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Nodulation, contribution of biological N_2 fixation, and productivity of the common bean (*Phaseolus vulgaris* L.) inoculated with rhizobia isolates

Leniany Patrícia Moreira[1], Ana Paula Santos Oliveira[2], Enderson Petrônio de Brito Ferreira[3*]

[1]Universidade Federal de Goiás, Rodovia GO-462, km 0, Campus Samanbaia, Goiânia, GO, 74001-970, Brazil.
[2]Instituto Federal Goiano, Rodovia GO-154 - Km 3, S/N - Zona Rural, Campus Ceres, Ceres, GO, 76300-000, Brazil
[3]Embrapa Arroz e Feijão, Rodovia GO-462, km 12, Santo Antônio de Goiás, GO, 75375-000, Brazil

***Corresponding author: enderson.ferreira@embrapa.br**

Abstract

The common bean has a great economic, social, and nutritional importance and requires high levels of nitrogen for its cultivation. The plant in symbiosis with rhizobia bacteria can benefit from biological nitrogen fixation (BNF). To assess the agronomic efficacy of rhizobia isolates on the nodulation, contribution of BNF and productivity of the common bean, two experiments were conducted in separate locations. Seventeen *Rhizobium tropici* isolates were selected and compared with three commercial *Rhizobium* spp. strains: CIAT 899 and H12 (*R. tropici*) and PRF 81 (*R. freirei*). The variables analyzed were: number of nodules (NN), nodules dry weight (NDW), root dry weight (RDW), leaf area (LA), shoot dry weight (SDA), total nitrogen in shoots (Total-N), number of pods (NP), number of grains (NG), grain yield (GY), percentage of nitrogen derived from BNF (Ndfa%), and total nitrogen derived from BNF in the shoots (NdBNF). Inoculation with *R. tropici* isolates promoted significant gains in nodulation, growth, and productivity of the common bean. About 50% of the isolates promoted GY rates equal to or superior to the nitrogen treatment (NT) and to the standard strains CIAT 899, PRF 81, and H12. Among the best isolates, the Ndfa% ranged from 64.2% to 75.8%, with NdBNF ranging from 802.91 to 1037.56 mg plant^{-1}. The isolate JPrG10A6 of *Rhizobium tropici* provided high values of NdBNF and GY, showing great potential to be used commercially as inoculant of the common bean crop.

Keywords: *Phaseolus vulgaris*, *Rhizobium*, symbiosis, BNF, symbiotic efficiency.

Introduction

The common bean (*Phaseolus vulgaris*L.) shows great social and economic importance worldwide (Builes et al., 2011). It is an excellent source of protein, carbohydrates, and minerals, constituting to the basic diet of many low-income populations (Mesquita et al., 2007). The crop shows a wide plasticity in adaptation to edaphoclimatic conditions, been cultivated in most of the production systems in Brazil, including small farms where it is used for household consumption and income generation. The common bean is also present in high-performance production systems, characterized by the use of some of the latest generation technologies and, by the cultivation under irrigation during the dry season. However, much of its cultivation is still associated with subsistence agriculture, characterized by the low use of technologies (Binotti et al., 2009).

Brazil is among the largest producers and consumers of the common bean in the world, but the average productivity is still considered low. The 2014/15 harvest resulted in 1,050 kg ha^{-1}, with 3.2 million metric tons being produced in an area of 3 million hectares (FAO, 2015). The low productivity of the common bean is associated, in large part, with nitrogen levels, which is the most absorbed nutrient and is required in larger quantities for plant growth (Farinelli and Lemos, 2010).

Commonly, N-fertilizers are used to meet the needs of the crop. However, the use of N-fertilizers increases the production costs and contributes to increased environmental risks, such as groundwater contamination with nitrite under irrigated production systems (Bortolotto et al., 2012) and the increase of greenhouse gas emission (Siqueira Neto et al., 2011). All of these indicate the need for alternative sources of N supply. Biological nitrogen fixation (BNF) provides nitrogen to the plant through the symbiotic association with *Rhizobium* spp., which fix atmospheric N_2. Studies on BNF in the common bean have reported very controversial results, showing either high rates of productivity, ranging from 2500 to 3500 kg ha^{-1} (Mostasso et al, 2002; Pelegrin et al, 2009) to very low yields, from 600 to 1500 kg ha^{-1} (Raposeiras et al, 2006; Souza et al, 2011 and Valadão et al, 2009). According to that, the complete replacement of N-fertilizers by inoculation is still a goal to be achieved for the common bean, as compared to the soybean crop, which is the most efficient model for BNF in the tropics. Currently, three strains of *Rhizobium* spp. used as inoculants for the common bean in Brazil are registered at the Ministry of Agriculture, Livestock and Food Supply. Two of them (CIAT 899 and H12) are *Rhizobium tropici* and one (PRF 81) is *Rhizobium freirei* (MAPA, 2011). However, the selection of new strains for the production of inoculants that combine the efficiency of the BNF, the adaptation to different edaphoclimatic conditions, and the competition for places of nodular infection must be carried out continuously, always seeking the best combination among the symbionts (Antunes et al. 2011). Based on the foregoing, the aim of this study was to assess the agronomic efficacy of rhizobia isolates on nodulation and productivity of the common bean, compared with the standard strains recommended as commercial inoculants for the crop.

