Article

Trichoderma: Harzianum Complex Clade Species Distribution in Soils of Central and South America

Adnan Ismaiel , Prashant P. Jambhulkar , Parimal Sinha , Dilip K. Lakshman *

Posted Date: 23 July 2024

doi: 10.20944/preprints202407.1786.v1

Keywords: biocontrol agent; biofertilizer; sustainable agriculture; Trichoderma species; Harzianum Complex Clade

Article

Trichoderma: Harzianum Complex Clade Species Distribution in Soils of Central and South America

Adnan Ismaiel [1], Prashant P. Jambhulkar [2], Parimal Sinha [3] and Dilip K. Lakshman [4,*]

[1] Environmental Microbial & Food Safety Laboratory, USDA-ARS, Beltsville, MD 20705, USA
[2] Department of Plant Pathology, Rani Lakshmi Bai Central Agricultural University, Jhansi, 284003, India
[3] Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[4] Molecular Plant Pathology Laboratory, USDA-ARS, Beltsville, MD 20705, USA
* Correspondence: dilip.lakshman@usda.gov

Abstract: As environmental and health concerns increase, the trend of sustainable agriculture moves toward using biological agents. About 60% of all biological fungicides have *Trichoderma* species as the active ingredient, with *T. harzianum* as the most common species in these products. However, the name *T. harzianum* has often been incorrectly used in culture collections, databases, and the scientific literature due to the division of the Harzianum Complex Clade (HCC) into more than 95 cryptic species with only one being named *T. harzianum*. In this study, strains previously identified as *T. harzianum* in three surveys of *Trichoderma* species from soils in South and Central America were reanalyzed using phylogeny based on *tef1α*, *rpb2,* and ITS loci and combined with the corresponding species in two other studies, where the species were correctly identified. Based on the results of the five surveys, *T. afroharzianum, T. lentiforme, T. endophyticum* followed by *T. azedevio* and *T. harzianum* were found to be the dominant species of the HCC in South and Central America. This is the first report of identifying dominant *Trichoderma* species within the HCC in South and Central American soil based on multiple studies, which will be useful in selecting strains within the complex clade for formulation of biocontrol and biofertilizer products in the continent.

Keywords: biocontrol agent; biofertilizer; sustainable agriculture; *Trichoderma* species; Harzianum Complex Clade

1. Introduction

A significant number of investigations documented beneficial microbes for disease suppression and plant growth enhancement. Species in the genus *Trichoderma* stand out for these plant-beneficial activities. *Trichoderma* as a genus was introduced in 1794 by Persoon [1]**.** The importance of *Trichoderma* in agriculture, specifically as a biocontrol agent (BCA) against fungal plant diseases has been known since the 1930s [2]. Then, in the 1980s, studies showed growth promotion of various crops by application of *Trichoderma* species [3,4]. However, only in the 1990s did commercial products with *Trichoderma* as an active ingredient become commercially available with reasonable success [5]. One of the most common species in those products is *Trichoderma harzianum*. Samuels and Hebbar [1] assembled a list of commercial *Trichoderma* biocontrol products that has *T. harzianum* as the active ingredient in 21 out of 55 products, which was higher than any other *Trichoderma* species, reflecting the importance of the species for biocontrol. Also, in a compiled list of publications, Zin and Badaluddin [6] showed investigations involving the effectiveness of *Trichoderma* species against fungal crop pathogens. Within the list, *T. harzianum* was the most studied species (11 out of 18) and showed high effectiveness against various crop diseases. Taxonomically, *T. harzianum* was only one of the nine aggregate species described by Rifai [7]. Aggregate species, per Rifai [7], means a group of more than one species that are morphologically identical but biologically different. Taxonomy based on DNA sequencing of specific markers started in the late 1990s which resulted in exponential expansion of the number of species in the genus of *Trichoderma*. The species, morphologically identified as *T. harzianum,* appeared to split into different clades. Those clades in some cases were marked by Roman numerals or Arabic numbers [8,9], without any coordination in numbering. These studies clearly showed that *T. harzianum* could represent several species that are morphologically indistinguishable. Therefore, the phrase Harzianum Complex Clade (HCC) started to replace *T. harzianum*. The confusion continued until 2015 when Chaverri et al. [10] described 14 species within the HCC, including a few that were already described. The number of species in the HCC continued to expand [11–15]. Unfortunately, the split of HCC did not resolve the confusion about the name completely. There are many sequences for strains deposited in databases including GenBank as *T. harzianum,* even though *T. harzianum* is only one of the uncommon species among more than 95 described species within the complex limiting the full value of the databases [10,16]. There is another problem with the HCC species. HCC boundaries are not clearly identified, and mistakes happen when new species are included in the HCC even though they are phylogenetically positioned outside the clade. For example, Chaverri et al. [10] did not include *T. tawa, T. tomentosum, and T. velutinum* within the complex clade of harzianum. However, Zheng et al. [13] included all the three species within the clade. There are several surveys exploring *Trichoderma* in soil in different parts of the world that have reported any species in HCC as *T. harzianum.*

In this study, strains identified as *T. harzianum* in three survey studies for isolation of *Trichoderma* strains from the soil of South and Central America were phylogenetically re-analyzed based on the available sequencing data of three loci namely, translation elongation factor 1α (*tef1α*), RNA polymerase subunit II (*rpb2*), and the Internal Transcribed Spacers (ITS). After re-identification, the number of strains for each species was added to the numbers of respective species in another two studies for which species in the HCC were correctly identified to determine the dominant soil resident species of the HCC in the continent. Knowing the exact *Trichoderma* species dominance in the soil from a geographical region could help biocontrol investigations to find species that may be Indigenous, compete well in soil, and may have the ability to establish endophytic relationships with plants, resulting in the better exploitation of plant beneficial activities by the *Trichoderma* species. Secondly, because of the importance of the species in HCC for biocontrol and plant growth promotion, accurate identification of *Trichoderma* species is critical as it reflects the properties associated with individual species.

2. Materials and Methods

2.1. Re-Identification of the Trichoderma Harzianum Strains Deposited in the GenBank

To evaluate the accuracy of identification of *Trichoderma harzianum* strains deposited at the National Center for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/genbank/) a search was carried out for "*Trichoderma harzianum* translation elongation factor" in the GenBank. The initial 100 sequence hits were downloaded in the FASTA file format. Sequences of sixteen ex-type species in HCC that were retrieved from GenBank were then added to this file as references. The FASTA file was aligned using Clustal Omega. (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). The alignment file was adjusted manually using the software Mesquite version 3.81 [17]. Then after, the file was used to construct phylogenetic trees using two methods: 1) parsimony tree was obtained using PAUP version 4.0a (https://phylosolutions.com/paup-test/). The tree was produced using a heuristic search with a starting tree obtained by 1000 random stepwise addition of sequences, tree-bisection-reconnection (TBR) as the branch-swapping algorithm with MULTREES in effect. Gaps were treated as missing characters. Supports for branches were assessed with 1000 replicates of bootstrap. 2) Maximum Likelihood tree was obtained using MEGA X with the substitution model predetermined using MEGA X [18]. Support for the clades was assessed with 1000 bootstrap replicates.

