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Diversity of *Phytophthora palmivora* and *Trichoderma* sp. strains in cocoa orchards in 3 regions of Côte d'Ivoire

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Abstract

Cocoa black pod disease, caused by *Phytophthora* sp. leads to yield losses. In order to remedy such losses, the use of an antagonistic fungus such as *Trichoderma* is recommended. This study aims at highlighting a diversity of *Phytophthora palmivora* and *Trichoderma* spp. strains in cocoa orchards in Côte d'Ivoire for better biological control of *Phytophthora palmivora*, the causative agent of cocoa black pod disease. To this end, soil from the cocoa rhizosphere, healthy cocoa pods, and cocoa pods affected by black pod disease were sampled. *Trichoderma* sp. and *Phytophthora* sp. were isolated from soil and black pod disease-affected pods, respectively. PCR, amplicon sequencing and phylogenetic demarcation analyses made it possible to characterize *Phytophthora* and *Trichoderma* species. Three *Phytophthora palmivora* morphotypes and three *Trichoderma* species (*T. harzianum, T. asperellum,* and *T. virens*) found in cocoa orchards were characterized. High nucleotide similarity (98.5 % to 99.55 %) was observed between the DNA sequences of these fungal strains and *P. palmivora* and *Trichoderma* spp. isolates reported in India and Côte d'Ivoire. Knowing the diversity of these fungal strains can contribute to improving cocoa yield in Côte d'Ivoire through the efficient control of *Phytophthora palmivora*, the causative agent of cocoa black pod disease.

Keywords : *cocoa, black pod, Phytophthora palmivora, Trichoderma spp., molecular diversity.*

Résumé

Diversité de Phytophthora palmivora et Trichoderma sp. souches dans les vergers de cacaoyers dans 3 régions de Côte d'Ivoire

La pourriture brune des cabosses de cacaoyer causée par *Phytophthora palmivora* entraine des pertes de productions. Pour remédier à ces pertes, l'utilisation d'un champignon antagoniste tel que *Trichoderma* est préconisée. Cette étude vise à mettre en évidence une diversité des souches de *Phytophthora palmivora* et de *Trichoderma* spp. dans les vergers de cacao en Côte d'Ivoire pour un meilleur contrôle biologique de *Phytophthora palmivora,* responsable de pourriture brune du cacao. Pour ce faire, des cabosses de cacao saines, atteintes de pourritures brunes et de sol issus de la rhizosphère des cacaoyers ont été prélevés. *Trichoderma* sp. et *Phytophthora* sp. ont été respectivement isolées à partir du sol et des cabosses atteintes

de la pourriture brune. Les analyses PCR, de séquençage des amplicons et des démarcations phylogéniques ont permis de caractériser les espèces de *Phytophthora* et de *Trichoderma*. Trois (3) espèces de *Trichoderma* (*T. harzianum*, *T. asperellum* et *T. virens*) et trois morphotypes de *Phytophthora palmivora* présents dans les vergers de cacao ont été caractérisés. Les séquences d'ADN de ces souches fongiques ont présenté les niveaux de similarité nucléotidique élevés (98,5 % à 99,55 %) avec les isolats de *P. palmivora* et de *Trichoderma spp.* décrits en Inde et en Côte d'Ivoire. Une connaissance de la diversité de ces souches fongiques peut contribuer à l'amélioration de la production de cacao en Côte d'Ivoire par le contrôle efficient de *Phytophthora palmivora,* agent responsable de la pourriture brune des cabosses de cacao.

Mots-clés : *cacao, pourriture brune, Phytophthora palmivora, Trichoderma spp., diversité moleculaire.*

1. Introduction

Theobroma cacao L. is the plant that yields cocoa, provided with nutrients and an excellent source of energy [1]. Cocoa beans undergo several processing in the food, cosmetic, pharmaceutical and especially in confectionery [2 - 4]. In Côte d'Ivoire, cocoa cultivation occupies an important place in the social and economic life of the population. It generates about 46 % of export revenue. Moreover, it constitutes a substantial source of income for thousands of cocoa farmers [5]. It alone represents 15 % of Ivorian GDP [6, 7]. The Ivorian orchard is threatened by various pest attacks in spite of its significant yield. These infections cause significant damage to plantations and economic losses for the producer. These include fungal infections, which can cause damage and result in yield losses ranging from 20 to 60 percent [8, 9]. In Côte d'Ivoire, cocoa black pod remains one of the main diseases in cocoa plantations [10]. In Côte d'Ivoire, cocoa black pod disease caused by *Phytophthora palmivora* results in 30 % yield losses. Control measures have been devised to lessen yield losses brought on by black pod disease. Although there have been solid research results, chemical management is still a controversial practice because of the high expense of fungicides and the labor-intensive nature of farming [11]. Integrated pest control is currently being investigated as a means of combating this disease. This includes cultivation practices, the selection of resistant cultivars, and the use of fungus antagonists, specifically *Trichoderma* species [12]. For the latter method, *Trichoderma* from the cocoa plantation must be isolated, a collection must be established, and their antagonistic potential must be assessed [13]. The purpose of this study was to highlight the variety of *Phytophthora palmivora* strains as well as those of the *Trichoderma* genus, which may act as *Phytophthora palmivora*'s antagonists for effective, long-lasting control of black pod disease [14, 15].

