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Agronomic efficiency of *Rhizobium* strains from the Amazon region in common bean

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ABSTRACT

To maximize the contribution of biological nitrogen fixation in common bean, it is necessary to use bacterial strains that are more adapted, competitive, and efficient in the symbiotic process. In this regard, the aim of this study was to evaluate the agronomic efficiency (AE) of three bacterial strains isolated from acid soils with high Al content from the Amazon region in an *Argissolo Vermelho Distrófico típico* soil (*Typic Rhodustults – USDA Classification)* from the municipality of Formiga, MG, Brazil. We compared their AE to that of the reference strain CIAT 899^T and of two controls without inoculation (one without and another with 80 kg ha^{-1} of N-urea). The results indicated that inoculation with the strains UFLA 02-100 and UFLA 02-127 provides grain yield equivalent to inoculation with the reference strain and to the control with mineral N. Thus, both have potential for recommendation as inoculants for common bean.

KEYWORDS: *Phaseolus vulgaris* L., *rhizobia*, biological nitrogen fixation, seed inoculation

Eficiência agronômica de estirpes de *Rhizobium* isoladas da Amazônia sobre o feijoeiro-comum

RESUMO

Para maximizar a contribuição da fixação biológica de nitrogênio no feijoeiro-comum é necessária a utilização de estirpes de bactérias mais adaptadas, competitivas e eficientes no processo simbiótico. Nesse sentido, objetivou-se avaliar, em um *Argissolo Vermelho Distrófico típico* do município de Formiga-MG, a eficiência agronômica (EA) de três estirpes isoladas de solos ácidos e com alto teor de Al da Amazônia e comparar suas EA à da estirpe referência CIAT 899^T e à de dois controles sem inoculação (um sem e outro com 80 kg ha^{-1} of N-ureia). Os resultados indicaram que a inoculação com as estirpes UFLA 02-100 e UFLA 02-127 propicia rendimento de grãos equivalente ao da estirpe referência e ao do controle com N mineral e que por isso, ambas têm potencial para ser recomendadas como inoculantes para o feijoeiro-comum.

PALAVRAS CHAVE: *Phaseolus vulgaris* L., rizóbio, fixação biológica de nitrogênio, inoculação na semente

Common bean is a legume capable of biological nitrogen fixation (BNF) through symbiosis with bacteria that fix atmospheric N2, which are able to supply at least part of the nitrogen (N) required by the plant for its development, leading to significant savings in the use of nitrogen fertilizers (Figueiredo *et al.* 2016; Oliveira *et al.* 2016; Soares *et al.* 2016). In spite of this ability, factors such as competition among the bacteria introduced and others already established in the soil, along with exposure to adverse environmental conditions such as high temperatures and soil acidity, can compromise the efficiency of inoculation, with reflections on the BNF of the crop (Rufini *et al.* 2011; Fonseca *et al.* 2013; Figueiredo *et al.* 2016; Oliveira *et al.* 2016). To maximize the contribution of BNF, above all in tropical soils where acidity conditions are more accentuated, it is necessary to use strains that are more adapted, competitive, and efficient in the infection process (Ferreira *et al.* 2009; 2012; Rufini *et al.* 2011; Fonseca *et al.* 2013). In this regard, the aim of this study was to evaluate the agronomic efficiency (AE) in the field of *Rhizobium* strains isolated from acid soils with high aluminum (Al) content from the Amazon region and compare their AE to that of the reference strain CIAT 899^T and of two controls without inoculation, with and without N from urea.

The field experiment was carried out in the rainy crop season in an *Argissolo Vermelho Distrofico típico* (*Typic Rhodustults – USDA classification*) of known fertility (Table 1) of the municipality of Formiga, State of Minas Gerais (20º 27' 26.408" S, 45º 26' 26.839" W). The climate of the area is Cwa, humid subtropical, with mean monthly rainfall of 272 mm in the period in which the trial was carried out (rainfall more concentrated after sowing, in the first month of the trial, reaching a mean value of 300 mm in this period) and a mean temperature of 23 ºC, according to the National Meteorology Institute (Instituto Nacional de Meteorologia). The experimental design was of randomized blocks with six replications and six treatments. The treatments involved the individual inoculation of strains UFLA 02-100, UFLA 02-127, and UFLA 02-86, isolated from soils of the Amazonian state of Rondônia and selected for their efficiency in Leonard jars, in pots with soil, and under field conditions (Soares *et al.* 2006; Ferreira *et al.* 2009; 2012; Rufini *et al.* 2011); the strain CIAT 899^T, approved by the Brazilian Ministry of Agriculture, Livestock, and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA) for production of commercial inoculants for bean seeds; and two controls without inoculation, one with 80 kg ha^{-1} of N-urea (half at sowing and the other half in topdressing at 20 days after emergence - DAE) and another without N-urea. The three strains of the bacteria with the UFLA code belong to the SBMPBS/UFLA (Biology, Microbiology, and Biological Processes Sector) collection of the Universidade Federal de Lavras. A more detailed description of the strains is shown in Table 2. The native rhizobial populations able to nodulate beans at the site were approximately 10^3 colony-forming units (CFU) g soil^{-1}. These most probable numbers were determined as described by Rufini *et al*. (2011).