2.2. Evaluation of the Dominant HCC Species from South and Central America

To determine the dominant HCC species in soils in South and Central America, strains identified based on translation elongation factor 1α (*tef1α*) in the studies of Hoyos-Carvajal et al., Smith et al., and Druzhinina et al. [19–21] as *T. harzianum* were re-analyzed phylogenetically based on the DNA sequencing data of three loci, *tef1α*, RNA polymerase subunit II (*rpb2*), and Internal Transcribed Spacers (ITS), respectively. The sequences for each locus were downloaded from the GenBank, and aligned with reference sequences particularly the ex-type species of known species in the clade described in Chaverri et al. [10] and del Carmen et al. [12] using the Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). All the strains used in the phylogenetic analysis are listed in Table 1. The alignment files for the three genes were concatenated and adjusted visually using the software Mesquite version 3.81 [17]. The alignment file was used to construct phylogenetic trees as described above. The trees obtained by both methods were essentially identical in topology and thus only the parsimony tree constructed by PAUP is presented.

After phylogenetic identification of the strains, the number of each species in the HCC from the three studies was added to respective species in two other studies by Inglis et al. and Barrera et al. [22,23] that had correctly identified species in the HCC. The results were tabulated to determine the most encountered species within the HCC in soils of South and Central America.

Table 1. Strains with their origin, strain number, and their sequences GenBank accession number and number of strains with identical *Tef1α, ITS and rpb*2 GenBank accession numbers.

***Trichoderma* species**	**origin**	**Strain #**	***Tef1α***	**ITS**	***rpb2***	**No. of strains**
***T. lentiforme*[a]**	**Brazil**	**CEN1428**	**MK666796**	**MK714909**	**MK696827**	
	Brazil	TUB F-746	AY857257	AY857216	X	
	Colombia	CIB T11	EU279976	EU280079	X	2[b]
	Colombia	CIB T02	EU279976	EU280079	X	4
	Mexico	DAOM 231417	AY605771	AY605728	X	
	Colombia	CIB T15	EU279982	EU280079	X	2
	Mexico	DAOM 231408	AY605773	AY605730	X	
	Mexico	DAOM 231402	AY605775	AY605732	X	
	Mexico	DAOM 231439	EU279994	AY605728	X	
	Colombia	CIB T91	EU279987	EU280079	X	
	Mexico	DAOM 231405	AY605774	AY605731	X	
	Colombia	CIB T56	EU279985	EU280079	X	4
	Peru	DAOM 237544	EU279993	EU280133	X	
	Mexico	DAOM 231425	AY605768	AY605725	X	

T. lentiforme	**Cameroon**	**E243**	**MK044089**	**X**	**MK044182**
	Mexico	TUB F-839	AY857283	AY857231	X
	Brazil	TUB F-1073	AY857295	AY857247	X
	Brazil	TUB F-1006	AY857286	AY857235	X
T. lentiforme*	**French Guiana**	**CBS 100542**	**AF469195**	**AF469189**	**X**
	Argentina	TUB F-1074	AY857296	AY857248	
T. inhamatum	**Peru**	**G.J.S. 09-697**	**KP115272**	**X**	**X**
T. inhamatum*	**Colombia**	**CBS 273.78**	**AF348099**	**FJ442680**	**FJ442725**
	Peru	DAOM 234005	EU279992	EU280091	X
T. hortense*	**Argentina**	**BAFC_cult_4291**	**MH253895**	**X**	**X**
	Colombia	CIB T136	EU279981	EU280078	X
	Colombia	CIB T127	EU279980	EU280078	X
T. afroharzianum	**Colombia**	**T22**	**AF469194**	**AF469188**	**X**
T. afroharzianum	**Brazil**	**CEN1414**	**MK696652**	**MK714894**	**MK696813**
T. afroharzianum*	**Peru**	**CBS 124620**	**FJ463301**	**FJ442265**	**FJ442691**
	Colombia	CIB T59	EU279986	EU280078	
T. pseudopyramidale*		**COAD 2420**	**MK044115**	**X**	**MK044208**
T. pseudopyramidale		**COAD 2439**	**MK044171**	**X**	**MK044264**
T. pyramidale		**S119**	**KJ665696**	**X**	**X**
T. atrobrunneum*	**France**	**CBS 548.92**	**AF443942**	**AF443924**	**X**
	Peru	CIB T52	EU279984	EU280077	
T. guizhouense*	**China**	**CBS 131803**	**JN215484**	**JN191311**	**JQ901400**
T. guizhouense	**Argentina**	**BAFC 4356**	**MG797485**	**X**	**X**
	Peru	TUB F-567	AY857267	AY857208	X
	Peru	TUB F-452	AY857266	AY857206	X
	Peru	TUB F-573	AY857268	AY857209	X
	Colombia	CIB T03	EU279977	EU280079	X
T. endophyticum*	**Ecuador**	**CBS 130729**	**FJ463319**	**FJ442243**	**X**
T. afarasin*	**Cameroon**	**CBS 130755**	**AF348093**	**AY027784**	**X**
	Guatemala	TUB F-693	AY857271	AY857211	X
	Mexico	TUB F-1083	AY857300	AY857253	X
	Colombia	CIB T139	EU279991	EU280075	X
	Colombia	Th051	AB568382	X	AB568476
	Colombia	CIB T131	EU279988	EU280075	X

