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CROP PRODUCTION

Inoculation with *Rhizobium tropici* can totally replace N-fertilization in the recently released BRSMG Uai bean cultivar

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ABSTRACT. The BRSMG Uai common bean cultivar (*Phaseolus vulgaris* L.) unites traits required by producers, such as high yield and upright stands, the latter of which is considerably improved in relation to previously released cultivars such as BRSMG Madrepérola and Pérola. However, the potential of the cultivar to form symbiotic relationships with rhizobia to fix N_2 has not yet been assessed. Here, field experiments were conducted to assess the efficiency of biological nitrogen fixation (BNF) in the cultivar, and to compare this to the efficiencies of other genetic materials widely grown throughout Brazil (BRSMG Madrepérola and Pérola). Experiments were conducted on two Oxisols in Minas Gerais State, Brazil (one during the dry season in the south, and the other during the winter season in the Alto-Paranaíba region), under a no-tillage system over maize stover. A randomized block experimental design was implemented in a 3 × 3 factorial arrangement with four replicates. Individual and combined Analysis of Variance were performed and the data were normalized. Homogeneity of the residual mean squares was observed. Significant effects of factors were identified by Scott-Knott tests and F tests. The BRSMG Uai cultivar responded to inoculation with the rhizobial strain CIAT 899, with yields similar to those achieved upon fertilization with 80 kg ha^{-1} of N-urea and to the yields of the BRSMG Madrepérola and Pérola cultivars inoculated with the same rhizobial strain. In terms of commercial use, this cultivar may be successful under either of the management practices adopted for N supply, and from the perspective of plant breeding, it has potential for inclusion in breeding programs directed toward improving BNF.

Keywords: *Phaseolus vulgaris*, management of N sources, biological nitrogen fixation, symbiotic efficiency.

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Introduction

There is increasing demand for genetic materials that combine high yield, disease resistance, and ease of growing and management. To meet these needs of crop producers in the state of Minas Gerais, Brazil, institutions that selectively breed dry edible bean/common bean (*Phaseolus vulgaris* L.) cultivars [*Embrapa Arroz e Feijão* (Embrapa), *Empresa de Pesquisa Agropecuária de Minas Gerais* (Epamig), the *Universidade Federal de Lavras* (UFLA), and the *Universidade Federal de Viçosa* (UFV)] registered BRSMG Uai cultivar in 2015 (Ramalho et al., 2016). The BRSMG Uai cultivar has carioca (beige with brown-streaked seed coat) grains and upright plant architecture, which reduces the severity of diseases and minimizes contact between pods and the ground, ensuring increased grain quality and facilitating mechanical harvesting operations. In spite of the good performance of this cultivar, information is lacking concerning its potential to establish effective symbiotic relationships with atmospheric N_2-fixing bacteria.

Plant growth-promoting rhizobacteria, which include symbiotic atmospheric nitrogen (N_2) fixers, are important in sustainable agriculture (Barea, 2015; Lagos et al., 2015; Larsen, Jaramillo-López, Nájera-Rincon, & González-Esquivel, 2015). Biological nitrogen fixation (BNF) has intensified in common bean crops in recent years, with positive and consistent results being achieved for various cultivars (Andraus, Cardoso, & Ferreira, 2016; Oliveira et al., 2018). Responsive cultivars and efficient rhizobia (native rhizobia or those introduced through inoculation) have led to satisfactory yields that are equivalent, or even superior, to those obtained from mineral nitrogen fertilization. Yet, there are materials that are more dependent on nitrogen fertilizers for their development than others (Grange, Hungria, Graham, & Martínez-Romero, 2007; Arf et al., 2011; Fageria, Ferreira, Melo, & Knupp, 2014; Pereira et al., 2015). Moreover, other factors, such as those inherent to the crop growing environment, soil fertility, edaphic factors, and climatic conditions, should not be ignored, due to their possible effects on the BNF symbiotic process.

Knowledge regarding the symbiotic potential of BRSMG Uai will therefore be able to direct breeding programs aiming to improve BNF, as well as help farmers determine the best source of nitrogen to adopt for their crop. Thus, the aim of this study was to evaluate the efficiency of inoculating the new common bean BRSMG Uai cultivar with the CIAT 899 nitrogen-fixing rhizobial strain, and to compare the BRSMG Uai cultivar with two other cultivars widely used in the state of Minas Gerais (BRSMG Madrepérola and Pérola) under two different field conditions.

