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Evaluation of Seed Yield and Yield Components of Common Bean Iranian Cultivars for Inoculation with Four Strains of *Rhizobium legominosarum* biovar *phaseoli*

[1]Abdollah Ghasemi Pirbalouti, [2]Ahamd Reza Golparvar and [1]Safar Ali Rostampoor
[1]Department of Agronomy and Plant Biology, Agriculture College,
Islamic Azad University of Shahrekord Branch, PO. Box 166. Shahrekord, Iran
[2]Department of Agronomy and Plant Breeding, Agriculture College,
Islamic Azad University of Khorasgan Branch, Isfahan, Iran

Abstract: In order to evaluate the effect of different bacterial strains on common bean yield and it's components and to estimate the best bacteria and cultivar's combination, field experiment was carried out at Shahrekord in Iran (latitude 32°44′ N, 2100 m asl) during May - October 2002 on a sandy -loam soil. The experiment was a spilt plot in randomized complete block design with four bacterial strains L-78, L-47, L-125, L-109 and non-inoculated controls including application of nitrogen fertilizer treatment (100 kg N ha^{-1}) and without application of nitrogen fertilizer (-N) as the main plots. Three cultivars included Shahrekord local (Cranberry bean), Talash (Cranberry bean) and Shahrekord local (Red Mexican type) were assigned as the subplots. The results revealed that high significant difference ($p<0.01$) in seed yield, seed and pod number per plant, LAI at 40-50% flowering (50 days after emergence), harvest index (HI) and dry matter yield among seed inoculated with different strains and non-inoculated controls (+N and -N treatment). Seeds inoculated with L-125 showed higher seed yield than other strains, but similar was observed in seed inoculated with L-109, L-78 strains and non-inoculated control (+N).

Key words: *Phaseolus vulgaris* L., inoculation, *Rhizobium legominosarum* biovar *phaseoli*, yield

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most important legume for human nutrition, major protein and calorie source in the world (Graham and Ranalli, 1997; Velazquez *et al*., 1988) It's now growing extensively in all major continental areas. In Iran, the crop occupies 0.125 million ha of area, the yield is moderate, on average 1470 kg ha^{-1}. *Phaseolus vulgaris* L. is a legume capable of symbiotically fixing N_2 atmospheric. Nodules formed on the bean roots contain bacteria that convert N_2 from the air to plant-available form. These bacteria can be purchased as an inoculum that is applied to the seed. A board range 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* (Segavio *et al*., 1993).

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 soils in Iran nitrogen have deficiency and N_2 fixation by *Rhizobium* bacteria can increase the yield at a low cost and preserve water resources from nitrate pollution.

Yield response of bean to inoculation with a specific *Rhizobium* is often variable and depend an environmental and agronomic factor (Giller and Cadisch, 1995; Handerson, 1993). The lack of response to inoculation can be attributed to intrinsic characteristics of both 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 soils (Rodriguez-Navarro *et al*., 1999). Genotype variation in bean for traits affecting nodulation and N_2 fixation has

Corresponding Author: Abdollah Ghasemi Pirbalouti, Department of Agronomy and Plant Biology, Agriculture College, Islamic Azad University of Shahrekord Branch, PO. Box 166. Shahrekord, Iran
Tel: 0098 381 331001 Fax: 0098 381 3346709

been found (Chavera and Graham, 1992). However, when highly effective *Rhizobium/Phaseol* combination have been selected, they provided bean yield of 60-70% of those obtained with N fertilizer control under field conditions (Santamaria *et al.*, 1997). Determination of N_2 fixation effectiveness in the process of strain selection is normally a several step procedure involving an initial selection under greenhouse conditions and a final testing in field trails (Rodriguez-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 (Santamaria *et al.*, 1997).

The aims of this research was to evaluate the effect of different bacterial strains obtained collection from Institute of Soil Biology Researches of Iran on common bean cultivars yield and it's components and to estimate the best *Rhizobium/Phaseol* combination in field condition.

MATERIALS AND METHODS

Field experiment was conducted at Shahrekord (latitude 32° 44′ N, 2100 m asl), located at about 100 km of Isfahan and 500 km of the capital town of Iran on spring and summer 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. Three cultivars included local cranberry bean; Talash cranberry and local red Mexican were assigned as the subplots. Each block consisted of six main plots spaced 2 m apart. The main plots had three subplots that consisted of five

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 study

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	ISWSBI
L-47	Feraydonshahr (Isfahan)-2002	"
L-125	Aleshtar (Lorastan)-2002	"

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^{-} for cranberry bean (Graham and Ranalli, 1997). Topsoil of the experimental plot area was kept moist throughout the growing season when necessary.