2. Material and methods

2-1. Sampling

A sample of 1 kg of soil was taken from the base of 10 cocoa trees bearing asymptomatic pods and cocoa trees showing black pod symptoms. Before each sampling, the soil surface was first cleared of dead leaves and plant detritus. The method of [16] was then used to sample at a depth of 30 cm from the surface layers. Asymptomatic pods as well as those showing black pod symptoms were also sampled. The pods were sampled at a rate of 20 pods per locality, including 10 asymptomatic pods and 10 others showing black pod symptoms. Sampling instruments were either cleaned with sodium hypochlorite or flambeed with alcohol to prevent contamination from one sampling location to another. Additionally, hands were sanitized with 70 % alcohol. Samples of soil and pods were kept in clear plastic bags and kept in coolers. The samples were brought back to the laboratory for isolation of the different fungal strains.

2-2. Isolating and purifying fungal strains

Cocoa pods showing black pod symptoms were used for isolating the fungi associated with the symptoms, according to the method of [17]. Explants of pod epidermal layer were taken from the growth front of the symptoms caused by the fungi and seeded on PDA medium. The cultures were carried out in Petri dishes at a rate of three dishes per pod and four explants per dish. Dishes sealed with plastic wrap were incubated at the laboratory's room temperature, approximately 27°C, for 3 days. Twenty grams of each soil sample were collected separately. To these different samples, a volume of 50 ml of distilled water was added. Using the method described by [18], four explants of roughly 1 cm^3 each were removed from healthy potato tubers (carefully cleaned as before) and buried in such soil slurry. Explants containing fungal colonies were gathered on blotting paper after a day, and water was drained from the area around them using a laminar flow cabinet After that, the infected explants were seeded in Petri dishes containing solidified PDA medium. Three Petri dishes filled with the soil slurry were used for each soil sample. Following their initial isolations, the different fungal colonies were subsequently subcultured independently on fresh PDA media. Pure, unique strains were obtained after three to four subcultures [16].

2-3. Morphological features of fungal isolates

At 800x magnification, the isolated fungal strains were characterized both macroscopically and microscopically. The coloration of mycelial colonies was the subject of macroscopic observations. Among the microscopic features were branching, mycelial partitioning, and spore shape. The identification keys of [19] were used to identify the fungal strains.

2-4. Molecular characterization of fungal isolates

The different fungal strains from the primary cultures were subsequently subcultured independently on fresh PDA media, with an emphasis on molecular analysis. Following three or four consecutive monospore or monocolony subcultures on the culture media, the DNA of the distinct, pure strains was extracted and amplified using PCR [16]. The PCR products were amplified and then electrophoresed on a 2 % agarose gel. For this, 30 mL of TAE 1X buffer (Tris, Acetate EDTA) was microwaved for two minutes to dissolve 0.6 g of agarose (Bioshop, Canada). After the agarose gel had cooled down, 1.5 µL of ethidium bromide (0.5 µg/mL) was added before it was poured into the mold. In a cell, the gel that had solidified was submerged in 0.5X WT buffer. (Wide Mini-Sub Cell GT). Next, 5 µL of every PCR result was added to the agarose gel's wells. A well was filled with a volume of 2 µL of size marker (BenchTop 100 bp DNA ladder, Promega, USA) in order to measure the various amplified DNA fragment sizes. For 60 minutes, migration was carried out at 70 V. Lastly, a gel reader (E-BOX VX5, France) was used to view the gel under a UV lamp. At Macrogène (Netherlands), PCR products were sequenced. MEGA version X was used to align the obtained nucleotide sequences, and Figtree version 3.2 was used to create phylogenetic trees. These nucleotide sequences were separated phylogenetically.

2-5. Data analysis

R studio version 4.3.0 was used to analyze the data from this study. The mean inhibition rates of various *Trichoderma* species on *Phytophthora palmivora* morphotypes were compared using the ANOVA test. To identify homogeneous groups in the event of significant differences, Fischer's LSD test was carried out.

3. Results

3-1. Isolated fungal strains

Phytophthora isolates totaling twelve were obtained from cocoa pods exhibiting black pod disease symptoms. Three morphotypes were identified for these isolates. *Phytophthora* spp. 1, 2, and 3 were among them. From the soil, twenty-one isolates of the genus *Trichoderma* were obtained. Based on their morphological features, these isolates were divided into three morphotypes : *Trichoderma* sp.1, *Trichoderma* sp.2, and *Trichoderma* sp. 3. These diverse fungal strains displayed a range of morphological traits ***(Figure 1)***.

