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Rhizobium strains selected from the Amazon region increase the yield of snap bean genotypes in protected cultivation

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ABSTRACT: Although the use of inoculants containing rhizobia is encouraged, there are no official recommendations for inoculation of snap bean. In this respect, the aim of this study was to evaluate the agronomic performance of *Rhizobium* strains in symbiosis with snap bean cultivars with different growth habits and crop cycles. The experiment was carried out in pots with soil in a greenhouse in the spring-summer season in Lavras, state of Minas Gerais, Brazil. A completely randomized experimental design was used, with 4 replications, in a 4 × 7 factorial arrangement involving four snap bean cultivars and seven treatments – five strains of *Rhizobium* (UFLA 02-100, UFLA 02-127, UFLA 04-173, CIAT 899, and PRF 81), plus two controls without inoculation (with and without 500 mg N-NH_4NO_3·dm^{-3}). We conclude that: i) while genetic differences were observed among cultivars with respect to nodulating capacity, in all cases significant benefits were observed from BNF inoculation and ii) inoculation with selected strains of *Rhizobium*, as well as fertilization with mineral N, favors plant growth, snap bean yields, and accumulation of N in the pods and, therefore, can fully substitute nitrogen fertilization in snap bean grown under protected cultivation.

Key words: biological nitrogen fixation, green bean cultivars, *Phaseolus vulgaris*, *Rhizobium* spp.

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Snap bean, of the same species as common bean (*Phaseolus vulgaris* L.), is a vegetable of great economic and social importance, produced and consumed in many countries. It has a wide variety of different genotypes with regard to growth habit and pod shape. Production is directed to consumption of tender pods *in natura* and, at lower volumes, to processing and export. Just as for common bean, nitrogen (N) is the most limiting nutrient for snap bean and the most costly for the vegetable grower (Peixoto and Cardoso 2016; Soares et al. 2016). As legumes, both have the capacity for biological fixation of atmospheric N (biological N fixation – BNF) through symbiosis with rhizobia, capable of providing at least part of the N required by the plant for its development, which translates into significant savings in the use of nitrogen fertilizer. The contribution of BNF to common bean has been emphasized of late, showing positive and consistent results from inoculation with selected strains of *Rhizobium* for diverse cultivars (Ferreira et al. 2009; 2012; Fonseca et al. 2013; Nogueira et al. 2017; Pádua Oliveira et al. 2017). For snap bean, however, there is no recommendation of specific strains, possibly as a result of research scarcity on the matter.

In Egypt, in one of the few studies involving *Rhizobium* in snap bean, Elbanna et al. (2009) observed high efficiency of *R. etli* and *R. leguminosarum* strains in symbiosis with the cvs. Paulista and Xera in a greenhouse, with results even superior to the control that received mineral N fertilization.

In Brazil, although the use of rhizobia inoculants is encouraged (Filgueira 2013; Peixoto and Cardoso 2016), there are no official recommendations for snap bean. In this respect, the aim of this study was to evaluate the agronomic performance of several *Rhizobium* strains, isolated from Amazon soils and recommended for common bean, in snap bean cultivars with different growth habits and crop cycles.

The experiment, with plants grown in pots of soil, was carried out in a greenhouse during the spring-summer crop season in Lavras, state of Minas Gerais, Brazil. A completely randomized 4 × 7 factorial experimental design was used with eight replications involving four snap bean cultivars of *Macarrão* type – cvs. Preferido, Favorito, Atibaia, and Conquista – and seven treatments – five strains of *Rhizobium* (UFLA 02-100 of *R. etli*, UFLA 02-127 of *R. leguminosarum* bv. *phaseoli*, UFLA 04-173 of *R. miluonense*, CIAT 899 of *R. tropici*, and PRF 81 of *R. freirei* sp. nov.), plus two controls without inoculation (without and with 500 mg $NH_4NO_3 \cdot dm^{-3}$, ½ applied at sowing and ½ in the V4 crop growth stage). Four replications were used for evaluations at full flowering. The other four replications were evaluated at harvest, when the plants had at least 50% marketable pods. The rhizobia strains CIAT 899 and PRF 81, which are recommended as commercial inoculants for common bean of the same species as snap bean, as well as the other strains, collected in the Amazon region, had previously shown high BNF efficiency for this species (Ferreira et al. 2009; Rufini et al. 2011; Nogueira et al. 2017).

