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Sweet Potato (*Ipomea batatas* L.): A New Alternative Host of Two Phytoplasmas Associated with Coconut Lethal Yellowing Disease in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Coconut Lethal Yellowing Disease has been threatening coconut plantations in Côte d'Ivoire for ten years and has destroyed more than 400 ha. The destruction of the coconut palm leads to phytoplasmas conservation on other plants grown in association or near of the disease outbreaks. Thus, the search for possible alternative host species was conducted.
Methodology: Surveys were carried out in infected coconut plantations, in order to describe symptoms associated with phytoplasma infections and to collect leaf samples of plant species other

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than coconut. Symptomatic and non-symptomatic leaves of sweet potato (*Ipomea batatas* L.) were collected for DNA extraction. The extracted DNA was subjected to molecular characterization.
Results: Mosaic and leaf reduction symptoms were observed on sweet potato leaves. From these samples, two phytoplasma strains were associated. Molecular analysis showed the presence of the endemic phytoplasma strain belonging to the 16SrXXII-B subgroup "*Candidatus* Phytoplasma palmicola". Sequencing and phylogenetic analysis also revealed the presence of a new phytoplasma strain, sharing 99% similarity with 16SrIV group strains "*Candidatus* Phytoplasma palmae".
Conclusion: The detection of these strains confirms sweet potato for the very first time, as an alternative host for coconut lethal yellowing phytoplasmas in Côte d'Ivoire.

Keywords: Sweet potato; coconut lethal yellowing disease; coconut; 16SrXXII-B phytoplasma subgroup; 16SrIV phytoplasma group.

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is an economically important crop for many producing countries, including Côte d'Ivoire. Côte d'Ivoire is the 24th country in the world and 5th in Africa [1], with coconut production of 124,810 tons/year [1]. However, in Côte d'Ivoire, coconut plantations have been threatened by lethal yellowing disease for nearly a decade, mainly in the Grand-Lahou locality [2,3,4]. Infected coconut trees show yellow or dried palms along the stipe, which eventually loses its crown [3]. The disease has destroyed more than 400 ha of producing coconut plantations, causing losses of more than 200 million CFA francs [5]. This has led to a considerable reduction in the economy of the producers. Furthermore, on an environmental aspect, destruction of coconut plantations in Côte d'Ivoire exposes the soil to sunlight and erosion [6].

Phytoplasma is a bacteria belonging to the class Mollicutes [7] and has been associated with lethal yellowing disease for the very first time, in the work of Parthasarathy et al [8]. The phytoplasma are known to live in both plants and insects [9]. Thus, in order to maintain favorable living conditions, phytoplasmas infect a large number of insect vectors and reservoir plants, both cultivated and non-cultivated, especially when the main host tends to disappear as a result of the infection.

In Côte d'Ivoire, the symptoms of lethal coconut yellowing disease have similarities to those of Cape St Paul Disease in Ghana and are associated with phytoplasmas belonging to several groups that are 16SrI, 16SrXXII-B and 16SrXXII-C [10,4]. Other studies have also identified phytoplasmas in several plant species, namely *Phyllantus muellerianus*, *Pennisetum pedicillatum* [9], palm (*Eleais guineensis* L.), roast palm (*Borassus aethiopium* L.) [4] and cassava (*Manihot esculenta* L.) [11]. This poses the problem of the transmission of phytoplasmas to other plant species present in coconut plantations. Indeed, insect vectors could feed on several plant species while transmitting the phytoplasma to them [12]. Recently, symptoms similar to those of coconut lethal yellowing disease have been observed in coconut-producing localities other than the original outbreak in Grand-Lahou. These symptoms were associated with the presence of the 16SrXXII-B subgroup phytoplasma and the presence of a new phytoplasma infecting coconut belonging to the 16SrIV group (MN545965) was even confirmed [13]. As a result, it is necessary to look for alternative host plants that could be involved in the spread of this disease. In Côte d'Ivoire, coconuts are often grown in association with sweet potato plant to meet the food needs of producing families. Sweet potato plants (*Ipomea batatas* L.) are grown on plots of land devastated by the disease for their tubers and leaves. It is also a source of income to compensate for the losses caused by the decline in production from coconut plantations infected by the lethal yellowing disease. In this context, sweet potato could be an alternative host for phytoplasmas associated with coconut lethal yellowing disease. In the present study, we aimed to identify and characterize phytoplasmas associated with coconut lethal yellowing disease in Côte d'Ivoire and new alternative hosts for these phytoplasmas.