Material and methods

Characterization of experimental sites and experimental design

Two field experiments were conducted under a no-tillage system over maize stover in 2017 in two different environments. One experiment was conducted during the dry season in a dystrophic Red Latosol (Oxisol) in the southern region of Minas Gerais State, Brazil [Lambari municipality (altitude 896 m, 21.9671° S, 45.3466° W)]. The second experiment was conducted during the winter in an Oxisol in the Alto Paranaíba region of the state [Patos de Minas municipality (altitude 940 m, 18.5794° S, 46.5184° W)]. According to the Köppen climate classification system, Lambari possesses a Cwa (tropical highland) climate, and Patos de Minas possesses an Aw (tropical savanna with a dry winter) climate (Sá Junior, Carvalho, Silva, & Alves, 2012). Samples of both soils were collected for fertility analysis 15 days before the experiment was set up (Table 1). Neither of the areas had a history of inoculating common bean crops with rhizobia. Rufini et al. (2011) estimated that the native rhizobial population able to nodulate beans at Lambari was 9.5×10^2 colony-forming units g soil^{-1} (CFU), and at Patos de Minas the populations rose to 2.5×10^3 CFU.

The soil of Patos de Minas received 1.5 ton ha^{-1} of dolomitic limestone incorporated to a depth of 10 cm in 2015. Soil acidity in Lambari was neutralized by applying 1.0 ton ha^{-1} of calcitic limestone incorporated with a plow in 2009, and later by reapplication at the same rate on the surface (without incorporation) in 2015.

Table 1. Chemical and physical characteristics of the soil samples collected from the 0-20 cm layer.

Characteristic[1]	Unit	Soil	
		Lambari	Patos de Minas
pH	-	6.1 WA[2]	5.7 MA
Available P	(mg dm^{-3})	12.50 M	38.78 VG
K	(cmol$_c$ dm^{-1})	0.25 G	0.076 VL
Ca	(cmol$_c$ dm^{-3})	1.09 L	0.86 L
Mg	(cmol$_c$ dm^{-3})	0.35 L	0.24 L
Al	(cmol$_c$ dm^{-3})	0.54 M	0.12 VL
H + Al	(cmol$_c$ dm^{-3})	7.37 HI	6.24 HI
SB	(cmol$_c$ dm^{-3})	1.69 L	1.18 L
T	(cmol$_c$ dm^{-3})	2.23 L	1.30 L
T	(cmol$_c$ dm^{-3})	9.06 G	7.42 M
M	(%)	24.22 L	9.23 VL
V	(%)	18.67 VL	15.86 VL
OM	(dag kg^{-1})	2.82 M	2.84 M
Zn	(mg dm^{-3})	4.72 HI	2.18 G
Fe	(mg dm^{-3})	41.35 G	54.46 HI
Mn	(mg dm^{-3})	14.92 HI	80.57 VHI
Cu	(mg dm^{-3})	1.03 M	13.74 HI
B	(mg dm^{-3})	0.02 VL	0.08 VL
S	(mg dm^{-3})	40.32 VG	85.09 VG
Physical Analysis			
Clay	(g kg^{-1})	450	340
Silt	(g kg^{-1})	150	410
Sand	(g kg^{-1})	400	250
Texture class	-	Clayey	Medium

[1]pH in water; H: hydrogen; SB: sum of exchangeable bases; t: cation exchange capacity; T: cation exchange capacity at pH = 7; m: exchangeable aluminum; V: base saturation; OM: organic matter. [2]Interpretation of soil analysis according to Alvarez, Novais, Barros, Cantarutti, and Lopes (1999): MA (Medium Acid), WA (Weak Acid), L (Low), G (Good), M (Medium), HI (High), VL (Very Low), VG (Very Good), VHI (Very High).

The experimental design consisted of randomized blocks, with four replications, in a 3 × 3 factorial arrangement involving three common bean cultivars from the carioca group and three sources of nitrogen (inoculated, non-inoculated, non-inoculated with mineral-N). Combined analyses also took environmental factors into consideration. Each experimental unit (12 m^2 in Lambari and 14.4 m^2 in Patos de Minas) consisted of six 4 m length rows, and the four innermost rows were used for data collection. All plots received base fertilization with single superphosphate (90 kg P_2O_5 ha^{-1} in Lambari and 70 kg P_2O_5 ha^{-1} in Patos de Minas) and potassium chloride (50 kg K_2O ha^{-1} in Lambari and 20 kg K_2O ha^{-1} in Patos de Minas), constituting Technological Level 4 (Chagas et al., 1999), to mitigate deficiencies revealed by soil analysis. Seeds were sown manually at a density of 15 seeds per meter.

