

VARIETIES OF LIMA BEAN SHOWS DIFFERENT GROWTH RESPONSES WHEN INOCULATED WITH *Bacillus* sp., A PLANT GROWTH-PROMOTING BACTERIA

VARIÉDADES DE FEIJÃO-FAVA EXIBEM DIFERENTES RESPOSTAS DE CRESCIMENTO QUANDO INOCULADAS COM *Bacillus* sp., UMA BACTÉRIA PROMOTORA DE CRESCIMENTO EM PLANTAS

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ABSTRACT: Plant growth-promoting bacteria (PGPB) comprise of soil microorganisms that cause positive effects on plant growth. The hypothesis according to which the inoculation of lima bean with PGPB *Bacillus* stimulates vegetative growth, which may result in a higher plant productivity, was tested. Thus, this study aimed to evaluate the effects of inoculation of two varieties of lima bean (branca and boca de moça) with *Bacillus* sp., UFPEDA 472 strain, based on plant growth responses. The experiment was conducted in a greenhouse using a completely randomized design. Three treatments were applied separately to each lima bean variety: the first used inoculation with *Bacillus*, one was submitted to nitrogen supplementation, and one was the absolute control (non-inoculated plants). The following variables were evaluated: absolute and relative growth rate, root length, stem diameter, fresh and dry mass of shoots and roots, shoot:root ratio and total chlorophyll. The results showed that lima bean displayed better growth responses when inoculated with *Bacillus* sp. UFPEDA 472 in relation to the treatment with nitrogen and/or non-inoculated plants. When inoculated with PGPB *Bacillus*, the lima bean var. branca was superior to boca de moça in terms of root length and root dry mass. The lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 was superior to branca in terms of absolute growth rate, stem diameter, fresh and dry mass of shoot, shoot:root ratio and total chlorophyll. In general, the lima bean var. boca de moça inoculated with *Bacillus* shows a better growth performance. Our results suggest that growth responses of lima bean varieties branca and boca de moça are related with a positive interaction with the PGPB *Bacillus*.

KEYWORDS: *Phaseolus lunatus*. PGPB. Absolute growth rate. Chlorophyll.

INTRODUCTION

Plant growth-promoting bacteria (PGPB) are beneficial microorganisms inhabiting the rhizosphere (FIGUEIREDO et al., 2011). They stimulate plant growth through by acting as bio-fertilizers and bio-stimulants, increasing the ability of the plant to resist stressful situations (GLICK, 2014; HUANG et al., 2014). PGPB act through complex mechanisms not yet fully explained (FIGUEIREDO et al., 2011; DUCA et al., 2014). PGPB may directly facilitate the supply of nutrients, produce siderophores and control phytohormone levels, particularly indole acetic acid (IAA), and consequently reduce the negative effects of environmental stress (DUCA et al., 2014; GLICK, 2014; GROBELAK et al., 2015). As indirect mechanisms, PGPB act by limiting the growth of pathogens through competitive processes (antibiosis and hyperparasitism) and the induction of systemic

resistance (EGORSHINA et al., 2012; DUCA et al., 2014).

PGPB are distributed in various genera, especially *Azospirillum*, *Bacillus*, *Herbaspirillum*, *Klebsiella* and *Pseudomonas* (FIGUEIREDO et al., 2011). The genus *Bacillus* includes bacteria with a remarkable ability to synthesize a wide range of beneficial substances such as IAA, gibberellins, cytokinins, siderophores, hydrolytic enzymes and antibiotics (MUDAY et al., 2012; HUANG et al., 2014). *Bacillus* species have other important mechanisms promoting plant growth such as the ability to fix nitrogen and solubilize phosphate and potassium (GROBELAK et al., 2015; PII et al., 2015). Furthermore, the presence of *Bacillus* microbial populations stimulates the uptake of nutrients by plant roots (DUCA et al., 2014). The inoculation with *Bacillus* results in a significant increase in the absorption of potassium, calcium and

magnesium by wheat roots grown in a limestone soil without fertilization (ÖĞÜT et al., 2011).

Several studies reported the efficiency of bacteria from the genus *Bacillus* alone or in combination with other microorganisms. Wheat seedlings treated with of *B. subtilis* spores, 11BM strain, showed a stimulating growth associated with a transient increase in IAA (EGORSHINA et al., 2012). The inoculation with *B. siamensis*, a bacterial species capable of producing and releasing gibberellins, increased the growth of banana plants (AMBAWADE; PATHADE, 2015). The MTCC 8983 strain of *B. circulans* promoted the growth of apple plants because they can solubilize phosphate and produce IAA and siderophores, and by an antagonistic activity against the fungus *Dematophora meatrix* (MEHTA et al., 2010). The co-inoculation of *Rhizobium phaseoli* with the Bx strain of *Bacillus* sp. resulted in an increase in common bean plant growth because of an increase in nitrogen fixation, phosphorus uptake and shoot dry matter (STAJKOVIĆ et al., 2011).