Results and Discussion

Effect of the treatments on nodulation and productivity of the common bean under family farming

The isolates NVSG2A2, UnPaG8A12, JPrG10A1, JPrG8A7, UnPaG6A8, UbALG3A5, NVSG7A7, JPrG8A3, JPrG6A8, ALSG5A4 and PCG4A2 of *R. tropici* did not differ from the strains CIAT 899 and H12 of *R. tropici* regarding NN, with both strains being superior to the other treatments. In the studies by Chagas Júnior et al. (2010), the authors found that 32% of new isolates showed more NN in the common bean compared to the standard strains CIAT 899 and H12. However, the quantity of nodules alone does not guarantee high efficiency of BNF (Cardoso et al., 2009; Fonseca et al., 2013). In this study we observed that the treatments differed among themselves concerning NN, but as for NDW, RDW and LA, no significant differences were observed (Table 1).
For SDW, the isolates JPrG1A1, JPrG8A7, JPrG10A6, UbALG3A5, UbALG7A7, JPrG8A3 and JPrG6A8 of *R. tropici* did not differ from the standard strain H12 and from the NT, with the strains being superior to the other treatments (Table 1). As for the total-N accumulated in the shoots, the isolates JPrG8A7, JPrG10A6, JPrG6A8, JPG1A1, PCG4A2, ALSG5A4, UBALG3A5 and UbALG7A7 of *R. tropici* stood out among the other treatments, matching the NT but differing significantly from the three standard strains (Table 1).

As for the components of productivity, the isolates JPrG1A1, JPrG10A1, PCG2A5, JPrG8A7, UnPaG6A8, JPrG10A6, UbALG3A5, NSVG7A7, UbALG7A7, JPrG11A7, JPrG8A3, JPrG6A8 and PCG4A2 of *R. tropici* and the NT showed higher NP concerning the strains CIAT 899 of *R. tropici* and PRF 81 of *R. freirei*. However, as for NG, these isolates and the NT were superior to the three standard strains (Table 1).
Concerning the GY, the isolates ALSG5A4, JPrG8A1, JPrG10A6, JPrG11A7, JPrG8A3, NVSG2A2, NVSG7A7, UbALG3A5, UnPaG8A12 and UnPaG8A1 of *R. tropici* did not differ from the standard strain PRF 81, which was superior to the other treatments. The isolate JPrG1A1 obtained greater GY, surpassing the NT and the three standard strains.

The productivity of the common bean, under irrigated conditions, can exceed 3,000 kg ha^{-1}. However, according to Ferreira et al. (2011), the conventional tillage system provides lower productivity of grain crops, compared with the no-tillage system. Since the field experiment in Guapó was carried out under conventional soil preparation, this fact may have influenced the low results observed for GY. In spite of that, the use of inoculation with new isolates and the standard strains promoted greater productivity in comparison with the use of nitrogen fertilizers. In addition, the obtained productivity was up to 26% higher than the Brazilian average.

Effect of the treatments on nodulation and productivity of the common bean under intensive agriculture

No significant differences were found among the treatments regarding NN; however, significant differences were found regarding NDW, though the strain PRF 81 was superior to the other treatments (Table 2). We verified in this study that all treatments showed NN above 15 nodules plant $^{-1}$, which indicate good symbiotic efficiency (Cardoso et al., 2009; Fonseca et al., 2013).

The high nodulation found in this study shows the nodulation ability of the isolates and of the standard strains assessed. However, the high nodulation observed in the C0 treatment indicates the presence of native rhizobia strains in the soil, capable of nodulating the common bean. Brito et al. (2015) found significant nodulation in plants of the common bean without inoculation in two areas near Santo Antônio de Goiás – GO. The high number of rhizobia native populations in these areas were indicated by the count of 10^5 cells g^{-1} of soil and concluded that previous crops of the common bean can facilitate the establishment of competitive native strains.
The NT showed best performance compared to the other treatments regarding RDW, LA, SDW, NP, and NG. For LA, the isolates PCG2A5, JPrG8A7 UnPaG6A8, UbALG3A5, NVSG7A7 and, UbALG7A7 of *R. tropici* were inferior to the NT but superior to standard strains and other treatments. In relation to the NP, the isolates NVSG2A2, UnPaG8A12, JPrG1A1, UnPaG8A1, NVSG7A7, JPrG11A7, JPrG6A8 and, PCG4A2 of *R. tropici* did not differ from the standard strains, being inferior to the NT, but superior to the other treatments. For NG, the isolates PCG4A2, JPrG6A8, JPrG11A7 and, UnPaG8A12 of *R. tropici* did not differ from the strains CIAT 899 and PRF 81, being inferior to the NT and superior to the strain H12 and other treatments. Though the NT showed good results on some parameters of growth and productivity, the isolate UnPaG8A1 stood out compared with other treatments as for Total-N in the shoots. However, the isolates JPrG10A6, JPrG8A3, JPrG6A8, NVSG2A2, NVSG7A7, PCG2A5, UbALG7A7 and, UnPaG8A1 of *R. tropici*, and the standard strains CIAT 899, PRF 81, and H12 obtained greater GY compared with other treatments (Table 2).

Ferreira et al. (2009) found that the inoculated treatments promoted GY similar to the treatment with 80 Kg N ha^{-1}. Brito et al. (2015) on the other hand, found that the treatments using different *Rhizobium* spp. strains showed grain yield superior to the treatment with 120 kg N ha^{-1}. Soares et al. (2006) found that about 75% of the previously selected strains promoted GY similar to the treatment with 70 kg N ha^{-1}.

The results of this study corroborate the ones by Barros et al. (2013), indicating that inoculation of the common bean with *Rhizobium* spp. strains can replace the nitrogen fertilization without loss of productivity. However, according to Ferreira et al. (2013), the BNF process requires optimal conditions to express its maximum efficiency, because several factors involved may reduce the macro or microsymbiont performance and consequently, the efficiency of BNF. The lower GY index was found in the control treatment (C0), indicating that, even in high number, the rhizobia native population was not efficient to the point of surpassing the GY observed with the standard strains and new the isolates (Table 2).

