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# Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization in two Cerrados soils

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**Abstract** Most soils sown with field beans (*Phaseolus vulgaris* L.) contain indigenous rhizobia which might interfere with the establishment of inoculated strains. As a consequence, the benefits of bean inoculation are usually questioned, and the use of N fertilizer is gradually becoming a common practice. The present study had the objective of evaluating the effectiveness of inoculation and N fertilization in field soil with (site 1) and without (site 2) a previous bean-cropping history. At site 1, which had a rhizobial population of $7 \times 10^2$ cells $g^{-1}$ soil, inoculation had no effect on nodulation or yield, whereas at site 2 ($<10$ cells $g^{-1}$ soil) inoculation increased nodulation, nodule occupancy by the inoculated strain and grain yield. N fertilizer decreased nodulation at both sites, but increased grain yield at site 1 but not at site 2, indicating that the response to inoculation and N fertilization depends on the cropping history. When bean was cultivated for the first time, indigenous populations of rhizobia were low and high yields were accomplished solely with seed inoculation, with no further response to N fertilizer. In contrast, previous cultivation of bean increases soil rhizobia, preventing nodule formation by inoculated strains, and N fertilizer may be necessary for maximum yields. A significant interaction effect between N fertilizer and inoculation was detected for serogroup distribution only at site 2, with N fertilizer decreasing nodule occupancy by the inoculated strain and increasing the occurrence of indigenous strains. Consequently, although no benefits were obtained by the combination of inoculation and N fertilizer, this practice may be feasible with the selection of appropriate N-tolerant strains from the indigenous rhizobial population.

**Key words** Rhizobial competitiveness · Inoculation · Dinitrogen fixation · Nodulation · *Phaseolus vulgaris*

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## Introduction

One-fifth of Brazil (204 million ha) constitutes an edaphic savanna known as "Cerrados" (Adámoli et al. 1986). With the development of economically viable fertilization practices, much of this area is now available for intensive agriculture. Common bean (*Phaseolus vulgaris* L.) is one of the major grain legumes cultivated in the Cerrados region, with its production centred in small farmers' fields where the use of N fertilizer is limited and average yields are low, usually $<500$ kg $ha^{-1}$. However, more recently, with the introduction of new bean cultivars and irrigation practices, some farmers have obtained yields $>3000$ kg $ha^{-1}$ with the use of high levels of fertilizers, including mineral N. Inoculation of beans is not adopted by the majority of the farmers due to doubts about the capacity of that practice to be translated into significant yield increases. Large-scale farmers, who usually make heavy investments, seek maximum yields that may not be accomplished with $N_2$ fixation as the main source of N. Under these circumstances, inoculation trials must emphasize not only the benefits of bean inoculation, but also of the combination of that practice with N fertilization, in order to achieve a decrease in mineral N input whilst still obtaining maximum yields.

In Brazil, yield response to bean inoculation under field conditions is variable, ranging from no responses to substantial increases. A lack of response to inoculation was reported, for example, by Vargas et al. (1983), Pereira et al. (1984) and Ramos and Boddey (1987).

However, under optimum conditions, with irrigation and in a gley humic soil without indigenous populations of *Rhizobium*, Mendes et al. (1994) observed that inoculation of bean cultivars Capixaba Precoce and CNPAF-178 promoted increases in grain yield similar to those obtained with 100 kg N $ha^{-1}$. Similar results were also reported in an Oxisol by Duque et al. (1985) with the Carioca and Negro Argel cultivars. Peres et al. (1994) observed, in well drained Oxisols of Cerrados without irrigation, that the yield gains due to inoculation were less pronounced, ranging from 63 to 290 kg $ha^{-1}$, than those in the non-inoculated treatments.

One of the major limiting factors for the success of the inoculation of common bean is the presence in the soil of a high population of indigenous rhizobia with the ability to nodulate that crop (Graham and Halliday 1977; Graham 1981; Moxley et al. 1986; Kucey 1989; Vargas et al. 1991; Lovato et al. 1991; Peres et al. 1994). The higher nodulation capacity of indigenous strains generally is a result of a numeric advantage in nodular infection sites, rather than competitiveness (Brockwell et al. 1988; Triplett and Sadowsky 1992). Thies et al. (1991) found that once the soil contained more than 10 bean rhizobia $g^{-1}$ soil, almost no response was obtained with rhizobial inoculation, but Vlassak et al. (1996) observed that reinoculation before the second bean crop with a very competitive *Rhizobium tropici* IIB strain resulted in a high percentage of nodule occupancy by the inoculated strain, even with a soil rhizobial population as high as $30 \times 10^3$ cells $g^{-1}$.

