

Molecular Identification of *Phytophthora palmivora* in the Cocoa Tree Orchard of Côte d'Ivoire and Assessment of the Quantitative Component of Pathogenicity*

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*Identification moléculaire de *Phytophthora palmivora* du verger cacaoyer de Côte d'Ivoire et évaluation de la composante quantitative du pouvoir pathogène

Abstract: Black pod rot is one of the most widespread diseases in the species *Theobroma cacao* L. In Côte d'Ivoire, it causes significant yield losses, estimated at about 10 to 60% depending on the ecological zones. This study was conducted in order to identify *Phytophthora* spp isolates in the cocoa orchard by the analysis of their molecular features and assess their pathogenicity on cocoa tree leaf disks having different sensitivity. Thirty-six isolates of *Phytophthora* spp., from 06 producing regions (Divo, Soubré, Daloa, Duékoué, Abengourou and Aboisso) were taken from immature pods naturally affected by black pod rot. Methods of molecular analysis based on PCR-RFLP techniques, sequencing the ITS1 region of ribosomal DNA and artificial inoculation tests on cocoa tree leaf disks were used to identify *Phytophthora* isolates and assess their aggressiveness. The results obtained enabled to identify *Phytophthora palmivora* and showed a difference of aggressiveness between isolates of this species with average grades of foliar sensitivity ranging from 0.90 to 3.93.

Keywords: Aggressiveness, Black Pod Rot, ITS-RFLP, Restriction Enzyme, *Theobroma cacao*

Résumé

La pourriture brune des cabosses est l'une des maladies les plus répandues chez l'espèce *Theobroma cacao* L. En Côte d'Ivoire, elle occasionne des pertes de production importantes, estimées à près de 10 à 60 % selon les zones écologiques. Cette étude a été réalisée en vue d'identifier les isolats de *Phytophthora* spp., du verger cacaoyers par l'analyse de leurs caractères moléculaires et d'évaluer leur pouvoir pathogène sur disques de feuilles de cacaoyers de sensibilité différente. Trente-six isolats de *Phytophthora* spp., provenant de 06 régions de production (Divo, Soubré, Daloa, Duékoué, Abengourou et Aboisso) ont été prélevées sur des cabosses immatures naturellement atteintes de pourriture brune. Les méthodes d'analyses moléculaires basées sur les techniques PCR-RFLP, de séquençage de la région ITS1 de

l'ADN ribosomal et les tests d'inoculation artificielle sur disques de feuilles de cacaoyers ont servi à identifier les isolats de *Phytophthora* et à évaluer leur agressivité. Les résultats obtenus ont permis d'identifier *Phytophthora palmivora* et ont montré une différence d'agressivité entre les isolats de cette espèce avec des notes moyennes de sensibilité foliaire variant de 0,90 à 3,93.

Introduction

Black pod rot, caused by different species of *Phytophthora* is one of the most economically important diseases of cocoa tree (*Theobroma cacao* L.). Production losses worldwide are estimated at nearly 30% (20). Côte d'Ivoire, the world's largest cocoa producer with more than 44% of the supply, is not exempt from such disease (26). Two species, *Phytophthora palmivora* and *P. megakarya*, are the

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most significant in Africa. The first one, *P. palmivora* is the most ubiquitous. It is rife in many cocoa producing countries, causing losses of about 20 to 30% (11). The second one, *P. megakarya* endemic in Central and West Africa, is the most aggressive species. This pathogen may, in some countries, cause the loss of the entire cocoa yield (9). Indeed, in the areas where it is present attack rates by 50% are commonly observed every year; with losses that may reach 80% when the environmental conditions are much favorable for disease development (16). To fight effectively against this disease, several means were used. Generally, the control strategy adopted against black pod integrates agronomic measures sometimes used alone or in combination with chemical and genetic control. Many studies on chemical control showed that a decrease in the workload is possible with the implementation of a response protocol of four fungicide applications per year (6), (15), but such technique is restricting and generally unprofitable; moreover, such method does not fit into a sustainable process of cocoa management because despite fungicide application every rainy season to control the disease, epidemics regularly break out in each growing season. The genetic control which consists in seeking resistant or tolerant varieties to the disease, is the only one that enables to fight efficiently against its long-term progress. But the variability in the behavior of cultivars deemed resistant from one cocoa growing area to another (3) is an indication of the insufficient knowledge of pathogens as well as host-parasite interactions. The identification of *Phytophthora* spp. species was based historically on morphological, cytological and biochemical features (2), (5). However, although such methods are easily achievable, the assessment of morphological features is based on average values. Many overlap, due to differences in measurement of those morphological features may lead to misidentifications or inaccurate identifications. Moreover, the majority of morphological features vary depending on the environments and culture media used. Advances in molecular biology have facilitated the development of investigation techniques of fungal genomes. The information generated by molecular tools such as RFLP profiles, specific or random amplification techniques, DNA sequencing have solved several taxonomic problems. The analysis of the gene sequences of nuclear rDNA and specifically ITS regions, in order to assess intraspecific and interspecific variability of several fungal genera is a good example. In light of the issues raised by black pod caused by *Phytophthora*, the fight against this disease represents a major challenge for the Ivorian cocoa cultivation. This study aims at identifying *Phytophthora* spp. isolates in the cocoa orchard from the analysis of their molecular features and to assess