The characteristics under investigation were seed yield, pod and seed number per plant, Dry Matter Total (TDM) and Leaf Area Index (LAI) at 40-50% flowering (50 days after emergence) and Harvest Index (HI). 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. Plant samples were dried to constant weight at 75°C at 72 h 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 ANOVA using the statistical computer package SAS (Release 6.12, Statistical Analysis System Institute, 1996) and treatment means separated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Seed and pods number per plant were influenced ($p \leq 0.01$) by seeds inoculated with strains and non-inoculated controls (Table 3). Non inoculated N-fertilizer treatment showed higher seed number per plant than control and seeds inoculated treatments, but similar seed number per plant was observed in seeds inoculated with strains L-47, L-125 and L-78 (Table 4). Seeds inoculated with L-125 showed higher pod number per plant than other treatments (Table 4), but similar result was observed in seed inoculated with L-109 and L-78 strains. There were

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

		Mean square							
SOV	df	Seed No. ($plant^{-1}$)	100 Seed weight (g)	Pod dry weight g ($plant^{-1}$)	Pod No. ($plant^{-1}$)	Dry matter total (kg m^{-2})	Seed yield (kg m^{-2})	Leaf area index (LAI)	Harvest index (%)
Block	4	0.27	13.60	37.17	5.81	0.005	0.005	0.54	0.0007
A	5	0.49**	16.86 ^{ns}	111.82**	38.42**	0.22**	0.016**	0.93**	0.036**
Error a	15	0.16	16.49	35.31	9.99	0.051	0.006	0.18	0.016
B	2	2.17**	2196.20 **	561.91**	23.68*	0.38**	0.053**	0.44**	0.002 ^{ns}
A*B	10	0.23 ^{ns}	19.51 ^{ns}	47.46**	7.09 ^{ns}	0.042**	0.002 ^{ns}	0.18 ^{ns}	0.008 ^{ns}
Error	36	0.13	15.29	16.21	5.97	0.019	0.003	0.10	0.01
cv (%)		12.17	9.82	25.43	23.59	22.16	25.78	10.53	30.68

^{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 DMRT at p≤0.05 level

Strains	Harvest index (%)	Leaf area Index (LAI)	Seed No. (plant)	pod dry weight g ($plant^{-1}$)	Pod No. ($plant^{-1}$)	Dry matter total (kg m^{-2})	Seed yield (kg m^{-2})
Control	0.39ab	2.59b	2.66c	11.67c	7.88c	3740b	1441c
N 100	0.34b	2.97ab	3.25a	19.44a	9.33bc	6530a	2121abc
L-47	0.45ab	2.88b	2.92abc	13.51bc	9.41bc	3960b	1771bc
L-109	0.49a	2.97ab	2.83bc	14.92abc	11.20ab	4280b	2103abc
L-125	0.49a	3.30a	3.08ab	18.92ab	11.55ab	5120ab	2506a
L-78	0.43ab	3.35a	3.00abc	16.53abc	12.77a	5110ab	2202ab

In each column means followed by same letter are not significantly different at the 5% level using DMRT

Table 5: Mean comparison of characteristic in strain *cultivar interaction effects using DMRT at p≤0.05 level