	Colombia	CIB T100	EU279978	EU280079	X
_T. harzianum_*	**U.K.**	**CBS 226.95**	**AF348101**	**AJ222720**	**AF545549**
	Mexico	TUB F-1078	AY857298	AY857250	X
T. lixii**	**Thailand**	**CBS 110080**	**AF443938**	**AF443920**	**X**
_T. camerunense_*	**Cameroon**	**CBS 137272**	**AF348107**	**AY027780**	**X**
T. botryosum	**Ethiopia**	**COAD 2526**	**MK044147**	**X**	**MK044240**
	Colombia	CIB T23	EU279989	EU280077	X
	Colombia	Th202	AB558911	X	AB558921
	Colombia	Th203	AB558912	X	AB558922
T. austroindianum	**Argentina**	**BAFC 3583**	**MH352421**	**X**	**X**
	Colombia	CIB T44	EU279983	EU280077	X
T. azevedoi	**Brazil**	**CEN1422**	**MK696660**	**MK714901**	**MK696821**
_T. rifaii_*	**Ecuador**	**CBS 130746**	**FJ463324**	**FJ442663**	**X**
T. rifaii	**Panama**	**CBS 130745**	**FJ463321**	**FJ442621**	**FJ442720**
	Colombia	CIB T99	EU279990	EU280103	X
_T. simmonsii_*	**USA, MD**	**CBS 130431**	**AF443935**	**AF443917**	**FJ442757**
_T. neotropicale_*	**Peru**	**G.J.S. 11-185**	**HQ022771**	**HQ022407**	**X**
T. aggressivum	**Northern Ireland**	**CBS 433.95**	**AF348097**	**FJ442605**	**FJ442704**
T. aggressivum	**U.K.**	**CBS 100525**	**AF348095**	**AF057600**	**AF545541**
_T. pleuroticola_*	**South Korea**	**CBS 124383**	**HM142381**	**HM142362**	**HM142371**
_T. pleuroti_*	**South Korea**	**CBS 124387**	**HM142382**	**HM142363**	**HM142372**
T. peberdyi	**Brazil**	**CEN1387**	**MK696619**	**MK714861**	**MK696781**
T. peberdyi	**Brazil**	**CEN1388**	**MK696620**	**MK714862**	**MK696782**
_T. tomentusom_*	**Canada**	**DAOM 178713a**	**AY750882**	**EU330958**	**AF545557**

[a], boldface strains are reference sequences. [b] numbers above one indicates more than one strain had the same *tef1α* GenBank accession number. *, ex-type species.

3. Results

3.1. Re-identification of the Trichoderma harzianum strains deposited in the GenBank

Initially, we searched GenBank for "*Trichoderma harzianum* translation elongation factor" and phylogenetically analyzed the first 100 sequences with the ex-type of *T. harzianum* strain CBS 226.95, GenBank accession number (AF348101). Based on the phylogenetic tree (Figure 1) only 22 out of 100 sequences clustered with the ex-type of *T. harzianum* strain CBS 226.95, GenBank accession number (AF348101); so, the respective 22 strains can be validated as *T. harzianum.* It also indicates that 78% of the sequences that were deposited had previously been erroneously identified.

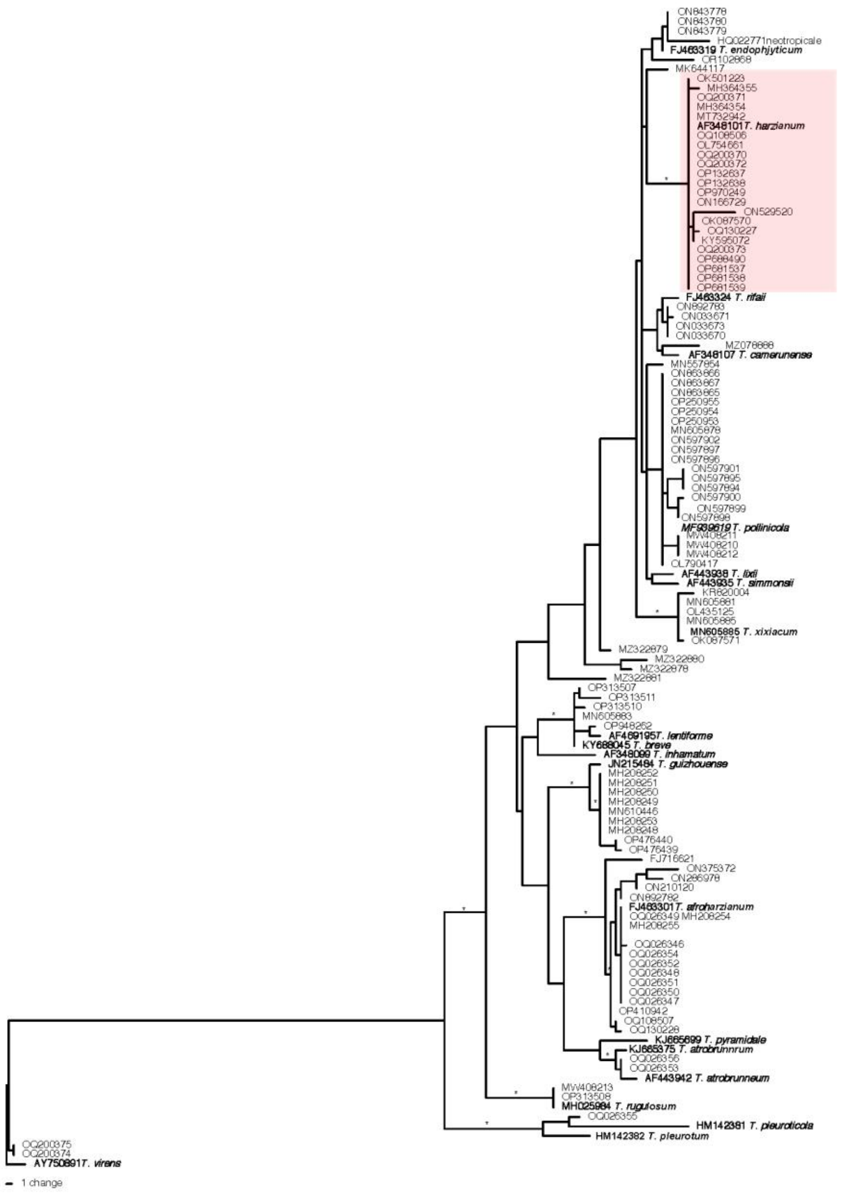

Figure 1. Phylogenetic tree based on *Tef1α* sequence data for 100 strains retrieved from GenBank which were earlier deposited as *Trichoderma harzianum*. The leaves are identified by GenBank accession numbers. * Given above the branches indicate bootstrap values of ≥ 70% obtained via 1000 replications. The boldface indicates reference strains for ex-type species included to identify the clades. Strains in pink highlighted clade are correctly deposited in GenBank as *T. harzianum.*

3.2. Evaluation of the dominant HCC species from South and Central America

In the three survey studies by Hoyos-Carvajal et al., Smith et al., and Druzhinina et al. [19–21] for isolation of *Trichoderma* species in the soil of Central and South America, all the strains in the Harzianum complex clade (HCC) were reported as *T. harzianum,* which does not reflect the current taxonomy of the genus that subdivided the clade into more than 95 species [10,16]. Precise species names are critical as some but not all the species in HCC may be associated with biocontrol and biofertilizer activities. Re-analyses of sequencing data of strains identified as *T. harzianum* in the three survey studies (Figure 2, Table 1) showed that 25 strains clustered with the type species of *T. lentiforme* in Clade 1 with high bootstrap values. Therefore, these strains are identified as *T. lentiforme*. Two other reference sequences in Clade 1 were also used. The reference strain with GenBank accession number MK896667 was placed in the tree to confirm the identity of the species in the study by Inglis, et al. [22], and the reference strain with MK044089 was placed in the tree to show that *T. lentiforme* was obtained in regions other than South America as this accession number belongs to a strain from Africa.

