Inoculation of rhizobia increases lima bean (*Phaseolus lunatus*) yield in soils from Piauí and Ceará states, Brazil[1]

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ABSTRACT

The inoculation of rhizobia in lima bean can increase its yield and contribute to smallholder farms. In this study, the potential of rhizobia in increasing the lima bean yield was evaluated in two regions from Piaui and Ceará states. The experiments were conducted in field comparing three rhizobia strains (UFPI32, UFPI38, UFPI59), one strain of reference to *Phaseolus vulgaris* (CIAT899) and two controls (with and without nitrogen). The parameters of nodulation, N accumulation and yield varied between treatments and locations. The nodule biomasses were higher in UFPI59 and UFPI32 (Piauí), and CIAT899 and UFPI32 (Ceará). The highest values of nodule size were found in treatments UFPI59 in both locations. Lima bean presented highest values of leaf N in UFPI59 and CIAT899. The highest values of grain yield were found in the treatment UFPI59 as compared to CIAT899 and the controls. The results confirmed the potential of UFPI59 in promoting nodulation, N accumulation, plant growth and yield of lima bean in both locations.

Keywords: *Phaseolus lunatus*; biological N fixation; *Rhizobium*; inoculation

INTRODUCTION

Lima bean (*Phaseolus lunatus*) is an important legume crop cultivated in some countries of North, Central and South America (Amorim *et al.*, 2019), representing a substantial source of protein (Araujo *et al.*, 2015). In addition, lima bean presents high rusticity and capacity to withstand long dry periods similar those found in the Northeastern region of Brazil (Santos *et al.*, 2011). Indeed, lima bean is cultivated by smallholder farms from the Northeastern region of Brazil, mainly the states of Piauí and Ceará, where the crop yield is estimated in 0.45 ton ha^{-1} (IBGE, 2018). Despite of its importance, lima bean yet presents low production and one reason is the low N availability in soil.

However, lima bean is a N-fixing crop and this characteristic can increase its growth and yield (Amorim *et al.*, 2019). Thus, the biological N fixation (BNF) is an important process that can increase the lima bean yield (Lopes *et al.*, 2015). Indeed, previous studies, under greenhouse conditions, have reported N-fixing rhizobia contributing, through BNF, to the growth of lima bean (Costa Neto *et al.*, 2017; Amorim *et al.*, 2019).

The responses of plants to BNF varies according to the rhizobia (Cardoso *et al.*, 2017) and their growing region (Leggett *et al.*, 2017). Thus, rhizobia isolated from a region and adapted to its specific soil conditions need to be evaluated in different locations and conditions, such as temperature, chemical properties and population of native bacteria (Koskey *et al.*, 2017; Irisarri *et al.*, 2019). It is important since indication of potential rhizobia should considerate their efficiency in different regions and soil conditions. So far, the studies evaluating potential N-fixing rhizobia are concentrated to soybean, common bean and cowpea (Merkeb *et al.*, 2016; Pinto *et al.*, 2007; Batista *et al.*, 2017), while there are not studies with lima bean, mainly

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under field conditions in different regions. As consequence, lima bean does not have any recommended rhizobia for inoculation, being necessary to advance the studies for selecting rhizobia to this crop.

Therefore, it is necessary and important the searching of suitable agricultural practices to increase the lima bean yield. The optimization of BNF, by the selection of efficient N-fixing rhizobia, could contribute for increasing the lima bean yield. Thus, the aim of this field study was to evaluate the potential of rhizobia in increasing the lima bean yield in two regions from Piaui and Ceará states.

MATERIAL AND METHODS

The experiments were simultaneously conducted at Piauí and Ceará states, Brazil, from May through September 2019. In Piauí state, the experiment was conducted in the Experimental Field from Universidade Federal do Piauí, Teresina (05º05'21"S; 42º48'07"W) under a mean of temperature of 28 ºC and 70% of humidity. In Ceará, the experiment was conducted at Instituto Federal do Ceará, Tiangua (3º43'55"S; 41º0'45W) under a mean of temperature of 22 ºC and 81% of humidity. The soils from Piauí and Ceará were classified as Fluvic and Quartzarenic Neosols, respectively, and the chemical properties (0-20 cm) are shown in Table 1. Particularly, soil from Ceará presented low soil pH (5.0) and it was corrected by using 650 kg ha^{-1} of dolomitic lime (17% CaO and 13% MgO) that was applied uniformly on the soil surface. The rate was calculated according to Bezerra *et al.* (2010).