2. MATERIAL AND METHODS

2.1 Collection Site

Sweet potato (*Ipomea batatas* L.) leaf samples were collected in cultivated fields near and inside infected coconut plantations, located in the

locality of Grand-Bassam in 2018. The locality of Grand-Bassam is located in Côte d'Ivoire, south-east of the coast, between latitude: 5°12′46″ North and longitude: 3°44′35″ West.

2.2 Observation and Description of Symptoms

In 2 sweet potato plantations in association with coconut plantations infected with coconut lethal yellowing disease, the general appearance of the sweet potato plants was observed. The symptoms present on the leaves of sweet potato plants were described by considering the different types and forms of symptoms observed [14].

2.3 Field Sampling

The plant material consisted of symptomatic and healthy-looking (asymptomatic) sweet potato leaves. The leaves were collected from plants at the 3 to 5 leaf stage. Sweet potato leaf samples were randomly collected within and adjacent to coconut plantations [9]. Leaf samples from symptomatic sweet potato plants exhibiting phytoplasma suspected symptoms were collected for further examination. Asymptomatic sweet potato plants from the same fields were also taken [15]. Then, all samples were placed in labelled plastic bags and were taken back in a cooler with ice to the laboratory at NANGUI ABROGOUA University (Côte d'Ivoire) and stored at -20 ℃ for molecular assays.

2.4 Nucleic Acid Extraction

Total DNA from 2 healthy-looking and 4 symptomatic sweet potato leaf samples was extracted according to the described procedure of Doyle and Doyle [16]. One hundred mg (100 mg) of sweet potato leaf midribs were ground into a fine powder using CTAB lysis buffer. The DNA samples was eluted in 25 µL TE. The concentrations and purity of extracted DNA were measured using a NanoDropOne (Thermo Scientific, USA) and stored at −20 °C until further use.

2.5 PCR Amplifications

The detection of phytoplasmas in sweet potato leaf samples was conducted using PCR assays. A direct PCR assay using the universal primer pair P1/P7 [17,18], was carried out in a 12.5 µL master mixture, including 2 µL of DNA template, 6.25 µL of GoTaq G2 Green buffer (Promega, USA), 1.25 µL of each primer and 1.75 µL of nuclease-free water (Promega, USA). Two µL of the products amplified by direct PCR were re-amplified in a nested PCR performed with the specific primer pair GH813f/AwkaSR [19], in a reaction solution with a total volume of 25 µL containing 12.5 µL of GoTaq G2 Green buffer (Promega, USA), 2.5 µL of each primer and 5.5 µL of nuclease-free water (Promega, USA). The reaction conditions of the primer pair P1/P7 were as follows: 94℃ for 3 min; 94℃ for 40 s, 56℃ for 40 s and 72℃ for 1 min 40 for 35 cycles in total; 72℃ for 10 min a for the final elongation. Nested PCR with the primer pair GH813f/AwkaSR was performed under the same conditions except at 53℃ for the hybridization temperature.