The cvs. Preferido, Favorito, and Atibaia have an indeterminate growth habit and *Macarrão* type pod, with tall plants that require trellises or supports. The three have a 70 to 100 day cycle, with harvest beginning around 75 days after emergence (DAE) and duration from 20 to 30 days. Only the cv. Conquista has a determinate growth habit and *Macarrão rasteiro* (dwarf) type pod, upright stem and low stature, early cycle, and harvest from 55 to 60 DAE. Selection of cultivars was made in accordance with good commercial acceptance and resistance to the main diseases that attack the crop. Furthermore, with the exception of cv. Conquista, all cultivars have some resistance to root-knot nematodes (Ferreira et al. 2010).

The inoculants were prepared with peat sterilized in an autoclave at the proportion of 3:2 (w:v) of peat and culture medium 79, adopting the procedures described by Ferreira et al. (2009). The quality of the inoculant was monitored by counting colony-forming units (CFU), meeting requirements for the minimum legal number of viable cells, which was about 10^9 CFU of *Rhizobium* per gram of inoculant. The resulting material was used at the rate of 10 g per kg of seed.

The soil used was classified as an Oxisol, collected in a pasture area without any record of previous inoculation. The native rhizobia population capable of nodulating the crop was approximately 10^3 CFU $g \cdot soil^{-1}$, estimated with the same methodology as Rufini et al. (2011). Chemical analyses of the soil sample taken at a depth of 0 to 0.2 m before beginning the experiment indicated pH in H_2O (1:2.5) 5.8; P 2.3 $mg \cdot dm^{-3}$; K^+ 0.16 $cmol_c \cdot dm^{-3}$; Al^{3+} 0.1 $cmol_c \cdot dm^{-3}$; H+Al 1.3 $cmol_c \cdot dm^{-3}$; Sum of Bases 2.3 $cmol_c \cdot dm^{-3}$; effective Cation Exchange Capacity (CEC) 2.4 $cmol_c \cdot dm^{-3}$; potential CEC pH 7 3.6 $cmol_c \cdot dm^{-3}$; aluminum saturation 4.2%; base saturation 63.5%; and organic matter 2.4 $dag \cdot kg^{-1}$. Liming was carried out to increase V to 60%. All plots received fertilization with 300, 300, 40, 0.8, 1.5, 3.6, 5.0, and 0.15 $mg \cdot dm^{-3}$ of P, K, S, B, Cu, Mn, Zn, and Mo, respectively.

The sources used were $3Ca(H_2PO_4)_2$, K_2SO_4, H_3BO_3, $CuSO_4$, $MnSO_4$, $ZnSO_4$, and Na_2MoO_4.

Before sowing, snap bean seeds were disinfected for 30 seconds in alcohol and for 2 minutes in 2% sodium hypochlorite (NaClO) and then washed with sterilized distilled water six times, according to the methodology of Soares et al. (2014). Three plants were sown per 3 dm^{-3} pot, later thinned to one seed per pot eight days after emergence. Pest and disease control was not necessary. Irrigation was performed manually, maintaining soil saturation of at least 60% field capacity.

The number of nodules (NN), dry matter of nodules (DMN), shoot dry matter (SDM), relative efficiency (RE), shoot nitrogen concentration (SNC), and shoot nitrogen accumulation (SNA) were evaluated at full flowering. Two harvests were made in the indeterminate cycle cultivars (cvs. Preferido, Favorito, and Atibaia) and a single harvest was made in cv. Conquista, due to its determinate growth habit. At that stage, determination was made of the number of pods (NP), pod fresh matter (PFM), pod dry matter (PDM), pod nitrogen concentration (PNC), and pod nitrogen accumulation (PNA). The RE was calculated through the expression (SDM of the treatment / SDM of the treatment with mineral N) × 100. The yield (NP, PFM, and PDM) was obtained by the sum total of production from all harvests. The SNC and PNC were determined by the semimicro-Kjeldahl method. The SNA was calculated multiplying the SDM by PNC and dividing by 100. The PNA was determined adopting the same methodology used for the shoot samples, substituting the values of SDM for those of PDM.

