Characterization and genetic diversity of *Trichoderma* strains isolated from tomato crop fields in Côte d'Ivoire

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Abstract

Trichoderma species possess numerous beneficial traits and hold significant potential for agricultural applications. However, recent studies indicate a general lack of knowledge regarding the prevalence of Trichoderma species in tomato fields in Côte d'Ivoire. This study aimed to identify Trichoderma species isolated in Côte d'Ivoire with potential agricultural benefits. Ten Trichoderma morphotypes were selected based on their biostimulant performance in relation to agromorphological parameters observed in a prior study. Macroscopic and microscopic analyses, PCR, amplicon sequencing, and phylogenetic inference were employed to characterize Trichoderma species isolated from tomato fields. Three species; *T. asperellum*, *T. virens*, and *T. hamatum* were identified as particularly promising. Assessment of the resulting phylogenetic tree revealed similarities between these three Trichoderma species and strains isolated elsewhere in Côte d'Ivoire, Ghana, Nigeria, Kenya, Morocco, and Egypt. Detailed understanding of these promising species could contribute to improved tomato yields in Côte d'Ivoire through their application as biofertilizers and biostimulants.

Key words: Tomato; *Trichoderma* spp.; morphological diversity; molecular diversity

Introduction

In Côte d'Ivoire, tomatoes are the most consumed vegetable after chilli peppers (Bancal & tano, 2019). This demand for tomatoes was estimated to amount to more than 46,628 tonnes in 2021, but the national production amount in 2022, 44,578.83 tonnes, was unable to cover this demand (FAOSTAT, 2023). The agricultural community is currently focused on

achieving sustainable environmentally friendly crop production (Kovács *et al*., 2021). Chemical inputs formerly used to accelerate crop production are a deterrent in this quest for sustainable production as they are very costly and—when used intensively—may cause atmospheric pollution and environmental damage, leaving harmful residues in the soil while also triggering resistance in the target organisms (Naseby *et al*., 2000; Atreya *et al*., 2012).

Research on microorganisms that promote sustainable crop production while safeguarding the environment is essential. The use of fungi as biocontrol agents is an effective alternative way of enhancing sustainable agricultural development without harming the environment (Filizola *et al*., 2019).

Trichoderma is a cosmopolitan fungal genus whose species are prevalent in farmland soils, rotting wood, and wood products (Thrane *et al*., 2021). *Trichoderma* spp. are classified as saphrotrophic fungi that often occur in the form of endophytes in woody plants (Kamala *et al*., 2015). According to Harman, the best strains are able to colonise the rhizosphere and may provide benefits to annual host plants throughout their lifecycles (Harman, 2000). Most *Trichoderma* species thrive at soil depths of 0–20 cm (Okoth *et al*., 2009), growing and proliferating best when the colonised host plants have abundant healthy roots. These strains have thus developed a range of mechanisms for both attacking other fungi and enhancing plant and root growth (Harman, 2006). Novel models representing the direct mechanisms of action of *Trichoderma* spp. against pathogenic fungi, including antibiosis, competition, mycoparasitism, and induced resistance (Giraud, 2018; Meriem, 2010), were proposed in the early 2000s (Harman *et al*., 2004). According to Giraud (2018), *Trichoderma* strains remotely secrete fungistatic substances (volatile antibiotics) that mainly affect the young hyphae of pathogenic fungi. *Trichoderma* spp. can tap the same environmental resources as pathogenic fungi, thereby enabling them to occupy host sites before the latter arrive (Meriem, 2010). Thanks to the skills they have developed, *Trichoderma* species are able to wrap themselves around a pathogen, penetrate its interior, and then inject enzymes (proteases and lipases) that read the host's cell contents (Leghlimi, 2013). Howell showed that *Trichoderma* strains can secrete hydrolytic enzymes such as glucanases, chitinases, and proteases to digest mycophyte cell walls (Howell, 2003). Moreover, once established, *Trichoderma* sp. colonies may have biostimulant effects on the morphological characteristics of the host plant (Sood *et al*., 2020). In a previous study involving an inventory and assessment of the effects of *Trichoderma* sp. morphotypes on tomato, 10 *Trichoderma* sp. morphotypes were found to stimulate tomato agromorphological parameters (Koffi *et al*., 2025). *Trichoderma* strains' rapid growth enables

them to gain a competitive advantage over harmful fungi while synergistically promoting host plant growth (Sharma *et al*., 2012).

Trichoderma spp. therefore have many positive features and high potential for use in agriculture (Waghunde *et al*., 2016), yet studies carried out in recent years have revealed that little is known about the prevalence of *Trichoderma* species in tomato crop fields. In the present study, promising *Trichoderma* species isolated in Côte d'Ivoire were identified through morphological characterization and molecular analysis.

Materials and methods

Thirty-eight *Trichoderma* morphotypes were isolated from tomato crop field soils in Côte d'Ivoire (Abidjan, Yamoussoukro, Bouaké et Korhogo) (Koffi *et al*., 2025). This study involved an inventory and selection of the best *Trichoderma* sp. morphotypes present in tomato crops in Côte d'Ivoire.

Selection of the best *Trichoderma* strains isolated from tomato rhizosphere

Setting up the nursery and transplanting

Thirty tomato seeds of the cobra 26 variety were sown in a 10 cm long pot by burying them to a depth of 2 to 3 mm for each of the isolates to be tested (Ouattara *et al*., 2018). This experiment was repeated 3 times. The seeds were covered with thin layers of sterile soil. The pots were placed in a screenhouse. Watering was carried out twice a day. *Trichoderma* spore suspension was sprayed on during sowing. The nursery period lasted 30 days. After these 30 days, only healthy, vigorous plants with a root ball around the roots were transplanted. Transplanting was carried out at a rate of 1 plant per pot, i.e., 3 pots per treatment. Spore suspension of each *Trichoderma* strain was sprayed while transplanting and then every 15 days.

Evaluation of *Trichoderma* Strains regarding Seed Germination, Tomato Leaf, and Root Biomass

The effectiveness of the *Trichoderma* spore suspension was assessed by comparing certain parameters between control plants sprayed with water and those treated with *Trichoderma*. Parameters were assessed using the method devised by Besnard & Davet (1993). The germination rate of the treated tomato plants was compared with that of the control plants. The number of plants in each pot was determined 30 days after sowing. After 60 days, the dry matter of the aerial and root parts was assessed (balance, HWA series).