Characterization of the factors studied

The cultivars used (BRSMG Uai, BRSMG Madrepérola, and Pérola) are recommended for the state of Minas Gerais, and BRSMG Uai (Ramalho et al., 2016), the most recently released, was registered in 2015. The BRSMG Madrepérola cultivar has a semi-early cycle of 80 days (Carneiro et al., 2012), and the other cultivars used have normal, variable cycle of between 85 and 95 days. All cultivars have indeterminate growth habits of types II (BRSMG Uai, upright architecture), III (BRSMG Madrepérola, prostrate), and II/III (Pérola, semi-upright).

The *Rhizobium tropici* strain used was CIAT 899, which is approved in Brazil by the *Ministério da Agricultura, Pecuária e Abastecimento* (Mapa) [Brazilian Ministry of Agriculture] for the production of commercial inoculants for the common bean. The inoculant was prepared with peat sterilized in an autoclave in a proportion of peat to 79 culture medium of 3:2 (w:v). The quality of the inoculant was monitored by counting the number of CFU, in order to meet the minimum legal number of viable *Rhizobium* cells (around 10^9 CFU) per gram of inoculant at sowing. The resulting material was used at a rate of 100 g per kg of seed.

In the mineral N treatment, 80 kg ha^{-1} of N-urea was applied manually (¼ in the planting furrow and then ¾ during topdressing, with continuous flowing distribution by sidedressing at 25 days after emergence - DAE). The N was incorporated by irrigation water.

Crop management practices

Crop treatments were those normally used for common bean crops in each region. In the dry season in Lambari, sprinkler irrigation was performed to complement rainfall (climate data shown in Figure 1) whenever soil moisture became critically low. In the winter season in Patos de Minas, this sprinkler form of irrigation was used throughout the crop cycle.

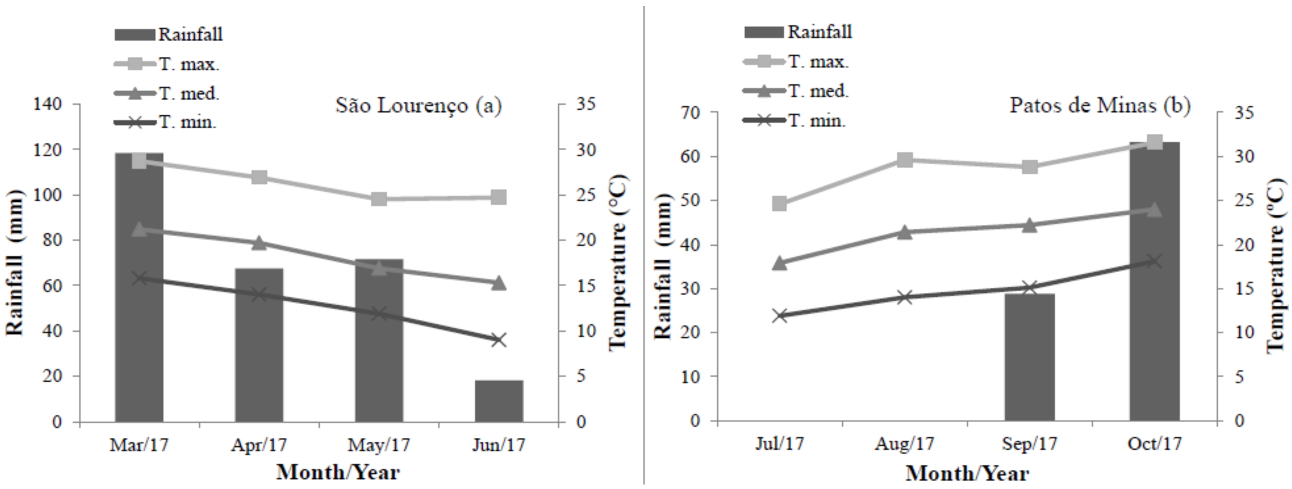

Figure 1. Monthly variation of maximum, mean, and minimum temperatures and rainfall recorded from 1 Mar. to 30 Jun. 2017 in (A) Environment 1*, and from 1 Jul. to 30 Oct. 2017 in (B) Environment 2. *São Lourenço – Inmet station closest to Lambari, state Minas Gerais. Source: National Meteorology Institute (*Instituto Nacional de Meteorologia* [Inmet], 2020).

