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Influence of common bean genotypes and rhizobia interaction for nodulation and nitrogen fixation

Influência da interação entre genótipos de feijoeiro e rizóbios na nodulação e fixação de nitrogênio

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ABSTRACT

Bacteria from the rhizobia group are able to associate symbiotically with bean crop, forming nodules in the root, in which the biological nitrogen-fixing process occurs. However, the efficiency of this process has been low and it can be attributed to genetic and environmental factors. Thus, the objective of this study was to evaluate the capacity of nodulation of local varieties and commercial common bean cultivars inoculated with a *Rhizobium tropici* strain used in commercial inoculants and rhizobia isolates from common bean root nodules. The experiment was carried out in a factorial scheme (2x4), in a randomized block design with four replicates. It was tested two local varieties and two commercial cultivars, inoculated with the reference *Rhizobium tropici* strain CIAT899 and the RBZ14 strain isolated from common bean nodules grown in soils of Southern Brazil, in adapted Leonard-type pots. The CIAT899 strain promoted either higher mass of viable nodules and higher nitrogen accumulation in the aerial part. The black group local variety showed better response than the cultivar TAA Dama for nodule viability, suggesting more efficiency for nodulation. The interaction between genotypes (local varieties and commercial cultivars) and bacteria showed the specificity of the complex symbiotic relationship of biological nitrogen fixation in common bean, requiring further studies of these interactions.

KEYWORDS: *Phaseolus vulgaris* L.; *Rhizobium tropici,* symbiosis; varieties.

RESUMO

As bactérias do grupo rizóbios estão associadas simbioticamente à cultura do feijão, formando nódulos na raiz, nos quais ocorre o processo biológico de fixação do nitrogênio. No entanto, a eficiência desse processo tem sido baixa e pode ser atribuída a fatores genéticos e ambientais. Assim, este estudo teve como objetivo avaliar a capacidade de nodulação de variedades locais e cultivares comerciais de feijão comum inoculadas com uma estirpe de *Rhizobium tropici* usada em inoculantes comerciais e isolados de nódulos da raiz do feijão comum. O experimento foi realizado em esquema fatorial (2x4), em delineamento de blocos ao acaso, com quatro repetições. Foram testadas duas variedades locais e duas cultivares comerciais, inoculadas com a estirpe de referência *Rhizobium tropici* CIAT899 e o isolado RBZ14 obtido de nódulos de feijão cultivados em solos do sul do Brasil, em vasos adaptados do tipo Leonard. A estirpe CIAT899 promoveu maior massa de nódulos viáveis e maior acúmulo de nitrogênio na parte aérea. A variedade regional do grupo preto apresentou melhor resposta que a cultivar TAA Dama, quanto à viabilidade dos nódulos, sugerindo resposta mais eficiente à nodulação. A interação entre genótipos e bactérias mostrou a especificidade da complexa relação simbiótica da fixação biológica de nitrogênio no feijoeiro, sendo necessário aprofundar os estudos nessas interações.

PALAVRAS-CHAVE: *Phaseolus vulgaris* L.; *Rhizobium tropici;* simbiose; variedades.

INTRODUCTION

Biological nitrogen fixation (BNF) occurs by the atmospheric N transformation of symbiotic bacteria associated to Fabaceae species providing the nutrient to the plants (HUNGRIA et al. 2001). Rhizobia form specific structures in the Fabaceae root, known as nodules, in which the BNF process occurs. In those nodules, the ammonia is combined to hydrogen ions (H^+) and transformed to ammonium (NH^{+4}) that will be distributed to the host plant and joined to ureides, amino acids and amides (HUNGRIA et al. 2001). Thus, BNF is one of the cheapest and most efficient ways to supply N for legume crops. In addition, there are no environmental impacts such as those caused by nitrogen fertilizers (HUNGRIA et al. 2001).

