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Characterization of Novel Brazilians Rhizosphere Soil *Trichoderma* to Select Effective Biocontrol Agents against *Sclerotinia sclerotiorum* on Beans

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Authors' contributions

This work was carried out in collaboration among all authors. Author MRCF did the substantial contribution in the concept and design of the study; in the data collection; in the data analysis and interpretation; in the manuscript preparation and in the critical revision, adding intellectual content. Author LRS did the contribution in the data analysis and interpretation, in the manuscript preparation and in the critical revision, adding intellectual content. Author PHPCM did the contribution in the manuscript preparation and in the critical revision, adding intellectual content. Author DDCC did the substantial contribution in the data analysis and interpretation; in the manuscript preparation; in the critical revision, adding intellectual content. Author SCMM did the substantial contribution in the concept and design of the study; in the data collection; in the data analysis and interpretation; in the manuscript preparation and in the critical revision, adding intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Trichoderma spp. are a fungi group considered among the most attractive for biological control due to their different mechanisms action against plant pathogens and as promoters of plant growth and productivity. The molecular characterization of this antagonists is of great importance for

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conducting studies in the area of biodiversity and to identify their potential use in biological control. The objective of this work was to identify 29 *Trichoderma* isolates based on molecular and phylogenetic analysis using sequences comparison of the ITS regions, in addition to selecting the isolates with the highest levels of antagonism against *S. sclerotiorum* and capable of promoting bean plant growth, using in vitro and greenhouse experiments. Among the sequences obtained, a total of five different *Trichoderma* species were identified: *T. asperellum*, *T. harzianum*, *T. koningiopsis*, *T. brevicompactum* and *T. tomentosum*, although this study should be complemented with the use of at least two other markers, TEF1 and RPBII, to definitively demarcate these species. *Trichoderma* isolates showed a variation in mycelial inhibition of the pathogen by non-volatile metabolites between 84.11% to 100%. Regarding the dry weight of plants treated with *Trichoderma* and the pathogen, it was observed that ten *Trichoderma* isolates promoted plant growth compared to the control with a variation of 2.6% to 34%. *Trichoderma asperellum* CEN201 proved to be the best candidate and should be used in more advanced tests, including field trials for the biological control of *S. sclerotiorum* and growth promotion in common bean, as it showed excellent results in laboratory and greenhouse conditions in suppressing the white mold disease and promoting the growth of bean plants.

Keywords: Biological control; mycelial inhibition; phylogenetic analysis; white mold.

1. INTRODUCTION

Beans are one of the most important crops based on harvested areas and global production of this legume [1]. Brazil is the world's largest producer of common bean (*Phaseolus vulgaris* L.) with an annual production of 3,250,000 t and an average grain yield of 1,024 kg/ha [2]. Although this crop is susceptible to several diseases, especially in large cultivated areas, few of them are as harmful as white mold [1,3], caused by the soil fungus *Sclerotinia sclerotiorum* [(Lib.) de Bary (1884)].

This pathogen is necrotrophic, capable of infecting more than 400 plant species, a fact that makes it an economic threat to world agriculture [4]. White mold epidemics are reported worldwide with estimated losses of 13% to 80%, depending on year and geographic region [5, 6].

In beans, white mold can affect the entire shoot, causing initially small, watery lesions that quickly increase in size. As the disease progresses, the affected parts lose color, becoming yellowish and then brown, producing soft tissue rot, followed by the white cottony mycelium characteristic of the fungus [7].

Sclerotinia sclerotiorum can produce survival structures by clumping hyphae with a high melanin content in the outer layer, called sclerotia. These structures are generally rounded, elongated or irregular in shape of different sizes which, depending on the host, range from a few millimeters to a few centimeters [8,9,10].

Modern agriculture is dependent on a number of synthetic fungicides that significantly contribute to increasing the economic efficiency of crop production to meet the food needs of the rapidly growing global population [11,12]. However, the unbalanced application of agrochemicals leads to environmental degradation and poses numerous challenges to agriculture, ecosystems and soil health [13].

Dozens of fungi and bacteria are widely described as acting in the biocontrol of *S. sclerotiorum* and promoting plant growth, including species of the genus *Trichoderma* Persoon (1794), a fungus considered one of the most attractive for biological control due to its different mechanisms action against plant pathogens and action to promote plant growth and productivity [14,15,16]. Several commercial *Trichoderma* based products are available in most of the world's best-developed large arable areas [17], so its use has become a viable and sustainable resource for reducing the use of agrochemicals in agriculture.

While correct species identification is important in the selection and validation of biocontrol agents, the assessment of infraspecific variation is also important to protect commercial strains and understand the genetic resources available in natural populations [18]. Regarding the identification and taxonomy of *Trichoderma*, until the 1990s, it was entirely based on morphological characteristics observed under optical microscopy and most *Trichoderma*

isolates cited in the literature were misidentified due to the difficulty in interspecific differentiation of reproductive structures [19]. The phylogenetic species concept, based on concordance of genealogies of multiple genes, revolutionized fungal taxonomy and exposed weaknesses in traditional identification based only on morphology [20].

After the 1990s, when molecular techniques began to be routinely employed, confirmation or correction of previously designated species occurred. In the past decade, a virtual database (Gen Bank) was established, where nucleotide sequences of most known species are deposited. In addition to this database, a website dedicated to the comparison of nucleotide sequences unique to *Trichoderma* species and their teleomorphs of the genus Hypocrea was created [21].

Given the above, the objective of this work was to identify 29 *Trichoderma* isolates based on molecular profiles and phylogenetic analysis, as well as to select the isolates that presented the highest levels of antagonism against *S. sclerotiorum* and that promoted growth in common bean using experiments *in vitro* and in a greenhouse.

2. MATERIALS AND METHODS

2.1 Microorganisms and Culture Conditions

Twenty-nine *Trichoderma* isolates (Table 1) and one of *Sclerotinia sclerotiorum* (CEN217) were supplied by the Fungal Collection for Biological Control of Plant Pathogens and Weeds of the Brazilian Agricultural Research Corporation (Embrapa), Brazil.

Table 1. rDNA-based molecular identification of *Trichoderma* strains from different Brazilian geographical origins and plant rhizospheres

