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# Evaluation of *Trichoderma atroviride* endophytes with growth-promoting activities on tomato plants and antagonistic action on *Fusarium oxysporum*

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## Abstract

In Brazil, tomato is one of the most consumed vegetables and the fungus *Fusarium oxysporum* is one of the most important phytopathogen of tomato plants (*Lycopersicon esculentum* Mill.). Thus, the search of beneficial microorganisms with growth-promoting and/or biological control properties represent an important tool for agricultural biotechnology. Herein, two *Trichoderma endophytes* (strains 36b and 164b) associated with *Coffea arabica* L. cultivar IAPAR-59 were investigated on their growth-promoting activities on plants and their antagonist effects and interactions against *F. oxysporum*. Molecular multigene (ITS- TEF-TUB-CAL) identification and phylogenetic analysis allowed the identification of these endophytes as belonging to *Trichoderma atroviride* species. When inoculated with the endophytic strain 36b, tomato plants reached the highest speed of seedling emergence (83.3%), but both endophytes increased the number of leaves, root length and dry biomass of treated plants. Regarding the in vitro antagonism assay, reduced phytopathogen growth by approximately 70 (strain 36b) and 52% (strain 164b) which indicates a partial replacement of endophytes after initial deadlock with mycelial contact. Scanning electron microscopy allowed to observe the presence of *Fusarium macroconidia* between endophytic hyphae and conidia, with the helicoidization of endophytic hyphae, which wrapped around the pathogen hyphae, suggesting a mechanical inhibition by strangulation.

**Keywords:** Molecular multigene identification; Growth-promoting endophytes; Scanning electron microscopy

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## 1 Introduction

Plant diseases are caused by various microorganisms and reduce the food productivity all over the world in approximately 10-16% (CHAKRABORTYA et al., 2000; STRANGE; SCOTT, 2005; GHINI et al., 2008; LO PRESTI et al., 2015). Fungal pathogens are the major causative agents of plant diseases that can infect roots, fruits, leaves, flowers and stems (HYDE et al., 2009; MANAMGODA et al., 2011; MAHARACHCHIKUMBURA et al., 2014; UDAYANGA et al., 2014). Tomato (*Lycopersicon esculentum* Mill.) is one of the most consumed vegetables in the world, with a harvested area and production estimated at 4.8 million ha and approximately 182 million tons, respectively in 2017 alone (FOOD AND AGRICULTURE ORGANIZATION, 2018). Brazilian tomato crops occupied an area of 61.5 million ha with an estimated production of approximately 4.2 million tons in 2017 (FOOD AND AGRICULTURE ORGANIZATION, 2018). However, this plant is highly susceptible to infection by various pathogens, including the fungus *Fusarium oxysporum* is known to cause the *Fusarium wilt*, one of the most important tomato diseases.

Sustainable agriculture are based on the reduction or elimination of fertilizers and agrochemicals (VINALE et al., 2008; CHOU, 2010). Concerning to minimize the detrimental effects of the conventional techniques of agriculture, innovative methods based on microbial inoculation are recently gaining more interest. In this context, the biocontrol is one of the promising tools to control plant pathogens with minimal environment impact. It is defined as the use of beneficial organisms, their genes, and/or products, such as metabolites, that reduce the negative effects of plant pathogens and promote positive responses by the plant (VINALE et al., 2008).

Endophytic microorganisms are potential biocontrol agents, capable of modulating interactions with pathogens and pests (AZEVEDO et al., 2000, 2002; POLONIO et al., 2015; BONGIORNO et al., 2016; RIBEIRO et al., 2018). Besides that, endophytes have some biocontrol strategies as triggering host resistance, prevention of infection, reduction of host tissues colonization and limitation of pathogen survival (AZEVEDO et al., 2002). The mechanism of antagonism is known for inhibiting pathogens by nutrients competition, induction of plant defense and production of enzymes and secondary metabolites; this diverse antagonistic mechanisms are described as mycoparasitism (DRUZHININA et al., 2011; HASAN et al., 2013; BUSBY et al., 2016).