Effect of the treatments on contribution of BNF in Santo Antônio de Goiás

The analysis of the contribution of BNF revealed that the lowest values of natural abundance of ^{15}N (δ^{15}N) were observed for the isolates UnPAG8A12, JPrG10A6, NVSG2A2, JPrG8A3, PCG2A5, JPrG1A1, UbALG7A7, PCG4A2 and NVSG7A7 of *R. tropici* and for the standard strains H12 and CIAT 899. These same treatments, except for

Table 1. Number of nodules (NN), nodules dry weight (NDW), root dry weight (RDW), leaf area (LA), shoot dry weight (SDA), total nitrogen (Total-N) in shoots, number of pods (NP), number of grains (NG) and grain yield (GY) of the common bean inoculated with new isolates and commercial *Rhizobium* spp. strains under field experiment in Guapó.

Isolate/standard strain	NN	NDW	RDW	LA	SDW	Total-N	NP	NG	GY
	nº $plant^{-1}$	mg $plant^{-1}$	g $plant^{-1}$	cm² $plant^{-1}$	g $plant^{-1}$	mg $plant^{-1}$	nº $plant^{-1}$	nº $plant^{-1}$	kg ha^{-1}
NT	22.20 b	32.00 a	0.63 a	595.01 a	8.88 a	214.19 a	16.75 a	78.33 a	766.65 b
C0	24.80 b	44.00 a	0.51 a	598.61 a	3.55 b	100.04 b	12.00 b	48.63 b	617.52 b
CIAT 899	63.25 a	72.00 a	0.47 a	596.30 a	4.50 b	115.99 b	10.75 b	48.63 b	897.35 b
H12	85.30 a	92.00 a	0.53 a	579.79 a	8.17 a	118.20 b	13.75 a	56.06 b	974.65 b
PRF 81	23.85 b	12.00 a	0.56 a	520.41 a	4.54 b	86.40 b	08.25 b	36.70 b	1302.33 a
ALSG5A4	65.20 a	88.00 a	0.50 a	643.50 a	5.84 b	175.32 a	11.75 b	54.60 b	1222.68 a
JPrG10A1	64.75 a	68.00 a	0.56 a	571.14 a	5.19 b	129.99 b	14.75 a	64.96 a	916.85 b
JPrG8A7	61.60 a	106.00 a	0.51 a	613.40 a	6.79 a	264.76 a	15.00 a	71.40 a	858.83 b
JPrG10A6	51.15 b	72.00 a	0.50 a	696.71 a	9.03 a	205.96 a	15.00 a	65.30 a	1093.09 a
JPrG11A7	44.45 b	40.00 a	0.53 a	586.09 a	5.41 b	152.78 b	13.50 a	56.03 b	1229.10 b
JPrG8A3	62.85 a	100.00 a	0.68 a	715.28 a	6.79 a	145.97 b	13.75 a	65.92 a	1134.09 a
JPrG6A8	60.85 a	48.00 a	0.60 a	672.89 a	8.39 a	218.69 a	17.00 a	80.33 a	1007.17 b
JPrG1A1	38.65 b	66.00 a	0.46 a	695.71 a	6.95 a	201.32 a	13.75 a	67.90 a	1325.47 a
NVSG2A2	58.80 a	124.00 a	0.60 a	457.94 a	5.28 b	108.98 b	11.00 b	44.42 b	1077.50 a
NVSG7A7	81.50 a	84.00 a	0.47 a	876.96 a	6.18 b	142.40 b	17.00 a	79.36 a	1175.65 a
PCG2A5	41.20 b	46.00 a	0.49 a	579.09 a	5.45 b	156.93 b	14.25 a	65.30 a	997.51 b
PCG4A2	86.95 a	74.00 a	0.50 a	543.53 a	6.19 b	194.46 a	16.75 a	78.33 a	909.61 b
UnPaG8A12	57.70 a	58.00 a	0.62 a	536.21 a	5.83 b	122.56 b	12.00 b	58.86 b	1201.28 a
UbALG3A5	78.05 a	80.00 a	0.66 a	755.34 a	7.60 a	251.68 a	15.00 a	71.73 a	1182.31 a
UbALG7A7	51.20 b	68.00 a	0.69 a	774.90 a	10.57 a	242.96 a	14.50 a	63.46 a	736.82 b
UnPaG6A8	70.65 a	74.00 a	0.63 a	446.23 a	4.80 b	118.22 b	17.00 a	90.46 a	740.10 b
UnPaG8A1	31.45 b	36.00 a	0.47 a	406.41 a	2.89 b	59.71 b	11.75 b	60.06 b	1088.01 a
CV* (%)	23.66	30.51	16.49	11.12	12.24	27.56	9.23	11.67	13.75

*Coefficient of variation. Values followed by the same letter in the same column are not significantly different by Skott-Knott test ($p<0.05$).

Table 2. Number of nodules (NN), nodules dry weight (NDW), root dry weight (RDW), leaf area (LA), shoot dry weight (SDA), total nitrogen (Total-N) in shoots, number of pods (NP), number of grains (NG) and grain yield (GY) of the common bean inoculated with new isolates and commercial *Rhizobium* spp. strains under field experiment in Santo Antônio de Goiás.

