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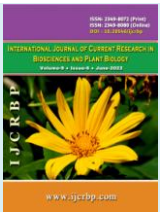

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Characterization of *Phytoplasmas* associated with cassava (*Manihot esculenta* Crantz) infection in Southern and Southeastern Côte d'Ivoire

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Abstract

In Côte d'Ivoire, cassava is an important source of food and income for the population. However, its development is confronted with the attack of numerous pathogens such as phytoplasmas as observed in several countries in recent years. The main aim of this study is to diagnose cassava leaf infection in Côte d'Ivoire in order to contribute to sustainable production. Thus, cassava leaf samples were collected from nine growing localities. Molecular analyses required the use of the universal primer pairs for the direct PCR and specific primers for Nested PCR in order to detect the presence of phytoplasmas in the DNA extracted from the samples. Mosaic, vein banding, chlorosis, shoestring and witches' broom symptoms were observed, sometimes alone and other times in complex symptoms. The prevalence of leaf infections was over 70%, with average severity scores ranging from 4.29 to 4.74 depending on the cassava variety. Of the samples tested, 42 (19.44%) were positive with the specific primer (AkwaSR/GH813f) and 32 (18.39%) with the universal primer (R16mF2n/R16mR2), thus demonstrating the presence of phytoplasmas in both symptomatic and non-symptomatic cassava leaves. The amplicons obtained with the specific primer pair allowed the detection of the phytoplasma "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B associated with the coconut lethal yellowing disease in Côte d'Ivoire in cassava grown in the different collection localities.

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Introduction

Cassava (*Manihot esculenta Crantz*) is grown in tropical and sub-tropical countries in Africa, the Americas and Asia (Ferguson et al., 2012), where it is part of the diet of several million people. It is most commonly grown for the consumption of its tuberized roots and its leaves. Cassava is a staple food and contributes 500 kcal/day/person when consumed. It is of great socio-economic importance, especially for small-scale farmers. Cassava ensures the subsistence and food security of millions of people in sub-Saharan Africa (Dixon et al., 2003).In Côte d'Ivoire, cassava is grown over a surface area of 866,452 ha with dominance in the southern, western and central zones of the country. In terms of yield, cassava is the second most important food crop after yam, with a yield around 5,238,244 tons in 2019 (FAO, 2021). Its cultivation and exploitation

generate various activities that help to fight poverty among the population.

In Côte d'Ivoire, these tuberous roots are consumed in various forms, including simple cooking in boiling water, flour transformed into finished products, heated pasta locally called "placali" and couscous locally called "attiéké" (Bakayoko et al., 2009).

Despite its socio-economic and cultural importance, cassava cultivation is subject to numerous biotic constraints such as nematodes, bacteria, fungi, and especially viruses which are responsible for a decline in quality and productivity. To this must be added the emergence of phytoplasma diseases also observed in cassava in several countries where cassava is produced.

Phytoplasma diseases can cause overgrowth, virescence, dwarfism, flower sterility and phyllody symptoms. They can reduce yield by 50-100% depending on the infection period or symptom onset (Bertaccini and Duduk, 2009). Thus, phytoplasma diseases are often confused with viral diseases as they induce virus-like symptoms (Diallo et al., 2017).

In Côte d'Ivoire, in the locality of Grand-Lahou, coconut cultivation has been abandoned in favor of cassava. Cassava cultivation has become an alternative source of income for women in Grand-Lahou. These cassava plantations have replaced coconut plantations destroyed by the lethal yellowing disease of the coconut tree caused by a phytoplasma. The work of Kra et al., (2017) showed that cassava was also infected with the same phytoplasma strain responsible for the coconut lethal yellowing disease observed in these coconut plantations in Grand-Lahou. This phytoplasma belongs to subgroup XXII-B. In addition, cassava in some countries is infected with several phytoplasma groups including the aster yellows (16SrI) in Cuba (Arocha et al., 2009) and the disease X (16SrII) groups in Brazil (Flores et al., 2013). Therefore, given the importance of cassava cultivation in food, economic and social needs for the population of Côte d'Ivoire, the identification of phytoplasma strains infecting cassava is essential to contribute to the development of a sustainable crop in southern and southeastern Côte d'Ivoire.

Collection site

Cassava leaf samples were collected from nine localities in southern and southeastern Côte d'Ivoire. These included Abengourou, Abidjan, Aboisso, Adzopé, Bonoua, Dabou, Grand-Bassam, Grand-Lahou and Jacqueville (Fig. 1).

Materials and methods

Plant material

The plant material consisted of 216 cassava leaf samples collected from nine localities, at a rate of 24 samples per locality. These samples were taken from different varieties grown in Côte d'Ivoire. These varieties are known as *bocou, bonoua, broni, yacé, samaké, zoglo, gloussi, glagban, mamawa* and *six mois*.

Symptomatology

The different types of symptoms observed in cassava fields were described according to the shape and coloring of cassava leaves on the plants in the field.