their pathogenicity on cocoa tree leaf disks having variable sensitivity.

Material and methods

Plant Material

Three cocoa tree clones having variable sensitivity with respect to black pod were chosen. They included a sensitive clone (NA32), a moderately resistant clone (PA150), and a resistant clone (SCA6), (23). These clones stemmed from block HO3 of the experimental plot of the CNRA research station in Divo. Healthy leaves were taken from semi-hardened branches located in the canopy of each tree in partial shade, from 6 trees per clone that is, 12 leaves per tree. A total of 216 leaves were collected that is, 72 leaves per clone. Using a sterile punch leaf discs of 15mm in diameter were taken from each leaf.

Methods

Isolation and culture of *Phytophthora* spp isolates.

Thirty-six (36) *Phytophthora* spp. isolates were used for study of aggressiveness. These isolates were obtained following a systematic collection organized as a phytosanitary survey in the 6 main cocoa producing areas of Côte d'Ivoire (Divo, Soubré, Daloa, Duékoué, Abengourou and Aboisso). In each region, the collection was carried out in 10 plantations and 3 pods naturally affected by black pod were taken from each plot. The isolates were isolated on immature pods naturally affected by black pod which necrosis were under development. The pods were cleaned with 95% alcohol and flamed out for 30 seconds. After selecting the sampling area, the superficial tissues of pods were stripped using a sterile scalpel. Three cubic fragments of 7 mm square were collected from the subcortical tissue with a sterile punch at the necrosis growth front. Pod disks were then deposited in water agar culture medium at 1.5% in 90 mm-diameter Petri dishes. The incubation was performed in darkness in an oven at 26 °C for 7 days. After a week of incubation, the mycelial strands taken from the growth front were deposited onto pea agar medium contained in Petri dishes. The purification of isolates was done by successive subculture using pea agar medium.

Cloning of *Phytophthora* spp, isolates by mono zoospore isolations

Monosporic isolates were obtained from cultures masses by mono zoospore isolation following the Babacauh (3) and Ortiz-Garcia (19) techniques. Isolates were grown on pea medium in Roux flasks. Incubation was carried out in total darkness at 26 °C for 5 days and Roux flasks were then exposed to a photoperiod of 12 hours for 5 days to induce the formation of sporocysts. In order to induce the release of zoospores by mature sporocysts, the cultures were flooded each with 40 ml of sterile

distilled water and placed in a refrigerator (4 °C) for 15 minutes. They were then returned to the light of an incandescent lamp (60W) for 45 minutes at laboratory room temperature (26 °C). The zoospore suspension obtained in the Roux flasks was counted with a Malassez cell. The concentration of 50 to 60 zoospores ml⁻¹ was obtained by successive dilution of an aliquot portion in which zoospores were immobilized by two drops of 10% hydrochloric acid. One hundred microliters of the calibrated suspension were spread on water agar medium at 1.5% with a bent glass rod, previously flamed and cooled in sterile distilled water. Incubation was performed in darkness at 26 °C for 12 to 24 hours. After incubation, the germinated zoospores were collected individually under binocular magnifying glass with a sterile micro lancet needle then seeded in carrot agar culture medium at a rate of 4 mono zoospores per isolate. Similarly, the mother isolate was seeded under the same conditions. After 7 days of incubation in darkness at 26 °C, the son facies identical to parental facies were selected for further studies.