Common bean species are associated mainly to the genera *Rhizobium*. At least six species are described as capable of fixing N to beans, including *R. legumonosarum* bv. *phaseoli*, *R. tropici*, *R. etli, R. gallicum* and *R. giardinii* (STOCCO et al. 2008). However, other genera and species were isolated from bean nodules because of their unspecific colonization by rhizobia (DALL'AGNOL et al. 2016, SHAMSELDIN & VELÁZQUEZ 2020). BNF can supply up to 70% of N requirement of common bean plants at suitable conditions (HARDARSON et al. 1993, MOREIRA et al. 2017, PACHECO et al. 2020). HUNGRIA et al. (2013) reported that CIAT899 is widely used in commercial inoculants, especially to stimulates South America and Africa common bean inoculation.

However, reports showed that this association often is not effective (FRANZINI et al. 2013). Common beans are described as the lowest BNF efficient legume at field conditions since symbiosis can be affected by different factors, and the nitrogen supply could not reach 50% of its requirement (CASSINI & FRANCO 2006). Among the limiting factors of BNF in common beans, poor nodules colonization by efficient rhizobia strains used in trade commercial inoculants can be an important issue. It can be explained by the large rhizobia community able to nodulate bean established in the soils (MERCANTE et al. 2017), some not effective to BNF. In addition, some reports described endogenous bacteria isolates ability to nodulate common beans as well as commercial inoculants strains, although the lower number of plant nodules (FIGUEIREDO et al. 2016). Nevertheless, recently, some reports showed indigenous rhizobia performs better than commercial rhizobia inoculants (WEKESA et al. 2021).

Moreover, the common bean is significantly susceptible to environmental stresses such as high temperatures, acidic soils, poor soil in organic matter and nutrients, water deficiency and several diseases (STRALIOTTO et al. 2002, RODIÑO et al. 2021) that can directly affect the symbiosis.

Low BNF efficiency can also be attributed to the short cycle and the large genetic variability among common bean cultivars, affecting BNF potential and genetic features of the fixing bacteria (ALCÂNTARA et al. 2009). ANDRIOLO et al. (1994) stated that wild lineages of beans accumulated a higher amount of nitrogen than cultivars thought efficient at *Rhizobium* symbiosis. BERTOLDO et al. (2015) also found greater genetic variability for nodulation traits associated with bean breed than commercial cultivars. GUNNABO et al. (2019) observed significant common bean genotype and rhizobia strain interaction that can be affected by the growing environment, representing both challenges and opportunities for improvement of nitrogen fixation.

Even though the importance of a well-developed and vigorous root system in plants, this feature has been neglected over time in bean breeding programs, which could decrease the symbiosis effectiveness (TSUTSUMI et al. 2015), due to elimination of alleles related to this character. Therefore, it is believed that regional bean genotypes, which have not undergone intense selection, may conserve elements for BNF. Moreover, there is great variability to nodulation capacity and efficiency among bean genotypes in general, as observed by ANDRAUS et al. (2016).

The objective of this work was to verify differences of nodulation ability among regional varieties and cultivars of common bean inoculated with *Rhizobium tropici* CIAT899 strain and an isolate (RBZ14) from the Southern part of Brazil.

MATERIAL AND METHODS

In a randomized block design with four replicates, the experiment was carried out in a factorial arrangement (2x4). Each replicate consisted of two pots. Four bean genotypes were tested: two local varieties (Traditional Black and Carioca) and two commercial cultivars (IPR Tuiuiú – black grain; and TAA Dama – brown grain). Farmers provided the regional varieties.

The two bacteria were CIAT899 strain of *Rhizobium tropici*, which the Brazilian Ministry of Agriculture currently recommends, Cattle and Supplying as standard strain for common bean inoculation and an isolate identified as RBZ14. The isolate was obtained from bean nodules grown in regional soils and after purification, its nodulation capacity was verified at aseptic system. HUNGRIA et al. (2013) reported that CIAT899 is widely used in commercial inoculants, especially stimulating South American and African common bean inoculation.

