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Seed inoculation with endophytic *Induratia* species on productivity of common beans

Inoculação de sementes com espécies endofíticas de *Induratia* na produtividade do feijoeiro

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ABSTRACT
Common bean (*Phaseolus vulgaris* L.) is a leguminous species of great economic and nutritional importance worldwide, and thus the development of management strategies to promote plant growth and increase grain yield of legumes is of great interest to farmers. The growth-promoting effects of the symbiosis of endophytic microorganisms in plants have been exploited in several crops. This study investigated the effects of *Induratia* sp. endophytic fungi on the growth and grain yield of seed-inoculated common bean plants. The experiments were conducted in a greenhouse during two growing seasons using a randomized complete block design with three replicates. Growth and yield-related parameters such as plant height, root length, leaf index, number of days for flowering, number of pods per plant, number of grains per pod, mass of 100 grains, and grain yield were evaluated. The grain yield of plants inoculated with *Induratia coffeana* strains CML 4018 and CML 4020 and *Induratia* sp. strain CML 4015 increased by 52.5%, 48.9%, and 56.4% compared to the non-inoculated control, respectively. Seed inoculation of common bean plants with endophytic fungi species of the genus *Induratia* showed a beneficial interaction as indicated by the recorded increase in grain yield. The present pioneering study could provide the means for developing crop management strategies for enhancing common bean crop productivity.

Index terms: Endophytic fungi; growth promotion; *Muscodor; Phaseolus vulgaris;* volatile organic compounds.

RESUMO
O feijoeiro comum (*Phaseolus vulgaris* L.) é uma leguminosa de grande importância econômica e nutricional em todo o mundo; portanto, o desenvolvimento de estratégias de manejo para promover o crescimento das plantas e aumentar a produtividade de grãos é de grande interesse para os agricultores. Os efeitos da promoção de crescimento por simbiose de microrganismos endofíticos com plantas têm sido explorados em várias culturas. Este estudo investigou os efeitos sobre o crescimento e a produtividade de grãos em feijoeiro por meio de fungos endofíticos *Induratia* inoculados em sementes. Os experimentos foram conduzidos em casa de vegetação no delineamento de blocos completos casualizados com três repetições. Foram avaliados a altura de planta, comprimento de raiz, índice foliar, número de dias para o florescimento, número de vagens por planta, número de grãos por vagem, massa de 100 grãos e produtividade de grãos. A produtividade das plantas inoculadas com *Induratia coffeana* (CML 4018, CML 4020) e *Induratia* sp. (CML 4015) aumentou 52,5%, 48,9% e 56,4%, respectivamente, em comparação com os controles. A inoculação de sementes de feijão com espécies de fungos endofíticos do gênero *Induratia* apresentou interação benéfica, aumentando a produtividade de grãos. Este estudo pioneiro é importante para o desenvolvimento de estratégias de manejo visando o aumento da produtividade do feijoeiro.

Termos para indexação: Fungos endofíticos; promoção de crescimento; *Muscodor*; *Phaseolus vulgaris;* compostos orgânicos voláteis.

INTRODUCTION

Symbiotic relationships with microorganisms are essential for numerous plant species. Endophytic microorganisms beneficial to the plants are frequently found within intercellular spaces, tissue cavities, or vascular bundles of their host. Inoculation of crop plants with endophytic fungi has resulted in multiple beneficial effects for host plants including enhanced plant fitness, increased crop yield, photosynthetic efficiency, nutrient and water use efficiency, and tolerance to abiotic and biotic stresses

(Rajamanikyam et al., 2017; Singh; Gill; Tuteja, 2011; Strobel, 2018). Increasing agricultural productivity is crucial for providing sustenance for the ever-increasing human population, highlighting the importance of exploring the use of endophytic microorganisms to improve crop yield.