Assessment of the phytosanitary status of the surveyed fields

The phytosanitary status of the fields visited was assessed on the basis of the prevalence and severity of the symptoms observed. These were assessed according to the collection localities and the varieties grown.

The prevalence of leaf infection was determined by the ratio of the number of symptomatic plants to the total number of plants observed in each field. The prevalence was determined according to the formula below.

$$P\ (\%) = \frac{Pi}{Pt} \times 100$$

P: prevalence; Pi: number of symptomatic plants; Pt: total number of plants observed in each locality.

Symptom severity was assessed according to Mignouna et al., (2001) 1 to 5 severity scale.

1: no symptoms;

2: symptoms on 25% of the leaf area;

3: symptoms on 26-50% of leaf area;

4: symptoms on 51-75% of leaf area;

5: symptoms on more than 75% of the leaf area.

Collection of leaf samples

Three fields were randomly selected per locality. Cassava leaves were sampled randomly on the basis of discoloration and leaf deformation symptoms in each field. Young cassava leaves from 3- and 6-month-old plants were collected from a total of 10 plants per field, at a rate of 7 symptomatic and 3 asymptomatic plants.

In each of the nine localities, 24 cassava leaf samples were collected, that is, 216 leaf samples. The leaf samples collected were placed in sterile plastic bags and labelled. Information about each sample (locality and sequence number) was put on the sterile plastic bags for testing and analysis in the laboratory.

Identification of phytoplasma associated with cassava leaf infections

Extraction of total DNA from cassava leaf samples

A total of 216 leaf samples were analyzed. One hundred (100) mg of collared asymptomatic and symptomatic leaf veins were used for total DNA extraction using Cetyl Trimethyl Ammonium Bromide (CTAB), following the method of Doyle and Doyle, 1990**.**

PCR analyses

The extracted total DNA was used for the different PCR tests.

A direct PCR was performed with the universal primer pair P1 and P7 (Deng and Hiruki, 1991; Schneider et al., 1995) for the amplification of the16S rRNA gene. It was performed in a total reaction volume of 12.5 µl comprising: 6.25 µl of GoTaq G2 PCR buffer (Promega, USA), 1.75 µl of ultrapure water, 1.25 µl of each primer P1 (10 µM) and P7 (10 µM), and 2 µl of pure DNA. The amplification was performed in a thermocycler (BIORAD T 100TM), according to the following program: an initial denaturation cycle at 94°C for 3 min, followed by 35 cycles comprising: denaturation at 94°C for 40 s, then hybridization at 56°C for 40 s and elongation at 72°C for 1 min 40 s. Finally a final elongation cycle at 72°C for 10 min.

After this PCR, a Nested PCR was performed with the specific primer pair AwkaSR and GH813f (Tymon et al., 1998) to specifically target the rRNA gene and part of the rRNA gene of the 16/23 S intergenic region. The total volume of the reaction medium was 50 µl. The composition of the reaction medium was 5 µl (10 µM) of each of the primers, 10 µl of ultrapure water, 25 µL of GoTaq G2 buffer (Promega, USA) and 5 µl of the PCR product of the primer pair (P1 / P7). The following amplification program was used for PCR with the AwkaSR / GH813f primer: an initial denaturation cycle at 94 °C for 3 minutes, followed by 35 cycles involving denaturation at 94 °C for 40 seconds, followed by hybridization at 53 °C for 40 seconds and elongation at 72 °C for 1 minute 40 seconds. Finally, a final elongation cycle at 72 °C for 10 minutes.

Subsequently, all samples that tested negative with the specific primer pair (AwkaSR / GH813f) were subjected to another Nested PCR. This Nested PCR with the primer pair R16mF2n / R16mR2 was performed according to the method of Gundersen and Lee (1996) in a reaction volume of 50 µl. The composition of the reaction medium was 5 µl (10 µM) of each of the universal primers R16mF2n and R16mR2, 10 µl of ultrapure water, 25 µl of GoTaq G2 buffer (Promega, USA) and 5 µl of each PCR product of the primer pair P1 / P7 used as template. The Nested PCR program was as follows: an initial denaturation cycle of 2 min at 94 °C; followed by 35 amplification cycles involving denaturation at 94 °C for 1 min, hybridisation at 55 °C for 2 min and elongation at 72 °C for 3 min, and a final elongation cycle at 72 °C for 10 min.

Electrophoresis of PCR products

The individual amplified Nested PCR products were separated in a 1% agarose gel incorporated with ethidium bromide in 1×TAE buffer (400 Mm Tris-Acetate, 10 Mm EDTA, Promega, USA). Two microliters of each Nested PCR product was used for electrophoretic migration at 100 volts for one hour. The gel was then visualized under a trans-illuminator (EBOX VX5, Vilbert Lourmat France).

