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ORIGINAL PAPER

Rhizobacteria control damping-off and promote growth of lima bean with and without co-inoculation with *Rhizobium tropici* CIAT899

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Abstract

Rhizoctonia solani compromises the production of lima bean, an alternative and low-input food source in many tropical regions. Inoculation of bacterial strains has been used, but research on their biocontrol and growth promotion potential on lima bean is scarce. The objective of this study was to evaluate the effects of inoculation with rhizobacterial strains of the genera *Bacillus*, *Brevibacillus, Paenibacillus*, *Burkholderia, Pseudomonas*, and *Rhizobium* in combination or not with N_2-fixing *Rhizobium tropici* on the control of damping-off disease and growth promotion in lima bean plants. Greenhouse experiments were conducted to evaluate the inoculation with bacterial strains with biocontrol potential in combination or not with *R. tropici* in substrate infected with *R. solani* CML 1846. Growth promotion of these strains was also assessed. Strains of *Brevibacillus* (UFLA 02-286), *Pseudomonas* (UFLA 02-281 and UFLA 04-885), *Rhizobium* (UFLA 04-195), and *Burkholderia* (UFLA 04-227) co-inoculated with the strain CIAT 899 (*Rhizobium tropici*) were the most effective in controlling *R. solani*, reducing the disease incidence in 47–60% on lima bean. The promising strains used in the biocontrol assays were also responsive in promoting growth of lima bean under disease and sterile conditions. A positive synergistic effect of co-inoculation of different genera contributed to plant growth, and these outcomes are important first steps to improve lima bean production.

Keywords *Phaseolus lunatus* · *Rhizoctonia solani* · *Thanatephorus cucumeris* · Rhizoctoniosis · PGPR · Biocontrol

Introduction

Lima bean (*Phaseolus lunatus* L.) is the second most commercialized crop from the genus *Phaseolus* (Fofana et al. 1999). Worldwide, lima bean shares the importance of leguminous crops with soybean, common beans, and cowpea as direct plant protein source. The USA is one of the most prominent producers with over 12,000 hectares cultivated (USDA 2019). In Brazil, lima bean is grown by small farmers mainly in the Northeastern region, under semi-arid climate as a subsistence crop. Yield and technological inputs are usually low, but there is always a demand for lima bean as an alternative protein source (Alves et al. 2008). The crop is severely affected by different groups of the *Rhizoctonia* complex, and symptoms are diverse (Assunção et al. 2011; Yang and Li 2012).

Damping-off caused by the fungus *Rhizoctonia solani* Kuhn is the most substantial cause of root disease in the world for several crops, such as maize, rice, wheat, soybean, peanut, dry bean, potato, cotton, etc. (Lamichhane et al. 2017; Tziros and Karaoglanidis 2022). *R. solani* severely compromises the production of beans from the genus *Phaseolus*. The damage caused by *R. solani* occurs up to three weeks after emergence, and symptoms include seed and root rot, stem canker, leaf blight, and seedling damping-off (Ajayi-Oyetunde and Bradley 2018).

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Control of *R. solani* can be very difficult due to its virulence, a wide host range, transmission through seeds, and overwintering as a saprotroph in the crop stubble or as sclerotia (Ajayi-Oyetunde and Bradley 2018). In many regions of the world, to prevent disease losses, lima bean farmers leave the infected areas, which has a significant economic impact and depreciates the land. The traditional control methods, such as the use of more resistant cultivars, fungicides, and crop rotation, might reduce the soil pathogen population but may not be satisfactory to control the disease or its adoption is rare for faba bean as it is for other legumes and pulses. Thus, other methods should be combined to keep *R. solani* below the damage threshold level (Lamichhane et al. 2017). In Brazil, the registration of biocontrol products for plant disease management is based on the target pathogen and not the crop. Some strains of *Thrichoderma* spp., *Bacillus pumillus*, and *Burkholderia cepacia* are available as commercial products (Bettiol et al. 2012). Therefore, there is no product specifically registered for lima bean damping-off caused by *R. solani* and there is no scientific endorsement with peer-reviewed publication for this matter. The selection of lima bean genotypes resistant to damping-off is also still a challenge, and the search for alternative methods is essential to limit disease damage, reduce the density of the pathogen population in the soil, and increase crop yield. There are no currently available lima bean cultivars that are resistant to *R. solani* in Brazil.

The use of plant growth-promoting rhizobacteria (PGPR) has been suggested as a biological control for damping-off (Noronha et al. 1995; Martins et al. 2018) and is used in Brazil as part of integrated disease management on other crops, such as common bean. For common bean plants, studies show that it is possible to control damping-off disease with PGPR that also have positive effects on nodulation and N fixation (Elkoca et al. 2010; Martins et al. 2018; Ferreira et al. 2020). The synergistic PGPR effects are especially advantageous considering organic, agroecological, and low-input production systems that do not use mineral N or synthetic fungicides. However, to the best of our knowledge, there is no report on control of damping-off with PGPR or on plant growth promotion mediated by beneficial bacteria for lima bean.

Previous studies from our laboratory identified several strains of different bacterial genera with various growth promotion traits (Ferreira et al. 2012, 2018; Costa et al. 2013). Some strains were tested previously for control of damping-off in common bean, and strains of the genera *Pseudomonas* and *Brevibacillus* were considered promising (Ferreira et al. 2020). Other studies also report these two genera for control of damping-off disease in common bean (Martins et al. 2018) and cowpea (Noronha et al. 1995). Strains from other genera, such as *Bacillus*, *Burkholderia,* and *Paenibacillus*, have also been reported as biocontrol of *R. solani* damping-off (Jung et al. 2003; Quan et al. 2006; Leite et al. 2013). Furthermore, the literature also reports that the biocontrol effect of PGPR can be enhanced when in co-inoculation with species of *Rhizobium,* including *R. tropici* (Jensen et al. 2002; Kalantari et al. 2018). The mechanisms promoted by these bacterial genera are diverse and include production of antibiotics, bacteriocins, siderophores, enzymes, plant hormones, and others (Jiao et al. 2021).

We hypothesized that 15 strains with growth promotion and damping-off control abilities that were promising in common beans would also be important biological assets for the disease control in lima bean since these plants are from the same genus. Furthermore, co-inoculation of *Rhizobium tropici* CIAT899 with these strains could promote a stronger biocontrol activity, as previously shown in other works for common-bean (Jensen et al. 2002; Ferreira et al. 2020). Therefore, the objective of this study was to evaluate the effects of inoculation with 15 rhizobacterial strains of the genera *Bacillus*, *Brevibacillus, Paenibacillus*, *Burkholderia, Pseudomonas*, and *Rhizobium* in combination or not with *Rhizobium tropici* CIAT899 on the control of damping-off disease and on growth promotion in lima bean plants.