DNA extraction

The isolates of *Phytophthora* spp. were seeded on a clarified V8 medium (500 ml V8 juice, 7.5 g CaCO₂, 37.5 g agar-agar, 2500 ml H₂O) contained in 90 mm-diameter Petri dishes. This agar medium had previously been covered with a sterile cellophane disk. Plates were incubated in darkness in an oven at 26 °C. After 7 days, the mycelium of each isolate was harvested by scraping the cellophane sheet with a sterile spatula. The mycelium obtained (20 mg) was transferred into sterile 2 ml Eppendorf microtubes. DNA Extraction was performed using a New Glucanex solution (18 ml H₂O, 42 ml NaCl at 1M, 1.8 g New glucanex); adjusted to pH 6 with a solution of 5M NaOH. The incubation took place in laboratory room temperature for 2 hours. Precipitation was conducted in 600 µl of isopropanol followed by 7-minute centrifugation. The resulting precipitates were washed with 70% ethanol and the DNA pellet was taken up in 100 µl of extraction buffer and then incubated in a water bath at 60 °C for 5 to 10 minutes for complete dissolution. The DNA concentration was assessed by agarose gel at 0.8% (2 µl DNA 2 µl bromophenol blue at 1% and 9 µl sterile H₂O).

DNA amplification by PCR and digestion of the ITS region using restriction enzymes

The polymerase chain reaction (PCR) of the ITS region of DNA extracts was performed with the nucleotide sequences of primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as described by White *et al.* (27). The DNA amplification reaction was performed in a Thermo Scientific Savant DNA

120-type thermocycler. It enabled to amplify by 30 successive cycles 2 µl of DNA in a reaction volume of 10 µl composed of 5.05 µl of deionized water, 1 µl of PCR buffer, 0.8 µl of DNTP at 5 mM each, 0.3 µl of MgCl₂ at 25 mM, 0.4 µl at 20 pmol each of primers ITS1 and ITS4, 0.05 µl Taq polymerase at 0.5 unit and a drop of mineral oil (SIGMA) was deposited on top of each reaction mixture before the amplification cycle. The amplification cycle parameters used were an initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, an hybridization at 55 °C for 45 seconds and an elongation at 72 °C for 2 min. At the end, a final elongation at 72 °C for 6 minutes was carried out. The PCR products were then digested with five restriction enzymes (*AluI*, *PvuII*; *HaeIII*, *HinfI* and *MspI*). The digestion with restriction enzymes was performed in a total volume of 25 µl composed of 11 µl of sterile deionized water, 3 µl of 10X buffer supplied with the enzyme, 1 µl of enzyme and 10 µl of PCR product. The reaction medium in sterile Eppendorf microtubes was incubated in a water bath at 37 °C overnight. The digestion products were separated by electrophoresis on 1.4% agarose gel in 0.5X TBE buffer (0.045M Tris-borate and 0.001 M EDTA, PH 8.0) at 50 V for 90 min. The gels were stained for 15 min in Ethidium Bromide (0.5 mg ml⁻¹ BET) and de-stained for 15 min in distilled water and then photographed under UV light. The sizes of restriction fragments generated were measured directly from the same gels and compared to a standard (1 kb) Invitrogen-type scale used as a molecular weight marker. PCR products (30 µl volume) of the 36 *Phytophthora* spp., isolates were sent to Eurofins MWG Operon DNA Sequencing Department (France)

Phylogenetic analyses

The obtained sequences of the ITS region of *Phytophthora* spp. isolates were compared with those listed in the GenBank of the National Center for Biotechnology Information (NCBI, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) using the option "BLAST search". Similarity rates were determined between the sequences obtained in the course of this study and those present in GenBank database. Sequences were considered similar only when they had a percentage homology of more than 97%.

Pathogenicity of *Phytophthora* spp isolates.

Inoculum preparation of *Phytophthora* spp, isolates and leaf disks test

The zoospore suspensions used for artificial inoculation of cocoa tree leaf disks was prepared from *Phytophthora* cultures contained in Roux flasks with pea agar medium. 5 day old cultures were exposed to a 12-hour photoperiod for 6 days so as to