Six treatments were compared under a randomized block design with four replicates. The treatments consisted of three rhizobia UFPI32 (*Bradyrhizobium sp*.), UFPI38 (*Rhizobium sp*.) and UFPI-59 (*Bradyrhizobium s*p.), isolated in soils from Piauí state (Antunes *et al.*, 2011) and selected from previous studies under greenhouse conditions. These rhizobia One strain of reference to *Phaseolus vulgaris* (CIAT899; *Rhizobium tropici*) and two controls (PC – with N; NC – without N). The strains

All strains were grown in Erlenmeyer flasks containing 50 mL of liquid culture broth (under orbital shaking at 200 rpm, 28 °C, 72 h). The bacterial growth was verified through a spectrophotometer (wavelength of 540 nm) considering a final concentration of 10^9 CFU mL^{-1}. Seeds of lima bean, genotype UFPI480, were disinfested with alcohol (70%) for 30 seconds and sodium hypochlorite (2%) for 60 seconds, being washed five times with sterile distilled water. Afterwards, seeds were inoculated (in the inoculated treatments) and sowed, immediately, in plots with 30 m^2 each, with 20 m^2 of usable area for analysis, and rows are spaced 1.0 m apart. In each row, plants were spaced 1.0m between them which provides a total of 10000 plants per hectare. The positive control (+N) received 20 kg ha^{-1} N (100 kg ha^{-1} NH_4SO_4) at sowing. All plots received chemical fertilization with P (60 kg ha^{-1} P_2O_5) and K (30 kg ha^{-1} K_2O). In both locations, lima bean was grown under rainfed conditions.

At 45 days after sowing (flowering stage), five plants from each plot were collected and excised at the cotyledonal node to separate shoots from roots. In the flowering stage, lima bean presents the highest number of active nodules (Santos *et al.*, 2009). Nodules were separates from the roots and counted to determine nodule number (NN). Afterward, nodules, shoots and roots were dried (65°C; 72 h) and weighed to determine nodules (NDW), shoot (SDW) and roots (RDW) dry weight. Nodules size (NS) was estimated by using the ratio between NDW and NN, expressed as mg per nodule (Rocha *et al*., 2019). Total N in plant was estimated by by Kjedahl method. At 70 days, lima bean yield was evaluated by sampling of five plants inside the plots and grains were dried for 13%.

The normality of data was analyzed by using the test of Shapiro-Wilk and treatments were assessed in two locations (Piauí and Ceará). Afterward, data were statistically analyzed by using ANOVA, and the means were compared using Scott-Knott test ($p \leq 0.05$%).

RESULTS AND DISCUSSION

Lima bean grown in Ceará showed the highest and lowest values of NN and NDW with CIAT899 and NC, respectively (Table 2). However, the inoculation of UFPI32 promoted similar value of NDW in lima bean as compared to CIAT899. Although CIAT899 has promoted higher NN and NDW in lima bean grown in Ceará, the value of NS was highest with UFPI59. Regarding to lima bean growth, the values of SDW and RDW presented small variation between treatments (Table 2). Thus, PC presented the highest values of SDW, being similar to UFPI32, UFPI59 and CIAT899, and higher than UFPI38 and NC. In contrast, UFPI32 promoted the highest RDW, being similar

Table 1: Chemical properties of the soils used in this study

	pH	OM	P	K	Ca	Mg
	(H_2O)	(g kg^{-1})	(mg kg^{-1})		(cmol$_c$ kg^{-1})	
Ceará	6.0	17.0	0.7	19.5	1.2	0.5
Piauí	6.5	8.3	0.5	15.6	1.3	0.7

OM – organic matter

to UFPI38, UFPI59, CIAT899 and PC, and different than NC. The inoculation of UFPI59 and CIAT899 promoted the highest accumulation of N in lima bean as compared to the other treatments (Table 2).