Materials and methods

Preparation of microbial inocula

In this study, 15 bacterial strains selected from the collection of the Soil Microbiology and Biological Processes Laboratory of the Universidade Federal de Lavras, Brazil, were used (Table 1). These strains had been isolated from different sites and land use systems in Brazil and were tested for several growth promotion traits in vitro and on common bean, cowpea, and siratro. The *Rhizobium tropici* strain CIAT 899 was also used in the experiments. *Rhizobium tropici* CIAT 899 is a genetically stable N_2-fixing strain adapted to tropical conditions and tolerant to abiotic stress conditions, such as high temperatures and acidity (Martínez-Romero et al. 1991; Graham 1992). The CIAT 899 strain has been approved by the Brazilian Ministry of Agriculture as an inoculant for common bean; however, it is not able to establish N_2-fixing symbiosis with lima bean. All bacterial strains were cultured separately in liquid 79 medium containing (g L^{-1}): K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; mannitol, 10.0, and yeast extract, 0.4; pH 6.8–7.0 (Fred and Waksman 1928). The inocula were cultivated at 28 °C under shaking at 110 rpm for 3 days for fast-growing strains and 7 days for slow-growing strains. Bacterial dose was applied at 10^{8-9} CFU ml^{-1}.

Rhizoctonia solani CML 1846 was provided by the Mycological Collection of the Universidade Federal de Lavras. We have used the Anastomosis group 4, which has a broad

Table 1 Identification and origin of the strains

Strain	Species	Origin/land use[a]	Growth characteristics in 79 medium		Accession number in GenBank (NCBI)	References
			GR[b]	pH		
UFPI B3-9	*Paenibacillus* sp.	PI/AGRI	Fast	Acidic	KF738126	Costa et al. (2013)
UFPI B4-9	*Paenibacillus* sp.	PI/AGRI	Fast	Alkaline	KF738122	Costa et al. (2013)
UFLA 02-281	*Pseudomonas* sp.	AM/P	Fast	Acidic	KU613382	Ferreira et al. (2018)
UFLA 02-286	*Brevibacillus* sp.	AM/P	Fast	Acidic	KU613387	Ferreira et al. (2020)
UFLA 02–290	*Bacillus megaterium*	AM/P	Fast	Acidic	KU613390	Ferreira et al. (2018)
UFLA 02-293	*Pseudomonas putida*	AM/P	Fast	Acidic	KU613392	Ferreira et al. (2018)
UFLA 03-10	*Paenibacillus kribbensis*	MG/SDTF	Fast	Acidic	JQ041885	Marra et al. (2012)
UFLA 03–107	*Bacillus subtilis*	MG/SDTF	Fast	Acidic	JQ041895	Marra et al. (2012)
UFLA 03-18	*Pseudomonas* sp.	AM/SF	Fast	Acidic	KC879697	Oliveira-Longatti et al. (2014)
UFLA 03–26	*Pseudomonas* sp.	AM/P	Fast	Acidic	KC879702	Oliveira-Longatti et al. (2014)
UFLA 04–122	*Burkholderia fungorum*	AM/PF	Fast	Acidic	JF412046	Ferreira et al. (2012)
UFLA 04–195	*Rhizobium miluonense*	AM/FA	Fast	Acidic	JF412048	Ferreira et al. (2012)
UFLA 04–227	*Burkholderia fungorum*	AM/AGRI	Fast	Neutral	JF412051	Ferreira et al. (2012)
UFLA 04–885	*Pseudomonas koreeensis*	RO/P	Slow	Alkaline	KC879711	Oliveira-Longatti et al. (2014)
629	*Bacillus subtilis*	BA/AGRI			JQ435867	Leite et al. (2013)
CIAT 899	*Rhizobium tropici*	Colombia	Fast	Acidic	–	Martínez-Romero et al. (1991)

[a]States of *AM* Amazônia, *MG* Minas Gerais, *PI* Piauí, *RO* Rondônia, *AGRI* agriculture, *P* pasture, *PF* primary forest, *SF* secondary forest, *FA* secondary forest in advanced stage of regeneration, *SDTP* semi-deciduous tropical forest

[b]*GR* growth rate, fast: 2–3 days, slow: 6–10 days

host range and is the most ubiquitous one. The inoculum was prepared in flasks containing 100 g of autoclaved hulled rice substrate (120 °C, 30 min, 101 kPa) and 40 mL of sterilized distilled water. Each flask received a 5-mm-diameter disk of the fungal culture, previously cultured in potato dextrose agar medium (200 g L^{-1} potato infusion, 20 g L^{-1} dextrose, and 17 g L^{-1} agar) for 7 days. After 10 days of incubation at 25 °C, the colonized substrate was placed in paper bags and dried for 48 h before being ground in a blender (Noronha et al. 1995).

Efficiency of bacterial strains for *R. solani* control in lima bean

The experiment to test the biocontrol of *R. solani* by the PGPR strains was carried out in a completely randomized experimental design with four replicates and the following treatments: single inoculation with the 15 PGPR strains; co-inoculation of the 15 PGPR strains with *R. tropici* CIAT 899; CIAT 899 inoculated alone; and a control without inoculation. The strain 629 of *Bacillus subtilis* was used as a positive control since it was previously identified as an antagonistic strain (Leite et al. 2013). The experiment was repeated 30 days afterward under the same conditions.

Two greenhouse experiments with the same treatments were conducted at the Universidade Federal de Lavras (21.2275° S, 44.9781° W) in a *Latossolo Vermelho distroférrico* soil (Oxisol, in approximation to the taxonomy of the United States Department of Agriculture) to test the efficacy of the 15 bacterial strains against *R. solani* on lima bean. The soil (pH = 5.6; N = 1,030 mg kg^{-1}; P = 3 mg kg^{-1}; K = 0.14 $cmol_c$ dm^{-3}; Al = 1.40 $cmol_c$ dm^{-3}; Ca + Mg = 0.80 $cmol_c$ dm^{-3}) was mixed with washed sand at a 2:1 (soil:sand) ratio. The substrate was autoclaved twice at 121 °C and 147 kPa for 1 h. After 15 days, the mixture was placed in 0.5-L plastic pots and the moisture was adjusted to 60% of field capacity. The substrate was inoculated with *R. solani* CML 1846 using the inoculum described above 24 h before sowing at a dose of 50 mg kg^{-1}. A previous test with the inoculum ensured that all plants were infected by the pathogen.

Seeds of lima bean variety Rajada, obtained in Floriano, Piauí, Brazil, were surface disinfected using 70% ethanol for 30 s and 2% sodium hypochlorite for 2 min. After disinfection, the seeds were thoroughly washed in sterilized distilled water. Four seeds were sown for each pot (4 pots per treatment) and each seed received 1 mL of bacterial inoculant, according to the respective treatment. The non-inoculated control treatment received 1 mL of sterilized water. Pots were constantly irrigated to maintain field capacity at 60%.

The temperature inside the greenhouse ranged from 22 to 25 °C for the first trial and 24–27 °C for the second.