Isolate/standard strain	NN	NDW	RDW	LA	SDW	Total-N	NP	NG	GY
	nº $plant^{-1}$	mg $plant^{-1}$	g $plant^{-1}$	cm² $plant^{-1}$	g $plant^{-1}$	mg $plant^{-1}$	nº $plant^{-1}$	nº $plant^{-1}$	kg ha^{-1}
NT	22.05 a	48.00 b	1.50 a	1471.63 a	15.88 a	267.67 c	30.25 a	146.20 a	2536.38 b
C0	68.05 a	102.00 b	0.61 b	672.71 c	7.47 b	202.11 d	21.25 b	101.30 b	2071.96 c
CIAT 899	36.20 a	64.00 b	0.60 b	874.09 c	8.39 b	189.84 d	19.75 b	97.73 b	3566.30 a
H12	40.35 a	86.00 b	0.74 b	902.66 c	8.32 b	272.68 c	19.25 b	84.72 c	3388.40 a
PRF 81	47.05 a	332.00 a	0.67 b	839.47 c	8.75 b	235.59 d	19.00 b	97.10 b	3381.62 a
ALSG5A4	39.85 a	60.00 b	0.71 b	848.20 c	7.26 b	300.64 b	14.25 c	79.00 c	2927.15 b
JPrG10A1	47.95 a	84.00 b	0.62 b	688.99 c	7.16 b	291.30 c	15.25 c	78.80 c	2988.12 b
JPrG8A7	63.85 a	112.00 b	0.89 b	1038.93 b	9.65 b	311.34 b	15.00 c	74.80 c	2834.91 b
JPrG10A6	55.45 a	114.00 b	0.72 b	871.03 c	8.07 b	301.48 b	18.00 b	91.03 c	3229.48 a
JPrG11A7	36.60 a	96.00 b	0.61 b	672.71 c	7.47 b	214.09 d	21.25 b	99.57 b	2917.95 b
JPrG8A3	37.80 a	68.00 b	0.63 b	681.31 c	6.74 b	200.28 d	15.75 c	75.95 c	3061.35 a
JPrG6A8	33.80 a	78.00 b	0.86 b	824.70 c	9.26 b	217.98 d	20.75 b	101.30 b	3097.71 a
JPrG1A1	52.05 a	108.00 b	0.72 b	895.43 c	7.45 b	264.44 c	18.25 b	92.30 c	2820.60 b
NVSG2A2	48.85 a	124.00 b	0.66 b	839.75 c	8.69 b	264.43 c	17.57 b	86.40 c	3438.40 a
NVSG7A7	45.95 a	90.00 b	0.81 b	1001.02 b	8.33 b	254.88 c	18.00 b	82.33 c	3194.06 a
PCG2A5	50.35 a	104.00 b	0.87 b	1039.52 b	9.34 b	218.41 d	17.00 c	87.70 c	3342.88 a
PCG4A2	39.80 a	88.00 b	0.63 b	850.18 c	8.40 b	221.42 d	19.00 b	111.60 b	2944.74 b
UnPaG8A12	27.35 a	62.00 b	0.63 b	752.64 c	7.61 b	268.74 c	18.00 b	108.13 b	2775.60 b
UbALG3A5	39.25 a	92.00 b	0.71 b	1050.83 b	8.47 b	260.06 c	13.50 c	69.00 c	2784.70 b
UbALG7A7	74.20 a	162.00 b	0.88 b	1077.35 b	10.39 b	325.93 b	16.50 c	86.06 c	3359.17 a
UnPaG6A8	47.25 a	106.00 b	0.67 b	967.73 b	8.09 b	257.92 c	15.25 c	78.36 c	2803.51 b
UnPaG8A1	46.60 a	138.00 b	0.61 b	840.48 c	8.29 b	374.36 a	18.50 b	90.36 c	3289.80 a
CV* (%)	19.40	28.09	31.40	12.37	8.32	13.97	8.95	10.15	12.61

*Coefficient of variation. Values followed by the same letter in the same column are not significantly different by Skott-Knott test ($p<0.05$).

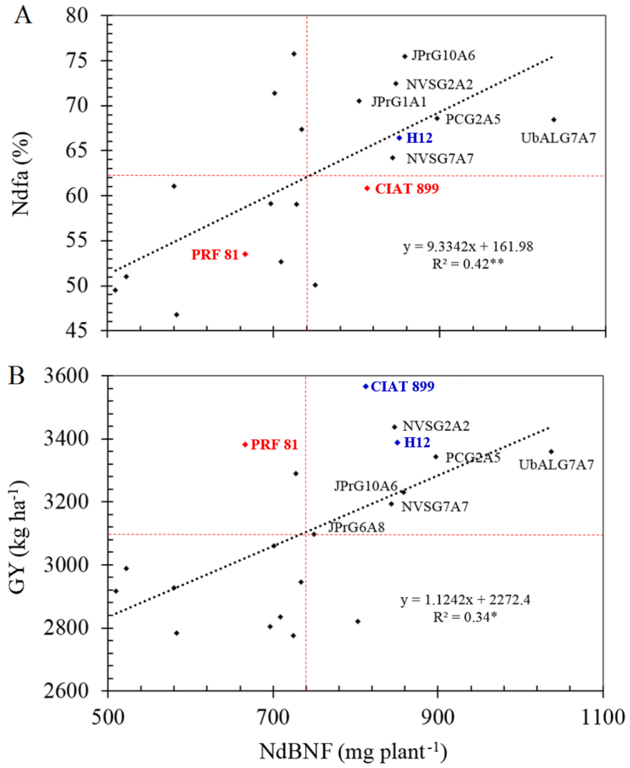

Fig 1. Pearson correlation between the percentage of nitrogen derived from BNF (Ndfa%) and total nitrogen derived from BNF (NdBNF) in the shoots (A) and between the grain yield (GY) and total nitrogen derived from BNF (B) in the shoots of the common bean at the flowering, inoculated with new isolates and commercial *Rhizobium* spp. strains. Red dotted lines correspond to the average of each parameter. * ($p \leq 0.05$); ** ($p \leq 0.01$).