The bacteria were grown in flasks containing 20 mL of LB medium. Four flasks were inoculated with CIAT899 and four with RBZ14 isolate and then incubated for 48 hours at 28 ºC. Seeds were disinfested by alcohol 96° GL for 30 min., 3% sodium hypochlorite solution for 3 minutes and seven washes with sterile distilled water, then placed in laminar flow to dry. After drying, sets of 20 seeds of each variety or cultivar were placed in each bacterial suspension for 40 minutes. Then, they were placed in laminar flow to dry. Numbers of bacterial cells in the suspensions with seeds were determined by serial dilution technique.

The substrate, a sterilized 2:1 mixture of commercial vermiculite and sand that was previously washed, dried and autoclaved. That mixture filled the modified Leonard type pots (SANTOS et al. 2009). The pots, experimental units, were kept throughout the experiment in a greenhouse with temperature control at 26 °C. Two seeds were sowed per pot, of each genotype inoculated with each bacterium. After sowing, watering was performed with sterilized distilled water. After emergence, one plant per pot was left. At cotyledons fall off, plants received sterilized nitrogen-free Hoagland nutrient solution once a week.

The analyses were performed at R6 stage (flowering) of each genotype. Roots were detached from shoot, gently washed with tap water to remove the substrate and after that nodules removed. Total nodule mass per plant (TNM), viable (or active) (VNM) and nonviable nodules masses per plant (NvNM) were weighted. The reddish color inside the nodule determined their viability. Percentages of viable (PVN) and nonviable nodules (PNN) were calculated by dividing each mass by the total nodules mass.

Shoot mass and wet root mass (mg) were determined. After drying at 45 ºC, their dry masses, Shoot Dry Mass (SDM) and Root Dry Mass (RDM), were verified.

Leaf nitrogen content (NAS) was assessed by Kjeldahl method and it was multiplied to shoot dry mass to calculate shoot N accumulation (mg of N g^{-1}).

All data were submitted to variance analysis by F test ($p<0.05$) for genotype factors (cultivars and varieties), for bacteria and for genotype x bacteria interaction. If significant, the means were compared by Tukey test ($p<0.05$). In addition, correlations among the nonviable nodule mass (NvNM), total nodule mass (TNM), root dry mass (RDM), shoot dry mass (SDM), total dry mass (TDM) and shoot nitrogen accumulation (NAS) were evaluated by t-test ($p<0.05$) at Pearson correlation analysis, and carried out separately for varieties and cultivars.

RESULTS AND DISCUSSION

The bacterial inoculated had 2.20×10^6 CFU (Colony Forming Unit)/mL for CIAT899 and 1.26×10^7 CFU/mL for RBZ14, indicating a larger number of cells in the isolate inoculum. In addition, the isolate ability to grow faster has been observed in previous tests. This could suggest that faster growth rate of the isolate could assist in the ability to colonize root infection sites, requiring further investigations.

There was statistical significance for viable nodule mass (VNM) and nitrogen accumulation in the shoot (NAS), comparing bacterial treatments (Table 1). Among the bean genotypes, mean square for nodule viability (NV) was significant. Significance was also detected at bacterium x genotype interaction for nonviable nodule mass (NvNM), total nodule mass (TNM) and shoot dry mass (SDM) (Table 1).

Table 1. Variance analysis of bean genotypes inoculated with CIAT899 and RBZ14.

VS	FD	VNM[1]	NvNM	TNM	NV	SDM	RDM	NAS
Bacteria (B)	1	1.76*	5.87*	14.08*	0.74ns	24.99*	0.22ns	7622.33*
Genotype(G)	3	0.11ns	0.72ns	1.03ns	824.09*	3.93*	0.13ns	1050.00ns
B x G	3	0.40ns	1.33*	2.62*	37.09ns	2.94*	0.02ns	192.11ns
Block	3	0.20ns	0.64ns	1.50ns	70.07ns	1.34ns	0.04ns	1515.18ns
Residue	20	0.14	0.33	0.66	239.14	0.93	0.07	463.13
Average		0.87	0.97	1.84	47.0	2.33	0.62	30.75
CV (%)		43.02	59.30	43.99	32.93	41.40	41.73	54.67

*Significance at F test ($p<0.05$); ns - not significant. VNM - viable nodule mass. NvNM - nonviable nodule mass. TNM - Total nodule mass. NV - nodule viability. SDM - shoot dry mass. RDM - root dry mass. NAS - nitrogen accumulation in the shoot.