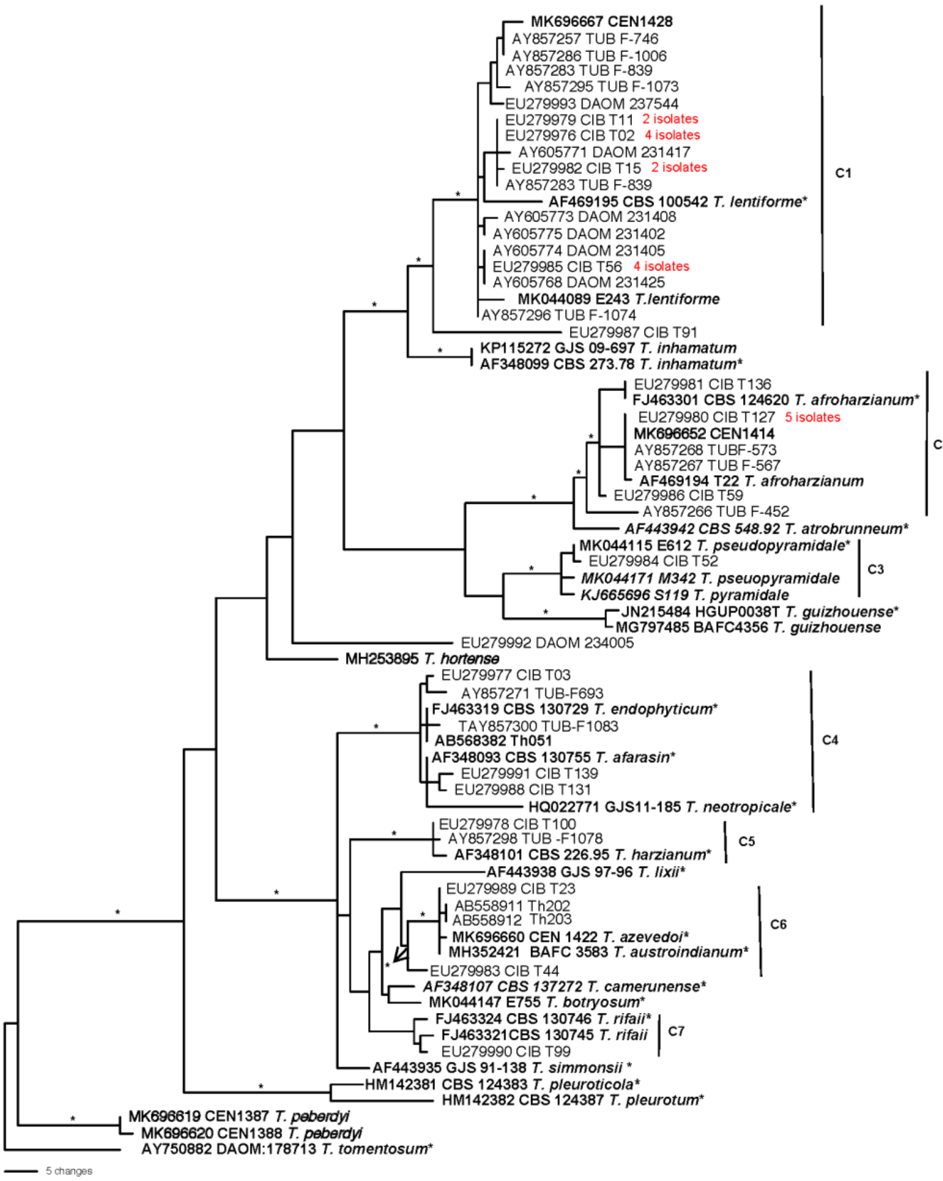

Figure 2. One of the most parsimonious trees generated by phylogenetic analysis of combined DNA sequences of *Tef1α*, *rpb2*, and ITS. * Given above the branches indicate bootstrap values of ≥ 70% obtained via 1000 replications. Leaves are identified by *Tef1α* GenBank accession numbers followed by strain numbers. The boldface indicates reference strains included to identify the clades. Vertical lines with numbers C1-C7 identify important clades with bootstrap values of ≥ 70. * After species names indicate the ex-type species.

In Clade 2, Figure 2, 10 strains from the three studies formed a highly supported clade with the type strain of *T. afroharzianum*. BLAST search for each isolate also confirmed that the most homologous sequences to the strains in this clade were sequences of *T. afroharzianum*. As a reference sequence, a sequence of the biocontrol strain known as T22 was aligned in this clade. T22 was re-identified as *T. afroharzianum* [10]. Also, the sequence of strain CEN414 from the study of Inglis et al. [22] fits in this clade, confirming the correct identification of this strain as *T. afroharzianum*.

In clade 3, Figure 2, one isolate from Peru [19], CIB T52 formed a clade with two species, *T. pyramidale* and *T. pseudopyramidale.* However, the CIB T52 stain is closer to *T. pseudopyramidale* than to *T. pyramidale.* The latter two species are closely related but one has been found only in Africa and the other in South America, respectively.

In clade 4, Figure 2, 6 strains formed a clade with the type species of *T. endophyticum*. However, the sequences of type species *T. neotropicale and T. afarasin* also fit into the clade. The type species *T. neotropicle* seems to be distantly related to the clade through a long branch, and *T. afarasin* is thus far only known to be an African fungus not found outside that continent. Therefore, it is most likely that the six isolates in the clade were T. *endophytcum*.

In Clade 5, Figure 2, two strains CIB T100 and TUB-F1078 from two different studies clustered with the type strain of *T. harzianum* CBS 226.95 with high bootstrap values by both methods, clearly identifying them as *T. harzianum*.

In clade 6, Figure 2, seven strains were nested with the ex-type strain of *T. azevedoi* (CEN1422 from Brazil); in most cases the sequences were identical. We also did a correction to the naming of a species. In the study of Barrera et al. [23], five strains from Argentina were identified as *T. austroindianum*. Four of the five strains have identical *tef1α* sequences. The other strain has 2 base differences with the other four isolates. *T. austroindianum* had sequences identical or highly homologous to *T. azevedoi* strains and fall in clade 6 and thus, we believe that the two species represent only one species. Thus, the strains of *T. austroindianum* were tabulated with the *T. azevedoi* as, on a priority basis, the latter species was described before *T. austoindianum* [24].

In clade 7, Figure 2, one isolate from Colombia CIB T52 formed a clade with the type species of *T. rifaii* and another reference strain of *T. rifaii*, suggesting the correct identification of the strain as *T. rifaii*.