All of the data were subjected to analysis of variance using the Sisvar version 4.0 software (Ferreira 2011), after being previously subjected to normality (Shapiro-Wilks test) and variance homoscedasticity (Bartlett test) tests, using the R software (R Development Core Team 2011). To meet the assumptions of analysis of variance, data of NN and DMN were $(x + 1)^{0.5}$ transformed. According to the official protocol to evaluate the variability and agronomic efficiency of 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 a differential response of cultivars with respect to all variables under study (Tables 1 and 2). The results confirm the existence of genetic variability among the cultivars, above all, in the potential for production of nodules and snap bean pod yields. The importance of the *Phaseolus vulgaris* L. genotype on the symbiosis process has been known for a long time, in addition to the importance of the strain (Nutman 1967). For common bean, it is well known that different varieties exhibit different potentials for nitrogen fixation, as well as different rhizobia inoculation requirements (Hardarson 1993; Kipe-Nolt et al. 1993; Franco et al. 2002), mainly depending on climate and soil conditions and interactions among all these factors (Soares et al. 2016). However, for snap beans, this is the first report on different symbiotic relationships with rhizobia strains in Brazil (the third report in the world). Because common bean and snap bean belong to the same species, we hypothesized that they would behave in the same way. Indeed, we found effects of both strains on the snap bean cultivars and of their interaction under the same environmental conditions.

Among the limiting factors for BNF in *Phaseolus vulgaris* L., the host plant may affect nitrogenase activity and the speed of nodule senescence (Hernández-Jiménez et al. 2002; Alcântara et al. 2009). As this senescence occurs naturally at the beginning of anthesis, due to generalized signal synthesis in the shoots or during grain filling, this process is accelerated in shorter cycle genotypes (Puppo et al. 2005). The early nodule senescence occurs without necessarily reducing the effectiveness of the nodules and the active period of biological fixation of N_2 and it does not indicate poor efficiency of the genetic material in symbiosis or low capacity of BNF (Fonseca et al. 2013; Andraus et al. 2016).

These arguments are consistent and may help explain the performance observed for cv. Conquista. In this context, the low nodulation of the cultivar may be a result of the reduced crop cycle and early maturation, typical characteristics of plants with a determinate growth habit. The effectiveness of the nodules, however, appears not to have been compromised, given that the mean values were equivalent (SNC, Table 2) or even greater (NP, PFM, and PDM, Tables 1 and 2) than those of the longer cycle cultivars. Furthermore, mechanisms inherent to short cycle cultivars, which have the ability to extend the BNF period to the R7 stage of pod formation (Andraus et al. 2016), may have been decisive for this contribution, allowing continued fixation in the remaining nodules.

The cv. Conquista plant type, due to morphological and physiological characteristics intrinsic to its growth habit,

Table 1. Mean values of number of nodules (NN), dry matter of nodules (DMN), shoot dry matter (SDM), shoot nitrogen accumulation (SNA), number of pods per plant (NP), pod fresh matter (PFM), and pod nitrogen accumulation (PNA) of snap bean inoculated with several rhizobial strains.