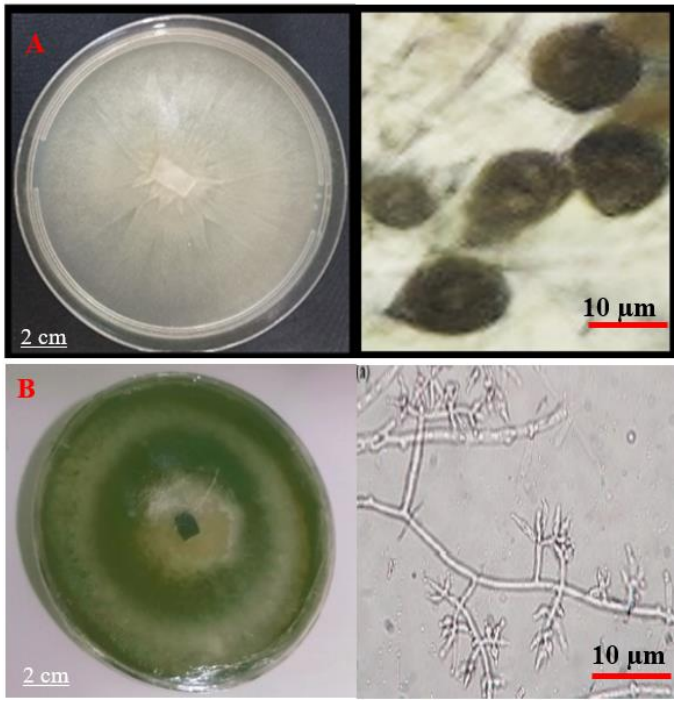

Figure 1 : *Morphological aspects of fungal strains*
A : Phytophthora sp.
B : Trichoderma sp.

3-2. Morphological features of fungal isolates

The macroscopic features of *Phytophthora* sp. 1 included a white thallus with a fibrous shape. Its appearance was broad, cerebriform relief on a cottony background. Sporangia were ovoid objects with rounded apices under the microscope. A smaller pedicel was used. A white aerial thallus was seen in *Phytophthora* sp. 2. This isolate grew irregularly and looked cottony. Under the microscope, the sporangia were spheroidal and the apex was pointed with a long pedicel. The macroscopic features of *Phytophthora* sp. 3 included a white, cottony-looking thallus that was fibrous and airy. Under the magnifying glass, the apex was rounded and the porocysts were ovoid. There were branches and an unpartitioned mycelium. *Trichoderma* sp. 1 grew quickly, exhibiting an eccentric circular band of growth for its dark green, cottony-looking thallus. At the tips of the conidiophores were Phialides, and the spores were smooth and globose. The macroscopic features of *Trichoderma* sp. 2 showed fast diffuse growth and a filamentous, dark green thallus. The smooth, globose Phialides spores at the tips of the conidiophores were among the microscopic features. Regarding *Trichoderma* sp. 3, it grew quickly and unevenly, exhibiting a powdery, green thallus that was dispersed throughout the Petri dish and evolved in an uneven way. Under a microscope, it revealed a smooth, globular conidia and a conidiophore with phialides.

3-3. Molecular diversity of fungal isolates

Amplicons with an estimated size of 600 bp were produced by the pathogenic (*Phytophthora* fungal strain) and antagonistic (*Trichoderma* fungal strain) fungi whose DNA was extracted and amplified by PCR ***(Figure 2)***.

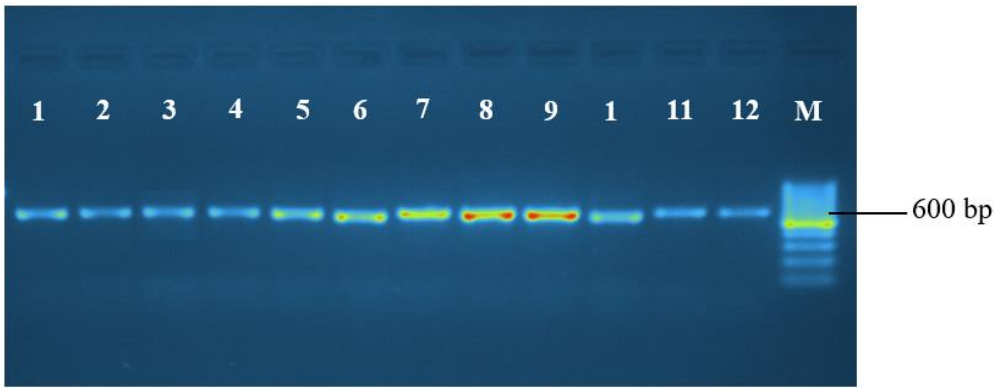

Figure 2 : *Electrophoretic profile for PCR products of the DNA of fungal strains with the primer pair ITS1 and ITS4*
M : molecular weight marker
1 to 12 : sample numbers

The sequencing results identified several *Trichoderma* species and a distinct diversity of fungi that cause cocoa black pod disease (*Phytophthora* sp.). The following fungal species were found by similarity searches between our sequences and those in the NCBI (National Center for Biotechnology Information) database: *Trichoderma virens*, *Trichoderma asperellum*, *Trichoderma harzianum*, and *Phytophthora palmivora* ***(Table 1)***.