Statistical analyses

Statistica 7.1 software was used to analyze the collected data. The Kruskal-Wallis rank test was used to compare the means of symptom prevalence and symptom severity depending on localities and cassava varieties grown. The multiple comparison of the mean ranks allowed the determination of the different homogeneous groups in case of significant differences at 5% threshold.

Results and discussion

Types of symptoms observed on infected cassava leaves

Various symptoms were observed on cassava leaves in the nine locations surveyed. These symptoms were of two types: leaf discoloration and deformation. Apart from these two types, symptom complexes (discoloration + deformation) were also observed on cassava leaves in all surveyed localities (Fig. 2).

The leaf discoloration showed: a mosaic characterized by alternating yellow and green patches of the leaf blade (Fig. 2A1); the leaf blade discoloring from green to yellow leaving the main and secondary veins green as vein banding. (Fig. 2A2). To this must be added a total discoloration of the leaves characteristic of chlorosis (Fig. 2A3).

Regarding the characteristic symptoms of leaf deformation, very marked reductions of the leaf blade in the shape of shoelaces, often leaving only the main vein visible, characteristic of Shoestring were observed (Fig. 2B1). In some cases, shortening of the internodes at the level of the witches' broom stem and reduction of the length of the stem (dwarfing) and leaves were observed (Fig. 2B2 and Fig. 2B3).

As for the symptom complexes on the same leaves, three forms were observed:

leaves with rounded helix-shaped edges with a mosaic of yellow and green colors were observed (Fig. 2C1);

a deformation of the leaf blade and a reduction of the leaf blade base with discoloration of the leaves (mosaic-leaf reduction) (Fig. 2C2);

infected plants also showed leaves curled downwards on themselves (convex) with blisters on the upper surface (embossing) with mosaic (Fig. 2C3).

Geographical distribution of symptoms observed on cassava leaves

The geographical distribution of symptoms observed on cassava leaves during this study varied according to the localities surveyed. Symptoms such as mosaic, vein banding, witches' broom, shoestring and mosaic associated with helical distortion were observed in all surveyed localities. These included the localities of Abengourou, Abidjan, Aboisso, Adzopé, Bonoua, Dabou, Grand-Bassam, Grand-Lahou and Jacqueville (Table 1). However, chlorosis symptoms and the mosaic-shoestring symptom complex were observed in all surveyed localities except Adzopé. As for the mosaic-witches' broom symptom complex, it was observed in all surveyed localities except Aboisso, Dabou and Grand-Bassam. Finally, the mosaic-leaf reduction symptom complex was only observed in Abengourou and Abidjan.

Mosaic, vein banding, witches' broom, shoestring and mosaic associated with helical distortion were the most observed symptoms on the leaves of cassava varieties grown in the nine localities. The least observed symptom was the symptom complex, mosaic - leaf reduction, seen only in Abengourou and Abidjan localities. In terms of symptom types, for discoloration symptoms, mosaic and vein banding were the most distributed symptoms (found in all 9 localities) and the least distributed was the chlorosis symptom (8 localities out of 9). For the distortion symptoms, witches' broom and shoestring, they were observed in all 9 localities. Concerning the symptom complexes, the mosaic associated with helical distortion was more distributed (found in all localities) and the mosaic - leaf reduction symptom complex was less distributed (2 localities out of 9).

Prevalence of cassava leaf infection in the cultivation localities visited

The prevalence of leaf infection in the surveyed localities was above 70%. The highest prevalence was observed in the locality of Grand-Lahou and the lowest in the locality of Abengourou. Statistical analysis, however, showed no significant difference ($H = 11.70$ and $P = 0.16$) between the mean prevalence of leaf symptoms in the different localities (Table 2).

Mean severity of leaf symptoms according to surveyed localities

On cassava plants, the observed severity level was 4 on the 1 to 5 scale showing that the infected leaf area ranged from 51 to 75%. Statistical analysis showed a significant difference ($P < 0.001$) between the symptom severity scores of the different localities. This foliar symptom severity ranged from 4.33 to 4.79 across localities (Figure 3). The symptom severity was divided

into four homogeneity groups. The first group, with more severe symptoms (4.72 - 4.79), was made up of symptoms observed on plants in Abidjan, Aboisso, Bonoua and Dabou. The second group was made up of symptoms from plants in the localities of: Adzopé and Grand-Bassam with symptoms of severe scores (4.60 - 4.61). The localities of Abengourou and Grand-Lahou formed the third group of symptom severity with low severity scores. The fourth group was represented by the locality of Jacqueville with the lowest symptom severity scores (4.33) (Fig. 3).

Mean severity of leaf symptoms depending on cassava varieties

The mean symptom severity observed on leaves was above 4 on a 1 to 5 scale. Statistical analysis showed a significant difference between symptom severity scores at the variety level ($P < 0.000$). It ranged from 4.29 to 4.74. Three severity groups were obtained. The highest severities (4.70- 4.75) were observed on the varieties *bocou*, *bonoua*, *broni*, *glangban* and *yacé* forming the first group. The second group was observed on the varieties zoglo, gloussi, *mamawa* and *samaké* with mean severities (4.42-4.46). The lowest mean severity (4.29) was observed in the six-month-old variety and formed the third group (Fig. 4).

