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Plant growth-promoting activity of wild-type and bromate-resistant mutant of the endophytic fungus *Colletotrichum karstii*

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ABSTRACT. Endophytes may play important roles in agriculture. Spontaneous or induced mutant strains may increase their biotechnological properties. Seventeen *Colletotrichum* endophytic fungi were investigated for their plant growth-promoting characteristics (*in vitro* phosphate solubilization, IAA, and siderophore production). The five best strains were inoculated into bean seeds, and the most prominent isolate was selected to obtain auxotrophic mutants by Potassium Bromate Resistance System (PBRS). The plant growth-promoting ability of the mutant was also investigated. Further, 41.17% of the evaluated endophytes presented promising results for *in vitro* assays (*C. karstii* SL10, *C. karstii* SL28, *C. karstii* SL57, *C. karstii* SL59, *C. karstii* SL12, *C. karstii* SL40, and *C. karstii* SL24). The endophyte *C. karstii* SL57 was statistically conspicuous for plant height and root length when compared to those in control plants. Bromate-resistant mutant *C. karstii* SL57 increased *in vitro* phosphate solubilization (23%) and chlorophyll levels (Chl*b* 0.607 mg g^{-1} and Chl*t* 0.973 mg g^{-1}) of bean plants when compared to the wild-type strain (Chl*b* 0.551 mg g^{-1} and Chl*t* 0.881 mg g^{-1}). This is the first time an auxotrophic mutant fungus has been obtained by PBRS with a biotechnological application for the agricultural field.

Keywords: Endophytes; bromate resistance; chlorophyll; bean.

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Introduction

In 2017, the global bean production was around 24 million tons planted in one million hectares, with approximately 109 million tons of nitrogen and 45 million tons of phosphate fertilizers (Food and Agriculture Organization of the United Nations [FAO], 2020). Nitrogen and phosphates were required during the planting stage to control plant nutritional deficiencies, since several micronutrients, such as phosphorus, are not easily absorbed by plants (Wang & Wang, 2016).

Microorganisms that asymptomatically inhabit the interior of plant tissues, called endophytes (Wenzel, Garcia, Filho, Prioli, & Pamphile, 2010; Garcia et al., 2012), may be an excellent replacement alternative for the application of chemical fertilizers without any adverse environmental effects. In fact, they are capable of solubilizing inorganic salts, produce phytohormones and antagonize phytopathogens (Polonio et al., 2015; Bongiorno et al., 2016; Ribeiro et al., 2018; Oliveira et al., 2020).

The *Colletotrichum* genus is among the most frequently isolated endophytic fungi. Although the genus has already been considered one of the eight pathogens of great agricultural importance (Cannon, Damm, Johnston, & Weir, 2012), the latter have also proved to be able to associate endophytically with different hosts and perform activities of biotechnological importance, such as enzyme production (Santos et al., 2019), phosphate solubilization (Hiruma et al., 2016) and the production of plant hormones (Robinson, Riov, & Sharon, 1998).

Another alternative is the isolation and identification of spontaneous or induced mutant strains, which maximize or introduce characteristics of biotechnological interest. For filamentous fungi, chlorate ($KClO_3$) and potassium bromate ($KBrO_3$) resistance systems are the most commonly described, especially the *nit* auxotrophic mutants (Kanan, 2002; Prado et al., 2007; Rosada et al., 2010; Kanan & Al-Najjar, 2010).

In current study, seventeen leaf endophytic fungi belonging to eight *Colletotrichum* species have been investigated for their plant growth-promoting agent characteristics. The ability to solubilize phosphate to produce IAA and *in vitro* siderophores of all endophytes was also evaluated. The five best strains were analyzed

for growth-promoting abilities on bean plants and the most prominent isolate was selected to obtain auxotrophic mutants by the potassium bromate resistance system. The plant growth-promoting characteristics of the mutant strain were also investigated.

Material and methods

Endophytic microorganisms

Seventeen *Colletotrichum* sp. strains, isolated as endophytes from their hosts *Justicia brandegeana* and *Serjania laruotteana* employed in current research, were retrieved from the Collection of Endophytic and Environmental Microorganisms (CMEA) of the Microbial Biotechnology Laboratory (LBIOMIC) of the State University of Maringá (UEM), Maringá PR Brazil) (Department of Biotechnology, Genetics and Cell Biology). The endophytes were grown on Potato-Dextrose-Agar (PDA; pH 6.6) for 7 days, at 28ºC, and evaluated for their plant-growth promotion capacity.