Phaseolus and *Vigna* are major genera of leguminous plants of the family Fabaceae, with a significant importance to the world (BITOCCHI et al., 2012). The *Phaseolus* genus includes approximately 50 species, especially *P. vulgaris*, *P. lunatus*, *P. dumosus*, *P. coccineus* and *P. acutifolius* (SERVÍN-GARCIDUEÑAS et al., 2014). *P. lunatus*, commonly known as lima bean, is the second most important leguminous of the genre *Phaseolus* (BITOCCHI et al., 2012; ARAUJO et al., 2015). It is used for food and cultivated as a green manure and as cover crop (DAHMER et al., 2008). Lima bean are cultivated in several regions in Brazil. However, their cultivation is greater in the Northeast region (DAHMER et al., 2008; ARAUJO et al., 2015). Although the cultivation of lima bean has an economic and social importance, especially for family farms in the Northeast region, it presents a low yield mainly due to the lack of domesticated genotypes and a low technological level applied to its cultivation (ARAUJO et al., 2015).

Lima bean stands out as one of the most adapted leguminous crops in rainfed agriculture. However, it has low levels of productivity and a large fluctuation in production (DAHMER et al., 2008; ARAUJO et al., 2015). Despite the greater rusticity and adaptability comparing to other legumes, studies focused on the characterization of lima bean and the search for genotypes with a high productivity in different environmental conditions are still incipient (ARAUJO et al., 2015). In this context, data helping to increase the development and the productivity of lima bean are useful to

increase the agronomic potential of this crop. The hypothesis according to which the inoculation of lima bean with PGPB *Bacillus* stimulates vegetative growth, which may result in a higher plant productivity, was tested in this study. Thus, this study aimed to evaluate the effects of inoculation of two varieties of lima bean with *Bacillus* sp. UFPEDA 472 on the growth of plants in a greenhouse.

MATERIAL AND METHODS

Preparation of the inoculant

The *Bacillus* sp. UFPEDA 472 was kindly supplied from the Microorganisms Culture Collection UFPEDA maintained by Department of Antibiotics of the Federal University of Pernambuco (Recife, Pernambuco state, Brazil). The strain UFPEDA 472 of *Bacillus* sp. was purified and multiplied in Petri dishes containing a Trypticase soy agar (TSA) solid medium. For the preparation of the inoculant, the *Bacillus* sp. UFPEDA 472 was collected in Erlenmeyer flasks with a Trypticase soy broth (TSB) liquid medium and incubated in a rotator shaker (220 rpm) at 28 °C for 96 h.

Conduction of the experiment

The experiment was conducted in a greenhouse (5°5'21" S and 42°48'07" W) at the Department of Crop Science in Federal University of Piauí (Teresina, Piauí state, Brazil). During the experiment, the temperature varied between 21 and 35 °C. The relative humidity, total annual insolation and the photoperiod were 65%, 2,625 hours and 12 h, respectively. Initially, seeds of lima bean var. boca de moça and branca were disinfected by immersing seeds into 70% alcohol (45 s) and 2.5% sodium hypochlorite (60 s), followed by nine washes with sterile distilled water (HUNGRIA; ARAÚJO, 1994). Prior to filling the pots, the soil was autoclaved (120 °C, 101 kPa, 1 h) and subjected to supplementation with phosphorus pentoxide and potassium oxide following the recommendations by Lopes et al. (2010) and the results of soil chemical analysis (Table 1).

Seeds of lima bean previously disinfected were sown in pots containing sieved and sterile soil as substrate (3.5 kg pot⁻¹), and simultaneously inoculated with 1.0 mL of TSB liquid medium containing the *Bacillus* sp. UFPEDA 472 at 10⁷ CFU mL⁻¹. For the inoculation, cell suspensions of *Bacillus* sp. (UFPEDA 472 strain) in TSB liquid medium were previously adjusted at 10⁷ CFU mL⁻¹ in a spectrophotometer at 560 nm. The thinning was carried out at seven days and two seedlings were

kept in each pot (an experimental unit). For the treatment with nitrogen, a nitrogen supplementation with ammonium sulfate (0.07 g pot^{-1}) was performed at 7, 14 and 21 days after thinning

following recommendations of soil chemical analysis. Non-inoculated plants were considered as the absolute control.

Table 1. Chemical analysis of the soil used in the experiment.

pH (H_2O)	P (mg dm^{-3})	K^+	Na^+	Ca^{+2}	Mg^{+2} ($\text{cmol}_c \text{ dm}^{-3}$)	Al^{+3}	H^+	CEC	SB
6.8	1.50	19.70	12.30	1.90	0.50	-	1.50	3.98	2.48

CEC = Potential cation exchange capacity at pH 7.0; SB = sum of bases.

During the experimental period, lima bean plants were watered every day until field capacity with distilled water and every two days with the nitrogen-free Hoagland & Arnon (1950) nutritive solution modified by Silveira et al. (1998) (7.0 mL pot^{-1}). The height of lima bean plants was evaluated every five days and the data were used to calculate absolute growth rate (BENINCASA, 2003). At harvest (45 days), root length, stem diameter and fresh mass of shoots and roots were evaluated. Total chlorophyll was determined using the Clorofilog® (Falker, Brazil). The dry mass of the shoots and roots was determined after drying in a forced-air ventilation oven at $65 \text{ }^\circ\text{C}$ until constant weight. The mass of shoots and roots were used to calculate the shoot:root ratio, whereas total dry mass was used to calculate relative growth rate (BENINCASA, 2003).