Another set of direct PCR assay was performed using the universal primer pair R16mF2/R16mR1 [20] for amplification of 2 µL of DNA in 12.5 µL master mixture containing 6.25 µL of GoTaq G2 Green buffer (Promega, USA), 1.25 µL of each primer and 1.75 µL of nuclease-free water (Promega, USA). A 1:30 diluted template generated by R16mF2/R16mR1 primers was used and subjected to nested PCR using the universal primer pair R16mF2n/R16mR2 [20,21]. The master mixture contained 5 µL of diluted product, 25 µL of Go Taq G2 Green buffer (Promega, USA), 5 µL of each primer and 10 µL of nuclease-free water (Promega, USA) for a final volume of 50 µL. The reaction conditions of the primer pairs R16mF2/R16mR1 and R16mF2n/R16mR2 were as follows: 94℃ for 2 min; 94℃ for 1, 50℃ for 2 min, 72℃ for 3 min for 35 cycles in total; 72℃ for 10 min for the final extension. A positive control (phytoplasma DNA from an infected coconut palm) and a negative control (nuclease-free water) were used in each PCR reaction.

The PCR products were visually detected by 1.5% agarose gel electrophoresis through ethidium bromide staining for 30 min at 80 volts. DNA bands were visualized using a trans-UV illuminator (EBOX VX5, Vilber Lourmat TM, France).

2.6 Sequencing and Phylogenetic Classification of the Phytoplasma

PCR products using the R16mF2n/R16mR2 primer pair were sequenced by the Eurofins laboratory (France). The consensus nucleotide sequence obtained in the study was assembled

using Genious Prime V 2019.1.3 software and saved on GenBank. The nucleotide sequence was compared and analyzed to identify the pathogen strain using the Basic Local Alignment Search Tool (BLAST) from NCBI (http://www.ncbi.nlm.nih.gov) [22]. Phylogenetic analysis was performed using MEGA X software [23]. The sequence obtained in this study was aligned along with the ones published on GenBank or not of phytoplasmas strains using the Clustal X V 2.0 algorithm [24]. Neighbour-joining method with 1000 bootstrap replications was then carried out to evaluate the stability of the phylogenetic tree. The 16S rRNA sequence of *Acholeplasma laidlawii* (M23932.1) was used as an outgroup for the phylogenetic tree construction.

3. RESULTS

3.1 Diversity of Symptoms Observed on Sweet Potato Leaves

Various types and forms of symptoms were observed on sweet potato leaves collected from plants located inside and around of coconut plantations (Fig. 1). Firstly, there were anatomical changes in terms of a reduction in the size of the apical leaves (A). Secondly, symptoms of color change, with infected leaves showing alternating light green and yellow colors, intermingled over the entire surface, characteristic of the mosaic (B), unlike the green leaves on non-infected control plants (C).

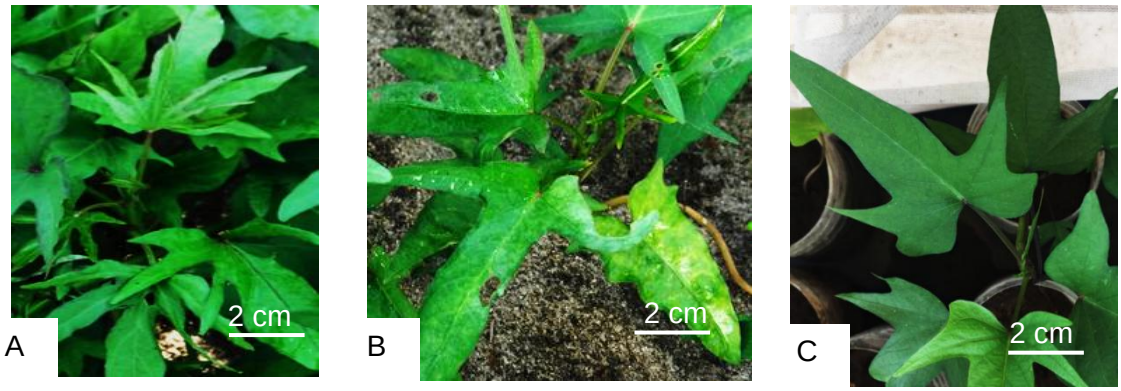

Fig. 1. Sweet potato (*Ipomea batatas* L.) leaves with or without symptoms
A: Leaves with leaf reduction symptoms; B: Leaves with mosaic symptoms; C: Leaves without symptoms.

