



Seed size influences the promoting activity of rhizobia on plant growth, nodulation and N fixation in lima bean

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ABSTRACT: *This study evaluated the activity of rhizobia isolates inoculated in large (18 mm) and small (11 mm) seeds on lima bean growth, nodulation and N fixation. Selected rhizobia isolates were compared with a reference strain CIAT899 and two controls without inoculation. Large seeds contributed for highest plant growth, nodulation and N fixation than small seeds. The isolates UFPI-59, UFPI-18 and UFPI-38 promoted the highest values of shoot and root dry weight, respectively. The isolates UFPI-32 promoted the highest values of nodule number, while UFPI-59 promoted the highest values of nodule dry weight. The isolates UFPI-38 and UFPI-59 promoted the highest accumulation of N. This study showed that seed size really influences lima bean growth, nodulation and BNF. Considering rhizobia isolates, UFPI-59, UFPI-38, and UFPI-18 contributed for plant growth, promoted better nodulation and effectiveness on biological N fixation.*

Key words: *Phaseolus lunatus, N-fixers, Rhizobium, Bradyrhizobium, plant-microbes interaction.*

O tamanho da semente influencia a atividade promotora dos rizóbios sobre o crescimento de plantas, nodulação e fixação de N em feijão-fava

RESUMO: *Este estudo avaliou a atividade de isolados de rizóbios inoculados em sementes pequenas (11 mm) e grandes (18 mm) sobre o crescimento, nodulação e fixação de N de feijão-fava. Isolados selecionados de rizóbios foram comparados com uma estirpe de referência CIAT899 e dois controles sem inoculação. Sementes grandes contribuíram para maior crescimento de plantas, nodulação e fixação de N do que sementes pequenas. Os isolados UFPI-59, UFPI-18 e UFPI-38 promoveram os maiores valores de massa seca de parte aérea e raízes. Os isolados UFPI-32 e UFPI-59 promoveram os maiores valores de número e massa de nódulos, respectivamente. Os isolados UFPI-38 e UFPI-59 promoveram maior acúmulo de N. Este estudo mostrou que o tamanho de sementes influencia o crescimento, nodulação e fixação de N em feijão-fava. Considerando os isolados de rizóbios, UFPI-59, UFPI-38 e UFPI-18 contribuíram para o crescimento de plantas, promoveram melhor nodulação e eficiência na fixação de N.*

Palavras-chave: *Phaseolus lunatus, fixadores de N, Rhizobium, Bradyrhizobium, interação plantas-microrganismos.*

INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) is an important crop for several countries, such as Peru, Colombia, Mexico, USA and Brazil (AMORIM et al., 2019). In Brazil, it is cultivated in the Northeastern region, mainly by small farmers, in an estimated area around 37,500 ha. The main characteristics of lima bean are the high rusticity and tolerance to abiotic factors, such as drought and high temperature (ARAÚJO et al., 2017).

Although, important for small farmers, lima bean still has low productivity and some reasons

contribute to this situation, such as the absence of commercial varieties and low availability of nutrients, mainly nitrogen (N). Nitrogen is essential to plants and its supply to lima bean could contribute for increasing the yield (LOPES et al., 2015). Importantly, lima bean can associate with N-fixing rhizobia and through biological N fixation (BNF) uptake N for its growth and yield (AMORIM et al., 2019).

However, the efficiency of the BNF varies according to the rhizobia strains and the selection of efficient rhizobia become an important step before recommendation to seed inoculation (IRISARRI et al., 2019). Several strains of rhizobia are already

recommended for different legumes species, such as soybean (MERKEB et al., 2016), and cowpea (BATISTA et al., 2017). So far, lima bean does not have any recommended rhizobia for inoculation and some previous studies have reported potential rhizobia isolates (ANTUNES et al, 2011; COSTA NETO et al, 2017). However, these studies were not conducted in soils, but under axenic conditions. Thus, it is necessary to advance the studies for selecting rhizobia under soil conditions.