induce the formation of sporocysts. Cultures were flooded with 40 ml of sterile distilled water and placed in a refrigerator (4 °C) for 15 minutes. To cause thermal shock, they were exposed to light from an incandescent lamp (60W) for 45 minutes. Such thermal shock enables the release of zoospores by ripe sporocysts. The zoospore suspension thus obtained was counted with a Malassez cell at a concentration of 3×10^5 zoospores ml^{-1} . Leaf disks having 15 mm diameter were arranged in complete randomization in 4 trays (repetition) containing moistened foams of sterile distilled water. Thus, 10 leaf disks per clone and per tray were inoculated for each isolate, that is, 30 washers for the 3 cocoa tree clones (SCA6; PA150 and NA32). For each isolate, leaf disks inoculation was performed using a micropipette (Pipetman) by deposit on abaxial side of the leaf up of 10 μl of a suspension calibrated at 3×10^5 zoospores/ml. The trays were tightly sealed and incubated at 26 °C in darkness for 7 days. After a week of incubation, the reading of symptoms was made according to a foliar sensitivity scale next: 0 (no symptoms), 1 (to penetration points), 2 (points in network), 3 (spots in network), 4 (mottled spot) and 5 (real spot).

Experimental design and statistical analysis

The leaf test was carried out in laboratory trays. Each series of inoculation was performed in 04 trays and each tray was a repetition. In each tray, 18 *Phytophthora* spp. isolates were tested. Each *Phytophthora* spp. isolate was tested on 30 leaf disks from 03 cocoa tree clones (01 sensitive clone; 01 moderately resistant clone and 01 resistant clone) at a rate of 10 leaf disks per clone. In each tray, 10 leaf disks from the same clone were placed on a same row, that is, a total of 54 lines of 10 leaf disks/tray. A random screening was carried out to determine the position of each row of 10 leaf disks in each tray. All the isolates were not tested both in a single series. Two series of inoculations were performed at different times with the introduction in each series of 03 control isolates which would be used to adjust the results obtained during both series of inoculation. The Statistica 7.1 software was used for statistical analysis. The notes of symptoms relating to the aggressiveness of isolates, one week after inoculation were subjected to a two-factor analysis of variance: isolate-clones. The comparison of averages was made by the Newman-Keuls test at 5% threshold in the event of significant differences.

Results

Molecular identification of *Phytophthora* spp. and evaluation of the quantitative component of pathogenicity

The amplification of DNA extracts from the 36 *Phytophthora* spp isolates with the pair of universal

primers ITS1 and ITS4 generated a DNA fragment which approximate size was 900 bp (Figure 1, lane 1). This value was confirmed by the calculation of the sum of the different restriction fragments generated by restriction enzymes. The ITS regions of all isolates were digested by restriction enzymes (*AluI*, *MspI*, *HinfI* and *PvuII*), which indicates that the ITS regions of *Phytophthora* spp. isolates studied contain recognition sites for such enzymes. In the analysis of restriction patterns, only enzyme *HaeIII* generated a restriction fragment (900 bp) whose size was equivalent to that of *Phytophthora* spp amplification profile. The analysis of resulting restriction patterns was used to determine the number and size of fragments generated by each enzyme. Thus, after digestion, enzymes *MspI* and *PvuII* recognized a single restriction site by generating two restriction fragments which size were respectively estimated at 600; 300 bp and 700; 200 bp (Figure 1). The digestion of ITS regions with enzyme *AluI* produced three fragments, of which the amount of digested fragments was less than the size of the PCR amplification profile. The nucleotide sequence analysis with the taxonomic browser of the server of the bank "GenBank" enabled to ascertain the affiliation of the isolates of *Phytophthora* studied. The most similar sequences to such isolates were identified as *Phytophthora palmivora* (Table 1) with a high rate of similarity ($\geq 97\%$).

The leaf disks of 3 clones of cacao trees inoculated with a zoospore suspension of *Phytophthora palmivora* produced brown spots, typical symptoms of *Phytophthora* attack. The dispersion of aggressiveness notes of *Phytophthora palmivora* isolates around the average ranged from 0.90 to 2.88; from 1.08 to 2.91 and from 1.29 to 3.93 respectively in clones SCA6; PA150 and NA32 (Figures 2, 3 and 4).

Discussion

The molecular identification of *Phytophthora* spp. confirmed that all 36 isolates were *P. palmivora*. The restriction enzyme experiment showed that all isolates had a 900 bp DNA fragment No variation in length was observed in this region, which means that no significant event (deletion/insertion) has appeared to intervene in the amplified intergenic regions. The size of the DNA profile obtained would correspond to the one expected from *Phytophthora palmivora* ITS regions as reported by Umayá (25). In the restriction analysis, only the enzyme *HaeIII* generated a restriction fragment equivalent to the approximate size of the undigested fragment of the ITS regions. This result was corroborated by the works of Ristaino *et al.* (21); in which the analysis of the restriction pattern revealed by enzyme *HaeIII* enabled to identify *P. palmivora* among nine other