Phytoplasmas associated with cassava leaf infections

Nested PCR with the primer pair AkwaSR/GH813f allowed the amplification of DNA fragments at the size of 1000 bp (Fig. 5) in 42 samples out of a total of 216 (i.e. 19.44%) of asymptomatic and symptomatic cassava leaves, thus demonstrating the presence of a phytoplasma in these samples. The phytoplasma in question is "*Candidatus Phytoplasma palmicola*", a subgroup XXII-B phytoplasma already identified as the phytoplasma responsible for coconut lethal yellowing in Côte d'Ivoire.

Nested PCR with the universal primer pair (R16mF2n / R16mR2) generated a fragment of approximately 1200 bp (Fig. 6), in 32 samples out of a total of 174 (i.e. 18.39%) of asymptomatic and symptomatic cassava leaves, thus highlighting the presence of other phytoplasma strain(s) in these samples. However, the presence of phytoplasma detected with the universal primer pair (R16mF2n / R16mR2) could not be sequenced. There is therefore a possibility of a diversity of phytoplasmas found on cassava in Côte d'Ivoire. "*Candidatus Phytoplasma palmicola* was found on all forms of cassava leaf discoloration and deformation symptoms except for leaves with the witches' broom associated mosaic symptom complex in Jacqueville locality.

The phytoplasma "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B was associated with all distortion and discoloration symptoms observed on cassava leaves. In contrast, the other unidentified phytoplasma strain was only associated with mosaic, chlorosis, vein banding, witches' broom, shoestring, mosaic associated with helical distortion and mosaic associated with witches' broom (Table 3).

In the symptomless leaf samples, "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B was also detected in 6 samples out of a total of 216 (i.e. 2.77%) from the localities of Abengourou, Adzopé and Aboisso in the leaves of the varieties *bocou, bonoua, yacé, glagban, zoglo and "six mois"*. On the other hand, the other unidentified phytoplasma strain was detected in 4 samples out of a total of 174 samples (i.e. 2.30%) from the localities of Abengourou, Aboisso and Grand-Bassam in the leaves of the *bonoua, yacé, zoglo and "six mois"* varieties.

Phytoplasmas associated with the leaves of cassava varieties and collection localities

Molecular analysis revealed the presence of "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B in symptomatic and non-symptomatic leaves of the cassava varieties *bocou, bonoua, broni, yacé, samaké, zoglo, gloussi, mamawa and glagban*. However, "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B could not be detected in the six-month variety from the localities of Grand-Bassam and Jacqueville. The unidentified phytoplasma was detected in all varieties except the *samaké* and *gloussi* variety (Table 4).

Leaves of cassava plants from the different localities surveyed showed a diversity of symptoms characterised by leaf discoloration and distortion with associated symptom complexes. This diversity of symptoms observed on the leaves in the localities could be explained by the presence of one or several pathogens acting alone or in co-infection in infected cassava in Côte d'Ivoire. It is worth mentioning that different viruses can induce similar symptoms in cassava, making

it impossible to distinguish between them. These include discoloration and distortion symptoms characterized by mosaic, vein banding and chlorosis of the leaves for discoloration, and shoestring, waffle leaves and reduction of the leaf blade for distortion. Cassava varieties grown by producers in Abengourou, Abidjan, Aboisso, Adzopé, Bonoua, Dabou, Grand-Bassam, Grand-Lahou and Jacqueville are reportedly susceptible to several causal agents. The phytopathogenic agent could have the capacity to alter the process of chorophyll formation, resulting in leaf chlorosis; to cause a modification of organ morphogenesis, resulting in deformation of infected cassava leaves. Indeed, the work of Zinga et al., (2008) showed that plant pathogens such as viruses have the capacity to alter the color or shape of leaves by destroying the chlorophyll, reducing photosynthesis by the plant and consequently the elaboration of carbohydrates. However, the work of Fernandez in 2018 also showed that phytoplasmas had the ability to cause leaf discoloration and deformation in cassava. Also, the work of Kra et al., in 2017 in Côte d'Ivoire on cassava had shown that cassava infected with the phytoplasma exhibited the above symptoms. Cassava leaves collected in these different localities could be infected by one or more phytoplasmas. Indeed, once the phytoplasma is inside the plant, it localizes in the phloem of the plant and multiplies rapidly in the conducting vessels, thus preventing the normal circulation of the sap (Cousin and Boudon, 2002). This also affects the photosynthetic mechanism by reducing chlorophyll pigments.

