Revista Brasileira de Ciência do Solo
ISSN: 0100-0683
revista@sbcs.org.br
Sociedade Brasileira de Ciência do Solo
Brasil

Martins Mercante, Fábio; Akio Otsubo, Auro; Rodrigues Brito, Osmar
New Native Rhizobia Strains for Inoculation of Common Bean in the Brazilian Savanna
Revista Brasileira de Ciência do Solo, vol. 41, 2017, pp. 1-11
Sociedade Brasileira de Ciência do Solo
Viçosa, Brasil

Revista Brasileira de Ciência do Solo

Division - Soil Processes and Properties | Commission – Soil Biology

New Native Rhizobia Strains for Inoculation of Common Bean in the Brazilian Savanna

Fábio Martins Mercante[(1)], Auro Akio Otsubo[(1)*] and Osmar Rodrigues Brito[(2)]

[(1)] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agropecuária Oeste, Dourados, Mato Grosso do Sul, Brasil.
[(2)] Universidade Estadual de Londrina, Departamento de Agronomia, Londrina, Paraná, Brasil.

ABSTRACT: Maximization of biological nitrogen fixation in the common bean (*Phaseolus vulgaris* L.) crop depends on the genetic characteristics related to the plant, the symbiotic efficiency of rhizobia, and environmental factors. The objective of this study was to evaluate the performance of rhizobia selected beforehand from *Cerrado* (Brazilian tropical savanna) soils in Mato Grosso do Sul. The experiments were conducted in 2007 in the municipalities of Aquidauana, Anaurilândia, Campo Grande, and Dourados, all located in the state of Mato Grosso do Sul. All procedures established followed the current recommendations of the Brazilian Ministry of Agriculture (Ministério de Agricultura, Pecuária e Abastecimento – MAPA), in accordance with the "official protocol for assessing the feasibility and agronomic efficiency of strains, and inoculant technologies linked to the process of biological nitrogen fixation in legumes". The program for selection of rhizobia for inoculation in bean plants resulted in identification of different strains with high symbiotic efficiency, competitiveness, and genetic stability, based on the Embrapa Agropecuária Oeste collection of multifunctional microorganism cultures. In previous studies, 630 isolates of *Rhizobium* were evaluated. They were obtained from nodules of leucaena (380) or dry beans (250) from 87 locations, including 34 municipalities in Mato Grosso do Sul. Three of them stood out from the others: CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 56.4 L2. Inoculation of these strains in bean plants demonstrated economic viability and high potential for obtaining a more effective inoculant suitable for trading purposes.

Keywords: *Phaseolus vulgaris*, *Rhizobium tropici*, microbial inoculants, biological nitrogen fixation.

*** Corresponding author:**
E-mail: auro.otsubo@embrapa.br

Received: September 14, 2015

Approved: May 16, 2016

How to cite: Mercante FM, Otsubo AA, Brito OR. New native rhizobia strains for inoculation of common bean in the Brazilian Savanna. Rev Bras Cienc Solo. 2017;41:e0150120.
https://doi.org/10.1590/18069657rbcs20150120

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), hereinafter "bean", is a crop of great social and economic importance in Brazil because, in addition to being one of the main sources of protein for the population, it is a source of income for thousands of rural producers, especially family farmers. Considering all three systems of bean production, at a low, medium, and high technological level, corresponding to the first, second, and third crop seasons, the average yield obtained in 2013 was 789, 1061, and 2466 kg ha^{-1}, respectively (IBGE, 2015). Among the different aspects related to the cropping systems and bean production, N management stands out due to high fertilizer costs (Cantarella, 2007). Moreover, the possibility of environmental contamination through adoption of unsuitable techniques and inadequate management must be considered, as shown by several authors, such as Straliotto et al. (2002) and Pelegrin et al. (2009). In this context, management of N fertilization is one of the main problems in the bean crop, since insufficient application rates may limit its productive potential and excessive N rates are not only costly, but can also promote negative environmental impact (Santos et al., 2003).

Biological nitrogen fixation (BNF) process can, at least partially, supply the requirements of this crop and ensure greater yield levels, up to 2500 kg ha^{-1} (Hungria et al., 2000; Pelegrin et al., 2009). Nevertheless, factors such as soil acidity, high concentrations of toxic Al and the competitive ability of rhizobia native to Brazilian soils can affect the symbiosis between rhizobia strains and bean crop, causing low efficiency in the inoculation process (Mercante et al., 1999; Vargas et al., 2000; Ferreira et al., 2013). Issues related to genetic instability of many rhizobia strains may cause a loss of efficiency or nodulation capacity under stress conditions, which may explain, in part, the considerable diversity in results obtained from inoculation of bean under field conditions (Peres et al., 1994; Hungria and Vargas, 2000; Hungria et al., 2003; Pelegrin et al., 2009).

Several species of *Rhizobium* have been described as capable of creating nodules and, in most cases, fixing N_2 in symbiosis with bean: *R. leguminosarum* bv. *phaseoli* (Jordan, 1984), *R. tropici* (Martínez-Romero et al., 1991), *R. etli* bv. *phaseoli* (Segovia et al., 1993), *R. gallicum* (bv. *gallicum* and bv. *phaseoli*), *R. giardinii* (bv. *giardinii* and bv. *phaseoli)* (Amarger et al., 1997), *R. leucaenae* (Ribeiro et al., 2012), *R. freirei* (Dall'Agnol et al., 2013), and *R. paranaense* (Dall'Agnol et al., 2014). An important feature of the bean microsymbiont rhizobia genome is the presence of DNA repeated sequences found in the symbiotic plasmid regions, pSym containing the structural nitrogenase gene, *nif*, or the nodulation gene, *nod* (Girard et al., 1991; Segovia et al., 1993). These copies are usually needed for expression of the BNF process and effectiveness, but they also represent recombination sites where genomic rearrangements occur, which can lead to loss of properties in these strains (Girard et al., 1991), providing the explanation for the frequent occurrence of loss of ability to create nodules and to fix N_2 in various strains.