Isolate Code	Geographical origin / substrate	ITS1-5.8S-ITS2	Genbank accession number
CEN162	Federal District / rhizosphere soil from rice	*T. asperellum*	KC561056
CEN201	Mato Grosso state / rhizosphere from native soil	*T. asperellum*	KC561057
CEN209	Federal District / rhizosphere from native soil	*T. koningiopsis*	KC561058
CEN210	Federal District / rhizosphere from native soil	*T. koningiopsis*	KC561059
CEN234	Federal District / rhizosphere soil from cotton	*T. harzianum*	KC561060
CEN250	Federal District / rhizosphere soil from cotton	*T. harzianum*	KC561061
CEN252	Federal District / rhizosphere soil from cotton	*T. tomentosum*	KC561062
CEN284	Federal District / rhizosphere soil from cotton	*T. harzianum*	KC561063
CEN287	Federal District / rhizosphere soil from cotton	*T. harzianum*	KC561064
CEN289	Federal District / rhizosphere soil from cotton	*T. harzianum*	KC561065
CEN503	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561066
CEN504	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561067
CEN509	Pernambuco state / rhizosphere soil from guava	*T. brevicompactum*	KC561068
CEN510	Pernambuco state / rhizosphere soil from guava	*T. harzianum*	KC561069
CEN511	Pernambuco state / rhizosphere soil from guava	*T. brevicompactum*	KC561070
CEN512	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561071
CEN514	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561072
CEN518	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561073
CEN519	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561074
CEN520	Pernambuco state / rhizosphere soil from guava	*T. asperellum*	KC561075
CEN522	Pernambuco state / rhizosphere soil from guava	*T. brevicompactum*	KC561076
CEN698	Federal District / rhizosphere soil from strawberry	*T. koningiopsis*	KC561077
CEN747	Federal District / rhizosphere soil from strawberry	*T. asperellum*	KC561078
CEN761	Federal District / rhizosphere soil from strawberry	*T. koningiopsis*	KC561079
CEN776	Federal District / rhizosphere soil from strawberry	*T. koningiopsis*	KC561080
CEN786	Federal District / rhizosphere soil from strawberry	*T. asperellum*	KC561081
CEN847	Federal District / rhizosphere soil from soybean	*T. asperellum*	KC561082
CEN854	Rio Grande do Sul state / native forest soil	*T. harzianum*	KC561083
CEN865	Rio Grande do Sul state / native forest soil	*T. asperellum*	KC561084

2.2 Genomic DNA Extraction, PCR Amplification and Sequencing

The *Trichoderma* strains were grown on potato dextrose agar (PDA, Acumedia, Michigan, USA) and the genomic DNA was obtained according to [22]. The rRNA gene ITS1-5.8S-ITS2 regions were amplified with universal primers ITS1 (5´-CCG TAG GTG AAC CTG CGG-3´) and ITS4 (5´-TCC TCC GCT TAT TGA TAT GC-3´) according to [23]. PCR reactions were performed in 25 µL containing: genomic DNA (5–25 ng), each primer (0.4 mM), dNTPs (0.2 mM, GE Healthcare, Connecticut, USA), $MgCl_2$ (1.5 mM, Healthcare, Connecticut, USA), Taq polymerase (2.0 U, Life Technologies, Ca, USA) and reaction buffer (10 µL, Invitrogen). Amplification was performed as described by [24] with an initial denaturation for 5 min at 94ºC, 30 cycles of 30 s at 94ºC, 30 s at 55ºC, 1 min at 72ºC, 10 min at 72ºC, and finally cooling to 4ºC. Purified PCR products were sequenced at Sanger Macrogen INC, South Korea, using the same primers described for PCR reactions.

2.3 Analysis of Sequence Data

DNA sequences were aligned using Clustal X v2.0.12. The terminal regions with poor alignment were manually removed using BioEdit v7.0.5.3, and the remaining portions were aligned together. Sequence analysis of the rRNA gene ITS1-5-8S-ITS2 amplicons was performed using the TrichOKEY 2.0 and TrichoBLAST tools available online at http://www.isth.info/. The sequences were deposited in GenBank.

2.4 Phylogenetic Analysis

The rRNA gene ITS1-5.8S-ITS2 sequences were analyzed by maximum parsimony (MP) and Bayesian phylogenetic Inference (BI) for taxonomic characterization and establishment of phylogenetic relationships among the *Trichoderma* strains. Sequences were aligned using the program MUSCLE - Multiple Sequence Alignment v3.5 [25] and the alignment manually corrected using BioEdit Sequence Alignment Editor v7.0.8.0, 1997-2007 [26]. Gaps present in the original alignment were preserved and coded separately using the program FastGap v1.2 [27]. MP analysis was performed using PAUP* program v4.0b10, 2001 [28]. The heuristic search consisted of 1,000 replicates using the random addition of taxa (Stepwise Addition). The exchange was carried out by the algorithm branches TBR (Tree Bisection and Reconnection) and the robustness of the tree topologies was evaluated by bootstrap analysis with 1,000 replicates. Bayesian approach was performed using the program MrBayes v3.1.2 [29]. The nucleotide substitution model more appropriate for alignment of the rDNA model was the GTR (General Time Reversible). The algorithm Monte Carlo Markov Chain (MCMC) was started from a random tree and six processed Markov chains for 1,000,000 generations, with samples collected every 100 generations. In the analysis, 25% were discarded of initial samples (burn-in) and the remainder used to determine the distribution of the posterior probability values. The indels were excluded from analysis. MP and IB trees were edited with Fig Tree - Tree figure drawing tool v1.3.1 [30] and Adobe Illustrator Cs5 v15.0.0.0 programs. The *Trichoderma longibrachiatum* CEN1067 was used as outgroup for rooting the tree.

2.5 Dual Culture

To evaluate the antagonism of twenty-nine *Trichoderma* strains against *Sclerotinia sclerotiorum* (CEN217), 5-mm diameter discs of PDA medium were taken from the edge of actively growing colonies of fresh fungal cultures, and placed on the surface of a fresh PDA plate at a spacing of 4 cm. The plates were kept in a BOD at 25 °C, with a 12-h photoperiod. The evaluation was performed when the pathogen completely covered the control plate without *Trichoderma*. The evaluation of antagonism was carried out according to the classification proposed by [31]: grade 1, *Trichoderma* grows on the pathogen and occupies the entire surface of the medium; grade 1.5, *Trichoderma* grows on 87.5% of the surface of the medium; grade 2, *Trichoderma* grows on 66.6% of the surface of the medium; grade 2.5, *Trichoderma* grows on 62.5% of the surface of the medium; grade 3, *Trichoderma* occupies 50.0% of the surface of the medium; grade 3.5, *Trichoderma* grows on 37.5% of the surface of the medium; grade 4, *Trichoderma* grows on 33.3% of the surface of the medium; and grade 5, *Trichoderma* does not grow and the pathogen occupies the entire surface of the medium. The experiment was replicated three times for each *Trichoderma* strains and conduced two times.

2.6 Non-volatiles Metabolites

To evaluate the *Trichoderma* no-volatile metabolite action against *S. sclerotiorum*, after cultivation of each *Trichoderma* isolate for seven

days, mycelial agar plugs (5 cm Ø) were inoculated in 50 ml sterilized potato dextrose broth (PDB) in 100 ml flasks and incubated at 25 ± 2ºC under orbital shaking at 150 rpm for seven days. Thereafter, the cultures were filtered through whatman N° 1 filter paper and filtered through a 0.45-µm membrane filter. Sterile PDA medium was distributed into tubes and the fungi filtrates were added to reach final filtrate concentrations of 25% (v/v). The mixtures were individually distributed in Petri dishes (9 cm Ø) and kept in UV light for solidification for 20 minutes. Five mm diameter of seven-days-old *S. sclerotiorum* culture was inoculated at the center of Petri dishes. The plates were then kept at 25± 2 °C for seven days with a 12-h photoperiod. Each test was repeated three times, and the efficiency of the filtrates was measured in terms of percentage of *S. sclerotiorum* mycelial growth inhibition calculated using the following formula: mycelial growth inhibition (%) = (dc- dt/dc) ×100 Where dc = mean diameter of control (untreated) and dt = mean diameter of treated mycelium. The experiment was replicated three times for each *Trichoderma* strains and conduced two times.