Under irrigated conditions it has been debated whether or not inoculation with *Rhizobium* is able to provide all the N necessary for high yields. Therefore, in this study we examined the response of field-grown, irrigated *P. vulgaris* to several levels of N fertilizer with and without inoculation. Another objective was to compare the responses in a soil cultivated for the first time with bean and another soil previously cultivated with this legume and with an established population of bean rhizobia.

## Materials and methods

### Field trials

Two field experiments were carried out in 1993 in the Brazilian Cerrados Research Centre (EMBRAPA CPAC; Planaltina, DF, Brazil) in a clay loam Red Yellow Oxisol. Experiment I was carried out at a site that had been cultivated for 5 years with several crops, and in 1992 was cultivated with bean inoculated with the *Rhizobium leguminosarum* bv. *phaseoli* strain UMR 1135 (from the University of Minnesota). Experiment II was carried out at a site that had been cultivated with soybean in 1991 and corn in 1992, and had never been cultivated with beans. The chemical analysis of the two soils before planting gave the following values for experiments I and II, respectively: pH ($H_2O$) 5.5 and 5.5; 0.04 $cmol_c$ Al $dm^{-3}$ and 0.05 $cmol_c$ Al $dm^{-3}$; 4.42 $cmol_c$ Ca plus Mg $dm^{-3}$ and 4.45 $cmol_c$ Ca plus Mg $dm^{-3}$; 14 mg P $dm^{-3}$, and 8 mg P $dm^{-3}$; 123 mg K $dm^{-3}$ and 33 mg K $dm^{-3}$. Ca, Mg and Al were extracted with 1 N KCl and determined through atomic absorption (Ca and Mg) and titration with 0.025 M NaOH (Al); P and K were extracted using the Mehlich 1 reagent (0.0125 M $H_2SO_4$ plus 0.05 M HCl) and determined through flame spectrophotometry (K) and the molybdenate blue method (P) (EMBRAPA 1979). In both soils, 500 kg NPK $ha^{-1}$ (0/20/20) were applied to correct for P and K deficiencies. At each site, 15 samples were taken randomly and mixed thoroughly. *Rhizobium* numbers were determined on these soil samples by the most probable number (MPN) technique (Vincent 1970). The indigenous rhizobial population capable of nodulating bean plants was estimated to be $7 \times 10^2$ rhizobia $g^{-1}$ soil in experiment I and $< 10$ rhizobia $g^{-1}$ soil in experiment II.

Both experiments had a completely randomized block experimental design with four replicates. Plots were 5.0-m long and consisted of nine rows planted 50 cm apart, with 20 seeds $m^{-2}$ of the bean cultivar Carioca. *R. tropici* strain CIAT 899 (original strain provided by Dr. Peter Graham, University of Minnesota; other denominations for this strain are UMR 1899, USDA 9030, TAL 1797, HAMBI 1163, SEMIA 4077 and ATCC 49672), which is recommended as a commercial inoculum in Brazil, was applied as a single-strain powdered-peat inoculum. The inoculum was prepared from a pure culture grown in yeast mannitol broth. Broth culture was applied to sterilized peat (the pH of which had been previously raised to 6.5 with $CaCO_3$) to reach about 50% of its water-holding capacity. The mixture was allowed to mature at room temperature for 30 days. Plate counts showed $1 \times 10^9$ cells $g^{-1}$ peat and a MPN of $2 \times 10^8$ cells $g^{-1}$. Immediately before planting, the seeds were inoculated by preparing a peat slurry with a 25% sucrose sticker solution, which was applied at a rate of 1 kg inoculum 40 $kg^{-1}$ seeds.

In experiment I, three levels of urea (20 kg N $ha^{-1}$, 40 kg N $ha^{-1}$, and 60 kg N $ha^{-1}$) were tested with and without inoculation, whereas in experiment II, four levels of urea (20 kg N $ha^{-1}$, 40 kg N $ha^{-1}$, 60 kg N $ha^{-1}$ and 80 kg N $ha^{-1}$) were tested with and without inoculation. The N fertilization was split into three side-dressings at 14, 26 and 40 days after emergence (DAE) in experiment I and 11, 26 and 43 DAE in experiment II. In both experiments there were two control treatments, one without inoculation and without N fertilizer and one with inoculation without N fertilizer.