Lima bean plants were collected at second true leaf stage (approximately 27 days after sowing), air-dried, and evaluated for plant height (PH); shoot, root, and total dry weight (SDW, RDW, and TDW); number of nodules (NN); nodule dry weight (NDW); germination rate (GR); and disease severity, according to Noronha et al. (1995). Disease severity was measured every 3 days over 15 days after emergence based on a 0 to 4 scale, where 0 = no symptoms; 1 = small lesions on the hypocotyl; 2 = large lesions on the hypocotyl; 3 = severely damaged hypocotyl, damping-off; 4 = non-germinated seeds. The disease severity was transformed to a disease index (DI), according to McKinney (1923), and used to calculate the area under the disease progress curve (AUDPC), according to the equation (Shaner and Finney 1977):

$$AUDPC = \sum_{i=1}^{n} \left[\left(Y_{i+1} + Y_i\right)/2\right]\left[X_{i+1} + X_i\right]$$

In which Y_i = disease severity (per unit) at the ith observation; X_i = time (days) at the ith observation, and n = total number of observations.

All data were assessed for normality and homoscedasticity of the residues and by analysis of variance. The two replications of the test were compared by the *F*-test since there were only two factors. All variables were presented as means of the two repetitions of the experiment. Since the repetitions were performed 30 days apart, environmental conditions could be the cause of differences in some variables. In this case, the means of both repetitions could approach a more realistic condition instead of just one set of data. Treatments were then compared by analysis of variance and means were grouped by the Scott–Knott test ($p < 0.05$). A principal component analysis (PCA) for the biocontrol experiment was performed with the means of the variables to calculate the scores. Results were presented in the form of biplots. The PCA was calculated with the package vegan (Oksanen et al. 2019). Statistical analyses were carried out in Sisvar 5.7 software (Ferreira 2011), R environment (R Core Team 2021), and the R Studio platform (RStudio 2021).

Severity of damping-off in relation to *R. solani* inoculum density in lima bean

The objective of the following experiment was to test the biocontrol effect of bacterial strains depending on the pathogen density of *R. solani* CML 1846. Strains with the best biocontrol effect observed in the first experiment were included in this experiment. A completely randomized experimental design was used in a 6 × 5 factorial arrangement. The factors consisted of five co-inoculations of strains UFLA 02-281, UFLA 02-286, UFLA 04-195, UFLA 04-227, and UFLA 04-885, with CIAT 899, plus the inoculation of CIAT 899 alone and five pathogen doses (0, 50, 100, 150, and 200 mg kg^{-1}). Four replications were carried out. Substrate, microbial inocula, and seeds were prepared as previously described. Five seeds were sown in 0.5-L pots with autoclaved substrate (2:1 mixture of soil:sand) with the respective dose of the pathogen inoculum. After emergence, pots were thinned to two plants. Plants were evaluated as described in the previous experiment. Data were assessed for normality and homoscedasticity of the residues, by analysis of variance, and by polynomial regression for the pathogen inoculum dose and the disease index (DI).

Plant growth promotion of lima bean under different N supply

The strains used in this work were previously tested for growth promotion on other crops, such as common bean. We hypothesized that they could also be able to promote growth of lima bean plants under axenic conditions and different N concentrations/supply, following the arrangement described in Ferreira et al. (2020). A completely randomized experimental design was used in a 15 × 3 factorial arrangement. The first factor consisted of inoculation of the 14 PGPR strains plus a control without inoculation. The second factor was the supply of N, composed of inoculation with the CIAT 899 strain of *R. tropici*, a low mineral N concentration of 5.25 mg L^{-1} (LN), and a high mineral N concentration of 52.5 mg L^{-1} (HN). The treatments that received CIAT 899 also received 5.25 mg L^{-1} of N. Different mineral N concentrations were provided through a nutrient solution using KNO_3 and $Ca(NO_3)_2$ as sources of N (Hoagland and Arnon 1950). Three replications were carried out.

The experiment was conducted in Leonard jars containing a 1:1 (v:v) mixture of sand and vermiculite as substrate and the nutrient solution. The jars were autoclaved at 121 °C and 147 kPa for 1 h. Four surface-sterilized lima bean seeds were sown in each jar, and each seed received 1 mL of the respective bacterial inoculant according to the treatments. After sowing, the jars were covered with a layer of paraffin sand (mixture of 10 kg of washed sand, 1 mL of chloroform, and 10 g of paraffin) to avoid contamination. After emergence, seedlings were thinned to leave one plant per jar. The nutrient solution was replenished periodically throughout the experiment.

Plants were harvested at the beginning of the flowering stage, approximately 40 days after sowing. Shoots and roots were air dried; and shoot, root, and total dry weight were assessed (SDW, RDW, and TDW, respectively). Shoots were then ground, and N concentration was determined by the semi-micro-Kjeldahl method (Liao 1981). Shoot N accumulation (SNA) was calculated as SDW × N concentration.

Statistical analyses were carried out in Sisvar 5.7 software (Ferreira 2011), R environment (R Core Team 2021), and the R Studio platform (RStudio 2021). Data were evaluated for normality and homoscedasticity of the residues, and analysis of variance and means were compared by the Scott–Knott test ($p < 0.05$). A PCA was performed for the plant growth promotion experiment with the means of the variables. Results were presented in the form of biplots. The PCA was calculated with the package vegan (Oksanen et al. 2019).

Results

Efficiency of bacterial strains for *R. solani* control in lima bean

Most of the treatments led to an increase in the germination rate (GR) of the seeds compared to the control without inoculation (Table 2). The inoculations UFLA 02-281, UFLA 02-286, and UFLA 02-286 + CIAT 899 resulted in 100% seed germination. Several co-inoculations led to superior results for plant height (PH) in comparison with plants inoculated with the efficient CIAT 899 strain alone (Table 2). TDW and SDW followed the same pattern, with almost the same treatments accumulating more biomass than the treatment with CIAT 899 alone (Table 2). Only plants inoculated with UFLA 02-293 were similar to the non-inoculated control. For RDW, several treatments led to an increase in root biomass compared to the control, including inoculation with CIAT 899.

All treatments reduced the disease index (DI) in comparison with the non-inoculated control (Table 2). Greater significant reductions in DI and AUDPC were found in the treatments UFLA 02-281, UFLA 02-286, UFLA 04-195, UFLA 04-885, and UFLA 04-227, all co-inoculated with CIAT 899. These treatments reduced the DI by up to 60%. The PCA (Fig. 1) confirms these results as these co-inoculations clustered and showed positive correlation with the plant-growth variables (except RDW), while being opposite to the disease variables. Other treatments, such as UFPI B4-9 + CIAT 899, UFLA 03-10, UFLA 04-122, also exhibited low values of AUDPC (Table 2). All treatments showed lower AUDPC than the control, except for the UFLA 02-290 and UFLA 02-290 + CIAT 899 treatments. There was a strong correlation between DI and AUDPC as showed by the PCA and, generally, treatments that showed low DI also showed low AUDPC.

The PCA (Fig. 1) also shows positive correlations among SDW, TDW, and PH, while germination and RDW were low or not correlated with these variables. As expected, DI and AUDPC were negatively correlated with the plant growth variables. It is presumed that higher disease incidence on the plants prevents their growth. Despite the abovementioned co-inoculations stood out, the other treatment scores were scattered throughout the components. Interestingly, the other co-inoculations were positively related to the PC1 and the disease indices, while some single inoculations were not. The control that did not receive any inoculation was closely related with the disease indices and negatively related to all other variables.