Endophytic fungi belonging to different genera have exerted growth promoting properties in several crops, such as tomatoes (*Solanum lycopersicum*), bananas (*Musa* sp.), and cucumbers (*Cucumis sativus*) (Hamayun et al., 2010; Suwannarach et al., 2015; Ting et al., 2008) although substantial work has been carried out on other aspects of plant growth promoting fungi (PGPF. However, few studies are available on the interactions between endophytic fungi and the common bean as a host plant. Common bean (*Phaseolus vulgaris* L.) is of considerable economic and nutritional value and is a major crop particularly in developing countries including Brazil (Nay et al., 2019)caused by Pseudocercospora griseola, is one of the most devastating diseases of common bean (Phaseolus vulgaris L.. Several endophytes have been used as biological agents exhibiting entomopathogenic activity such as *Beauveria bassiana*, *Isaria fumosorosea, Lecanicillium lecanii,* and *Metarhizium anisopliae* (Dash et al., 2018; Parsa et al., 2018), while *B. bassiana* has also exhibited plant growth promoting properties when inoculated in common bean plants (Afandhi et al., 2019).

Endophytes of the genus *Muscodor* produce volatile organic compounds (VOCs) that have lethal effects on various phytopathogenic fungi. *Muscodor albus* was the first identified species of this genus and has been approved as a biocontrol agent for agricultural use in the USA (Strobel, 2018; Worapong et al., 2001). Other *Muscodor* species have also been evaluated as promising microbial agents suitable for myco-fumigation in agriculture and crop protection, pharmaceutical and environmental applications, and food preservation (Kudalkar et al., 2012; Meshram et al., 2017; Meshram; Gupta; Saxena, 2015; Monteiro et al., 2017; Saxena; Strobel, 2020). Samarakoon et al. (2020) showed the close phylogenetic relationship of *Muscodor* species to the xylarialean genera *Emarcea* and *Induratia* using a polyphasic taxonomic approach, resulting in all *Muscodor* species being transferred to the genus *Induratia*. Therefore, this new nomenclature was used for reporting this genus in the present study.

Induratia spp. isolated from coffee plants in Brazil produce enzymes capable of biodegrading polysaccharides and volatile and non-volatile metabolites with modulating activity in the blood coagulation process, acting as inhibitors or potentiators of the catalytic activity exerted by different classes of proteases (Bastos et al., 2020; Monteiro et al., 2020; Guimarães et al., 2021). Mota et al. (2021) reported the potential of *Induratia* spp. to control the phytopathogens *Colletotrichum lindemuthianum*, *Sclerotinia sclerotiorum,* and *Pseudocercospora griseola*, reducing their disease severity and incidence in common bean.

The antimicrobial activity of *Induratia* species is mainly attributed to the production of VOCs (Strobel et al., 2018; Guimarães et al., 2021). However, little is known about the potential of these species to be used as plant growth promoting fungi. Thus, the objective of this study was to investigate the effects of *Induratia* species, isolated from coffee plants and inoculated on common bean plants seeds, on the growth parameters and grain yield of common bean. This is the first study investigating the use of *Induratia* spp. as plant growth promoters of common bean, focusing on their potential for agricultural applications.

MATERIAL AND METHODS

Common bean cultivars

Two Brazilian common bean (*P. vulgaris*) cultivars, BRSMG Madrepérola and BRMG União, were used in this study. BRSMG Madrepérola is a Mesoamerican cultivar with a carioca type of grain and indeterminate growth. BRSMG União is a cultivar from the Andean gene pool, with a jalo type of grain and determinate growth. These cultivars are representatives of small and large grain lines of the common bean.

Endophytic fungi

Twelve fungal endophytic strains isolated from fresh healthy leaves and stems of wild *C. arabica* plants growing in secondary forests in Mata do Paraíso (20°48’03.4’’S 42°51’42.6”W), Zona da Mata region, Viçosa, Minas Gerais, Brazil, were evaluated. Pure cultures obtained by hyphal tipping were cryopreserved and deposited in the Coleção Micológica de Lavras (CML) fungal collection at the Departamento de Fitopatologia, Universidade Federal de Lavras, Brazil. The fungi were identified by sequencing using DNA’s internal transcribed spacer regions and their sequences were deposited in the GenBank database under the following accession numbers: *I. coffeana* (CML 4009) MN658674, *I. coffeana* (CML 4010) MN658675, *I. coffeana* (CML 4011) MN658676, *I. coffeana* (CML 4012) MN658677, *Induratia* sp. (CML 4013) MN658681, *I. yucatenensis* (CML 4014) MN658683, *Induratia* sp. (CML 4015) MN658682, *I. yucatenensis* (CML 4016) MN658684, *I. yucatenensis* (CML 4017) MN658685, *I. coffeana* (CML 4018) MN658678, *I. coffeana* (CML 4019)