CIAT899 strain inoculation of bean genotypes provided greater viable nodule mass, total plant dry mass and shoot nitrogen accumulation compared to RBZ14 isolate inoculation (Figure 1). Bacterium and genotype interaction were significant to nonviable nodule mass (NvNM), total nodule mass (TNM) and shoot dry mass (SDM) parameters (Table 1), suggesting there was genotypes differential behavior to bacteria inoculation (Table 1). CIAT899 strain inoculation at bean genotypes provided greater viable nodule mass, total plant dry mass and shoot nitrogen accumulation, comparing to RBZ14 isolate (Figure 1). Results matched to those that described greater viable nodule mass of several bean genotypes inoculated with CIAT899 strain, ranging from 0.2 to 0.6 g (KNUPP et al. 2017). RIZZARDI et al. (2017) also observed CIAT899 efficiency comparing to UFLA 02-100 isolate (*Rhizobium etli*).

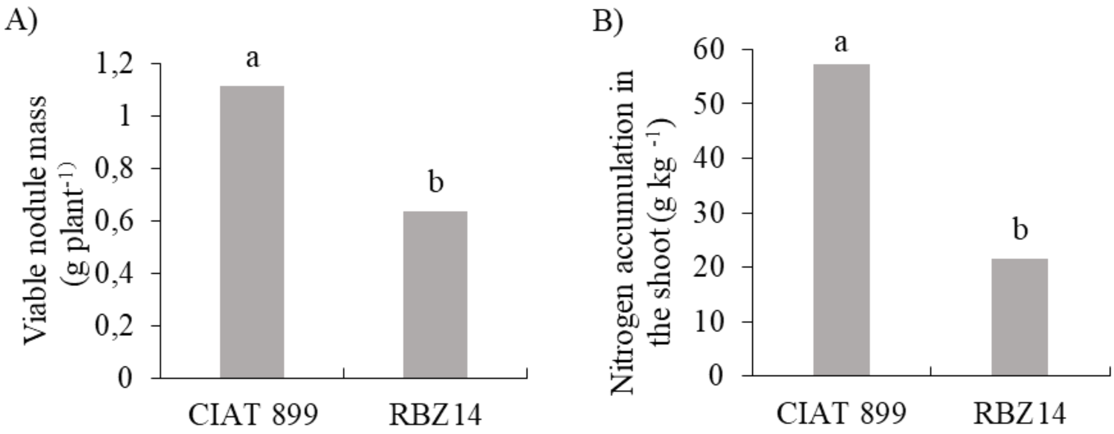

Figure 1. Effect of CIAT899 strain and RBZ14 inoculation on common bean genotypes. A) viable nodule mass. B) shoot nitrogen accumulation. *Means followed by the same letter do not differ at Tukey's test ($p<0.05$).

According to HERRIDGE & DANSO (1995), nodule number and size are not considered measures for nodule functionality. In this sense, the nodule viability was defined as a correspondence of nodule leghemoglobin activity (reddish color) to BNF. Regarding bean genotypes, there were differences in the nodule viability (VN%). Black Traditional showed viability of 55.5%, differing from TAA Dama cultivar that reached 32.3% (Figure 2).

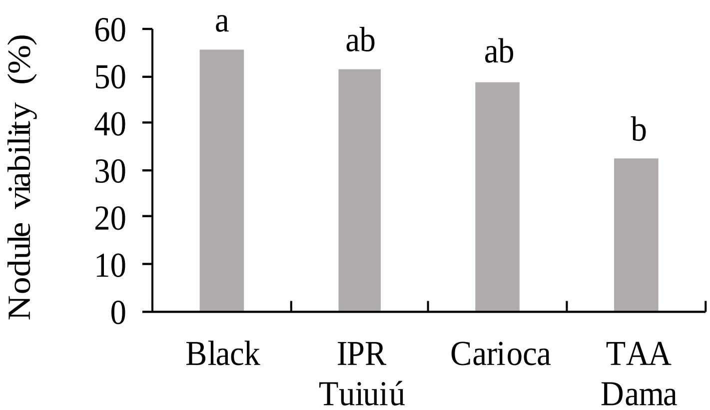

Figure 2. Nodule variability of bean genotypes inoculated with CIAT899 strain and RBZ14 isolate. *Means followed by the same letter do not differ at Tukey's test ($p<0.05$).

