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*Full Length Research Paper*

# Effectiveness of native *Rhizobium* on nodulation and growth properties of dry bean (*Phaseolus vulgaris* L.)

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**This study was conducted in order to evaluate the symbiotic effectiveness of native *Rhizobium* isolated strains on number of nodule, weight, and morphological properties of dry bean. To realize this purpose, fresh and healthy root nodules were collected from major dry bean-growing districts of Konya, Turkey. These nodules were used to determine the effects of native *Rhizobium* isolated strains in the experiments. The first experiment was conducted to the determination of the most effective native isolated strains, by using Leonard bottles. Six (1, 3, 5, 23, 69 and 85) isolated strains of 94 native Rhizobium bacteria were found the most effective as compared with reference strain *Rhizobium tropici* CIAT899. The second experiment was conducted as pot experiment and arranged in a complete randomized block design with three replicates per bacteria. The subjects of the treatment subjects are control, nitrogen control, native isolated strain 1, native isolated strain 3, native isolated strain 5, native isolated strain 23, native isolated strain 69, native isolated strain 85, reference strain (*R. tropici* CIAT899) and mix strain. It has been shown that the used strains were significantly (P<0.05) increased nodulation and other morphological parameters. Significant differences existed in the symbiotic potential of the investigated strains. These results indicate that effective strains which were isolated from dry bean roots had potential for use as inoculants on beans (*Phaseolus vulgaris* L.). However, *Rhizobium* strains should be genetically identified before they are being subjected to recommend as commercial inoculations.**

**Key words:** Native *Rhizobium*, inoculation, bean, nitrogen fixation, yield, yield components.

## INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.), also called common bean or kidney bean, is one of the major leguminous crops grown in the world. The legume seeds are widely consumed around the world and their acceptability depends on the climatic conditions predominating around the cultivation area. In Turkey, *P. vulgaris* L. is the most widely consumed legume, supplying considerable amounts of nutrients to the diet of nourished people. The popularity of dry bean may be due to its high protein content and availability at relatively reasonable prices (Elshheikh and Elzidany, 1997; Uyanöz and Karaca, 2011). Native rhizobia are adapted to their soil environments. However, the environmental factors which result in promotion or stress on natural populations are poorly understood. Most published works on the ecology of rhizobia deals with introduced organisms as affected by crop-induced change in soil factors such as moisture, temperature, pH, and soil toxicity (Ham, 1980; Lowendorf, 1980; Schmidt, 1978). Inoculation of faba bean has been found to increase the shoot and root dry weights, number of nodules per plant, as well as yield and yield components. Undoubtedly, organic, inorganic and bio-fertilizers are important factors in increasing the yield of legumes (Salih and Elmuburak, 1986). The compatible rhizobia induce root nodules, where atmospheric nitrogen (N) is fixed. The number of the nodules and their rate of N fixation are determined by the effectiveness and the number of rhizobia in the soil (Lupwayi and Mkandawire, 1996; Amijee and Giller, 1998; Aynabeba et al., 2001). Furthermore, the level of effectiveness of Rhizobia results in variation in the color