Table 1 : *Caractéristiques des séquences des souches fongiques*

Code	Name of similar sequences	Identity Percentage	Similar accession number
CK11	*Phytophthora palmivora*	100 %	MH401199
CK13	*Phytophthora palmivora*	99,64 %	KY447326
CK14	*Phytophthora palmivora*	100 %	JX155790
CK7	*Trichoderma virens*	93 %	MN102106
CK2	*Trichoderma asperellum*	100 %	MT529846
C5	*Trichoderma harzianum*	80,45 %	OL604510

Fungal strain sequencing produced six consensus sequences from ten DNA samples. These sequences were extracted from soil and pods affected by black pod disease. Three of the sequences (isolated from pods affected by black pod disease) were found to be *Phytophthora palmivora* isolates, according to DNA sequence analysis ***(Figure 3)***. *Phytophthora palmivora* isolates found in exhibit the highest nucleotide similarity (98.5 % to 99.44 %) with isolates of the same species that were described in India in 2012 (Accession number JX155790) and 2020 (Accession number MT052675). The nucleotide similarity between these *P. palmivora* isolates and those reported in Côte d'Ivoire in 2014 is also present (accession numbers: KJ144804, KJ144805, and KJ144792). The remaining three DNA sequences, which were extracted from soil, belonged to the fungal genus *Trichoderma* (*T. harzianum, T. asperellum* and *T. virens*) ***(Figure 4)***. There was an 86.5 % nucleotide similarity between these sequences. These sequences shared the greatest nucleotide similarities (98.5 %, 99.39, and 99.55 %, respectively) with *T. harzianum*, *T. asperellum*, and *T. virens*, which were discovered in Côte d'Ivoire and have accession numbers KY315597, ON121957, and KY315596 on GenBank.

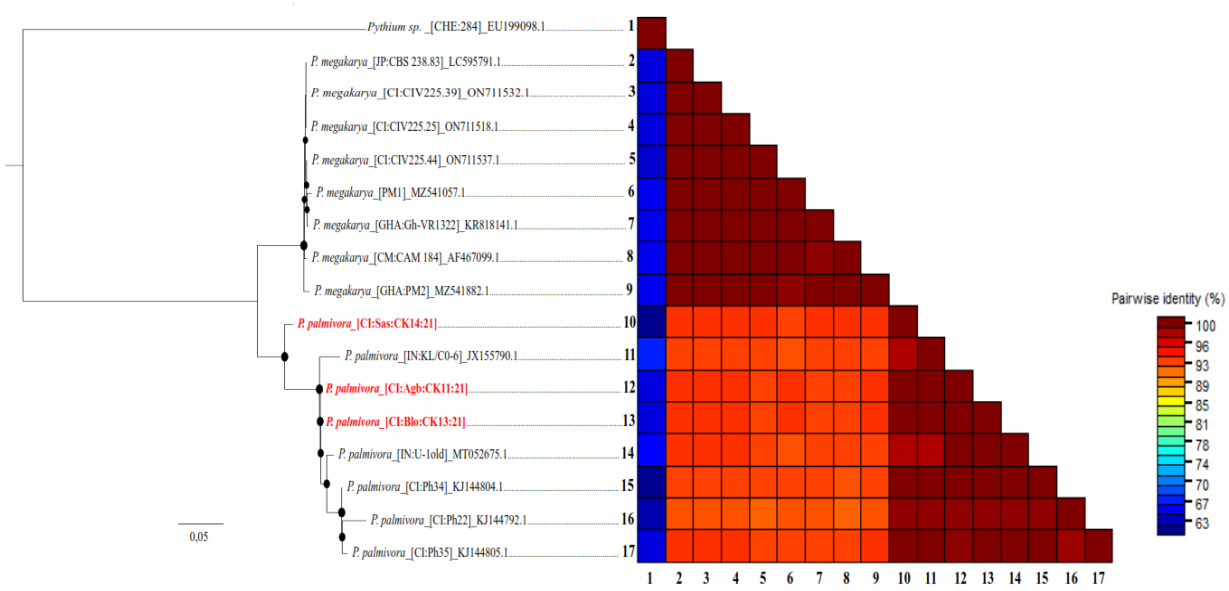

Figure 3 : *Maximum likelihood phylogenetic tree and color-coded matrix of pairwise nucleotide identity deduced from partial nucleotide sequence alignments of the ITS region of rDNA from Phytophthora palmivora isolates*

The tree was rooted on a partial nucleotide sequence of the ITS region of *Pythium* sp. The matrix uses a discontinuous range of three color shades (red, green and blue) differentiating two values representing the demarcation thresholds of fungal strain and species.