MN658679, and *I. coffeana* (CML 4020) MN658680 (Guimarães et al., 2021).

Seed inoculation

Fungi were grown on Potato Dextrose Agar (PDA) culture medium for 15 days at 25 °C. Undisinfected seeds from each common bean cultivar were spread in a single layer on top of each full-grown fungal mycelium. The control was prepared with seeds spread on top of non-inoculated PDA medium. Petri dishes with seeds were incubated at 25 °C until the emergence of the radicles. Three seeds were sown per pot containing soil and Tropstrato HA® substrate at a ratio of 2:1 and NPK fertilizer (04-14-08). N-urea topdressing was subsequently applied. Potted plants were grown in a greenhouse with irrigation until harvest.

Experimental design

Two experiments were conducted in a greenhouse each initiated by sowing seeds in May 2016 and September 2016. A randomized complete block design with three replicates was used in the factorial scheme (2×13), consisting of the two common bean cultivars treated with the 12 endophytic fungi and one untreated control. Each plot consisted of a pot with three plants. The following agronomical properties were evaluated: plant height (cm), root length (cm), leaf length (cm), number of days to flowering, number of grains per pod, number of pods, 100-grain weight (g), and grain yield (g).

Statistical analyses

Individual and joint analyses of variance (ANOVA) were performed. When the residuals of a measured variable did not follow the non-normal distribution, the data were properly transformed according to the Box-Cox transformation (Osborne, 2010)multiple regression, ANOVA in order to meet the ANOVA assumptions. Dunnett's test was used to compare the means of treatments with that of the control for each trait. Analyses were performed using the statistical genetic software Genes (Cruz, 2013) and R software (Version 3.3.2) (R Core Team, 2016).

RESULTS AND DISCUSSION

Achieving a high grain yield in a sustainable manner is one of the main goals of common bean producers. Various strategies for sustainable crop production have mainly focused on the selection of microorganisms to promote plant growth and control pathogens. Entomopathogenic fungi and endophytic rhizobacteria inoculated in *P. vulgaris* have been reported to provide multiple benefits to their host plants, including direct and indirect plant growth promotion and plant defense against plant pathogens and herbivores (Afandhi et al., 2019; Dash et al., 2018; Martins et al., 2018; Parsa et al., 2018). As discussed by Parsa et al. (2018), the success in obtaining beneficial interactions between plant hosts and endophytic fungi relies on a combination of different factors, including the compatibility of plant and endophytic species, inoculation method, and environmental conditions such as soil type and microclimate.

In the present study, we evaluated the effects of 12 endophytic fungi of the *Induratia* genus on the growth and grain yield of the common bean. Our results showed that two isolates of *I. coffeana* and one isolate of *Induratia* sp. positively affected the grain yield. *Induratia coffeana* strains CML 4018 and CML 4019 increased grain yield in a manner which was associated with an increase in the number of pods per plant. In contrast, *I. coffeana* CML 4020 and *Induratia* sp. CML 4015 strains also increased grain yield, but no significant difference in the number of pods per plant was observed between inoculated and non-inoculated plants (Figure 1).

The results of the joint ANOVA showed significant interactions between the number of pods per plant and grain yield (Table 1), suggesting that differences in grain yield were predominantly influenced by the increase in the number of pods per plant. The plasticity of the primary components (number of pods per plant, number of grains per pod, and 100-weight grain) can explain the effects on grain yield. The performance of these endophytes in common bean plants was not dependent on the Mesoamerican or Andean gene pool as no significant difference was observed in the interaction between cultivar and endophytes.