2.7 Sporulation in Solid Substrate

To determine the sporulation of *Trichoderma* isolates, 1000 mL Erlenmeyer flasks were filled with 100 g of parboiled rice with 60% distilled water (V/V), sealed (gauze and cotton plug) and then autoclaved at 121ºC for 25 minutes. After autoclaving, under aseptic conditions, 4 ml of *Trichoderma* conidia suspension (10^8 conidia/mL) were transferred onto the parboiled rice. The cultures were thereafter incubated at 25± 2°C for seven days under alternate cycles of 12 h light and 12 h darkness. To determine the mean sporulation of each of the 29 *Trichoderma* isolates, each Erlenmeyer was shaken by hand and one gram was collected from each repetition of the treatments to then count each sample in a Neubauer chamber. The treatments with each *Trichoderma* strain were repeated three times and the experiment was conducted twice.

2.8 Greenhouse Experiments

The experiments to verify plant growth promotion and white mold action in bean plants (cultivar carioca) were carried out in a greenhouse with temperature variation from 17.2 °C to 21.78 °C and humidity with variation from 85.8% to 88.0%. The treatments were set up with all *Trichoderma* isolates and the pathogen, as follows: *Trichoderma* + Pathogen; Pathogen and absolute witness (no fungi).

The experiment was set up in polypropylene trays with 72 cells (120 cm^3/cell) containing substrate based on pine bark and vermiculite (Plantmax®). After filling the cells with substrate, 1g of rice + pathogen was transferred to each corresponding cell and, 48 hours later, 10 ml of the spore suspension was inoculated ($1.0x10^6$ spores.ml^{-1}). Plants previously germinated for three days in the laboratory were planted individually in each cell of the tray. The treatments used for the experiment were elaborated in the following three ways: *Trichoderma* + Pathogen; only the pathogen is an absolute witness (no fungi). Dry weight and disease intensity caused by the pathogen were evaluated 21 days after planting.

The experiment was conducted with four replications per treatment in a completely randomized design and repeated twice. Results were subjected to analysis of variance and to the Scott‑Knott test at 5% probability, using the Sisvar software [32].

3. RESULTS AND DISCUSSION

3.1 Identification of *Trichoderma* Isolates

Recently, approach errors were detected in the identification of some *Trichoderma* species, using only ITS markers. Many of the sequences deposited in databases and used as specific patterns would actually be components of species complexes. That said, the *Trichoderma* isolates were identified in this work to species level based upon Blast and Trichokey comparisons with ITS1-5.8S-ITS2 rRNA gene sequence data for known species within the genus *Trichoderma* (Table 1). Among the sequences obtained, a total of five different *Trichoderma* species or species complex were identified: *T. asperellum*, that is *Asperellum* Sect. Trichoderma, according to Samuels, (13), *T. harzianum* Rifai (7) that is, representatives of the *Harzianum* species complex, *T. koningiopsis* Samuels (5), *T. brevicompactum* Kraus (3) that is, representatives of the *Brevicompatum* species complex (3) and *T. tomentosum* Bisset (1).

Table 2. rDNA-based molecular identification of *Trichoderma* strains from different Brazilian geographical origins and plant rhizospheres

Isolate Code	Geographical origin/substrate	Species/complex	Accession number
CEN162	Distrito Federal/rhizosphere soil from rice	*Asperellum (Sect.* Trichoderma*)*	KC561056
CEN201	Mato Grosso / rhizosphere soil from native vegetation	*T. asperellum*	KC561057
CEN209	Distrito Federal / rhizosphere soil from native vegetation	*T. koningiopsis*	KC561058
CEN210	Distrito Federal / rhizosphere soil from *copaiba*	*T. koningiopsis*	KC561059
CEN234	Distrito Federal / rhizosphere soil from cotton	*Harzianum*	KC561060
CEN250	Distrito Federal / rhizosphere soil from cotton	*Harzianum*	KC561061
CEN252	Distrito Federal / rhizosphere soil from cotton	*T. tomentosum*	KC561062
CEN284	Distrito Federal / rhizosphere soil from cotton	*Harzianum*	KC561063
CEN287	Distrito Federal / rhizosphere soil from cotton	*Harzianum*	KC561064
CEN289	Distrito Federal / rhizosphere soil from cotton	*Harzianum*	KC561065
CEN503	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561066
CEN504	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561067
CEN509	Pernambuco / rhizosphere soil from guava	*Brevicompactum*	KC561068
CEN510	Pernambuco / rhizosphere soil from guava	*Harzianum*	KC561069
CEN511	Pernambuco / rhizosphere soil from guava	*Brevicompactum*	KC561070
CEN512	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561071
CEN514	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma)	KC561072
CEN518	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561073
CEN519	Pernambuco / rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561074
CEN520	Pernambuco/rhizosphere soil from guava	*Asperellum (Sect.* Trichoderma*)*	KC561075
CEN522	Pernambuco / rhizosphere soil from guava	*Brevicompactum*	KC561076
CEN698	Distrito Federal / rhizosphere soil from strawberry	*T.koningiopsis*	KC561077
CEN747	Distrito Federal / rhizosphere soil from strawberry	*Asperellum (Sect.* Trichoderma*)*	KC561078
CEN761	Distrito Federal / rhizosphere soil from strawberry	*T. koningiopsis*	KC561079
CEN776	Distrito Federal / rhizosphere soil from strawberry	*T. koningiopsis*	KC561080
CEN786	Distrito Federal / rhizosphere soil from strawberry	*Asperellum (Sect.* Trichoderma*)*	KC561081
CEN847	Distrito Federal / rhizosphere soil from soybean	*Asperellum (Sect.* Trichoderma*)*	KC561082
CEN854	Rio Grande do Sul / native forest soil	*Harzianum*	KC561083
CEN865	Rio Grande do Sul/ native forest soil	*Asperellum (Sect.* Trichoderma*)*	KC561084

Phylogenetic trees generated from analysis of ITS1-5.8S-ITS2 rRNA gene sequences (Fig. 1) using Maximum Parsimony and Bayesian analyses exhibited similar topologies with four well-defined groups with branches strongly supported by posterior probability higher than 0.9 and bootstrap value higher than 80%. These groups corresponded to Hamatum, Viride, Brevicompactum and Harzianum clades, each of which contained a reference sequence for the species. The Hamatum clade was composed of 13 isolates of *T. asperellum (Sect. Trichoderma),* six of which (CEN503, CEN504, CEN512, CEN514, CEN519 and CEN520) forming a monophyletic group, supported with posterior probability of 0.95 and a bootstrap value of 92%. These isolates were all collected from guava rhizosphere soil samples in Pernambuco State (Brazil). The Viride clade consisted of five isolates of *T. koningiopsis* and infer that CEN776

and CEN761 isolates, collected in strawberry crop in Distrito Federal (Brazil), show high similarity. The CEN698 isolate, collected in the same geographic origin/substrate, was shown to be phylogenetically distinct to the two other strains. Two monophyletic clades within the Harzianum clade were observed which were very well defined and strongly supported with posterior probability higher than 0.80 and bootstrap value higher than 75%: one containing seven *T. harzianum* isolates (species complex) and the other with the *T. tomentosum* isolate. This is informative data that show good separation of the *T. harzianum* and *T. tomentosum* isolates.