Studies carried out in large group strains of *R. tropici,* including *R. leucaenae*, *R. freirei*, and *R. paranaense* showed that this specie had greater genetic stability than the other bean microsymbiont, probably due to a single nifH gene copy (Piha and Munns, 1987; Martínez-Romero et al., 1991). Estimates of rearrangement frequency show that *R. tropici* can be 100 times more stable than *R. etli* and, when considering plasmid loss, the stability of *R. tropici* can be a hundred times higher than *R. etli* (Ferreira et al., 2013). Recent studies involving analysis of symbiotic and non-symbiotic genes of 15 rhizobia strains selected in programs of four distinct Brazilian geographical regions showed that all these strains clustered with *R. tropici* type A or type B and suggested that the strain selection in this species could reflect evolutionary strategies to maximize nodulation and BNF in bean (Pinto et al., 2007).

In this context, programs for continuous selection of genetically more stable native strains, adapted to local climatic conditions, with greater competitiveness in Brazilian soils and tolerant to environmental stresses, can significantly contribute to achieve more efficient fixation systems and, consequently, increases in bean yield and even contribute to reduction in N fertilizer application that increases production costs and negatively affects the environment.

In previous works several native rhizobia strains capable of fixing nitrogen in bean plants have already been selected by the Embrapa in *Cerrado* soils in the state of Mato Grosso do Sul. This study was carried out with the objective to evaluate the strains agronomic efficiency in order to indicate commercial inoculants compositions to the bean culture.

MATERIALS AND METHODS

Experimental Conditions

The experiments were carried out in the 2007 crop year in bean production areas in four municipalities in the state of Mato Grosso do Sul: Aquidauana (20° 28′ S; 55° 47′ W; 147 m); Anaurilândia (22° 11′ S; 52° 43′ W; 312 m); Campo Grande (20° 26′ S; 54° 38′ W, 532 m); and Dourados (22° 16′ S; 54° 49′ W; 408 m). The areas selected to set up the trials belonged to the Experimental Station of the Universidade Estadual do Mato Grosso do Sul (UEMS), *Fazenda São José*, the Experimental Station of the Universidade para o Desenvolvimento do Estado e da Região do Pantanal (UNIDERP), and the Experimental Station of Embrapa Agropecuária Oeste, respectively. The soils at these different sites were classified as *Argissolo Vermelho-Amarelo Distrófico* (Santos et al., 2013), a sandy Paleudalf (Soil Survey Staff, 2010), *Argissolo Vermelho-Amarelo* (sandy loam Paleudalf), *Latossolo Vermelho Distrófico* (clayey Rhodic Hapludox), and *Latossolo Vermelho Distroférrico* (clayey Rhodic Hapludox), respectively. Chemical analyses of soil samples collected from the 0.00-0.20 m layer are shown in table 1.

The tillage procedures for setting up the experiments in the Aquidauana, Anaurilândia, and Campo Grande areas combined one plowing with two harrowings; in the Dourados area, the no tillage system was used. The cultivar *Pérola* of the *Carioca* commercial group was used in the four experiment locations. Seeds were sown manually by distributing 15 seeds per meter, aiming to obtain approximately 10 plants m^{-1}. Seeds were sown on April 4, April 17, April 19, and May 5, 2007 in Dourados, Anaurilândia, Campo Grande, and Aquidauana, respectively. Fertilization in the municipalities of Anaurilândia, Dourados, and Campo Grande consisted of the application of 300 kg ha^{-1} of the formulation 00-20-20 (N-P-K), whereas in Campo Grande, the fertilization was 270 kg ha^{-1} of the same formulation. For the control with N fertilization, 40 kg ha^{-1} N (urea) was applied at sowing and the same amount manually in topdressing at 30 days after emergence.

A randomized block experimental design was used with 10 treatments and four replications. The following treatments were used: control, without inoculation and without N fertilization; N fertilization with 80 kg ha^{-1} of N; strain CIAT 899 (SEMIA 4077) + 20 kg ha^{-1} of N at sowing; strain CIAT 899 (SEMIA 4077); strain PRF 81 (SEMIA 4080); strain H 12 (SEMIA 4088); strain CPAO 2.11 L; strain CPAO 12.5 L2; strain CPAO 17.5 L2; and strain CPAO 56.4 L2.

Table 1. Soil chemical properties of experimental areas

Location	pH(H_2O)	OM	P	Al^{3+}	Ca^{2+}	Mg^{2+}	K^+	V
		g kg^{-1}	mg dm^{-3}	———	$cmol_c$ dm^{-3}	———		%
Aquidauana	5.6	15.9	47.2	0.0	2.7	0.6	0.26	50.0
Anaurilândia	6.0	21.0	11.0	0.0	1.7	0.6	0.48	62.0
Campo Grande	6.1	31.2	9.0	0.0	3.9	1.7	0.57	58.0
Dourados	5.9	35.1	23.2	0.0	4.8	2.0	0.71	56.0

pH in water; OM: organic matter, Walkley-Black method; P and K: extractor Mehlich-1; Ca^{2+}, Mg^{2+}, Al^{3+}: extractor 1 mol L^{-1} KCl; V: base saturation.