Some *Trichoderma* strains are used in commercial formulations for induce plant growth promotion and the biological control of phytopathogenic fungi being considered an attractive option for management of plant diseases and plant growth promotion (HARMAN et al., 2004; DRUZHININA et al., 2011; MARTÍNEZ-MEDINA et al., 2014; GUPTA et al., 2016). Considering the high capability of the *Trichoderma* genus as biocontrol agent and its capacity of inducing the plant growth, the current study evaluated the biological activity of two Trichoderma endophytes of isolated from *Coffea arabica L. cultivar* IAPAR-59.

## 2 Material e Methods

### 2.1 Biological material

The fungal strains *Trichoderma* sp. 36b (GenBank code KP256766.1) and *Trichoderma* sp. 164b (KP256783.1), isolated as endophytes from healthy leaves of *Coffea arabica* L. cultivar IAPAR-59, were retrieved from the Collection of Endophytic and Environmental Microorganisms (CMEA/LBIOMIC-UEM) from the Laboratory of Microbial Biotechnology, Universidade Estadual de Maringá, Paraná State, Brazil. Previous molecular identification was based on sequence of ITS1-5.8S-ITS2 regions of rDNA (BONGIORNO et al., 2016). The plant pathogenic fungus *Fusarium oxysporum* (ATCC 2163) was provided by the Fundação André Tosello, Campinas, SP, Brazil.

Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivar Santa Clara (Top Seed Garden, Agristar) were purchased in an agricultural products store in Maringá, Paraná State, Brazil. This cultivar belongs to the blue line segment – it did not receive chemical treatments – and it has no resistance to any phytopathogens. Seeds were disinfected with alcohol 70% (2 min) and 1% sodium hypochlorite solution (3 min), and then rinsed in sterile distilled water for 10 times.

### 2.2 Molecular taxonomy of fungal endophytes

Genomic DNA was extracted using the Power Soil DNA Isolation kit (MoBio Laboratories, USA) in accordance with manufacturer instructions. Genes/loci selected for multilocus sequences analysis (MLSA), their respective oligonucleotide primers and the PCR conditions are listed in Table 1. PCR products were analyzed by electrophoresis on 1.5% agarose gel.

Table 1 – Genes/loci used for multilocus sequences analysis, their respective oligonucleotide primers and the amplification conditions

| Gene/ Loci | Primers | Sequences (5'→ 3') | PCR conditions | References |
|---|---|---|---|---|
| **ITS** | ITS1<br>ITS4 | TCCGTAGGTGAACCTGCGG<br>TCCCCGCTTATTGATATGC | 92 °C: 4 min; (92 °C 40s, 52 °C, 1 min and 30s, 72 °C, 2 min) x 35 cycles; 72 °C, 5 min | White et al. (1990) |
| **TEF 1-α** | EF1-728F<br>EF1-986R | CATCGAGAAGTTCGAGAAGG<br>TACTTGAAGGAACCCTTACC | 94 °C: 4 min; (94 °C 30s, 55 °C, 1 min, 72 °C, 1 min) x 40 cycles; 72 °C, 3 min | Carbone and Kohn (1999) |
| **TUB** | T1<br>Bt2bR | AACATGCGTGAGATTGTAAGT<br>ACCCTCAGTGTAGTGACCCTTGGC | 94 °C: 4 min; (94 °C 30s, 55 °C, 1 min, 72 °C, 1 min) x 40 cycles; 72 °C, 3 min | O'Donnell and Cigelnik (1997) Glass and Donaldson (1995) |
| **CAL** | CAL-228F<br>CAL-737R | GAGTTCAAGGAGGCCTTCTCCC<br>CATCTTTCTGGCCATCATGG | 94 °C: 4 min; (94 °C 30s, 55 °C, 1 min, 72 °C, 1 min) x 40 cycles; 72 °C, 3 min | Carbone and Kohn (1999) |

ITS = ITS1-5.8S-ITS2 regions of rDNA; TEF 1-α = translation elongation factor gene 1-α; TUB = β-tubulin gene; CAL = calmodulin gene

Samples were sequenced using the ABI-PRISM 3100 Genetic Analyzer equipment (Applied Biosystems, USA) and results were analyzed using the software BioEdit version 7.2.2. Sequences were compared to those deposited in the GenBank (http://www.ncbi.nlm.nih.gov) using the BLASTn tool with a restricted search to type strains. Closely related species were also selected based on data available in TreeBASE database (http://www.treebase.org). Recovered sequences were aligned and compared by using the MAFFT online interface (KATOH; TOH, 2008). Then the phylogenetic analysis of the concatenated sequences was performed; multigenic assembly was performed by using SequenceMatrix software (VAIDYA et al., 2011).