Plant traits also influence nodulation and BNF in legumes. Among these traits, seed size contributes for increasing or decreasing the process of N fixation (DOBER & TBLEVINS, 1993; SINGH and WRIGHT, 2002; ERDEMCI et al., 2017). According to ERDEMCI et al. (2017) seed size influences seedling vigor and plant growth and large seeds usually produce more vigorous seedlings than small ones. In a previous study with *Pisum sativum*, SINGH and WRIGHT (2002) reported that large seeds had a higher increase in nodulation than small ones. In lima bean, DOBERT and BLEVINS (1993) found a positive correlation between seed size and nodulation. However, they did not evaluate the correlation between seeds size and rhizobia on plant growth and BNF. Thus, it remains unclear how are the interaction between rhizobia in seed size on plant growth, nodulation, and BNF of lima bean. The aim of this study was to evaluate the influence of seed size in promoting the activity of rhizobia on plant growth, nodulation and BNF in lima bean.

MATERIALS AND METHODS

The study was conducted in a greenhouse located at the Agricultural Science Center, Federal University of Piauí, Brazil, from January to March 2019. A sandy soil was used in this study and it was collected at 0-20 cm depth. The soil presented the following physical and chemical properties: sand – 832 g kg⁻¹, silt – 64 g kg⁻¹, clay - 104 g kg⁻¹, pH – 6.2, organic matter – 0.57 g kg⁻¹, P – 1.8 mg kg⁻¹, K – 38.9 mg kg⁻¹, Ca – 1.06 cmol_c kg⁻¹ and Mg – 0.3 cmol_c kg⁻¹.

For the experiment, five rhizobia isolates, selected according to their high efficiency under axenic condition (ANTUNES et al., 2011), were inoculated in small (genotype UFPI-1120; 11 mm and 350 mg of diameter and dry weight) and large (genotype UFPI-480; 18 mm and 800 mg of diameter and dry weight) seeds of lima bean (*Phaseolus lunatus* L). The isolates were: UFPI-18 (*Enterobacter sp.*), UFPI-32 (*Bradyrhizobium sp.*), UFPI-38 (*Rhizobium sp.*), UFPI-50 (*Bradyrhizobium sp.*), and

UFPI-59 (*Bradyrhizobium sp.*). These isolates were compared against a reference strain to *Phaseolus* (CIAT 899 - *Rhizobium tropici*; FLORENTINO et al., 2018), and two controls without inoculation: negative (NC; without N fertilization) and positive (PC; with N fertilization).

The isolates 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 10⁹ CFU mL⁻¹. The experimental unit consisted of 64 pots (diameter 18 cm, length 16 cm) filled with 5 kg of soil (previously air-dried at room temperature and sieved in a 2 mm mesh). Before inoculation, seeds were disinfected with alcohol (70%) for 30 seconds and sodium hypochlorite (2%) for 60 seconds, being washed five times with sterile distilled water. Five seeds were sowed per pot and each one was directly inoculated with 1 mL of the suspension containing the rhizobia. The positive control (+N) received 50 mg N per pot, in accordance with N requirement by lima bean. Five days after germination, plants were thinned, leaving one plant per pot. Pots were irrigated daily with sterilized water to maintain soil moisture at 80% of field capacity.

The experiment was harvested 45 days after sowing (flowering stage). Plants were excised at the cotyledonal node to separate shoots from roots. Nodules were separated 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. Total N content in shoot was estimated by Kjeldahl method and the accumulation of N (AcN) in shoot was estimated by the shoot dry matter. The effectiveness of BNF was estimated comparing the inoculated treatments against negative control, being the values expressed in %, according to the expression: Effectiveness = (SDW_i / SDW_{ni}) x 100, in which SDW_i and SDW_{ni} are the values of shoot dry weight reported in inoculated and non-inoculated plants, respectively.

The treatments were arranged in a randomized design, under an 8 x 2 factorial scheme with four replications, corresponding to eight treatments (rhizobia and controls) and two seed sizes (small and large). The normality of data was analyzed by using the test of Shapiro-Wilk. Afterward, data were statistically analyzed by using ANOVA, and the means were compared using Scott-Knott test ($p \leq 0.05\%$) through the statistical program SISVAR, version 5.6 (FERREIRA, 2014). In addition, to