The trials were carried out following the technical control recommendations for pests and diseases, without irrigation. The following characteristics were analyzed: number of nodules (NN), following the methodology proposed by Cardoso et al. (2009); dry matter of nodules (DMN) and dry matter of shoots (DMS) of plants in full flowering, after drying in an oven at 60 °C until achieving constant weight; N content in the shoots determined by the Kjeldahl method (semi micro), according to Malavolta et al. (1997); 100 grain weight; and crop yield. Estimates were made on the basis of experimental plots using the two central rows, eliminating the first two plants from the ends of each row. Yield was calculated after correcting for moisture of 13 %.

Rhizobia strains used as reference for comparison

Rhizobia strains commercially recommended in Brazil and used in all the experiments belong to the group of *Rhizobium tropici*, CIAT 899^T (=UMR 1899, =USDA 9030, = TAL 1797, =HAMBI 1163, =SEMIA 4077, =ATCC 49672); PRF 81 (= SEMIA 4080) of *R. freirei*; and H 12 (=SEMIA 4088) of *R. leucaenae*.

Native *Cerrado* rhizobia selected for bean plant inoculation

Rhizobia selection, based on the Embrapa Agropecuária Oeste collection of cultures of multifunctional microorganisms for bean plant inoculation, resulted in the identification of several isolates with high symbiotic efficiency when inoculated in bean. From assessment of 630 *Rhizobium* isolates obtained from nodules of leucaena (380) or dry beans (250) from 87 sites including 34 municipalities of Mato Grosso do Sul, four of them stood out from the others based on previous studies: CPAO 2.11 L, CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 56.4 L2.

Inoculation with bacteria was performed using a product prepared at a density of 10^9 cells g^{-1}, which was applied to the seeds at the rate of 500 g per 50 kg of seeds. Before inoculation, 300 mL of sugar solution at 10 % (m/v) were added to improve inoculant adhesion on the seed surface.

Economic analysis

In economic analysis, only the costs of the inoculant, the fertilizer (urea), and application operations (in the planting furrow and in topdressing) were considered. Other costs, such as the operation of soil tillage, planting, fertilization with P and K, and crop treatments were not considered because they were common to all treatments. The price of urea in Mato Grosso do Sul in December 2014, was US$ 518.08 Mg^{-1} or US$ 1.15 kg^{-1} of N (Conab, 2015). The application cost was estimated considering 0.1 Mh ha^{-1} (machine hour per hectare) or US$ 1.15 ha^{-1} and the inoculant cost was US$ 1.89 ha^{-1}. The bean price considered the average price of different regions of the state of Paraná because Paraná is a producing region and a traditional provider of product price. The average price on February 6, 2015 was US$ 54.59 per 60 kg bag of *Carioca* beans or US$ 0.91 per kg (Agrolink, 2015).

Using these data as a reference, comparisons were made (considering increase in yield, cost of production, increase in gross returns, and increase in net returns) between the treatments and the absolute control (without N fertilization and without inoculation with rhizobia). The increase in yield corresponded to the difference in grain production achieved in the treatments considered and in the absolute control. The increase in gross returns was determined by the increase in yield multiplied by the value paid for the commercial product. The increase in net returns was the difference between the growth of gross return minus the costs of inoculant and nitrogen fertilizer and their application.

The results were subjected to simple analysis of variance by location and to joint analysis in situations in which the relation of residues of each variable mean squares was less than or equal to 7.0, as recommended in Banzato and Kronka (1992). When necessary, the averages were compared by the Tukey test and the Scott-Knott test at 5 % probability.

RESULTS AND DISCUSSION

The environmental variability among the experimental sites was evident (Table 2). This characteristic, as determined by the Brazilian Ministry of Agriculture (Ministério de Agricultura, Pecuária e Abastecimento - MAPA) by Normative Instruction DAS 13 of March 25, 2011, is one of the fundamental criteria used to assess the feasibility and agronomic efficiency of inoculants and technologies associated with the biological nitrogen fixation (BNF) process in legumes for the purpose of selection and recommendation of bean crop inoculants in Brazil.

The NN were lower in areas with N fertilization (Table 3), indicating a negative effect of N fertilization on nodulation. The BNF would be an alternative, however due to high energy cost, it is not the preferable path to N assimilation. The availability of mineral N can affect the rhizobium-legume interaction, producing no nodules or even stopping BNF in plants with nodules (Schultze et al., 1994; Mercante et al., 1999).

The ability to increase formation of nodules is an important factor in the selection process aiming to increase BNF, although it is not considered as a measure of the nodule efficiency. In this regard, CPAO 17.5 L2, CPAO 12.5 L2, and CIAT 899 stood out in Aquidauana, H 12 in Anaurilândia, CPAO 56.4 L2 in Campo Grande, and CPAO 12.5 L2, CIAT 899, CPAO 17.5 L2, and H12 in Dourados. In general analysis, the strains H12, CPAO 17.5 L2, CPAO 12.5 L2, CPAO 2.11 L, CIAT 899, and CPAO 56.4 L2 were the ones that stood out best (Table 3). It should be emphasized that the potential of the native strains evaluated were similar to the commercial strains H12 and CIAT 899, and superior to PRF 81 (Table 3).