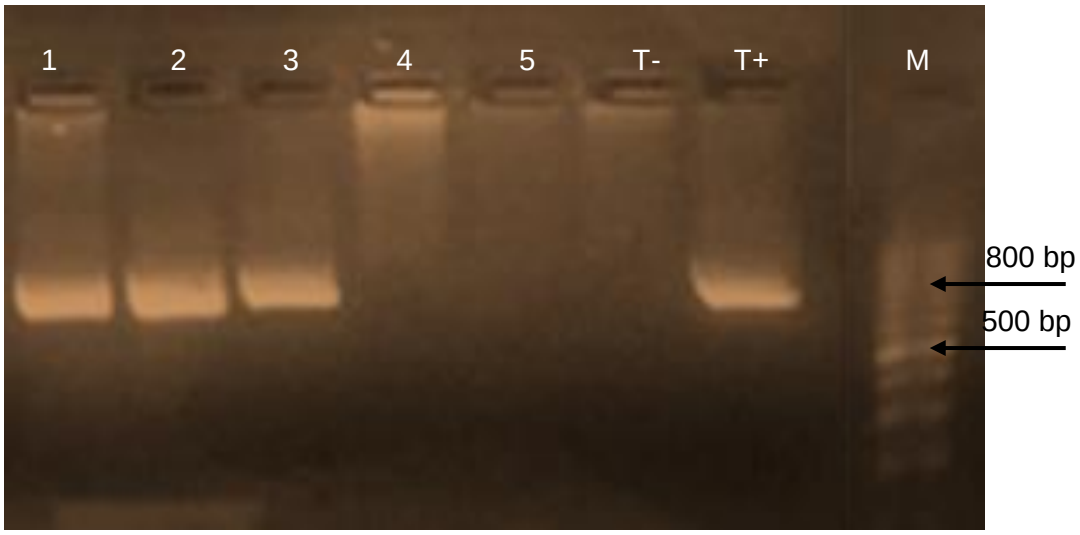

Fig. 2. Agarose gel electrophoretic profile of Nested PCR products of phytoplasma DNA in sweet potato (*Ipomea batatas* L.) leaf samples with the specific primer GH813F/AwkaSR
M = molecular weight marker (100 bp); 1-2: positive sweet potato leaf samples showing mosaic symptom; 3: sweet potato leaf sample showing leaf reduction symptom; 4-5: asymptomatic sweet potato leaf samples; T+: positive control (sample of trunk boring collected from an infected coconut tree); T-: negative control (sterile water)

3.2 Amplification of Phytoplasmas DNA Associated with Coconut Lethal Yellowing Disease in Sweet Potato Leaves

Amplicons in the expected size bands of approximately 800 bp were obtained with the GH813f/Awka SR primer (Fig. 2) from 2 samples of sweet potato leaves showing mosaic symptoms and in sweet potato leaves showing leaf reduction symptoms. Amplicons in the expected size bands of approximately 1250 bp were obtained with the R16mF2n/ R16mR2 primer (Fig. 3) from one sweet potato leaf sample showing symptoms of leaf reduction. No amplicons were obtained from the sample of asymptomatic leaves or sterile water.

3.3 Identification of Phytoplasmas Associated with Coconut Lethal Yellowing Disease in Sweet Potato Leaves

The 800 bp amplicons obtained from the 2 symptomatic sweet potato leaf samples are specific to the 16SrXXII-B phytoplasma sub-group belonging to "*Candidatus* Phytoplasma palmicola" already identified in coconut in Côte d'Ivoire and Ghana.