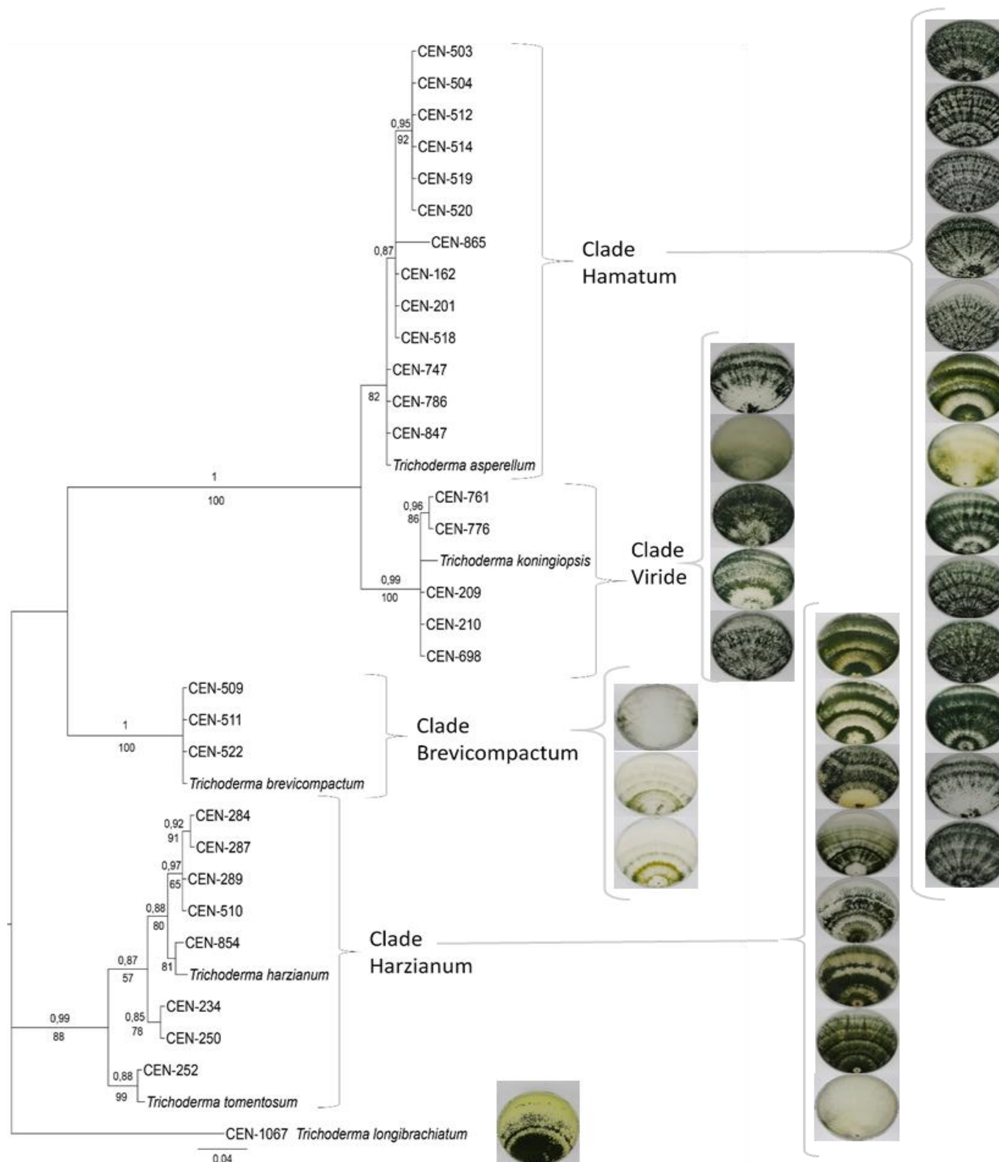

Fig. 1. Phylogenetic tree based on ITS1-5.8S-ITS2 rRNA gene of 29 *Trichoderma* isolates. The tree topology was inferred from Bayesian analysis. Numbers above branches indicate Bayesian posterior probabilities (value > 0.9) and numbers below branches indicate bootstrap values (value > 50%) for 1,000 replicates derived from maximum parsimony analysis. The isolates *T. asperellum* (JN004182), *T. brevicompactum* (JQ040334), *T. harzianum* (JN179079), *T. koningiopsis* (HQ857120), and *T. tomentosum* (HQ857116) are external reference standard isolates obtained from GenBank. *Trichoderma longibrachiatum* CEN1067 was used as outgroup. The images aligned in each clade are the colonies of the respective *Trichoderma* isolates cultivated in PDA medium for 7 days at 25°C in a 12h photoperiod

3.2 Evaluation of *Trichoderma Isolates In vitro against Sclerotinia sclerotiorum*

The selection of 29 Trichoderma isolates for testing was based on previous experiments that showed the potential for biological control of the pathogen.

Regarding the laboratory experiments, the *Trichoderma* isolates used showed a variation in the mycelial inhibition of the pathogen by non-volatile metabolites (Table 2) between 84.11% (CEN511) to 100% (CEN201). Isolates CEN201, CEN503, CEN504, CEN509, CEN512, CEN514, CEN518, CEN519, CEN520, CEN698 and CEN786 showed the highest mycelial inhibition rates against *S. sclerotiorum*, ranging from 90% to 100%.

In the pairing of cultures (Table 2), according to the scale by [31], only grades 01 and 02 were observed. Trichoderma isolates considered as grade 01 were: CEN162, 201, 504, 511, 512, 514, 518, 519, 520.522 and 747. The other isolates were considered as with grade 02.

Analyzing the sporulation of *Trichoderma* isolates in parboiled rice (Table 2) was observed discrepancy between the production of spores ranging from 4.35×10^6 spores. mL^{-1} (CEN210) to 2.20×10^8 spores mL^{-1} (CEN511). Four isolates (CEN209, CEN210, CEN776, CEN786) produced around 10^6 spores. mL^{-1}. Nineteen isolates (CEN201, CEN252, CEN284, CEN287, CEN503, CEN504, CEN509, CEN510, CEN514, CEN518, CEN519, CEN520, CEN522, CEN698, CEN747, CEN761, CEN847, CEN854, CEN865), presented sporulation rate of 10^7 spores. mL^{-1}. For the others, the sporulation rate was 10^8 spores. mL^{-1}.