The TAA Dama cultivar showed higher nonviable nodules mass (NvN) when inoculated with CIAT899 (Table 2). Also produced greater nonviable nodules mass than IPR Tuiuiú cultivar and the traditional Black. To the other genotypes, there was no difference for bacterial inoculation. Genotypes inoculated with RBZ14 isolate did not significantly diverge. For total nodule mass (TNM), CIAT899 inoculation at TAA Dama cultivar and traditional Black provided higher results (Table 2), while RBZ14 inoculation improved this on IPR Tuiuiú, compared to TAA Dama and Traditional Black. Black Traditional showed nodule viability of 55.5%, differing from TAA Dama cultivar that reached 32.3% (Figure 2), suggesting greater nodulation effectiveness to varieties than to cultivars. ANDRAUS et al. (2016) found by 20 to 70% variation of nodule viability in commercial cultivars. ANDRIOLO et al. (1994) corroborated our results that implied the advantage of varieties for nodule viability. Authors observed that wild bean with higher nitrogen accumulation compared to cultivars considered efficient to *Rhizobium* symbiosis. A significant higher response of variety than cultivar suggested that genetic improvement of common bean that promoted shoot attributes, eventually neglected traits related to BNF. Thus, regional varieties could be more responsive because they have not been intensively selected. BERTOLDO et al. (2015) found a wide variability for traits inherent to nodulation evaluating germplasm bank accessions and commercial common bean cultivars. However, FONSECA et al. (2013) observed no difference among several cultivars inoculated with CIAT899 for nodules dry mass.

Table 2. Mean comparisons of bean genotypes inoculated with CIAT899 and isolate RBZ14.

Genotypes	NvNM (mg. Plant^{-1})		TNM (mg. Plant^{-1})		SDM (mg. Plant^{-1})	
	CIAT899	RBZ14	CIAT899	RBZ14	CIAT899	RBZ14
TAA Dama	2.30Aa	0.35Ba	3.43Aa	0.85Bab	4.05Aa	1.45Ba
Carioca	1.27Aab	0.57Aa	2.40Aa	1.27Aab	4.62Aa	1.59Ba
IPR Tuiuiú	1.04Ab	1.08Aa	2.02Aa	2.16Aa	2.65Aab	2.20Aa
Trad. Black	1.02Ab	0.20Aa	2.26 Aa	0.47Bb	2.05Ab	0.60Ba

*Significance at Tukey´s test ($p<0.05$); NvNM - nonviable nodule mass. TNM - Total nodule mass. SDM - shoot dry mass. Upper case letters compare the bacterial inoculation effect on each genotype. Lowercase letters compare inoculation effect of each bacterium on bean genotypes.

For total nodule mass (TNM), CIAT899 increased nodulation at TAA Dama and Traditional black compared to RBZ14, while to Carioca and IPR Tuiuiú there was no difference between bacteria inoculations. MATOSO & KUSDRA (2014) found prevalence of native bacteria over CIAT899 strain inoculated at IPR 139 cultivar, for dry nodules mass and found no difference for total nodules number. FONSECA et al. (2013) verified that Madrepérola cultivar had higher affinity to CIAT899, for nitrogen content and shoot accumulation analysis, demonstrating that the strain had different relationship with the cultivar. HUNGRIA & NEVES (1986) observed affinity of Negro Argel cultivar and the CO5 strain, whose combination reached higher total nitrogen accumulation in leaves, stem, and pods.