BLAST analysis of homologous gene sequences of 16Sr RNA gene sequence revealed that the sweet potato leaf reduction isolate obtained in this study and identified on in the Grand-Bassam locality (MN549454.1.), shares 99% homology with phytoplasmas in the 16SrIV group "*Candidatus* Phytoplasma palmae", associated with Coconut Lethal Yellowing disease in Mexico (KX982667.1). Phylogenetic analysis of partial 16Sr RNA sequences confirmed the sequence analysis. The phytoplasma isolate PATBAS identified in Grand-Bassam (MN549454.1 were clustered along with the phytoplasmas associated with Coconut Lethal Yellowing in Mexico (KX982667.1) and the previous one in Côte d'Ivoire (MN545965.1) and which belong to the 16Sr IV group "*Candidatus* Phytoplasma palmae" (Fig. 4). However, this sequence obtained on sweet potato (MN549454.1.) is in a different clade from that which includes the lethal yellowing disease associated strains obtained at Grand-Lahou in Côte d'Ivoire on coconut (MN540266), oil palm (KY767914.1), raffia (KY711302.1) and roast palm (KY711392.1).

The molecular results indicated that two distinct phytoplasma isolates, related to 16SrXXII-B and 16SrIV groups, were associated with observed symptoms on sweet potato.

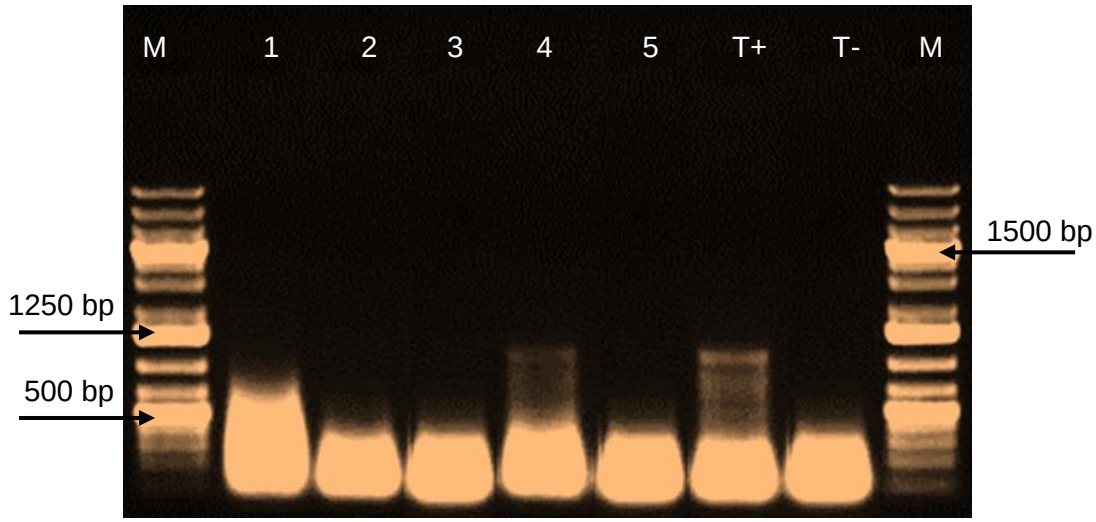

Fig. 3. Agarose gel electrophoretic profile of Nested PCR products of phytoplasma DNA in sweet potato (*Ipomea batatas* L.) leaf samples with the universal primer R16mF2n/ R16mR2

M = molecular weight marker (1 kb); 1-2: asymptomatic sweet potato leaf samples; 3 and 5: sweet potato leaf samples showing mosaic symptom; 4: positive sweet potato leaf sample showing leaf reduction symptom; T+: positive control (sample of trunk boring collected from an infected coconut tree); T-: negative control (sterile water)