Phytophthora species. The restriction enzyme *AluI* generated fragments whose sum of restriction fragments was lower (520; 170; 150 bp) than the size of the PCR products fragments (900 bp). This could be attributed to the difficulty in viewing fragments of less than 50 bp or the migration of the fragments of the same size on the gel. The second possible reason could be due to the loss of small fragments during electrophoresis on the gel. Similar results were obtained by Appiah *et al.* (2). Similar work conducted by Lee and Taylor (12) has enabled the identification of species *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. megakarya* and *P. palmivora* by the use of RFLP on the PCR products of ITS regions. These results were confirmed by Cooke *et al.* (7) and Crawford *et al.* (8). The restriction patterns generated by the different enzymes showed no polymorphism. This reflects the strong conservation of the nucleotide structure of the ITS regions between populations of this species. Similar results were obtained by Martin and Tooley. (13) in which, the analysis of nuclear DNA by the PCR-RFLP technique showed strong interspecific variation between different species of *Phytophthora*.

The aggressiveness of *Phytophthora palmivora* isolates was tested in the laboratory by artificial inoculation of leaf disks. Foliar sensitivity grades showed a significant variation of aggressiveness level of the isolates on the 3 cocoa tree clones. This study showed a spectrum of isolates, ranging from less aggressive which essentially characterized isolates having an aggressiveness grade between 0 and 2 to a moderately aggressive level (grade between 2 and 3); then to a high level of aggressiveness whose average aggressiveness grade varied from 3 to 4. Such aggressiveness variations were noted among isolates collected in the same plot/locality and different plots/localities. Several authors have established identical rankings of aggressiveness levels between isolates of *Phytophthora* spp. on leaves and pods detached from cocoa trees (10), (17). The effect of the isolate factor and the interaction isolate x clone has been studied and the analysis of variance of the average aggressiveness grades recorded on the clones showed a significant difference ($P < 0.0001$) of the isolate factor but not the interaction isolate x clone ($P = 0.098$). This analysis showed that the aggressiveness level of the isolates is a characteristic of their own. Isolates would have therefore kept their level of aggressiveness vis-à-vis the three clones. This result confirms work of Zei (28) who observed a non-significant between the *Phytophthora palmivora* isolates tried and the cocoa tree clones tested. However, this result is contrary to the works of Nyassé *et al.* (17), in which an effect isolate x clone although less significant was observed between the *Phytophthora megakarya* isolates and the cocoa tree

clones tested. For these authors, such interaction would be linked to the low number of repetitions performed during his study. The different highly significant aggressiveness profiles have shown that *P. palmivora* isolates do not have the same aggressiveness level. Some studies have shown a similar variation in the aggressiveness of *P. palmivora* isolates from different geographical origins Surujdeo *et al.* (22). The development of symptoms depends on the host, the pathogen and the environment. In this study, the cocoa tree clones were grown under similar environmental conditions and the selection of leaves at the same stages of development have helped reduce the influence of leaf age and environmental factors. Moreover, the environment in which the trial was performed (constant temperature at 25 °C) reduced the influence of the environment on the aggressiveness of *P. palmivora* strains. These observations have highlighted the genetic nature of isolates aggressiveness. According to Blaha & Lotodé (4), colonization of host leaf area is characterized by the presence of symptoms, is the result of transmission at differential speed of germ tubes by zoospores through the stomata of leaves. This differential pressure of inoculum might influence the time for onset and development of symptoms Abdelhadi (1). Under the conditions of our trial, the leaf disks were inoculated with deposits of zoospore suspension on the lower surface. This arrangement facilitated the penetration of zoospore germ tubes through the opening of stomata during the infection process. Similar results were obtained by Torto-Alalibo *et al.* (24) on *Phytophthora pisi* and *Phytophthora Sojae*. These authors after having inoculated pea roots with a zoospore suspension followed by stereomicroscopic observations confirmed the importance of zoospores as infectious agents of root rot. In addition, the different levels of aggression observed, despite the success of the infection, justify the use of that criterion as a factor expressing the aggressiveness of isolates. The appearance of the symptoms characterizing the disease after deposit of the suspension of zoospores reflects the response of the host tissue to the penetration of the pathogen infectious strands. Indeed, plant pathogens, and especially *phytophthora* species after the installation phase, generally grow via hyphae by modulating the morphological, biochemical and physiological process by secretion of proteins and other molecules also known as effectors. This analysis is consistent with the different levels of attack observed in all countries where the disease is rife. These results are similar to those obtained by Montarry *et al.* (14), in the pathosystem *P. infestans* / potato.