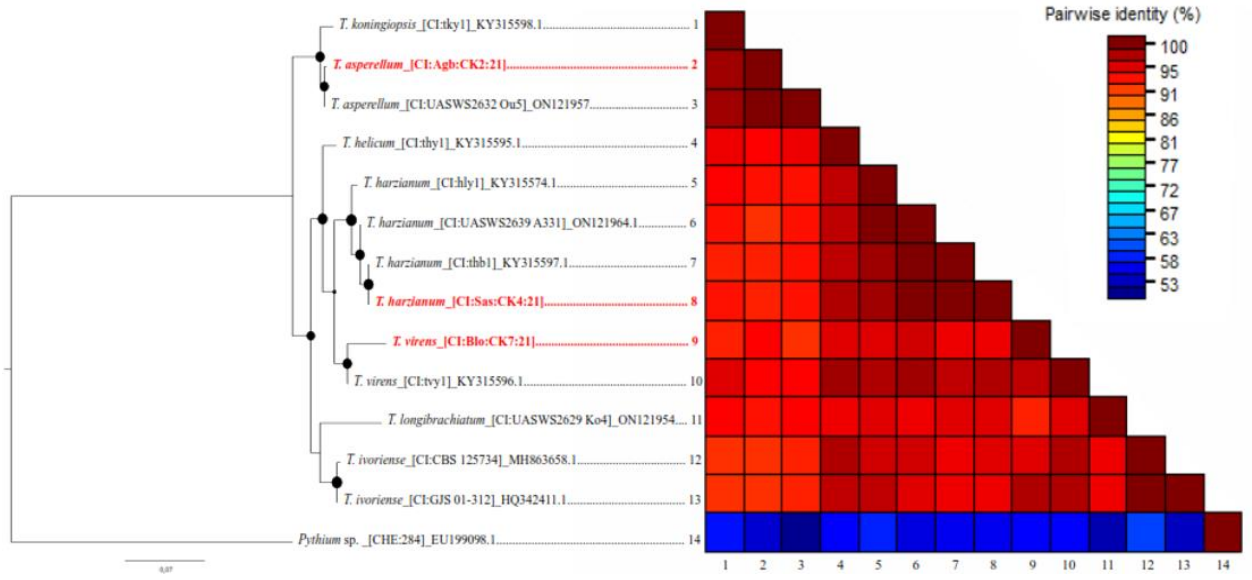

Figure 4 : *Maximum likelihood phylogenetic tree and color-coded matrix of pairwise nucleotide identity deduced from partial nucleotide sequence alignments of the ITS region of rDNA from Trichoderma isolates*

The tree was rooted on a partial nucleotide sequence of the ITS region of *Pythium* sp. The matrix uses a discontinuous range of three color shades (red, green and blue) differentiating two values representing the demarcation thresholds of fungal strain and species.

4. Discussion

4-1. Symptoms description

Necrosis, or the alteration of organs, is one of the symptoms of black pod disease, according to observations made on cocoa pods taken from plantations. This rot could be the result of tissue damage brought on by a *Phytophthora* sp. infection. Indeed, sporangia, zoospores, oospores, and chlamydospores are the four types of spores that can cause infection either directly or indirectly in *Phytophthora* sp. Sporangia are produced on infected fruits, leaves, stems or roots. They have the ability to produce zoospores and can germinate both in the soil and directly on the surface of the plant. To enter a plant or get to the cocoa pods, zoospores can swim in soil water or water on the plant's surface. Brown color and wet decomposition are present in infected pods. Abiotic stress and enzymatic reactions may be the cause of the brown hue of cocoa pod rot symptoms. It is true that abiotic stresses like temperature changes, high humidity, nutrient shortages, and extended sun exposure can affect cocoa pods. These stressors have the potential to change the pods' physiology and cause chemical reactions that lead to brown discoloration. Furthermore, using pesticides or fungicides to stop fungal infections can react chemically with the compounds in cocoa pods, causing browning of the rot symptoms [20]. These findings support the claim made by [13] that *Phytophthora palmivora* secretes toxins and enzymes that allow it to attach to and penetrate the tissues of cocoa plants. Cell walls and extracellular matrix components are broken down by these enzymes, which include cellulases and pectinases. *P. palmivora* uses the nutrients found in plant cells to grow and spread once it has entered plant tissues. The oomycete secretes lipases and proteases, two types of enzymes that degrade proteins and lipids in plant tissue to liberate nutrients vital to the plant's growth. It also generates toxins that harm the cells of plants. Browning of the cocoa pod is one of the signs of stress and deterioration caused by these toxins, which interfere with normal cell functions. The appearance and distribution of these symptoms on the fruit varied.

4-2. Morphological diversity of *Phytophthora palmivora*

From cocoa pods affected by cocoa black pod disease and from cocoa rhizosphere soils in six cocoa production areas in Côte d'Ivoire, a variety of *Phytophthora* morphotypes were isolated. The many reproductive strategies of the fungal genus *Phytophthpora*, which add to the morphological diversity of the species, may account for the diversity of *Phytophthora* sp. morphotypes seen in these various production zones. By producing asexual spores known as zoospores, which can be distinguished morphologically, the oomycete is able to reproduce asexually. Furthermore, the fungus *Phytophthora* possesses the ability to reproduce sexually, which can result in the development of specialized reproductive structures like oospores, which can also exhibit morphological diversity. Additionally, research of [21] indicates that there is some genetic diversity present in the *Phytophthora palmivora* population. Genetic recombination, horizontal gene transfer events, or spontaneous mutations can all contribute to this genetic diversity. The observed morphological diversity can be attributed to the presence of distinct genotypes or strains of *P. palmivora* within a given geographic area, as indicated by genetic studies. Additionally, [22] showed that there are two different morphological and chromosomal types of *Phytophthora palmivora* on cocoa in West Africa. Differences in chromosomal trim are linked to morphological variations.