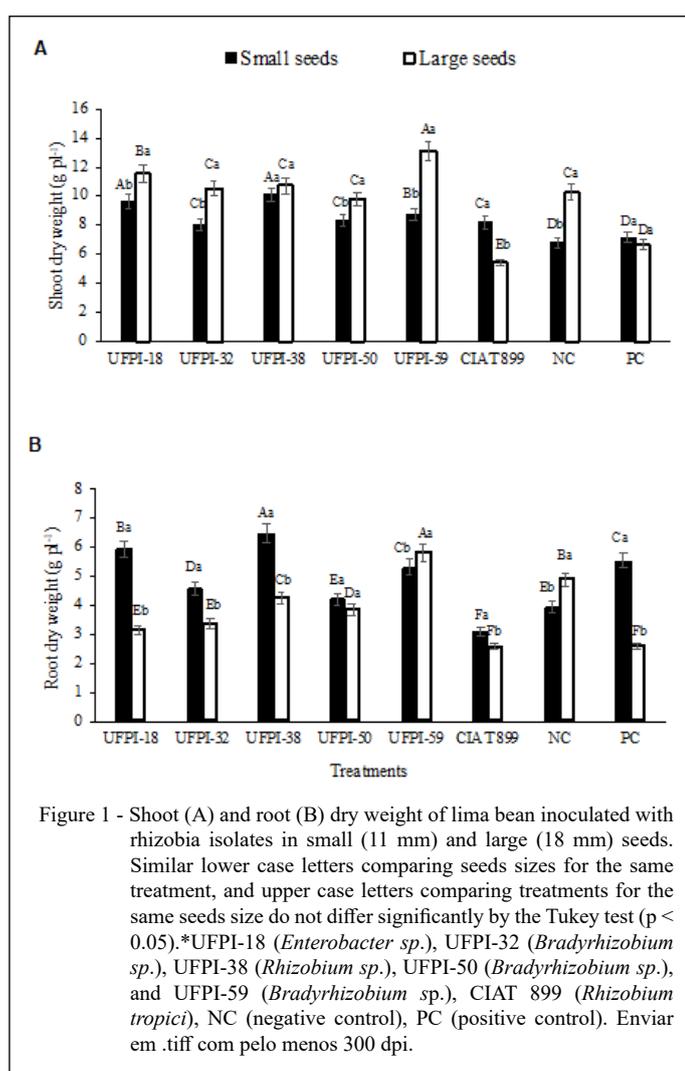
compare the isolate profiles we conducted a principal component analysis biplot (PCA) based on the determined characteristics. For this, the data matrix was initially analyzed using detrended correspondent analysis (DCA) to evaluate the distribution of the data, which indicated the best-fit model PCA. Then, PCA plots were generated using the Canoco 4.5 software (BIOMETRICS, WAGENINGEN, THE NETHERLANDS).