All inoculated treatments promoted greater nodulation than the control and the treatments that used mineral fertilization in Aquidauana, although the latter two did not differ between themselves (Table 4). In Anaurilândia, the strains H 12 and CPAO 2.11 L stood out in plant nodulation. In Campo Grande, no differences were observed between the treatments studied, whereas in Dourados, the strains CPAO 12.5 L2, CIAT 899, CIAT 17.5 L2, and CPAO 56.4 L2 stood out. When analyzed together (Table 3), all the strains and CIAT 899 and H 12 had higher nodule weight, and the lowest values were observed in the treatment of inoculation with the strain PRF 81, the control, and the treatment that contained mineral N. Silva et al. (2009) also observed a linear decrease in DMN as the mineral N supply increased. According to these authors, there is a range of nitrate leaf concentrations where large reductions in nodule size are observed. The presence of nodules in the control treatment indicates the existence of native strains of *Rhizobium spp*. in all places; however, both the number of nodules and dry weight of nodules were lower than in plants inoculated with the strains tested, indicating that selection of efficient strains is required to achieve maximization of BNF in the bean crop.

Table 2. Agronomic variables and common bean grain yield (cv. *Pérola*) in four locations in the state of Mato Grosso do Sul, in 2007 crop season

Variant	Aquidauana	Anaurilândia	Campo Grande	Dourados
Nodules per plant[(1)]	10.84 b	20.24 a	1.05 c	1.24 c
Dry matter of nodules (mg per plant)[(1)]	10.82 a	11.16 a	1.68 b	3.11 b
Dry matter of shoots (g per plant)	4.05 b	3.21 c	5.91 a	2.88 c
Nitrogen in shoots (g kg^{-1})	39.17 ab	40.22 a	37.57 b	38.31 b
100 grain weight (g)	29.36 b	28.09 c	36.82 a	24.00 d
Yield (kg ha^{-1})	1.088 b	1.154 b	2.447 a	1.172 b

[(1)] Data processed by $(x+1)^{1/2}$ for statistical analysis. Means followed by the same letter in the line do not differ by the Tukey test at 5 % probability.

Table 3. Average of number of nodules per plant, dry matter (DMN) of nodules per plant, dry matter of shoots (DMS) per plant, shoot nitrogen (N), 100 grain weight, and common bean (cv. *Pérola*) grain yield in relation to the different treatments, in four locations in the state of Mato Grosso do Sul (Aquidauana, Anaurilândia, Campo Grande, and Dourados), in 2007 crop season

Treatment	Nodules(1)	DMN(1)	DMS	Shoot N	100 grain weight	Yield
	NPP	mg	g	g kg^{-1}	g	kg ha^{-1}
Control(2)	5.4 b	3.0 b	4.2 a	38.7 a	27.8 b	1,002 c
Nitrogen fertilization(3)	2.0 c	1.7 b	4.0 a	38.9 a	30.9 a	1,600 a
CIAT 899 + 20 kg of N	5.5 b	3.1 b	3.6 a	38.4 a	30.3 a	1,367 b
CIAT 899	8.7 a	7.0 a	3.9 a	38.0 a	30.0 a	1,412 b
PRF 81	7.6 b	5.3 b	4.3 a	39.7 a	29.3 a	1,409 b
H 12	13.6 a	13.1 a	4.3 a	39.1 a	29.7 a	1,394 b
CPAO 2.11 L	10.2 a	9.2 a	4.3 a	39.1 a	29.8 a	1,522 b
CPAO 12.5 L2	10.7 a	8.5 a	3.9 a	39.4 a	29.1 a	1,667 a
CPAO 17.5 L2	12.0 a	9.3 a	4.0 a	37.9 a	29.4 a	1,530 b
CPAO 56.4 L2	7.9 a	6.7 a	3.7 a	39.0 a	29.4 a	1,755 a
CV (%)	21.3	31.5	22.0	8.4	6.6	17.6

(1) Data processed by $(x+1)^{1/2}$ for statistical analysis. (2) Without nitrogen fertilization and without inoculation. (3) 80 kg ha^{-1} of N (40 kg at planting and 40 kg in topdressing). Means followed by the same letters in the columns do not differ by the Scott-Knott test at 5 % probability. CV: coefficient of variation. NPP: number per plant.

The production of DMS was equal among sites and among treatments (Table 4). In the treatments inoculated with different strains, the production of shoot dry matter was similar to the treatment with N fertilization (80 kg ha^{-1} of N), although they did not differ from the control. These results showed the efficiency of the native rhizobia population for nodule creation and N fixation, and the unfeasibility of N fertilization, which increases the cost of production and leads to possible environmental problems.

Regardless of the location, the treatments did not show differences in the N contents of shoot dry matter (Tables 2 and 3). In this regard, Lemos et al. (2003) also found no differences in N concentrations in the dry matter between genotypes of bean which received or did not receive inoculation with rhizobia. The lack of difference between the treatments and the control with N fertilization (80 kg ha^{-1} of N) differs from the results of Binotti et al. (2010), who observed an increase in N concentrations in dry matter. However, this result indicates that there was restriction in the use of the N applied, possibly from losses due to other processes, such as leaching and volatilization, among others, or the efficiency of native strains in biological N fixation and also in supplying plant needs.

With the exception of Dourados, the 100 grain weight of the control treatment was lower compared to the other treatments (Table 4). These results are similar to multiple studies that have demonstrated the positive effect of N on 100 grain weight (Rapassi et al., 2003; Silva et al., 2004; Alvarez et al., 2005; Teixeira et al., 2005; Binotti et al., 2009). However, Bassan et al. (2001) observed higher values in the inoculated treatments in relation to the non-inoculated ones and attributed the result to the non-existence of efficient native strains. Thus, it can be inferred that the strains evaluated in this study were also competitive and efficient in supplying N, enabling achievement of 100 grain weight equivalent to that obtained in the treatment with N fertilization at 80 kg ha^{-1}.