The strain DAOM 234005 did not fit into any known clade and represents a distinct lineage distantly related to other species of the Harzianum complex. Moreover, based on BLAST search, the *Tef1α* sequence of this strain had no close homologous sequence to it in the GenBank; thus, we consider it a possible new species in the HCC. As to *T. pedryi*, this species was described as a new species in the HCC [22]. However, the position of this species in the phylogenetic tree (Figure 2) showed that this species is close to *T. tomentosum,* which is not included in the HCC [10]. Thus, we did not include this species in the tabulations of HCC species in Table 2.

Table 2. Trichoderma species in the Harzianum Complex Clade in soils of South and Central America based on data of five survey studies.

Species	**Strain number**	**Number of isolates**	***tef1α* accession number**	**Country**	**Ref.**
T. afroharzianum	CIB T136	1	EU279981	Colombia	19
	CIB T07, CIB T63, CIB T61, CIB T53, CIB T127	5	EU279980*	Colombia	19
	CIB 59	1	EU279986	Colombia	19

	CEN, 1410, CEN1414, CEN1417	3	MK696648*	Brazil	22
	TUB F-567, TUB F-573, TUB F-452	3	AY857267, AY857268, AY857266	Peru	21
	BAFC 4374, BAF 4392, for the rest see the reference	35	MH395411, MH395415	Argentina	23
Total		48			
T. lentiforme	DAOM 237544	1	EU279993	Peru	19
	CIB T02, CIB T112, CIB T35, JB M10-2	4	EU279976*	Mexico, Colombia	19
	CIB T15, CIB T41	2	EU279982*	Colombia	19
	DAOM 231417	1	AY605771	Mexico	19
	DAOM 231439	1	EU279994	Mexico	19
	DAOM 231408	1	AY605773	Mexico	19
	DAOM 231405	1	AY605774	Mexico	19
	DAOM 231425	1	AY605768	Mexico	19
	CIB T56, CIB T60, CIB T16, DAOM 229985	4	EU279985*	Panama, Colombia	19
	CIB T91	1	EU279987		
	CIB T11, CIB T102	2	EU279979*	Colombia	19
	DAOM 231402	1	AY605775	Mexico	19
	CEN1412, CEN1415, CEN1416, CEN1428, CEN1429	5	MK696650, MK696653, MK696654, MK696668, MK696667	Brazil	22
	TUB F-839, TUB F-1073, TUB F-1006, TUB F-746, TUB F-1074	5	AY857283, AY857295, AY857286, AY857257, AY857296	México, Brazil, Brazil, Brazil, Argentina	21

	BAFC 4391, BAFC 4394, for the rest see the reference	9	MH036883, MH036885	Argentina	23
Total		39			
T. endophyticum[a]	CIB T03, CIB T31, CIB T139	3	EU279977, EU279988, EU279991	Colombia	19
	TUB F-1083, TUB F-693	2	AY857300, AY857271	Mexico, Guatemala	21
	Th051	1	AB568382	Colombia	20
	BAFC 4358, BAFC 4372, for the rest see the reference	18	MH371393, MH371397	Argentina	23
Total		24			
T. azevedoi	CIB T23, CIB T24, CIB T126, CIB T128	4	EU279989*	Colombia	19
	CIB T44	1	EU279983	Colombia	19
	CEN1422, CEN1423, CEN1403	3	MK696638*	Brazil	22
	Th202, Th203	2	AB558911*	Colombia	20
	BAFC 3583, BAFC 3844, GJS 08-128, GJS 08-181, VAB-T051	5	MH352421, MG822709, MH352423, MH352422, MH352424	Argentina	23
Total		15			
T. harzianum	CIB T100, PER4-2	2	EU279978*	Colombia, Peru	19
	TUB F-1078	1	AY857298	Mexico	21
	GJS 08-172, GJS 08-173, VAB-T032, VAB-T052, VAB-T053	5	KT275197, KT275198, KT275199, MH364354, MH364355	Argentina	23
Total		8			

T. guizhouense	BAFC 4356, BAFC 4370, GJS 08-102, GJS 08-121, VAB-T047	5	MG797485, MG797486, MG797484, MG797483, MG797482	Argentina	23
T. neotropicale	GJS 08-182, GJS 08-183, VAB-T049	3	MG822718, MG822719, MG822720	Argentina	23
T. syagri	BAFC 4357, BAFC 4371	2	MG227714, MG227710	Argentina	23
T. pseudopyramidale	CIB T52	1	EU279984	Peru	19
T. hortense	GJS 08-116	1	MH253895	Argentina	23
T. rifaii	CIB T99	1	EU279990	Colombia	19
T. sp.	JB PER6-2	1	EU279992	Peru	19

*Strains have identical *tef1α* GenBank accession numbers.

The results of our re-identification of species of HCC were combined with the previously identified species in two other studies [22,23] and presented in Table 2 and summarized in Figure 3. *T. afroharzianum* is the most common species in Central and South America with 44 stains found in four out of five studies and in countries from North to South of the continent. This is a well-known biocontrol species and the strain T22 is an example [10,25].

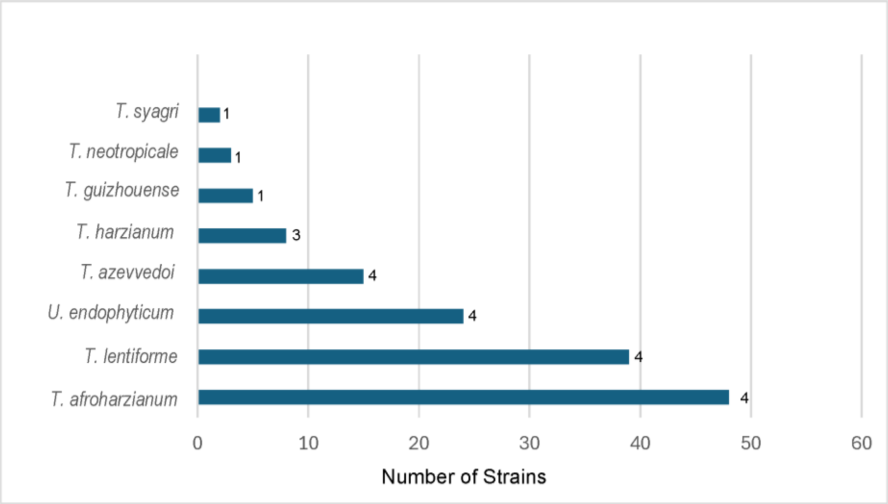

Figure 3. Dominant species of *Trichoderma* in the Harazianum Complex Clade in soil in Central and South America. The numbers at the end of the bars represent the frequency of presence of a species in five studies.

doi:10.20944/preprints202407.1786.v1

T. lentiforme was the second most common species with 39 strains from four studies out of five. The strains were distributed in all the regions from North to South of the continent. Chaverri et al. [10] reported that this species was found only in South America and mainly as an endophyte. However, as the tree (Figure 2) and Table 2 show, this species is commonly isolated from soil and the strain E243 (accession MK044182) was also found as an endophyte in coffee plants in Cameroon, Africa.