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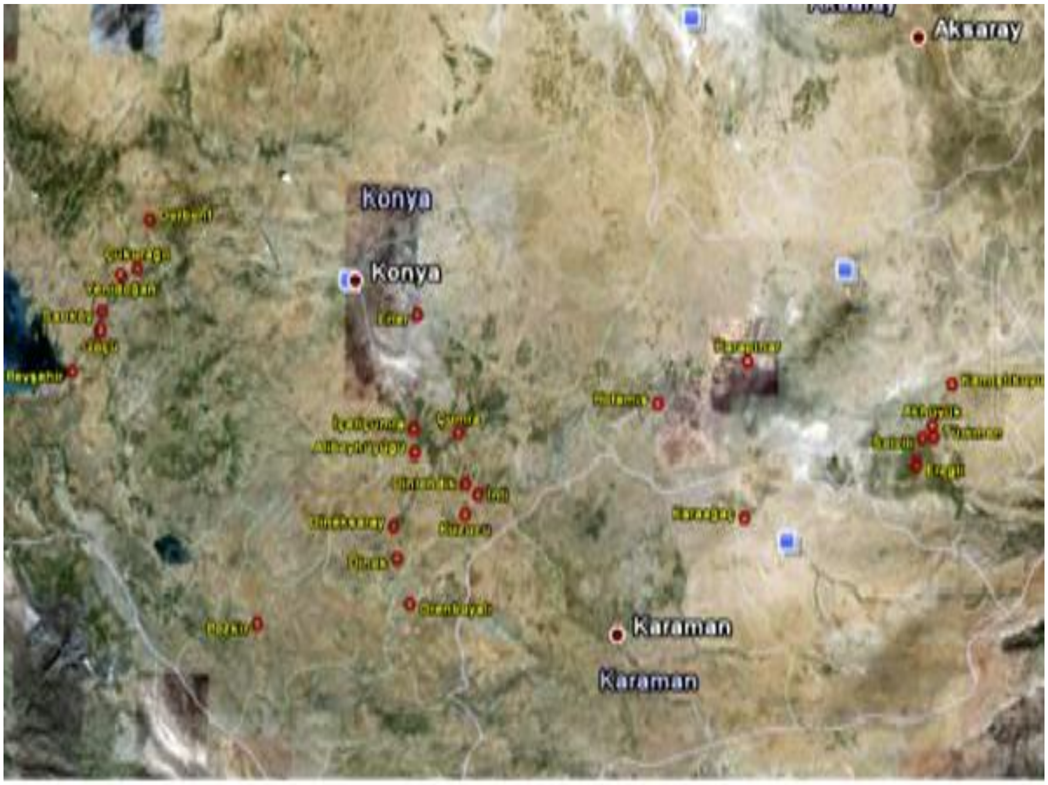


**Figure 1.** The locations where the nodule samples were taken from.

of nodules (pink indicates effective while white indicates ineffective in nitrogen fixation). The variation in nodule number and color in turn induce variation in growth and yield of the host due to variation in fixed N (Lupwayi and Mkandawire, 1996; Amijee and Giller, 1998). *Rhizobium* is a common soil bacterium. *Rhizobium* is not toxic to humans, plants or animals. It is one of the most beneficial bacteria in agricultural practices. Some *Rhizobium* is specific and nodulate only specific legumes, while others may nodulate several legumes. Native *Rhizobium* may be in sufficient numbers to nodulate both native and introduced legumes. In general, the native *Rhizobium* are low in numbers, are the wrong species or strain are being introduced with legume, or they are not efficient nitrogen fixers. Native rhizobia are adapted to their soil environments. However, the environmental factors have effects on native populations. This study was conducted in order to evaluate the symbiotic effectiveness of native *Rhizobium* isolated strains on number of nodule, weight, and the morphological properties of dry bean.

## MATERIALS AND METHODS

### Site description

The locations where the nodule samples were taken are illustrated in Figure 1. The soils in the study area were all in classic sediments which were brought from the uplands. The soil formation was mainly depended only texture and lime content of this sedimentary parent material, and also on climate and topography. The terraces, Colluvial Slopes, Alluvial Plains and Bajadas were all loam or clay and had a lime content ranging from 10% to 25%. The Konya Basin is one of the driest regions in Turkey. The climate is semiarid with cold moist winters and hot dry summers. Most of the time, evaporation exceeds total precipitation. The mountain in the south and west causes local variations especially in the wind and precipitation. The Çumra county of Konya province produces important amount of winter wheat besides other products such as sugar beet, dry bean, corn, melons and vegetables.

### Nodule sampling

Fresh and healthy root nodules and soil samples (a total of 94) were collected from farmers' dry bean fields in which dry bean has been grown with no history of inoculation with Rhizobia in Konya (Figure 1). In the present study, the area was searched for root nodules of plants during flowering period. *Rhizobium* sp. isolated from nodules was compared with reference bacteria (CIAT899) under greenhouse conditions in terms of their activities. The root nodule samples were randomly taken into sterilized polyethylene tubes from root per plant in each sampling site. Soil samples were also collected from root area of dry bean field.