4-3. Morphological diversity of *Trichoderma* genus

The sexual reproduction mechanism of the *Trichoderma* genus, which produces spores and exhibits a wide range of morphological diversity in terms of size, color, and shape, is another factor contributing to the diversity of fungal strains within the genus. *Trichoderma* can also reproduce sexually, which can result in the

development of specialized reproductive structures like ascospores, which can also have a variety of morphological features. The emergence of morphological variants in *Trichoderma* sp. can be attributed to environmental influences on its morphological diversity, as demonstrated by [22]'s work. *Trichoderma*, for instance, exhibits morphological changes in response to variations in pH, temperature, substrate composition, and other environmental factors. These morphological changes could be adaptive, helping the fungus thrive and colonize in particular environments.

4-4. Molecular characteristics of fungal strains

Amplification of DNA extracts using the universal primer pair ITS1 and ITS4 resulted in the migration of DNA fragments containing roughly 600 base pairs. The findings of [23 - 25] are corroborated by this result. [26] reports that products of approximately 900 base pairs are produced by PCR amplification of the ITS region of *Phytophthora* sp. isolates, with minor variations in certain isolates. The study yielded nucleotide sequences that were identical to those found in the NCBI GenBank databases. This indicates that other nations may also harbor the strains of *Phytophthora* sp. that were discovered in Côte d'Ivoire. [27] state that fungi are found throughout the world and have a wide variety of host plants. *Phytophthora palmivora* and *Trichoderma* species isolates were identified as *Phytophthora* sp. strains by sequence alignment using NCBI's BLASTn. *Phytophthora palmivora* strains were isolated from soil samples and pods exhibiting black pod disease symptoms. These findings demonstrate that Côte d'Ivoire is home to *Phytophthora palmivora*. These findings are consistent with those of [25], who separated these two *Phytophthora* species that were isolated from black pod disease-affected pods using restriction enzymes. The *P. palmivora* species was identified by these authors' study using the restriction enzyme Hae III. Additionally, *P. palmivora* was identified through restriction analysis of ITS regions using the Alu I restriction enzyme [27, 29]. However, after using these two restriction enzymes to perform restriction analysis on ITS regions, [30] were able to identify the species *P.* megakarya. According to [31], this species (which is thought to be the most aggressive in the *Phytophthora* genus) was discovered in Côte d'Ivoire. *Trichoderma* is a genus of filamentous fungi, and the three species of *Trichoderma* that were found (*T. harzianum*, *T. asperellum*, and *T. virens*) indicate that there is some genetic diversity among the species. Genetic recombination, horizontal gene transfer events, or spontaneous mutations could all contribute to this genetic diversity. Different strains or species of *Trichoderma* have been found in the same geographic area, according to genetic studies. Genetic variation within the *Trichoderma* population can result from spontaneous or induced genetic mutations, as demonstrated by studies conducted by [32]. These mutations can affect genes that code for particular traits, resulting in the phenotypic and morphological variations seen in different strains. During *Trichoderma*'s sexual reproduction, genetic recombination mechanisms like homologous recombination can also take place. The population's genetic diversity can be increased by these recombination events by resulting in the exchange of DNA segments between parental chromosomes. Furthermore, [33] has shown that *Trichoderma* can also pick up new genes through a process known as horizontal transfer, in which genetic material from other organisms is acquired. Mechanisms like spontaneous genetic mutation or hybridization with different fungal species or genera can cause this. *Trichoderma*'s genetic diversity can be increased through horizontal gene transfer by introducing novel alleles. The interest of these results is that certain fungal strains of biological control such as *Trichoderma* sp. may be more effective against specific strains of *Phytophthora palmivora*, thus improving biological control [14].

5. Conclusion

This study aimed at illustrating the fungal diversity amongst *Trichoderma* sp. and *Phytophthora palmivora* strains. To this end, samples of cocoa pods were taken. Purified and isolated fungal strains were obtained. Phylogenetic demarcation was done after a molecular characterization inventory of strains of *Phytophthora* and *Trichoderma* was completed. By the time our study concluded, the rhizosphere of cocoa orchards and cocoa pods exhibiting black pod disease symptoms had yielded twelve (12) *Phytophthora* isolates and twenty-one (21) *Trichoderma* isolates. These fungal genera showed a variety of morphological characteristics and were divided into three (3) morphotypes each. Three species of *Trichoderma* and three strains of *Phytophthora palmivora* were identified by molecular characterization. (*T. harzianum*, *T. asperellum* and *T. virens*). In addition, isolates from the same fungal genera share nucleotide levels with *Phytophthora palmivora* and *Trichoderma* species found in Côte d'Ivoire, according to DNA sequence analysis.