Morphological characterization of 10 *Trichoderma* sp. morphotypes

Pure cultures were identified on the basis of their macroscopic and microscopic characteristics. Thallus texture and colour were the macroscopic characteristics assessed visually. A more detailed classification was carried out with respect to microscopic characteristics using alight microscope (OPTIKA, Italy) at x40 magnification. This involved scraping a portion of PDA medium containing the *Trichoderma* sp. strain and placing it on a slide with a coverslip. The slide was then mounted and viewed under the light microscope. The mycelium type (concerning mycelium partitioning and branching) and the conidia shape and colour were recorded. The fungal strains were identified based on the identification keys reported by Gams and Bissett (1998). Interventional studies involving animals or humans, and other studies that require ethical approval, must list the authority that provided approval and the corresponding ethical approval code.

Molecular analysis of 10 *Trichoderma* sp. morphotypes

DNA extraction

DNA of the different *Trichoderma* morphotypes was extracted using a modified version of the method described by Bélé *et al*. (2018). In this phase, 2 g samples of a 7-day-old *Trichoderma* fungal colony were placed in Eppendorf tubes, 1 mL of preheated (65 °C) cethyltrimethyl ammonia bromide (CTAB) buffer was added, and the tubes were vortexed. The tubes were then incubated for 30 min in a water bath at 65 °C, with homogenization via tube inversion occurring at 10 min intervals. The tubes were left at room temperature for 2 min, and then 800 µL of chloroform isoamyl alcohol was added per tube. The tubes were vortexed and then centrifuged at 13,000 rpm for 10 min. The supernatant was collected and precipitated with 650 µL of isopropanol and then stored in a freezer (−18 °C). The tubes were again centrifuged at 13,000 rpm for 10 min. After centrifugation, the supernatant was removed. A total of 500 mL of ethanol (70%) was added per tube, and the tubes were then vortexed and centrifuged for 5 min at 13,000 rpm. The supernatant was removed after centrifugation, and the tubes were drained and left to dry at room temperature. Finally, the DNA pellet was recovered in 50 µL of Tris EDTA (TE) and left to stand for 30 min before being stored in a freezer at −20 °C.

PCR Amplification

Polymerase chain reaction (PCR) was carried out to amplify fungal rDNA internal-transcribed-spacer (ITS) regions using an ITS1 (5′ TCCGTAGGTGAACCTGCGC 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) universal primer pair according to a modified version of the method developed by White *et al*. (1990). PCRs were performed with 12.5 µL

of GotTaq G2 Hot Start Green Master Mix, 4 µL of DNA template, and 2 µL of ITS1 and ITS4 primer solutions, with 4.5 µL of ultrapure water added to obtain a 25 µL final reaction volume. Amplifications were performed using a Bio-Rad T100 PCR Thermal Cycler. The amplification process involved an initial denaturation cycle for 5 min at 95 °C, followed by a series of 30 cycles, each consisting of 35 s denaturation at 95 °C. This was followed by annealing for 35 s at 60 °C, extension for 1.5 min at 72 °C, and final extension for 7 min at 72 °C.

Gel Electrophoresis

Following amplification, 1% agarose gel electrophoresis was used for PCR product migration. A total of 1 g of agarose (Bioshop, Canada) was thus dissolved in 100 mL of Tris-acetate EDTA (TAE 1X) buffer for 2 min in an iWave microwave oven. Then, 5 µL of ethidium bromide (0.5 µg/mL) was added to the supercooled agarose gel before being poured into a mould. The resulting solidified gel was immersed in 1X TAE buffer in a Wide Mini-Sub Cell GT chamber. Then, 5 µL of each PCR product was deposited in the agarose gel wells. A 5 µL volume of DNA marker (BenchTop 100bp DNA Ladder, Promega, USA) was added to a well to determine the different amplified DNA fragment sizes. Migration was run for 65 min at 70 V. Finally, the gel was analysed under UV light using a gel documentation imaging system (E-BOX VX5, France).

Sequencing and phylogenetic analysis

The PCR products were sent to Macrogen (Amsterdam, Europe) for sequencing. The resulting sequences were edited, and consensus sequences were generated using the BioEdit 7.2.5 package. Multisequence alignment of the resulting sequences was performed to generate consensus sequences using the BioEdit 7.2.5 package. The resulting consensus sequences were then compared to those in the GenBank database via BLAST on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST accessed on 31 August 2023) (Altschul *et al*., 1990) for fungal species identification. Sequence alignments were carried out using the method described by Kumar et al. to determine the similarities or differences between the genomic sequences (i.e., consensus sequences and those listed in GenBank) (Kumar *et al*., 2018). The sequences were stacked and aligned using the ClustalW package via the MEGA X tool (version 11.0.13). A phylogenetic tree for sequence replacement was constructed using FigTree and then classified using the method described by Muhire *et al*. (2014). The Sequence Demarcation Tool (version 1.2) was used for this classification based on pairwise sequence identity calculations (SDTv1.2).

Statistical Analyses

To analyse the effect of *Trichoderma* morphotypes on tomato root and leaf dry matter, generalised linear models were run with a Poisson distribution for germination rate and a gamma distribution for the other parameters. After ensuring that the residues did not over disperse (fitted model), an ANOVA was performed. In the event of significant differences, a multiple comparison of means with a Bonferroni correction was performed using the 'emmeans package'. The contrast function enabled us to see which strains had significant effects on the variable studied in comparison with the mean effect. In this study, all data obtained were analysed using RStudio software version 4.2.2 (R Core Team, 2022).

Results

Selection based on the effect of *Trichoderma* morphotypes on germination and leaf and root dry matter

Effect of *Trichoderma* on Seed Germination

The first six groups (with a germination rate greater than or equal to 73.33%) were selected to assess their effect on tomato root and leaf biomass, i.e., 20 morphotypes of *Trichoderma* (Table 1).