In vitro solubilization of inorganic phosphate

A plug (6 mm diameter) from the endophyte colony previously grown on PDA medium (7 days) was transferred and incubated in a medium with insoluble phosphate (10 g L^{-1} glucose; 5 g L^{-1} NH_4Cl; 1 g L^{-1} $MgSO_4.7H_2O$; 0.8 g L^{-1} $CaHPO_4$; 15 g L^{-1} agar; pH 7.2) for 5 days, at 28ºC. The experiment was carried out in triplicate, whilst solubilization was characterized by the formation of halos around the fungal colonies. Solubilization Index (SI) was calculated by the following equation: diameter of fungal colony/halo/ diameter of fungal colony.

Production of 3-indoleacetic acid (IAA)

Isolates were grown in a 10% Potato-Dextrose (PD; pH 6.8) liquid medium, supplemented with 5 mM L-tryptophan, for 7 days, at 28°C, in the dark. Cultures were then centrifuged at 15,000 xg for 5 minutes. Further, 2 mL of Salkowski's Reagent (Bric, Bostock, & Silverstone, 1991) were added in 1 mL aliquots of the supernatant, reacting for 30 min. in the dark. Qualitative analysis was verified by the colorimetric chemical reaction of the oxidation of indolic compounds, with a yellowish color for negative and reddish-pink for positive testing. In the case of quantitative analysis (Husen, 2003), readings were made on a Spectrophotometer with a wavelength of 520 nm, and normalized by a standard curve (R^2 = 0.98) obtained with different concentrations of 3-indoleacetic commercial acid (Sigma-Aldrich).

Siderophores Production

Endophytic fungi were tested for the production of siderophores by Chrome Azurol Agar medium (Schwyn & Neilands, 1987) following methodology by Milagres, Machuca, and Napoleão (1999). The assay was carried out in triplicate and disks were kept at 28ºC for 15 days. A positive reaction would make the agar-CAS medium turn blue.

Endophyte inoculation in common bean seeds (*Phaseolus vulgaris* L.)

Seeds were superficially disinfected with 70% alcohol (1 min.), 2% sodium hypochlorite (2 min.), and 70% alcohol (30 s), followed by three rinses with sterile distilled water. They were then transferred to fungal suspensions adjusted for an Optical Density (OD) of 0.2 (600 nm) in 0.85% sterile saline solution in a spectrophotometer, and kept for 30 min. without shaking. Subsequently, the seeds were transferred to flasks with 90g of autoclaved soil (120ºC, for 1h) followed by incubation at 28ºC, with a photoperiod of 8h light/ 16h dark. The experiment was carried out with four replicates for each treatment, one seed per flask, as described by Sánchez-Cruz et al. (2019). As a control group, untreated seeds were used and incubated under the same conditions. The plant´s height, root length and number of leaves were determined after 25 days.

Toxicity of the *Colletotrichum karstii* SL57 wild strain to potassium bromate

The endophyte *C. karstii* SL57 with a statistical emphasis for *in vitro* assays and plant growth-promotion on common bean plants was used to obtain spontaneous auxotrophic mutants resistant to potassium bromate.

Bromate toxicity (0, 1.5, 3.12, 6.25, 12.5, 25, 50, and 100 mM) for the wild-type strain was analyzed by inoculating a plug (6 mm diameter) from the endophyte colony previously grown on PDA medium (7 days) into a Complete Medium (CM) (1.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.001 g L^{-1} $FeSO_4$, 0.001

g L^{-1} $ZnSO_4$, 10.0 g L^{-1} glucose, 2 g L^{-1} yeast extract, 2 g L^{-1} peptone, 1.5 g L^{-1} hydrolyzed casein, 1 mL of 5 mM N-source Solution [methionine, valine, cysteine, leucine, alanine, lysine, proline, arginine, cystine, uric acid and tryptophan], 15 g L^{-1} agar, 1000 mL distilled water; pH 6.8) supplemented with the aforementioned concentrations of bromate. Plates were incubated at 28ºC, for 7 days, and their mycelial growth was measured by ImageJ.

Isolation of *C. karstii* SL57 bromate resistant mutants

Mutant strains were isolated based on their resistance to bromate (25 mM) with a sole source of nitrogen, as described by Cove (1976). Eleven nitrogen sources were used: uric acid (UA), proline (Pro), arginine (Arg), tryptophan (Trp), lysine (Lys), methionine (Met), valine (Val), alanine (Ala), leucine (Leu), cysteine (Cys), and cystine (Cyst). A standardized 5 mL suspension in 0.85% sterile saline solution at 0.2 OD was prepared. Aliquots of 200 µL were plated in a Minimum Medium (MM) (1.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} KCl, 0.5 g L^{-1} $MgSO_4.7H_2O$, 0.001 g L^{-1} $FeSO_4$, 0.001 g L^{-1} $ZnSO_4$, 10 g L^{-1} Glucose, 15 g L^{-1} Agar, 1000 mL distilled water; pH 6.8) containing potassium bromate (25 mM) with a single source of N (5 mM). All plates were cultured for 48h at 28ºC (Kanan & Al-Najjar, 2010).