Table 3. Delta $^{\delta 15}$N (‰), percentage of nitrogen derived from BNF (Ndfa%) and total nitrogen derived from BNF in the shoots (NdBNF) of common bean at the flowering, inoculated with new isolates and commercial *Rhizobium* spp. strains under field experiment carried out in Santo Antônio de Goiás.

Isolate/standard strain	δ15N(%)		Ndfa%		NdBNF (mg plant^{-1})	
UnPaG8A12	0.10	b	75.76	a	724.93	b
JPrG10A6	0.10	b	75.48	a	858.15	a
NVSG2A2	0.26	b	72.42	a	847.34	a
JPrG8A3	0.30	b	71.39	a	700.98	b
PCG2A5	0.37	b	68.59	a	897.06	a
JPrG1A1	0.42	b	70.51	a	802.91	a
UbALG7A7	0.50	b	68.42	a	1037.56	a
H12	0.57	b	66.43	a	850.78	a
PCG4A2	0.57	b	67.33	a	733.94	b
NVSG7A7	0.71	b	64.22	a	843.39	a
CIAT 899	0.78	b	60.85	b	812.39	a
ALSG5A4	0.89	a	61.05	b	579.85	b
UnPaG8A1	0.93	a	59.02	b	727.53	b
UnPaG6A8	1.02	a	59.09	b	696.64	b
PRF 81	1.22	a	53.52	c	665.59	b
JPrG8A7	1.29	a	52.69	c	708.78	b
JPrG6A8	1.33	a	50.10	c	749.89	b
JPrG10A1	1.35	a	51.02	c	522.46	b
JPrG11A7	1.52	a	49.54	c	509.95	b
UbALG3A5	1.68	a	46.80	c	583.30	b
CV* (%)	12.75		11.96		11.07	

*Coefficient of variation. Values followed by the same letter in the same column are not significantly different by Skott-Knott test (p<0.05).

Table 4. Identification, wild genotype of common bean used as trap plant and origin of the soil samples to obtain new isolates of *Rhizobium tropici* evaluated on field experiments.

Isolate	Wild genotype	Origin of soil samples		
		State	City	Commercial farm
PCG4A2	G12912	PR	Prudentópolis	Campi
PCG2A5	G23490	PR	Prudentópolis	Campi
ALSG5A4	G23475	PR	Araucária	Lagoa Suja
JPrG8A3	G12904	GO	Jussara	Primavera
UnPaG8A1	G12904	MG	Unaí	Paris
UbALG7A7	G23460	MG	Uberlândia	Água Limpa
NVSG7A7	G23460	GO	Nova Veneza	Souza
JPrG8A7	G12904	GO	Jussara	Primavera
JPrG10A6	PHA VUL 8141	GO	Jussara	Primavera
UnPaG6A8	G12858	MG	Unai	Paris
NVSG2A2	G23490	GO	Nova Veneza	Souza
JPrG1A1	G23499A	GO	Jussara	Primavera
UnPaG8A12	G12904	MG	Unaí	Paris
UbALG3A5	G23500A	MG	Uberlândia	Água Limpa
JPrG6A8	G12858	GO	Jussara	Primavera
JPrG11A7	PHA VUL 8122	PR	Araucária	Lagoa Suja
JPrG10A1	PHA VUL 8141	GO	Jussara	Primavera

Source: Sampaio, 2013.

Table 5. *Rhizobium* spp. strains used as reference on the field trials for the evaluation of new *Rhizobium tropici* isolates.

Specie	Strain	Synonymy	State/Country	Reference
R. tropici	CIAT 899	SEMIA 4077	Colombia	Martínez-Romero et al., 1991
R. tropici	H12	SEMIA 4088	Distrito Federal, Brazil	Mostasso et al., 2002
R. freirei	PRF 81	SEMIA 4080	Paraná, Brazil	Hungria et al., 2000

Table 6. Chemical soil attributes of the experimental areas before sowing in two municipalities of the state of Goiás.

Municipality	pH	Ca	Mg	Al	H+Al	P	K	Cu	Zn	Fe	Mn	OM
	H_2O	$mmol_c dm^{-3}$				$mg\ dm^{-3}$						$g\ kg^{-1}$
Guapó	5.6	18	11	1	18	3.2	81	1.7	2.1	33.1	48.1	30.6
Santo Antônio de Goiás	6.1	18	14	0	9	12.1	109	1.0	2.6	20.5	9.2	38.3