Table 1: Germination rates of tomato seeds depending on the different *Trichoderma* sp. morphotypes

Morphotypes	Germination Rate (%)
Y7	23.33 ± 3.33^{a}
Y6	33.33 ± 12.01^{ab}
Y3	36.66 ± 3.33^{abc}
Y1	36.66 ± 3.33^{abc}
A6	36.66 ± 3.33^{abc}
A2	40 ± 15.27^{abcd}
Y8	46.66 ± 23.33^{bcde}
K4	46.66 ± 3.33^{bcde}
A20	46.66 ± 3.33^{bcde}
A11	50 ± 26.45^{bcdef}
A18	50 ± 15.27^{bcdef}
A7	53.33 ± 3.33^{bcdefg}
K6	53.33 ± 17.63^{bcdefg}
A8	53.33 ± 17.63^{bcdefg}
A21	56.66 ± 23.33^{cdefgh}
A12	56.66 ± 21.85^{cdefgh}
A13	60 ± 5.77^{defghi}
A5	60 ± 26.45^{defghi}
Y2	60 ± 20^{defghi}
B1	63.33 ± 6.66^{defghij}
K1	$66.66 \pm 28.48^{efghijk}$
M-2	$66.66 \pm 20.27^{efghijk}$

A14	70 ± 5.77^{efghijk}
A1	73.33 ± 3.33^{fghijk}
A16	73.33 ± 8.81^{fghijk}
A15	73.33 ± 6.66^{fghijk}
Y5	73.33 ± 17.63^{fghijk}
Control	73.33 ± 17.63^{fghijk}
B2	76.66 ± 12.01^{ghijk}
A9	80 ± 5.77^{hijk}
B3	83.33 ± 12.01^{hijk}
K2	83.33 ± 12.01^{hijk}
A4	86.66 ± 13.33^{ijk}
Y9	86.66 ± 13.33^{ijk}
Y4	86.66 ± 13.33^{ijk}
A19	90 ± 5.77^{jk}
A17	90 ± 5.77^{jk}
K5	90 ± 5.77^{jk}
K3	93.33 ± 6.66^{k}

Values marked with letters are statistically different at the 5% threshold.

Above-Ground Biomass Dry Matter

Trichoderma morphotypes K3, A19, and B2 increased the mass of the above-ground part compared with the mean mass of the treated subjects and the control mass. *Trichoderma* morphotypes K5, A9, and B1 decreased the mass of the above-ground parts of the tomato plants compared with the mean mass. Statistical analysis also enabled classification into three homogeneous groups.

Root Dry Matter

Trichoderma morphotypes Y5, K3, A19, and B2 improved the root mass compared with that of the control. In contrast, *Trichoderma* morphotypes K5, A9, B1, A12, M2, A17, Y4, A16, A1, and A15 decreased the mass of the root matter of tomato plants compared with the mean mass in this study. A homogeneous group test was used to classify the *Trichoderma* morphotypes into three groups.

The effect on leaf and root dry matter therefore enabled us to identify 10 promising morphotypes for future development: ***Trichoderma* morphotypes A19, B2, A16, Y2, K2, A14, A1, Y9, K1, and Y5** (Table 2).

Table 2: Tomato leaf and root biomass of tomato plants depending on the different *Trichoderma* sp. morphotypes

Morphotypes	Leaf dry matter	Root dry matter
Control	135.7 ± 44.04 [a]	123.8 ± 26.75 [a]
K5	281.0 ± 82.87 [ab]	219.2 ± 50.94 [ab]
A9	383.2 ± 55.87 [ab]	263.3 ± 33.2 [ab]
B1	364.2 ± 103.56 [ab]	279.0 ± 50.64 [ab]
A12	561.4 ± 88.46 [ab]	329.6 ± 37.24 [ab]
M2	484.6 ± 120.97 [ab]	336.4 ± 68.32 [ab]
A17	521.1 ±112.25 [ab]	362.1 ± 86.98 [ab]
Y4	546.7 ± 101.6 [ab]	405.3 ± 65.36 [ab]
A16	828.7 ± 51.70 [ab]	448.9 ± 46.09 [ab]
A1	783.6 ± 149.53 [ab]	485.4 ± 86.78 [ab]
A15	586.1 ± 178.16 [ab]	492.1 ± 112.03 [ab]
A4	706.1 ± 153.6 [ab]	503.1 ± 115.6 [ab]
K1	797.8 ± 215.09 [ab]	509.8 ± 115.13 [ab]
Y9	801.8 ± 157.5 [ab]	520.3 ± 116.1 [ab]
K2	874.6 ± 118.71 [ab]	523.3 ± 50.96 [ab]
A14	732.9 ± 147.16 [ab]	547.7 ± 92.35 [ab]
B3	653.1 ± 183.17 [ab]	591.3 ± 238.82 [ab]
Y5	831.4 ± 184.4 [ab]	612.6 ± 107.45 [b]
K3	1094.2 ± 197.97 [b]	670.8 ±104.09 [b]
A19	1144 ± 221.6 [b]	704.3 ± 116.26 [b]
B2	920.3 ± 201.37 [b]	776.4 ± 224.32 [b]

Values bearing the same letter in the same column are statistically identical.

Morphological characteristics of 10 *Trichoderma* sp. morphotypes

The selected *Trichoderma* morphotypes A19, B2, A16, K1, K2, A14, A1, Y9, Y2, and Y5 formed white fungal colonies after 3 days of culturing. As of the 5th day of culture, the colonies of *Trichoderma* morphotypes A14, A16, A19, B2, Y5, and K2 had changed colour from white to green. *Trichoderma* morphotype A1 exhibited a white fungal colony topped by a few green colonies. Morphotypes Y2, Y9, and K1 formed a whitish-beige colony (Table 3). In addition to the fungal colony colours, some morphotypes showed concentric rings in the centres of their colonies along with the presence of pustules (Table 4).

Table 3: Macroscopic and microscopic features of elite *Trichoderma* sp. morphotypes

