Nitrogen Fixation on Common Bean Cultivars as Affected by Inoculation of Different Strains of *Rhizobium leguminosarum* biovar *phaseoli* in Shahrekord Region


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Abstract:
Common bean (*Phaseolus vulgaris* L.) is a legume capable of symbiotically fixing atmospheric nitrogen gas (N₂). Nodules formed on the bean roots contain bacteria that convert N₂ from the air to plant – available form. These bacteria can be used as an inoculum that is applied to the seed. A bored range of *Rhizobium* species are able to form nodule and fix N₂ with beans including *R. leguminosarum* biovar *phaseoli*, *R. tropic* and *R. etli*. In Iran, the usual practices of bean cultivation do not involve inoculation of seeds with a specific rhizobial inoculation, and farmers rely on the application of inorganic nitrogen fertilizer to sustain growth for yield improvement yield. This experiment was carried out at Shahrekord (latitude 32° 44' N, 2100 m asl) during May – October 2002 on a sandy-loam soil to (i) evaluate the effect of different bacterial strains on common bean yield and N₂ fixation rate (ii) estimate the best bacteria and cultivar's combination. 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 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 among seed inoculated with different strains and non-inoculated controls (N fertilizer treatment and without N fertilizer 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 fertilizer treatment). Also the results showed that seed inoculation with strain increased nodule dry weight, N total (shoot) and percent of fixed N₂ in relation to bean rhizobia population naturalized and N fertilizer treatment. However, strain *L*-109 gave highest nodule dry weight, N total and percent of fixed N₂.

The study of interaction between cultivars and strains showed that the highest grain yield were associated with Talash * L*-125 strain, Talash * L*-109 strain, Shahrekord local (Red Mexican) * L*-125 strain, Shahrekord local (Red Mexican)* L-78 plus Talash * N100 treatment. Furthermore, the results of investigation showed that Shahrekord local (Red mexican) received a mixed inoculum, *R. leguminosarum* biovar *phaseoli* strain *L*-78 (Shahrekord) showed higher percent of fixed N₂ than other treatments.

**Key words:** Bean (*Phaseolus vulgaris* L.), Cultivars, Inoculation, Strains, *Rhizobium leguminosarum* biovar *phaseoli*, Yield and N₂ fixation rate.
Introduction:
The common bean (*Phaseolus vulgaris* L.) is the most important legume for human nutrition and major protein and calorie source in the world (Graham, 1981; Velazaquez 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 atmospheric nitrogen gas (N₂). Nodules formed on the bean roots contain bacteria that convert N₂ 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 N₂ with beans including *R. leguminosarum* biovar *phaseoli*, *R. tropic* and *R. etli*. 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. For example in Shahrekord condition high rate of levels of inorganic nitrogen fertilizer used at the rates of 100- 300 kg N ha⁻¹.

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 et al., 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 N₂ fixation per se, like the high rate of levels used in intensive agriculture and the residual N remaining in soils (Rodriguez – Navaro et al., 1999). Genotype variation in bean for traits affecting nodulation and N₂ fixation has been found (Chara 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 N₂ 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 (Bergrson, 1980; Rodriguez – Navaro 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 aim of this work was to study the response of bean cultivars to inoculation with different *R. leguminosarum* biovar *phaseoli* strains obtained Collection from Institute of Soil and Biology Research Iran for best *Rhizobium / Phaseol* combination in field condition.

Materials and Methods
Field experiment was conducted in Shahrekord (latitude 32 ° 44' N, 2100 m asl). on spring and summer 2002. The mean annual rainfall is about 337 mm. The average annual temperature is 11.9 °C. Soil samples were collected to a depth of 0-30 cm before planting. The experiment was performed on soil that has not been cultivated with beans for at least 5 years. C, N, P and K content, 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 electrical conductivity (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, 1987).
The experiment was arranged in a randomized complete block design with a split plot layout and four replications. Four bacterial strains of rhizobia, N fertilizer (40 kg N ha\(^{-1}\) as urea at sowing and 60 kg N ha\(^{-1}\) at 35 days after sowing) and plus a control were assigned as factor A to main plots (Table. 2). Inoculation was added to the seeds with 15% (w/v) source solution to increase adherence. Factor B to subplots was bean Iranian cultivars including Shahrekord local (Cranberry bean), Talash (Cranberry bean) and Shahrekord local (Red Mexican type). 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 spotted bean (Graham, 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), nitrogen concentration of leaf and stem and percentage of nitrogen fixation. Percent nitrogen fixed was estimated according to Rannie(1984) with following formula:

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

Plants were sampled at 25 DAE, 45-50 DAE and at physiological maturity (70-80 DAE). 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 ANOVA using the statistical computer package SAS and treatment means separated using Duncan’s multiple range test at \(P < 0.05\) level.

**Results:**

**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, but similar was observed in seed inoculated with \(L-109, L-78\) strains and non-inoculated control (N fertilizer treatment) (Fig.1). Therefor, 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 (Giller, 2001; Hungria et al., 2000). 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 (Peres et al., 1994; Hungria et al., 1997b).

Hungria et al., 2000 reported that the common bean inoculated with \(PRF31, PRF55\) and \(PRF81\) showing high rates on N2 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 by Shisania, 2002 revealed that tepary bean inoculated with R3254 and non-inoculated (control without inoculated and N fertilizer) the highest and lowest seed yield, respectively. The results of analysis of variance indicated that there were high significant differences between cultivars in seed yield (Table 3). Talash cultivar showed higher seed yield than other cultivars (Table 4). The interaction between strain * cultivar effect was no significant difference (Table 3), but the results of mean comparison by DMR1 (at 5% level) showed the highest and lowest seed yield were associated to Talash (spotted bean) * N fertilizer (non-inoculated strain) and Shahrekord local (red bean) * control (Table 4). However, Talash (spotted bean) * L-125, Shahrekord local (red bean) * L-125 and Shahrekord local (red bean) * L-109 combinations similar were observed in seed yield with N fertilizer treatment (non-inoculated strain) (Table 4).

A significant interaction Rhizobium strain * common bean cultivar for seed yield was found (Rodriguez-Navarro et al., 1999). While, other authors (Buttery et al., 1997; Shisania 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.

**Shoot and pods dry matter**

Shoot and pods dry matter were influenced by seeds inoculated with strains and non-inoculated controls ($P<0.01$) (Table 3). Rodriguez-Navarro et al., 1999 have also reported that significant differences in shoot and pods growth were observed among common bean inoculated difference strains and non-inoculated controls (-N and +N treatments). 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). 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 (Fig. 2 and 3).

The results of study by Tamimi et al., 2002 showed that shoot dry matter was the highest for common bean inoculated with isolate JOVI. 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 (Shisania, 2002).

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 4). A significant interaction Rhizobium strain * common bean cultivar for shoot and pods dry matter were found by Rodriguez-Navarro and Buendia, 2000. 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.

1 -Duncan Multiple Range Test
N fixation rate
Table 3 shows that nodule dry weight (at 40-50% flowering), N total (shoot) and percent of fixed N\textsubscript{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 (Table. 3). The results revealed that all treatments were capable of nodulation, however, strains L-109 gave highest nodule dry weight, N total (shoot) and percent of fixed N\textsubscript{2} (Fig. 4 and 5). Furthermore, seed inoculation with strain increased nodule dry weight, N total (shoot) and percent of fixed N\textsubscript{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 that of plants supplied with N-fertilizer treatment.