In the second season, plants inoculated with *I. yucatenensis* CML 4014 had a higher 100-grain weight than the controls (Dunnett's test) (Figure 2). In plants inoculated with *I. coffeana* CML 4018 and CML 4020 and *Induratia* sp. CML 4015, grain yield increased by 52.5%, 48.9%, and 56.4%, respectively, compared with that of the controls (Figure 3). The number of pods in plants inoculated with *I. coffeana* CML 4018 and *Induratia* sp. CML 4015 was 52.6% and 43.8% higher than that in the controls, respectively (Figure 3). Although no statistical difference was found in the traits evaluated in the plants inoculated with *I. coffeana* CML 4011, we visually observed differences in plant vegetative growth compared to the control (Figure 4).

Different inoculation techniques have been reported to promote endophytic colonization in common beans, directly associated with the ability of the fungus to colonize different parts of the plant. Afandhi et al. (2019) successfully enhanced the growth of common bean plants by inoculation with *B. bassiana* via soil drenching or leaf spraying. These methods facilitate the attachment of conidia to the surface of plant roots or leaves and their consequent translocation to other parts of the plant. *B. bassiana, I. fumosorosea, L. lecanii, M. anisopliae*, and *Trichoderma* spp. also induced an increase in plant height and other growth parameters when inoculated into the seeds of *P. vulgaris* (Dash et al., 2018; Mayo-Prieto et al., 2020; Parsa et al., 2018). In our study, undisinfected seeds were spread on top of the fungal mycelium and incubated until radicle emergence. The effectiveness of this inoculation technique was evident by the observed positive effects of *Induratia*-inoculation into seeds on grain yield and number of pods in common bean plants. The role of surface disinfection of crop seeds before inoculation with microorganisms has been discussed in various studies. Parsa et al. (2016) reported that after excluding endophytic bacteria with the use of antibiotics, 394 fungal endophytes were isolated from 584 seedlings of 11 common bean cultivars.