Conclusion

The study of the quantitative component aggressiveness of pathogenicity showed a difference

of aggressiveness among *P. palmivora* isolates. The number of isolates studied in this trial is certainly not representative of the entire existing population in Côte d'Ivoire. However, this investigation is an evidence of the highly heterogeneous nature of the aggressiveness level of isolates in this population. This heterogeneity is noticed in isolates belonging either to the same plot of land or plots located in different areas. Our results have shown that it is possible to use this criterion to assess the aggressiveness of *P. palmivora* isolates but also to quantify the level of resistance as part of an early breeding program of cocoa tree to the disease.

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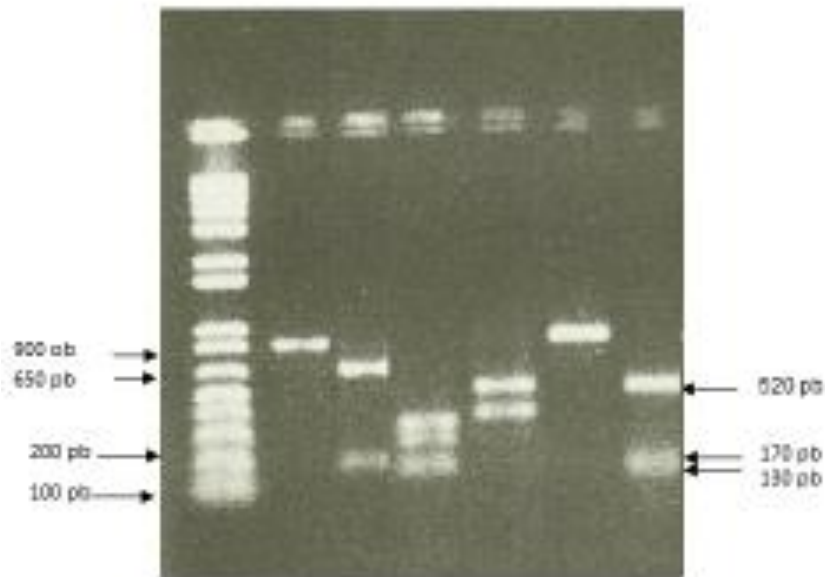


Figure 1: PCR-RFLP profiles of *Phytophthora* spp. isolates after digestion with restriction enzymes. M: Molecular marker 1 kb (Invitrogen); Undigested DNA (1); *Pvu*II (2), *Hin*fI (3), *Msp*I (4), *Hae*III (5); *Alu*I (6).