the standard strain CIAT 899, showed higher percentage values of nitrogen from the BNF (Ndfa%), with values ranging from 64.2% to 75.8%. Also, the isolates JPrG10A6, NVSG2A2, PCG2A5, JPrG1A1, UbALG7A7 and NVSG7A7 of *R. tropici* and the standard strains H12 and CIAT 899 showed the highest values of N derived from the BNF (NdBNF), with values ranging from the 802.91 to 1037.56 mg $plant^{-1}$ (Table 3). Pearson correlation analysis revealed that the percentage of N derived from BNF (Fig 1A) and the grain yield (Fig 1B) showed highly significant correlation ($p<0.01$) with the total N derived from BNF. We observed that among the standard strains assessed, only the H12 showed values of Ndfa and NdBNF higher than the average of the treatments (red dotted lines in Fig 1), along with the isolates JPrG10A6, NVSG2A2, JPrG1A1, PCG2A5, NVSG7A7 and UbALG7A7 of *R. tropici*. In addition, the isolate JPrG10A6 showed the highest value of Ndfa and the isolate UbALG7A7 the highest value of NdBNF (Fig 1A). We observed that among the standard strains assessed, CIAT 899 and H12 showed values of GY and NdBNF superior to the average of the treatments (red dotted lines Fig 1), along with the isolates JPrG10A6, JPrG6A8, NVSG2A2, PCG2A5, NVSG7A7 and UbALG7A7 of *R. tropici*. Results by Brito et al. (2009) showed that common bean obtained a maximum accumulation of 298 mg N $plant^{-1}$, with 83% of these from the symbiotic fixation. The results showed that the symbiotic fixation provided most of the N accumulated in plants and consequently, contributed to the GY. The correlation analysis between the Ndfa% and the GY, indicates that the isolates JPrG10A6, NVSG2A2, PCG2A5, NVSG7A7 and UbALG7A7 of *R. tropici*, and the standard strain H12 were more symbiotically efficient, providing the highest rates of fixed N and grain production. However, considering all assessed parameters together (nodulation, growth, and production), according to the inoculation of the common bean, the results of this study indicate that the isolate JPrG1A6 of *R. tropici* and the standard strain H12 provided the best performance indices in both locations. Therefore, the isolate JPrG10A6 of *R. tropici* shows a great potential to be used commercially as an inoculant for the common bean crop. Even showing these good results, the isolates JPrG1A6, NVSG2A2, PCG2A5, NVSG7A7 and UbALG7A7 of *R. tropici* must be assessed in a greater number of field experiments for a clear recommendation of their use as commercial inoculants for the common bean.

Materials and Methods

Isolates and strains evaluated

Seventeen *Rhizobium tropici* isolates were selected from nodules of wild genotypes of the common bean grown in soils of three Brazilian states (Table 4). The selection was based on the morphological characterization and use of C sources performed by Sampaio (2013) and on the characterization regarding tolerance to salinity, temperature, and symbiotic efficiency performed by Cardoso (2014). The standard strains used were: CIAT 899 and H12 of *R. tropici*, and PRF 81 of *R. freirei*, all registered with the Ministry of Agriculture, Livestock and Food Supply as commercial inoculants of the common bean crop (Table 5).

Description of the experimental areas

The experiments were conducted under field conditions at the Palmeiras Farm, Assentamento Canudos, in Guapó state of Goiás, Brazil, whose geographical coordinates are: latitude 16°52'52.69"S, longitude 49°42'12.56"W, and at an altitude of 573 m; and at Embrapa's Rice and Beans research facility, in Santo Antônio de Goiás, Goiás, Brazil, whose coordinates are: latitude 16°28'00"S, longitude 49°17'00"W, and with an altitude of 823 m. According to the Köppen classification, the climate in Santo Antônio de Goiás is Aw, a tropical savanna, megathermal, and in Guapó, it is Aw, tropical, semi-humid. In both locations, the pluvial regime is well defined, with a rainy season from October to March and a dry season from April to September, with an average annual rainfall of 1300 mm. The evaluated experimental areas differ in the type of management. In Guapó, the common bean crop was conducted under a family farming management system in which there was a low supply of inputs, such as fertilizers, insecticides, and fungicides. In Santo Antônio de Goiás, the common bean crop was conducted according to the practices used in the intensive agriculture management system, where there is a high use of inputs. The soil in Guapó is classified as Red Latosol, with clay = 267 g kg^{-1}; sand = 487 g kg^{-1}, and silt = 246 g kg^{-1}, while in Santo Antônio de Goiás, the predominant soil is Dark Red Latosol, with clay = 349 g kg^{-1}; sand = 440 g kg^{-1}, and silt = 211 g kg^{-1} (EMBRAPA, 2006). Before the installation of the experiments, chemical analysis of the soil was conducted in accordance with the methodology proposed by EMBRAPA (1997) and the results are shown in Table 6.

Installation of the experiments

The experimental design used was random blocks with four repetitions, and the treatments consisted of 17 *Rhizobium tropici* isolates (Table 4), three standard strains (Table 5), one nitrogen treatment (NT, equivalent to 80 kg ha^{-1} of N, using urea as the N source, divided in two applications: 20 kg ha^{-1} of N applied in furrow at sowing and 60 kg ha^{-1} applied as a topdressing 32 days after the emergence), and one absolute control (C0, without inoculation or fertilization), resulting in 22 treatments. The plots consisted of eight rows of four meters in length, with 0.45 m spacing, totaling an area of 12.6 m². The seeds of the common bean cultivar Pérola were disinfested superficially by immersion in alcohol (70%, 30 s), followed by immersion in sodium hypochlorite (2%, 5 min) and washed successively 10 times with autoclaved distilled water. Before planting, seed inoculation was conducted using the peat inoculant, containing 10^9 cells g^{-1} of peat, prepared with each isolate and standard strain in the proportion of 500 g of inoculant for 50 kg of seeds. Before sowing in Guapó, the soil was conventionally prepared twice with disc harrowing operations, while in Santo Antônio de Goiás sowing was performed in a no tillage system. Sowing was carried out mechanically, distributing 15 seeds per meter. The fertilization of sowing was carried out according to soil chemical analysis, following the technical recommendations for the crop. Phytosanitary procedures were carried out according to the recommendation for the common bean and the area was irrigated by center pivot sprinklers, depending on the water needs of the crop.

Assessment of nodulation and growth of the common bean

When the plants reached growth stage R5, the beginning of flowering, at 45 days after emergency (DAE), five plants were collected from each plot with a straight shovel, by removing the roots along with a soil block of 25x25x25 cm depth x width x length. The roots were separated from the shoots, washed under running water, and dried on paper towels. The nodules were separated from the roots and counted to determine the number of nodules (NN). After the count, the nodules and roots were dried (65°C; 72 h) and weighed to determine nodule dry weight (NDW) and root dry weight (RDW). The leaves were separated from the stems to determine the leaf area (LA) using a LI-COR model 3100 meter (LI-COR, 1996). Then, the shoots were dried (65°C; 72 h) and weighed to determine the shoot dry weight (SDW). The shoots were milled to determine the total N (N-Total) using the Kjedahl method, as described by Silva and Queiroz (2006).