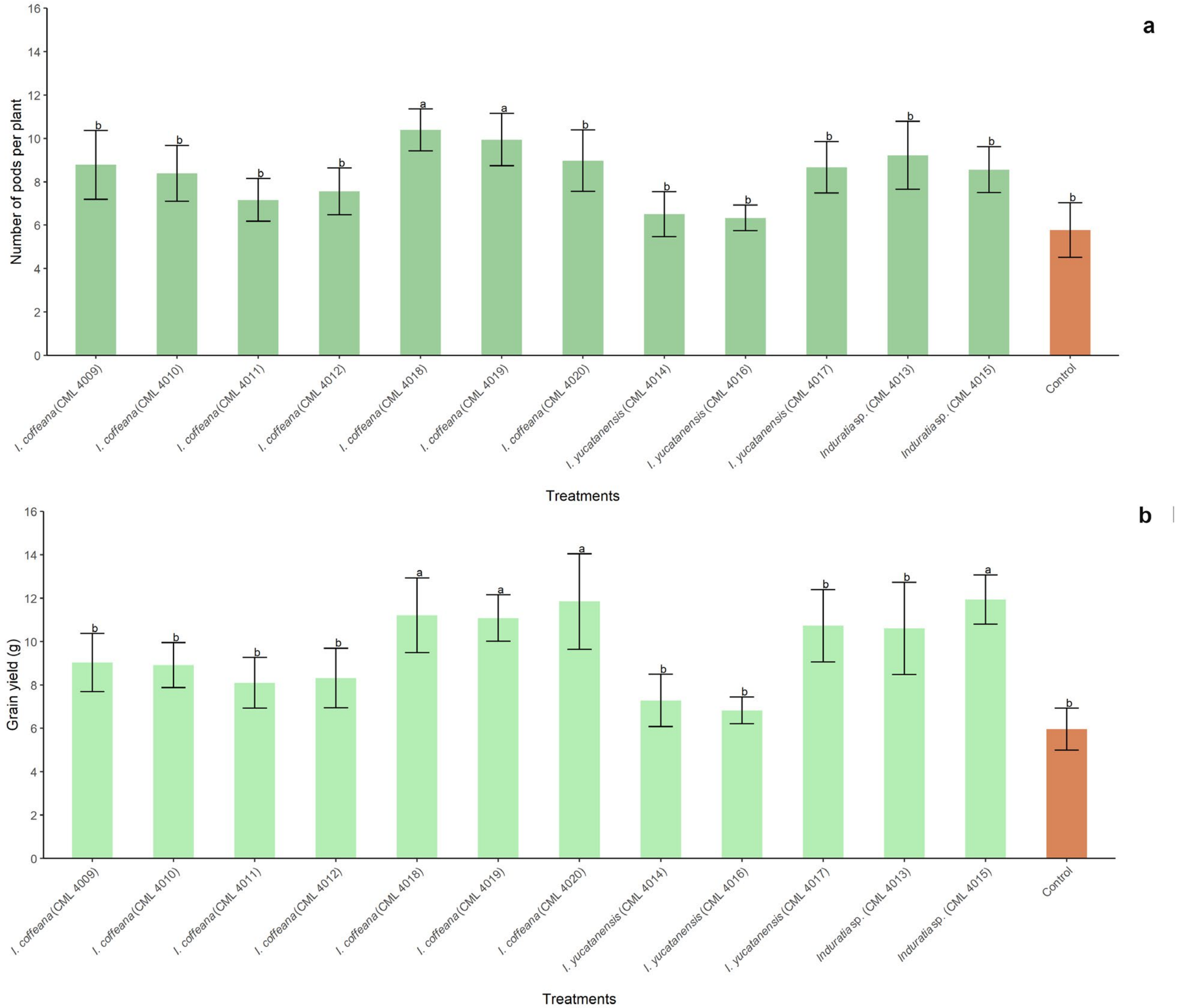

Figure 1: Effect of *Induratia* spp. inoculated in common bean plants in the first growing season (May). (a) Mean of number of pods per plant (X) and (b) grain yield (W) in grams (g) of treatments. a and b show the mean separations according to the Dunnett's test ($p < 0.05$). Bars indicate standard deviations of the means.

Table 1: Summary of ANOVA (individual and joint) for number of pods per plant (pods) and grain yield (yield) evaluated in the first season and 100-grain weight (weight) in the second season.

Source of Variation	First Season		Second Season
	pods	yield	weight
Individual Analyses			
Cultivars (C)	154.70*	85.26*	2511.9*
Endophyte(F)	11.95*	22.25*	15.53
Among	9.64	17.64*	12.87
F vs T[1]	37.31*	73.02*	44.78*
CxF	4.73	9.98	10.64
Error	5.50	8.56	8.99
Mean	8.17	9.31	31.31
CV (%)	28.70	31.41	9.57
Joint Analysis			
Season (S)	211.6**	92.6**	
Cultivar (C)	376.6**	320**	
Endophyte(F)	8.9**	21.4**	
Among	7.5*	19.8 *	
F vs T[1]	25.3**	39.7 *	
ExC	3.3ns	16.7ns	
ExF	5.82ns	8.6ns	
CxF	3.82ns	9.6ns	
ExCxF	5.18ns	10.9ns	
Error	3.81	7.18	
Mean	7.01	8.60	
CV (%)	27.84	31.36	

* and ** Significant at the 0.05 and 0.01 probability level, respectively by test F.

[1] Control.

In the present study, the inoculated seeds after radicle emergence were sown in a non-sterile substrate and soil. The effect of sterile or non-sterile substrates for sowing endophyte-inoculated seeds on the success of endophytic establishment in the common bean was reported to be dependent on the fungal genus (Dash et al., 2018; Parsa et al., 2018). Although sterile substrates allow for more controlled conditions, non-sterile substrates have the advantage of better mimicking natural field conditions, which was our intention in the present study. In addition, interactions with natural soil microbial communities can be beneficial for endophytic colonization.