Colonies with no growth on MM with bromate were considered as putative auxotrophic mutants. The site where they were inoculated in the MM solid medium was cut and transferred to CM. After 7 days of incubation at 28°C, each isolate was tested for nutritional conditions, as described by Cordeiro, Lima, and Azevedo (1995), in a selective medium containing bromate (25 mM) and one single nitrogen source (5 mM).

Plant growth-promotion capacity of the mutant strain

The *in vitro* solubilization of inorganic phosphate and IAA assays were tested according to the previously mentioned methodologies for the wild-type strain. Bromate-resistant mutant strain in common bean seeds was also inoculated as previously described. However, plants were incubated for 15 days at 28ºC, with a photoperiod of 8h light/ 16h dark and four replications. In addition to the plant height, root length and number of leaves, all the plants´ fresh root, dry weight and chlorophyll were also determined.

Further, 1 g of fresh matter from each plant treatment was macerated in 10 mL of 80% acetone solution to determine chlorophyll concentration (mg g^{-1}). The acetone extract was centrifuged at 3000 xg for 20 min., and the supernatant was analyzed in a spectrophotometer at 645, 652, 654, and 663 nm. Calculations to determine the milligrams of chlorophyll per gram of leaf fresh tissue were based on equations by Whitham, Blaydes, and Devlin (1971): Chlorophyll *a* (Chl*a*) = (12.7 x A663 – 2.69 x A645) V $1000W^{-1}$, Chlorophyll *b* (Chl*b*) = (22.9xA654 – 4.68 x A663) V $1000W^{-1}$, and total chlorophyll (Chl*t*) = (A652 x 1000 x V $1000W^{-1}$)/ 34.5; where A = Absorbance at the indicated wavelength; V = final volume of chlorophyll - acetone extract; W = Fresh leaf matter in grams of the plant material used.

Statistical analysis

All tests were performed in triplicate, except the inoculation of endophytes in bean seeds, which were analyzed in four replicates for each treatment. Averages of data underwent ANOVA (analysis of variance) and statistically compared by Scott-Knott test at $p < 0.05$, employing the SISVAR statistical analysis program (Ferreira, 2011).

Results and discussion

Although the genus *Colletotrichum* has been commonly described as an agricultural important phytopathogen (Cannon et al., 2012), these fungi have also had their endophyte role successfully reported in different plant species (Bongiorno et al., 2016; Ribeiro et al., 2018; Santos et al., 2019; Golias et al., 2020; Oliveira et al., 2020).

Several studies with endophyte promoting plant-growth have focused on endophytic bacteria (Sánchez-Cruz et al., 2019) and the identification and selection of endophytic fungi have also increased. Two important characteristics for plant growth-promotion by microorganisms are the ability to solubilize inorganic phosphate and to produce plant hormones, such as IAA.

Table 1 shows *in vitro* assays for mechanisms that may indicate growth-promoting agent characteristics. For the quantification of IAA using the commercial hormone curve (R^2=0.98), significant statistical differences were observed for strains *C. gigasporum* JB168 (144.32 µg mL^{-1}) and *C. cacao* JB41 (205.87 µg mL^{-1}) when compared to remaining strains. Although the endophytes *C. truncatum* JB277, *C. karstii* SL57, *C. plurivorum*

JB47, *C. karstii* SL40, *C. phyllanthi* SL24, and *C. gigasporum* JB160 were assembled into a different statistical group, significant amounts higher than 70 µg mL^{-1} were also reported.

Table 1. *In vitro* activity of phosphate solubilization, IAA production, and siderophores of endophytic fungi of the *Colletotrichum* genus isolated from *Justicia brandegeana* (JB) and *Serjania larutteana* (SL).

Endophyte	IAA*	SI**	SD***
Colletotrichum truncatum JB276	16,37^{c}	0,00^{d}	0,50^{a}
Colletotrichum truncatum JB277	79,94^{b}	0,00^{d}	0,25^{a}
Colletotrichum. karstii SL57	90,52^{b}	2,53^{b}	0,62^{a}
Colletotrichum karstii SL59	49,43^{c}	2,34^{c}	0,50^{a}
Colletotrichum phyllanthi SL10	45,44^{c}	2,61^{b}	0,65^{a}
Colletotrichum karstii SL28	2,77^{c}	2,28^{c}	0,55^{a}
Colletotrichum plurivorum JB47	95,67^{b}	0,00^{d}	0,08^{a}
Colletotrichum phyllanthi SL12	0,00^{c}	2,55^{b}	0,37^{a}
Colletotrichum karstii SL40	70,67^{b}	2,30^{c}	0,50^{a}
Colletotrichum cacao JB295	58,84^{c}	0,00^{d}	0,50^{a}
Colletotrichum plurivorum JB12	3,50^{c}	0,00^{d}	0,00^{a}
Colletotrichum phyllanthi SL24	106,06^{b}	2,44^{c}	0,50^{a}
Colletotrichum gigasporum JB160	90,09^{b}	2,12^{c}	0,00^{a}
Colletotrichum cacao JB41	205,87^{a}	0,00^{d}	0,00^{a}
Colletotrichum gigasporum JB168	144,32^{a}	0,00^{d}	0,00^{a}
Colletotrichum boninense JB131	0,00^{c}	0,00^{d}	0,00^{a}
Colletotrichum karstii SL60	0,00^{c}	2,91^{a}	0,45^{a}