Grain yield (Table 4) obtained in the different treatments at each experimental location can be regarded as satisfactory because sowing was carried out at the end of the period

Table 4. Number of nodules per plant, dry matter of nodules (DMN) per plant, dry matter of shoots (DMS) per plant, shoot nitrogen (N), 100 grain weight, and common bean (cv. *Pérola*) grain yield as a function of the supply of mineral nitrogen and/or inoculation of seeds with different strains of commercial rhizobia and strains selected from soils of Central Brazil (Mato Grosso do Sul), in 2007 crop season

Treatment	Nodules(1)	DMN(1)	DMS	Shoot N	100 grain weight	Yield
	NPP	mg	g	g kg^{-1}	g	kg ha^{-1}
			Aquidauana			
Control(2)	6,5 c	6,2 b	3,81 a	39,08 a	25,4 b	649 b
Nitrogen fertilization(3)	2.6 d	1.6 b	4.14 a	37.55 a	31.0 a	1,307 a
CIAT 899 + 20 kg of N	6.0 c	4.5 b	3.65 a	40.11 a	28.4 b	1,160 a
CIAT 899	15.7 a	15.2 a	4.35 a	38.13 a	32.3 a	1,299 a
PRF 81	7.3 c	10.6 a	4.80 a	39.60 a	28.2 b	945 b
H 12	12.1 b	19.1 a	4.19 a	37.94 a	28.6 b	927 b
CPAO 2.11 L	13.1 b	9.2 a	4.65 a	38.34 a	30.5 a	968 b
CPAO 12.5 L2	16.3 a	15.8 a	2.99 a	39.80 a	31.1 a	1,187 a
CPAO 17.5 L2	18.5 a	16.3 a	4.02 a	40.79 a	30.1 a	1,257 a
CPAO 56.4 L2	10.3 b	9.9 a	3.91 a	40.32 a	27.9 b	1,182 a
CV (%)	13.8	24.9	21.8	5.7	8.6	24.1
			Anaurilândia			
Control(2)	14.4 c	5.2 b	3.5 a	39.6 a	27.0 b	1,267 a
Nitrogen fertilization(3)	4.7 d	1.4 b	3.1 a	41.7 a	29.8 a	982 a
CIAT 899 + 20 kg of N	14.6 c	7.0 b	2.9 a	38.9 a	30.9 a	1,067 a
CIAT 899	16.2 c	6.1 b	3.3 a	41.9 a	26.6 b	1,137 a
PRF 81	22.2 b	8.4 b	3.8 a	42.3 a	28.6 b	1,056 a
H 12	39.5 a	27.5 a	3.1 a	40.5 a	27.1 b	1,048 a
CPAO 2.11 L	25.3 b	23.9 a	3.3 a	38.5 a	27.8 b	1,213 a
CPAO 12.5 L2	23.4 b	10.9 b	2.8 a	38.5 a	28.0 b	1,071 a
CPAO 17.5 L2	26.6 b	14.7 b	3.1 a	39.6 a	27.9 b	1,195 a
CPAO 56.4 L2	15.3 c	6.8 b	3.2 a	40.7 a	27.2 b	1,505 a
CV (%)	18.9	27.2	28.8	9.5	4.7	17.5
			Campo Grande			
Control(2)	0.3 b	0.3 a	6.4 a	38.8 a	34.6 b	1,082 b
Nitrogen fertilization(3)	0.4 b	3.3 a	5.9 a	38.5 a	37.8 a	2,803 a
CIAT 899 + 20 kg of N	1.2 b	0.7 a	5.1 a	36.7 a	37.9 a	2,108 a
CIAT 899	0.3 b	0.3 a	5.6 a	33.5 a	37.8 a	2,252 a
PRF 81	0.3 b	0.6 a	5.3 a	38.5 a	35.6 b	2,599 a
H 12	0.7 b	3.1 a	7.1 a	38.4 a	38.6 a	2,587 a
CPAO 2.11 L	1.3 b	1.7 a	6.0 a	38.6 a	36.9 a	2,686 a
CPAO 12.5 L2	0.3 b	0.5 a	6.6 a	38.6 a	35.2 b	2,664 a
CPAO 17.5 L2	0.7 b	0.9 a	5.7 a	34.5 a	35.1 b	2,506 a
CPAO 56.4 L2	5.0 a	5.5 a	5.4 a	39.6 a	38.7 a	3,187 a
CV (%)	31.4	46.1	25.3	9.6	5.9	14.7

Continue

Continuation

			Dourados			
Control(2)	0.2 b	0.2 b	3.0 a	37.6 a	24.3 a	1,010 b
Nitrogen fertilization(3)	0.1 b	0.4 b	2.9 a	37.8 a	24.8 a	1,303 b
CIAT 899 + 20 kg of N	0.2 b	0.3 b	3.0 a	37.8 a	24.1 a	1,134 a
CIAT 899	2.5 a	6.4 a	2.5 a	38.5 a	23.3 a	960 b
PRF 81	0.5 b	1.8 b	3.2 a	38.3 a	24.8 a	1,036 a
H 12	2.1 a	2.6 b	2.6 a	39.3 a	24.3 a	1,012 b
CPAO 2.11 L	1.0 b	2.3 b	3.2 a	40.9 a	24.1 a	1,221 a
CPAO 12.5 L2	2.8 a	7.0 a	3.3 a	40.8 a	22.0 a	1,745 a
CPAO 17.5 L2	2.1 a	5.3 a	3.1 a	36.9 a	24.6 a	1,161 b
CPAO 56.4 L2	1.2 b	4.7 a	2.1 a	35.3 a	23.7 a	1,145 b
CV (%)	28.1	38.2	25.3	8.2	6.5	14.1