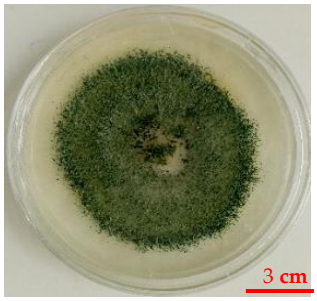
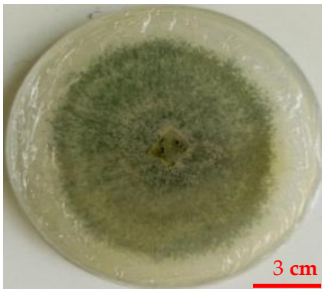
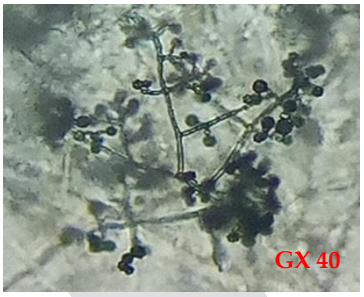
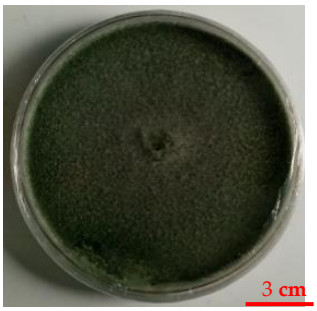
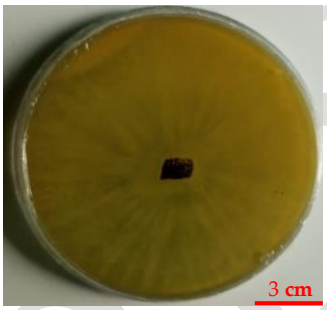
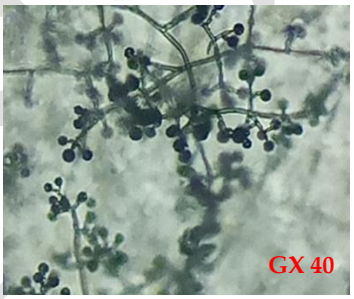
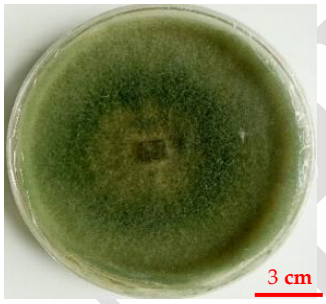
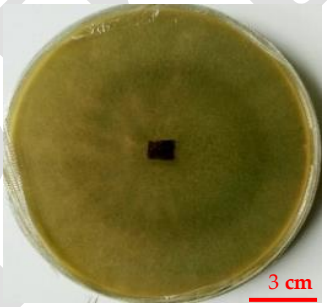
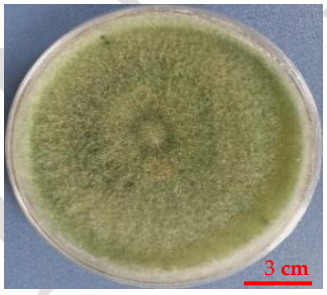
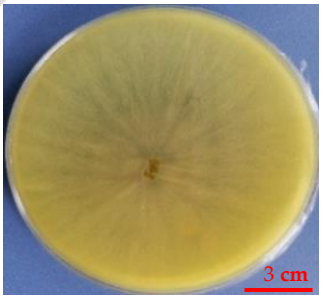
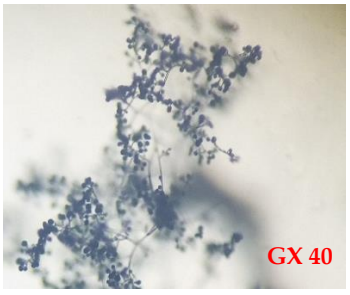
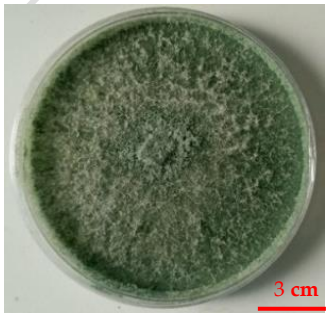
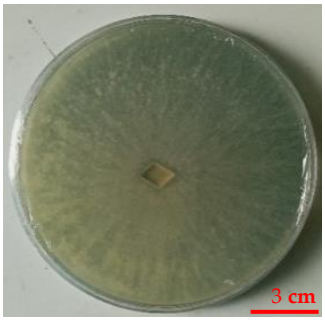
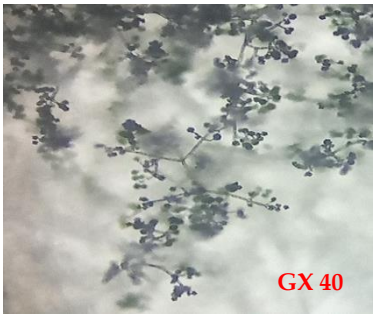
Trichoderma sp. morphotypes	Macroscopic features (after 5 days of culture)		Microscopic structures (after 5 days of culture)
	Face	Back	
A14			
A16			
A19			
B2			
Y5			

Table 4 (Continuation and end): Macroscopic and microscopic features of elite *Trichoderma* sp. Morphotypes