*IAA= Indoleacetic acid. Results expressed in µg mL^{-1}; **SI= Solubilization phosphate index. Results expressed in cm; SD= Siderophores. Results expressed in Unit of Activity (AU). The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

Several ascomycete fungi have been capable of producing IAA, a hormone for plant growth and development of paramount importance. *In vitro* biosynthesis of IAA using tryptophan as a precursor is the most commonly reported type. Robinson et al. (1998) quantified the IAA amount production by 18 strains of *Colletotrichum* sp., describing results ranging between 2 and 32 µg mL^{-1}. In current research, *Colletotrichum* strains which were capable of producing 2 to 144 µg mL^{-1} amounts of IAA have been described. These rates are higher than those reported in the study by Robinson et al. (1998) and also in other research works such as that by Ye, Li, Yi, Zhang, and Zou (2019), who employed different endophytic fungi, with the best isolate producing about 85 µg mL^{-1}.

In a medium containing inorganic phosphate ($CaHPO_4$), 52.94% of the endophytic fungi presented a solubilization halo around the fungal colony, suggesting a solubilizing activity. Nine isolates showed a solubilization index ranging between 2.12 and 2.91 cm. According to statistical analysis (Scott-Knott test at 5% significance), the endophyte *C. karstii* SL60 was conspicuous by presenting a solubilization index of 2.91 cm (Figure 1). Table 1 shows the production of siderophores expressed in AU (Activity Unit). Twelve strains showed a positive result, with an activity index between 0.08 and 0.65 AU.

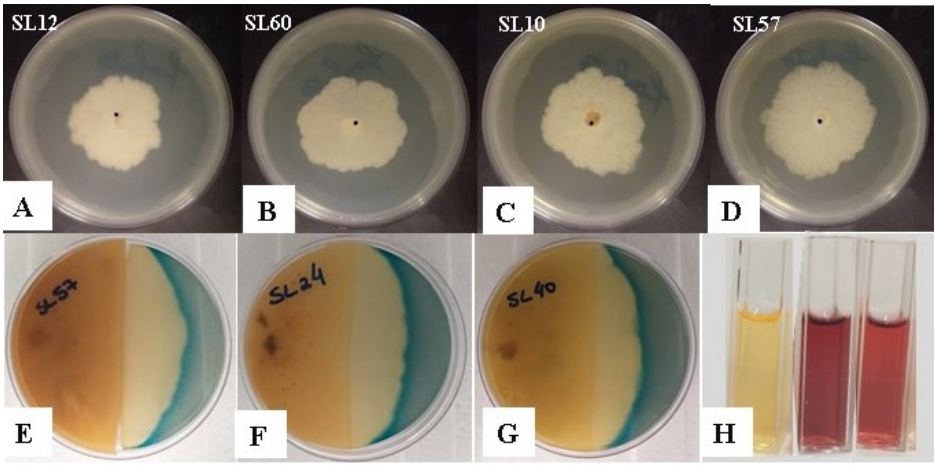

Figure 1. From A-D phosphate solubulization, *Colletotrichum phyllanthi* SL12 (A), *C. karstii* SL60 (B), *C. phyllanthi* SL10 (C), and *C. karstii* SL57 (D). From E-F production of siderophores, *C. karstii* SL57 (E), *C. phyllanthi* SL24 (F), and *C. karstii* SL40 (G). (H) production of indoleacetic acid by *C. cacao* JB41 and *C. gigasporum* JB168 (both red) and control (yellow).

The activity of solubilizing phosphate performed by endophytes is also important for their hosts, since phosphate is one of the three macronutrients that limit plant growth. However, most phosphorus present in many soils exists in insoluble and unavailable forms for plants (Wang & Wang, 2016). Hiruma et al. (2016) studied the colonization of *C. tofieldiae* in *Arabidopsis thaliana* plants and suggested, through *Colletotrichum* transformants with the GFP gene, that colonization by this genus of fungi begins through the root system and is then systematically distributed to other tissues. Another important result reported by Hiruma et al. (2016) is the ability of *C. tofieldiae* of transferring phosphate to the treated plants and thus contributing to their development.