The values of NN did not vary between treatments in lima bean grown in Piauí, while that the inoculation of UFPI59 and UFPI32 promoted higher values of NDW (Table 3). Interestingly, the value of NS was found in lima bean inoculated with UFPI59. Lima bean grown in Piauí presented the highest values of SDW with the inoculation of UFPI38 and UFPI59 (Table 3). However, the highest values of RDW were found in lima bean inoculated with UFPI32 and UFPI59. Regarding to leaf N, the highest values were observed with the inoculation of UFPI59 and CIAT899.

The highest values of grain yield were found in lima bean inoculated with UFPI59 as compared to CIAT899, PC and NC in both Ceará and Piauí state (Table 4). In addition, the inoculation of UFPI38 also contributed to increase the grain yield as compared to PC and NC in both states.

In this study, four potential rhizobia strains to lima bean inoculation were evaluated under field conditions in both Ceará and Piauí states. These strains were compared against a strain of reference to commom bean (CIAT899) and two controls (NC and PC). Although both states were not statistically compared, the results showed higher nodulation in Ceará than Piauí, and it can be explained for the lowest soil temperature found in Ceará that contributed for a better environment to rhizobia.

Anyway, the results showed different responses to nodulation, i.e. nodule number and dry weight, in lima bean grown in Ceará and Piauí. In Ceará, CIAT899 stimulated a greater number of nodule than other rhizobia, and it suggest that the rhizobia from Piauí (UFPI32, UFPI38, UFPI59) do not present efficiency in stimulating nodulation in lima bean in Ceará. However, UFPI32 promoted similar values of nodule biomass as compared to CIAT899 in lima bean grown in Ceará. Since nodule biomass and size are the most important parameters to estimate the efficiency of BNF in comparison to nodule number (Oðutcu *et al.*, 2010), these results suggest potential efficiency of UFPI32 in nodulating lima bean in Ceará.

On the other hand, UFPI59 presented better performance in increasing the nodule size in lima bean in both states. The better performance of UFPI-59 in stimulating larger nodules could be important to increase the biological N fixation, since higher nodules contribute

Table 4: Values of yield (kg ha^{-1}) of lima bean inoculated with rhizobia and grown in Piauí and Ceará states. At 70 days of lima bean sowing

	Ceará	Piauí
UFPI32	437^{c}	816^{b}
UFPI38	801^{ab}	998^{ab}
UFPI59	1005^{a}	1150^{a}
CIAT899	410^{c}	758^{b}
PC	650^{b}	350^{c}
NC	400^{c}	175^{c}

PC – positive control; NC- negative control;

Table 2: Values of NN (nodule pl^{-1}), NDW (mg pl^{-1}), NS (mg nodule^{-1}), SDW (g pl^{-1}), RDW (g pl^{-1}), and leaf N (%) in lima bean inoculated with rhizobia and grown in Ceará state. At 45 days of lima bean sowing

	NN	NDW	NS	SDW	RDW	Leaf N
UFPI32	150^{b}	144^{ab}	1.2^{b}	2.2^{ab}	2.3^{a}	1.3^{b}
UFPI38	153^{b}	109^{c}	0.9^{b}	1.4^{b}	2.0^{ab}	1.4^{b}
UFPI59	148^{b}	94^{c}	1.5^{a}	1.8^{ab}	1.9^{ab}	1.8^{a}
CIAT899	239^{a}	157^{a}	0.7^{b}	2.2^{ab}	2.0^{ab}	1.9^{a}
PC	147^{b}	132^{b}	0.9^{b}	2.4^{a}	1.8^{ab}	1.2^{b}
NC	39^{c}	42^{d}	1.0^{b}	1.4^{b}	0.8^{c}	1.4^{b}

PC – positive control; NC- negative control

Table 3: Values of NN (nodule pl^{-1}), NDW (mg pl^{-1}), NS (mg nodule^{-1}), SDW (g pl^{-1}), RDW (g pl^{-1}), and leaf N (%) in lima bean inoculated with rhizobia and grown in Piaui state. At 45 days of lima bean sowing