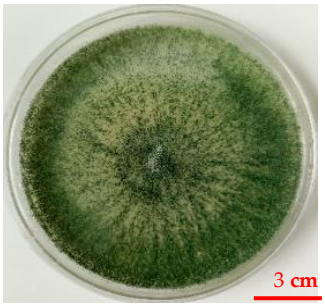
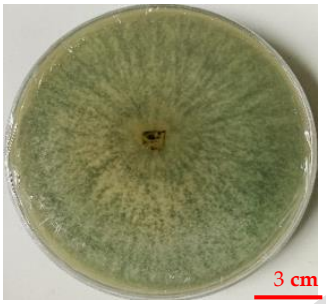
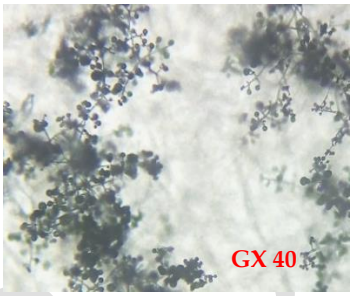
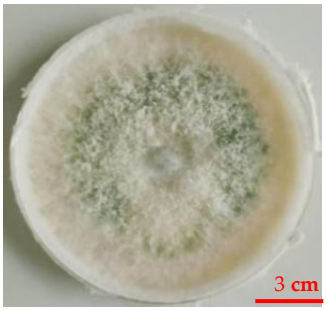
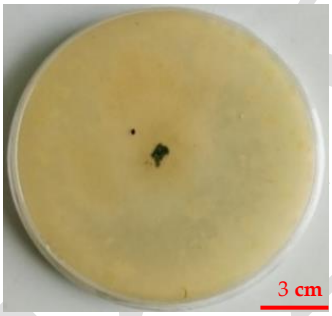
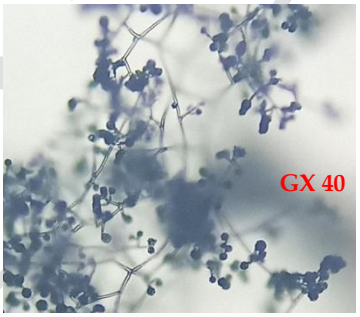
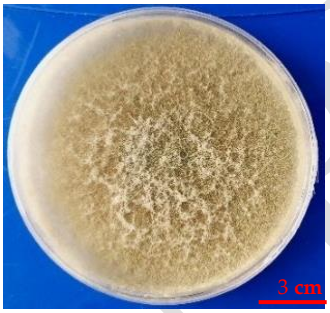
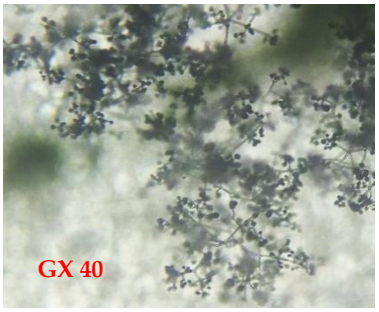
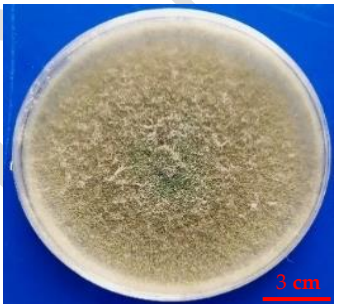
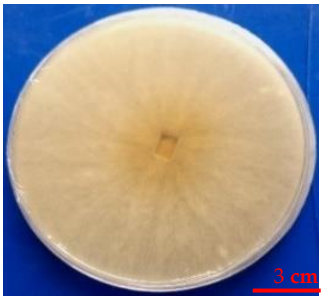
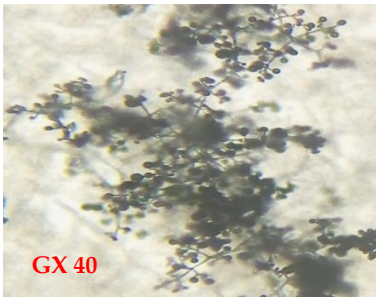
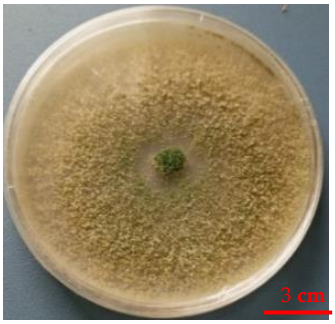
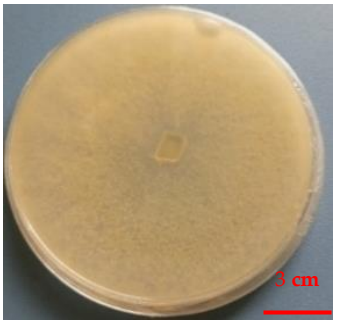
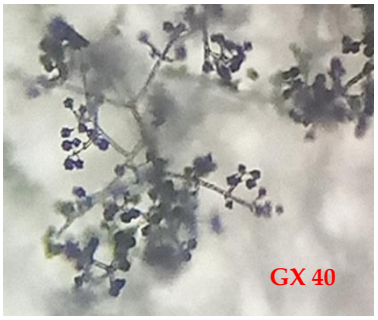
Trichoderma sp. morphotypes	Macroscopic features (after 5 days of culture)		Microscopic structures (after 5 days of culture)
	Face	Back	
K2			
A1			
Y2			
Y9			
K1			

Table 4: Description of *Trichoderma* sp. morphotypes.

Morphotypes	**Description of *Trichoderma* sp. Morphotypes**
Trichoderma sp. A19	Thallus colour: green; presence of dark-green rings; core colour: greenish yellow; underside colour: yellow; appearance: powdery.
Trichoderma sp. A16	Thallus colour: dark green; underside colour: yellow; appearance: fluffy.
Trichoderma sp. A14	Thallus colour: green; core fungal colony condensation; underside colour: transparent; appearance: powdery.
Trichoderma sp. A1	Thallus colour: white; presence of green rings; presence of beige pustules; underside colour: transparent; appearance: fluffy.
Trichoderma sp. B2	Thallus colour: green; presence of green rings; underside colour: transparent; appearance: fluffy.
Trichoderma sp. K2	Thallus colour: green topped with white filaments; underside colour: transparent; appearance: powdery.
Trichoderma sp. Y9	Thallus colour: beige topped with white filaments; core colour: green; underside colour: beige with a yellow core; appearance: fluffy.
Trichoderma sp. Y2	Thallus colour: yellowish-beige topped with white filaments; core colour: green topped with white filaments; underside colour: beige with a yellow core; appearance: fluffy.
Trichoderma sp. Y5	Thallus colour: green topped with white filaments; underside colour: transparent; appearance: powdery.
Trichoderma sp. K1	Thallus colour: beige; presence of green pustules; underside colour: beige; appearance: fluffy.

Microscopic observation of the 10 *Trichoderma* morphotypes revealed similarities in the shape and coloration of the conidia of each morphotype. Branched mycelia with the presence of phialides and conidia were also noted (Figure 1). These conidia were produced at the tips of the phialides. The conidia were smooth, globular, and green.