In current assay, 41.17% of the evaluated *Colletotrichum* endophytic fungi presented promising results for IAA production, phosphate solubilization, and siderophores synthesis (*C. karstii* SL10, *C. karstii* SL28, *C. karstii* SL57, *C. karstii* SL59, *C. karstii* SL12, *C. karstii* SL40 and *C. karstii* SL24). However, it may be underscored that, although endophytes *C. gigasporum* JB168 and *C. cacao* JB41 have not presented a phosphate solubilization index and siderophores production, they were still able to synthesize large amounts of IAA in a L-Tryptophan-supplemented medium (5 mM) (Table 1).

Table 2 outlines the results obtained for the biometric parameters of inoculated and non-inoculated bean seedlings with the endophytes *C. karstii* SL57, *C. phyllanthi* SL24, *C. karstii* SL40, *C. cacao* JB41 and *C. gigasporum* JB168. No visible symptoms of diseases were observed in the plants treated with the suspension of endophytes and their respective control plants, suggesting an endophytic association.

Table 2. Biometric parameters of bean plants (*Phaseolus vulgaris* L.) inoculated with five endophytic fungi *Colletotrichum* sp. isolated from *Justicia brandegeana* and *Serjania larutteana*. The evaluation was performed 21 days after inoculation. Photoperiod: 8h light/ 16h dark.

Treatments	Number of leaves	Plant heigh	Root length
Untreated plants	3,5^{b}	15,35^{b} cm	16,50^{b} cm
Colletotrichum karstii SL57	2,75^{b}	20,87^{a} cm	21,0^{a} cm
Colletotrichum phyllanthi SL24	5,0^{a}	18,25^{a} cm	15,0^{b} cm
Colletotrichum karstii SL40	5,0^{a}	19,85^{a} cm	15,37^{b} cm
Colletotrichum cacao JB41	3,5^{b}	21,12^{a} cm	17,0^{b} cm
Colletotrichum gigasporum JB168	2,0^{b}	17,87^{b} cm	17,50^{b} cm

The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

Significant statistical results were reported on the number of leaves for treatments with *C. phyllanthi* SL24 (5.0) and *C. karstii* SL40 (5.0) when compared to control plants (3.5). There was also a statistical difference in shoot height for treatments *C. karstii* SL57 (20.87 cm), *C. phyllanthi* SL24 (18.25 cm), *C. karstii* SL40 (19.85 cm) and *C. cacao* JB41 (21.12 cm) in relation to the untreated control plants (15.35 cm) (Figure 2).

When length of roots (Figure 2) was assessed, *C. karstii* SL57 (21.0 cm) was outstanding among the other endophytes' treatments and control plants (16.50 cm). In fact, three endophytes (*C. phyllanthi* SL24, *C. karstii* SL40 and *C. karstii* SL57) stood out among the biometric parameters analyzed. The above indicates their potential as growth-promoting agents, especially in bean plants (Table 2).

Figure 2. (A) Roots of plants treated with a fungal suspension of the endophytes and control plants (no treatment). (B) Shoot of the treated and untreated plants.

When the positive roles presented by endophytic microorganisms for their hosts are taken into account, genetic improvement using endophytic fungi or bacteria, such as the isolation of spontaneous or induced mutants, may lead to the discovery of new strains with the improvement or introduction of new biotechnological characteristics of importance for their hosts (Freeman & Rodrigues, 1993; Prusky, Freeman, Rodriguez, & Keen, 1994; Pamphile, Rocha, & Azevedo, 2004).

Since endophyte *C. karstii* SL57 has shown significant statistical results for *in vitro* assays (Table 1) and has stood out in two biometric parameters when compared to the bean control plants and other endophytes treatments (Table 2), the strain was selected for isolating auxotrophic mutants resistant to potassium bromate aiming at obtaining mutant strains capable of maximizing their activities as a plant growth-promoting agent.

The toxicity of potassium bromate to the wild strain of *C. karstii* SL57 was achieved at a concentration of 25 mM, with complete inhibition occurring at 50 mM (Table 3). The concentration of 25 mM was chosen for isolating the bromate resistant mutants considering the toxicity of 87%, since 50 and 100 mM were very restrictive (100% toxicity).

Table 3. Evaluation of mycelial growth and toxicity to potassium bromate from the wild strain *Colletotrichum karstii* SL57 in different concentrations.