Experimental design and statistical analysis

The experimental design was completely randomized. Three treatments were applied separately to each lima bean variety (branca and boca de moça): one was inoculated with *Bacillus*, one was submitted to nitrogen supplementation, and one was the absolute control (non-inoculated plants). It were used four replications. Data obtained were tested for normality using a Shapiro-Wilk test ($P < 0.05$). The variables were subjected to analysis of variance (ANOVA) with an F test ($P < 0.05$). The comparison of means was performed using a Tukey's test ($P < 0.05$). All analyses described were performed with the ASSISTAT® statistical software (SILVA, 2012).

RESULTS

The varieties of lima bean had different responses to the inoculation with *Bacillus* sp., UFPEDA 472 strain, compared with the treatments with nitrogen and without inoculation (Figure 1). The variety boca de moça had a growth rate higher than the variety branca in all treatments (Figure 1A). Comparing with non-inoculated plants, lima bean

inoculated with *Bacillus* sp. UFPEDA 472 showed a growth rate higher than 177% and 111% for lima bean var. branca and boca de moça, respectively. Non-inoculated lima bean, varieties branca and boca de moça, had an absolute growth rate lower than 60% and 47%, respectively, compared with nitrogen-supplied plants (Figure 1A). When lima bean var. branca and boca de moça grown in a soil with nitrogen (nitrogen-supplied plants) were compared to plants inoculated with *Bacillus* sp. UFPEDA 472, it was possible to note that the absolute growth rate was lower in nitrogen-supplied plants regarding both lima bean varieties.

Non-inoculated lima bean var. branca and boca de moça had a lower relative growth rate in relation to other treatments (Figure 1B). The relative growth rate of lima bean var. branca and boca de moça inoculated with *Bacillus* sp. UFPEDA 472 was higher than non-inoculated lima bean plants by approximately 40% (Figure 1B). Also regarding non-inoculated plants, lima bean varieties grown in a soil with nitrogen (nitrogen-supplied plants) increased by 18% and 22% the relative growth rate for branca and boca de moça varieties, respectively. An increase of 15% in relative growth rate was recorded for the lima bean var. branca when inoculated with *Bacillus* sp. UFPEDA 472 in relation to plants grown in a soil with nitrogen (nitrogen-supplied plants). For the lima bean var. boca de moça, this increase was less expressive (approximately 10%) (Figure 1B).

There were no significant differences in root length between lima bean varieties of non-inoculated plants (Figure 2A). As shown in figure 2A, the lima bean var. branca had a greater root length than boca de moça when fertilized with nitrogen (34% superior) or inoculated with *Bacillus* sp. UFPEDA 472 (16% higher). Non-inoculated lima bean, var. branca, had a root length 36% smaller when compared to nitrogen-supplied plants. For the lima bean var. boca de moça, no significant differences were recorded (Figure 2A).

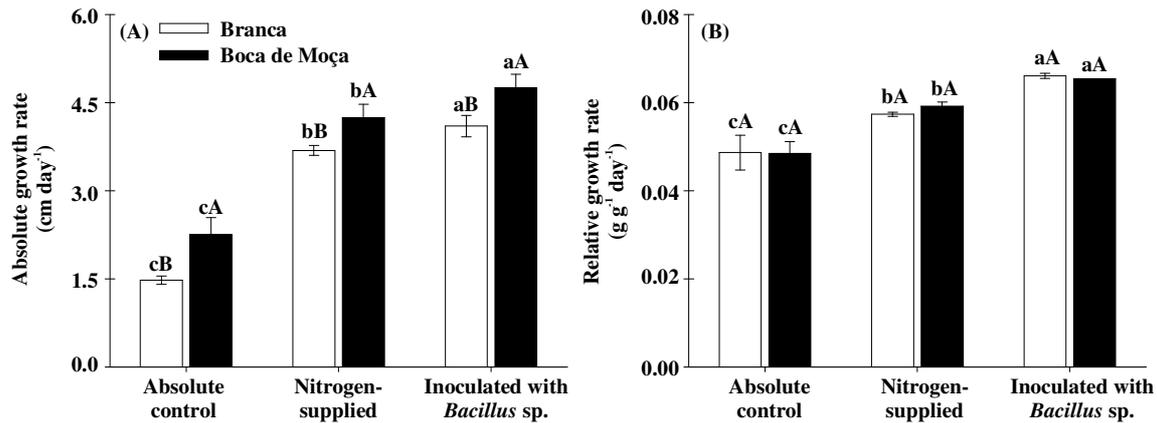


Figure 1. Growth parameters of lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472: (A) absolute growth rate and (B) relative growth rate. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Different lowercase letters represent significant differences among treatments in each variety, while different uppercase letters indicate significant differences between the two varieties by Tukey's test ($P < 0.05$).