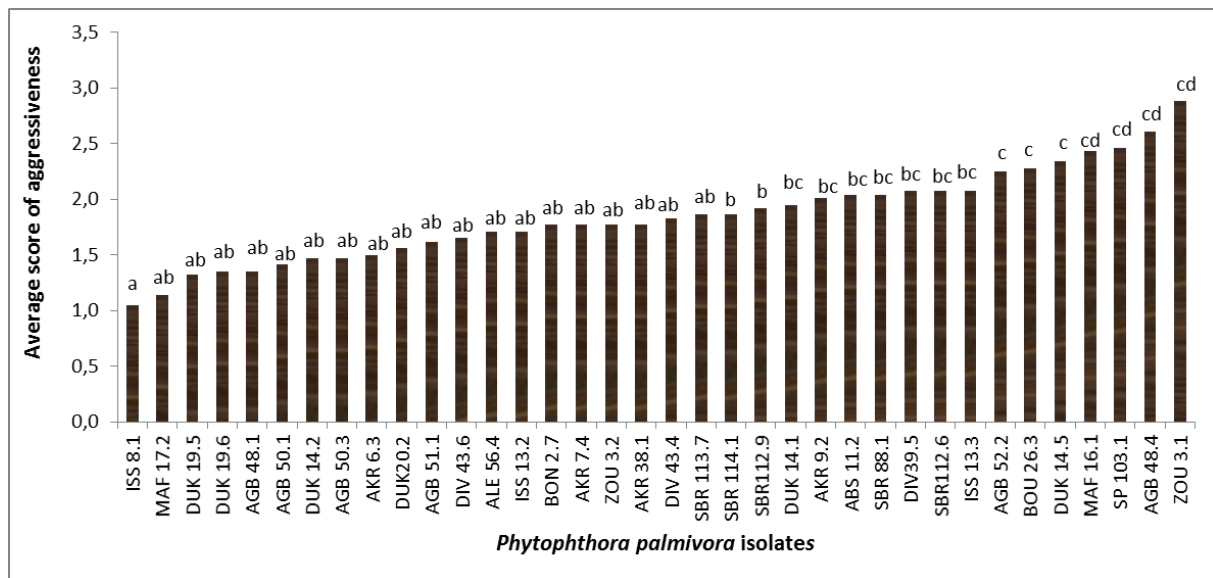


Figure 2: Foliar sensitivity of cocoa tree clone SCA 6 with regard to *Phytophthora palmivora* isolates. The bars followed by the same letter are not significantly different according to the Newman and Keuls test at 5% threshold.

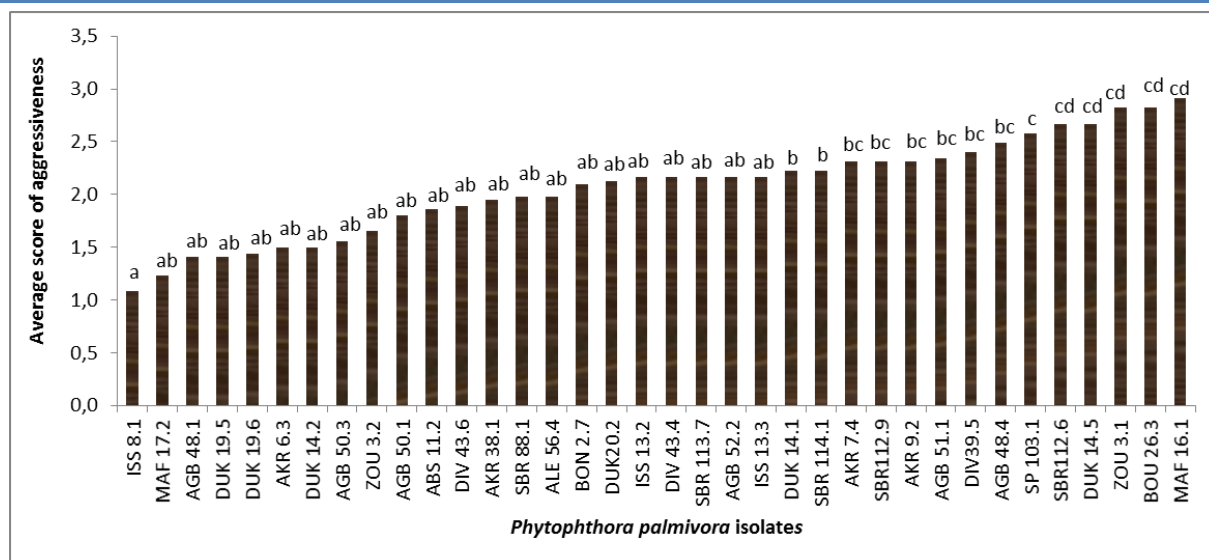


Figure 3: Foliar sensitivity of cocoa tree clone PA150 with regard to *Phytophthora palmivora* isolates. The bars followed by the same letter are not significantly different according to the Newman and Keuls test at 5% threshold.