(1) Data processed by $(x+1)^{1/2}$ for statistical analysis. (2) Without nitrogen fertilization and without inoculation. (3) 80 kg ha^{-1} of N (40 kg at planting and 40 kg in topdressing). Means followed by the same letters in the columns do not differ by the Scott-Knott test at 5 % probability. CV: coefficient of variation. NPP: number per plant.

recommended for bean cultivation in accordance with edaphoclimatic zoning. Joint analysis of data (Table 3) showed that inoculation with the strains CPAO 56.4 L2, CPAO 12.5 L2, and N fertilization (80 kg ha^{-1} of N) were the treatments that showed the highest yields. There is yet another group formed by the commercial strains (CIAT 899, PRF 81, and H 12) and the native strains (CPAO 17.5 L2 and CPAO 2.11 L), as well as CIAT 899 + 20 kg of N, that differed from the control.

An increase in bean yield was determined by inoculation with strains collected from Central Brazil in relation to the average of the commercial strains CIAT 899 (SEMIA 4077), PRF 81 (SEMIA 4080), and H 12 (SEMIA 4088). All new strains evaluated were superior to those used in the commercial inoculants. The most outstanding were CPAO 56.4 L2 and CPAO 12.5 L2, which exhibited a 25 and 19 % increase in bean yield, respectively. There were significant results shown by CPAO 17.5 L2 and CPAO 2.11 L, with increases of 9 and 8 %, respectively. In accordance with the protocol established by the MAPA for analysis of results for indication of strains and their recommendation in Brazil, the native strains CPAO 56.4 L2, CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 2.11 L can be indicated for production of a new bean plant inoculant in Brazil since they resulted in yields higher than those obtained with the inoculants currently marketed.

Due to large variations in the price of N fertilizers and the different forms of supplying N, the use of financial analysis in bean research studies is becoming increasingly common (Binotti et al., 2009; Pelegrin et al., 2009; Binotti et al., 2010). However, an increase in net returns, which should be the ultimate goal of rural producers, is not always linked to technology or a process that enables an increase in yield, as noted by Binotti et al. (2009). These authors found that application rates of ammonium sulfate that led to the highest bean yields did not result in higher values in net return, due to the high costs of N fertilizers. Pelegrin et al. (2009) observed that an increase of 795 kg ha^{-1} in bean yield with 160 kg ha^{-1} of N fertilization was financially equivalent to an increase of only 373 kg ha^{-1} provided by inoculation (*Rhizobium tropici* CIAT 899- SEMIA4077 sign) associated with only 20 kg ha^{-1} of N from N fertilization applied at sowing.

Data concerning financial analysis of the treatments used in this study are shown in table 5. It should be noted that all treatments provided an increase in net return compared to the control treatment. The largest increases were obtained when the bean seeds were inoculated with the strains CPAO 56.4 L2, CPAO 12.5 L2, CPAO 17.5 L2, and

Table 5. Variation in financial income in relation to the application of mineral nitrogen and inoculation of bean seeds with different strains of rhizobia in relation to the treatment without inoculation and without fertilization (control), average of sites: Aquidauana-MS, Anaurilândia-MS, Campo Grande-MS, and Dourados-MS, Brazil, in 2007 crop season

Treatment	Yield increase(1)	Gross return(2)	Total cost of fertilization(3)	Increase in net return
	kg ha^{-1}	US$ ha^{-1}		
Mineral N (80 kg ha^{-1})	598	544.04	93.15	450.89
CIAT 899 + 20 kg de N	365	331.91	24.15	307.76
CIAT 899	410	372.85	1.89	370.96
PRF 81	407	370.12	1.89	368.23
H 12	392	356.47	1.89	354.58
CPAO 2.11 L	520	473.30	1.89	471.41
CPAO 12.5 L2	665	604.86	1.89	602.97
CPAO 17.5 L2	528	480.39	1.89	478.50
CPAO 56.4 L2	753	685.10	1.89	683.21

(1) Yield increase in relation to the control. (2) Considering US$ 54.59 per bag of 60 kg of *Carioca* beans. (3) Cost of inoculant per application of US$ 1.89; cost of N – US$ 1.15 kg^{-1}; application of urea for topdressing – US$ 1.15.

CPAO 2.11 L, which led to increases in net return per hectare of US$ 683.21, 602.97, 478.50 and 471.41, respectively. In this situation, the increases in net return were higher than those obtained from the application of 80 kg ha^{-1} of N, which was only US$ 450.89 ha^{-1}. It is interesting to note that the strains used in commercial inoculants (CIAT 899, PRF 81 and H 12) showed increases in net return lower than those obtained from nitrogen fertilization. These results indicate that the new strains evaluated in this study (CPAO 56.4 L2, CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 2.11 L) may be used in the preparation of commercial inoculants for the bean crop. The indication for use of these new strains for all bean producing regions in Brazil also depends on completion of validation tests in all regions.

CONCLUSIONS

Bean inoculation with the native strains CPAO 56.4 L2 and CPAO 12.5 L2 resulted in grain production similar to that obtained with fertilization of 80 kg ha^{-1} of N.