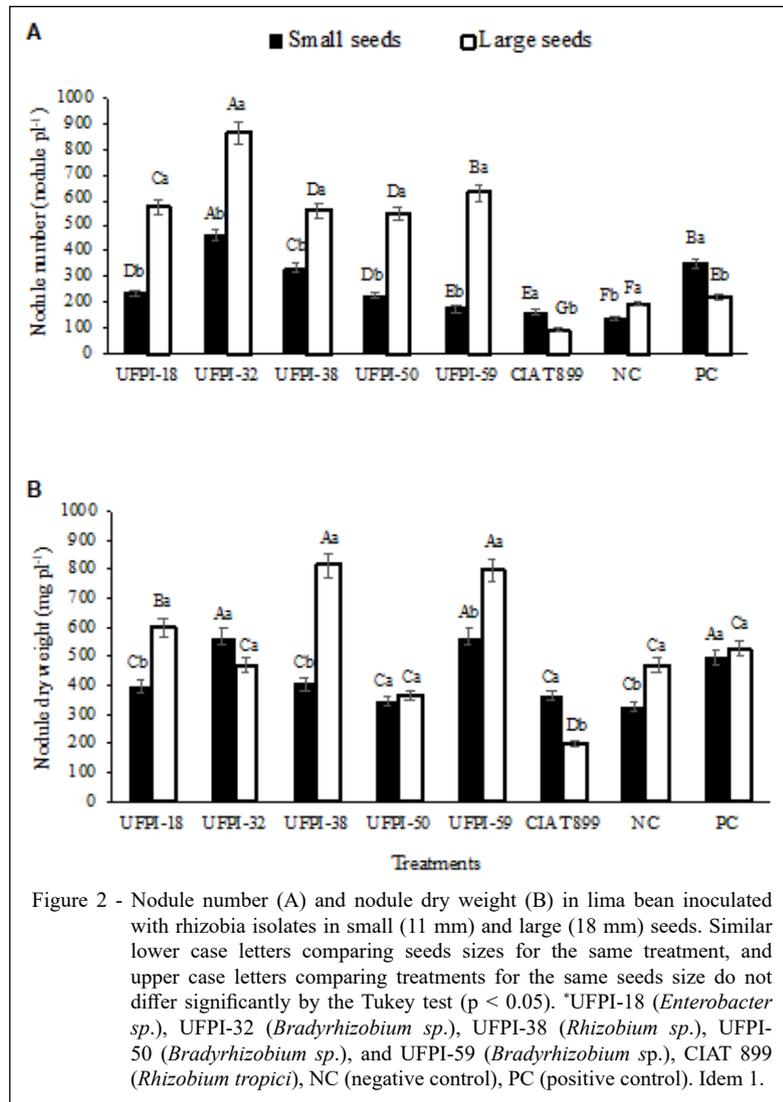
RESULTS

Treatments, seed size and interactions between treatments and seed size significantly influenced all variables. In general, the values of SDW were highest with large seeds than small

ones (Figure 1A). The exception was CIAT899 that showed the highest SDW with small seeds. For this plant parameter, UFPI-59 promoted the highest values when inoculated in large seeds. For small seeds, UFPI-18 and UFPI-38 promoted the highest values of SDW. In contrast, the values of RDW were highest with small seeds (Figure 1B). The exception was UFPI-59 and NC that promoted the highest RDW with large seeds. For this plant parameter, UFPI-38 and UFPI-59 promoted the highest values, when inoculated in small and large seeds, respectively. Interestingly, UFPI-38 and UFPI-18, in small seeds, and UFPI-59, in large seeds, increased the root growth when compared to NC, PC and CIAT899.

The values of NN and NDW were highest with large seeds (Figure 2). The exception was



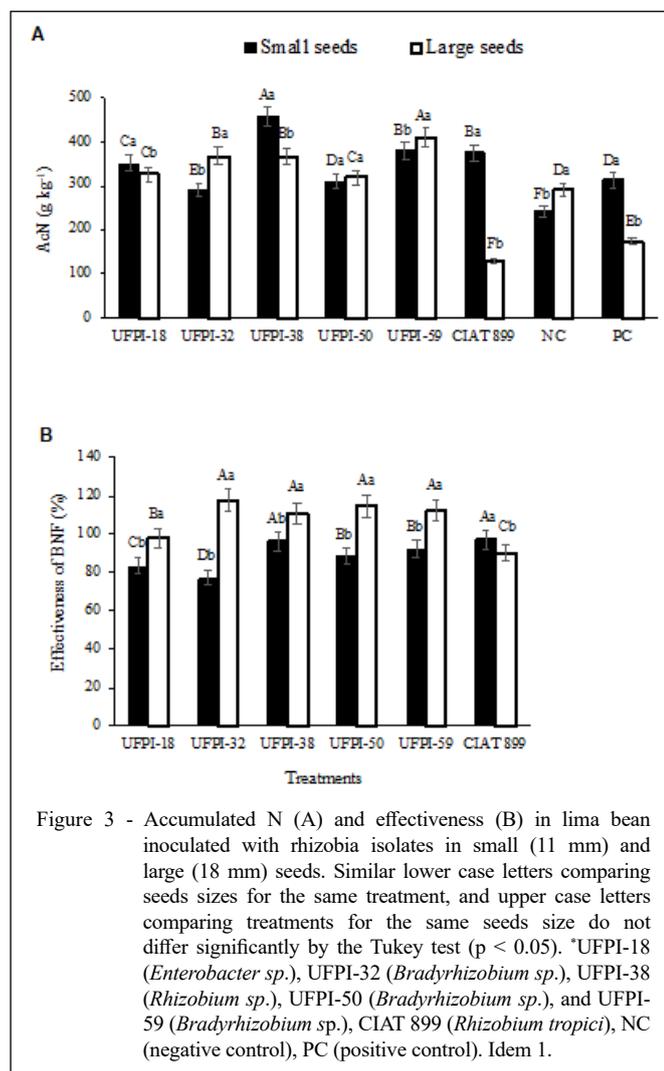


CIAT899 and PC, for NN, and CIAT899 and UFPI-32, for NDW, that presented the highest values with small seeds. The isolates UFPI-32 increased NN in both small and large seeds as compared to other treatments. For NDW, UFPI-59 and UFPI-38 promoted the highest values when inoculated in large seeds. In small seeds, UFPI-59, UFPI-32 and PC presented the highest values of SDW (Figure 2).