No significant differences were observed in plant height and root length; however, there was an increase in the number of pods per plant and grain yield compared to that of the control. Studies reporting the effect of endophytic microorganisms on these traits are scarce in the literature because, in most studies, the evaluation is performed before the reproductive growth stages of the plants. For example, the colonization of common bean with some entomopathogenic fungi showed positive effects on plant height, fresh shoot, and root weight at 7- and 14-days post inoculation (Dash et al., 2018); plant height, number of leaves, and root length at 10 and 20 days post inoculation (Afandhi et al., 2019); and hypocotyl diameter, root system length, wet weight, and dry weight of the aerial parts and root system at 45 days post inoculation (Mayo-Prieto et al., 2020).

Endophytes can promote plant growth by improving nutrition by solubilizing nutrients from the substrate, producing phytohormones, increasing metabolic activity, and fixating nitrogen (Berg, 2009; Rai et al., 2014). Positive effects on common bean plants were observed when seeds were inoculated with *Induratia* strains, but the mechanisms underlying the increase in grain yield and number of pods per plant were not elucidated. Although we assumed that fungal endophytic colonization was successful in the common bean plants inoculated by the tested endophytes, we did not confirm this hypothesis by isolating *Induratia* strains from grown plants. Our findings suggest that *Induratia* spp. might belong to Class 2 endophytes, since this class includes endophytes that colonize roots, stems, and leaves; are capable of translocating inside the plant; and exhibit a high frequency of colonization in plants under stress (Rodriguez et al., 2009). Further studies will be conducted to confirm the endophytic colonization of *P. vulgaris* plants, to determine the mechanism of plant growth promoting action of promising endophytic isolates, and to evaluate their effectiveness and applicability under field conditions.

The distribution and survival of endophytic fungi inoculated into host plants are also affected by biotic and abiotic stresses. Some species of endophytic fungi can activate host defense mechanisms against pathogens, such as fungi, bacteria, and viruses, as well as insects and nematodes, thereby acting as biological control agents (Hardoim et al., 2015; Kumar et al., 2019). *Induratia* species, including the strains evaluated in this study, have been reported to produce VOCs, which have antifungal properties against various phytopathogenic fungi causing common bean diseases such as anthracnose, white mold, and angular leaf spot diseases (Hongsanan et al., 2015; Macías-Rubalcava et al., 2010; Monteiro et al., 2017; Mota et al., 2021; Saxena; Strobel, 2020; Strobel, 2018, 2006). Considering our results and those published by Mota et al. (2021), some strains of *Induratia* could have significant potential to enhance common bean production and control disease in this crop. Future work should focus on seed inoculation trials of a selected strain, for example *I. coffeana* CML 4019, which can promote an increase in grain yield as well as an increase in the number of pods per plant and exhibit biological control activity against common bean diseases. Overall, results have demonstrated that seed inoculation of common bean with coffee's endophytic *Induratia* spp. strains can improve grain yield.