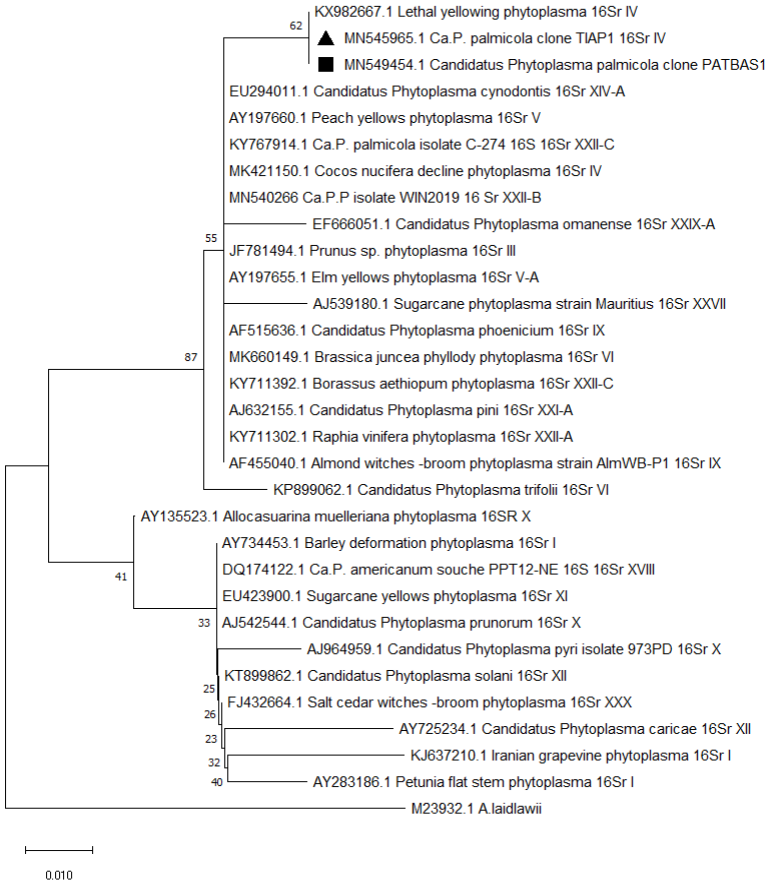

Fig. 4. Phylogenetic tree based on the R16mF2n/ R16mR2 sequences of the phytoplasma associated with leaf reduction in sweet potato (*Ipomea batatas* L.) and the 16S rRNA gene of phytoplasma reference sequences constructed using the neighbour-joining method with MEGA X

The species Acholeplasma laidlawi was chosen to root the tree. Bootstrap values from 1000 replicates are indicated on the branches. GenBank accession numbers for each sequence are given before the phytoplasma name. ■ *The strain obtained in this study* ▲*The strain identified in coconut*

4. DISCUSSION

Observation of sweet potato (*Ipomea batatas* L.) plants growing within and adjacent to coconut plantations revealed the presence of different types and forms of symptoms on the leaves. These symptoms included reduction leaf size called "little leaf" and leaf mosaic. The expression of these symptoms by the sweet potato could be explained by the action of phytoplasmas. However, these symptoms have also been associated with the presence of viruses [25] and therefore are not specific. However, the presence of viruses was not screened since the main focus was the identification of the phytoplasmas that were already reported associated to the disease in this area. Indeed, the "little leaf" symptom observed on sweet potato leaves was already associated with the presence of a different phytoplasma strain belonging to the *Candidatus* Phytoplasma aurantifolia, 16SrII group in Australia [26]. Identification of different groups of phytoplasmas associated with the same symptom or disease in different areas has already been reported around the world. For example, the Napier grass stunting disease of *Cenchrus purpureus* (Schumach.) Morrone in Kenya, was found to be associated with a group 16SrXI phytoplasma [27], while in Ethiopia it was 16SrIII phytoplasma group that was associated with the same symptoms in Napier grass [28]. Thus, different groups of phytoplasmas can be associated with the same symptoms on the same host, as has been reported previously with coconut [13].

Diversity of symptoms observed on sweet potato leaves could be probably due to the action of different phytoplasma strains they could harbor. These symptoms observed on the leaves could

suggest a hormonal imbalance [29]. Indeed, when phytoplasma infects a plant, it causes several physiological and morphological disorders represented by organ discoloration and deformation [30].