To choose the best evolutionary model of Bayesian inference and maximum likelihood, the MrModeltest v. 2.3 (NYLANDER, 2004) software was used. The MrBayes v. 3.2.5 (RONQUIST et al., 2012) was used to generate the phylogenetic tree, considering the parameters generated by MrModelTest, with MCMC (Markov Chain Monte Carlo) lasting until the average standard deviation of the frequencies reached below 0.01. The tree was edited using FigTree v.1.4.2 software (RAMBAUT, 2009).

## 2.3 Production of tomato seedlings, inoculation and re-isolation of *Trichoderma endophytes*

To obtain the mycelial suspension, 6-mm mycelial plugs of seven days-old cultures of endophytes were macerated in microtubes containing 1.5 mL of autoclaved distilled water, which were shaken until complete homogenization. Tomato seeds were disinfected with alcohol 70% for 2 min, 1% sodium hypochlorite for 3 min, and then rinsed 10 times in sterile distilled water.

Two substrates were used for sowing the tomato seeds: (i) MECPLANT® substrate (composed of Pinus bark, vermiculite, acidity regulator, macro and micronutrients) previously autoclaved at 121ºC for 120 min; (ii) soil sample (previously autoclaved at 121 °C for 120 min) collected at the State University of Maringá, classified by chemical and physical analyses as type 3 (clay/sandy). Granulometric analysis determined the content of sand (42.5%), silt (7.5%) and clay (50%). The complete analyses of the chemical attributes are seen in the Supplementary Tables 1 and 2.

The in vivo assays were performed in the Greenhouse of the Agronomy Department, in the State University of Maringá, Brazil (23°24'12.18"S and 51°56'30.54"W). Aliquots (1 mL) from the mycelial solution of endophytic strains 36b (T1) and 164b (T2) were inoculated into 300-mL polystyrene cups containing the MECPLANT® substrate and each cup received one tomato seed (at the same point of fungal inoculation). Controls consisted of plants without fungal inoculation.

Twenty-one days after endophytic inoculation, five tomato seedlings were selected for re-isolation of *Trichoderma* strains. Tomato roots were washed in distilled sterile water, cut into fragments and deposited on PDA dishes (five fragments per dish) and incubated at 28 °C for seven days.

## 2.4 Assessment of germination percentage, speed of seedling emergence

The test of speed of tomato seedlings emergency consisted of the following treatments: plants inoculated with endophyte 36b (T1), plants inoculated with endophyte 164b (T2), plants without fungal inoculation (Control), 30 totaling plants per treatment.

On the 5th day, the first germination count was obtained by counting the number of normal seedlings. After 7 days, the final germination count was performed, and the results were expressed as germination per-

centage, according to the criteria established by the Rules for Seed Analysis (BRASIL, 2009). To calculate the emergence speed index (ESI), the formula proposed by Maguire (1962) was used Equation 1:

$$ESI = \frac{E1}{N1} + \frac{E2}{N2} + \frac{En}{Nn} \quad (1)$$

Where: E1, E2, En = number of plants that had emerged on the respective day; N1, N2, Nn = number of days after sowing, from the first to the last counting.

## 2.5 Evaluation of the biometric parameters

This assay consisted of the following treatments: plants inoculated with endophyte 36b (T1), plants inoculated with endophyte 164b (T2), plants without fungal inoculation (Control), 15 totaling plants per treatment.

For each plant, the number of leaves was counted, and the plant height (in cm) was obtained by measuring (with a graduated ruler) the distance from the stem base to the apical bud. Similarly, a graduate ruler was used to measure root length from the base of the stem. To obtain the dry biomass, plants were placed into identified paper bags and taken to a forced-air circulation oven at 60 °C for 72 hours, then the material was weighed.