### Isolation of native bacteria

Fresh and healthy root nodules, that collected from dry bean plants, were thoroughly washed with sterile distillated water and then surface sterilized for 3 to 4 min with a sodium hypochlorite solution (0.5% W/V) and again, rinsed six times with sterile

**Table 1.** Some physical and chemical properties of the soil used in the experiment.

| Property | Value |
|---|---|
| pH | 6.81 |
| Class | Sandy, clay, loam |
| Organic matter % | 1.59 |
| $CaCO_3$ (%) | 25.54 |
| N (mg $kg^{-1}$) | 42.14 |
| P (mg $kg^{-1}$) | 154.26 |
| K (mg $kg^{-1}$) | 63.5 |
| Fe (mg $kg^{-1}$) | 2.65 |
| Cu (mg $kg^{-1}$) | 1.99 |
| Mn (mg $kg^{-1}$) | 51.11 |
| Zn (mg $kg^{-1}$) | 0.41 |

distilled water. The nodules were then crushed in drop sterile water on a sterile glass slide using a scalpel. The suspension was streaked on Petri-dishes which was containing Yeast Extract Mannitol Agar (YEM) composed of the following which was dissolved in 1000 ml of distilled water: NaCl 0.1 g; mannitol 10.0 g; $MgS0_4.7\ H_2O$ 0.2 g; $CaCO_3$, 0.5 g; yeast extract, 0.5 g; agar 15.0 g, and $FeCl_26H_2O$ 0.002 M. The plates were incubated for 3 to 6 days at 28°C (Somasegaran and Hoben, 1994). After incubation for 3 to 4 days at 28°C, single colonies were selected and re- streaked on YEM agar for purity. The stock culture of pure *Rhizobium* was inoculated on YEM plate for 48 h at room temperature (26± 2°C) and then stored at 4 to 6°C (Vincent, 1970) for use in the experiments of the study.

**Test plant**

The Yunus 90 dcultivar (Anadolu Agricultural Research Institute, Eskisehir, Turkey) was dry bean used as the plant material. This genotype is one of the most popular common varieties which is grown in Central Anatolia, Turkey.

**Soil analyses**

The pH and electrical conductivity were measured in soil water extract (1/5 w/v) (Richards, 1954). Available P was extracted with sodium bicarbonate and determined by using the method which was developed by Olsen et al. (1954). Available K (extracted with ammonium acetate) was measured by flame photometry (Knudsen et al., 1982). Total N was determined by the Kjeldahl method (Bremner, 1965), $CaCO_3$ was determined by using the method which was developed by Hizalan and Ünal (1965) and organic matter was extracted as described by Smith and Weldon (1941). Texture was obtained by the hydrometer method (Bouyocous, 1951). In addition, Fe, Cu, Mn, and Zn contents of the soil were determined using the samples treated with the DTPA by ICP-AES (Soltanpour and Workman, 1981) (Table 1).

**Experimental design 1**

The first experiment was carried out under control to determine the effective of *Rhizobium* strain. For this purpose, 94 fresh root nodules were used. Dry bean seeds (*P. vulgaris L.*) were grown under greenhouse conditions for 8 weeks in Leonard jars. Surface sterilized seeds of dry beans were transferred aseptically to Leonard jars (five seeds per jar) and inoculated with 10 ml ($10^5$ to $10^6$ cfu $ml^{-1}$) of bacterial culture of *Rhizobium tropici* CIAT899 and native *Rhizobium* strains. Uninoculated plants were also included in each plant test. Each jar was containing three plants and they were grown under supplemental lighting (14 h/10 h light/dark cycles) and temperatures were maintained at 24/20°C. According to the results of the experiment by Leonard bottle, 1, 3, 5, 23, 69 and 85 strains were determined as effective compared with reference strain (*R. tropici* CIAT899) in the 94 *Rhizobium* bacteria which were isolated from different parts of the Konya region. Then, the 1, 3, 5, 23, 69 and 85 strains and reference strain were kept refrigerated at +4°C to use for the second experiment.