The highest values of AcN were reported with UFPI-38 and UFPI-59 in small and large seeds, respectively (Figure 3A). In addition, these isolates promoted higher AcN than NC, and PC. In small seeds, UFPI-38 and CIAT899 presented the highest effectiveness, while that UFPI-32, UFPI-38, UFPI-

50 and UFPI-59 presented higher effectiveness when inoculated in large seeds (Figure 3B).

The PCA showed the relationship between treatments and the evaluated parameters, according to seeds size (Figure 4). This analysis explained 96.8 % of the total variation of which 87.2% and 9.6% are displayed on the horizontal and vertical axes, respectively. Large seeds inoculated with rhizobia correlated with the plant and nodules parameters than small ones. The exception was the treatments NC, PC and CIAT899. Thus, when large seeds were inoculated, isolates UFPI-38 and UFPI-59 correlated with SDW, AcN, NDW and effectiveness, while UFPI-32 correlated with NN.

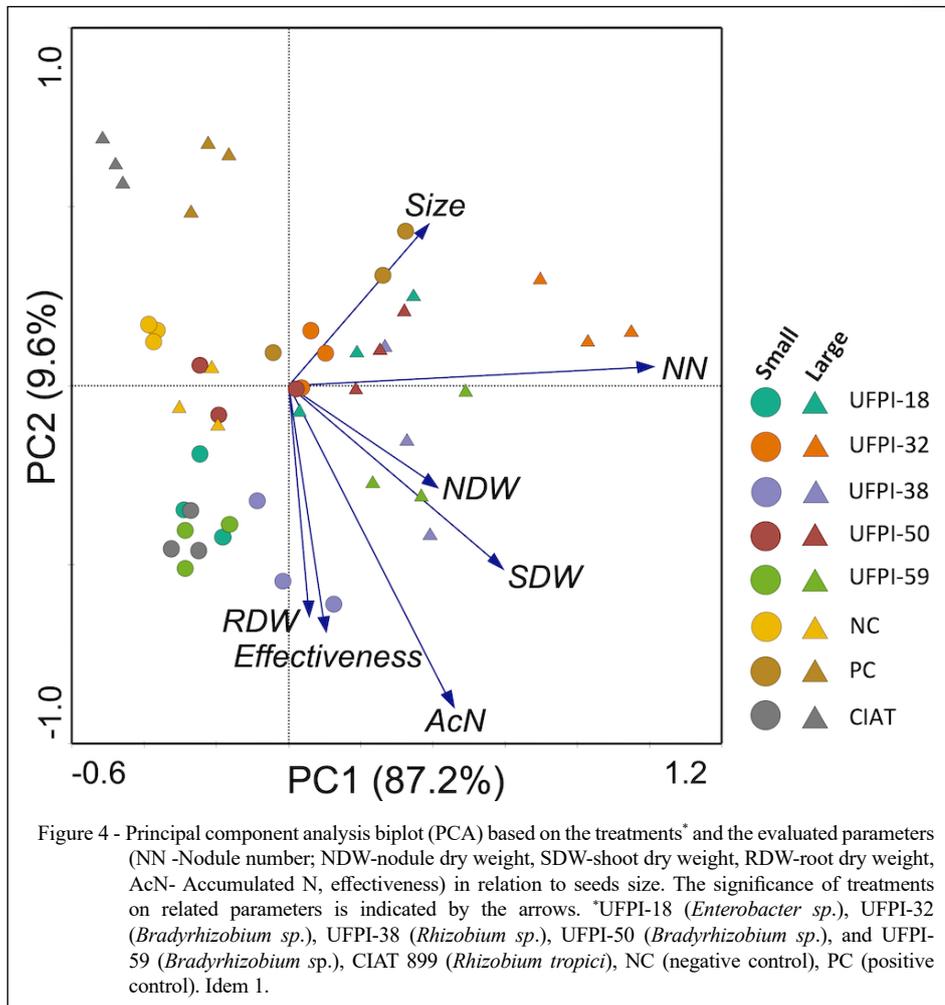


DISCUSSION

Results showed that lima bean growth, nodulation and BNF varied according to rhizobia. These results are in line with previous studies that showed differences on nodulation and symbiotic efficiency by rhizobia in soybean (BARBOSA et al., 2017) and common bean (MERCANTE et al., 2017). In addition, results showed that, in sandy soil with low fertility, the inoculation in lima bean should be strongly recommended. In general, the inoculation of UFPI-59, UFPI-18 and UFPI-38 promoted the highest plant growth, when inoculated in large and small seeds, as compared with NC, PC and CIAT899. The plant growth, i.e. shoots and roots dry weight, is an important parameter

for selecting rhizobia to inoculation. Thus, these isolates present potential for further studies in field and recommendation as inoculant.