The inoculant was prepared with peat (sterilized in a autoclave), mixed at a ratio of 3:2 (w:w) with log phase cultures in a semi-solid 79 medium (Fred and Waksman 1928), according to the procedures described by Soares *et al.* (2006). The resulting material was applied at a ratio of 10 g per kg of seeds. Inoculant quality was monitored by counting the number of CFU and comparing it to the minimum legal number of viable cells, approximately 10^9 *Rhizobium* cells

Table 1. Chemical characteristics of a soil sample collected in 0 to 20 cm in Formiga, Minas Gerais, Brazil.

Characteristics											
pH	P available	K	Ca	Mg	Al	SB	t	T	m	V	OM
(H_2O)	mg dm^{-3}	cmolc dm^{-3}							%		dag kg^{-1}
5.6	6.5	160.0	2.8	1.5	Undetected	4.7	4.7	8.3	0.0	56.7	2.2

Table 2. Origin, characteristics and identification of *Rhizobium* strains used as inoculants for the common bean (*Phaseolus vulgaris* L.) in the study.

Strains	*LUS/ Location	Growth characteristics in 79 medium						Identification
		GR[a]	CD[b]	GP[c]	pH[d]	Al[e]	CC[f]	
UFLA 02-100[g]	Agriculture, RO-Brasil	F	>2	Low	Neutral	Yes	White	*Rhizobium etli*
UFLA 02-86[g]	Agriculture, RO-Brasil	F	>2	High	Neutral	Yes	White	*R. etli* bv. *phaseoli*
UFLA 02-127[g]	Agriculture, RO-Brasil	F	>2	Medium	Neutral	Yes	White	*R. leguminosarum* bv. *phaseoli*
CIAT 899^T [g]	Colômbia	F	>2	High	Acidic	Yes	Yellow	*R. tropici*

*LUS: Land use system from which the strain was isolated. RO=Rondônia state. Growth characteristics in 79 medium: [a]GR. Growth rate - F: Fast (3 days); [b]CD. Colony diameter; [c]GP: Gum production; [d]pH: pH of the culture medium after growth; [e]Al. Absorption indicatior; [f]CC. Colony colour. [g] Source: Oliveira-Longatti *et al*. (2013). The three strains of the bacteria with the UFLA code belong to the SBMPBS/UFLA (Biology, Microbiology, and Biological Processes Sector) collection of the Universidade Federal de Lavras.

per gram of inoculant. The final concentration was about 10^6 *Rhizobium* cells per seed.

The experimental plot consisted of twelve 6-m-long bean rows, with 0.5 m between rows. Two rows on each side of the plot were considered borders and the eight central rows were used for data collection. Soil tillage included plowing, harrowing, and demarcation of rows. The planting furrows of all plots were fertilized with 90 kg ha^{-1} of P_2O_5 (triple superphosphate source) and 20 kg ha^{-1} of K_2O (potassium chloride source). Immediately after *Rhizobium* inoculation, manual sowing was carried out of the 'Pérola' cultivar of carioca bean, with 17 seeds per meter. Manual weeding was performed whenever necessary. Preventive disease control was performed with applications of triphenyltin hydroxide (Mertin® 400 g L^{-1}) at 25 DAE and azoxystrobin (Amistar® 50 g kg^{-1}) at 30 DAE, both at a spray volume of 400 L ha^{-1}. There was no need for pest control. The trial plot received supplementary irrigation by conventional sprinkler.

At the R6 stage of the bean cycle (flowering, 45 DAE), 10 plants were collected from each plot (rows 3 and 10) for assessments of number of nodules, nodule dry matter, and shoot dry matter. At the R9 stage (plant maturity), final stand (FS) and grain yield (GY) were determined (rows 4-9). The FS was obtained by counting the number of plants, which was expressed as number of plants per hectare. The GY was expressed in kg ha^{-1} at 130 g kg^{-1} moisture.