Furthermore, the prevalence of leaf infection in all surveyed localities was high. The prevalence of symptoms of up to 70% shows that cassava leaf infection is an important disease in Côte d'Ivoire. However, statistical analysis did not show any significant difference at the locality level. This could be explained by the fact that these localities belong to the same agro-ecological zone and therefore have the same climatic conditions. This could also be related to the phytosanitary status of the cuttings grown in the localities.

In addition, the average severity of the symptoms was different from one locality to another and also between the different varieties grown. This variation would be due to the nature of the interaction between the host and the pathogen. This difference in symptom expression could be linked to the action of various factors such as the virulence of the strains found, the probable presence of at least two strains (their synergistic action), but above all to the cultivation practices carried out, in particular the use of infected plant material. The presence of several strains that act in co-infection in addition to their virulence. This variation in severity could also be explained by a high parasite pressure (presence of phytoplasma and insects) that differs between localities.

The observed difference in symptom expression between varieties could also be explained by the biotype of the insect vector (Camara et al., 2013). According to Owor et al., (2004), the high severity could be due to the virulence of the strain circulating in the epidemiological region or to the repeated cutting of contaminated plant material, which would thus increase the concentration of the pathogen in the plant.

In this study, the different molecular tests revealed the presence of "*Candidatus Phytoplasma palmicola*" in 19.44% of leaf samples tested with the specific primer pair AwkaSR/GH813f. This subgroup XXII-B phytoplasma is the one associated with the lethal yellowing disease identified on coconut trees in Grand-Lahou (Arocha et al., 2014). This same result was obtained by Kra et al., (2017) in their work on phytoplasma diseases of cassava. In addition 18.39% of the samples tested with the universal primer pair R16mF2n/R16mR2 had within them other phytoplasma strain(s) different from that associated with coconut lethal yellowing disease.

From these results, it can be deduced that cassava in Côte d'Ivoire hosts several phytoplasma strains regardless of the variety grown. In addition, the presence of phytoplasma is associated with a diversity of symptoms observed on cassava leaves. Alvarez et al., in 2013, showed in their work that different phytoplasmas were associated with cassava in Vietnam.

The phytoplasma "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B was also detected in some symptomless samples, showing that they are healthy carriers. The absence of symptoms on the plant following infection could be explained by the fact that the infection of the plant by the phytoplasma is recent or that the strain present is even less virulent. Indeed, when a plant is infected, there is a latent period during which the phytoplasma replicates in the phloem tissues and subsequently spreads throughout the plant.

Table 1. Distribution of symptoms observed on cassava leaves depending on the localities surveyed

Observed symptoms		**Surveyed localities**								
Types of symptoms	**Forms of symptoms**	**Aben**	**Abi**	**Aboi**	**Adz**	**Bo**	**Da**	**GB**	**GL**	**Ja**
Leaf discoloration	M	+	+	+	+	+	+	+	+	+
	C	+	+	+	-	+	+	+	+	+
	VB	+	+	+	+	+	+	+	+	+
Leaf distortion	BS	+	+	+	+	+	+	+	+	+
	SS	+	+	+	+	+	+	+	+	+
Symptom complexes	M – ADH	+	+	+	+	+	+	+	+	+
	M – BS	+	+	-	+	+	-	-	+	+
	M – G	+	+	+	-	+	-	-	+	-
	M - SS	+	+	-	+	+	+	+	+	+
	M - RF	+	+	-	-	-	-	-	-	-

VB: vein banding; SS: shoestring; G: blistering; C: chlorosis; M: mosaic; BS: witches' broom; ADH: helical distortion; RF: leaf reduction. Aben: Abengourou; Abi: Abidjan. Aboi: Aboisso; Adz: Adzope; Bo: Bonoua, Da: Dabou; GB: Grand-Bassam (Vitre), GL: Grand-Lahou; Ja: Jacqueville. +: presence; - : absence

Table 2. Prevalence of leaf infection depending on cassava growing localities in 2020 in southern and southeastern Côte d'Ivoire.

Localities	Prevalence (%)
Grand-Lahou	100±00a
Jacqueville	99.33±0.66a
Adzopé	90±7.63a
Abidjan	83.33±8.33a
Dabou	83.33±8.33a
Bonoua	83.33±8.33a
Aboisso	81.66±6.66a
Abengourou	73.33±13.01a
Grand-Bassam	70±10.40a
H	11.70
P	0.164

a: Values with the same letter are statistically identical at 5% threshold, according to the Kruskal Wallis test, H: Kruskal Wallis value, P: Probability

Table 3. Phytoplasma detected depending on symptoms observed on cassava leaves.