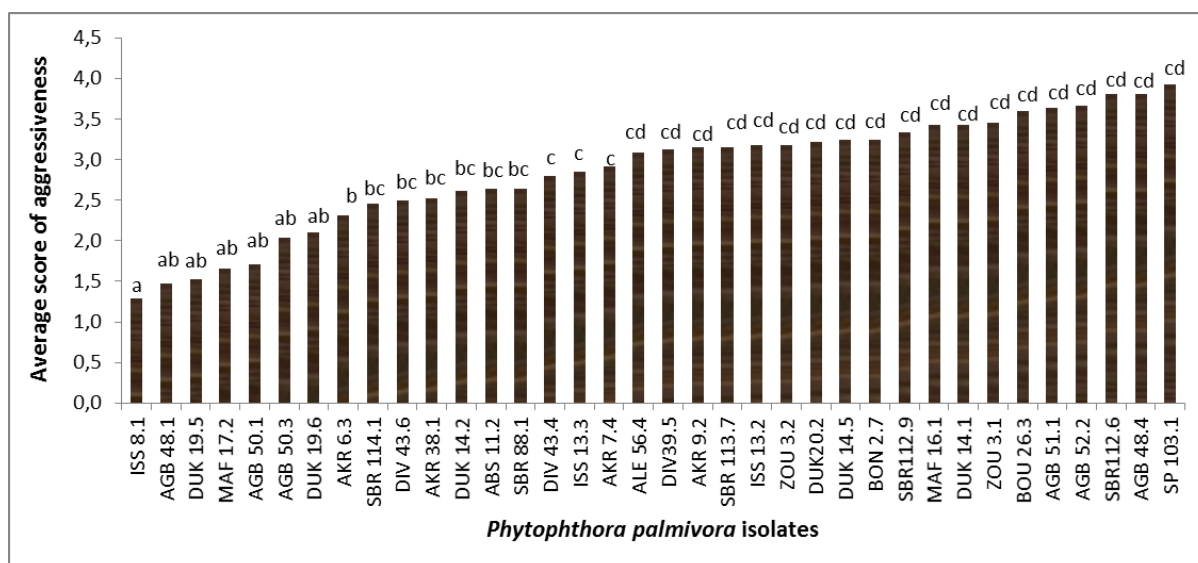


Figure 4: Foliar sensitivity of cocoa tree clone NA32 with regard to *Phytophthora palmivora* isolates. The bars followed by the same letter are not significantly different according to the Newman and Keuls test at 5% threshold.

Table 1: DNA sequences of *Phytophthora palmivora* in cocoa tree orchard

Species.	Codes	Geographical Origin	Acc.No. GenBank
<i>Phytophthora palmivora</i>	Seq1	San-pédro	KJ144771
<i>Phytophthora palmivora</i>	Seq2	Soubré	KJ144772
<i>Phytophthora palmivora</i>	Seq3	Duékoué	KJ144773
<i>Phytophthora palmivora</i>	Seq4	Duékoué	KJ144774
<i>Phytophthora palmivora</i>	Seq5	Divo	KJ144775
<i>Phytophthora palmivora</i>	Seq6	Maféré	KJ144776
<i>Phytophthora palmivora</i>	Seq7	Niablé	KJ144777
<i>Phytophthora palmivora</i>	Seq8	Divo	KJ144778
<i>Phytophthora palmivora</i>	Seq9	San-pédro	KJ144779
<i>Phytophthora palmivora</i>	Seq10	Duékoué	KJ144780
<i>Phytophthora palmivora</i>	Seq11	Divo	KJ144781
<i>Phytophthora palmivora</i>	Seq12	Soubré	KJ144782
<i>Phytophthora palmivora</i>	Seq13	Maféré	KJ144783
<i>Phytophthora palmivora</i>	Seq14	Niablé	KJ144784
<i>Phytophthora palmivora</i>	Seq15	Duékoué	KJ144785
<i>Phytophthora palmivora</i>	Seq16	Issia	KJ144786
<i>Phytophthora palmivora</i>	Seq17	Divo	KJ144787
<i>Phytophthora palmivora</i>	Seq18	Maféré	KJ144788
<i>Phytophthora palmivora</i>	Seq19	Soubré	KJ144789
<i>Phytophthora palmivora</i>	Seq20	Zoukougbeu	KJ144790
<i>Phytophthora palmivora</i>	Seq21	Saioua	KJ144791
<i>Phytophthora palmivora</i>	Seq22	Divo	KJ144792
<i>Phytophthora palmivora</i>	Seq23	Zoukougbeu	KJ144793
<i>Phytophthora palmivora</i>	Seq24	Bonoua	KJ144794
<i>Phytophthora palmivora</i>	Seq25	Duékoué	KJ144795
<i>Phytophthora palmivora</i>	Seq26	San-pédro	KJ144796
<i>Phytophthora palmivora</i>	Seq27	Saioua	KJ144797
<i>Phytophthora palmivora</i>	Seq28	Issia	KJ144798
<i>Phytophthora palmivora</i>	Seq29	Issia	KJ144799
<i>Phytophthora palmivora</i>	Seq30	Bonoua	KJ144800
<i>Phytophthora palmivora</i>	Seq31	Alépé	KJ144801
<i>Phytophthora palmivora</i>	Seq32	Saioua	KJ144802
<i>Phytophthora palmivora</i>	Seq33	Bonoua	KJ144803
<i>Phytophthora palmivora</i>	Seq34	Soubré	KJ144804
<i>Phytophthora palmivora</i>	Seq35	Alépé	KJ144805
<i>Phytophthora palmivora</i>	Seq36	Zoukougbeu	KJ144806