The third most common encounter in soils of Central and South America is *T. endophyticum* with 18 stains that was found in four out of five studies. This species was reported to be found as endophytes of tropical trees [10]. However, this study showed that the species is a common encounter in soil as well. The fourth highest encounter with 15 strains is *T. azevedoi* which was first described in 2020 by Inglis et al [22]. However, based on BLAST search of *Tef1α* sequence of type species of *T. azevedoi* (accession # MK696660, strain CEN 1422) we found several sequences that are highly homologous and have been deposited under the same species name from the soil in Australia (e.g. strain BRIP 74284; accession number OR801290). *T. harzianum* is the fifth in order of most common species in Central and South America, with 8 strains found in three out of five studies. The strains were found in North Mexico, Colombia, and South Argentina. Even though this species is mainly found in north temperate regions, Europe, and North America [1], the results here show that it is also found in Central and South America. *T. guizhouense* with 5 strains made the 6th most common species in the HCC in Central and South America. However, the species was observed only in one out of five studies and found only in Argentina. This may indicate a biogeographic restriction of the species in the continent of South and Central America. Other species in order were *T. neotrpicale* (3 stains), *T. syagri* (2 strains), *T. Hortense* (1 strain), *T. pseudopyramidale* (1 strain) and one isolate remained unidentified and placed in the table as *Trichoderma* sp.

4. Discussion

Biocontrol programs are in place in South and Central America, notably in nations like Brazil, Argentina, Colombia, and Mexico, to battle plant diseases and encourage crop development with biological agents [26]. One of the most important fungal names in biocontrol products of plant diseases and / or plant health promotion are species in the genus of *Trichoderma* and in particular, the species name of *T. harzianum* [1,10,27]. However, confusion exists about the name *T. harzianum* being used for all the species within Harzianum Complex Clade (HCC) despite the revision of the phylogeny of the clade and description of more than 95 species that started in 2015 [10,16]. In fact, the problem is widespread and continuous in scientific publications, databases, and commercial platforms. As an example, after searching the GenBank for "*Trichoderma harzianum* translation elongation factor" and identifying the first 100 sequences (this study), it turned out that only 22 out of 100 clustered with the ex-type of *T. harzianum* strain CBS 226.95 GenBank accession number (AF348101) and could be identified as *T. harzianum,* indicating 78% misidentification in the name of strains deposited as *T. harzianum*. What is disturbing about this is the fact that 100 % of the sequences used in Figure 1 were deposited between 2018-2023, at least three years after the major revision of the HCC [10]. Precise naming of species of HCC is critical as beneficial properties of biocontrol and plant growth promotion are species specific or even strain specific. Due to improper usage of the name, there is a lack of studies exploring which species in the clade are dominant species in soil despite the HCC species being isolated in many survey investigations. We hypothesize that the dominance in the soil is an important criterion for selecting any biocontrol strain as it reflects the fungus's high ability to compete, outgrow, and suppress other species, leading to a higher potential for the fungus to establish endophytic relationship with plants and possibly induce systemic resistance. Through inducing systemic resistance, the *Trichoderma* species provides other benefits to the plants such as resistance to abiotic stress and high efficiency in using nitrogen [5].

This study used data from five investigations to identify dominant *Trichoderma* species within the HCC in the soil of South and Central America. To determine the correct species name and identify the prevalent species in soils of Central and South America, in this study we re-analyzed with multi-locus phylogeny the data from three survey studies published in 2005-2013 [19–21]. The number of re-identified species was added to their respective species from two other studies where HCC species were identified correctly [22,23]. *T. afroharzianum* and *T. lentiforme*, respectively, were the top two dominant species in the continent and were obtained from north to south. *T. afroharzianum* is cosmopolitan and a well-known species of biocontrol agents, and the strain T22 is reported as the most known BCA [28]. *T. lentiforme* was reported [10] to be mainly an endophytic fungus. The dominance of *T. lentiforme* in soil is a new report of our findings. Having this ability in soil and the ability to establish endophytic relations with plants are top criteria for selection with biocontrol and biofertilizer properties. In a recent publication [29] *T. lentifore* was found to have biocontrol activity against *Sclerotinia sclerotiorum* and had growth stimulant property for cotton.

T. endophyticum and *T. azevedoi* are the third and fourth most common species, respectively, on the continent. *T. endophyticum* was shown to be exclusively endophytic based on the strains available for the study [10]. However, this study also showed that this species is a soil fungus as well (Table 2). *T. azevedoi* was described in the study of Inglis et al. [22] and has been obtained only in the South American continent. In this context, we corrected the name of a species described by Barrera et al. [23] as *T. austroindianum.* Five strains of *T. austroindianum* appear to have identical or highly homologous *tef1α* sequences to those of *T. azevedoi* and both clustered in one highly supported clade. Thus, we renamed all five strains of *T. austroindianum* as *T. azevedoi* in Table 1. Here we stress the importance of (1) BLAST search of the sequences of *tef1α* or *rpb2* loci of unknown *Trichoderma* strains before describing them as new species to avoid duplication of species naming and (2) to include the most homologous species to it in the phylogenic analyses. The phylogenetic trees in Barrera et al. [23] did not include *T. azevedoi* in the analyses, which is probably the reason that the authors overlooked this error.

The boundaries of the species within the HCC are equally unclear. For example, Chaverri et al. [10] did not include *T. tawa, T. tomentosum, and T. velutinum* within the complex clade of Harzianum. However, Zheng et al. [13] included all the above three species within the clade. In another example, Chaverri et al. [10] excluded *T. amazonicum* and *T. pleuroticola* from HCC. Yet, Chen and Zhuang [30] placed both the species inside the HCC. In this regard, Inglis et al. [22] described a new species named *T. peberdyi* as part of the HCC. Based on BLAST search at NCBI GenBank, and our phylogenetic tree (Figure 2), this species is closely related to *T. tomentosum.* Based on Chaverri et al. [10], *T. tomentosum* is not part of the clade. Therefore, *T. perbedyi* is not part of the HCC clade and was excluded from our list of HCC species (Table 2, Figure 3).