Twelve plants per plot were collected at 20 and 15 DAE to evaluate nodule number and dry weight, in experiments I and II, respectively. Six plants per plot were collected for the determination of number and dry weight of nodules, serological analysis and plant dry weight, at 40 and 35 DAE, in experiments I and II, respectively. Neighbouring plants were removed from near the end of the third and seventh rows of each plot. Plant tops were dried immediately after harvest by placing them in a forced-air dryer for 72 h at 72 C. The root systems were rinsed with tap water, the nodules were detached, dried at 72 °C for 72 h, weighed and counted.

At harvest, a 4.0-m section was removed from the four central rows of each plot. Harvested seeds were cleaned and weighed. Yields were adjusted to 13% moisture content.

### Nodule serotyping

Serotyping of nodules was done by immuno-agglutination (Vincent 1970), and antisera were prepared against strains CIAT 899, CENA C05, UMR 1135 and CPAC V23, as described by Somasegaram and Hoben (1994). The last three strains were formerly used in common bean inocula produced in Brazil, and for this reason were included in this analysis. Background levels of the inoculum serogroup (CIAT 899) and the serogroups UMR 1135, CPAC V23 and CENA C05 were determined by serotyping the uninoculated plots. Nodule suspensions were prepared in sterile 0.8% NaCl and boiled for 1 h at 100 °C. For each nodule, agglutination was performed in microtiter trays. Three hundred microlitres of the nodule preparation (antigen) was mixed with one drop of the appropriate antiserum, besides the antigen control which contained only the antigen in physiological saline. Positive reactions viewed through a light source were determined by the

formation of a white precipitate. Percentage recovery of strains CIAT 899, UMR 1135, CPAC V23 and CENA C05 was determined by serotyping 50 nodules selected at random from each plot.

### Statistical analyses

Data were analysed using the SAS software package (SAS, Cary, N.C.). Percentage data were subjected to arc sin $\sqrt{x}$ transformations prior to analysis and retransformed means are presented.

The experiment was analysed as a factorial with two levels for inoculation (with and without) and three (experiment I) and four (experiment II) levels for N fertilizer rates. The main interest in these experiments were the differences between treatments with and without inoculation, among N rates, and the interaction between inoculation and N fertilizer levels. If interactions were significant ($P<0.05$) they are presented, otherwise only main effects are shown. Comparisons among N rates were made by using Duncan's multiple range test.

## Results and discussion

Inoculation response was variable in the two areas. In experiment I, which was cultivated with beans in the previous year and presented a bean rhizobial population of $7\times10^2$ cells $g^{-1}$ soil, plants were well nodulated in the control treatment and inoculation had almost no effect on the parameters evaluated (Table 1). The serological analysis confirmed that the inoculated treatment failed to increase the nodule occupancy by strain CIAT 899 in nodules, and that the strain inoculated in the previous bean crop, UMR 1135, also established poorly in the soil (Table 2). Consequently, in this experiment, 90% of the nodules were occupied by indigenous strains (Table 2). A taxonomic study performed in the same area showed that 70% of the bean rhizobial strains could be classified as *R. tropici* IIA and 19% as *R. tropici* IIB (Hungria et al. 1997b).

Most soils in which field beans are commonly grown contain populations of indigenous rhizobia (Graham 1981; Rennie and Kemp 1983; Moxley et al 1986; Lovato et al. 1991; Peres et al. 1994) which compete for nodulation sites with rhizobia added as inocula. With a high population of rhizobia, it is likely that the indigenous rhizobia have a numeric advantage for nodular infection sites in relation to the inoculated strain. Thies et al. (1991) found that once the soil contained more than 10 rhizobia $g^{-1}$ soil, almost no response was obtained with rhizobial inocula, and Kucey (1989) also failed to obtain any positive response in soils with native populations $>8\times10^3$ cells $g^{-1}$ soil. However, Vlassak et al. (1996), reinoculating a very competitive bean rhizobia, reported a large percentage of nodules occupied by the inoculated strain before the second crop, when the soil had a population of $30\times10^3$ cells $g^{-1}$, but not before the third crop, when the soil had $10^4$ cells $g^{-1}$. In experiment I, although CIAT 899 was used as a highly enriched inoculum ($10^9$ cells $g^{-1}$), the strain was not able to compete against the soil rhizobial population estimated at $7\times10^2$ cells $g^{-1}$.

