for nodule number and weight at 40-50% flowering, N total in shoot and percentage of N_2 fixed were found (Table 3). Table 5 shows the highest nodule number, nodule weight and percentage of N_2 fixed were associated to local cranberry cultivar * L-78 strain, local red bean * L-125 and local red bean * L-78, respectively. Hungria *et al.*, 2000 reported that under controlled greenhouse condition, inoculation with strains PRF81 and CIAT899 enhanced nodulation, compared to other treatments, resulting in accumulation of more N in common bean tissues. An efficient symbiotic was achieved with strains PRF81 and CIAT899. Rates of N_2 fixation achieved with strain R. tropici type IIB CIAT 899 were 112% greater than with *R. legominosarum* biovar *phaseoli* strain USDA 2671 and could reflect the adapted symbiosis of Brazilian cultivar with R. tropici species (Hungria *et al.*, 2000).

These results confirm a report also with Iranian cultivar (Asadi Rahmani, 2000) and disagree with obtained in other countries (Olivera and Graham, 1990, Streit *et al.*, 1992). However, the different strains substantially in symbiotic properties, such as, nodulation and N_2 fixation capacity, as well as in the synthesis of nod factors after inoculation.

Further research is warranted to determine the success of this technology across the broad range of environmental conditions used for the culture of common bean around the world. Various bean nodulating strains may be used in different parts of the world in order to provide an inoculums well adapted to specific soil conditions.

ACKNOWLEDGMENTS

This work received support from the vice chancellor for research at the University of Tehran, Aboorayhan campus and the Soil Biology Part of Institute of Soil and Water of Iran. We thank Dr. Ali Sharif Zadeh, Dr. Asadi Rahmani, Msc. Mehnat Kesh, Msc. Yadegari and Mc. Farzan for present study help.

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