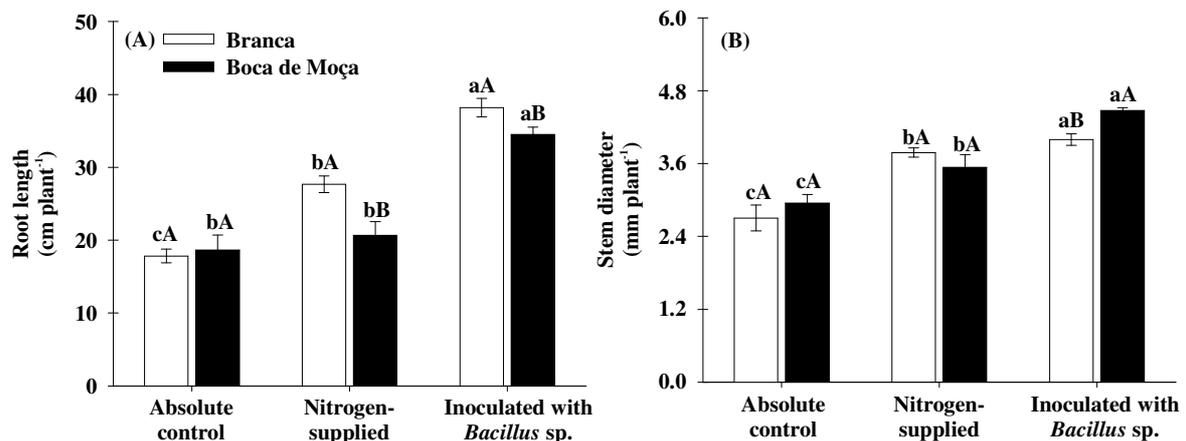


Figure 2. Root length (A) and stem diameter (B) of the lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Different lowercase letters represent significant differences among treatments for each variety, while different uppercase letters indicate significant differences between the two varieties by Tukey's test ($P < 0.05$).

The varieties branca and boca de moça inoculated with *Bacillus* sp. UFPEDA 472 showed an increase in root length of approximately 120% and 80% when compared to non-inoculated plants, respectively (Figure 2A). In addition, an expressive increase in root length (60%) was observed for lima bean varieties inoculated with *Bacillus* sp. UFPEDA 472 compared to lima bean varieties fertilized with nitrogen.

There were no significant differences between lima bean varieties regarding stem diameter in a same treatment. However, significant differences among the different treatments were observed (Figure 2). As shown in figure 2B, the stem diameter of lima bean plants inoculated with

Bacillus sp. UFPEDA 472 was superior to the others treatments. A higher increase of 45% in stem diameter was observed for lima bean var. branca and boca de moça inoculated with *Bacillus* sp. UFPEDA 472 in relation to non-inoculated plants (Figure 2B). Lima bean plants grown in a soil with nitrogen (nitrogen-supplied plants) had 40% and 20% increases in stem diameter for the varieties branca and boca de moça, respectively, compared to non-inoculated plants. Lima bean inoculated with *Bacillus* sp. UFPEDA 472 had a higher stem diameter in relation to nitrogen-supplied lima bean plants, mainly the lima bean var. boca de moça, which had increases of approximately 30%.