We also attempted to compare the dominant species in Central and South America with other continents. However, there is currently insufficient data from survey studies that quantitatively show the number of strains of HCC within the total number of strains of *Trichoderma* isolated. Thus, comparing South and Central America with other continents was not feasible. Nevertheless, we found individual reports to which we could compare our results. For example, the study of a survey of *Trichoderma* isolates from the soil of India [31] showed that among 15 strains in HCC that were identified, 11 were *T. afroharzianum*. This data agrees with the high encounter of *T. afroharzianum* in the soil in South and Central America. On the other hand, a survey study from Iran showed that *T. afroharzianum* was not a dominant HCC species from soil in the western region of the country [32]. In fact, T. *harzianum* was the most frequently isolated species. In another study, Tang et al. [33] showed that species of HCC were the most encountered *Trichoderma* species from soil in China in the region of Zoige Alpine. Among species of the HCC, *T. harzianum* was the most dominant species representing about 72% of the species in HCC (37/51). This is different from what we obtained in South and Central America. *T. harzianum* is known to be a species of cold temperature region and the temperature range of soil in the region where samples were obtained is -10 C in winter to 15 C in summer [34]. This may have caused a bias in species dominance. In South Africa, du Plessis et al. [35] showed that species of *T. afroharzianum, T. atrobrunneum* and *T. camerunense* were the only species within HCC obtained from the soil in South Africa; however, the authors did not report the quantitative data of the respective strains.

5. Conclusions

Within the genus *Trichoderma*, *T. harzianum* is one of the most well-known species for biocontrol and plant growth promotion. The Harzianum Complex Clade's (HCC) split into more than 95 species has led to the improper usage of this species' name in scientific publications, databases, and commercial platforms. This study used data from five investigative reports to identify dominant *Trichoderma* species within the HCC in the soil of South and Central America. Based on the multilocus (*tef1α, rpb2,* and ITS) phylogenetic analyses of the five published surveys, *T. afroharzianum, T. lentiforme, T. endophyticum, T. azedevio,* and *T. harzianum* were found to be the dominant species of the HCC in South and Central America. However, the soil sampling in those five studies was not obtained in a statistical manner and order. Thus, we consider that the findings from this study are a rough approximation of the dominating species of HCC in Central and South America. Currently, the HCC is known to have roughly 95 species. Selecting strains or species for biocontrol and growth promotion from the HCC could be an expensive and difficult undertaking. Identifying the dominant strains can expedite the commercialization process by reducing the time and expense associated with strain selection. We further emphasize the need for additional research to be done in the future from different continents, as we were unable to locate any comparable data with which to compare our findings. Moreover, we emphasize the significance of accurately identifying *Trichoderma* species prior to entering them into the HCC database.

Author Contributions: A.I. conceptualization, data generation, analysis, interpretation, writing—original draft preparation and editing; D.K.L. conceptualization, analysis, interpretation, writing, and editing; P.P.J. data collection and analyses, and P.S. feedback, and formatting of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by USDA-ARS in-house projects 8042-21220-259-000D and 8042-22000-320-000D.

Data Availability Statement: Data is contained within the article and supplementary materials.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Samuels, G.J.; Hebbar, P.K. *Trichoderma: Identification and Agricultural Applications*; APS Press, 2015.
2. Weindling, R. *Trichoderma lignorum* as a Parasite of Other Soil Fungi. *Phytopathology* **1932**, *22*, 837–845.
3. Lindsey, D.L.; Baker, R. Effect of Certain Fungi on Dwarf Tomatoes Grown under Gnotobiotic Conditions. *Phytopathology* **1967**, *57*, 1262–1263.
4. Chang, Y.-C.; Chang, Y.-C.; Baker, R.; Kleifeld, O.; Chet, I. Increased Growth of Plants in the Presence of the Biological Control Agent *Trichoderma harzianum*. *Plant Dis*. **1986**, *70*, 145–148.
5. Harman, G.E. *Trichoderma*—Not Just for Biocontrol Anymore. *Phytoparasitica* **2011**, *39*, 103–108, https://doi.org/10.1007/s12600-011-0151-y.
6. Zin, N.A.; Badaluddin, N.A. Biological Functions of *Trichoderma* Spp. for Agriculture Applications. *Ann. Agric. Sci.* **2020**, *65*, 168–178. https://doi.org/10.1016/j.aoas.2020.09.003
7. Rifai, M.A. A Revision of the Genus *Trichoderma*. *Mycol Pap*. **1969**, *116*, 1–56.
8. Chaverri, P.; Castlebury, L.A.; Samuels, G.J.; Geiser, D.M. Multilocus Phylogenetic Structure within the *Trichoderma harzianum/Hypocrea lixii* Complex. *Mol. Phylogenet. Evol.* **2003**, *27*, 302–313. https://doi.org/10.1016/s1055-7903(02)00400-1
9. Kubicek, C.P.; Bissett, J.; Druzhinina, I.; Kullnig-Gradinger, C.; Szakacs, G. Genetic and Metabolic Diversity of *Trichoderma*: A Case Study on South-East Asian Isolates. *Fungal Genet. Biol.* **2003**, *38*, 310–319. https://doi.org/10.1016/s1087-1845(02)00583-2
10. Chaverri, P.; Branco-Rocha, F.; Jaklitsch, W.; Gazis, R.; Degenkolb, T.; Samuels, G.J. Systematics of the *Trichoderma harzianum* Species Complex and the Re-Identification of Commercial Biocontrol Strains. *Mycologia* **2015**, *107*, 558–590. https://doi.org/10.3852/14-147
11. Gu, X.; Wang, R.; Sun, Q.; Wu, B.; Sun, J.-Z. Four New Species of *Trichoderma* in the Harzianum Clade from Northern China. *MycoKeys* **2020**, *73*, 109-132. https://doi.org/10.3897/mycokeys.73.51424
12. del Carmen H. Rodríguez, M.; Evans, H.C.; de Abreu, L.M.; de Macedo, D.M.; Ndacnou, M.K.; Bekele, K.B.; Barreto, R.W. New Species and Records of *Trichoderma* Isolated as Mycoparasites and Endophytes from Cultivated and Wild Coffee in Africa. *Sci. Rep.* **2021**, *11*, 5671. https://doi.org/10.1038/s41598-021-84111-1