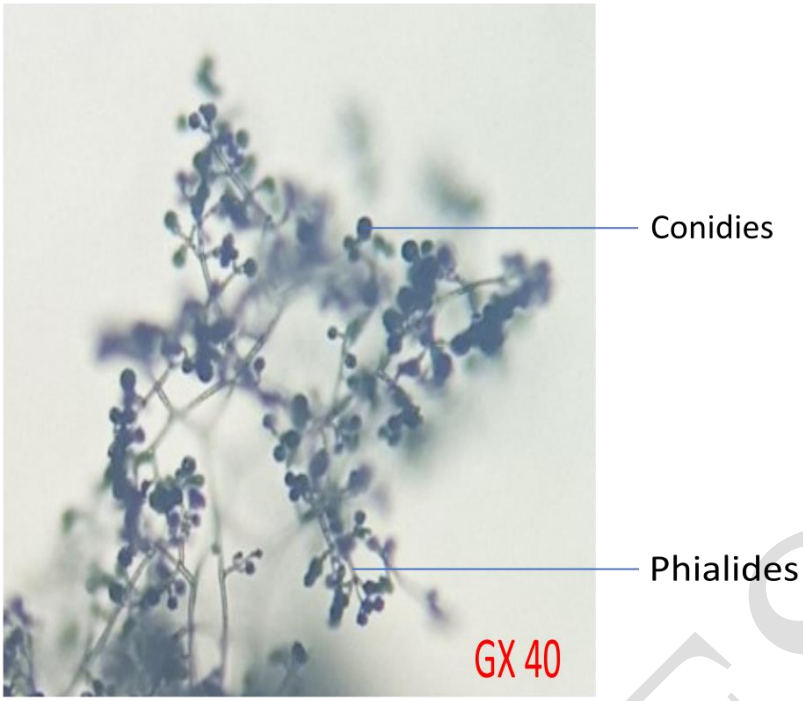


Figure 1: Microscopic features of elite *Trichoderma* sp. morphotypes

Molecular diversity of elite *Trichoderma* species

DNA was extracted from 10 *Trichoderma* morphotypes and successfully amplified using the ITS1/ITS4 universal primer pair. Amplification of the ITS region using this primer pair generated a DNA fragment of ~600 bp, corresponding to the size expected for *Trichoderma* strains (Figure 2). The sequencing results enabled us to obtain nine DNA consensus sequences from 10 DNA samples. A comparison of these nine sequences with those contained in the National Center for Biotechnology Information (NCBI) database highlighted similarities with *Trichoderma virens*, *Trichoderma hamatum*, and *Trichoderma asperellum* sequences (Table 5). The phylogenetic tree constructed from the nine DNA consensus sequences and the sequence available in the NCBI database was based on a *Trichoderma virens* sequence that had been identified in Spain (BK006704) (Figure 3). The phylogenetic tree configuration revealed two clades. The nine sequences obtained were part of the second clade, which consisted of three subclades. The *Trichoderma asperellum 5*, *Trichoderma* virens 3, *Trichoderma virens 2*, and *Trichoderma virens 1* sequences of strains isolated in Abidjan, Yamoussoukro, and Bouaké soils were grouped with *Trichoderma virens* subclade strains. The *Trichoderma asperellum 1*, *Trichoderma asperellum 2*, *Trichoderma asperellum 3*, *Trichoderma asperellum 4*, and *Trichoderma hamatum* sequences belonged to *Trichoderma asperellum* subclade strains. Based on phylogenetic distance and membership to the same

clade, the *Trichoderma virens 3* sequence was closer to that described in Ghana (OP597667). However, the Sequence Demarcation Tool (SDT) revealed a low level of homology between these sequences, i.e., 60%. The *Trichoderma virens 1* and *Trichoderma virens 2* sequences were closer to the *Trichoderma* sequences of strains identified in Côte d'Ivoire (KY315596) and Ghana (OP597666). The SDT revealed 100% sequence homology, thereby confirming these results. The *Trichoderma asperellum 1*, *Trichoderma asperellum 2*, *Trichoderma asperellum 3*, and *Trichoderma asperellum 4* sequences were closer to those identified in Nigeria (LC590856), Kenya (KJ584627), and Egypt (OQ355648). The SDT revealed 100% sequence homology. The *Trichoderma hamatum* sequence was also close to the sequences of strains from Nigeria (LC590856), Kenya (KJ584627), and Egypt (OQ355648), with 100% sequence homology.

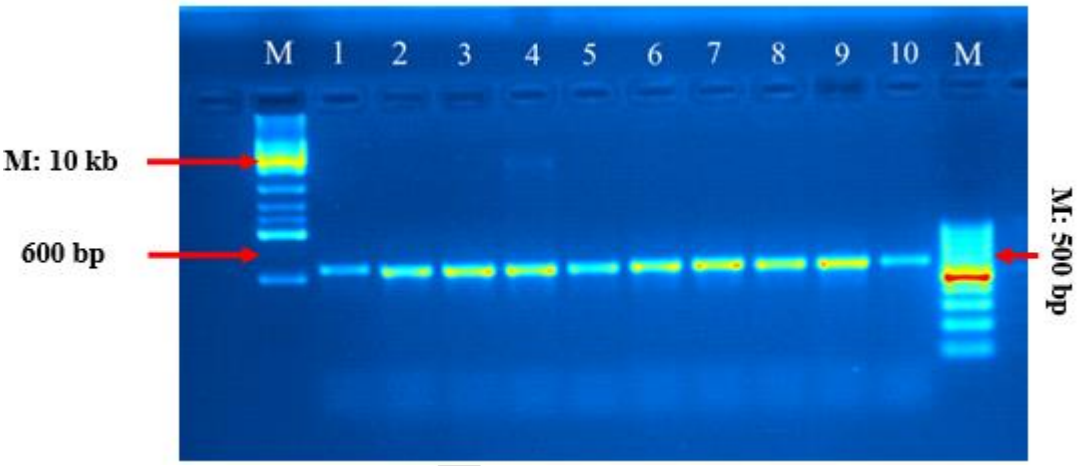


Figure 2: Electrophoresis profile of fungal DNA amplification products. M: molecular-weight marker (500-10 kb and 100 bp); 1-10: B2, A16, K1, K2, A14, A1, A19, Y9, Y2, and Y5.