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References

[1] - N. S. ELIAS, C. R. DANILO, M. M. LÍGIA, O. S. ALEXILDA, C. S. L. SUZANA & V. S. MARCONDES, Nutritional value and antioxidant capacity of "cocoa honey" (*Theobroma cacao* L.). *Food Science and Technology, Campnas,* 34 (4) (2014) 755 - 759

[2] - Y. C. T. TERENCE, Y. L. XIN, H. H. Y. JULIE, W. H. L. SHAUN & M. L. NAI, The Health Effects of Chocolate and Cocoa : A Systematic Review. *Nutrients*, 13 (9) (2021) 2909 - 2934

[3] - T. M. MARIA, D. GIUSY, T. FRANCESCO, R. C. GIUSY, D. G. OSVALDA, C. GIUSEPPINA, D. C. AGOSTINO & P. PIERO, Chocolate, "Food of the Gods": History, Science, Human Health. *International Journal of Environmental Research and Public Health,* 16 (24) (2019) 4960 - 4981

[4] - K. KOUAKOU, Diversité moléculaire du cssv (Cocoa Swollen Shoot virus) et épidémiologie de la maladie du swollen shoot du cacaoyer (*Theobroma cacao* L.) en Côte d'Ivoire. Mémoire de thèse, Université Félix Houphouët-Boigny. Abidjan, Côte d'Ivoire, (2014) 153 p.

[5] - A. DEMBELE, A. COULIBALY, S. K. TRAORE, K. MAMADOU, N. SILUE & A. A. TOURE, Détermination du niveau de contamination de l'ochratoxine A (OTA) dans les fèves de cacao à l'exportation. *Tropicultura.*, 27 (1) (2009) 26 - 30

[6] - N'GUESSAN, K. F. N'GUESSAN, K. P. KOUASSI, N. N. KOUAME & P. W. N'GUESSAN, Dynamique des populations du foreur des tiges du cacaoyer, Eulophonotus myrmeleon Felder (Lépidoptère : Cossidae) dans la région du Haut-Sassandra en Côte d'ivoire. *Journal of Applied Biosciences*, 83 (2014) 7606 - 7614

[7] - M. DUFUMIER, L'adaptation de la cacaoculture ivoirienne au dérèglement climatique : L'agroécologie pourrait-elle être une solution ? Rapport de mission Marc Dufumier - Sept 2016. *Plate-Forme pour le Commerce Equitable,* (2016) 16 p.

[8] - K. COULIBALY, B. I. KEBE, A. R. AKA, K. KOUAKOU, W. P. N'GUESSAN, G. M. TAHI, K. E. KASSIN, S. B. GUIRAUD, M. E. ASSI, B. KONE & K. F. N'GUESSAN, Bien lutter contre la pourriture brune des cabosses du cacaoyer en Côte d'Ivoire. Fiche technique cacaoyer n° 6. *Centre National de Recherche Agronomique,* Abidjan, Côte d'Ivoire, (2017) 2 p.

[9] - S. H. KOUA, N. A. M. D. COULIBALY & W. A. M. ALLOUEBORAUD, Caractérisation vergers et des maladies de cacao de la Côte d'Ivoire : cas des départements d'Abengourou, Divo et Soubré. *Journal of Animal & Plant Sciences*, 35 (3) (2018) 5706 - 5714

[10] - K. KOUAKOU, B. I. KÉBÉ, N. KOUASSI, A. P. ANNO, S. AKÉ & E. MULLER, Impact de la maladie virale du swollen shoot du cacaoyer sur la production de cacao en milieu paysan à Bazré (Côted'Ivoire). *Journal of Applied Biosciences*, 43 (2011) 2947 - 2957

[11] - A. YANELIS, H. ANNIA, H. MAYRA, E. J. MONDHER & N. H. ANA, Management of black pod rot in cacao (*Theobroma cacao* L.) : a review. *Fruits*, 67 (1) (2011) 41 - 48

[12] - J. MPIKA, B. K. ISMAËL, S. IRINA, M. K. DRUZHININA, P. CHRISTIAN & SEVÉRIN AKÉ, Inhibition de *Phytophthora palmivora*, agent de pourriture brune des cabosses de cacaoyer en Côte d'Ivoire, par *Trichoderma* sp. *Sciences et Nature*, 6 (**1**) (2009) 49 - 62

[13] - L. SANTOS, O. MANUEL, H. ELGAR, C. BEIMER, R. KAROL, G. FLOR & T. MAGDIEL, Assessment of the Potential of *Trichoderma* spp. Strains Native to Bagua (Amazonas, Peru) in the Biocontrol of Frosty Pod Rot (*Moniliophthora roreri*). *Agronomy*, 10 (9) (2020) 1376 - 1391

[14] - S. N. VISHNU, S. J. NEETHA, P. A. INDIRA, M. H. VINAYAKA, L. J. MUTHULEKSHMI, S. M. RAJ & S. V. SYAMALA, Characterization of *Trichoderma* spp. antagonistic to *Phytophthora colocasiae* associated with leaf blight of taro. *Annals of Microbiology*, 64 (2014) 1513 - 1522

[15] - M. NAZIA, S. K. ABHIJEET, S. G. RAVI, V. S. R. MAHENDRA, K. S. PAWAN, K. S. SUSHIL & V. S. HARSH, *Trichoderma*: Advent of Versatile Biocontrol Agent, Its Secrets and Insights into Mechanism of Biocontrol Potential. *Sustainability*, 14 (19) (2022) 12786 - 12818

[16] - P. DAVET & F. ROUXEL, Détection et isolement des champignons du sol. *TNRA*, Paris, France, (1997) 194 p.