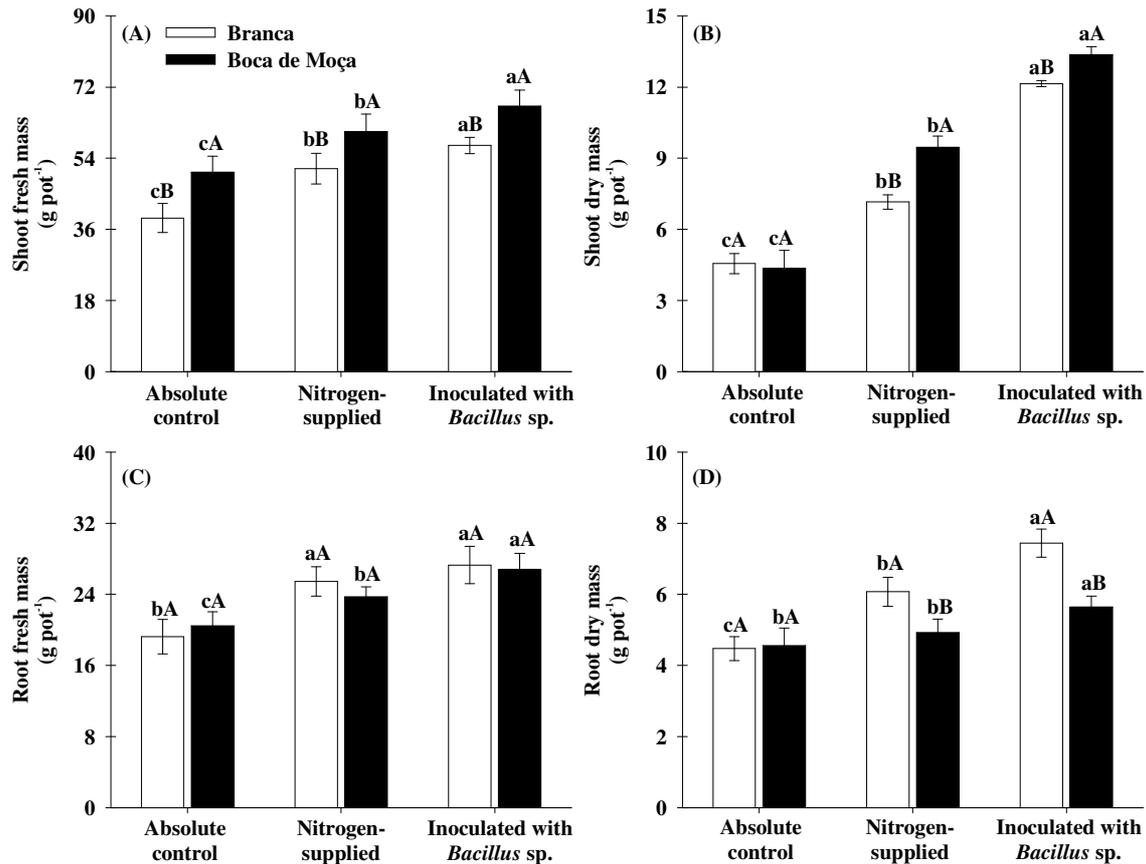


Figure 3. Shoot and root fresh (A, C) and dry (B, D) mass of lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Different lowercase letters represent significant differences among treatments for each variety, while different uppercase letters indicate significant differences between the two varieties by Tukey's test ($P < 0.05$).

The fresh and dry mass of shoots and roots increased in the two lima bean varieties inoculated with *Bacillus* sp. UFPEDA 472 (Figure 3). The shoot fresh mass increased in lima bean, especially the lima bean var. boca de moça, which stood out in all treatments (Figure 3A). The lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 showed an increase in fresh mass of shoots of approximately 10% compared to nitrogen-supplied plants, and 47% compared to the absolute control (non-inoculated plants). There was an increase of approximately 20% in the shoot fresh mass of lima bean var. boca de moça in relation to the lima bean var. branca when inoculated with *Bacillus* sp. UFPEDA 472 or when nitrogen-supplied lima bean plants (Figure 3A). Additionally, lima bean var. branca and boca de moça inoculated with *Bacillus* sp. UFPEDA 472 had 42% and 31% increases, respectively, in root fresh mass compared to non-inoculated plants (Figure 3B).

Changes in the dry mass of shoots and roots were recorded for lima bean varieties inoculated

with *Bacillus* sp. UFPEDA 472, mainly the shoot dry mass of the lima bean var. boca de moça (Figure 3C and 3D). Lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 had an increase in shoot dry mass of 208% and 41% compared to non-inoculated plants and nitrogen-supplied plants, respectively (Figure 3C). As shown in the Figure 3D, lima bean var. branca has a root dry mass 23% and 32% higher than boca de moça grown in a soil with nitrogen or when inoculated with *Bacillus* sp. UFPEDA 472, respectively. In relation to non-inoculated plants, an increase of 66% in root dry mass was observed for the lima bean var. branca inoculated with *Bacillus* sp. UFPEDA 472 (Figure 3D). Lima bean var. branca inoculated with *Bacillus* sp. UFPEDA 472, compared to nitrogen-supplied plants, had a 23% increase in root dry mass.

Shoot and root dry weight of all treatments were compared with non-inoculated lima bean plants and data are shown as percentages in the Figure 4. There was an increase in the percentage of

shoot and root dry weight of the lima bean var. branca and boca de moça, mainly when lima bean varieties were inoculated with *Bacillus* sp. UFPEDA 472. The increase in shoot dry weight in lima bean var. branca inoculated with *Bacillus* sp. UFPEDA 472 was 183%, while lima bean var. boca de moça had increases higher than 231% (Figure 4). There was a significant difference regarding shoot dry weight when lima bean varieties were grown in a soil with nitrogen. The lima bean var. boca de moça

was higher than lima bean var. branca by approximately 60% regarding shoot dry weight. Regarding root dry weight, the lima bean var. branca was higher than the lima bean var. boca de moça when fertilized with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Due to the inoculation with *Bacillus* sp. UFPEDA 472, lima bean var. branca had increases higher than 65% in root dry weight when compared to non-inoculated plants.

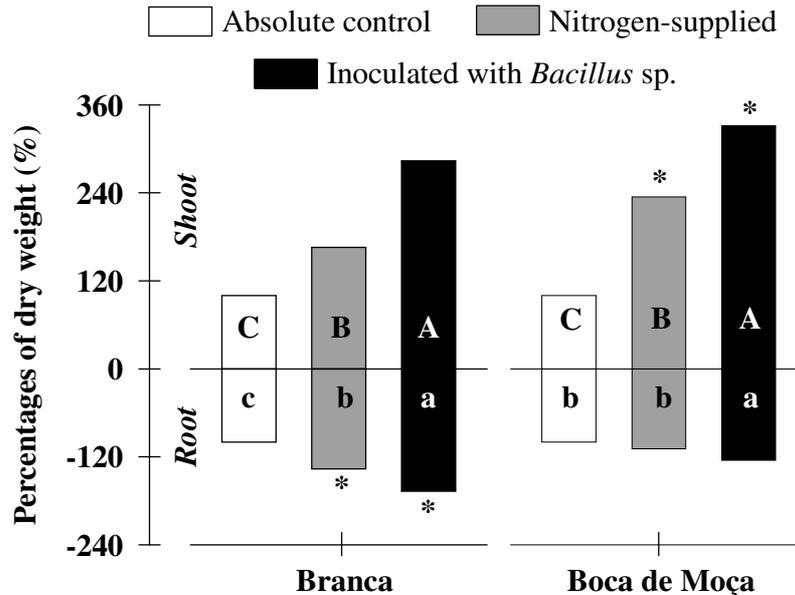


Figure 4. Distribution of dry weight between shoots and roots of lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Means followed by the same lowercase (shoot) or uppercase (root) letters do not differ statistically ($P < 0.05$) among treatments with each variety. The asterisk (*) indicates significant differences between the two varieties by Tukey's test ($P < 0.05$).