Observed symptoms		***Candidatus* Phytoplasma palmicola subgroup XXII-B (AwkaSR/GH813f primers)**	**Nested PCR (AwkaSR/GH813f primers) a**	**unidentified phytoplasma**	**Nested PCR (R16mF2n / R16mR2) b**
Leaf discoloration	*Mosaic*	+	6/26	+	6/20
	Chlorosis	+	3/16	+	3/13
	Vein banding	+	4/22	+	4/18
Leaf distortion	Witches' broom	+	3/17	+	4/14
	Shoestring	+	3/17	+	5/14
Symptom complexes	*Mosaic - Witches' broom*	-	0/12	+	3/12
	Mosaic - Distortion	+	6/25	+	3/19
	Mosaic - Blistering	+	3/10	-	0/7
	Mosaic - *Shoestring*	+	4/16	-	0/12
	Mosaic - Foliar reduction	+	4/10	-	0/6
		Total	36/171		28/135

+: presence, -: absence; a and b: number of positive samples/ number of samples tested per symptom

Table 4. Varieties of cassava and associated phytoplasmas depending on localities.

Varieties	Localities	*Candidatus phytoplasma palmicola subgroup XXII-B*	Unidentified phytoplasma
Bocou	Adzopé	+	+
	Grand-Lahou	+	-
	Abengourou	+	+
	Dabou	+	+
	Bonoua	+	+
	Aboisso	+	+
Bonoua	Bonoua	+	+
	Aboisso	+	+
	Grand-Lahou	+	-
	Grand-Bassam	+	+
	Abidjan	+	+
Yacé	Grand-Lahou	+	+
	Dabou	+	+
	Bonoua	+	+
	Aboisso	+	+
Broni	Abidjan	+	+
Bagban	Abidjan	+	+
Zoglo	Abengourou	+	+
Gloussi	Abengourou	+	-
Samaké	Dabou	+	-
	Jacqueville	+	-
Six mois	Adzopé	+	+
	Jacqueville	-	+
	Grand-Bassam	-	+
Mamawa	Adzopé	+	+

+: presence,-: absence

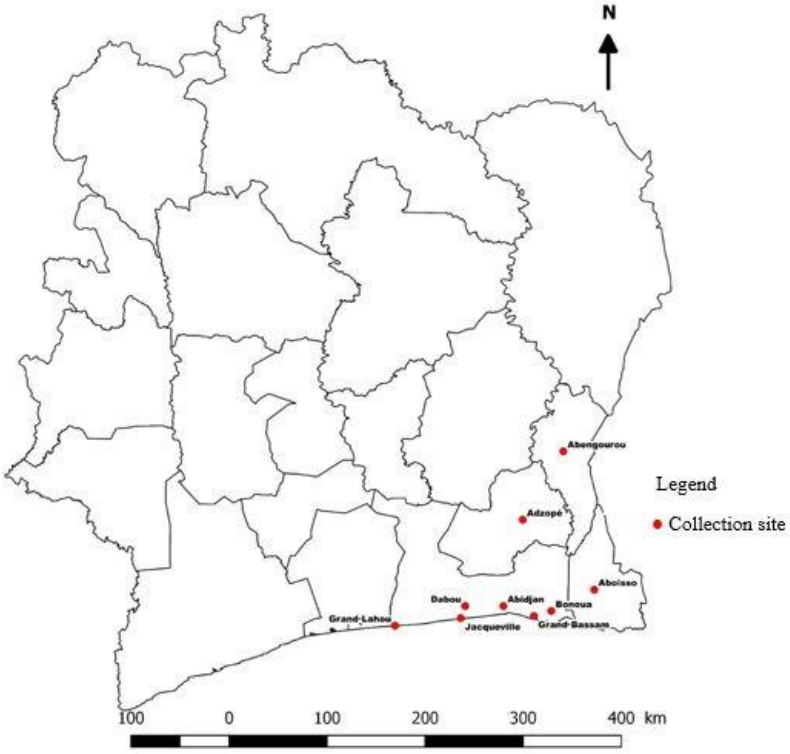

Fig. 1: Cassava leaf collection sites in southern and southeastern Côte d'Ivoire in 2020

Fig. 2: Different symptoms observed on infected cassava leaves. A: discoloration: (A 1: mosaic, A 2: vein banding, A 3: chlorosis); B: leaf deformation: B 1 Shoestring; B 2 and B 3: witches' broom; C: symptom complex: C 1: mosaic - deformation, C 2: mosaic - leaf reduction, C 3: mosaic - blistering