In Lambari, pest and disease control measures were not necessary. In Patos de Minas, preventive measures were taken through applications of lambda-cyhalothrin + lambda-cyhalothrin + thiamethoxam (Engeo Pleno®, 250 mL ha^{-1}) and thiamethoxam (Actara 250®, 100 g ha^{-1}) insecticides and two applications of the thiophanate-methyl (Cercobin®, 150 mL ha^{-1}) and azoxystrobin (Amistar 500®, 100 g ha^{-1}) fungicides. In both environments, weeds were controlled using a mixture of 0.9 L of Flex® + 1.7 L of Fusilade® per hectare, and through complementary weeding when necessary. A cobalt + molybdenum mixture (CoMo®, 120 mL ha^{-1}) was applied on the leaves at 20 DAE in both environments. The solutions were distributed with a manual backpack sprayer at a spray volume of 400 L ha^{-1}.

Parameters analyzed and techniques used

At full flowering (R6 stage), a six-plant sample was taken at random from each plot (from rows 2 and 3) to determine the number of nodules (NN), nodule dry matter (NDM), shoot dry matter (SDM), and shoot N accumulation (SNA). At maturity (R9 stage), yield and grain N accumulation (GNA) were determined using samples from rows 4 and 5. Yield was obtained from the total weight of grain produced per plant, adjusted to a grain moisture content of 130 g kg^{-1}, and the results were expressed for an average stand of 200,000 plants ha^{-1}. The SNA was calculated by multiplying SDM by the percentage of N, which was obtained through the semi-micro Kjeldahl method pioneered in Sarruge and Haag (1979) and described in Ferreira et al. (2009); the results of which were then divided by 100. The GNA was determined through the same method used for the shoot samples, replacing the values of SDM with those of yield.

Statistical analyses

All data were subjected to individual and combined Analysis of Variance using the Sisvar 4.0 software (Ferreira, 2019), after undergoing tests to confirm normality (Shapiro-Wilk's test), homoscedasticity of variances (Bartlett Test), and homogeneity of the residual mean squares. The NN and NDM variables were previously transformed by: $(x+1)^{0.5}$. According to the official protocol for evaluating the variability and agronomic efficiencies of plant stocks, inoculants, and technologies related to the BNF process in legumes (Brasil, 2011), in the event of a significant effect of a factor being determined by the F test ($p < 0.05$ or $p < 0.10$), clustering of mean values was performed by the Scott-Knott test (for cultivars or for sources of N) or the F test (for 'Environments', for having two levels) at the same level of significance.

Results and discussion

There were significant main effects of environment (E; on NN, NDM, SDM, and SNA), source of N (SN; on all variables), and cultivar (C; on SDM). The following interactions were also significant: E × SN (on NDM and GNA), E × C (on NN), SN × C (on NDM), and E × SN × C (on SNA).

The highest NN and NDM values were observed in Patos de Minas (Table 2). The effects of inoculation on NDM were more evident in this environment, as the nodules under this treatment were heavier than the native population (data inferred based on the control treatment). In Lambari, there was no difference in NDM between the native rhizobia and the introduced rhizobia (Table 2). However, mineral N fertilization limited nodulation of the native rhizobia in both environments [Table 2 (NDM) and Table 3 (NN)].

Table 2. Mean values of nodule dry matter (NDM) and of grain nitrogen accumulation (GNA) in relation to environments; NDM in relation to sources of N and to cultivars; and number of nodules (NN) in relation to environments and to the cultivars of common bean.

Factor	NDM (mg plant^{-1})					GNA (g kg^{-1})	
	Environment		Cultivar			Environment	
Source of N[1]	Lambari	Patos de Minas	BRSMG Uai	BRSMG Madrepérola	Pérola	Lambari	Patos de Minas
Mineral N	13.7 Bb	48.3 Ca	35.2 Ba	35.5 Ba	22.3 Bb	108.2 Aa	127.2 Aa
Inoculation	59.9 Ab	243.2 Aa	134.7 Aa	180.7 Aa	130.3 Aa	118.2 Aa	97.9 Ba
Control	58.7 Ab	159.6 Ba	155.2 Aa	56.5 Bb	115.7 Aa	103.0 Aa	78.6 Bb

Cultivar	NN	
	Environment	
	Lambari	Patos de Minas
BRSMG Uai	9 Ab	27 Aa
BRSMG Madrepérola	12 Ab	24 Aa
Pérola	15 Aa	19 Ba

Within each factor, mean values followed by the same uppercase letter in the column and lowercase letter in the row belong to the same group ($p < 0.05$). Discrimination of the mean values performed by the F test for 'Environment' (for having two levels) and the Scott-Knott test for 'Source of N and Cultivar'. [1]Fertilization with mineral N; Inoculation with rhizobia; Control without inoculation and without N-fertilization.