Table 3. Antagonism, growth inhibition of *S. sclerotiorum,* and sporulation by *Trichoderma* isolates

Isolate Code	Inhibition by V.M.%	Dual culture	Conidia mL^{-1}
CEN162	88.89 d	1	1.25E+08 d
CEN201	100.00 a	1	8.65E+07 f
CEN209	86.11 f	2	7.75E+06 m
CEN210	88.33 e	2	4.35E+06 m
CEN234	88.00 e	2	125E+08 d
CEN250	85.78 f	2	1.65E+08 b
CEN252	87.78 e	2	1.80E+07 m
CEN284	85.78 f	2	9.65E+07 e
CEN287	86.11 f	2	1.35E+07 l
CEN289	88.89 d	2	1.45E+08 c
CEN503	92.22 b	1	8.55E+07 f
CEN504	92.22 b	1	2.80E+07 j
CEN509	90.00 d	2	1.45E+07 l
CEN510	87.22 e	2	1.80E+07 l
CEN511	84.11 g	1	2.35E+08 a
CEN512	91.56 b	1	1.25E+08 d
CEN514	92.22 b	1	2.55E+07 j
CEN518	92.22 b	1	7.80E+07 f
CEN519	92.22 b	1	8.55E+07 f
CEN520	89.78 d	1	9.65E+07 e
CEN522	88.56 d	1	3.45E+07 i
CEN698	92.22 b	2	8.75E+07 f
CEN747	88.00 e	1	6.65E+07 g
CEN761	88.56 d	2	7.65E+07 f
CEN776	85.00 g	2	8.75E+06 m
CEN786	90.56 c	2	8.65E+06 m
CEN847	87.89 e	2	5.25E+07 h
CEN854	87.89 e	2	3.75E+07 i
CEN865	87.89 e	2	1.60E+07 l
CEN217	0.0 h	**	**
C.V.	5.71	**	8.91

3.2 Growth Promotion and Pathogen Control in Common Bean with *Trichoderma* in a Greenhouse

Regarding the dry weight of the plants (Fig. 2) treated with *Trichoderma* and the pathogen, it was observed that ten *Trichoderma* isolates promoted plant growth in relation to the control with a variation of 2.6% (CEN510) to 34% (CEN201). Of these isolates, nine are of the species complex Asperellum and one of Harzianum. Isolates CEN201 and CEN162 promoted the greatest development of plants, followed by isolates CEN786, CEN512, CEN503, CEN518, CEN519, CEN854, CEN847 and CEN510. The rest of the 19 isolates tested showed a decrease in plant growth in relation to the control, which ranged from -0.6% (CEN209) to -67.3% (CEN284).

Regarding the control of the pathogen in bean plants under greenhouse conditions, there was an absence of symptoms and signs in treatments that showed an increase in dry weight (Fig. 2). In treatments where the dry weight was negative, strong symptoms and signs of the pathogen were observed. Plants treated with isolates CEN209, CEN250 and CEN504 had lower dry weight compared to the control, which ranged from -0.6% (CEN209) to -4.5% (CEN504), however, they did not show symptoms or signs of the pathogen.

When analyzing the correlations between the results of the laboratory experiments and the results in the greenhouse (data not shown), a positive correlation was observed between the tests of "non-volatile metabolites" x "Dry weight of plants in a greenhouse" ($r = 0.45$; $p = 0.34$).

Fungi of the *Trichoderma* genus can act by different mechanisms against phytopathogens and as plant growth promoters. However, it is difficult to be precise about which mechanisms and to what extent they use them to benefit plants and reduce the population of phytopathogenic fungi. These parameters depend on the physical-chemical conditions of the environment. For this reason, the action of *Trichoderma* isolates can be unpredictable [33].
It is important to understand the genetic diversity of *Trichoderma* species, their biocontrol mechanisms and reproductive potential in order to be more successful in applying the best *Trichoderma* isolates in the pathosystem in which the problem is observed [33,34]. Information extracted from the ITS1-5.8S-ITS2 rRNA gene showed the formation of four clades after analysis. In the future, at least two more specific molecular markers such as translation Elongation Factor (TEF-1α) and RNA polymerase II (RPBII) should be used to definitively demarcate these *Trichoderma* species. Despite this, thirteen *T. asperellum* isolates, one *T. tomentosum* isolate, seven *T. harzianum* isolates, five *T. koningiopsis* isolates and three *T. brevicompactum* isolates were identified based on similarity with the identified representative species. The molecular taxonomy of *Trichoderma* isolates is in accordance with the pre-existing designs [35,36], by which the species were grouped in the same clades (Hamatum, Viride, Brevicompactum, and Harzianum), according to the groupings in Fig. 1.

Results from other authors using the same regions of the DNA gene corroborate this work, as shown by the clusters obtained with *T. asperellum* (Hamatum clade), *T. harzianum* (Harzianum clade) and *T. longibrachiatum* in a study with 13 isolates [37]. Similarly, was found in another study with six different species [38] which, however, showed ambiguous identifications for *T. asperellum* and *T. brevicompactum*, contrary to the situation in Fig. 1, where the difference between *Trichoderma* species is clear. This shows, once again, the need to use complementary markers for correct identification of *Trichoderma* species.

Trichoderma spp. have a natural ability to inhibit the attack of phytopathogens by different mechanisms, mainly direct parasitism, antibiosis and competition, although with different intensities depending on the strain used, as well as other factors, including biological targets, climate, and matter content soil organic matter [39,40,41].

In this work, all *Trichoderma* isolates showed a high rate of mycelial inhibition of the pathogen in the paired culture and volatile metabolites tests. One of the most prominent results was with CEN201 in both trials. In experiments carried out with *T. harzianum* inhibition of mycelial growth of *S. sclerotiorum* was observed in 56.3% and 48%, respectively, using tests of paired cultures and production of non-volatile metabolites [42]. Similar results have been reported [43] with *T. asperelloides* [31].