## 2.6 Evaluation of indole acetic acid (IAA) production

Endophytes were grown in PDB (Potato Dextrose Broth) with 10% L-tryptophan (5 mM) and incubated in darkness at 28 °C for 7 days. Cultures were centrifuged (15000 × g for 5 min), then aliquots (1 mL) of supernatants were added to 2 mL of the Salkowski's reagent described by Patten and Glick (2002). Solutions were kept in dark at room temperature for 30 min. The assay was performed in triplicate and absorbance was measured in a Microplate Reader EZ-Read 2000 (Biochrom, Cambourne, CBE, United Kingdom) at 520 nm. The standard curve was produced by plotting standard IAA (Sigma Aldrich) concentration (10, 25, 50, 100, 150, 200, 250, 300 µg/mL).

## 2.7 In vitro antagonism of endophytes against F. oxysporum

The antagonistic action of endophytes against the phytopathogen *F. oxysporum* was evaluated by the dual culture method described by Campanile et al. (2007) and outlined by Orlandelli et al. (2015). Triplicates remain incubated at 28 °C for 7 days, and then were photographed and assessed for pathogen area; results were then compared to those obtained for the controls.

The inhibition index percentage of mycelial growth (IM) was evaluated using the ImageJ software (v. 1.46r). The inhibition index percentage was calculated as Equation 2:

$$IM(\%) = \left(1 - \frac{MT}{MC}\right) \times 100 \quad (2)$$

Where: IM = inhibition index percentage of mycelial growth, MT = mean of triplicate area measured for treatment in $cm^2$, and MC = average of triplicate control area in $cm^2$.

The competitive interactions (CI) between endophytes and phytopathogens were determined according to the Badalyan rating scale (BADALYAN et al., 2002), which considers three main types (A, B and C) and four subcategories (CA1, CB1, CA2 and CB2) of interactions. Types A and B consist of deadlock (mutual inhibition) at mycelial contact (A) or at a distance (B); type C represent the replacement or overgrowth without initial deadlock, and its subtypes consist of partial (CA1) or complete (CA2) replacement after initial deadlock with mycelial contact, and partial (CB1) or complete (CB2) replacement after initial deadlock at a distance.

### 2.8 Scanning electron microscopy (SEM)

To study the endophyte-pathogen interaction, seven days-old cultures of fungi were used for the microculture technique, in which dishes containing PDA medium remain incubated at 28 °C for 7 days. Then, culture fragments were transferred to 24-well plates for the beginning of material fixation. Samples were fixed with 2.5% glutaraldehyde and 0.2 M cacodylate buffer, and dehydrated in graded alcohol series (30, 50, 70, 80, 90, 95 and 100%). Drying was done with carbon dioxide using a BAL-TEC CPD 030 critical point dryer, with 10 cycles. After drying, the material was adhered to stubs with conductive carbon adhesive tapes and covered with a thin layer of gold, in a Sputter Coater BAL-TEC SCD 050 metallizer. Samples were observed under FEI Quanta 250 Scanning Electron Microscope.

### 2.9 Statistical analyses

Results of emergence speed index (ESI), germination of tomato seedlings, biometric parameters, and antagonism test were statistically analyzed by ANOVA and the mean values were compared by Scott-Knott test ($p < 0.05$), using the program SISVAR 5.6 (FERREIRA, 2011).

## 3 Results and Discussion

### 3.1 Multilocus sequence analysis (MLSA)

The ITS gene represents a robust tool for identification at genus level and it was previously chosen by Bongiorno et al. (2016) for the identification of strains 36b and 164b, belonging to Trichoderma sp. However, the inclusion of additional specific genes for species identification is required for some taxonomic groups. In this context, the MLSA allows the concomitant analysis of two or more genes, and the resulting genealogy concordance provide a successfully resolution at the species level.