	NN	NDW	NS	SDW	RDW	Leaf N
UFPI32	17^{a}	66^{a}	3.5^{b}	2.9^{b}	4.3^{a}	1.8^{c}
UFPI38	10^{a}	25^{b}	2.3^{c}	4.9^{a}	2.1^{b}	2.5^{b}
UFPI59	18^{a}	74^{a}	4.6^{a}	4.5^{a}	3.5^{a}	3.4^{a}
CIAT899	13^{a}	10^{b}	0.7^{d}	3.0^{b}	2.1^{b}	3.3^{a}
PC	15^{a}	11^{b}	0.6^{d}	3.1^{b}	2.2^{b}	1.6^{c}
NC	9^{a}	25^{b}	2.4^{c}	2.8^{b}	2.5^{b}	1.9^{c}

PC – positive control; NC- negative control with more N fixed in the whole root system (Tajima *et al.*, 2007). For instance, previous studies have reported that soybean presenting larger nodules exhibits high N fixation as compared to plants with smaller nodules (Sato *et al.*, 2003; Yashima *et al.*, 2003). Interestingly, UFPI59 has been recognized as potential in a previous study with lima bean under laboratory and greenhouse conditions (Antunes *et al.*, 2011). In addition, this strain, classified as *Bradyrhizobium* sp., presents interesting biochemical features, i.e. it is positive for catalase, gelatinase and solubilization of P (Chibeba *et al.*, 2020).

Indeed, UFPI59 contributed for increasing the N content in lima bean and it can be correlated to the higher nodule size found with this strain. There is a strong correlation between nodule size and N accumulation in plants (Deliæ *et al.*, 2010; Voisin *et al.*, 2015; Hamawaki & Kantartzi, 2018) and it corroborates with the results observed in this study. It can indicate that UFPI59 presents high effectiveness in contributing with N to lima bean. Previously, Antunes *et al.* (2011) also found UFPI59 increasing N accumulation in lima bean under laboratory conditions.

Regarding to lima bean growth, the results showed variation between treatments and it can suggest that shoot and root biomass could not be suitable parameters to evaluate rhizobia strain in lima bean. Anyway, UFPI59 promoted high values of shoot and root dry weight in lima bean. In line with the results found on nodule size and N accumulation, the inoculation of UFPI59 promoted the higher grain yield in lima bean. This grain yield is about twice as high as the average found in Ceará and Piauí states (IBGE, 2018). Thus, UFPI59 seems to present potential to be recommended as inoculant to lima bean. The results also showed lower grain yield in soil without fertilization and even in in fertilized plots, and these values are within the range found in previous studies with lima bean (Santos *et al.*, 2002; Oliveira *et al.*, 2011).

Finally, this study showed that, in general, nodules and plant parameters varied between Ceará and Piauí states. It means that there were differences in the responses of lima bean according to rhizobia and location. These differences between rhizobia on all evaluated parameters agree with some studies with cowpea (Farias *et al.*, 2016), soybean (Barbosa *et al.*, 2017) and common bean (Mercante *et al.*, 2017). Regarding to location, the results showed that lima bean presented better growth and yield in Piauí than Ceará, and it can be explained due to the genotype of lima bean used in this study is originated from Piauí, and it contributed for the better performance of this state crop. Anyway, the results have shown that UFPI59 presents high potential to be recommended as inoculant to lima bean in Ceará and Piauí. However, further studies should be done to evaluate this strain under different N rates, lima bean genotypes, and at least for two years.

CONCLUSIONS

The strain UFPI59, classified as *Bradyrhizobium* sp., promotes larger nodules, higher N accumulation and plant growth in lima bean. In addition, this strain increases the grain yield of lima bean in both Ceará and Piauí states which indicates this bacterium as a potential candidate to be recommended to inoculation in lima bean for the states of Piauí and Ceará. It is important to lima bean producers that can be benefited with higher grain yield through of a sustainable and biological technology.

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