Cultivar[1]	NN	DMN (mg·plant⁻¹)	SDM (g)	SNA (mg·plant⁻¹)	NP	PFM (g·plant⁻¹)	PNA (mg·pod⁻¹)
Preferido	182 a	230 a	9.1 a	322.9 a	5.7 b	29.8 b	75.6 b
Favorito	89 b	90 b	7.9 b	291.9 a	7.4 b	26.7 b	88.0 a
Atibaia	168 a	190a	7.2 b	204.0 b	6.9 b	27.4 b	97.6 a
Conquista	54 c	60 b	5.7 c	221.3 b	12.0 a	37.9 a	62.7 b
Treatment	**NN**	**DMN (mg·plant⁻¹)**	**SDM (g)**	**SNA (mg·plant⁻¹)**	**NP**	**PFM (g·plant⁻¹)**	**PNA (mg·pod⁻¹)**
UFLA 02-100	226 a	227 a	6.7 aB	214.4 b	8.0 a	36.7 aA	100.4 a
UFLA 02-127	134 a	189 a	7.6 aA	252.5 b	7.8 a	29.3 aA	81.1 a
UFLA 04-173	82 b	78 b	7.7 aA	267.2 b	7.6 a	24.3 aB	61.3 b
CIAT 899	173 a	216 a	7.2 aB	236.8 b	7.4 a	31.8 aA	93.1 a
PRF 81	122 a	142 a	7.8 aA	243.5 b	8.3 a	30.1 aA	62.9 b
Cont. w/N	43 b	30 b	8.0 aA	323.9 a	9.9 a	34.1 aA	99.0 a
Cont. wo/N	85 b	123 b	7.5 aB	281.9 a	7.9 a	27.2 aB	68.9 b
Mean	123	143	7.5	281.7	8.0	30.4	81.0

Within each factor, mean values (four replications) followed by the same lowercase letter ($p < 0.05$) and uppercase letter ($p < 0.10$) within columns belong to the same group, according to the Scott-Knott test. [1]Two pod harvests were made in the indeterminate cycle cultivars (Preferido, Favorito, and Atibaia) and a single harvest in the cv. Conquista, due to its determinate growth habit. Cont. wo/N and Cont. w/N = without and with 500 mg $NH_4NO_3 \cdot dm^{-3}$, respectively.

Table 2. Relative efficiency (RE), shoot nitrogen concentration (SNC), pod dry matter (PDM), and pod nitrogen concentration (PNC) of snap bean cultivars as a function of inoculation treatments with several rhizobial strains.

Treatment	RE (%)	SNC (%)				PDM (g·plant⁻¹)				PNC (%)			
		Pref.	Fav.	Atib.	Conq.	Pref.	Fav.	Atib.	Conq.	Pref.	Fav.	Atib.	Conq.
UFLA 02-100	85.1 aB	3.2 aA	4.1 aA	2.4 aA	3.7 aA	4.0 aA	2.2 aA	3.3 aA	4.8 aA	2.4 aA	3.7 aA	3.9 aA	2.0 aA
UFLA 02-127	96.7 aA	3.5 aA	3.7 aA	2.2 aA	4.4 aA	1.9 aB	3.5 aA	2.6 aA	3.6 aB	2.5 aA	3.9 aA	3.2 aA	1.7 aA
UFLA 04-173	100.3 aA	3.7 aA	3.9 aA	3.5 aA	2.3 aB	0.9 aB	2.3 aA	2.7 aA	4.3 aA	2.8 aA	3.5 aA	3.8 aA	1.3 aA
CIAT 899	90.5 aB	3.8 aA	3.0 aA	2.4 aA	4.3 aA	2.5 aB	2.8 aA	1.9 aA	5.3 aA	3.2 aA	4.0 aA	3.3 aA	2.0 aA
PRF 81	99.2 aA	3.2 aA	3.0 aA	2.6 aA	4.1 aA	3.4 aA	1.7 aA	2.9 aA	4.7 aA	2.1 aA	2.2 aB	3.8 aA	0.8 aA
Cont. w/N	101.3 aA	4.0 aA	4.1 aA	3.6 aA	4.8 aA	3.5 aA	3.5 aA	3.2 aA	2.6 aB	3.3 aA	2.7 aB	4.0 aA	2.2 aA
Cont. wo/N	91.7 aB	3.5 aA	4.4 aA	2.9 aA	4.6 aA	2.6 aB	3.6 aA	2.4 aA	3.0 aB	2.9 aA	4.7 aA	3.1 aA	1.3 aA
Mean*	84.0	3.6 a	3.8 a	2.8 b	4.0 a	2.7 b	2.3 b	2.7 b	3.4 a	3.0 b	3.9 a	3.9 a	1.8 c