Significant differences between the shoot:root ratio of the two lima bean varieties were recorded for plants supplied with nitrogen and plants inoculated with *Bacillus* sp. UFPEDA 472 (Figure 5). A maximum value of shoot:root ratio (2.38 g g^{-1}) was recorded for the lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 (Figure 5). Lima bean var. boca de moça was 55% and 45% superior than the lima bean var. branca when cultivated with nitrogen in a nutritive solution

and when inoculated with *Bacillus* sp. UFPEDA 472, respectively. As illustrated in Figure 5, the shoot:root ratio of lima bean var. branca and boca de moça inoculated with *Bacillus* sp. UFPEDA 472 increased by 62% and 203% compared to non-inoculated plants, respectively. It is possible to note that lima bean var. branca and boca de moça increase by 38% and 30% the shoot:root ratio when inoculated with *Bacillus* sp. UFPEDA 472 in relation to nitrogen-supplied plants (Figure 5).

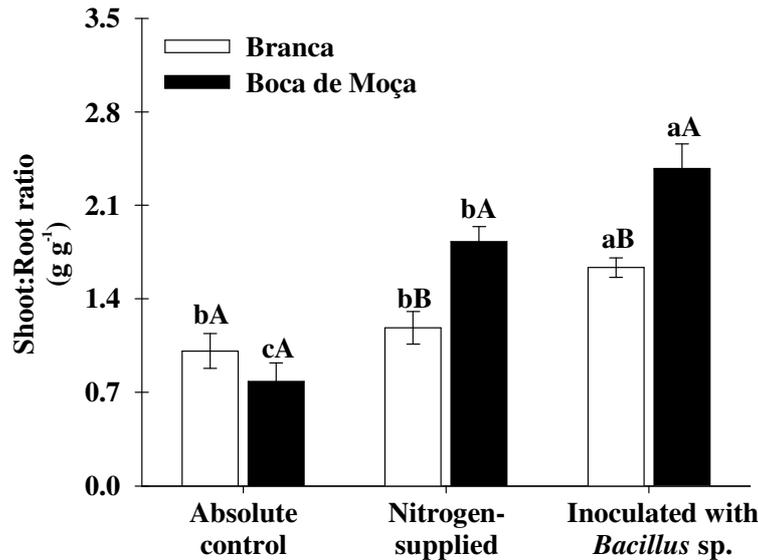


Figure 5. Shoot:root ratio of lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Different lowercase letters represent significant differences among treatments for each variety, while different uppercase letters indicate significant differences between the two varieties by Tukey’s test ($P < 0.05$).

The total chlorophyll was measured in both lima bean varieties submitted to different treatments. Significant differences were recorded (Figure 6). As shown in Figure 6, the lima bean var. boca de moça was superior than the lima bean var. branca when inoculated with *Bacillus* sp. UFPEDA 472 or when supplied with nitrogen. Compared to non-inoculated plants, the lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 stood out in relation to the lima bean var. branca. Increases of 62% for total chlorophyll were

recorded (Figure 6). Lima bean varieties grown in a soil with nitrogen (nitrogen-supplied plants) shows increases in total chlorophyll of 26% and 36% for the varieties branca and boca de moça, respectively, in relation to non-inoculated plants. An increase higher than 15% in total chlorophyll was recorded for lima bean var. branca and boca de moça when inoculated with *Bacillus* sp. UFPEDA 472 in relation to plants grown in a soil with nitrogen (nitrogen-supplied plants).

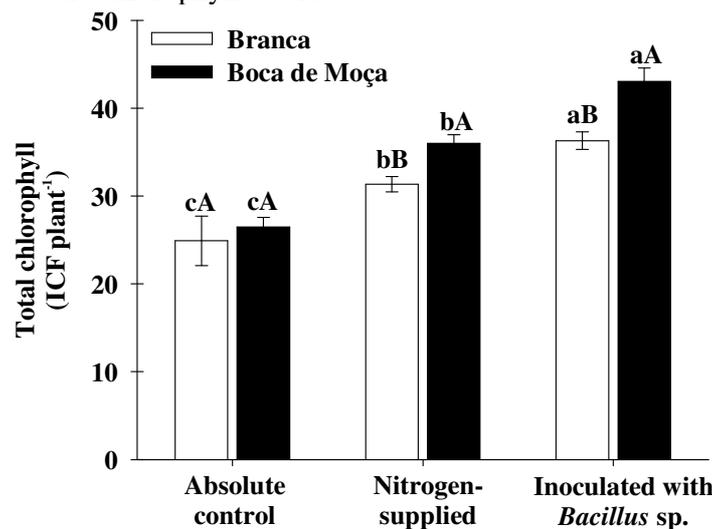


Figure 6. Total chlorophyll of lima bean var. branca and boca de moça supplied with nitrogen or inoculated with *Bacillus* sp. UFPEDA 472. Absolute control plants are non-inoculated plants cultivated without nitrogen supplementation. Different lowercase letters represent significant differences among treatments for each variety. Different uppercase letters indicate significant differences between the two varieties by Tukey’s test ($P < 0.05$).