**Experimental design 2 (plant growth promotion experiments)**

The experiment was made in a randomized complete block design with three replicates. There were 10 treatments that consisted from six native *Rhizobium* strains, reference strain (*R. tropici* CIAT899), mix strain, uninoculated plants and nitrogen control. Dry bean cv. was grown in plastic pots (20 cm diameter and 15 cm length) that were filled up with sterile washed sand, perlite and soil. Pots were watered and allowed to free drain to field capacity for over two days. Seeds were surface-sterilized for 3 to 4 min with a sodium hypocloritte solution (0.5 % W/V) and then washed in clean running deionised water. Three healthy seeds were sown per pot at 4 to 5 cm depth and inoculated with the required inoculum (bacterial strain). Uninoculated pots were sown firstly to avoid cross-contamination. Each pot was inoculated with 10 ml of liquid inoculant ($10^5$ to $10^6$ cfu $ml^{-1}$) of *R. tropici* CIAT899 and native *Rhizobium* strains. All plants were watered with distill water every other day. Plants were grown in greenhouse conditions (day/night temperature: 28/20°C; 16 h light/8 h dark period; relative humidity 40/60%). During planting, mineral fertilizers such as nitrogen, phosphorus, and potassium (40 kg N, 60 $P_2O_5$, 50 kg $K_20$ $ha^{-1}$, respectively) were applied. In addition, Fe, Mn, Cu and Zn were applied as sulphates at 54, 100, 4.5 and 8.5 kg $ha^{-1}$, respectively. Plants were harvested during flowering period (8 weeks after sowing) and symbiotic effectiveness was estimated by comparing the shoots' dry weights (dried at 70°C) with those of uniinoculated control plants. Nodulation was evaluated by the number and dry weight of nodules. Besides, agronomic properties such as plant height, root length biomass, root weight, total dry matter, total nitrogen, total symbiotic efficiency and efficiency rates were determined. The dry weights were recorded once the nodules were

**Table 2.** Different isolates inoculation of Yunus 90 bean kind of plant greenhouse experiment to obtain yield component and dry matter values.

| Strain number | | Plant height (cm) | Root length (cm) | Shoot weight (g) | Root weight (g) | Total dry matter (g) |
|---|---|---|---|---|---|---|
| Control | | 6.47 ± 1.66 | 14.53 ± 5.52 | 1.06 ± 0.70 | 0.36 ± 0.05 | 1.42 ± 0.75 |
| Nitrogen control | | 9.67 ± 1.16 | 22.97 ± 0.58 | 3.10 ± 0.25 | 1.68 ± 0.40 | 4.78 ± 0.62 |
| Native isolate 1 | | 12.47 ± 1.08 | 23.97 ± 0.35 | 3.50 ± 0.40 | 1.62 ± 0.43 | 5.12 ± 0.82 |
| Native isolate 3 | | 13.57 ± 1.03 | 22.90 ± 1.71 | 3.62 ± 0.20 | 1.70 ± 0.18 | 5.32 ± 0.31 |
| Native isolate 5 | | 11.57 ± 0.23 | 24.30 ± 2.65 | 3.97 ± 0.27 | 1.96 ± 0.19 | 5.93 ± 0.26 |
| Native isolate 23 | | 9.33 ± 0.35 | 24.23 ± 1.57 | 2.63 ± 0.63 | 1.12 ± 0.30 | 3.75 ± 0.92 |
| Native isolate 69 | | 12.90 ± 0.17 | 23.57 ± 0.98 | 3.12 ± 0.11 | 1.48 ± 0.09 | 4.60 ± 0.13 |
| Native isolate 85 | | 10.20 ± 0.85 | 22.67 ± 0.65 | 3.56 ± 0.82 | 1.43 ± 0.29 | 4.99 ± 1.10 |
| CIAT899 | | 12.57 ± 1.40 | 26.70 ± 5.11 | 3.57 ± 0.22 | 1.78 ± 0.17 | 5.35 ± 0.06 |
| Mix strain | | 11.47 ± 0.40 | 22.43 ± 1.03 | 3.44 ± 0.71 | 1.37 ± 0.32 | 4.81 ± 0.99 |
| Source | DF | ANOVA | ANOVA | ANOVA | ANOVA | ANOVA |
| Isolate | 9 | * | * | * | * | * |

*=P < 0.01.

detached and roots dried in oven (70°C, 48 h). Symbiotic effectiveness (%) and efficient rate (%) were calculated as follows using the formula (Beck et al., 1993; Holding and Kong, 1963; Materon et al., 1995):

S.E. (%) = (A / B)*100

Where, S.E. = symbiotic effectiveness, A = the amount of nitrogen in the plant isolate inoculated, B = the amount of nitrogen with nitrogen control, E.R. (%) = (C / D)*100, E.R. (%) = efficient rate, C = the average weight dry matter of test plant and D = the average weight dry matter of with nitrogen control plant.

### Statistical analyses

All varieties were analyzed by using analysis of variance (ANOVA). Multiple comparisons of means were done by LSD method. All the data obtained were tested at the 1 and 5% confidence levels (Düzgüneş et al., 1987).