Severity of damping-off in relation to *R. solani* inoculum density in lima bean

There was no evidence of damping-off in lima bean plants when the pathogen was not inoculated in the substrate. When plants were grown in the substrate with different doses of *R. solani*, plants with the UFLA 02-286 + CIAT 899, UFLA 04-195 + CIAT 899, UFLA 04-227 + CIAT 899, and UFLA 04-885 + CIAT 899 treatments were able withstand the pathogen at the dose of 50 mg kg^{-1} than plants in the treatment with CIAT 899 alone (Fig. 2). At the dose of 100 mg kg^{-1}, plants co-inoculated with UFLA 04-195 + CIAT 899, UFLA 04-227 + CIAT 899, and UFLA 04-885 + CIAT 899 showed moderate pathogen management. At the highest pathogen doses, 150 and 200 mg kg^{-1}, none of the treatments were able to reduce the disease, and the DI approached 100%. Although they did not perform as much as the other treatments, inoculations with UFLA 02-281 + CIAT 899 and CIAT 899 alone were able to promote some disease management at the lowest inoculum dose (50 mg kg^{-1}).

Plant growth promotion of lima bean under different N supply

Inoculation of the PGPR strains on lima bean plants showed diverse responses in the three forms of N supply (Table 3). Inoculation with UFLA 02-290, UFLA 02-293, UFLA 03-107, and UFLA 04-885 led to similar SDW in all the forms of N supply. Plants inoculated with UFPI B3-9 had greater SDW than plants that received the low N concentration (LN). In contrast, plants inoculated with UFLA 03-18 developed more SDW in the LN than when co-inoculated with CIAT 899. All other inoculations were more responsive when associated with a high N concentration (HN). Within each form of N supply, responses varied according to the strain inoculated. UFLA 02-286, UFLA 02-293, UFLA 04-195, and UFLA 04-227 promoted greater shoot growth when co-inoculated with CIAT 899 and in LN concentration than the controls inoculated with CIAT 899 alone and the non-inoculated control. Among the plants fertilized with HN concentration, the best shoot growth was promoted by UFLA 03-18. For RDW, UFLA 02-286, UFLA 02-293, UFLA 03-10, UFLA 04-227, and the control did not differ among the forms of N supply. The other inoculations accumulated more root dry weight when supplied with HN. In

Table 2 Germination (GERM), plant height (PH), total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), disease index (DI), and area under the disease progress curve (AUDPC) of lima bean plants inoculated with PGPR and co-inoculated with PGPR and *Rhizobium tropici* CIAT 899 grown on soil infected with *Rhizoctonia solani*

	GERM	PH	TDW	SDW	RDW	DI[a]	AUPDC[a]
	%	cm	g plant^{-1}			%	
Experiment repetitions[b]							
1st	81.9	5.05	0.37*	0.26*	0.10*	71.03	4205.7
2nd	80.8	5.29	0.29*	0.23*	0.07*	73.37	4321.7
Treatment[c]							
UFPI B3-9	60.0 e	3.97 d	0.52 a	0.41 a	0.10 a	66.72 d	3925.7 e
UFPI B3-9 + CIAT 899	65.0 e	5.63 b	0.39 b	0.27 b	0.12 a	88.52 b	4333.4 d
UFPI B4-9	77.5 d	4.41 d	0.48 a	0.35 a	0.13 a	68.52 d	4659.5 c
UFPI B4-9 + CIAT 899	83.1 c	6.88 a	0.45 a	0.35 a	0.10 a	85.50 c	3328.2 f
UFLA 02-281	100.0 a	5.45 b	0.39 b	0.33 a	0.06 b	79.88 c	4132.1 d
UFLA 02-281 + CIAT 899	75.0 d	6.03 a	0.45 a	0.39 a	0.06 b	42.35 g	3431.8 f
UFLA 02-286	100.0 a	4.98 c	0.36 b	0.32 a	0.05 b	82.54 c	4563.6 c
UFLA 02-286 + CIAT 899	100.0 a	4.38 d	0.44 a	0.37 a	0.07 b	35.83 g	4651.7 c
UFLA 02-290	85.0 c	4.35 d	0.23 c	0.16 c	0.06 b	83.25 c	5457.9 a
UFLA 02-290 + CIAT 899	80.0 c	4.65 c	0.32 c	0.26 b	0.06 b	81.67 c	5423.0 a
UFLA 02-293	65.0 e	5.10 c	0.16 d	0.10 d	0.06 b	82.75 c	4722.1 c
UFLA 02-293 + CIAT 899	65.0 e	5.41 b	0.30 c	0.20 c	0.10 a	70.63 d	5225.7 b
UFLA 03-10	95.0 b	4.76 c	0.25 c	0.17 c	0.08 b	71.83 d	3437.4 f
UFLA 03-10 + CIAT 899	80.0 c	4.46 d	0.28 c	0.17 c	0.11 a	73.79 d	4296.5 d
UFLA 03-107	92.5 b	5.44 b	0.32 c	0.26 b	0.06 b	83.89 c	4543.1 c
UFLA 03-107 + CIAT 899	75.0 d	4.23 d	0.30 c	0.21 c	0.09 a	62.08 e	4325.9 d
UFLA 03-18	80.0 c	5.00 c	0.24 c	0.15 c	0.08 b	84.67 c	3108.5 f
UFLA 03-18 + CIAT 899	65.0 e	5.18 b	0.31 c	0.18 c	0.13 a	80.52 c	4245.1 d
UFLA 03-26	80.0 c	5.43 b	0.40 b	0.31 b	0.10 a	76.54 d	4523.6 c
UFLA 03-26 + CIAT 899	80.0 c	5.00 c	0.34 b	0.21 c	0.13 a	71.33 d	4375.9 d
UFLA 04-122	85.0 c	5.20 b	0.35 b	0.28 b	0.06 b	66.79 d	3137.1 f
UFLA 04-122 + CIAT 899	82.5 c	6.11 a	0.25 c	0.17 c	0.07 b	83.54 c	4960.3 b
UFLA 04-195	92.5 b	5.60 b	0.35 b	0.28 b	0.07 b	84.10 c	4562.1 c
UFLA 04-195 + CIAT 899	95.0 b	6.10 a	0.41 b	0.32 a	0.09 a	38.96 g	3531.5 f
UFLA 04-227	65.0 e	5.55 b	0.27 c	0.21 c	0.06 b	80.63 c	4316.5 d
UFLA 04-227 + CIAT 899	92.5 b	6.40 a	0.44 a	0.33 a	0.10 a	48.54 f	2347.8 g
UFLA 04-885	83.8 c	4.58 c	0.28 c	0.18 c	0.10 a	87.29 b	4244.8 d
UFLA 04-885 + CIAT 899	98.8 a	4.63 c	0.45 a	0.36 a	0.08 b	40.83 g	3545.2 f
629	85.0 c	5.70 b	0.23 c	0.15 c	0.08 b	81.42 c	4326.5 d
629 + CIAT 899	85.8 c	5.83 b	0.24 c	0.15 c	0.08 b	69.17 d	4744.0 c
CIAT 899	75.0 d	5.25 b	0.37 b	0.25 b	0.12 a	60.69 e	4455.9 c
Control	60.0 e	3.95 d	0.12 d	0.07 d	0.05 b	95.67 a	5556.3 a