Bromate concentration	Mycelial growth*	Toxicity
0 mM	51,74 cm^2	---
1,5 mM	50,17 cm^2	3%
3,12 mM	48,57cm^2	6%
6,25 mM	36,69 cm^2	48%
12,5 mM	24,98 cm^2	51%
25 mM	6,49 cm^2	87%
50 mM	0,0 cm^2	100%
100 mM	0,0 cm^2	100%

The evaluation was carried out in Complete Agar Medium supplemented with different concentrations of Bromate. All concentrations were tested in triplicate and incubated at 28ºC for 7 days.*Average growth in triplicates.

Results presented in Table 4 demonstrate the putative mutants of *C. karstii* SL57 strain resistant to the concentration of 25 mM of potassium bromate. Although 40 putative mutants were screened for five N-sources (Cys, Met, Lys, UA, and Cyst), only one mutant was confirmed for uric acid (*C. karstii* SL57$^{mut\ BR\text{-}UA}$).

Table 4. Putative and confirmed mutants resistant to Potassium Bromate (25 mM) obtained from the wild strain *Colletotrichum karstii* SL57.

N-sources	Putative mutants	Confirmed mutants
Cysteine (Cys)	01	00
Methionine (Met)	18	00
Lysine (Lys)	01	00
Uric acid (UA)	03	01
Cystine (Cyst)	17	00

A 6 mm-diameter plug of the putative mutant colonies were grown on Minimum medium + 25 mM bromate + each N-source. Those fungi that failed to grow on MM supplemented with bromate + N-source but failed on unsupplemented MM were considered an auxotrophic mutant. All plates were incubated at 28ºC.

The description of auxotrophic mutants of the *Colletotrichum* genus has been previously described, especially strains resistant to potassium chlorate ($KClO_3$) (Prado et al., 2007; Rosada et al., 2010; Santanna et al., 2010). In 2011, Carvalho and Costa (2011) studied the genetic variability and vegetative compatibility of *nit* mutant strains of *C. lindemuthianum* and reported, similar to the authors of current study, auxotrophic mutants for uric acid (UA). Most studies aimed at isolating *Colletotrichum* mutants use the chlorate resistance system, while in current assay the authors are reporting for the first time retrieval of *Colletotrichum* mutants through the potassium bromate resistance system ($KBrO_3$), similar to the one previously mentioned for the *Aspergillus* species (Kanan, 2002; Kanan & Al-Najjar, 2010).

Significant statistical results were observed according to Scott-Knott test (5% significance) for the phosphate solubilizing activity of *C. karstii* SL5 mutant strain when compared to the wild-type strain. Results in Table 5 demonstrate a 23% increase in the solubilization index by the mutant fungus. In the case of IAA production (Figure 3B), there was a decrease in the ability to synthesize this hormone *in vitro* by the mutant strain. However, the mutant strain continued to produce a significant amount of auxin when compared to other *Colletotrichum* wild-type strains described in Table 1, with similar indices.

Table 5. *In vitro* inorganic phosphate solubilization index and IAA production by the auxotrophic mutant fungus *Colletotrichum karstii* SL57$^{mut\ BR\text{-}UA}$. Statistical analysis performed with the wild-type fungus *C. karstii* SL57.

Treatments	SI*	IAA*
Colletotrichum karstii SL57 $^{Wild\text{-}type}$	2,53^{b} cm	90,52^{a} µg mL^{-1}
Colletotrichum karstii SL57 $^{mut\ BR\text{-}UA}$	2,76^{a} cm	44,46^{b} µg mL^{-1}

*SI: Solubilization phosphate index; **IAA: 3-Indoleacetic acid. The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