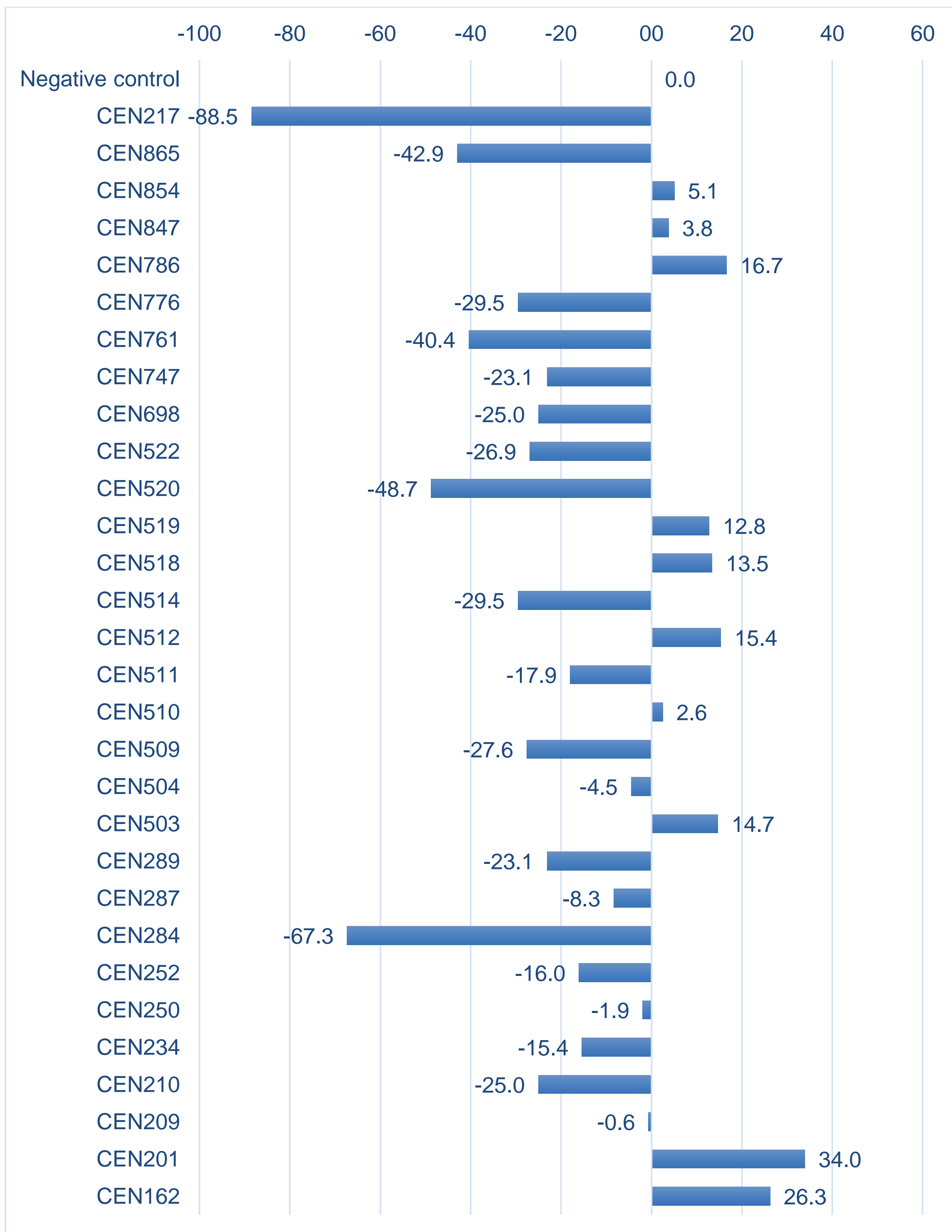

Fig. 2. Relative efficiency (%) of dry weight evaluated at 16 days after planting of bean inoculated with *Trichoderma* spp. Coefficient of variation: 10.29

Isolate CEN201 showed satisfactory sporulation in parboiled rice. However, the highest sporulation rate was verified with CEN511, which also presented good results in the mycelial inhibition test in paired cultures, as well as volatile metabolites. The difference between this and CEN201 was the behavior in a greenhouse. Isolate CEN201 totally controlled the pathogen and promoted plant growth by 26%, while isolate CEN511 did not prove to be a good agent for

biocontrol of the pathogen, allowing the expression of signs and symptoms of *S. sclerotiorum* in common bean.

In the selection of *Trichoderma* for biological control of phytopathogens and promotion of plant growth, it is very important that the microorganism has a high sporulation rate in the chosen culture medium, because in large-scale commercial production, high spore productivity directly influences the reduction the costs of the entire production chain of commercial biological products [44]. Furthermore, the high spore production significantly affects the formulation, shelf life and dose of the product applied in the field [45], generating a product of higher quality and lower price for the farmer. From a biological point of view, the large production of spores can influence disease management and increase the agronomic aspects of the plant, given that one of the main biocontrol mechanisms used by *Trichoderma* is competition for space and nutrients. However, the best isolate for pathogen control was CEN201, which sporulated 26% less than the best isolate in relation to this aspect (CEN511). This was demonstrated [46] in the selection of *Trichoderma* for biological control of phytopathogens and promotion of plant growth, it is very important that the microorganism has a high sporulation rate in the chosen culture medium, because in large-scale commercial production, high spore productivity directly influences the reduction the costs of the entire production chain of commercial biological products [44]. Furthermore, the high spore production significantly affects the formulation, shelf life and dose of the product applied in the field [45]. From a biological point of view, the large production of spores can influence disease management and increase the agronomic aspects of the plant, given that one of the main biocontrol mechanisms used by *Trichoderma* is competition for space and nutrients. However, the best isolate for controlling of the pathogen was CEN201, with less sporulation capacity than CEN511. Therefore, sporulation cannot always serve as parameters to indicate that a *Trichoderma* isolate is better or worse for disease control. This was demonstrated by [46] who evaluated the potential for volatile metabolites of *Trichoderma* spp. to inhibit the mycelial growth of *S. sclerotiorum*. These authors demonstrated that the species that sporulated the most was the one that least inhibited the growth of the pathogen's mycelium and inferred that the pathogen's mycelial inhibition seems to be more related to the qualitative profile of the volatile metabolites emitted by *Trichoderma* spp.

Another isolate that stood out in laboratory and greenhouse tests was CEN162, that reached a high level of mycelial inhibition by non-volatile metabolites (88.9 %), a maximum score on the scale of [31] against the pathogen, and greater sporulation in parboiled rice than CEN201. The great difference between these two isolates was in the promotion of bean growth in a greenhouse, CEN162 promoted a 26.3% increase in dry mass of the bean plant, 7.69% more than isolate CEN201.

Several authors use greenhouse trials to analyze disease control and plant growth promotion. These works are highly relevant, as they indicate that microorganisms can be more successful in the field than those tested only in the laboratory. [47] used 19 *Trichoderma* isolates and selected the best four according to laboratory tests. One of them showed better plant growth promotion and the another showed the highest rate of inhibition of *Fusarium* wilt in tomato. Also [48] selected 29 *Trichoderma* isolates in laboratory and greenhouse showing that an isolate UFT201 (*T. asperelloides*) was the most efficient, both in laboratory tests and in a greenhouse, for the promotion of growth in caupi beans. The same isolated was used [49] to verify the growth promotion in soybean under field conditions in the state of Tocantins, Brazil. The authors concluded that its use provided greater development of soybean plants and significantly increased grain yield, and showed that the selection of *Trichoderma* isolates in laboratory and greenhouse had positive results in the field.

The ability of microorganisms to promote plant growth has often been associated with the emission of volatile and non-volatile metabolites near plant roots. Positive correlation, although low, was verified between the tests of “Volatile Metabolites” vs. “Dry weight of plants in a greenhouse”. This correlation makes a lot of sense, as some metabolites emitted by Trichoderma are known to act as plant growth promoters in addition to having direct effects on phytopathogens [50,51]. This reinforces the theory that metabolites produced by Trichoderma spp. and other microorganisms act as mediators of their biochemical relationships with plant roots and, consequently, can trigger responses capable of stimulating plant growth [52,53,54], favoring the synthesis of plant hormones such as auxins and ethylene [55].

4. CONCLUSION

The results obtained in this work with 29 *Trichoderma* isolates demonstrate that the isolate CEN201 is the best candidate to participate in a broader selection that would include field tests for the biological control of *S. sclerotiorum* and growth promotion in common beans, as in the laboratory and greenhouse testes.