Means of the treatments refer to the mean of the two repetitions of the experiment for all variables

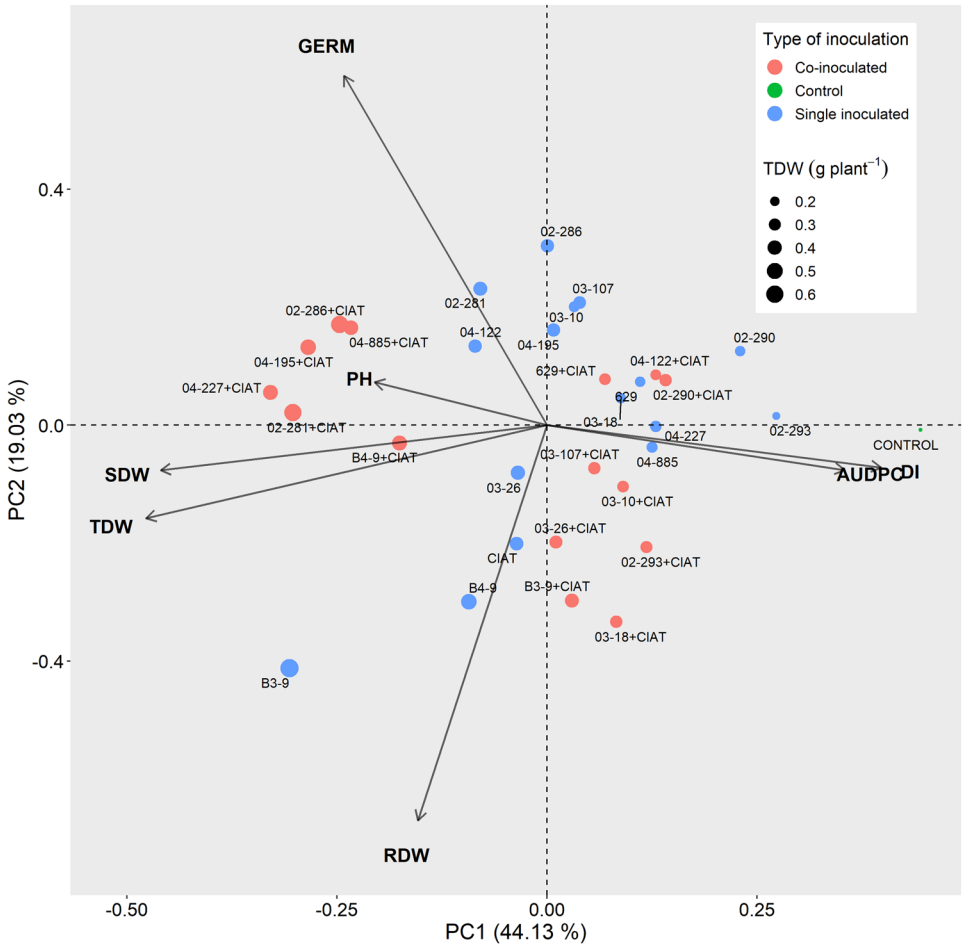
[a]Higher values indicate lower plant resistance to *Rhizoctonia solani*

[b]Mean values followed by * in the column differ by the *F* test ($P < 0.05$) for the experiment replications

[c]Mean values followed by the same letter in the column do not differ by the Scott–Knott test ($P < 0.05$) for the treatments

addition, there was no difference in RDM within each form of N supply.

Considering the TDW accumulated by the plants, similar results were found for UFLA 02-293, UFLA 03-107, UFLA 04-227, and UFLA 04-885, which did not differ among the forms of N supply, and for the other treatments that developed more in HN (Table 3). Plants inoculated with UFLA 02-290 accumulated similar TDW as in the HN concentration when they were co-inoculated with CIAT 899. Unfolding the inoculation factor in each form of N supply, the treatments with UFPI B3-9, UFLA 02-281, UFLA 02-286, UFLA 02-290, UFLA 02-293, UFLA 04-195, and UFLA 04-227 exhibited greater plant growth than inoculation with CIAT 899 alone. Among the



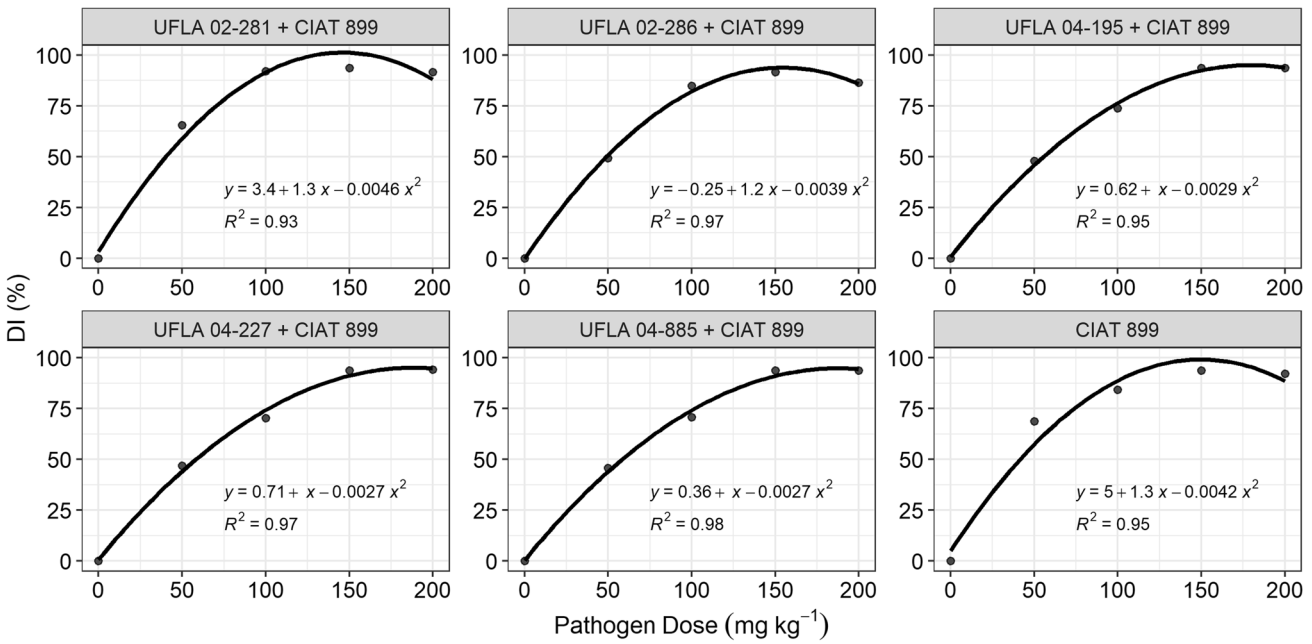


Fig. 1 Principal component analysis summarizing the results of the biocontrol experiment. Scores of the treatments means are displayed. Diameter size indicates plant growth in terms of total dry weight (TDW). PH, GERM, RDW, SDW, TDW, DI, and AUDPC stand for plant height, germination index, root dry weight, shoot dry weight, total dry weight, disease index, and area under the disease progress curve, respectively. The types of inoculation stand for the inoculation, co-inoculation (with CIAT 899 strain), or non-inoculated (control) with the studied strains