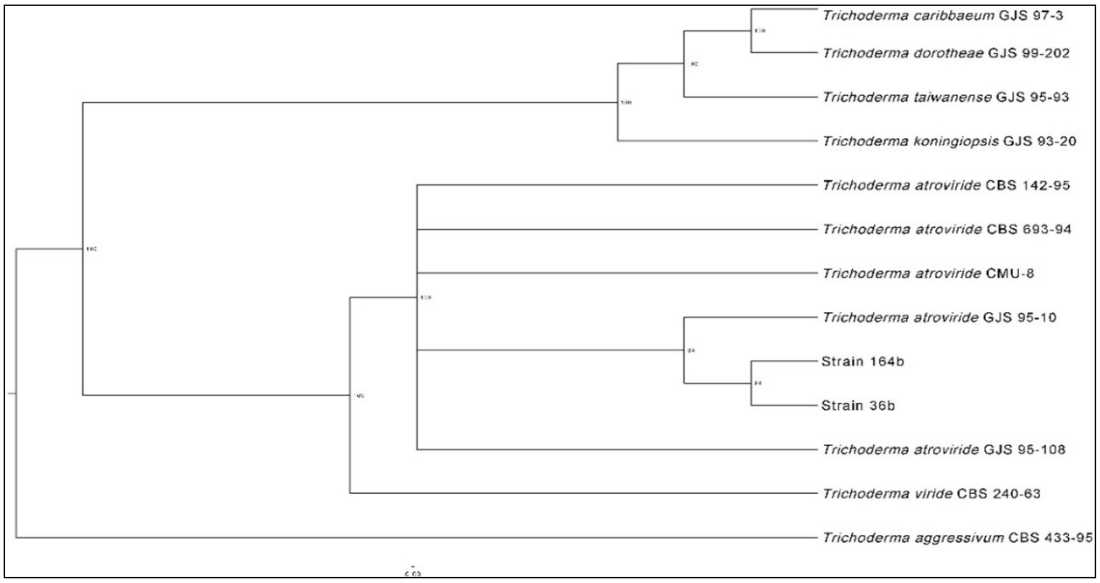
Herein, MLSA considered the ITS region and genes encoding translation elongation factor 1-α (TEF 1-α), β-tubulin (TUB) and calmodulin (CAL) genes. The comparison of DNA sequences with others available in GenBank and TreeBASE databases revealed that endophytes had high similarity to *Trichoderma atroviride*, which was confirmed by the Bayesian analysis with a specific alignment at 100% of Bayesian probability between endophytic strains 164b and  and *T. atroviride* strains (Table 2, Figure 1). Consistent with our results, the recent literature underscored the MLSA for the precise identification of different species of endophytes belonging to genera *Diaporthe* (RIBEIRO et al., 2018; SOARES et al., 2018; FELBER et al., 2019), *Colletotrichum, Phyllosticta* and *Xylaria* (RIBEIRO et al., 2018).

Table 2 – Trichoderma endophytes and sequences with greatest identity when aligned to GenBank database (NCBI)

| Gene | Endophytic strain | Fungus with highest similarity | GenBank code | Identity (%) |
|---|---|---|---|---|
| **ITS** | 164b | *Trichoderma atroviride* | AF456917.1 | 91 |
| | 36b | *Trichoderma atroviride* | AF456917.1 | 87 |
| **TEF 1-α** | 164b | *Trichoderma atroviride* | AY376051.1 | 98 |
| | 36b | *Trichoderma subviride* | KU529131.1 | 97 |
| **CAL** | 164b | *Trichoderma atroviride* | DQ122166.1 | 87 |
| | 36b | *Trichoderma atroviride* | DQ122166.1 | 97 |
| **TUB** | 164b | *Trichoderma atroviride* | XM_014083680.1 | 86 |
| | 36b | *Trichoderma viride* | Z15055.1 | 79 |

ITS = ITS1-5.8S-ITS2 regions of rDNA; TEF 1-α = translation elongation factor gene 1-α; TUB = β-tubulin gene; CAL = calmodulin gene

Figure 1 - *Cladogram obtained* from a *Bayesian inference* based on the alignment of four (ITS, TEF1-α, CAL and TUB) combined genes. Bayesian probability was demonstrated at the nodes between each organism. The strain *Trichoderma aggressivum* CBS 433-95 represents an external group




## 3.2 Growth promoting-activity and re-isolation of endophytes from tomato plants

The endophyte *T. atroviride* 36b influenced the speed of seedling emergence (Table 3) with higher results (83.3%) in comparison to the control, whereas the isolate 164b had an inferior result in comparison to the control. No symptoms were observed for plants inoculated with endophytes, indicating that their presence did not cause deleterious effects. Regarding ESI and height of tomato plants, statistical analyses highlighted the growth promoting-activity of *T. atroviride* 36b; both endophytes increased the number of leaves, root length and dry biomass in comparison to the control. Both endophytes were also positive for IAA production when cultured in PD broth supplemented with 0.5 mM L-tryptophan. The results, calculated using the standard curve constructed for IAA ($R^2$=0.9987), indicated the higher efficiency of strain 164b (7.10 µg/mL) in comparison with strain 36b (1.0 µg/mL).