The taxonomy of *Trichoderma* evolved rapidly and today additional markers are recommended to discriminate species within previously designated species complexes. Therefore, the data shown here will be complemented using at least two markers (TEF-1α and RPB2 sequences) shortly.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lima RC, Teixeira PH, Souza AFF, Paula Júnior TJ,Teixeira H, Lehner MS, Carneiro JES, Marçal TS, Vieira RF.Strategy to identify resistance to white mould associated with high yield for irrigated common bean in Brazil. Plant Pathol. 2020;69:1172-1184.
Available:https://doi.org/10.1111/ppa.13181
2. CONAB. Acompanhamento da safra brasileira. Grãos. Safra v. 8, n. 10, 2020/21. 10º Levantamento; 2021.
Accessed 20 July 2021
Available:https://www.conab.gov.br/info-agro/safras/graos. Portuguese
3. Chauhan S, Katoch S, Sharma SK, Sharma PN, Rana JC, Singh K, Singh M. Screening and identification of resistant sources against *Sclerotinia sclerotiorum* causing white mold disease in common bean. Crop Sci. 2020;60:1986-1996.
Available:https://doi.org/10.1002/csc2.20160
4. Taylor A, Rana K, Handy C, Clarkson JP. Resistance to *Sclerotinia sclerotiorum* in wild *Brassica* species and the importance of *Sclerotinia subarctica* as a *Brassica* pathogen. Plant Pathol. 2018;67:433-444.
Available:https://doi.org/10.1111/ppa.12745
5. Kerr ED, Steadman JR, Nelson LA. Estimation of white mold disease reduction of yield and yield components of dry edible beans. Crop Sci. 1978;18:257-279.
Available:https://doi.org/10.2135/cropsci1978.0011183X001800020020x
6. Bakhshi S, Zadeh HRZ, Atghia O. Molecular identification of bean genotypes for partial resistance to *Sclerotinia sclerotiorum* based on SCAR markers. J Plant Pathol. 2019;102:1-7.
Available:https://doi.org/10.1007/s42161-019-00432-1
7. Fagundes‑Nacarath IRF, Debona D, Brás VV, Silveira PR, Rodrigues FA. Phosphites attenuate *Sclerotinia sclerotiorum*-induced physiological impairments in common bean. Acta Physiol. Plant. 2018;40:1-14.
Available:https://doi.org/10.1007/s11738-018-2776-7
8. Le Torneau D. Morphology, cytology and physiology of *Sclerotinia* especies in culture. Phytopathology. 1979;69:887-890.
Available:https://doi.org/10.1094/Phyto-69-887
9. Choi IY, Kim, JH, Kim J, Han KS, Galea V, Shin HD. Confirmation of *Sclerotinia sclerotiorum* as the causal agent of stem rot of stock in Korea. Australasian Plant Dis. Notes. 2017;12:1-3.
Available:https://doi.org/10.1007/s13314-017-0247-4
10. Smolińska U, Kowalska B. Biological control of the soil-borne fungal pathogen *Sclerotinia sclerotiorum* - a review. J Plant Pathol. 2018;100:1-12.
Available:https://doi.org/10.1007/s42161-018-0023-0
11. Mao XW, Li JS, Chen YL, Song XS, Duan YB, Wang JX, Chen CJ, Zhou MG, et al. Resistance risk assessment for fluazinam in *Sclerotinia sclerotiorum*. Pestic Biochem Physiol. 2018;144:27-35.
Available:https://doi.org/10.1016/j.pestbp.2017.10.010
12. Mandal A, Sarkar B, Mandal S, Vithanage M, Patra AK, Manna MC. Impact of agrochemicals on soil health. In: Prasad

MNV, editor. Agrochemicals Detection, Treatment and Remediation. Oxford: Butterworth-Heinemann. 2020:161–187. Available:https://doi.org/10.1016/B978-0-08-103017-2.00007-6.
13. O'Brien PA. Biological control of plant diseases. Australasian Plant Pathol. 2017; 46:293-304. Available:https://doi.org/10.1007/s13313-017-0481-4
14. Kredics L, Chen L, Kedves O, Büchner R, Hatvani L, Allaga H, Nagy VD, Khaled JM, et al. Molecular tools for monitoring *Trichoderma* in agricultural environments. Front. Microbiol. 2018;9:1-17. Available:https://doi.org/10.3389/fmicb.2018.01599
15. Ojaghian S, Wang L, Xie GL, Zhang JZ. Effect of volatiles produced by *Trichoderma* spp. on expression of glutathione transferase genes in *Sclerotinia sclerotiorum*. Biol Control. 2019;136:1-6. Available:https://doi.org/10.1016/j.biocontrol.2019.103999
16. Raymaekersa K, Poneta L, Holtappels D, Berckmans B, Cammue BPA. Screening for novel biocontrol agents applicable in plant disease management – A review. Biol Control. 2020;144:1-18. Available:https://doi.org/10.1016/j.biocontrol.2020.104240
17. Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G, Lorito M. *Trichoderma*-based products and their widespread use in agriculture. Open Mycol J. 2014;8:71-126. Available:https://doi.org/10.2174/1874437001408010071]
18. Chaverri P, Branco-Rocha F, Jaklitsch W, Gazis R, Degenkolb T, Samuels GJ. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia. 2015;107:558-590. Available:https://doi.org/10.3852/14-147
19. Robbertse B, Strope PK, Chaverri P, Gazis R, Ciufo S, Domrachev M, Schoch CL. Improving taxonomic accuracy for fungi in public sequence databases: applying one name one species in well-defined genera with *Trichoderma*/Hypocrea. Database. 2017;1-14. Available:https://doi.org/10.1093/database/bax072
20. Inglis PW, Mello SCM, Martins I, Silva JBT, Macêdo K, Sifuentes DN, Valadares-Inglis MC. *Trichoderma* from Brazilian garlic and onion crop soils and description of two new species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. Plos One. 2020;15: 1-23. Available:https://doi.org/10.1371/journal.pone.0228485
21. Druzhinina I, Kopchinskiy A. International Subcommission on *Trichoderma* and Hypocrea Taxonomy. ISTH; 2018. Accessed 1 June 2021 Available:http://isth.info/biodiversity/index.php
22. Raeder U, Broda P. Rapid preparation of DNA from filamentous fungi. Lett. Appl. Microbiol. 1985;1:17-20. Available:https://doi.org/10.1111/j.1472-765X.1985.tb01479.x
23. White TJ, Bruns TD, Lee S Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand MAIDH, Sninsky JJ and White TJ (eds.) PCR Protocols: A Guide to Methods and Applications. New York: Academic Press; 1990.
24. Bonugli-Santos RC, Durrant LR, Da Silva M, Sette LD. Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. Enzym Microbiol Technol. 2010;46: 32-37. . Available:https://doi.org/10.1016/j.enzmictec.2009.07.014
25. Edgar RC. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 2004;32:1792-1797. Available:https://doi.org/10.1093/nar/gkh340
26. Hall TA. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows95/98/nt. Nucleic Acids Symp. Ser. 1999;41:95-98. Available:https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29
27. Borchsenius F. FastGap 1.0.8. 2007. Software distributed by the authors. Accessed 15 June 2021 Available:http://192.38.46.42/aubot/fb/FastGap_home.htm)
28. Swofford DL. Paup: Phylogenetic analysis using parsimony (*and other methods) version 4.0b10. Sunderland, Massachussetts: Sinauer Associates; 2003.
29. Huelsenbeck JP, Ronquist F. Bayesian inference of phylogenetic tree.

Bioinformatics Application Note. 2001;17: 754-755.