The strains CPAO 56.4 L2, CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 2.11 L increased bean plant net returns in relation to the absolute control treatment (without N fertilization and without inoculation), to the N control (80 kg ha^{-1} of N and without inoculation), and to the commercial strains.

The strains CPAO 12.5 L2, CPAO 17.5 L2, and CPAO 56.4 L2 have high potential for obtaining more effective and economical inoculants for use in the bean crop.

ACKNOWLEDGMENTS

The first author is grateful to the CNPq for a research productivity scholarship, and the second author, to Fundect for a scholarship during doctoral studies.

REFERENCES

Agrolink. Cotações. 2015 [acesso em 6 fev 2015]. Disponível em: http://www.agrolink.com.br/cotacoes/graos/feijao.

Alvarez ACC, Arf O, Alvarez RCF, Pereira JCR. Resposta do feijoeiro à aplicação de doses e fontes de nitrogênio em cobertura no sistema plantio direto. Acta Sci Agron. 2005;27:69-75.

Amarger N, Macheret V, Laguerre G. *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* Nodules. Int J Syst Bacteriol. 1997;47:996-1006. https://doi.org/10.1099/00207713-47-4-996

Banzato DA, Kronka SN. Experimentação agrícola. 2a ed. Jaboticabal: Funep; 1992.

Bassan DAZ, Arf O, Buzetti S, Carvalho MAC, Santos NCB, Sá ME. Inoculação de sementes e aplicação de nitrogênio e molibdênio na cultura do feijão de inverno: produção e qualidade fisiológica de sementes. Rev Bras Sementes. 2001;23:76-83.

Binotti FFS, Arf O, Cardoso ED, Sá ME, Buzeti S, Nascimento V. Fontes e doses de nitrogênio em cobertura no feijoeiro de inverno irrigado no sistema plantio direto. Biosci J. 2010;26:770-8.

Binotti FFS, Arf O, Sá ME, Buzetti S, Alvarez ACC, Kamimura KM. Fontes, doses e modo de aplicação de nitrogênio em feijoeiro no sistema plantio direto. Bragantia. 2009;68:473-81. https://doi.org/10.1590/S0006-87052009000200022

Cantarella H. Nitrogênio. In: Novais RF, Alvarez V VH, Barros NF, Fontes RLF, Cantarutti RB, Neves JCL, editores. Fertilidade de solos. Viçosa, MG: Sociedade Brasileira de Ciência do Solo; 2007. p.376-470.

Cardoso JD, Gomes DF, Goes KCGP, Fonseca Junior NS, Dorigo OF, Hungria M, Andrade DS. Relationship between total nodulation and nodulation at the root crown of peanut, soybean and common bean plants. Soil Biol Biochem. 2009;41:1760-3. https://doi.org/10.1016/j.soilbio.2009.05.008

Companhia Nacional de Abastecimento - Conab. Séries Históricas: feijão total (1a, 2a e 3a safras) - Brasil. 2015 [acesso em 3 abr 2015]. Disponível em: http://www.conab.gov.br/conteudos.php?a=1252&ordem=produto&Pagina_objcmsconteudos=2#A_objcmsconteudos.

Dall'Agnol RF, Ribeiro RA, Delamuta JRM, Ormeño-Orrillo E, Rogel MA, Andrade DS, Martínez-Romero E, Hungria M. *Rhizobium paranaense* sp. nov., an effective N_2-fixing symbiont of common bean (*Phaseolus vulgaris* L.) with broad geographical distribution in Brazil. Int J Syst Evol Microbiol. 2014;64:3222-9. https://doi.org/10.1099/ijs.0.064543-0

Dall'Agnol RF, Ribeiro RA, Ormeño-Orrillo E, Rogel MA, Delamuta JRM, Andrade DS, Martínez-Romero E, Hungria M. *Rhizobium freirei* sp. nov., a symbiont of *Phaseolus vulgaris* that is very effective at fixing nitrogen. Int J Syst Evol Microbiol. 2013;63:4167-73. https://doi.org/10.1099/ijs.0.052928-0

Ferreira EPB, Mercante FM, Hungria M, Mendes IC, Araújo JLS, Fernandes Júnior PI, Araújo AP. Contribuições para a melhoria da eficiência da fixação biológica do nitrogênio no feijoeiro comum no Brasil. Tópicos Cienc Solo. 2013;8:251-91.

Girard ML, Flores M, Brom S, Romero D, Palácios R, Dávila G. Structural complexity of the symbiotic plasmid of *Rhizobium leguminosarum* bv. *phaseoli*. J Bacteriol. 1991;173:2411-9. https://doi.org/10.1128/jb.173.8.2411-2419.1991

Hungria M, Vargas MAT. Environmental factors impacting N_2 fixation in legumes grown in the tropics, with an emphasis on Brazil. Field Crop Res. 2000;65:151-64. https://doi.org/10.1016/S0378-4290(99)00084-2

Hungria M, Andrade DS, Chueire LMO, Probanza A, Guttierrez-Mañero FJ, Megías M. Isolation and characterization of new efficient and competitive bean (*Phaseolus vulgaris* L.) rhizobia from Brazil. Soil Biol Biochem. 2000;32:1515-28. https://doi.org/10.1016/S0038-0717(00)00063-8

Hungria M, Campo RJ, Mendes IC. Benefits of inoculation of the common bean (*Phaseolus vulgaris*) crop with efficient and competitive *Rhizobium tropici* strains. Biol Fertil Soil. 2003;39:88-93. https://doi.org/10.1007/s00374-003-0682-6

Instituto Brasileiro de Geografia e Estatística - IBGE. Banco de Dados Agregados. 2015 [acesso em 6 mar 2015]. Disponível em: http://www.sidra.ibge.gov.br/bda/tabela/protabl.asp?c=1002&z=p&o=28&i=P.