Assessment of yield components and grain yield of the common bean

At the physiological maturation, in the phenological stage R9, ten plants were collected from each plot to determine the number of pods (NP) and number of grains (NG). Grain Yield (GY) was determined using plants from the central area of the plot with 10.6 m², being expressed in kg ha^{-1}, with the values corrected to 13% moisture.

Quantification of BNF contribution

In the experiment conducted in Santo Antônio de Goiás, the estimation of the contribution of BNF was calculated in the phenological stage R5 (flowering). Samples of the plants' shoots were pulverized in a rolling mill (Smith and Um 1990).
The estimation of the contribution of BNF was calculated by the ^{15}N natural abundance technique ($\delta^{15}N$), as described in Shearer and Kohl (1986) and Peoples et al. (2002), obtained according to the formula:

$$\%BNF = \left\{ \frac{\left(\delta^{15}\text{N non - fixing plant}\right) - \left(\delta^{15}\text{N fixing plant}\right)}{\left(\delta^{15}\text{N non - fixing plant - B}\right)} \right\} x100$$

In which: $\delta^{15}N$ of the non-fixing plant is the $\delta^{15}N$ value of the soil obtained through non-fixing plants, used as reference; $\delta^{15}N$ of the fixing plant is the $\delta^{15}N$ value of N_2 fixing plant; B is the value of the isotopic discrimination of ^{15}N made by the plants during the BNF process. The non-nodulating genotype of the common bean NORH 54 was considered as non-fixing plant and the B value -1.2 obtained by Pacheco (2014) was adopted. The analysis determined Delta $\delta^{15}N$ (‰), percentage of nitrogen from the BNF (Ndfa%), and total nitrogen derived from BNF (NdBNF) in the shoots of plants.

Statistical analysis

Data were subjected to analysis of variance and when a significant value of "F" was observed, the means of the treatments were compared by the Skott-Knott test at 5% probability using the SISVAR software (Ferreira, 2011). Pearson correlation analyses were also performed for Ndfa% and NdBNF and for GY and NdBNF using free statistics software (Wessa, 2016).

Conclusions

Inoculation with rhizobia isolates promotes significant gains on nodulation and productivity of the common bean, since about 50% of the isolates provided grain yield equal to or superior to the nitrogen treatment and to the standard strains CIAT 899, PRF 81, and H12. Among the best isolates, the percentage of N derived from BNF ranges from 64.2% to 75.8%, with total nitrogen derived from BNF in the shoots of the common bean ranging from 802.91 to 1037.56 mg plant^{-1}. The isolate JPrG10A6 of *Rhizobium tropici* provided high values of total nitrogen derived from BNF and grain yield, showing great potential to be used commercially as inoculant of the common bean crop.

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References

Antunes JEL, Gomes RLF, Lopes ACA, Araújo ASF, Lyra MCCP, Figueiredo MVB (2011) Symbiotic efficiency of rhizobia isolated from nodules of lima bean (*Phaseolus lunatus* L.). Rev Bras Cienc Solo. 35(3):751-757.

Barros RLN, Oliveira LB, Magalhães WB, Médici LO, Pimentel C (2013) Interaction between rizobial inoculation and sowing nitrogen fertilization on yield of common bean crop at dry and rainy seasons. Semin-Cienc Agrar. 34(4):1443-1450.

Binotti FFS, Arf O, Sá ME, Buzetti S, Alvarez ACC, Kammura KM (2009) Effect of sources, doses and nitrogen split in winter common bean in no tillage system. Bragantia. 68(2):473-481.

Bortolotto RP, Bruno IP, Reichardt K, Timm LC, Amado TJC, Ferreira AO (2012) Nitrogen fertilizer (^{15}N) leaching in a central pivot fertigated coffee crop. Rev Ceres. 59(4):466-475.

Brito MMP, Muraoka T, Silva EC (2009) Uptake rate of nitrogen from soil and fertilizer, and n derived from symbiotic fixation in cowpea (*Vigna unguiculata* (L.) WALP.) and common bean (*Phaseolus vulgaris* L.) determined using the^{15}N isotope. Rev Bras Cienc Solo. 33(4):895-905.

Brito LF, Pacheco RS, Souza Filho BF, Ferreira EPB, Straliotto R, Araújo AP (2015). Response of common bean to *Rhizobium* inoculation and supplemental mineral nitrogen in two Brazilian biomes. Rev Bras Cienc Solo. 39(4):981-992.

Builes VHR, Porch TG, Harmsen EW (2011) Genotypic differences in water use efficiency of common bean under drought stress. Agron J. 103(4):1206–1215.

Cardoso AA (2014) Caracterização polifásica de isolados bacterianos obtidos de nódulos de feijoeiro-comum. Dissertation, Universidade Federal de Goiás, Goiânia, 2014.

Cardoso JD, Gomes DF, Goes KCGP, Fonseca Júnior NS, Dorigo OF, Hungria M, Andrade DS (2009) Relationship between total nodulation and nodulation at the root crown of peanut, soybean and common bean plants. Soil Biol Biochem. 41(8):1760-1763.

Chagas Júnior AF, Oliveira LA, Oliveira NA (2010) Caracterização fenotípica de rizóbio nativos isolados de solos da Amazônia e eficiência simbiótica em feijão caupi. Acta Sci-Agron. 32(1):162-169.