13. Zheng, H.; Qiao, M.; Lv, Y.; Du, X.; Zhang, K.-Q.; Yu, Z. New Species of *Trichoderma* Isolated as Endophytes and Saprobes from Southwest China. *J. Fungi* **2021**, *7*, 467. https://doi.org/10.3390/jof7060467
14. Qin, W.-T.; Zhuang, W.-Y. Seven New Species of *Trichoderma* Hypocreales in the Harzianum and Strictipile Clades. *Phytotax* **2017**, *305*, 121–139. https://doi.org/10.11646/phytotaxa.305.3.1
15. Zhang, Y.-B.; Zhuang, W.-Y. New Species of *Trichoderma* in the Harzianum, Longibrachiatum and Viride Clades. *Phytotaxa* **2018**, *379*, 131–142. https://doi.org/10.11646/phytotaxa.379.2.1
16. Cao, Z.-J.; Qin, W.-T.; Zhao, J.; Liu, Y.; Wang, S.-X.; Zheng, S.-Y. Three New *Trichoderma* Species in Harzianum Clade Associated with the Contaminated Substrates of Edible Fungi. *J. Fungi* **2022**, *8*, 1154. https://doi.org/10.3390/jof8111154
17. Maddison, W.P. Mesquite: A Modular System for Evolutionary Analysis. Version 3.18. **2007**. http://www.mesquiteproject.org
18. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol*. **2021**, *38*, 3022–3027. https://doi.org/10.1093/molbev/msab120
19. Hoyos-Carvajal, L.; Orduz, S.; Bissett, J. Genetic and Metabolic Biodiversity of *Trichoderma* from Colombia and Adjacent Neotropic Regions. *Fungal Genet. Biol.* **2009**, *46*, 615–631. https://doi.org/10.1016/j.fgb.2009.04.006
20. Smith, A.; Beltrán, C.A.; Kusunoki, M.; Cotes, A.M.; Motohashi, K.; Kondo, T.; Deguchi, M. Diversity of Soil-Dwelling *Trichoderma* in Colombia and Their Potential as Biocontrol Agents against the Phytopathogenic Fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. *J. Gen. Plant Pathol.* **2013**, *79*, 74–85. https://doi.org/10.1007/s10327-012-0419-1
21. Druzhinina, I.S.; Kopchinskiy, A.G.; Komoń, M.; Bissett, J.; Szakacs, G.; Kubicek, C.P. An Oligonucleotide Barcode for Species Identification in *Trichoderma* and Hypocrea. *Fungal Genet. Biol.* **2005**, *42*, 813–828. https://doi.org/10.1016/j.fgb.2005.06.007
22. Inglis, P.W.; Mello, S.C.; Martins, I.; Silva, J.B.; Macêdo, K.; Sifuentes, D.N.; Valadares-Inglis, M.C. *Trichoderma* from Brazilian Garlic and Onion Crop Soils and Description of Two New Species: *Trichoderma azevedoi* and *Trichoderma peberdyi*. *PloS One* **2020**, *15*, e0228485. https://doi.org/10.1371/journal.pone.0228485
23. Barrera, V.A.; Iannone, L.; Romero, A.I.; Chaverri, P. Expanding the *Trichoderma harzianum* Species Complex: Three New Species from Argentine Natural and Cultivated Ecosystems. *Mycologia* **2021**, 1–20. https://doi.org/10.1080/00275514.2021.1947641
24. Hawksworth, D.L.; Crous, P.W.; Redhead, S.A.; Reynolds, D.R.; Samson, R.A.; Seifert, K.A.; Taylor, J.W.; Wingfield, M.J.; Abaci, Ö.; Aime, C.; et al. The Amsterdam Declaration on Fungal Nomenclature. *IMA Fungus* **2011**, *2*, 105–111, https://doi.org/10.5598/imafungus.2011.02.01.14
25. Kubicek, C.P.; Steindorff, A.S.; Chenthamara, K.; Manganiello, G.; Henrissat, B.; Zhang, J.; Cai, F.; Kopchinskiy, A.G.; Kubicek, E.M.; Kuo, A.; et al. Evolution and Comparative Genomics of the Most Common *Trichoderma* species. *BMC Genomics* **2019**, *20*, 485, https://doi.org/10.1186/s12864-019-5680-7
26. van Lenteren, J. C.; Bueno, V. H. P.; Luna, M. G. Biological control in Latin America and the Caribbean: information sources, organizations, types and approaches in biological control. In J. C. V. Lenteren, V. H. P. Bueno, M. G. Luna, & Y. C. Colmeneraz (Eds.), *Biological control in Latin America and the Caribbean: its rich history and bright future.* (pp. 1-20). CABI **2020**. https://doi.org/10.1079/9781789242430.0001
27. Xiao, Z.; Zhao, Q.; Li, W.; Gao, L.; Liu, G. Strain Improvement of *Trichoderma harzianum* for Enhanced Biocontrol Capacity: Strategies and Prospects. *Front. Microbiol.* **2023**, *14*, 1146210. https://doi.org/10.3389/fmicb.2023.1146210
28. Han, W.; Wu, Z.; Zhong, Z.; Williams, J.; Jacobsen, S.E.; Sun, Z.; Tang, Y. Assessing the Biosynthetic Inventory of the Biocontrol Fungus *Trichoderma afroharzianum* T22. *J. Agric. Food Chem.* **2023**, *71*, 11502–11519, https://doi.org/10.1021/acs.jafc.3c03240
29. Silva, L.G.; Camargo, R.C.; Mascarin, G.M.; Nunes, P.S. de O.; Dunlap, C.; Bettiol, W. Dual Functionality of *Trichoderma*: Biocontrol of *Sclerotinia sclerotiorum* and Biostimulant of Cotton Plants. *Front. Plant Sci.* **2022**, *13*, 983127. https://doi.org/10.3389/fpls.2022.983127
30. Chen, K.; Zhuang, W.-Y. Discovery from a Large-Scaled Survey of *Trichoderma* in Soil of China. *Sci. Rep.* **2017**, *7*, 9090. https://doi.org/10.1038/s41598-017-07807-3
31. Jambhulkar, P.P., Singh, B., Raja, M. *et al.* Genetic diversity and antagonistic properties of *Trichoderma* strains from the crop rhizospheres in southern Rajasthan, India. *Sci Rep* **2024**, *14*, 8610. https://doi.org/10.1038/s41598-024-58302-5

32. Mirzaeipour, Z.; Bazgir, E.; Zafari, D.; Darvishnia, M. Isolation and identification of Harzianum clade species of *Trichoderma* from Khorramabad County. *Mycologia Iranica* **2023**, *10*, 67-78. https://doi.org/10.22043/MI.2023.362910.1264
33. Tang, G.T.; Li, Y; Zhou, Y; Zhou, Y.; Zhu, Y.H.; Zheng, X.J.; Chang, X.L.; Zhang, S. R.; Gong, G.S. Diversity of *Trichoderma* species associated with soil in the Zoige alpine wetland of Southwest China. *Sci. Rep*. **2022**, *12*, 21709. https://doi.org/10.1038/s41598-022-25223-0
34. Bai, J.; Lu, Q; Zhao, Q.; Wang, J.; Ouyang, H. Effects of alpine wetland landscapes on regional climate on the Zoige Plateau of China. *Advances in Meteorology*. **2013**, 972430. 7pp. https://doi.org/10.1155/2013/972430
35. du Plessis, I.L.; Druzhinina, I.S; Atanasova, L.; Yarden O.; Jacobs, K. The diversity of *Trichoderma* species from soil in South Africa, with five new additions, *Mycologia*, **2018**, https://doi.org/10.1080/00275514.2018.1463059