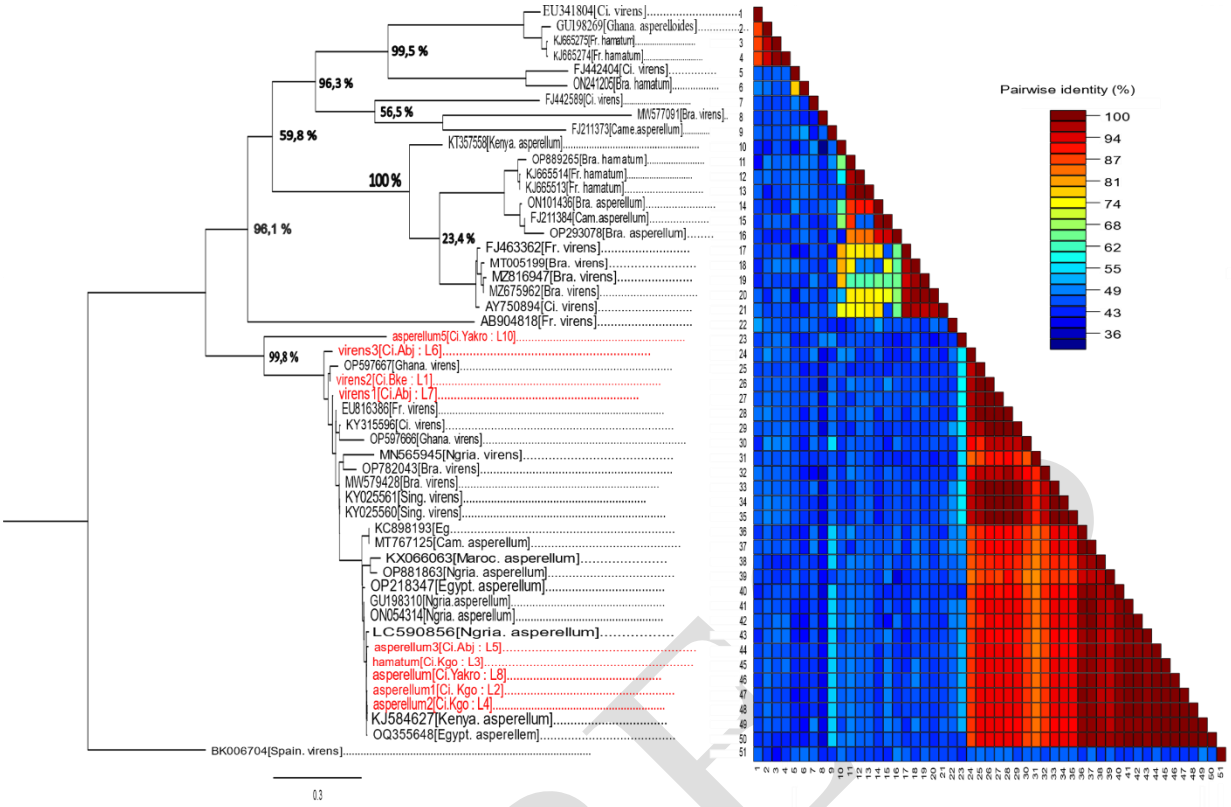


Figure 3: Maximum-likelihood-based phylogenetic tree and colour-coded matrix of pairwise nucleotide identity inferred from partial nucleotide sequence alignments of the ITS regions of *Trichoderma* rDNA isolates.

Table 5: Percentage homology of DNA sequences of *Trichoderma* strains obtained from GenBank

Morphotypes	Sampling code	Identified species	Similar Genbank accession number	Coverage level (%)	Percentage homology (%)	GenBank accession number
Trichoderma sp. *A19*	L7	*Trichoderma virens*	MT889750	100	99.66	PQ159277
Trichoderma sp. *B2*	L1		MK791709	100	99.83	PQ159271
Trichoderma sp. *A1*	L6		MH781006	100	97.29	PQ159276
Trichoderma sp. *A16*	L2	*Trichoderma asperellum*	MK841003	100	100	PQ159272
Trichoderma sp. *K2*	L4		MT133310	99	100	PQ159274
Trichoderma sp. *A14*	L5		OP794016	100	99.50	PQ159275
Trichoderma sp. *Y9*	L8		MT133310	100	100	PQ159278
Trichoderma sp. *Y5*	L10		MW165417	85	97.01	NI
Trichoderma sp. *K1*	L3	*Trichoderma hamatum*	MT111894	100	100	PQ159273
Trichoderma sp. *Y2*	L9	*Trichoderma* sp.	NI	NI	NI	NI

Discussion

We identified 10 *Trichoderma* sp. morphotypes (A19, B2, A16, Y2, K2, A14, A1, Y9, K1, and Y5) that could enhance tomato agromorphological parameters (germination rate and root and leaf dry matter). The stimulation of germination observed could be explained by the production of growth regulators (Petrisor *et al*., 2019). Ali *et al*. (2014) have shown that the enhancement of cucumber seed germination by *Trichoderma* application occurs through the secretion of hormones such as gibberellins. The effects of *Trichoderma* species on seedling growth and vigour are consistently dependent on the species/isolate applied (Azarmi *et al*., 2011). According to Zhao *et al*. (2014), the increase in plant growth induced by *Trichoderma* strains could be explained by the fact that they are capable of producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, phytohormones, and siderophores. Azarmi *et al*. (2011) showed that *Trichoderma harzianum* species significantly increased shoot dry weight and root dry weight. Woo *et al*. (2023) have shown that for eco-sustainable agriculture, it is important to use *Trichoderma* spp., which, through its colonization of plants, either on roots or as an endophyte, has developed the ability to communicate with plants and provide many multifaceted benefits for its host.

We present the specific molecular features of these 10 elite *Trichoderma* sp. morphotypes, all of which were morphologically characterised by the presence of phialides and conidiophores typical of *Trichoderma* sp. strains. Du Plessis *et al*. (2018) showed that *Trichoderma* strains feature conidiophores, while Dourou and La porta (2023) documented highly branched conidiophores and clusters of divergent phialides, typical microscopic characteristics of *Trichoderma* sp. strains. Colonies of these 10 morphotypes exhibited different colouring (green or white) and had different aspects (fluffy or powdery), with the presence or absence of rings and pustules. Dourou and La porta (2023) obtained *Trichoderma* strains that initially formed white or transparent colonies, followed by several colours within a week, while having a fluffy texture. These morphological changes could be a means of adapting to variations in pH, temperature, genetic factors, and other environmental factors (Yao *et al*., 2024; Shah & Afiya, 2019).

Trichoderma spp. show high genetic diversity in crop fields in Côte d'Ivoire. In this study, the different *Trichoderma* morphotypes were characterised at the molecular level. Amplification of the ITS region via the ITS1/ITS4 primer pair generated a ~600 bp DNA fragment that matched the size expected for *Trichoderma* strains. This finding is in line with that of Boat *et al*. (2019) who screened, identified, and assessed *Trichoderma* spp. for the potential biocontrol of common bean damping-off pathogens. Castrillo *et al*. (2016) obtained a 591 bp

DNA fragment in the molecular typing of *T. koningiopsis*. In addition, Chakraborty *et al*. (2010) showed that the internal transcribed spacer (ITS) region enabled the detection of polymorphisms in the rDNA ITS region in *Trichoderma* isolates. In their molecular characterization of *T. virens*, Alfiky & Eldenary (2019) obtained a DNA fragment ranging from 520 to 590 bp in size.