Table 3. Mean values of number of nodules (NN) and grain nitrogen accumulation (GNA) in relation to the factors studied.

Factor	NN
Source of N	
Fertilization with mineral N	7.9 B
Inoculation with rhizobia	23.6 A
Control	19.6 A
Cultivar	GNA (g kg^{-1})
BRSMG Uai	101.6 A
BRSMG Madrepérola	109.5 A
Pérola	105.5 A

Within each factor, mean values followed by the same uppercase letter in the column belong to the same group by the Scott-Knott test ($p < 0.05$).

The mean values of most of the parameters were similar between the inoculated treatment and the mineral N fertilization treatment (Tables 2, 3, 4, and 5). Plants inoculated or fertilized with mineral nitrogen showed increased SDM (Table 4) and grain yield (Table 4) compared to the control.

Table 4. Mean values of shoot dry matter (SDM) and yield in relation to the factors studied.

Factor	SDM	Yield
Environment	(g $plant^{-1}$)	(1000 kg ha^{-1})
Lambari	10.7 A	3.1 A
Patos de Minas	7.6 B	2.8 A
Source of N		
Fertilization with mineral N	11.5 A	3.3 A
Inoculation with rhizobia	10.8 A	3.1 A
Control	8.2 B	2.6 B
Cultivar		
BRSMG Uai	8.8 B	2.8 A
BRSMG Madrepérola	11.0 A	3.1 A
Pérola	9.7 B	2.7 A

Within each factor, mean values followed by the same uppercase letter in the column belong to the same group ($p < 0.05$). Discrimination of the mean values performed by the F test for 'Environment' (for having two levels) and the Scott-Knott test for 'Source of N and Cultivar'.

Table 5. Mean shoot nitrogen accumulation in common bean in relation to environments, sources of N, and cultivars.

Environment[1]	Source of N[1]	SNA (mg $plant^{-1}$) Cultivar: BRSMG Uai	BRSMG Madrepérola	Pérola
Lambari	Mineral N	292.8 Ab	434.7 Aa	421.1 Aa
	Inoculation	379.1 Aa	464.8 Aa	379.8 Aa
	Control	200.8 Bb	352.2 Aa	219.2 Bb
Patos de Minas	Mineral N	498.4 Aa	311.2 Ab	328.7 Ab
	Inoculation	225.6 Ba	324.8 Aa	285.1 Aa
	Control	184.7 Ba	218.3 Aa	186.5 Ba

Cultivar	Source of N	SNA (mg $plant^{-1}$) Environment: Lambari	Patos de Minas
BRSMG Uai	Mineral N	292.8 b	498.4 a
	Inoculation	379.1 a	225.6 b
	Control	200.8 a	184.7 a
BRSMG Madrepérola	Mineral N	434.7 a	311.2 b
	Inoculation	464.8 a	324.8 b
	Control	352.2 a	218.3 b
Pérola	Mineral N	421.1 a	328.7 a
	Inoculation	379.8 a	285.1 a
	Control	219.2 a	186.5 a

Within each factor, mean values followed by the same uppercase letter in the column and lowercase letter in the row belong to the same group ($p < 0.05$ or $p < 0.10$). Discrimination of mean values performed by the F test for 'Environment' (for having two levels) and the Scott-Knott test for 'Source of N and Cultivar'. [1]Fertilization with mineral N; Inoculation with rhizobia; Control without inoculation and without N-fertilization.

Genetic variability of the cultivars affected nodulation ability (NN and NDM) and growth (SDM), as well as plant nutrition (SNA), without compromising grain yield or grain nitrogen accumulation (GNA, Table 3). The Pérola cultivar produced fewer nodules in Patos de Minas, but produced similar NN to other cultivars when grown in Lambari (Table 2). The greatest SDM was observed in the cultivar BRSMG Madrepérola (Table 4); however, BRSMG Madrepérola did not produce superior NDM (Table 2), SNA (Table 5), grain yield

(Table 4), or GNA (Table 3) values in either environment. Higher grain yield in BRSMG Uai occurred under seed inoculation and with mineral N fertilization treatments (Tables 5 and 6).

Table 6. Mean yields that are part of the decomposition of the 'Cultivar × Environment' and 'Cultivar × Source of N' interactions.