30. Rambaut EM. FigTree v1.3.1 [http://tree.bio.ed.ac.uk/software/Figtree/] webcite Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, United Kingdom; 2009.
31. Bell DK, Wells HD, Markham CR. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phyto-pathology. 1982;72:379-382.
32. Ferreira DF. Sisvar: A computer statistical analysis system. Ciênc. agrotec. 2011;35: 1039-1042. Available:https://doi.org/10.1590/S1413-70542011000600001
33. Benítez T, Rincón AM, Limón MC Codón AC. Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol. 2004;7:249-260.
34. Hermosa MR, Grondona I, Monte E, Iturriaga EA, Diaz-Minguez JM, Castro C, Monte E, Garcia-Acha JM. Molecular characterization and identification of biocontrol isolates of *Trichoderma* spp. Appl. Environ. Microbiol. 2000;66:1890-1898.
35. Druzhinina IS, Kopchinskiy AG, Komon M, Bissett J, Szakacs G, Kubicek CP. An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. Fungal Genet. Biol. 2005;42: 813-828. Available:https://doi.org/10.1016/j.fgb.2005.06.007
36. Druzhinina IS, Kubicek CP, Komoń-Zelazowska M, Mulaw TB, Bissett J. The *Trichoderma harzianum* demon: complex speciation history resulting in coexistence of hypothetical biological species, recent agamospecies and numerous relict lineages. BMC Evol Biol. 2010;10:1-14. Available:https://doi.org/10.1186/1471-2148-10-94
37. Filizola PRB, Luna MAC, de Souza AF, Coelho IL, Laranjeira D, Campos-Takaki GM. Biodiversity and phylogeny of novel *Trichoderma* isolates from mangrove sediments and potential of biocontrol against *Fusarium* strains. Microb Cell Fact. 2019;18:1-14. Available:https://doi.org/10.1186/s12934-019-1108-y
38. Xia X, Lie TK, Qian X, Zheng Z, Huang Y, Shen Y. Species diversity, distribution, and genetic structure of endophytic and epiphytic *Trichoderma* associated with banana roots. Microb Ecol. 2011;61:619-625. Available:https://doi.org/10.1007/s00248-010-9770-y
39. Cooney JM, Lauren DR. *Trichoderma*/ pathogen interactions: measurement of antagonistic chemicals produced at the antagonist/pathogen interface using a tubular bioassay. Lett Appl Microbiol. 1998; 27:283-286. Available:https://doi.org/10.1046/j.1472-765x.1998.t01-9-00449.x.
40. Zeilinger S, Grubera S, Bansalb R, Mukherjee PK. Secondary metabolism in *Trichoderma* e chemistry meets genomics. Fungal Biol. Rev. 2016;30:74-90. Available:https://doi.org/10.1016/j.fbr.2016.05.001
41. Haddad PE, Leite LG, Lucon CMM, Harakava R. Selection of *Trichoderma* spp. strains for the control of *Sclerotinia sclerotiorum i*n soybean. Pesq. agropec. bras. 2017;52:1140-1148. Available:https://doi.org/10.1590/S0100-204X2017001200002
42. Zhang F, Ge H, Zhang F, Guo N, Wang Y, Chen L, Ji X, Li C. Biocontrol potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum* in soybean. Plant Physiol Biochem. 2016;100:64-74. Available:https://doi.org/10.1016/j.plaphy.2015.12.017
43. Sumida CH, Daniel JFS, Araújo APCS, Peitl DC, Abreu LM, Dekker RFH, Canteri MG. *Trichoderma asperelloides* antagonism to nine *Sclerotinia sclerotiorum* strains and biological control of white mold disease in soybean plants. Biocontrol Sci Technol. 2018;28:142-156. Available:https://doi.org/10.1080/09583157.2018.1430743
44. Sala A, Barrena R, Artola A, Antoni S. Current developments in the production of fungal biological control agents by solid-state fermentation using organic solid waste. Crit. Rev. Environ. Sci. Technol. 2019;49:655-694. Available:https://doi.org/10.1080/10643389.2018.1557497
45. Mishra S, Kumar P, Malik A. Suitability of agricultural by-products as production-medium for spore production by *Beauveria bassiana* HQ917687. Int J Recycl Org Waste Agricult. 2016;5:179-184. Available:https://doi.org/10.1007/s40093-016-0127-5

46. Silva LR, Muniz PHPC, Peixoto GHS, Luccas BEGD, Silva JBT, Mello SCM. Mycelial inhibition of *Sclerotinia sclerotiorum* by *Trichoderma* spp. volatile organic compounds in distinct stages of development. Pak. J. Biol. Sci. 2021;24: 527-536.
Available:https://doi.org/10.3923/pjbs.2021.527.536
47. 47. Bader NA, Salerno GL, Covacevich F, Consolo VF. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). J. King Saud Univ. Sci. 2020;32:867-873.
Available:https://doi.org/10.1016/j.jksus.2019.04.002
48. Chagas LFB, De Castro HG, Colonia BSO, Carvalho Filho MR, Miller LO, Chagas Júnior AF. Efficiency of *Trichoderma* spp. as a growth promoter of cowpea (*Vigna unguiculata*) and analysis of phosphate solubilization and indole acetic acid synthesis. Braz. J. Bot. 2016;39:437-445.
Available:https://doi.org/10.1007/s40415-015-0247-6
49. Chagas LFB, Chagas Junior AF, Fidelis RR, Carvalho Filho MR, Miller LO. *Trichoderma asperellum* efficiency in soybean yield components. Comun. Sci. 2019;10:165-169.
Available:https://doi.org/10.14295/cs.v8i1.1754
50. Mohanta TK, Occhipinti A, Atsbaha Zebelo S, Foti M, Fliegmann J, Bossi S, Maffei ME, Bertea CM. *Ginkgo biloba* responds to herbivory by activating early signaling and direct defenses. Plos One. 2012;7:1-14.
Available:https://doi.org/10.1371/journal.pone.0032822
51. Llorens E, Camañes G, Lapeña L, García-Agustín P. Priming by hexanoic acid induce activation of mevalonic and linolenic pathways and promotes the emission of plant volatiles. Front. Plant Sci. 2016;7:1-12.
Available:https://doi.org/10.3389/fpls.2016.00495
52. Kanchiswamy CN, Malnoy M, Maffei ME. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends Plant Sci. 2015;20:1-7.
Available:https://doi.org/10.1016/j.tplants.2015.01.004
53. Kaddes A, Fauconnier ML, Sassi K, Nasraoui B, Jijakli MH. Endophytic fungal volatile compounds as solution for sustainable agriculture. Molecules. 2019; 24:1-16.
Available:https://doi.org/10.3390/molecules24061065
54. Silva LR, Valadares-Inglis MC, Peixoto GHS, Luccas BEG, Muniz PHPC, Magalhães DM, Moraes MCB, Mello SCM. Volatile organic compounds emitted by *Trichoderma azevedoi* promote the growth of lettuce plants and delay the symptoms of white mold. Biol Control. 2021;152:1-10.
Available:https://doi.org/10.1016/j.biocontrol.2020.104447
55. Estrada-Rivera M, Rebolledo-Prudencio OG, Pérez-Robles DA, Rocha-Medina MADC, González-López MDC, Casas-Floresa S. *Trichoderma* Histone Deacetylase HDA-2 modulates multiple responses in *Arabidopsis*. Plant Physiol. 2019;179:1343-1361.
Available:https://doi.org/10.1104/pp.18.01092.

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