## RESULTS

### Pot experiment

The variance analysis of the obtained data showed that the strains had significantly affects on agronomic properties, plant height, root length of biomass, root weight, total dry matter, total nitrogen, total symbiotic efficiency and efficiency rates (Tables 2 and 3). All the strains that used in the experiment were effective on plant height compared with the control pots. The lowest values which were related to these parameters were obtained from the control treatment. However, the longest plants were taken from inoculants of native isolated strain 3, native isolated strain 69, reference strain, native isolated strain 1, native isolated strain 5 and mix strain (with the values of 13.57, 12.90, 12.57, 12.47, 11.57, 11.47 cm), respectively. It shows that almost half of the strains were more effective than reference strain. The variance analysis results showed that the difference between inoculations was significant in terms of root weight, but not for root length. Although native isolated strains were more effective than control, there was no significant difference among the native isolated strains. However, all the treatments differed significantly (P<0.05) in terms of root weight in dry bean plants (Yunus 90) compared to control. The increase in native *Rhizobium* and other inoculation (reference and mix), were 4 to 5 folds for root weight when compared to the control. Furthermore, inoculations with *R. tropici* CIAT899, native *Rhizobium* and their mixture were more effective on shoot weight of dry bean compared to the control. The shoot weight was 2.48 to 3.36 folds more than the control (non-inoculation). Nodules were determined on dry bean in all the treatment cases. However, total nodule numbers in dry bean significantly increased (P<0.05) compared to the control, but no nodule was found in the control treatments. The number of nodules differed significantly among native isolated strains. The number of nodules in the roots was found to be over 100 except for native isolated strain 85 and nitrogen treatment. Nitrogen treatment was effective in formation inhibiting of nodulation. Inoculation led to occurrence of significantly higher nodule number compared to the control. However, it was observed that nodules also formed in the uninoculated plants. The highest nodule number was obtained from reference strain (*R. tropici* CIAT899), mixture (co-inoculants) and native strain 1, respectively. Nodule weights of roots increased in line with the nodule number. However, the same correlation was not valid for *R. tropici* CIAT899. On the other hand, the nodule weights of isolated strains 1, 3, 5 and 69 were higher than those of the other isolated strains. Plant total dry weight in the pot experiment did not differ significantly among the inoculations but, the microbial inoculations

**Table 3.** Different isolates inoculation of Yunus 90 bean kind of plant greenhouse experiment to obtain yield component with nitrogen and nodulation values.

| Strain number | | N % (leaf+root) | Growth media N (mg kg$^{-1}$) | Nodule number (number/plant) | Nodule weight (number/g) | S. E. (%) | E. R. (%) |
|---|---|---|---|---|---|---|---|
| Control | | 2.72 ± 0.11 | 42.47 ± 9.11 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.00 ± 0.00 | 29.64 ± 15.70 |
| Nitrogen control | | 3.54 ± 0.05 | 53.57 ± 3.15 | 12 ± 1.73 | 0.05 ± 0.03 | 100.14 ± 1.34 | 99.93 ± 12.87 |
| Native isolate 1 | | 5.01 ± 0.24 | 71.87 ± 3.15 | 165 ± 65.18 | 1.32 ± 0.87 | 141.59 ± 6.75 | 107.05 ± 17.12 |
| Native isolate 3 | | 4.45 ± 0.20 | 61.09 ± 3.15 | 110 ± 25.00 | 1.56 ± 0.28 | 125.77 ± 5.69 | 111.43 ± 6.51 |
| Native isolate 5 | | 4.41 ± 0.25 | 75.46 ± 5.19 | 110 ± 15.00 | 1.41 ± 0.29 | 124.70 ± 7.20 | 124.06 ± 5.34 |
| Native isolate 23 | | 4.86 ± 0.30 | 65.66 ± 5.88 | 108 ± 40.29 | 0.70 ± 0.17 | 137.39 ± 8.46 | 78.52 ± 19.28 |
| Native isolate 69 | | 4.86 ± 0.06 | 65.01 ± 9.05 | 229 ± 46.29 | 1.31 ± 0.21 | 137.15 ± 1.69 | 96.31 ± 2.65 |
| Native isolate 85 | | 4.45 ± 0.61 | 79.71 ± 5.40 | 85 ± 50.27 | 0.41 ± 0.17 | 125.64 ± 17.15 | 104.39 ± 23.09 |
| CIAT899 | | 4.82 ± 0.17 | 92.45 ± 4.08 | 226 ± 63.79 | 0.93 ± 0.41 | 136.20 ± 4.84 | 111.92 ± 1.16 |
| Mix strain | | 4.03 ± 0.21 | 96.37 ± 7.36 | 215 ± 68.02 | 0.93 ± 0.06 | 113.90 ± 6.02 | 100.63 ± 20.78 |
| Source | DF | ANOVA | ANOVA | ANOVA | ANOVA | ANOVA | ANOVA |
| Isolate | 9 | * | * | * | * | * | * |