[17] - G. K. KOUAME, F. SORHO, D. KONE, L. E. BOMISSO, S. AKE & J. YATTY, Activité pathologique comparée de deux isolats de *Colletotrichum gloeosporioïdes* (Penz.) sur deux varietes de mangues (*Mangifera indica* L.). *Agronomie Africaine*, 23 (1) (2011) 33 - 41

[18] - B. HUGUENIN, B. BOHER, A. HAURY & E. LAVILLE, Etude de *phytophthora cinnamomi* de l'avocatier au Cameroun. *Fruits,* 30 (1975) 525 - 533

[19] - B. BOTTON, A. BRETON, M. FEVRE, S. GAUTHIER, P. H. GUY, J. P. LARPENT, P. REYMOND, J. J. SANGLIER, Y. VAYSSIER & P. VEAU, Moisissures utiles et nuisibles, importance industrielle. *Collection Biotechnologies. 2è édition, Masson*, Paris, 1 (1990) 34 - 206

[20] - E. O. AFOAKWA, A. PATERSON, M. FOWLER & A. RYAN, Flavor formation and character in cocoa and chocolate : a critical review. *Critical reviews in food science and nutrition*, 49 (9) (2008) 840 - 857

[21] - D. X. ZHANG & Q. YANG, Genetic diversity and population structure of Phytophthora palmivora isolates from rubber tree in Hainan Province, China. *Journal of Phytopathology,* 164 (10) (2016) 720 - 727

[22] - D. JENNIFER, R. S. ROBERTO, G. E. SAMUEL, S. B. DAHYANA, S. P. ALINA, R. AILTON, A. F. S. RILDO, H. J. JAIME, D. M. N. L. EDNA, B. P. DANILO & J. P. MARELLI, *Phytophthora theobromicola* sp. nov.: A New Species Causing Black Pod Disease on Cacao in Brazil. *Frontiers in Microbiology,* (2021) 12 p. https://doi.org/10.3389/fmicb.2021.537399

[23] - C. BRASIER, B. SCANU, D. COOKE & T. JUNG, *Phytophthora*: an ancient, historic, biologically and structurally cohesive and evolutionarily successful generic concept in need of preservation. *IMA Fungus,* 13 (1) (2022) 12 - 37

[24] - S. O. CACCIOLA, N. A. WILLIAMS, E. L. DAVID, J. COOKE & M. DUNCAN, Molecular identification and detection of *Phytophthora* species on some important Mediterranean plants including sweet chestnut. *Forest snow and landscape Research,* 76 (3) (2001) 351 - 356

[25] - T. W. DARMONO, I. JAMIL & D. A. SANTOSA, Development of molecular marker for the detection of *Phytophthora palmivorain* cacao. *Menara Perkebunan,* 74 (2) (2006) 86 - 95

[26] - A. A. APPIAH, J. FLOOD, S. A. ARCHER & P. D. BRIDGE, Molecular analysis of the major *Phytophthora* species on cocoa. *Plant Pathology*, 53 (2004) 209 - 219

[27] - K. S. BURGESS & B. C. HUSBAND, Habitat differentiation and the ecological costs of hybridization: the effects of introduced mulberry (*Morus alba*) on a native congener (*M.rubra*). *Journal of Ecology*, 94 (2006) 1061 - 1069

[28] - K. COULIBALY, I. B. KEBE, N. K. KOFFI, J. MPIKA & D. KONÉ, Caractérisation des isolats de *Phytophthora* spp du verger cacaoyers de Côte d'Ivoire. *Journal of Applied Biosciences*, 70 (2013) 5567 - 5579

[29] - A. UMAYAH & A. PURWANTARA, Identification of isolates of *Phytophthora* from cocoa. *Menara Perkebunan,* 74 (2) (2006) 75 - 85

[30] - J. J. SMITH & J. FLOOD, Epidemiology and population structure of *Phytophthora* species causing disease of coconut in Indonesia. *CABI Bioscience.* UK Centre, Egham, Surrey, Royaume-Uni, (2001) 83 p.

[31] - S. P. ALINA, I. BRIAN, A. S. TOMAS, W. SARAH & G. OSMAN, Effect of cacao black pod rot screening method on disease reaction determination. *Chemistry Proceedings*, (2022) 71 p.

[32] - S. GRUBER, C. P. KUBICEK & V. SEIDL-SEIBOTH, Differential regulation of orthologous chitinase genes in mycoparasitic *Trichoderma* species. *Applied and environmental microbiology*, 77 (21) (2011) 7217 - 7226

[33] - S. FAJON, Relations génome, transcriptome et épigénome chez le champignon filamenteux *Trichoderma reesei. Thèse de doctorat. Université Paris-Saclay,* Orsay, France, (2023) 283 p.