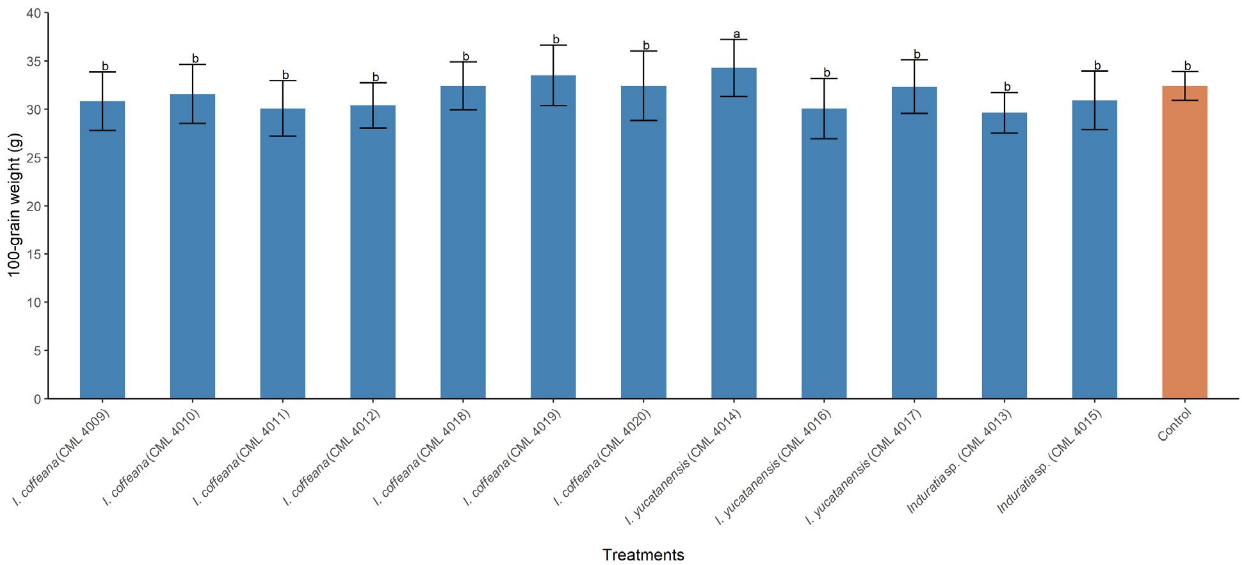

Figure 2: Mean 100-grain weight (Z) of common bean plants in the second growing season (September). a and b show the separation of means according to the Dunnett's test ($p < 0.05$). Bars indicate standard deviations of means.