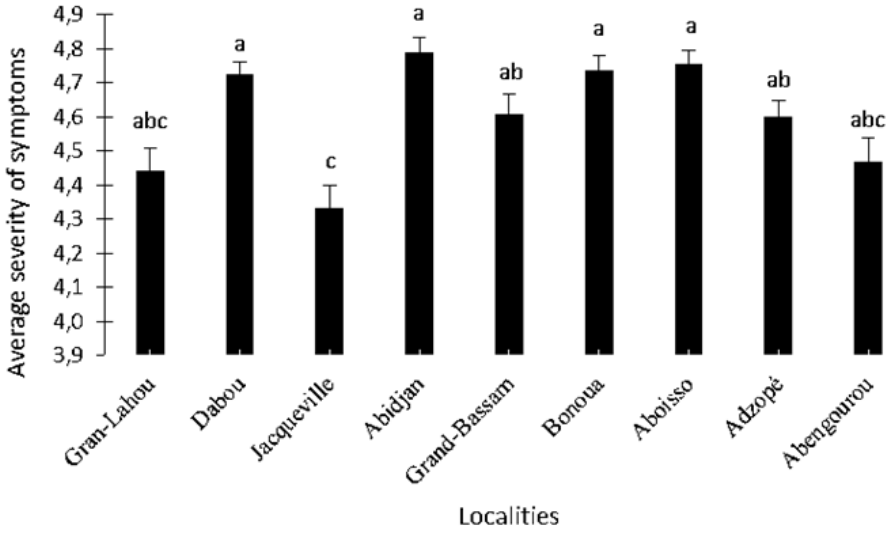

Fig. 3: Mean severity of leaf symptoms according to cassava growing localities in 2020 in southern and southeastern Côte d'Ivoire. Scores with the same letter are statistically identical at 5% threshold according to Kruskal Wallis

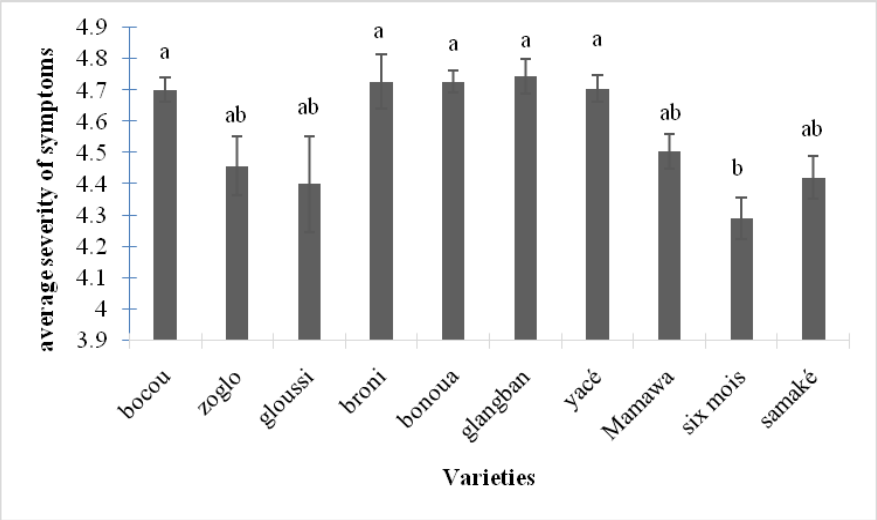

Fig. 4: Mean severity of leaf symptoms depending on cassava varieties grown in southern and southeastern Côte d'Ivoire. Severity scores with the same letter are statistically identical at 5% threshold according to Kruskal Wallis

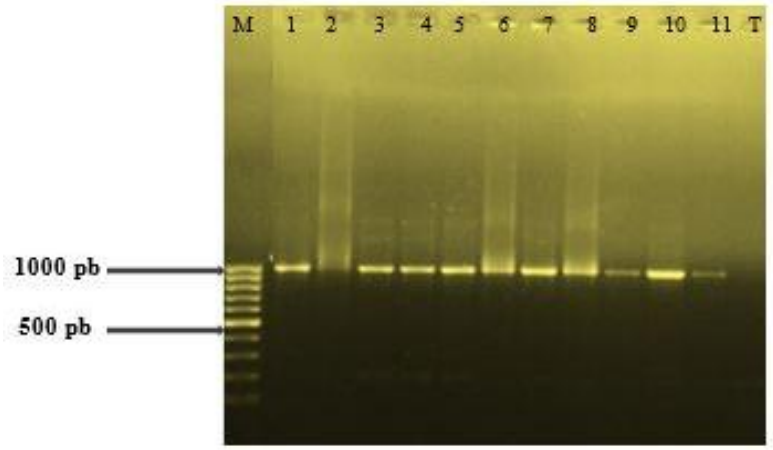

Fig. 5: Electrophoresis profile (1%) for Nested PCR products with the AkwaSR/GH813f primer pair. M: molecular weight marker= 100 bp, 1-11: cassava leaf samples, T: negative control (water)