* = P < 0.01 S. E. : Simbiotic efficient E. R. : Efficient rate

increased the dry weight of dry bean compared to the among the inoculations but, the microbial inoculations increased the dry weight of dry bean compared to the control treatments. In particular, inoculations of the isolated strain 1 increased cumulative plant N. Total symbiotic efficiency, efficiency rate and total nitrogen content increased due to inoculations. Almost all the native isolated strains had significant effects in terms of symbiotic efficiency and efficiency rate.

## DISCUSSION

In this study, the increases in the number and weight of dry nodules, plant height, root length, biomass, root weight, total dry matter, total nitrogen, total symbiotic efficiency and efficiency rate were investigated after bacteria inoculations. However, it is a well known fact that factors such as salinity, temperature, water supply, pH, mineral nutrition and combined nitrogen have a great importance in the symbiosis process (Elsheikh and Elzidany, 1998). A favorable rhizosphere environment is highly important for the interaction between root hairs and *Rhizobium* as it does not only encourage the growth and multiplication of Rhizobia, but also ensures the healthy development of root hairs. Any environmental stress that affecting these processes is also likely to effect on infection and nodulation (Alexander, 1984; Cordovilla, et al., 1999). Also the present study, showed that there are differences among the inoculated strains in terms of some properties of dry bean such as dry nodule weight, plant height, root length biomass, root weight, total dry matter, total nitrogen, and total symbiotic efficiency and efficiency rate. Moreover, other studies with native inoculation or *Rhizobium sp.* and dry bean (Cebel, 1988; Chaverra and Graham, 1992; Özdemir, 2002; Slattery, et al., 2004) have shown that isolated strains used significantly ($P<0.05$) increased nodulation and other morphological parameters. Significant differences existed in the symbiotic potential of the isolates examined. In terms of shoot dry matter and $N_2$ fixation ratio only four of the isolated strains (1, 3, 5, and 23) possessed as promising symbiotic efficiency. In this study, the differences among isolated strains were also found such distinction could be explained with environmental condition in the experimental soil. That is to say that the soil properties have main importance in such microorganism-related studies. The match between rhizobia and the legume host is particularly important. The soils (*Rhizobium* was isolated and on which dry bean was grown) had alkaline pH, clay loam texture, high amounts of $CaCO_3$ and low organic matter.

The results were also showed that the inoculations were significantly different from each other with respect to plant agronomic properties. Moreover, the results of agronomic and symbiotic efficiency indicated that *Rhizobium* isolated strains which were isolated from soils that grown dry bean can be in harmony with *P. vulgaris L.*

The results of the present studies reveal that the native strains (1, 3, 5, 23, reference and mix) had significant effect on the plant height. Besides, the same strains had significant ($P<0.05$) effects on number of nodule and nodule weight. In addition, the isolated strains had positive effect on root weight, total dry matter, total nitrogen, total symbiotic efficiency and efficiency rate. However, the strains 69 and 85 did not have helpful effect on morphological properties of dry bean. An attribution could be made about to the possibility that the condition for the strains of 69 and 85 were unsuitable for favorable matching between rhizobia and the legume host. It suggests that nitrogenous fertilizers may have been used excessively on these soils. Naturally formed *Rhizobium* populations were large in number, their ability to fix nitrogen was high and they could therefore compete strongly with introduced *Rhizobium* inoculants.

## Conclusion

We consider that the isolated strains of *Rhizobium* have an ability to fix nitrogen and thus have a commercial potential. However, *Rhizobium* strains are to be genetically identified before they are being recommended for commercial inoculations.

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