Embrapa - Empresa Brasileira de Pesquisa Agropecuária (1997) Manual de métodos de análise de solo (2 ed), Rio de Janeiro, 1997.

Embrapa- Empresa Brasileira de Pesquisa Agropecuária (2006) Sistema brasileiro de classificação de solos (2 ed), Rio de Janeiro, 2006.

FAO – Food and Agricultural Organization of the United Nations. Available at: <http://faostat3.fao.org/download/Q/QC/E> Accessed in: May 19th, 2015.

Farinelli R, Lemos LB (2010) Yield, agronomic efficiency, technological and nutritional characteristics of the common bean with nitrogen fertilization in no-tillage and conventional tillage. Bragantia. 69(1):165-172.

Ferreira PAA, Silva MAP, Cassetari A, Rufini M, Moreira FMS, Andrade MJ (2009) Inoculação com cepas de rizóbio na cultura do feijoeiro. Cienc Rural. 39 (7):2210-2212.

Ferreira EPB, Stone LF, Partelli FL, Didonet AD (2011) Grain yield of common bean as affected by cover crops and soil management systems. Rev Bras Eng Agric Ambient. 15(7):695–701.

Ferreira EPB, Mercante FM, Hungria M, Mendes IC, Araújo JLS, Fernandes Junior PI, Araújo AP. Contribuições para melhoria da fixação biológica de nitrogênio no feijoeiro-comum no Brasil. In: Araújo AP, Alves BJR. Tópicos em Ciência do Solo, Viçosa, 2013.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Cienc Agrotec. 35(6):1039-1042.

Fonseca GG, Oliveira DP, Soares BL, Ferreira PAA, Teixeira CM, Martins FAD, Moreira FMS, Andrade MJB (2013) Common bean cultivars response to seed inoculation with two rhizobium strains. Biosci J. 29 (6): 1778-1787.

LI-COR. LI 3100 area meter instruction manual. Lincoln: LI-COR, 1996. 34p.

MAPA – Ministério da Agricultura, Pecuária e Abastecimento (2011) Instrução normativa nº13. Available in: <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=recuperarTextoAtoTematicaPortal&codigoTematica=1229256>. Assessed on july 18th, 2016.

Mesquita FR, Corrêa AD, Abreu CMP, Lima RAZ, Abreu AFB (2007) Bean (Phaseolus vulgaris L.) lines: Chemical composition and protein digestibility. Cienc Agrotec. 31:1114–1121.

Mostasso L, Mostasso FL, Dias BG, Vargas MAT, Hungria M (2002) Selection of bean (*Phaseulous vulgaris* L.) rhizobial strains for the Brazilian Cerrados. Field Crop Res. 73(2-3):121-132.

Pacheco RS (2014) Fixação biológica de nitrogênio em cultivares de feijoeiro estimada pela abundância natural de ^{15}N. Thesis, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, 2014.

Peoples MB, Boddey RM, Herridge DF (2002) Quantification of Nitrogen Fixation. In: Leigh GJ Nitrogen fixation at the millennium. Boston, Elsevier Science.

Pelegrin R, Mercante FM, Otsubo IMN, Otsubo AA (2009) Response of common bean crop to nitrogen fertilization and rhizobium inoculation. Rev Bras Cienc Solo. 33(1):219-226.

Raposeiras R, Marriel IE, Muzzi MRS, Paiva E, Pereira Filho IA Carvalhais LC, Passos RVM, Pinto PP, Sá NMH (2006) *Rhizobium* strains competitiveness on bean nodulation in Cerrado soils. Pesqui Agropecu Bras. 41(3):439-447.

Sampaio FB (2013) Isolados de rizóbios capturados por genótipos silvestres de feijoeiro: obtenção, morfologia e uso de fontes de carbono. Dissertation, Universidade Federal de Goiás, Goiânia, 2013.

Shearer G, Kohl DH (1986) N2 fixation in field settings: estimations based on natural ^{15}N abundance. Aust J Plant Physiol. 13(6):699-756.

Silva DJ, Queiroz AC (2006) Análise de alimentos: métodos químicos e biológicos (3 ed.) Viçosa, 2006.

Siqueira Neto M, Piccoli MC, Costa Junior C, Cerri CC, Bernoux M (2011) Greenhouse gas emission caused by different land-uses in Brazilian Savannah. Rev Bras Cienc Solo. 35(1):63-76.

Smith JL, Um MH (1990) Rapid procedures for preparing soil and KCl extracts for ^{15}N analysis. Commun Soil Sci Plant. 21(17-18):2173-2180.

Soares ALL, Ferreira PAA, Pereira PAR, Vale HMM, Lima AS, Andrade M JB, Moreira MS (2006) Agronomic efficiency of selected rhizobia strains and diversity of native nodulating populations in Perdões (MG - Brazil). II – beans. Rev Bras Cienc Solo. (30):803-811.

Souza EFC, Soratto RP, Pagani FA (2011) Nitrogen fertilization and rhizobium inoculation in common bean cultivated after corn intercropped with palisade grass. Pesqui Agropecu Bras. 46(4):370-377.

Valadão FCA, Jakelaitis A, Conus LA, Borchartt L, Oliveira AA, Valadão Junior DD (2009) Seeds inoculation and nitrogen and molybdenum fertilization of common bean in Rolim de Moura, RO. Acta Amaz. 39(4):741-748.

Wessa P (2016) Pearson correlation (v1.0.6) in free statistics software (v1.1.23- r7). Office for Research Development and Education. 2014. Available at:<www.wessa.net/rwasp_correlation.wasp/> Accessed on: June 10th 2016.