DISCUSSION

This study evaluated the inoculation of lima bean, varieties branca and boca de moça, with *Bacillus* sp., UFPEDA 472 strain, on parameters related to plant growth and development. *Bacillus* are considered plant growth-promoting bacteria (PGPB) and, jointly with *Pseudomonas* and *Azospirillum*, are the most studied group of PGPB in the world (DUCA et al., 2014; GROBELAK et al., 2015). This genus comprises of species with plant growth-promoting characteristics occupying the rhizosphere of many plant species. It provides beneficial effects to the host plant (FIGUEIREDO et al., 2011; HUANG et al., 2014). In general, the varieties of lima bean studied herein increase plant growth parameters when inoculated with *Bacillus* sp. UFPEDA 472. The promotion of plant growth by PGPB *Bacillus* involves a large number of mechanisms, such as nitrogen fixation, mineralization of nutrients, production of siderophores and hormones such as auxins and gibberellins (HUANG et al., 2014; PII et al., 2015).

The absolute growth rate was calculated based on the plant height of lima bean, and therefore is strongly related to photosynthesis. According to Lopez et al. (2012), bacterial endophytes, such as some *Bacillus* strains, may increase the photosynthetic activity. Lima bean plants inoculated with *Bacillus* sp. UFPEDA 472, mainly the lima bean var. boca de moça (Figure 1A), were capable of growing in a nitrogen-free medium. These results confirmed that *Bacillus* sp. UFPEDA are capable of fixing nitrogen and therefore promoting plant growth, as observed by Xu et al. (2014) upon studying the plant growth-promoting activity of *B. subtilis* HYT-12-1 on tomato seedlings. If nitrogen influences the structure and the composition of the photosynthetic apparatus (ANDREWS; LEA, 2013), its absence may result in a decreased photosynthetic rate and therefore in less plant growth, as observed for the absolute control.

When inoculated with *Bacillus* sp. UFPEDA 472, the lima bean var. branca was superior than lima bean var. boca de moça in terms of root length and root dry mass (Figure 2A and 3B). According to Figueiredo et al. (2011), the growth-promoting ability of some bacteria may be highly specific to certain plant species, varieties, cultivars and genotypes. This is probably the reason why PGPB *Bacillus* sp. UFPEDA 472 effectively interacted with the lima bean var. branca, resulting in the promotion of root development. The increase in root length of groundnut inoculated with *B. licheniformis* MML2501 was associated with production of IAA

(PRASHANTH; MATHIVANAN, 2010). In wheat treated with spores of *B. subtilis* 11BM, a stimulation to growth was observed associated with a transient increase in IAA (EGORSHINA et al., 2012). IAA is the most commonly phytohormone studied worldwide. It belongs to the auxin group, which acts mainly on the formation of lateral roots and root hair cells (MUDAY et al., 2012; HUANG et al., 2014).

Bacterial isolates that produces IAA, when inoculated in mung bean (*Vigna radiata*), increase their shoot length and stem diameter (MATSUOKA et al., 2015). Similarly, it was reported in this study increases in stem diameter for the lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472. This is probably because the growth response of this plant is related with a synergistic interaction between IAA and 1-aminocyclopropane-1-carboxylate (ACC) deaminase (MUDAY et al., 2012; MATSUOKA et al., 2015). ACC is a direct ethylene precursor, and ACC deaminase metabolizes ACC reducing the volume of ethylene (PII et al., 2015). Ethylene in low concentrations facilitates plant growth, whereas high ethylene concentrations cause suppression of growth (HUANG et al., 2014). Glick (2014) stresses that PGPB secreting IAA and producing ACC deaminase may efficiently promote plant growth due to the action of IAA simultaneously with the control of an excessive ethylene production.

IAA, abscisic acid, gibberellin, cytokinins and ethylene are growth regulators produced by PGPB acting in shoots and roots, increasing the intake of nutrients by plants (HUANG et al., 2014). For example, *Bacillus megaterium* promoted the growth of *Arabidopsis thaliana* and this response requires an intact cytokinin-signaling pathway (ORTÍZ-CASTRO et al., 2008). The authors reported that the bacterial inoculation caused a threefold increase in shoot fresh mass in wild-type *A. thaliana* (Col-0 and C-24 ecotypes) in relation to non-inoculated plants. In this study, lima bean inoculated with *Bacillus* sp. UFPEDA 472 increased the fresh and dry mass of shoots, mainly the lima bean var. boca de moça (Figure 3 and 5). Similar findings were recorded for shoot dry mass of rice plants inoculated with *Bacillus* sp. SVPR30 (BENEDUZI et al., 2008), quailbush inoculated with *B. pumilus* ES4 or RIZO1 grown in a soil with mine tailings (DE-BASHAN et al., 2010), and *Brachypodium* inoculated with *B. subtilis* B26 (GAGNÉ-BOURQUE et al., 2015).