The nodulation, i.e. nodule number and dry weight, differed between rhizobia and seed size. The inoculation of UFPI-32 stimulated the nodule number, while that UFPI-59 increased the nodule biomass. Although, nodule number has been stimulated by inoculation of UFPI-32, this isolate did not stimulate the nodule growth as compared to UFPI-59. According to ÖĞÜTÇÜ et al. (2010), nodule dry weight is more reliable than nodule number as indicator of effective symbiosis since larger nodules fix more N than smaller ones. Therefore, UFPI-59 presents high potential for increasing the lima bean growth and nodulation. According to PARRA-



COLMENARES and KAHN (2005), nodulation and plant growth are key parameters commonly used to estimate the potential of rhizobia. In a previous study, the isolate UFPI-59 also promoted higher nodule biomass in lima bean under axenic condition (ANTUNES et al., 2011). Thus, results suggested that this isolate is also effective under soil condition.

The nodulation observed in uninoculated plants (NC and PC) suggested the presence of native rhizobia nodulating lima bean. However, these native rhizobia were not more effective than the isolates. The results showed that CIAT899 presented highest nodulation in small seeds and it confirmed the recommendation of this strain for *Phaseolus vulgaris* (common bean) which presents small seeds.

The isolate UFPI-59, that promoted highest nodule biomass, contributed to higher accumulation of N by lima bean. However, the isolate UFPI-38, that

did not present highest nodulation, also contributed to higher accumulation of N. Although, UFPI-38 did not present high nodulation, this isolate promoted higher root growth by which can contribute to the high N uptake due to the increased root surface. In addition, UFPI-38 presents urease activity (CHIBEBA et al., 2020) and it can provide greater efficiency in the use of N by the plants (MOBLEY & HAUSINGER, 1989). Results have shown that the inoculation of UFPI-59 and UFPI-38 potentially increased plant growth, nodulation and N fixation independent of seeds size. This finding agrees with ANTUNES et al. (2011) who reported a strong response of UFPI-59 on nodulation and accumulation of N in lima bean under axenic conditions. Usually, higher nodule biomass increases the accumulation of N in plants (HAMAWAKI and KANTARTZI, 2018). Also, the isolate UFPI-59 presents protease activity

and the capability of solubilize P (CHIBEBA et al., 2020), being two important biochemical properties to increase plant growth. Thus, both isolates can be selected as potential for increasing N fixation and contributing for lima bean growth.

The multivariate analysis (PCA) showed differences between large and small seeds on all parameters. It confirmed the variation found on plant growth, nodulation and BNF according to seeds size. The results confirmed UFPI-38 and UFPI-59 as more efficient rhizobia, mainly with large seeds. In addition, the highest values of parameters were reported in large seeds and it agrees with previous studies assessing the effect of seed size on plant growth and nodulation (DOBERT & BLEVINS 1993; SINGH and WRIGHT, 2002; ERDEMCI et al, 2017). Since large seeds present higher reserve content, it could promote faster emergence and more vigorous seedling (SINGH and WRIGHT, 2002). Although, this study did not evaluate seedling emergence and vigor, these factors may have contributed for highest plant growth and nodulation. Indeed, DOBERT & BLEVINS (1993) have reported an influence of seed-factors on lima bean nodulation.

Since the lima bean presents importance mainly to smallholders who use different types of seeds (PENHA et al., 2017), this study brings important information regarding to the potential rhizobia isolate to be inoculated in small or large seeds. In addition, the practice of inoculation is important to lima bean since this crop presents low yield and, at the same time, it can contribute for decreasing the dependency of N fertilizers and increasing the environmental sustainability.

CONCLUSION

Seed size influences plant growth, nodulation and BNF, being a key trait to determine the success for inoculation in lima bean. Thus, large seeds contributed with better lima bean performance on all evaluated parameters. Considering rhizobia isolates, UFPI-59 and UFPI-38 contributed for plant growth, nodulation and BNF. Therefore, these isolates present high potential for evaluation under field experiments.

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DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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