Jordan DC. Family III. Rhizobiaceae. In: Krieg NR, Holt JG, editors. Bergey's manual of systematic bacteriology. 9th ed. Baltimore, London: Williams & Wilkins Co; 1994. p.234-42.

Lemos LB, Fornasieri Filho D, Camargo MB, Silva TRB, Soratto RP. Inoculação de rizóbio e adubação nitrogenada em genótipos de feijoeiro. Agronomia. 2003;37:26-31.

Malavolta E, Vitti GC, Oliveira SA. Avaliação do estado nutricional das plantas: princípios e aplicações. 2a ed. rev e atual. Piracicaba: Potafos; 1997.

Martínez-Romero E, Segovia L, Mercante FM, Franco AA, Graham P, Pardo MA. *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* L. beans and *Leucaena spp*. trees. Int J Syst Bacteriol. 1991;41:417-26. https://doi.org/10.1099/00207713-41-3-417

Mercante FM, Teixeira MG, Abboud ACS, Franco A. Avanços biotecnológicos na cultura do feijoeiro sob condições simbióticas. Rev Univ Rural, Ser Cien Vida. 1999;21:127-46.

Pelegrin R, Mercante FM, Otsubo IMN, Otsubo AA. Resposta da cultura do feijoeiro à adubação nitrogenada e à inoculação com rizóbio. Rev Bras Cienc Solo. 2009;33:219-26. https://doi.org/10.1590/S0100-06832009000100023

Peres JRR, Suhet AR, Mendes IC, Vargas MAT. Efeito da inoculação com rizóbio e da adubação nitrogenada em sete cultivares de feijão em solo de Cerrados. Rev Bras Cienc Solo. 1994;18:415-20.

Piha MI, Munns DN. Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature. Plant Soil. 1987;98:183-94. https://doi.org/10.1007/BF02374822

Pinto FGS, Hungria M, Mercante FM. Polyphasic characterization of Brazilian *Rhizobium tropici* strains effective in fixing N_2 with common bean (*Phaseolus vulgaris* L.). Soil Biol Biochem. 2007;39:1851-64. https://doi.org/10.1016/j.soilbio.2007.01.001

Rapassi RMA, Valério Filho WV, Sá AAB, Sá ME, Carvalho MAC, Buzetti S, Arf O. Níveis e fontes de nitrogênio sobre o feijoeiro de inverno. Rev Cult Agron. 2003;12:103-15.

Ribeiro RA, Rogel MA, López-López A, Ormeño-Orrillo E, Barcellos FG, Martínez J, Thompson FL, Martínez-Romero E, Hungria M. Reclassification of *Rhizobium tropici* type A strains as *Rhizobium leucaenae* sp. nov. Int J Syst Evol Microbiol. 2012;62:1179-84. https://doi.org/10.1099/ijs.0.032912-0

Santos AB, Fageria NK, Silva OF, Melo MLB. Resposta do feijoeiro ao manejo de nitrogênio em várzeas tropicais. Pesq Agropec Bras. 2003;38:1265-71. https://doi.org/10.1590/S0100-204X2003001100003 Embrapa, 2013

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Oliveira JB, Coelho MR, Lumbreras JF, Cunha TJF. Sistema brasileiro de classificação de solos. 3a ed. Rio de Janeiro: Embrapa Solos; 2013.

Schultze M, Kondorosi E, Ratet P, Buiré M, Kondorosi A. Cell and molecular biology of *Rhizobium*-Plant. Int Rev Cytol. 1994;156:1-75. https://doi.org/10.1016/S0074-7696(08)62252-4

Segovia L, Young JPW, Martinez-Romero E. Reclassification of American *Rhizobium leguminosarum* biovar phaseoli type I strains as *Rhizobium etli sp. nov*. Int J Syst Bacteriol. 1993;43:374-7. https://doi.org/10.1099/00207713-43-2-374

Silva EF, Marchetti ME, Souza LCF, Mercante FM, Rodrigues ET, Vitorino ACT. Inoculação do feijoeiro com *Rhizobium tropici* associada à exsudato de *Mimosa flocculosa* com diferentes doses de nitrogênio. Bragantia. 2009;68:443-51. https://doi.org/10.1590/S0006-87052009000200019

Silva MG, Arf O, Sá ME, Rodrigues RAF, Buzetti, S. Nitrogen fertilization and soil management of winter common bean crop. Sci Agric. 2004;61:307-12. https://doi.org/10.1590/S0103-90162004000300012

Soil Survey Staff. Keys to soil taxonomy. 11th ed. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service; 2010.

Straliotto R, Teixeira MG, Mercante FM. Fixação biológica de nitrogênio. In: Aidar H, Kluthcouski J, Stone LF, editores. Produção de feijoeiro comum em várzeas tropicais. Santo Antônio de Goiás: Embrapa Arroz e Feijão; 2002. p.122-53.

Teixeira CM, Carvalho GJ, Andrade MJB, Furtini Neto AE, Marques ELS. Palhadas e doses de nitrogênio no plantio direto do feijoeiro. Acta Sci Agron. 2005;27:499-505.

Vargas MAT, Mendes IC, Hungria M. Response of field grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and N fertilization in two Cerrados soils. Biol Fertil Soils. 2000;32:228-33. https://doi.org/ 10.1007/s003740000240