In contrast, site 2, which had not been previously cropped with bean and showed a bean rhizobial population of $<10$ cells $g^{-1}$ soil, non-inoculated plants were poorly nodulated at 15 DAE. Inoculation had a statistically significant effect on nodulation parameters at 15 and 35 DAE and on grain yield (Table 3), and also increased the occurrence of the inoculated strain in nodules from 0 to 91% (Table 4). Mendes et al. (1994) and Peres et al. (1994) also reported significant increases in bean nodulation with inoculation of Cerrados soils in the first year of cultivation. These data are an indication that the rhizobial population capable of nodulating bean is small in Cerrados soils, but that the indigenous population may increase with the cultivation of bean, interfering with nodule formation by the ino-

**Table 1** Effects of inoculation with *Rhizobium tropici* strain CIAT 899 and N fertilization on nodulation [nodule number (*NN*) and nodule dry weight (*NDW*)], shoot dry weight and grain yield of bean cultivar *Phaseolus vulgaris* Carioca grown in a soil previously cropped with this legume and showing a bean rhizobial population of $7\times10^2$ cells $g^{-1}$ soil (site 1). Means at 20 days after emergence (*DAE*) ($n=12$) and at 40 DAE ($n=6$) followed by *different letters* were statistically different at $P<0.05$ (Duncan's test). *ns* Statistically not significant

| | Nodulation (mg plant$^{-1}$) | | | | Shoot dry weight (g plant$^{-1}$) | Yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| | NN | NDW | NN | NDW | | |
| | 20 DAE | | 40 DAE | | 20 DAE | |
| N rate | | | | | | |
| 0 kg N ha$^{-1}$ | 50 | 19.6 a | 40 a | 71 a | 2.89 b | 2976 c |
| 20 kg N ha$^{-1}$ | 41 | 11.7 b | 37 a | 49 ab | 2.91 b | 3339 b |
| 40 kg N ha$^{-1}$ | 47 | 15.3 b | 28 ab | 31 bc | 3.57 a | 3292 bc |
| 60 kg N ha$^{-1}$ | 38 | 8.8 c | 22 b | 15 c | 4.08 a | 3695 a |
| $P<F$ | ns | *** | ** | *** | *** | *** |
| Inoculation | | | | | | |
| Without | 39 b | 11.3 b | 30 | 42 | 3.21 | 3292 |
| With | 49 a | 16.4 a | 32 | 41 | 3.51 | 3360 |
| $P<F$ | *** | *** | ns | ns | ns | ns |

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

**Table 2** Effects of inoculation with strain CIAT 899 and N fertilization on serogroup distribution of bean nodules of plants grown in a soil previously cropped with this legume and showing a rhizobial population of $7 \times 10^2$ cells $g^{-1}$ soil (site 1). Means of plants harvested at 40 DAE ($n = 6$). For abbreviations, see Table 1

| | Serogroup distribution in nodules (%) | | | | |
|---|---|---|---|---|---|
| | CIAT 899 | UMR 1135 | CPAC V23 | CENA C05 | No reaction |
| N rate | | | | | |
| 0 kg N $ha^{-1}$ | 4.0 | 3.0 | 0.6 | 0.0 | 82.3 |
| 20 kg N $ha^{-1}$ | 1.8 | 2.0 | 4.4 | 0.2 | 90.1 |
| 40 kg N $ha^{-1}$ | 1.0 | 0.8 | 8.0 | 0.0 | 90.3 |
| 60 kg N $ha^{-1}$ | 1.3 | 1.0 | 3.2 | 0.2 | 94.3 |
| $P < F$ | ns | ns | ns | ns | ns |
| Inoculation | | | | | |
| Without | 2.4 | 11.8 | 7.9 | 0.2 | 88 |
| With | 1.6 | 5.9 | 5.2 | 0.0 | 91 |
| $P < F$ | ns | ns | ns | ns | ns |