Fig. 2 Effect on disease index (DI) of lima bean co-inoculated with PGPR strains and *Rhizobium tropici* CIAT 899 in substrate infected with progressive *Rhizoctonia solani* inoculum doses of 0, 50, 100, 150, and 200 mg kg^{-1}. Higher values indicate lower plant tolerance to damping-off disease

Table 3 Total dry weight (TDW), shoot dry weight (SDW), root dry weight (RDW), and shoot nitrogen accumulation (SNA) of lima bean plants inoculated with PGPR under different forms of N supply

Treatment	TDW			SDW		
	g jar^{-1}					
	CIAT	LN	HN	CIAT	LN	HN
UFPI B3-9	4.51 aB	3.14 bB	7.18 bA	3.37 aB	1.88 bC	4.88 cA
UFPI B4-9	3.14 bB	3.18 bB	7.56 bA	1.94 bB	1.94 bB	5.35 bA
UFLA 02-281	3.74 aB	3.03 bB	6.58 bA	2.34 bB	1.91 bB	4.39 cA
UFLA 02-286	4.27 aB	4.77 aB	6.30 bA	3.24 aB	3.73 aB	4.86 cA
UFLA 02-290	3.61 aA	2.33 bB	4.26 cA	2.15 bA	1.55 bA	1.94 dA
UFLA 02-293	4.57 aA	4.70 aA	5.85 bA	2.95 aA	3.52 aA	4.04 cA
UFLA 03-10	1.64 bB	2.24 bB	5.11 cA	0.85 bB	1.54 bB	3.65 cA
UFLA 03-107	2.91 bA	2.26 bA	3.80 cA	1.77 bA	1.42 bA	1.95 dA
UFLA 03-18	3.38 bB	4.57 aB	9.52 aA	1.99 bC	3.41 aB	6.27 aA
UFLA 03-26	2.54 bB	3.57 bB	7.34 bA	1.55 bB	2.25 bB	5.26 bA
UFLA 04-122	3.04 bB	3.09 bB	7.18 bA	1.67 bB	1.98 bB	5.06 bA
UFLA 04-195	3.91 aB	4.74 aB	6.75 bA	2.98 aB	3.37 aB	4.64 cA
UFLA 04-227	3.78 aA	4.20 aA	6.52 bA	2.59 aB	3.28 aB	4.68 cA
UFLA 04-885	2.63 bA	3.34 bA	3.70 cA	1.55 bA	2.16 bA	1.64 dA
Control	3.12 bB	2.68 bB	6.27 bA	1.78 bB	1.29 bB	4.64 cA
Treatment	RDW			SNA		
	g jar^{-1}			mg jar^{-1}		
	CIAT	LN	HN	CIAT	LN	HN
UFPI B3-9	1.13 aB	1.26 aB	2.30 aA	93.12 aB	87.01 aB	162.59 bA
UFPI B4-9	1.19 aB	1.25 aB	2.21 aA	67.62 aB	63.35 aB	200.09 aA
UFLA 02-281	1.40 aB	1.12 aB	2.18 aA	29.34 bB	54.46 bB	96.46 cA
UFLA 02-286	1.03 aA	1.06 aA	1.44 aA	60.48 bA	34.50 bA	81.66 dA
UFLA 02-290	1.47 aB	0.78 aB	2.32 aA	73.57 aA	56.90 aA	94.34 cA
UFLA 02-293	1.62 aA	1.18 aA	1.81 aA	80.58 aA	36.59 bB	110.61 cA
UFLA 03-10	0.79 aA	0.70 aA	1.46 aA	15.57 bB	38.75 bB	127.90 cA
UFLA 03-107	1.13 aB	0.84 aB	1.86 aA	59.77 bA	34.09 bA	60.20 dA
UFLA 03-18	1.39 aB	1.16 aB	3.25 aA	105.54 aB	58.15 aB	230.27 aA
UFLA 03-26	0.99 aB	1.32 aB	2.09 aA	56.62 bB	69.69 aB	180.86 bA
UFLA 04-122	1.37 aB	1.11 aB	2.11 aA	45.82 bB	68.72 aB	147.00 bA
UFLA 04-195	0.94 aB	1.37 aB	2.11 aA	74.00 aB	77.61 aB	180.48 bA
UFLA 04-227	1.20 aA	0.93 aA	1.83 aA	79.94 aB	68.49 aB	176.59 bA
UFLA 04-885	1.08 aB	1.18 aB	2.07 aA	46.27 bA	31.50 bA	59.81 dA
Control	1.33 aA	1.39 aA	1.63 aA	48.21 bB	43.85 bB	147.50 bA

CIAT inoculation with *Rhizobium tropici* CIAT 899 at low mineral *N* concentration (5.25 mg L^{-1} N), *LN* low mineral N concentration (5.25 mg L^{-1} N), *HN* high mineral N concentration (52.5 mg L^{-1} N)

Means followed by lowercase letters in the same column compare the inoculation factors of a single N supply by the Scott–Knott test ($P < 0.05$). Means followed by uppercase letters in the same row compare the N supply factors of a single inoculation by the Scott–Knott test ($P < 0.05$)

plants fertilized with HN, only inoculation with UFLA 03-18 increased TDW. Notably, some inoculations (UFLA 02-290, UFLA 03-10, UFLA 03-107, and UFLA 04-885) reduced plant growth in HN compared to the non-inoculated control.

Shoot N accumulation (SNA) was similar among the forms of N supply for UFLA 02-286, UFLA 02-290, UFLA 03-107, and UFLA 04-885 (Table 3). Interestingly, plants inoculated with UFLA 02-293 had similar SNA when co-inoculated with CIAT 899 as when N was supplied at the HN concentration. Within the treatments of co-inoculation with CIAT 899 and LN concentration, the PGPR strains varied in their response regarding SNA, and some strains accumulated more N than the respective controls. In the HN

concentration, however, only inoculations with UFPI B4-9 and UFLA 03-18 were more effective than the non-inoculated control; in some cases, plants inoculated with some strains even accumulated less N than the control.

The PCA (Fig. 3) shows that almost all treatments that received a high dose of mineral N clustered and were positively correlated with SNA, SDW, and TDW. Exceptions are for the strains UFLA 02-290, UFLA 04-885, UFLA 03-107. Co-inoculated treatments and treatments that received the low N dose grouped separately from those that received the high N dose. Presumably, the plant growth variables were correlated with nitrogen accumulation, but correlations with RDW were low or null.