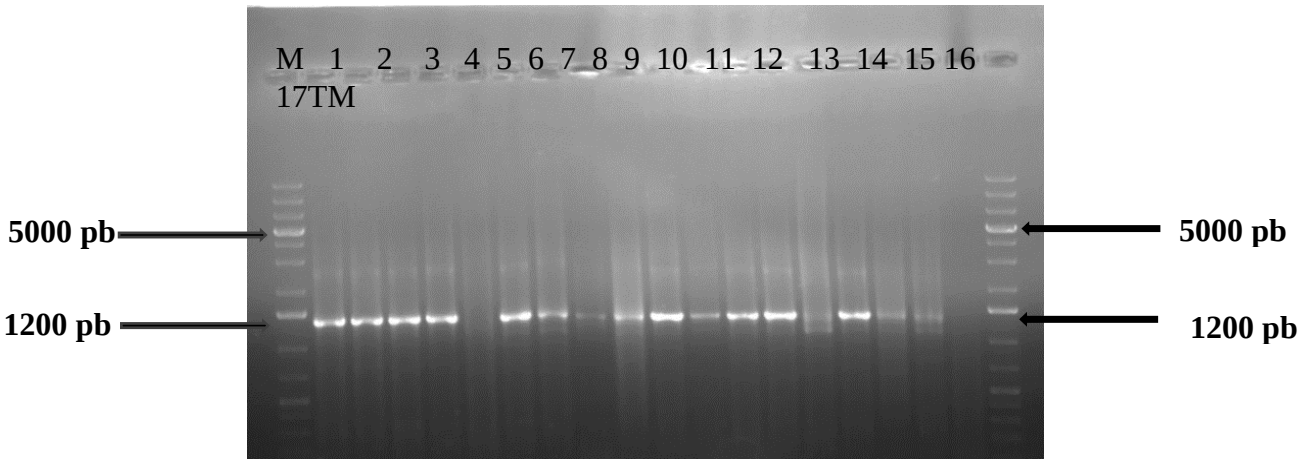

Fig. 6: Electrophoresis profile (1%) for Nested PCR products with primer pair R16mF2n/R16mR2; M: molecular weight marker = 1kb, 1-16: leaf samples, T: negative control (water)

Symptoms appear when the phytoplasma has had time to multiply and infect its host (Streten and Gibb, 2006). This could also be due to the fact that infection occurs late in the plant's development cycle, and the accumulation of phytoplasma may not be sufficient to cause visible symptoms. Some plant species are tolerant or resistant to phytoplasma infection. Once infected, these plants may show only mild symptoms or none at all. Indeed, Marcone (2010) has also shown in his work that the development of a symptom depends on the virulence of the phytoplasma strain present and the resistance of the host. "*Candidatus Phytoplasma palmicola* of subgroup XXII-B was detected in cassava leaf samples collected in all surveyed localities. This could be explained by the use of infected plant material during the establishment of the crops. Indeed, the plant material used and its sanitary condition play an important role in the spread and persistence of plant diseases. Also, the use of susceptible plant material increases the risk of contamination of this material and the persistence of the disease during cultivation. According to Souza et al., (2014), the use of cassava stems as the source of planting material for the next crop cycle makes it difficult to control the spread of pathogens, when these are infected. In addition, in Côte d'Ivoire, farmers obtain cuttings mainly from previous fields and also by purchasing cuttings from the National Center for Agronomic Research (CNRA). This is due to the fact that there is no mechanism for the rational distribution of healthy cuttings to farmers. All these factors would explain the presence of "*Candidatus Phytoplasma palmicola*" in the samples collected in all survey locations. According to Tian et al., (2000), the most common pathway for the spread of emerging phytoplasma diseases is the propagation or micro-propagation of infected material during cultivation.

In addition, "*Candidatus Phytoplasma palmicola*" has been identified in the leaves of the cultivated cassava varieties *yacé, bonoua, broni, samaké, glagban, bocou, zoglo, mamawa* and *gloussi*. Similar observations were made in Côte d'Ivoire by Diallo et al., (2017), their work showed that certain varieties *bocou*, *yacé* and *bonoua* would host this phytoplasma after molecular tests. Phytoplasmas can infect several cassava varieties. But also that different phytoplasmas can infect one cassava variety. This could be related to the presence and feeding habits of the insect vectors, which are mostly polyphagous (Weintraub and Wilson, 2010).

Cassava leaves from the nine collection sites showed several forms of discoloration and distortion such as; mosaic, vein banding and chlorosis, witches' broom, Shoestring, for distortion symptoms. The symptom complexes were: mosaic-wrecking, mosaic-leaf reduction, mosaic-helical distortion and mosaic-witches' broom. Molecular analysis confirmed the presence of phytoplasma strains including "*Candidatus Phytoplasma palmicola*" of subgroup XXII-B in leaf samples of cassava varieties grown in the different collection localities.

It emerged from this work that the cassava varieties grown in the localities surveyed in Côte d'Ivoire would host other phytoplasma strains in addition to "*Candidatus Phytoplasma palmicola*", the phytoplasma responsible for coconut lethal yellowing. In view of the unidentified phytoplasma strain(s), it would be important to further identify the phytoplasma associated with cassava foliar symptoms in Côte d'Ivoire and to carry out transmission tests in order to know their mode of transmission.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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Author contributions

The experiments were carried out and the data analysed by C.A.K., under the supervision of H.A.D. and A.I.Z.B. The manuscript was drafted by C.A.K., and edited and revised by all authors.

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