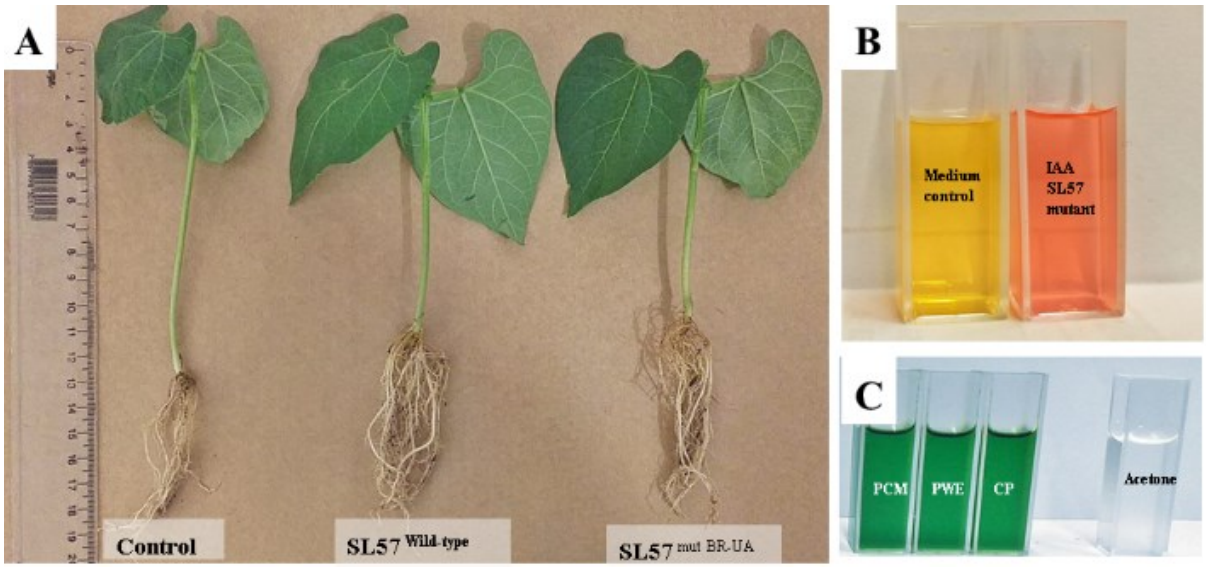

Figure 3. Evaluation of the bean growth promotion by the auxotrophic mutant *Colletotrichum karstii* SL57$^{mut\ BR\text{-}UA}$. (A) Plants evaluated after 15 days of inoculation; (B) Production of indoleacetic acid. On the left, PD 10% medium supplemented with 5 mM L-tryptophan and on the right IAA produced by *C. karstii* SL57$^{mut\ BR\text{-}UA}$; (C) Chlorophyll extract in acetone 80% of control plants (CP), plants treated only with the endophyte *C. karstii S*L57 wild-type (PEW) and plants treated with *C. karstii* SL57$^{mut\ BR\text{-}UA}$ (PCM).

When inoculated in common bean seeds (Figure 3A), *C. karstii* SL57$^{mut\ BR\text{-}UA}$ was able to demonstrated the permanence of their plant growth-promoting characteristics. Although no statistical differences were observed between the wild-type and mutant strain for the biometric parameters analyzed, both *C. karstii* SL57 wild and *C. karstii* SL57$^{mut\ BR\text{-}UA}$ statistically stood out among the control plants (Table 6 and 7), especially for the shoot fresh weight and root fresh/dry weight (Table 8).

Table 6. Biometric parameters of common bean plants (*Phaseolus vulgaris* L.) inoculated with the wild-type *Colletotrichum karstii* SL57 endophyte isolated from *Serjania larutteana* and its auxotrophic mutant *Colletotrichum karstii* SL57$^{mut\ BR\text{-}UA}$. The evaluation was performed 15 days after inoculation. Photoperiod: 8h light/ 16h dark.

Treatments	Number of leaves	Plant height	Root length
Control plants	2,0^{a}	10,65^{a} cm	10,62^{a} cm
Colletotrichum karstii SL57 $^{Wild\text{-}type}$	2,0^{a}	9,72^{a} cm	10,77^{a} cm
Colletotrichum karstii SL57 $^{mut\ BR\text{-}UA}$	2,0^{a}	8,55^{b} cm	10,35^{a} cm

The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

Table 7. Biometric parameters of common bean plants (*Phaseolus vulgaris* L.) inoculated with the wild-type *Colletotrichum karstii* SL57 endophyte isolated from *Serjania larutteana* and its auxotrophic mutant *Colletotrichum karstii* SL57$^{mut\ BR\text{-}UA}$. The evaluation was performed 15 days after inoculation. Photoperiod: 8h light / 16h dark.

Treatments	Root fresh weight	Shoot fresh weight	Root dry weight
Control plants	0,650^{b} g	1,315^{c} g	0,051^{b} g
C karstii SL57 $^{Wild\text{-}type}$	0,889^{a} g	1,849^{a} g	0,067^{a} g
C. karstii SL57 $^{mut\ BR\text{-}UA}$	0,875^{a} g	1,600^{b} g	0,063^{a} g

The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

We have inoculated the wild-type strain and *Colletotrichum* mutant by superficially disinfected seeds. According to findings by Hiruma et al. (2016), these strains may have initiated colonization by their roots, or rather, one of the first stages of bean development, suggesting that the statistical differences found in the root lengths of the plants treated with *C. karstii* SL57 wild strain (21 cm) after 21 days may be related to this colonization mechanism. Further, the phosphate solubilization index of wild *C. karstii* SL57 (2.53 cm)

increased in *C. karstii* SL57$^{mut\ BR\text{-}UA}$ (2.76 cm), which would reinforce the fresh and dry biomass of the plants analyzed after 15 days when compared to control plants (0.65 g), may also be related to the authors' findings.