It is knowledge that *Trichoderma* growth-promoting activity can occurs through distinct mechanisms, including the synthesis of phytohormones and analogues of auxins (Martínez-Medina et al., 2014), suggesting that there is a relation between IAA secretion and growth of tomato plants (BADER et al., 2019); however, the clear correlation between IAA synthesis and growth promotion in soil-based systems is not fully understood (NIETO-JACOBO et al., 2017). Interestingly, literature reported that IAA production by *T. atroviride* under *in vitro* culture supplemented with L-tryptophan represents a possible mechanism to increase fresh weight of shoots and roots on tomato (GRAVEL et al., 2007).

Table 3 – Emergence speed index (ESI) and germination of tomato seedlings, and biometric parameters of tomato plants after treatments with fungal endophytes

| Treatment | ESI | Germination (%)* | Number of leaves** | Height (cm)** | Root length (cm)** | Dry biomass (g)** |
|---|---|---|---|---|---|---|
| T1 | 2.27[a] | 83,3% | 5.47[b] | 21.29[a] | 17.07[a] | 0.59[a] |
| T2 | 0.95[b] | 50% | 6.30[a] | 15.80[b] | 13.05[b] | 0.38[b] |
| Control | 1.40[b] | 60% | 4.07[c] | 13.36[b] | 9.63[c] | 0.28[c] |

T1 = plants inoculated with T. *atroviride* 36b; T2 = plants inoculated with T. *atroviride* 164b; Control = plants without fungal inoculation. *Mean values from 30 seeds per treatment. **Means values from 15 plants per treatment. The mean values followed by different letters indicate that results are significantly different according to the Scott-Knott test ($p < 0.05$)

### 3.3 Antagonism and interaction between *Trichoderma endophytes* and *F. oxysporum*

*In vitro* assays may not accurately represent what occurs in the *in vivo* conditions, however, these studies are necessary for screening potential biocontrol candidates that may help to elucidate the mechanisms of reducing the pathogen infection (MEJÍA et al., 2008). In this context, both *T. atroviride* endophytes tested were effective for reducing the growth of *F. oxysporum.* ANOVA showed differences among the in vitro antagonistic actions; results highlighted the activity of *T. atroviride* 36b, which exhibited an IM of 70.56%, while a reduction of 52.37% was obtained for the strain 164b. More details regarding antagonistic activity are shown in Table 4. According to the Badalyan rating scale, CI between endophytes and pathogen were defined as CA1, which indicates a partial replacement of endophytes after initial deadlock with mycelial contact.

Table 4 – Antagonism of *Trichoderma atroviride* endophytes *against Fusarium oxysporum* assessed by average of pathogen area (A), inhibition index percentage of *F. oxysporum* mycelial growth (IM) and competitive interactions (CI)

| Endophytes | A ($cm^2$)* | IM (%)* | CI** |
|---|---|---|---|
| *T. atroviride* 36b | 12.48[a] | 70.56 | CA1 |
| *T. atroviride* 164b | 20.19[b] | 52.37 | CA1 |
| Control | 42.39[c] | - | - |

*Means of triplicates. The mean values followed by different letters indicate that results are significantly different according to the Scott-Knott test ($p < 0.05$). **Badalyan rating scale (BADALYAN et al., 2002): CA1 = partial replacement after initial deadlock with mycelial contact.