**Table 3** Effects of inoculation with strain CIAT 899 and N fertilization on nodulation (NN and NDW), shoot dry weight and grain yield of bean cultivar Carioca grown in a soil that had not been previously cropped with this legume and showing a rhizobial population $< 10$ cells $g^{-1}$ soil (site 2). Means of plants at 15 DAE ($n = 12$) and of plants at 35 DAE ($n = 6$) followed by *different letters* were statistically different at $P < 0.05$ (Duncan's test). For abbreviations, see Table 1

| | Nodulation (mg $plant^{-1}$) | | | | Shoot dry weight (g $plant^{-1}$) | Yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|
| | NN | NDW | NN | NDW | | |
| | 15 DAE | | 35 DAE | | 35 DAE | |
| N rate | | | | | | |
| 0 kg N $ha^{-1}$ | 26 | 10.0 | 22 | 50.0 | 1.89 b | 3030 |
| 20 kg N $ha^{-1}$ | 36 | 14.4 | 29 | 44.6 | 2.08 ab | 3214 |
| 40 kg N $ha^{-1}$ | 32 | 12.1 | 26 | 33.1 | 2.15 ab | 3347 |
| 60 kg N $ha^{-1}$ | 27 | 11.4 | 22 | 25.6 | 2.42 a | 3494 |
| 80 kg N $ha^{-1}$ | 34 | 10.8 | 23 | 22.4 | 2.38 a | 3402 |
| $P < F$ | ns | ns | ns | ns | * | ns |
| Inoculation | | | | | | |
| Without | 21 b | 7.9 b | 14 b | 28.0 b | 2.20 | 3196 b |
| With | 41 a | 15.6 a | 34 a | 43.5 a | 2.19 | 3444 a |
| $P < F$ | *** | *** | *** | ** | ns | * |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

**Table 4** Effects of inoculation with strain CIAT 899 and N fertilization on serogroup distribution of bean nodules of plants grown in a soil that had never been cropped previously with this legume (site 2)

| | Serogroup distribution in nodules(%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CIAT 899 | | UMR 1135 | | CPAC V23 | | CENA C05 | | No reaction | |
| | Non-inoculated | Inoculated | Non-inoculated | Inoculated | Non-inoculated | Inoculated | Non-inoculated | Inoculated | Non-inoculated | Inoculated |
| N rate | | | | | | | | | | |
| 0 kg N $ha^{-1}$ | 0.0 | 91.4 | 0.0 | 0.5 | 0.0 | 3.5 | 0.0 | 0.0 | 100 | 4.5 |
| 20 kg N $ha^{-1}$ | 0.6 | 54.4 | 0.0 | 0.0 | 3.8 | 1.5 | 0.0 | 0.0 | 98.0 | 43.9 |
| 40 kg N $ha^{-1}$ | 0.6 | 39.9 | 0.6 | 0.0 | 1.3 | 0.5 | 0.0 | 0.0 | 97.6 | 59.5 |
| 60 kg N $ha^{-1}$ | 2.3 | 42.5 | 0.0 | 0.0 | 1.0 | 1.5 | 0.5 | 0.5 | 94.6 | 55.2 |
| 80 kg N $ha^{-1}$ | 0.0 | 17.8 | 0.0 | 1.0 | 2.5 | 3.8 | 0.0 | 0.5 | 90.4 | 76.7 |

culated strains. An increase in the bean rhizobial population in soils with continuous cultivation of this legume was previously reported in Brazil by Vlassak et al. (1996) and Hungria et al. (1997a, 1997b).

At site 1 (previously cultivated with bean), application of N fertilizer (starting 14 DAE) had no effect on early nodule formation (number of nodules at 20 DAE), but decreased nodule number at 40 DAE and nodule dry weight at 20 and 40 DAE, particularly at high levels of N (Table 1). N fertilizer also increased both shoot dry matter of plants at 40 kg N $ha^{-1}$ and 60 kg N $ha^{-1}$ and grain yield, particularly with 60 kg of