Bacteria affinity to bean genotypes of different grain types was not observed. For TNM, there was no significant difference among genotypes inoculated with CIAT899 (Table 2). For RBZ14 inoculation, highest means were observed at IPR Tuiuiú (black grain) and Carioca (brown grain). YAGI et al. (2015) observed that brown grain group cultivars were more susceptible to nodulation, either by native rhizobia or inoculated strain, compared to black group cultivars, different from the results obtained in this study.

For shoot dry mass (SDM), CIAT899 inoculation improved TAA Dama, Traditional Carioca and Traditional Black genotypes (Table 2). For IPR Tuiuiú cultivar, there was no difference to CIAT899 or the isolate inoculation. Comparing the genotypes, TAA Dama cultivar and Carioca showed higher shoot dry mass than the traditional Black (Table 2). For RBZ14 inoculation, the genotypes did not differ (Table 2). In general, Dama cultivar and Traditional Carioca reached the highest averages when inoculated with CIAT899, whereas bean genotypes did not differ from each other with RBZ14 inoculation. However, FONSECA et al. (2013) found no difference for plant dry mass between CIAT899 and UFLA 04-173 strain, suggesting the bacteria-genotype interaction. This interaction demonstrated a differential response: the specificity among them that can increase the selection complexity. Both selections, viable nodular bean genotypes and efficient strains/isolates, can contribute to the biological nitrogen fixation.

According to AMBROSANO et al. (1997), the critical level of N content to the common bean is below 30 g kg^{-1}. Endogenous isolate promoted N accumulation lower than this value. Indeed, such behavior confirmed the standard strain efficiency. According to ORMEÑO-ORRILLO et al. (2012) *R. tropici* (CIAT899) compared to other rhizobia species, is genetically more stable and tolerant to several stresses, such as low pH and high temperatures, common to tropical environments, and to antimicrobials, such as pesticides. However, some results showed no significance between the inoculation of the strain and native rhizobia. FERREIRA et al. (2009) found no difference to shoot dry mass and shoot nitrogen accumulation with the inoculation of CIAT899 or Amazon native rhizobia to bean Talismã cultivar.

The single correlation performed for bean cultivars and varieties allowed to verify the differential responses (Table 3). Viable nodules mass was high and positively associated ($p<0.01$) to nonviable nodules mass for varieties and not significant for cultivars. Positive correspondences were observed at VNM and TNM, VNM and SDM, indicating that viable nodule production contributed significantly to plant shoot growth for cultivars and varieties. However, VNM showed positive and significant correspondence to RDM (root dry mass) only for cultivars. NvNM and TNM, NvNM and SDM were positive for varieties and cultivars (Table 3).

High ($p<0.01$) and negative correlation was observed to NMI and VN (nodule viability) for cultivars (Table 3). However, this relationship was not significant for varieties, possibly due to the increase in viable nodule mass. Nonviable nodules mass showed positive correlation to root dry mass and to shoot nitrogen accumulation only for varieties. Similar response was observed for correspondences of NTM, indicating analogous differences to varieties and cultivars. In fact, early nodules senescence of bean varieties and cultivars (Table 3) could explain positive relationships effect showed by nodules (nonviable) on plant dry mass and nitrogen accumulation. ALCÂNTARA et al. (2009) reported differences in the biological nitrogen fixation potential among legume species and stated that these could be affected also by early nodule senescence. STRALIOTTO et al. (2002) pointed out that the presence of at least 20 reddish-colored nodules, with approximately 4 to 5 mm diameter, could indicate nodule activity and ability to supply almost all nitrogen requirements to the plant.

Table 3. Pearson correlation at local varieties and cultivars inoculated with CIAT899 and RBZ14.