Strain * Cultivar	Seed No. (plant)	100 Seed weight (g)	Pod No. ($plant^{-1}$)	Pod dry weight (g $plant^{-1}$)	Dry matter total (kg m^{-2})	Harvest index (%)	Seed yield (%)
LRM[1] * Co	2.0d	26.3c	6.89e	9.23ef	0.41fg	0.25bc	0.09d
TC [2] * Co	3.0bc	44.70ab	7.02e	8.63f	0.45efg	0.31abc	0.14cd
LC [3] * Co	3.0bc	45.20ab	9.72bcde	17.13bcd	0.63bcdef	0.32abc	0.20abc
LRM * N 100	3.75a	30.1c	8.50de	11.45def	0.65defg	0.26bc	0.15bcd
TC * N 100	3.0bc	49.3a	8.91cde	28.40a	1.12a	0.22c	0.29a
LC * N 100	2.5cd	45.5ab	10.57bcde	18.45bc	0.84b	0.26bc	0.22abc
LRM * L-47	2.75bc	29.9c	8.46e	7.890f	0.4g	0.33abc	0.13cd
LC * L-47	3.0bc	46.8ab	9.86bcde	15.24bcde	0.65bcde	0.32abc	0.21abc
TC * L-47	3.0bc	43.5ab	9.92bcde	17.40bcd	0.53defg	0.36abc	0.19abc
LC * L-109	3.0bc	29.6c	8.76cde	7.58f	0.37g	0.36abc	0.13cd
TC * L-109	3.0bc	49.60a	10.74abcde	18.1bc	0.59cdefg	0.43ab	0.24ab
LRM * L-109	3.0bc	41.8b	14.10a	19.02bc	0.75bcd	0.35abc	0.27a
LC * L-125	3.0bc	27.5c	12.83abc	13.68cdef	0.52defg	0.43ab	0.21abc
TC * L-125	3.25ab	47. 8ab	11.91abcd	21.83b	0.82bc	0.33abc	0.27a
LRM * L-125	3.0bc	44.4ab	13.55ab	21.25b	0.71bcd	0.40ab	0.27a
TC * L-78	2.5cd	29.9c	10.93abcde	11.63def	0.57defg	0.30abc	0.17bcd
LRM * L-78	3.25ab	41.4b	13.30ab	19.77bc	0.66bcde	0.44a	0.26a
LRM * L-78	3.25ab	43.6ab	10.40abcde	18.19bc	0.68bcde	0.34abc	0.22abc

In each column means followed by same letter are not significantly different at the 5% level using DMRT., [1:] Local red Mexican bean, [2:] Talach Cranberry bean and [3:] Local Cranberry

no significant differences between cultivars and strains in seed and pods number per plant as well as an interaction strain * cultivar for both parameters (Table 3), but the results (DMRT) at p≤0.05 level showed that the highest seed and pod number were associated to local red Mexican bean * +N and local red Mexican bean * L-109 strain, respectively (Table 5). Also, 100-Seed weight showed no response to seeds inoculated with strains and non-inoculated controls (N-fertilizer and without N-fertilizer treatments) (Table 3).

Table 3 showed that shoot and pods dry matter were influenced (p≤0.01) by seeds inoculated with strains and non-inoculated controls. Non-inoculated (+N) showed higher shoot and pods dry matter than other treatments (Table 4). There were high significant differences (p≤0.01) 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 cranberry cultivar (Table 5).

In the present study, high significant difference (p≤0.01) in seed yield was observed among seeds inoculated with different strains and non-inoculated controls (+N and -N treatments) (Table 3). Seeds inoculated with L-125 showed higher seed yield than other treatments (Table 4), but similar result was observed in seed inoculated with L-109, L-78 strains and non-inoculated control (N fertilizer treatment). The results of ANOVA 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.

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 (Hungria *et al*., 2000). 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 other countries (Olivera and Graham, 1990; Streit *et al*., 1992). However, the strains different 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 a high significant difference ($p \leq 0.01$) between seed inoculated and control treatments in Harvest Index (HI) (Table 3). Seeds inoculated with L-125 and L-109 strains showed higher and N fertilizer treatment lower harvest index than other treatments, respectively. Leaf Area Index (LAI) was influenced ($p \leq 0.01$) by seeds inoculated with strains and non-inoculated controls (Table 3). In thin research revealed that seed inoculated with L-78 and non-inoculated (control without inoculated and N fertilizer) the highest and lowest LAI, respectively (Table 4).

The difference in vegetative and reproductive (pods) growth between treatments reflected changes in assimilate partitioning, which led to significant differences in harvest index (Rodriguez-Navarro *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 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. Also, Harvest Index (HI) was the highest for common bean inoculated with strain ISP-1.

In tepary bean, inoculated with strain R3254 and non-inoculated control (-N) demonstrated the highest and lowest shoot and pods dry matter, respectively (Shisanya, 2002). A significant interaction *Rhizobium* strain* common bean cultivar for seed yield, shoot and pods dry matter were found (Rodriguez-Navarro *et al*., 1999). While, other authors (Buttery Brian *et al*., 1998; Shisanya, 2002) found no interaction strain* cultivar effects on plant growth and seed yield. Therefore, plant breeding of bean can be done with and effective strain. 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.