The data were subjected to analysis of variance using the Sisvar 4.0 software (Ferreira 2011). To fulfill the assumptions of analysis of variance, the data of NN and DMN were first transformed in $(x+1)^{0.5}$. According to the official protocol for evaluation of the variability and agronomic efficiency of the plant stocks, inoculants, and technologies related to the BNF process in legumes (Brasil 2011), when there was a significant effect of cultivars or of inoculation treatments by the F test ($p<0.05$ or $p<0.10$), clustering of the mean values was performed by the Scott-Knott test at the same level of significance.

There was no significant variation in nodulation and shoot dry matter production of the inoculated and non-inoculated treatments, which did not differ from the controls that were fertilized or not fertilized with mineral N (Table 3), which has also been observed in other studies (Fonseca *et al.* 2013; Figueiredo *et al*. 2016; Oliveira *et al*. 2016). Fertilization with 40 kg ha^{-1} of N-urea at planting did not affect nodulation because the low application rate was insufficient to suppress nodulation, as also indicated by Figueiredo *et al*. (2016). Another possible explanation for this occurrence is that high rainfall, in the order of 300 mm, leached part of the mineral N and stimulated nodulation of the plant in response to the nutritional deficiency induced (Moreira and Siqueira 2006; Figueiredo *et al*. 2016). It is possible that this same moisture available in the soil during establishment of the crop was decisive so that no saline effect would be manifested on plant density with an increase in nitrogen fertilization (Table 3). For that reason, any difference in grain yield cannot be attributed to differences in FS.

Although the performance of the native population on nodulation and plant growth in this study was similar to that of the other treatments, its contribution to grain yield was equivalent only to that of the strain UFLA 02-86, with the mean values remaining below the values of the other inoculations (Table 3). In contrast, the selected strains, UFLA 02-100 and UFLA 02-127, exhibited yields higher than 2,800 kg ha^{-1}, which represents around three times the Brazilian mean value obtained in the 2015/16 crop season (Conab 2016). Furthermore, both strains provided a grain yield equivalent to that of the already recommended CIAT 899^T strain and to the control with 80 kg ha^{-1} of N-urea. The good activity of these strains, just as that of UFLA 02-86 of *R. etli* bv. *phaseoli***,** had already been mentioned in other studies, such as in Soares *et al*. (2006), who also obtained an increase in common bean yield in an *Argissolo Vermelho distrófico* (*Typic Rhodustults – USDA classification*) in Perdões, Minas Gerais, with savings in the use of nitrogen fertilizer. In a *Latossolo*

Tabela 3. Mean values of number of nodules (NN), nodule dry mass (NDN), shoot dry mass (SDM), final stand (FS) and grain yield (GY) of common bean cv. Pérola, regarding to sources of N.

Source of N	NN	NDN (mg $plant^{-1}$)	SDM (g $plant^{-1}$)	FS (10^3 plants ha^{-1})	GY (kg ha^{-1})
UFLA 02-100	29 a	22 a	9.39 a	246 a	2,883 A
UFLA 02-127	29 a	25 a	9.32 a	263 a	2,897 A
UFLA 02-86	36 a	27 a	10.92 a	246 a	2,620 B
CIAT 899^T	31 a	20 a	11.03 a	246 a	3,007 A
*CwN	28 a	17 a	12.99 a	264 a	3,136 A
**CwtN	28 a	20 a	9.86 a	274 a	2,531 B
Means	30	22	10.58	256	2,845

Within each column, mean values followed by the same lowercase letters ($p<0.05$) and uppercase letters ($p<0.10$) belong to the same group, according to the Scott-Knott test. Control with no inoculation: *CwN-with fertilization (80 kg ha^{-1} of N-ureia), **CwtN-without N fertilization.

Vermelho eutroférrico (Eutric Acrudox – USDA classification) in Lavras, Minas Gerais, the strain UFLA 02-127 not only promoted a grain yield similar to that of the control fertilized with mineral N, but also exceeded CIAT 899^T (Ferreira et al. 2009), reaffirming the symbiotic capacity of these strains with common bean. Based on these results and the results of this study, the UFLA 02-100 and UFLA 02-127 strains have potential for recommendation as commercial inoculants for common bean seeds, contributing to reduce the amount of crop fertilizer applied.

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