Discussion

This study provided evidence of the ability of PGPR to control rhizoctoniosis damping-off disease and to improve lima bean growth under different nitrogen sources. In general, PGPR act as a biological control by competing with unfavorable microorganisms or producing different compounds that inhibit or eliminate them. Inoculation of lima bean plants with strain CIAT 899 of *Rhizobium tropici* was able to reduce the incidence of the disease on the plants; however, co-inoculation of lima bean with other PGPR of different genera along with CIAT 899 was more efficient in controlling the disease and promoting plant growth in soil infected with the pathogen. The same effect was observed in common bean plants co-inoculated with CIAT 899, a strain that nodulates common bean, and different PGPR genera (Ferreira et al. 2020). *Rhizobium* strains can act as a biocontrol of pathogenic fungi (Buonassisi et al. 1986; Volpiano et al. 2018). Since CIAT 899 is a strain well adapted to weathered tropical soils and is able to provide N through biological N fixation in nodulating species, such as common bean, it could be an important asset for biological control of *Rhizoctonia solani*.

Among the PGPR strains tested, the treatments that exhibited lower DI also resulted in the highest accumulation of biomass and a low AUDPC. This is particularly notable for the co-inoculation treatments UFLA 02-281 + CIAT 899, UFLA 02-286 + CIAT 899, UFLA 04-195 + CIAT 899, UFLA 04-227 + CIAT 899, and UFLA 04-885 + CIAT 899. The literature reports that co-inoculation of PGPR with rhizobia strains may exert more efficient control on

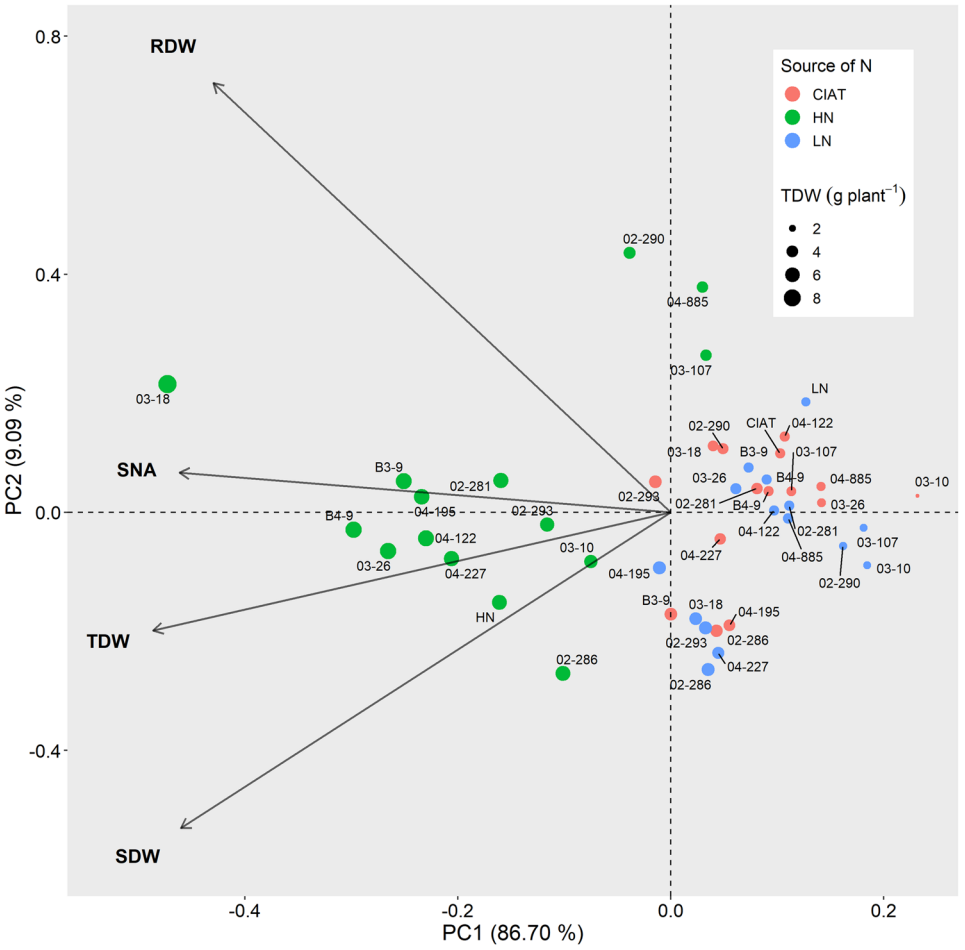


Fig. 3 Principal component analysis summarizing the results of the growth promotion experiment. Scores of the treatment means are displayed. Diameter size indicates plant growth in terms of total dry weight (TDW). RDW, SDW, TDW, and SNA stand for root dry weight, shoot dry weight, total dry weight, and shoot N accumulation, respectively. The colors of the circles indicate the source of N for the plants: CIAT stands for (co)inoculation with CIAT 899 strain; LN received 5.25 mg L^{-1} of N; and HN received 52.5 mg L^{-1} of N

pathogenic fungi than inoculation with PGPR alone. Our research group confirmed similar results on common bean with these co-inoculation combinations (Ferreira et al. 2020). *Rhizobium tropici* also limited the development of *R. solani* root rot in common bean when co-inoculated with *Bacillus subtilis* and improved growth and yield (Jensen et al. 2002). Co-inoculation of *Rhizobium* with PGPR from the genera *Bacillus* and *Pseudomonas* also helps other leguminous plants thrive against pathogenic fungi: chickpea (Hameeda et al. 2010), lentil (Akhtar et al. 2010), and white bean (Kalantari et al. 2018). However, there are no reports of co-inoculation of lima bean with *Rhizobium* and other PGPR strains. The effectiveness of biocontrol promoted by different PGPR indicates an important approach in bean production and may reduce the need for seed chemical treatments.

Lima bean plants were subjected to a progressive increase in the dose of the soil pathogen and withstood up to the dose of 100 mg kg^{-1} when treated with UFLA 04-885 (*Pseudomonas*), UFLA 04-195 (*Rhizobium*), and UFLA 04-227 (*Burkholderia*) co-inoculated with CIAT 899 (*Rhizobium*). The PGPR strains used in this experiment in co-inoculation with CIAT 899 also showed significant biocontrol activity in the previous experiment, with the lowest disease indices, as well as an increase in plant biomass. Similar results were achieved in common bean subjected to increasing soil pathogen doses (Ferreira et al. 2020).

There are different mechanisms that PGPR use to act as a biocontrol. Direct mechanisms are related to suppression of pathogenic microorganisms through production of antibiotics, bacteriocins, cyanhydric acid, metabolites, toxins, and enzymes (Raaijmakers et al. 2002; Compant et al. 2005; Jiao et al. 2021). Siderophores are considered another effective mechanism for microorganism biocontrol. The rationale is that the extracellular siderophore chelates iron and prevents the pathogen from acquiring that essential nutrient (Radziki et al. 2013). Antibiotics, bacteriocins, and siderophores are considered the most effective mechanisms for identifying potential biocontrol strains (Kloepper et al. 1980). Other indirect mechanisms also help control pathogens: rhizosphere colonization (Chiarini et al. 1998), induction of systemic resistance (Raza et al. 2016) and acquired resistance (Gao et al. 2015), and hormone interaction, such as auxins and gibberellins (Kazan and Manners 2009).