Nine DNA consensus sequences were obtained from the 10 DNA samples. By comparison with sequences in the National Center for Biotechnology Information (NCBI) database, the sequences of *Trichoderma* morphotypes A19, B2, and A1 were identified as belonging to *T. virens*, with 99.66%, 99.83%, and 97.29% homology, respectively. This comparison likewise enabled us to infer *Trichoderma* morphotypes A16, K2, A14, Y9, and Y5 belonged to *T. asperellum*, with 97.01% and 100% homology. Through sequence comparisons with the NCBI database, the *K1* morphotype was also identified as a *T. hamatum* strain. Raja *et al*. (2017) showed that species name assignment based on GenBank BLAST search results resulted in ≥80% query coverage and ≥97% sequence homology. Boat *et al*. (2019) successfully identified *T. viride, T. hamatum*, and *T. harzianum* strains through the NCBI-GenBank BLAST search tool, with 90–100% homology. *T. asperellum* and *T. virens* strains have also been isolated from cocoa orchard soils by Mpika *et al*. (2009). This finding could be explained by the cosmopolitan distribution of *Trichoderma* strains (Thrane *et al*., 2001). Yet, few studies on the diversity of *Trichoderma* strains isolated from tomato crop field soils have been carried out in Côte d'Ivoire.

The phylogenetic tree assessment revealed sequence similarities between the *T. asperellum* strains isolated from soils in Côte d'Ivoire and those from Morocco (KX066063), Nigeria (OP881863 and LC590856), Kenya (KJ584627), and Egypt (OQ355648). These sequence similarities could be explained by the crop type, and tomato crop field soil seems to be a prime habitat for *T. asperellum* strains. Kubicek *et al*. (2003) showed that strains of some *Trichoderma* spp., such as *T. virens*, could prevail in cultivated soils, indicating that this is a preferred habitat. This similarity could also be explained by the fact that some factors, such as pH and the presence of metal ions and pesticides, had no effect on *T. asperellum* strains. According to Kredics *et al*. (2003), abiotic and biotic environmental parameters impact *Trichoderma* spp. Mpika *et al*. (2009) reported that *T. asperellum* strains are generally regarded as being cosmopolitan.

The *Trichoderma virens* strains identified in this study showed sequence similarities with those described in Ghana (OP597667 and OP597666) and Côte d'Ivoire (KY315596). The similarities between the identified sequence and that of strains prevailing in Côte d'Ivoire

could be evidence of the presence of *T. virens* in Côte d'Ivoire crop fields. In their studies, Mpika *et al*. (2009) demonstrated that *T. virens* strains are widespread throughout cocoa-growing areas of Côte d'Ivoire. Factors such as wind and rain could therefore be vehicles for the spread of *T. virens* spores from cocoa orchards to tomato crop fields. The sequence similarities between the strains identified in this study and those present in Ghana could be explained by exchanges of plant material or products between these two neighbouring countries. Nakkeeran *et al*. (2021) showed that the native microflora is increasingly threatened by the marked increase in imports of foreign microbes in the form of commercial biocontrol products or for research.

The phylogenetic tree assessment revealed a relationship between the *T. hamatum* strains identified in this study and the *T. asperellum* strains sequenced in Nigeria (LC590856), Kenya (KJ584627), and Egypt (OQ355648). Kullnig *et al*. (2002) showed—based on the phylogenetic tree—that *T. hamatum* and *T. asperellum* belong to the same clade and are closely related. This similarity could be explained by gene mutation. Jaklitsch & Voglmay (2015) also showed, via phylogenetic tree assessment, that *T. hamatum* and *T. asperellum* share a common subclade. Moreover, Chaverri and Samuels (2013) noted that the ecological evolution of *Trichoderma* was the result of a combination of mutations, duplications, and the loss of genes from the common ancestors of Ascomycota and Basidiomycota.

Conclusion & prospects

This study aimed to identify—through morphological characterization and molecular analysis—*Trichoderma* sp. strains isolated in Côte d'Ivoire that could be beneficial for agricultural applications.

Ten *Trichoderma* morphotypes have shown promise in improving germination and leaf and root biomass. These *Trichoderma* sp. morphotypes formed white and green fungal colonies with or without concentric rings and pustules. Microscopic observation highlighted the presence of phialides and conidia (smooth, globular, and green) in these *Trichoderma* sp. morphotypes. Following molecular analysis, the 10 best *Trichoderma* morphotypes selected were identified as corresponding to the species *Trichoderma asperellum*, *Trichoderma hamatum*, and *Trichoderma virens*, with a percentage of similarity ranging from 97.01 to 100% with respect to those already existing in the gene bank (National Center for Biotechnology Information). Moreover, the phylogenetic tree assessment highlighted a relationship between the *Trichoderma hamatum* strains identified in this study and the sequences of *Trichoderma asperellum* strains described in Nigeria, Kenya, and Egypt.

Homology was also noted between the sequences of the identified *Trichoderma virens* strains and those described in Ghana and Côte d'Ivoire. Finally, the phylogenetic tree revealed similarities between the sequences of *Trichoderma asperellum* strains isolated from soils in Côte d'Ivoire and those described in Morocco, Nigeria, Kenya, and Egypt.

The results of this study broaden the scope for further research, including the assessment of *Trichoderma* spp. (*T. hamatum, T. virens and T. asperellum*) in tomato crop fields and the identification of the substances produced by each species. These three *Trichoderma* species could therefore be promising candidates for addressing this region's problems.

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Cover letter

In the present study, Macroscopic and microscopic monitoring, PCR, amplicon sequencing and phylogenetic inference were used to characterize *Trichoderma* species isolated from tomato cropfields, three of which seemed highly promising (*T. asperellum, T. virens and T. hamatum*). Assessment of the resulting phylogenetic tree revealed similarities between these three *Trichoderma* species and strains isolated elsewhere in Côte d'Ivoire, Ghana, Nigeria, Kenya, Morocco and Egypt.

This study gained insight into the most efficient *Trichoderma* spp. present in cropfields in Côte d'Ivoire. The findings should facilitate the use of these species to enhance tomato

production throughout this African subregion. This is particularly interesting when we know that in Africa biofertilizers and biostimulants are not developed yet.

We believe this paper will interest your readers. Actually, there is an increase in the number of publications related to molecular and cell biology. This is likely because of the overall interest for environmentally safe agriculture.

With the submission of this manuscript, I would like to verify that:

* All authors of this research paper have directly participated in the planning, execution, or analysis of the study;

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Declarations

Author contributions. Conceptualization, A.K., S.K., and E.D.; methodology, A.K., S.K., and E.D.; investigation, A.K.; analysis, A.K., and W.Y.; writing—original draft preparation, A.K..; writing—review and editing, S.K., and E.D

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