Chlorophyll contents demonstrated the best results. The *C. karstii* SL57$^{mut\ BR\text{-}UA}$ strain were statistically prominent both for chlorophyll *b* (0.6078 mg g^{-1}) and total chlorophyll (0.9734 mg g^{-1}) when compared to control plants and plants treated with wild-type endophyte (Table 8, Figure 3C).

Table 8. Chlorophyll contents of bean plants (*Phaseolus vulgaris* L.) inoculated with the endophyte *Colletotrichum karstii* SL57 wild isolated from *Serjania larutteana* and its respective auxotrophic mutant *C. karstii* SL57$^{mut\ BR\text{-}UA}$.

Treatments	Chl*a**	Chl*b***	Chl*t****
Control plants	0,3718[a]	0,5257[b]	0,8667[b]
C. karstii SL57 $^{Wild\text{-}type}$	0,3223[b]	0,5517[b]	0,8817[b]
C. karstii SL57 $^{mut\ BR\text{-}UA}$	0,366[a]	0,6078[a]	0,9734[a]

*Chl*a*: Chlorophyll a; **Chl*b*: Chlorophyll b; ***Chl*t*: Total Chlorophyll. Results expressed in mg g^{-1}. The Same lowercase letters do not differ statistically when compared with the Scott-Knott test with 5% significance.

Chlorophyll is a molecule of extreme importance for plants. It is related to photosynthesis and is responsible for capturing and transmitting light energy during the first stage of the photosynthetic process (Song et al., 2020). The most common types of chlorophyll found in plants are type Chl*a* and Chl*b* (Costa et al., 2019).

The binding of Chl*b* with antenna proteins is crucial for the correct assembly of antenna complexes on thylakoid membranes. Therefore, Chl*b* levels affect the capture of light and thermal energy dissipation processes and the electron transport in the thylakoid membrane (Hoober, Eggink, & Chen, 2007; Voitsekhovskaja & Tyutereva, 2015). Further, Chl*b* is related to delay of cell aging and photosynthetic processes, making them longer. The latter are characteristics previously reported in transgenic and non-transgenic plants for the production of this type of chlorophyll (Tyutereva & Voitsekhovskaja, 2011; Biswal et al., 2012).

Longer photosynthesis periods could lead to higher biomass accumulation in plants, which is a particularly interesting characteristic for agriculture, since it would allow an increase in productivity (Voitsekhovskaja & Tyutereva, 2015). These ideas corroborate our findings with the *C. karstii* wild-type and mutant strains inoculated and analyzed at 15 days in common bean plants. The Chl*b* levels in plants treated with *C. karstii* SL57$^{mut\ BR\text{-}UA}$ suggest that the strain may have provided longer photosynthetic periods to plants and favored the accumulation of biomass, which statistically differed from that in control plants.

In current assay, the isolation of a bromate-resistant auxotrophic mutant with a single nutritional requirement has been reported. Detected mutations, probably in one single locus to acid uric metabolism, were capable of producing advantageous mutations. This may be observed in a study by Freeman and Rodriguez (1993) with *C. magna* mutant resistant to potassium chlorate, which reduced the virulence and pathogenicity in watermelon when compared to its wild-type strain, without losing important characteristics such as sporulation and the ability to infection/colonization. Prusky et al. (1994) corroborate results by Freeman and Rodriguez (1993) regarding the positive mutation in *C. magna* chlorate-resistant strains, now in avocado plants. The authors' findings corroborate results in our study for *Colletotrichum* mutants, especially the *C. karstii* SL57$^{mut\ BR\text{-}UA}$ auxotrophic mutant, which presented an increase in solubilization activity of inorganic phosphate (23%) and the Chl*b* and Chl*t* contents in common bean plants.

López-González et al. (2017) and Oliveira et al. (2020) have reported the *Colletotrichum* fungi playing an endophyte role in plants of *Phaseolus* sp. genus, similar to reports by Parsa et al. (2016), including the *C. karstii* species analyzed in current study and described as a bean endophyte by the authors. Thus, the above authors' findings and results of current assay foreground the hypothesis that the genus of fungi may associate itself endophytically and act as plant growth-promoting agent, especially in bean plants.

Conclusion

Current assay showed that endophytic fungi of the *Colletotrichum* sp. genus were able to solubilize inorganic phosphate, producing IAA/siderophores, and promoting bean growth. Further, we have described for the first time an auxotrophic mutant strain for isolated uric acid using the potassium bromate resistance system, with a biotechnological application for the agricultural field. The mutant *C. karstii* SL57$^{mut\ BR\text{-}UA}$ *in vitro* increased the phosphate solubilizing capacity and the chlorophyll levels of bean plants when compared to the wild-type strain.

Strains of spontaneous or induced mutant microorganisms, especially the endophytes, are important because these microorganisms will actually use their hosts' nutritional sources to supply their auxotrophic needs. If released into the environment, they will not cause any harmful effects.

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