Cultivar	Factor	Yield
BRSMG Uai	Environment	(1000 kg ha^{-1})
	Lambari	2.9 A
	Patos de Minas	3.0 A
	Source of N	
	Fertilization with mineral N	3.1 A
	Inoculation with rhizobia	2.9 A
	Control	2.3 B

Within each factor, the mean values followed by the same uppercase letter in the column belong to the same group ($p < 0.05$).

There was a negative effect of nitrogen fertilization on the NDM of the three cultivars. This may be due to the treatment creating excess mineral N in the soil, which does not favor nodulation as there is no nutritional deficiency (Moreira, 2006). In both environments, the mineral N treatment limited nodulation of the native rhizobia; a fact which is corroborated by Oliveira et al. (2016; 2018) and Oliveira et al. (2017). In the present study, the performance of the introduced rhizobia was superior to that of the native population for some parameters, with or without interactions among factors. As they were better nourished (SNA, Table 5), the plants under the inoculated and N-fertilized treatments grew an average of 36% more than the non-inoculated control treatment (SDM, Table 4), which resulted in an average increase in yield of 21% (Table 4). Grange et al. (2007) and Andraus et al. (2016) presented similar results in studies of the same nature focusing on other genotypes of *Phaseolus vulgaris*. High yields from inoculation (3,100 kg ha^{-1}) are generally only achieved in technologically-advanced agricultural systems, and these high yields were around three times the mean yield obtained in Brazil for the crop in 2017 (*Companhia Nacional de Abastecimento* [Conab], 2018), because majority of farmers do not apply neither rhizobia inoculation nor other advanced technologies. It should be considered that rhizobia inoculation can also benefits small farmers, however it is recommended at least phosphorus fertilization due to the very low levels of this nutrient in tropical soils (Soares et al., 2006). It should be highlighted that these yields are greater than the mean yield of the 28 sites in which BRSMG Uai was tested by Ramalho et al. (2016) (2,201 and 2,249 kg ha^{-1} from sowing in November and in May-July, respectively). Therefore, based on these results, inoculation with rhizobia is feasible for farmers and may even replace nitrogen fertilization This not only results in monetary savings on fertilizer, but may also have important ecological benefits.

Plant architecture and growth habits affected production of plant biomass, and contributed to greater SDM, in BRSMG Madrepérola (Table 4). The BRSMG Madrepérola cultivar possesses a type III growth habit, characterized by more developed and numerous lateral branches than type II (BRSMG Uai) and II/III (Pérola) cultivars.

Since BRSMG Uai is a more recently-produced genetic material, its BNF potential had not been evaluated. This highlights the importance of the current study, in which this genotype showed high nodulation ability (NN and NDM, Table 2) and good symbiotic efficiency (SNA, Table 5), responding well to inoculation with efficient rhizobia, as was the case in this study with the CIAT 899 strain. Above all, when this cultivar is grown in fertile soil with adequate moisture for common bean (as in Lambari), BNF accumulates the same amount of N in its shoots as when fertilized with 80 kg N-urea ha^{-1}. The only time this was not achieved was when the soil had chemical restrictions, such as very high levels of Mn and Cu (as in Patos de Minas), which probably directly affected plant-rhizobia symbiosis regarding N_2 fixation. There may also have been a negative effect of the fungicides applied in Patos de Minas on symbiosis. In spite of these limitations in Patos de Minas, the N that was fixed had a positive effect on yield (Table 4) and on grain enrichment (Table 3), resulting in mean values similar to those of the BRSMG Madrepérola and Pérola cultivars, which are grown throughout Brazil.

Another relevant finding concerning the new cultivar is in regard to its yield capacity (Yield, Tables 4 and 6), which was not associated with specific environmental conditions, as SNA was. Although this genotype was developed under mineral nitrogen fertilization during the selection cycle, the yield capacity was able to vary based on the use of N mineral fertilization or inoculation with selected rhizobia (Tables 4 and 6). This adaptability has positive connotations from the commercial market perspective. The suitability of this cultivar for inclusion in breeding programs as a source of BNF target traits has been confirmed in the present study.

Conclusion

The BRSMG Uai cultivar responded to inoculation with rhizobial strain CIAT 899 in a manner similar to fertilization with 80 kg ha^{-1} of N-urea, and its results were similar to those of the BRSMG Madrepérola and Pérola cultivars, which are widely grown throughout Brazil, when inoculated with the same rhizobial strain.

The BRSMG Uai cultivar has the potential for inclusion in breeding programs directed towards improving BNF.

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