In the columns, mean values followed by the same lowercase letter ($p < 0.05$) and uppercase letter ($p < 0.10$) belong to the same group according to the Scott-Knott test. *In the "mean" line, cultivars with the same lowercase letter belong to the same group according to the Scott-Knott test ($p < 0.05$). Cultivars: Pref. = Preferido; Fav. = Favorito; Atib. = Atibaia; Conq. = Conquista. Cont. wo/N and Cont. w/N = without and with 500 mg $NH_4NO_3 \cdot dm^{-3}$, respectively.

explains the low SDM, which, consequently, resulted in N accumulation 30% lower than the accumulation observed in the cvs. Preferido and Favorito, which have indeterminate growth and climbing habit. This affirmation and the fact that the cv. Conquista was efficient in BNF were reaffirmed by the high concentrations of N observed in the shoots (4%), surely used in the formation of many pods and in the high fresh matter yield obtained. If we compare cv. Conquista (the lowest SDM and SNA) with cv. Preferido (the highest SDM and SNA), we notice that Conquista was more efficient in producing pod fresh matter with the same nitrogen accumulation, probably indicating more efficient nitrogen use. Other experiments could confirm this trait.

It should be emphasized that even with the different production potentials, all genotypes had satisfactory yield and snap bean pod quality characteristics within the parameters given by Filgueira (2013), and additional harvests could further increase the yield of cvs. Preferido, Favorito, and Atibaia. From a practical perspective, however, based on the harvests carried out, the cv. Conquista may be more →

advantageous for the vegetable grower. Advantages of cv. Conquista include early maturity, ease of growing, no requirement for trellises or supports, high yields, good pod commercial quality standards, and lower production costs. The presence of nodules in the treatment without inoculation and without mineral N (Table 1) confirms the occurrence of native strains of rhizobia in the soil, which, however, were not as effective as those introduced by the inoculum. Apparently, the excess of mineral N did not prevent the establishment of symbiosis of the native rhizobia, leading to nodulation equivalent to that of the treatment without inoculation and without mineral N (Table 1). In spite of this similarity, the efficiency of the absolute control with respect to N accumulation was lower, which, however, did not reduce the yield in this treatment (Table 1).

Good performance of most introduced strains was observed in all of the snap bean genotypes tested (Tables 1 and 2). With common bean cultivars, previous studies had already shown efficiency not only of strains recommended for the crop (CIAT 899 and PRF 81), but also of strains UFLA 02-100, UFLA 02-127, and UFLA 04-173, of high competitive capacity and equivalent (Ferreira et al. 2009; Fonseca et al. 2013; Figueiredo et al. 2016) or better performance (Soares et al. 2006; Nogueira et al. 2017; Pádua Oliveira et al. 2017) than native strains. With snap bean genotypes in a greenhouse, Elbanna et al. (2009) likewise observed the contribution of *Rhizobium* isolates to the establishment, development, and operation of symbiosis. Beshir et al. (2015) reported that inoculation with efficient strains is a feasible and less costly alternative for production of snap beans in Ethiopia. In this study, the similarity of the results obtained with strains UFLA 02-100 and UFLA 02-127 compared to CIAT 899 and PRF 81 (recommended for common bean) and, in some cases, the superiority of these UFLA strains to the control with mineral N indicate their potential use as strains for nitrogen fixation in snap bean. The good performance of the inoculants contributed to N nutrition suitable for growth and development, which resulted in high yields of fresh snap beans.

Therefore, considering the greenhouse conditions under which this study was conducted, the inoculation of snap bean (with responsive cultivars and selected *Rhizobium* strains) provides a possible alternative to increase crop yields. Although the results are preliminary and still require field investigations, these results are encouraging and represent not only potential savings in fertilizer costs, especially for small producers with limited resources, but may also minimize possible environmental problems from excessive application of nitrogen fertilizers. Furthermore, based on this study, the UFLA 02-100 and UFLA 02-127 strains have potential for recommendation as commercial inoculants for common bean seeds and can contribute to reduce the amount of crop fertilizer applied. Additional research under different soil types and field environmental conditions to identify particular genotype × rhizobial strain combinations are necessary and are being carried out.

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