



Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)
<https://pjp.pakps.com>



RESEARCH ARTICLE

First Detection of *Candidatus phytoplasma asteris* in Papaya Orchards in Ivory Coast

Néhémie P. A. Lobognon*, Kouamé D. Kra, Marie-Noel Y. Toualy

Natural Science Department, Laboratory of Plant Protection, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Ivory Coast

Corresponding Author:

Néhémie Pierre Anicet Lobognon, Email: lobognonanicet@gmail.com

Article History:

Submitted: August 10, 2024; Revised: October 25, 2024; Accepted for Publication: November 07, 2024.

ABSTRACT

Papaya is an important food and economic crop in Ivory Coast. However, its cultivation is facing persistent virus problems. Recent studies carried out around the world have shown that phytoplasmas cause symptoms similar to viruses and are increasingly affecting various crops. This study was carried out with the aim of identifying the phytoplasma(s) likely to be associated with leaf deformation symptoms in papaya trees in Ivory Coast. Leaf deformation symptoms were observed on plants of the solo. Phytoplasmas were detected in leaf samples collected from December 2022 to February 2023 by nested polymerase chain reaction with primer pairs amplifying the 16Sr phytoplasma genes. The results showed a wide range of leaf symptoms. The prevalence of foliar symptoms varied from 1.85% to 60%. Sequencing of the amplicons and phylogenetic analysis revealed that *Candidatus phytoplasma asteris* of the 16SrI-B subgroup was the phytoplasma detected in 11 leaf samples out of 38 (28.94%) using Nested PCR carried out with the R16F2n/R16R2 primer. However, no traces of the phytoplasma responsible for the fatal coconut yellowing disease, which affects coconut and manioc in Ivory Coast, were found in these samples. The results obtained have improved the diagnosis of papaya diseases in Ivory Coast, as they have made it possible to associate an agent other than viruses and fungi, which have always been associated with papaya infections in all the studies carried out in Ivory Coast.

Keywords: Papaya, Phytoplasmas, 16SrI-B Subgroup, Ivory Coast, PCR.

INTRODUCTION

The papaya tree (*Carica papaya* L) is a fruit tree native to Latin America, particularly Mexico (Fuentes and Santamaría, 2013). Papaya is primarily cultivated for its fruit, which has a flesh rich in vitamins (A and C), sugars, and potassium. It is eaten as a dessert during meals but is also made into jam and puree after processing. Its seeds have vermifuge properties (Toure *et al.*, 2020). It is the third most-produced fruit in Ivory Coast and is exported to the European market.

According to FAOSTAT 2022, Ivory Coast is the second-largest African exporter of papaya to the European market, with export volumes declining from 658 tonnes

in 2020 to 358 tonnes in 2023. This drop in production is due to significant pressure from pathogens and pests affecting papaya plants (Touré *et al.*, 2020). Papaya diseases commonly encountered in papaya fields are generally caused by viruses and fungi (N'Da *et al.*, 2008). In fact, according to Séka *et al.* (2023), it is nearly impossible to cultivate papaya for local consumption and export due to the resurgence of viral papaya diseases since 2003, notably papaya ringspot disease and papaya mosaic disease. The appearance of virus-like diseases in papayas in Ivory Coast, with incidence rates reaching up to 100% in some fields, has led several farmers to abandon papaya

cultivation (Diallo *et al.*, 2008). In Ivory Coast, the Solo n8 and Sunrise Solo 7212 varieties are the most commonly grown and exported N'Da *et al.* (2008) several studies on viral diseases have been conducted on these varieties, and control methods have been proposed and implemented by farmers. Despite these efforts, the cultivated area is in decline (Iqbal *et al.*, 2022).

Several phytoplasma diseases have been reported in papaya, presenting symptoms nearly identical to those of viral diseases (Arocha *et al.*, 2009; Ahmad *et al.*, 2024; Valadez *et al.*, 2024). Phytoplasmas are wall-less, bacteria-like organisms that live exclusively within plant vascular tissues and rely on insect vectors for transmission. Their specific localization within plant tissues and their ability to induce symptoms similar to those of viruses make their detection challenging and require specialized diagnostic methods (Bertaccini and Duduk, 2009). Phytoplasma attacks in papaya orchards can lead to incidence levels of up to 75%, causing substantial yield losses (Ventura *et al.*, 2004; Tahir *et al.*, 2023). The above leads us to ask whether one or more phytoplasmas might be associated with the persistence of diseases in papaya trees in Ivory Coast. The aim of this work is therefore to find out which phyoplasma or phyoplasmas could be associated with the various forms of leaf deformation in papaya.