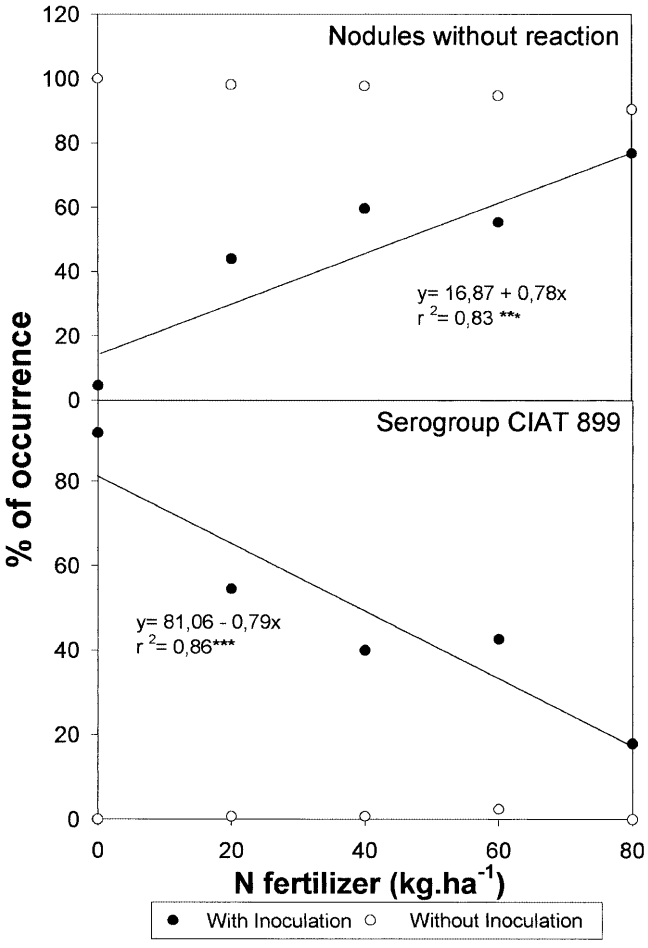


**Fig. 1** Effects of N fertilizer (kg $ha^{-1}$) on nodule occupancy in *Phaseolus vulgaris* L. by unidentified native rhizobia and *Rhizobium tropici* strain CIAT 899

N $ha^{-1}$. For site 2, statistical differences in nodulation and grain yield were not detected due to the application of N fertilizer, although nodule dry weight at 40 DAE decreased by up to 55% under the higher levels of mineral N (Table 3).

The negative effects of N fertilizer on bean nodulation have been well described in the literature since early studies were made (Fred and Graul 1916). However, the use of N fertilizer with bean crops has been gradually adopted by many farmers, aiming to maximize grain yield, particularly when irrigation systems are used. Under these conditions, rhizobial inoculation must be complemented by N fertilization to reach higher yields. Some studies have reported the inhibition of bean symbiosis with the application of N fertilizer doses normally recommended for this legume, i.e. 40–60 kg N $ha^{-1}$ (Ruschel et al. 1979; Graham 1981). However, synergistic effects between low levels of mineral N and inoculation have been shown (Silva et al. 1993; Tsai et al. 1993), and Silva et al. (1993) observed that the use of 10 kg N $ha^{-1}$ after plant emergence increased bean grain yield, whereas 50 kg N $ha^{-1}$ inhibited nodulation and failed to increase yield.

There was a statistically significant interaction between mineral N and nodule occupancy by strain CIAT 899, and by the indigenous strains (nodules without reaction) at site 2. As shown in Fig. 1, in the inoculated plots, increasing levels of N fertilizer decreased nodule occupancy by the inoculated strain CIAT 899 from 91% to 17%. In constrast, nodule occupancy by the indigenous strains (nodules without reaction) followed an opposite trend, indicating that the latter are more tolerant to mineral N. These indigenous strains may be valuable in programs for the selection of rhizobial strains tolerant to mineral N.

The results of the present study indicate that the response to inoculation and N fertilization differs according to cropping history. When bean is cultivated for the first time, indigenous rhizobial populations are low and yields >3000 kg $ha^{-1}$ may be accomplished exclusively with seed inoculation, with no further response to N fertilizer levels as high as 80 kg N $ha^{-1}$. In contrast, previous cultivation of bean increases populations of indigenous soil rhizobia and prevents nodule formation by the inoculated strain, therefore N fertilizer may be necessary in order to reach maximum yields. When inoculation and N fertilizer were applied to a site previously cultivated with bean, no benefits were obtained by the combination of inoculation with N fertilization, but this practice may be feasible with the selection of N-tolerant strains and appropriate strategies of N-fertilizer management.

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