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\textsubscript{2} fixation achieved with strain R. tropici type IIB CIAT 899 were 112% greater than with R. leguminosarum biovar phaseoli strain USDA 2671 and could reflect the adapted symbiosis of Brazilian cultivar with R. tropici species (Hungria et al., 2000). The study by Tamimi, 2002 showed that common bean inoculated with isolates JOV1, JOV3 and JOV10 the highest nodule number per plant and nodule mass. N content and N fixed % were also the highest for plants inoculated with these isolates. Isolate JOV3, however was the most effective followed by isolates JOV1 and JOV10. 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 percent of N\textsubscript{2} fixed. However, the highest nodule dry weight, nodule number and N total were achieved with Shahrekord local (spotted bean) cultivar (Table. 4). In the present study, no significant interaction Rhizobium strain * common bean cultivar for nodule number and weight at 40-50 % flowering, N total in shoot and percent of N\textsubscript{2} fixed were found (Table. 3). But the results of mean comparison by DMR\textsuperscript{2} (at 5% level) showed the highest nodule number, nodule weight, N total and percent of N\textsubscript{2} fixed were associated to Shahrekord local (spotted bean) cultivar * L-78, local (red bean) * L-109, local (red bean) * L-109 and local (red bean) * L-78, respectively (Table. 4). Further, the lowest nodule number, nodule weight and N total were achieved with Talash * N fertilizer treatment, local (red bean) * control (without N fertilizer) and Talash * control (without N fertilizer), respectively.

The results of investigation by Hungria et al., 2000 showed that the Brazilian cultivar Carioca received a mixed inoculum, R. tropici strains PRF 81, PRF 54 and PRF 35 showed higher nodule occupancy than R. etli strain CFN 42 and R. leguminosarum biovar phaseoli strain USDA2671. These results confirm a report also with Iranian cultivar (Asadi Rahmani, 2000) and disagrees with obtained in other country by Olivera and Graham (1990) and Streit et al., (1992). However, the strains different substantially in symbiotic properties, such as, nodulation and N\textsubscript{2} fixation capacity, as well as in the

\textsuperscript{2}Duncan Multiple Range Test
synthesis of Nod factors after inoculation with naringenin. Consequently, important symbiotic differences can be detected among strains showing genetic relatedness using DNA fingerprints obtained by PCR with these primers (Hungria et al., 2000).

**Discussion**

The results revealed that there were significant differences in seed yield, pods and shoot dry matter, nodule dry weight, N total and percent of fixed N\(_2\) were observed among seeds inoculated with different strains and non-inoculated controls (N fertilizer treatment and without N fertilizer treatment). Treatment of inoculated seeds with \(L-125\) strain and control demonstrated the highest and lowest grain yield, respectively. The results revealed that seed inoculation with strain increased nodule dry weight, N total (shoot) and percent of fixed N\(_2\) in relation to bean rhizobia population naturalized and N fertilizer treatment. However, strains \(L-109\) gave highest nodule dry weight, N total and percent of fixed N\(_2\). 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.

Study of interaction between cultivar and strain showed that the highest grain yield were associated to Talash (spotted bean) * \(L-125\) strain, Talash * \(L-109\) strain Shahrekord local (red bean) * \(L-125\) strain Shahrekord local (red bean) * \(L-78\) plus Talash (spotted bean) * N100 treatment. While the lowest grain yield was associated to Shahrekord local (red bean) * control treatment. Furthermore, The results of investigation showed that the cultivar of Shahrekord local (red bean) received a mixed inoculum, \(R.\) leguminosarum biovar phaseoli strain \(L-78\) (Shahrekord) showed higher percent of fixed N\(_2\) than other treatments.

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**Reference:**


5-Giller, KE (2001) Nitrogen fixation in tropical cropping systems. 2\(^{nd}\) edn. CABI publishing.


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<thead>
<tr>
<th>Strain</th>
<th>Year and space</th>
<th>Source</th>
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<td>Institute of soil and water of soil biology part in Iran</td>
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<td>Touysercan (Hamadan)-2002</td>
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<td>L-47</td>
<td>Feraydonshahr (Isfahan)-2002</td>
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<tr>
<td>L-125</td>
<td>Aleshtar (Lorastan)-2002</td>
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Fig 1. Mean grain yield as affected by different levels of main effects
Columns with the same letter are not significant at the 5% level (DMRT)

Fig 2. Mean pod dry matter as affected by different levels of main effects
Columns with the same letter are not significant at the 5% level (DMRT)
Fig 3. Mean shoot dry matter as affected by different levels of main effects
Columns with the same letter are not significant at the 5% level (DMRT)

Fig 4. Mean nod weight per plant as affected by different levels of main effects
Columns with the same letter are not significant at the 5% level (DMRT)