Interestingly, previous investigation with *T. atroviride* 36b and 164b reported the interaction type A between endophytes and *Sclerotinia sclerotiorum*, CA2 interaction for *Glomerella* sp., CB2 (strain 36b) and CA1 (strain 164b) interactions with the pathogen Colletotrichum sp. (BONGIORNO et al. 2016). Similar to obtained herein, replacement after initial deadlock with mycelial contact (CA1 or CA2 interactions) was found as main CI between Trichoderma antagonists and *Phytophthora cactorum* and *Phytophthora plurivora* (BERGER et al., 2015).

*F. oxysporum* is responsible for causing the *Fusarium* wilt that mainly affects tomato cultivars. The pathogen invades the root system, causing the withering of the aerial parts and the plant death (VOS et al., 2014). This fungus produces two types of reproductive structures (macro and microconidia) which, in the absence of a host, can survive in the environment for more than six years and may extend for more than 10 years if other resistance structures, such as chlamydospores, occur (ZHANG et al., 2015).

In the present study, SEM allowed an in-depth knowledge on the *in vitro* interactions between *Trichoderma* endophytes and *F. oxysporum*. As highlighted by Pamphile et al. (2008), SEM represents an important tool for better understanding the endophytic interactions and offers unique advantages as high resolution and large depth of field. Regarding the interaction between pathogen and *T. atroviride* 164b, we found the presence of *Fusarium* macroconidia between endophytic hyphae and conidia (Figure 3). In addition, the helicoidization of endophytic hyphae, which wrapped around *Fusarium* hyphae, suggested a mechanical inhibition by strangulation (Figure 2). These results corroborated with literature reports on Trichoderma interactions with *Fusarium solani, F. oxysporum*, *Ceratocystis paradoxa* and *S. sclerotiorum* (EZIASHI et al., 2007; LOUZADA et al., 2009; VILLAMIZAR-GALLARDO et al., 2017; JULIATTI et al., 2019; SONKAR, 2019). This strangulation process indicates the hyperparasitic behavior of *Trichoderma*, in which this fungus detects the pathogen hyphae due to chemical stimuli produced by the host hypha itself (JULIATTI et al., 2019; SONKAR, 2019).

Figure 2 – Scanning electron microscopy showing the interaction between *Fusarium oxysporum* and *Trichoderma atroviride* 164b. a) endophytic hyphae (arrow with asterisk) wrapping around pathogen hyphae (arrow without asterisk).

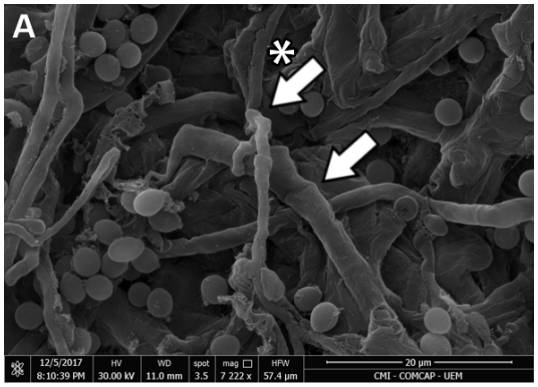
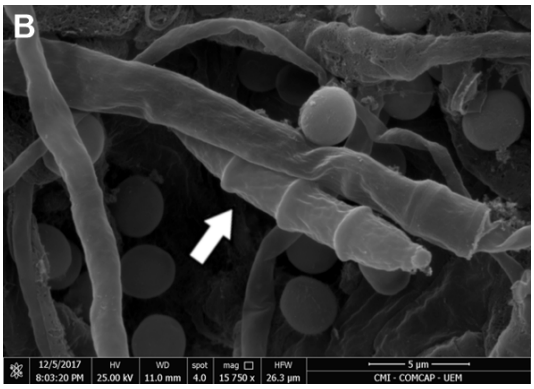


Figure 3 – Scanning electron microscopy showing the interaction between Fusarium oxysporum and Trichoderma atroviride 164b. b) F. oxysporum macroconidia (arrow) between endophytic hyphae and conidia.




## 5 Conclusions

The *Trichoderma atroviride* strains tested in the current study showed an *in vitro* inhibition of *F. oxysporum*, possibly caused by a mechanical inhibition by strangulation. The *in vivo* tests indicated the growth-promoting potential of these endophytes. Once both fungi were IAA-positive, the results could suggest a relation between IAA secretion and growth of tomato plants.

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