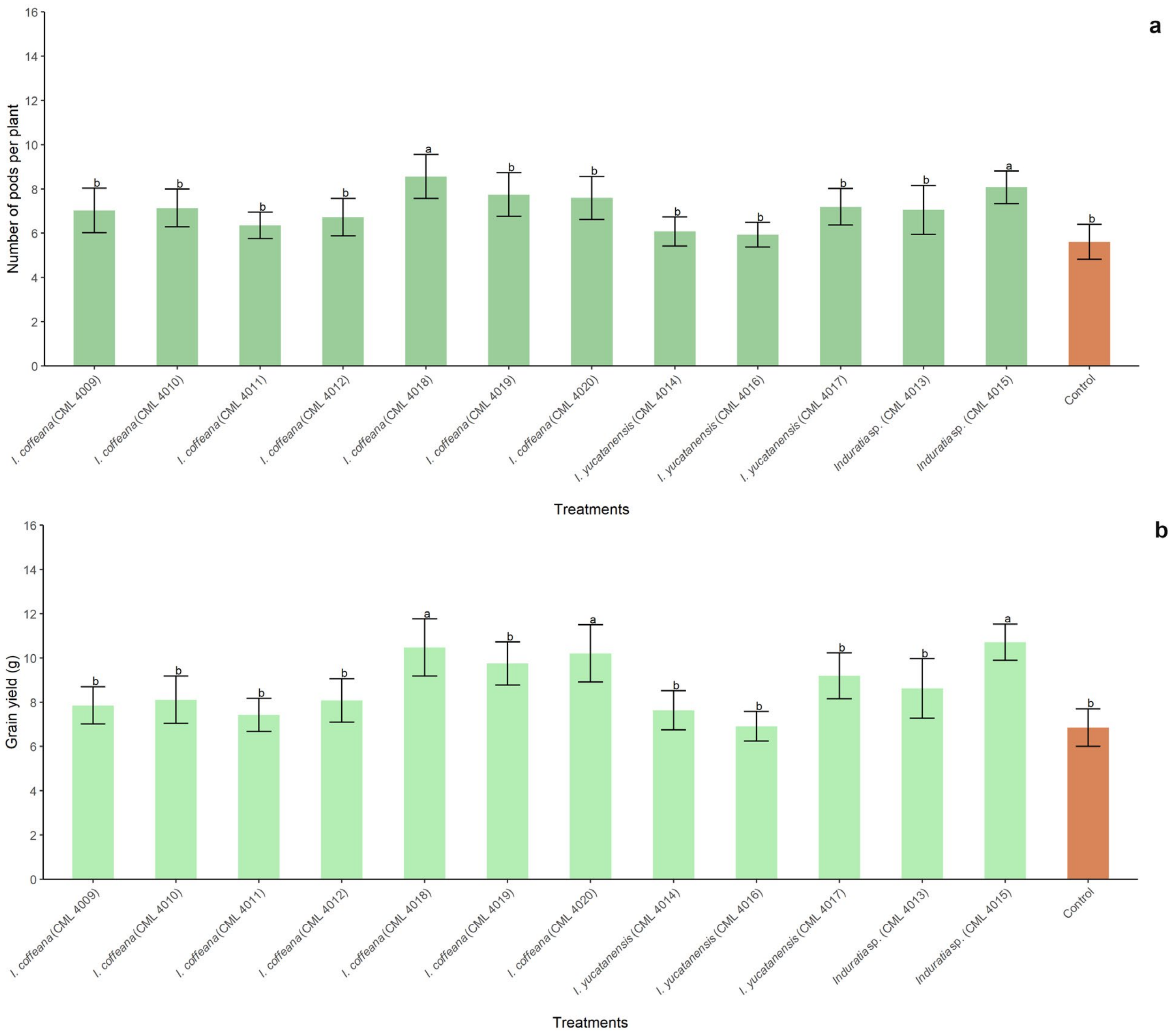

Figure 3: Effect of *Induratia* spp. inoculated in common bean plants**.** (a) Mean number of pods per plant (X) and (b) grain yield (W) in grams (g) of all treatments from joint ANOVA. a and b show the separations of means according to the Dunnett's test ($p < 0.05$). Bars indicate standard deviations of means.

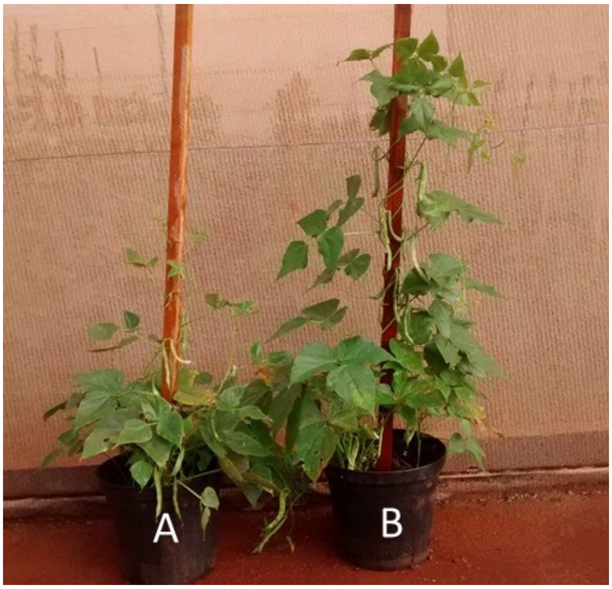

Figure 4: Vegetative growth of common bean plants, cultivar 'BRSMG Madrepérola': (A) control and (B) inoculated with endophyte *I. coffeana* CML 4011.

CONCLUSIONS

Endophytic fungi isolated from coffee plants showed great potential in promoting common bean growth, especially *I. coffeana* CML 4018, CML 4020, and *Induratia* sp. CML 4015 isolates which showed the best results. Inoculation of common bean seeds with endophytic fungi can be effective for enhancing grain yield and is a promising crop management strategy for improving yield and plant health of common beans.

AUTHOR'S CONTRIBUTION

Conceptual Idea: Souza, E.A.; Cardoso, P.G.; Pereira, O.L.; Methodology design: Souza, E.A.; Cardoso, P.G.; Hayashibara, C.A.A.; Data collection: Hayashibara, C.A.A.; Costa, L.C.; Data analysis and interpretation: Souza, E.A.; Cardoso, P.G.; Hayashibara, C.A.A.; Costa, L.C.; and Writing and editing: Souza, E.A.; Cardoso, P.G.; Hayashibara, C.A.A.; Costa, L.C.; Pereira, O.L.

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