Rhizobium strains are able to produce inhibitory compounds or promote plant resistance through several ways. CIAT 899 strain was reported to produce volatile compounds and siderophore to control the pathogenic fungi *Sclerotium rolfsii*, while other *Rhizobium* strains produced considerable amounts of indole-acetic acid (Volpiano et al. 2018). Innumerous volatile compounds affect mycelial growth and virulence enzymes such as laccase, a virulence factor that protects the pathogen from plant defense molecules (Mayer and Staples 2002; Wheatley 2002). In addition to the well-stablished growth promotion traits of indole-acetic acid, this hormone can indirectly trigger plant immune responses, inhibit, or even stimulate fungal development (Kulkarni et al. 2013; Fu et al. 2015). Volpiano et al. (2018) found a weak, but significant correlation ($r = 0.447$, $p = 0.011$) between indole-acetic acid produced by *Rhizobium* spp. and *Sclerotium rolfsii* growth inhibition, but the suppression of collar rot disease in field trials could also be a consequence of the growth promotion abilities of *Rhizobium* strains on common bean.

The other genera were also reported to produce compounds that promote biocontrol activity. Strains of *Pseudomonas* produced siderophore, indole-acetic acid, proteolytic enzymes, such as chitinase, β-1,3-glucanase, and protease, and significantly reduced the disease index of tomato plants in the presence of *R. solani* on both glasshouse and field trials (Solanki et al. 2014). Chitinase and β-1,3-glucanase are directly involved in the degradation of fungal cell walls and insect cuticles, thus being considered important biocontrol enzymes produced by PGPR (Pereira et al. 2007). Species of *Burkholderia* have been considered as biocontrol agents for a long time. The genus is able to produce several hydrolytic enzymes such as chitinase and β-1,3-glucanase and induce systemic resistance (Ahmad et al. 2022), as well as to produce secondary metabolites with biocontrol activity, such as pyrrolnitrin, cepacin, and burkholdin (Biessy et al. 2022). *Bacillus, Brevibacillus,* and *Paenibacillus* spp. are also capable of producing hydrolytic enzymes and antibiotics (Budi et al. 2000; Raza et al. 2008; Jamali et al. 2020), and secondary metabolites (Arguelles-Arias et al. 2009; Canova et al. 2010; Jiang et al. 2015) to control *Rhizoctonia solani* and other soil pathogens.

The genera used in this study, i.e., *Bacillus*, *Brevibacillus Burkholderia*, *Paenibacillus*, *Pseudomonas*, and *Rhizobium*, are often described as biocontrol agents against several plant pathogens (O'Sullivan and O'Gara 1992; Depoorter et al. 2016; Rybakova et al. 2016; Das et al. 2017; Miljaković et al. 2020). These genera are also well known as plant growth promoters and are already recommended worldwide for diverse growth promotion traits in different plant species (Bhattacharyya and Jha 2012). Furthermore, to the best of our knowledge, research on growth promotion and biocontrol in lima beans is scarce, and there is a need to verify positive effects of PGPR on this plant species since *Phaseolus lunatus* is the second most important species in the *Phaseolus* genus (Fofana et al. 1999).

Plant growth promotion by the strains varied in each form of N supply. Overall, inoculations with UFPI B3-9 (*Paenibacillus* sp.), UFLA 02-286 (*Brevibacillus* sp.), UFLA 02-290 (*Bacillus megaterium*), UFLA 02-293 (*Pseudomonas putida*), UFLA 04-195 (*Rhizobium miluonense*), and UFLA 04-227 (*Burkholderia fungorum*) combined with CIAT 899 (*R. tropici*) led to an increase in plant biomass and N accumulation

in comparison with plants inoculated with CIAT 899 alone. In addition to biocontrol, other plant growth promotion traits from strains of these genera have been reported, and positive results on growth, nodulation, and N fixation in leguminous plants have been found from co-inoculation of *Rhizobium* with *Bacillus* (Rajendran et al. 2008, Korir et al. 2017), *Brevibacillus* (Abbas et al. 2018), *Burkholderia* (Oliveira-Longatti et al. 2013), *Paenibacillus* (Korir et al. 2017), and *Pseudomonas* (Tilak et al. 2006).

In some cases, the combinations of PGPR strains with CIAT 899 were able to increase plant biomass and accumulated N as much as in the treatments that received mineral nitrogen. The co-inoculations UFLA 02-290 + CIAT 899 (*Bacillus megaterium* + *R. tropici*), UFPI B3-9 + CIAT 889 (*Paenibacillus* sp. + *R. tropici*), and UFLA 02-293 + CIAT 899 (*Pseudomonas putida* + *R. tropici*) were able to achieve values of total dry weight, shoot dry weight, and accumulated N, respectively, equal to application of the highest mineral N concentration and higher than application of the low mineral N concentration. Nodulation, however, was not found in this study. Lima bean is able to nodulate especially with *Bradyrhizobium* (Ormeño-Orrillo et al. 2006), as well as efficiently fix N_2 through symbiosis with this genus (Costa et al. 2017). Since nodulation with *Rhizobium* is not possible, the growth promotion by the *Rhizobium* strains were other than symbiotic N_2 fixation, and the positive effect was enhanced by co-inoculation with other PGPR genera. Nevertheless, the combination of CIAT 899 and PGPR strains from other genera enabled more plant N acquisition despite the lack of nodulation, indicating a favorable synergistic effect of these strains, with positive consequences for lima bean growth and an asymbiotic N_2 fixation effect.

Conclusions

Most strains were able to manage damping-off and promote plant growth in substrate infected with *Rhizoctonia solani* CML 1846. Strains of *Brevibacillus* (UFLA 02-286) *Pseudomonas* (UFLA 02-281 and UFLA 04-885), *Rhizobium* (UFLA 04-195), and *Burkholderia* (UFLA 04-227) co-inoculated with the CIAT 899 strain of *Rhizobium tropici* were considered most effective in controlling the disease. The co-inoculations UFLA 04-195 + CIAT 899, UFLA 04-227 + CIAT 899, and UFLA 04-885 + CIAT 899 were able to increase the plant disease management under increased soil pathogen doses. Diverse responses were found for growth promotion among the inoculated PGPR strains when N was supplied in different forms (low or high mineral N concentration or inoculation with CIAT 899), but the promising strains used in the biocontrol assays were also responsive in promoting growth of lima bean under these conditions. There was a synergistic effect of co-inoculation of some PGPR strains on lima bean, and further research is necessary to identify the mechanisms of biocontrol of these strains as well as their effects under field conditions. We believe that these outcomes are important first steps to improve lima bean production either by promoting plant growth or by managing *R. solani* damping-off, especially considering the low cost of inoculation for a crop mostly produced by small farmers.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors have no conflicts of interest to declare.

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