			Local varieties			
Traits	NvNM	TNM	NV	SDM	RDM	NAS
MNV	0.86**	0.97**	0.23	0.67**	0.45	0.29
MNI		0.96**	-0.22	0.85**	0.60*	0.63*
MNT			0.01	0.78**	0.54*	0.47
VN				-0.30	-0.38	-0.46
MSPA					0.61*	0.68**
MSR						0.39
			Cultivars			
Traits	NvNM	TNM	NV	SDM	RDM	NAS
VMN	0.52	0.68**	-0.47	0.76**	0.72**	0.16
NvNM		0.98**	-0.92**	0.67**	0.32	0.15
TNM			-0.90**	0.75**	0.44	0.17
NV				-0.71**	-0.38	-0.19
SDM					0.56*	0.33
RDM						0.48

Significance at t test ** $p<0.01$ and * $p<0.05$. VMN - viable nodule mass. NvNM - nonviable nodule mass. TNM - total nodule mass (TNM). NV - nodule viability. SDM - shoot dry mass. RDM - root dry mass. NAS - nitrogen accumulation in the shoot.

The nodules viability was negatively correlated to shoot dry and total dry masses for cultivars. Shoot dry mass was positively associated to root dry and total dry masses for varieties and cultivars. However, shoot dry mass only showed a positive correspondence to shoot nitrogen accumulation for varieties.

The single correlation performed for bean cultivars and regional genotypes allowed to verify the differential response. Viable nodules mass was positively associated to nonviable nodules mass for varieties and not significant for cultivars. Some reports suggested that native strains could nodulate as much as commercial inoculants strains because of common bean nodular promiscuity (FIGUEIREDO et al. 2016, SHAMSELDIN & VELÁZQUEZ 2020, WEKESA et al. 2021). Varieties should be easier to develop nodules, in general, with soil rhizobia community. This could be positive for genetic improvement, but negative considering BNF efficiency. Common bean easily forms nodules with any rhizobia, even when inoculated. The rhizobia soil community is more adapted to the edaphic conditions and then can stand out over the inoculated bacteria. However, some individuals could be not efficient to BNF, despite forming large number of nodules.

Number of nodules and nitrogen accumulation are related to bean plants as important parameters. However, the correspondence to higher nodulation and the N fixation increase is not always linear. HANSEN et al. (1993) tested a bean mutant that could duplicate nodule mass and increase six times the number of nodules. However, they observed that BNF was almost like others, suggesting that the super nodulation results produced smaller nodules and did not affect their efficiency.

The complex interaction between genotype and bacteria has been limiting genetic improvements for nodulation efficiency and biological nitrogen fixation to common bean. The identification of efficient symbiotic pairs (bean genotype and rhizobia) is one of the main conditions to obtain the greatest benefits of this biological process to bean crop. Thus, the *Rhizobium tropici* species is promising for BNF in common bean stresses such as high temperatures and soil acidity because it resists stresses such as high temperatures and soil acidity, and these bacteria have some high-competitive strains. In this sense, it is important to search for new strains, especially in soils with high rhizobia diversity, (STOCCO et al. 2008, GUNNABO et al. 2021, RAMÍREZ et al. 2021). STOCCO et al. (2008) reported high rhizobia intraspecific diversity of isolates from different regions of Santa Catarina and observed that 17.1% of them belonged to the *Rhizobium tropici* species. The authors also reported that isolates from Curitibanos region did not correlate genotypically to this reference rhizobia species. This reinforces that other bacterial groups could be related to BNF (DALL'AGNOL et al. 2016). It is also suggested to evaluate the bean genetic variability among the regional genotypes because they could have co-evolved with soil nitrogen-fixing bacteria and then more efficient symbiotic relationship could be established. In addition, observing the period between early nodule development and nodular senescence is an important strategy for selecting responsive bean genotypes to rhizobia and its relationship with the BNF.

CONCLUSION

The CIAT899 strain provided higher viable nodules mass and shoot nitrogen accumulation to common bean genotypes. Nevertheless, RBZ14 isolate and CIAT899 had similar effect on total nodule mass and shoot dry mass of IPR Tuiuiú. Furthermore, the regional bean variety from the black grain group showed higher nodule viability than the TAA Dama cultivar from the brown grain group, suggesting nodulation efficiency.

The nodulation efficiency results from the specific interaction between genotype and bacteria, as can be seen by the differential correlation observed for regional bean variety and cultivars.

Different effects of bacterial inoculation on nonviable nodules, total nodules and shoot masses indicated specific interaction among rhizobia and bean genotypes, requiring further studies.

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