The inoculation of lima bean with *Bacillus* sp. UFPEDA 472 resulted in a significant increase in shoot:root ratio. When inoculated with *Bacillus*

sp. UFPEDA 472, the lima bean var. boca de moça achieved the maximum value of shoot:root ratio in relation to the other treatments. Shoot:root ratio increased by 20% in red pepper under salt stress when inoculated with *B. aryabhatai* RS341, which is an ACC deaminase-producing halotolerant bacterium (SIDDIKEE et al., 2012). Furthermore, the inoculation of teak with *B. subtilis* KED-4 and the co-inoculation simultaneously with *B. subtilis* KED-4 and *Pseudomonas fluorescens* TCO-6, two bacteria with a phosphate-solubilizing capacity, increased the shoot:root ratio probably because of increases in the phosphate availability in the soil solution (MOHAN; RADHAKRISHNAN, 2012). In general, the change in the shoot:root ratio is an adaptive response to changes in the nutrient availability (PÉRET et al., 2011; MOHAN; RADHAKRISHNAN, 2012).

An increase in shoot:root ratio is expected when plants show adequate amounts of nutrients in their tissues, especially nitrogen and phosphate (PÉRET et al., 2011; SIDDIKEE et al., 2012). Nitrogen is an essential nutrient strongly related with chlorophyll, a pigment found in plants and vital for photosynthesis (HOHMANN-MARRIOTT; BLANKENSHIP, 2011). The data presented in Figure 6 show that the inoculation of lima bean with *Bacillus* sp. UFPEDA 472 results in a significant increase in total chlorophyll. Similar results were recorded for radish inoculated with *B. subtilis* (MOHAMED; GOMAA, 2012), for potato inoculated with *B. cereus* (DAWWAM et al., 2013) and for mustard inoculated with *B. megaterium* mj1212, a soil microorganism able to solubilize phosphate (KANG et al., 2014). In addition, Stefan et al. (2013) observed that *B. pumilus* or *B. mycoides* increased chlorophyll levels of runner bean (*P. coccineus*), a specie closely related to lima bean.

An adequate plant-bacteria interaction may trigger an increase in the synthesis of chlorophyll and thus lead to an increase in photosynthesis (KANG et al., 2014). Thereby, the flow of photoassimilates may be directed to the growth of shoots, aiming develop more leaf areas photosynthetically active. In consequence, the shoot:root ratio increases. This is probably due to the presence of *Bacillus* sp. UFPEDA 472 in the rhizosphere of lima bean, especially in the lima bean var. boca de moça. This stimulates the photosynthetic process and a more efficient absorption of nutrients (MOHAMED; GOMAA, 2012; DAWWAM et al., 2013). This statement is based on the strong increase in total chlorophyll shown by the lima bean var. boca de moça when inoculated with *Bacillus* sp. UFPEDA 472 in relation to non-inoculated plants. In addition, the lima bean var. boca de moça inoculated with *Bacillus* sp. UFPEDA 472 has a higher shoot dry mass and shoot:root ratio compared to the other treatments.

CONCLUSION

The increase in plant growth of the lima bean var. boca de moça, when inoculated by PGPB *Bacillus*, is the consequence of a positive and adequate interaction between this variety of lima bean and the strain UFPEDA 472 of *Bacillus* sp. However, further studies are needed to explain what are the additional characteristics and/or factors involved in the promotion of plant growth induced by PGPB *Bacillus*.

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RESUMO: Bactérias promotoras de crescimento em plantas (BPCP) compreendem microrganismos do solo que causam efeitos positivos no crescimento vegetal. A hipótese de que a inoculação de feijão-fava com BPCP *Bacillus* estimula o crescimento vegetativo, o qual pode resultar em uma maior produtividade vegetal, foi testado. Assim, este estudo objetivou avaliar o efeito da inoculação de duas variedades de feijão-fava (branca e boca de moça) com *Bacillus* sp., estirpe UFPEDA 472, baseado em respostas de crescimento vegetal. O experimento foi conduzido em casa de vegetação usando um delineamento completamente ao acaso. Três tratamentos foram aplicados separadamente à cada variedade de feijão-fava: o primeiro utilizou a inoculação com *Bacillus* sp. UFPEDA 472, um foi submetido à suplementação com nitrogênio e um foi o controle absoluto (plantas não inoculadas). As seguintes variáveis foram avaliadas: taxa de crescimento absoluto e relativo, comprimento das raízes, diâmetro do caule, massa fresca e seca da parte aérea e raízes, relação parte aérea:raiz e clorofila total. Os resultados mostram que o feijão-fava exibiu melhores respostas de crescimento quando inoculadas com *Bacillus* sp. UFPEDA 472 em relação ao tratamento com nitrogênio e/ou às plantas não inoculadas. Quando inoculado com *Bacillus* sp. UFPEDA 472, o feijão-fava var. branca foi superior à boca de moça em termos de comprimento e massa seca das raízes. O feijão-fava var. boca de moça inoculado com *Bacillus* sp. UFPEDA 472 foi superior à branca na taxa de crescimento absoluto, diâmetro do caule, massa fresca e seca da parte aérea, relação

parte aérea:raiz e clorofila total. No geral, o feijão-fava var. boca de moça inoculado com *Bacillus* sp. UFPEDA 472 mostrou melhor performance de crescimento. Nossos resultados sugerem que as respostas de crescimento das variedades de feijão-fava branca e boca de moça são relacionadas com uma interação positiva com o *Bacillus* sp. UFPEDA 472.

PALAVRAS-CHAVE: *Phaseolus lunatus*. BPCP. Taxa de crescimento absoluto. Clorofila.

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