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REFERENCES

Asadi Rahmani, H., 2000. Evaluation of N_2 fixation efficient with different strains of Iranian Rhizobia on common bean (*Phaseolus vulgaris* L.). J. Iranian Soil and Water Sci., 9: 93-97.

Buttery Brian, R., P. Soont and P.B. Van Berkum, 1998. Effects of cultivar and strain of *Rhizobium* on growth bean yield and nitrogen content of common bean (*Phaseolus vulgaris* L.). Tektran Agriculture Research Service.

Chavera, M.H. and P.H. Graham, 1992. Cultivar variation in traits affecting early nodulation of common bean. Crop Sci., 32: 1432-1436.

Giller, K.E., 2001. Nitrogen Fixation in Tropical Cropping Systems. 2nd Edn., CABI Publishing.

Giller, K.E. and G. Cadisch, 1995. Future benefits from biological nitrogen fixation-an ecological approach to agriculture. Plant Sci., 174: 225-277.

Graham, P.H. and P. Ranalli, 1997. Common bean (*Phaseolus vulgaris* L). Field Crops Res., 53: 131-146.

Graham, P.H., 1990. Problemas de la nudulacion y la fijacion de nitrogeno en *Phaseolus vulgaris* L. Una reevaluacion Terra, 8: 71-82.

Handerson, G., 1993. Methods for enhancing symbiotic nitrogen fixation. J. Plant Soil, 152: 1-17.

Hungria, M., S. Andrade, L. Chueire, M. Probanza, F.J. Guttierrez-Manero and M. Megias, 2000. Isolation and characterization of new efficient and competitive bean (*Phaseolus vulgaris* L.) rhizobia from Brazil. J. Soil Biol. Biochem., 32: 1515-1528.

Jordan, D.C., 1984. *Rhizobium* Conn. In: Krieg, N.G., J.G. Holt, (Eds.). Bergeys Manual of Systematic Bacteriology. Williams and Wikins, Baltimore, USA.

Martinez-Romero, E., Segavio, F.M. Mercante, A.A. Feranco, P.H. Graham and M.A. Pardo, 1991. *Rhizobium tropici* a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena* sp. trees. Intl. J. Sys. Bacteriol., 41: 417- 426.

McKeague, J.A., 1978. Manual of soil sampling and methods of analysis. Soil Survey Committee, Canadian Society of Soil Science, Ottawa, Ont., Canada.

Olivera, L.A. and P.H. Graham, 1990. Speed of nodulation and competitive ability among *Rhizobium legominosarum biovar phaseoli*. Arch. Microbiol., 153: 311-315.

Rodriguez-Navarro, D.N., C. Santamaria, F. Temprano and E.O. Leidi, 1999. Interaction effects between *Rhizobium* strain and bean cultivar on nodulation, plant growth, biomass partitioning and xylem sap composition. Eur. J. Agron., 11: 131-143.

Santamaria, C., D.N. Rodriguez, M. Camacho, A. Daza, F. Temprano and E.O. Leidi, 1997. Selection de cepas de *Rhizobium legominosarum* bv. *phaseoli* effective con cultivars comercial de judia verda. Consejeria Agriculture y Pesca, Junta de Andalucia, Seville. Segavio E, Young JPW, Martinez-Romero E. 1993. Reclassification of American *Rhizobium leguminosarum* biovar *phaseoli* type I strain as *Rhizobium etli* sp. nov. I. Intl. J. System. Bacteriol., 43: 347-377.

Shisanya, C.A., 2002. Improvement of drought adapted tepary bean (*Phaseolus acutifolius* A. Gray var. *latifolius*) yield through biological nitrogen fixation in semi-arid SE-Kenya. Eur. J. Agron., 16: 13-24.

Streit, W., K. Kosch and D. Werner, 1992. Nodulation competitiveness of *Rhizobium legominosarum bv. Phaseoli* and *R. tropici* strains measured by glucoronidase gene fusions. J. Biol. Fertil. Soils., 14: 140-144.

Tamimi, S.M., 2002. Genetics diversity and symbiotic effectiveness of rhizobia isolated from root nodules of common bean (*Phaseolus vulgaris* L.) grown in the soils of the Jordan valley. J. Applied Soil Ecol., 19: 183-190.

Velazquez, Y.A., R.A. Kluson and E.C. Scroder, 1988. *Rhizobium* inoculation of *Phaseolus vulgaris* in Lajas, Puerto Rico. J. Agric. Univ. Porto Rico, 72: 247-436.