During the current study, phytoplasmas were found in 4 symptomatic sweet potato leaf samples and not in the asymptomatic ones. This suggests that these phytoplasmas are associated with the various symptoms observed. The 800-bp amplicon obtained with the specific primer pair suggests the presence of the 16SrXXII-B subgroup "*Candidatus* Phytoplasma palmicola" phytoplasma associated with coconut lethal yellowing disease [2,9]. This phytoplasma strain has been discovered on infected coconut trees in other production localities including Grand-Bassam [13] and is identical to the phytoplasma associated with Cape St Paul disease (CSPWD) in Ghana [31]. It is the most widespread phytoplasma group to date in Côte d'Ivoire [13].

Sequence and phylogeny analyses have also identified another strain associated with leaf reduction in sweet potato (*Ipomea batatas* L.) and belonging to 16SrIV group "*Candidatus* Phytoplasma palmae". This strain appears to be very closely related to strain detected in infected coconut trees in Côte d'Ivoire [13]. The presence of this group is a discovery in both coconut and sweet potato in west Africa and more specifically in Côte d'Ivoire, as it was limited to Tanzania and countries in America and the Caribbean [32]. This suggests a wide distribution of similar strains in different countries and diverse crops. Thus, the presence of group 16SrIV phytoplasma could be linked to the existence of an insect vector different or to *Nedotepa curta* species already suspected to be the vector of the 16SrXXII-B subgroup phytoplasma strain. In both cases, these insects could apparently feed on both coconut and sweet potato. However, no insects were observed feeding on these 2 plants at the same time during the surveys.

The presence of these two strains belonging to the species "*Candidatus* Phytoplasma palmicola" and "*Candidatus* Phytoplasma palmae" in the species sweet potato (*Ipomea batatas* L.), thus indicates the adaptability of these strains to other cultivated species. Indeed, recent studies by Kra et al. [11] have shown that the 16SrXXII-B subgroup strain has been identified in other crops such as cassava (*Manihot esculenta* Crantz) in Côte d'Ivoire, showing that cassava is a reservoir host for this phytoplasma. Like cassava, sweet potato could constitute an alternative host and a potential reservoir for phytoplasmas associated with Coconut Lethal Yellowing disease in Côte d'Ivoire. However, here is currently no evidence to explain the epidemiological role of sweet potato in the spread of the phytoplasma associated with Coconut Lethal Yellowing disease, although this plant species has been present in coconut plantations infected by this disease. Moreover, diseases caused by phytoplasmas are strongly influenced by the number, abundance and diversity of alternative host plants and insect vectors [33]. Thus, sweet potato could be involved in the spread of these phytoplasmas and in the increase in the disease in Côte d'Ivoire.

The results obtained in this study show for the very first time that sweet potato (*Ipomea batatas* L.) can be infected by both phytoplasmas associated with Coconut Lethal Yellowing disease in Côte d'Ivoire, belonging to the 16SrXXII-B "*Candidatus* Phytoplasma palmicola" and 16SrIV "*Candidatus* Phytoplasma palmae" groups. Studies will be needed to identify the role played by sweet potato in the spread of Coconut Lethal Yellowing phytoplasmas in Côte d'Ivoire.

5. CONCLUSION

Mixed cultivation of sweet potato and infected coconut palms could contribute to an exchange of agents associated with or responsible for infection. The present study reveals for the first time that phytoplasmas of 16SrXXII-B and 16SrIV groups associated with Coconut Lethal Yellowing disease affect sweet potato in Côte d'Ivoire. Once infected, sweet potato plants show symptoms of leaf deformation and discoloration. However, further studies will be needed to identify the vector and other alternative hosts of these phytoplasmas for better management of Coconut Lethal Yellowing disease in Côte d'Ivoire.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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