Specifically, it will involve: Make an inventory of the symptoms of leaf deformations in papaya, Detect the presence of phytoplasmas in leaves showing symptoms and Identify the phytoplasma(s) associated with papaya leaf deformation symptoms.

MATERIALS AND METHODS

Study Area: The study was conducted in four (4) localities that were once the main cultivation areas and are currently being abandoned due to the persistence of disease. These included three (3) cities in the southern part of the country: Adzopé, Agboville, and Tiassalé. Additionally, the city of Yamoussoukro in the center of the country was included.

Field Sampling: Sampling was carried out in December 2022, January an April 2023. In each of the four localities, three fields were chosen. Ten (10) papaya trees were randomly selected in each field by following a Z-shaped path after leaf observation. On each selected papaya tree, fresh young leaves were taken at the top were collected on the basis of whether or not symptoms were observed. Samples consisting of three (3) leaves per plant were collected. A total of 360 samples were collected. All the

collected samples were placed separately in khaki envelopes and labelled. Each sample was identified by a number including the collection site, the field and the sample number. The samples were transported to the laboratory of the phytosanitary Unit of the University of Nangui Abrogoua, where they were kept in a freezer for later analysis. The leaf samples were all collected from the solo variety, grown in all the fields surveyed. The plants collected were between 4 and 12 months old.

Description of symptoms: Descriptions of the symptoms observed in each field were based on the shape, size and appearance of the symptoms on the papaya leaves. The symptoms were described in the field after the leaf samples had been collected. This description was based on the different forms of leaf deformation observed on the symptomatic plants.

Evaluation of the phytosanitary status of the plots visited: The phytosanitary status of the fields visited was assessed on the basis of the prevalence and severity of the symptoms observed. This was done according to the locations visited.

Symptom prevalence by locality: The prevalence of the disease was determined by the ratio of the number of symptomatic plants to the total number of plants visited in each field. This prevalence was assessed on 30 randomly selected plants following a zigzag path within pre-defined subplots (150 m x 150 m) in each field. It was calculated using the Ogara and Bina (2010) formula below:

$$Pr = \frac{Nps}{Npt} \times 11111$$

Nps: Number of plants showing a symptom form; Npt:

Total number of sample plants;

Pr: Prevalence of a symptom.

Symptom severity: Severity was determined using a rating scale from 1 to 5 Mignouna *et al.* (2001) on 30 randomly selected plants following a zigzag path within pre-defined subplots (150 m x 150 m) in each field.

1= plants with symptomless leaves;

2=1-25% of leaves showing symptoms;

3=26-50% of leaves showing symptoms;

4=51-74% of leaves showing symptoms;

5= more than 75% of leaves showing symptoms

Detection of phytoplasmas: Extraction of total DNA from collected leaves: Total DNA was extracted for each sample analyzed from one hundred (100) mg of leaf veins collected using the method described by Doyle and Doyle (1990). One hundred (100) mg of leaf samples were ground with a CTAB solution, incubated at 65°C and then

purified with chloroform-isoamyl alcohol. The supernatant obtained was precipitated with isopropanol at -20°C, followed by centrifugation and washing with ethanol. The DNA pellet was dried, recovered in TE with RNAase and stored at -20°C for future analysis.

PCR: Total DNA obtained after extraction was used for several PCR tests. This technique was used to detect the presence of phytoplasma sequences in the extracted DNA. A first direct or classical PCR was carried out using the universal primer pair P1/P7 (Schneider *et al.*, 1995). The amplification reaction was carried out in a total reaction volume of 12.5 µL. The reaction medium consisted of 6.25 µL GoTaq PCR buffer 2× (GoTaq G2 Green Master mix, 2× (2×Green Go Taq G2, PH 8.5; 400 µM (of ATP, dGTP, Dttp, CTP); 3Mm MgCl₂); 1.25 µL for primers P1 (10 µM) and P7 (10 µM); 1.25 µL for primers F2 (10 µM) and R1 (10 µM), 1.75 µL ultrapure water and 2 µL DNA. A negative control containing all the components of the reaction medium except the DNA, which was replaced by water (2 µL of water), was added to the samples to check the accuracy of the results. The program used was as follows: An initial denaturation cycle at 94°C for 3 min followed by 35 reaction cycles each comprising: denaturation at 94°C for 40 seconds, hybridization at 56°C for 40 seconds and elongation at 72°C for one 100 secs; and finally a cycle for final elongation at 72°C for 10 min.

After this direct PCR, two Nested PCRs were performed using the Gh813f/AwkaSR specific primer pairs according to the method of Tymon *et al.* (1998) and the universal primer pairs R16mF2n/R16mR2 and according to the method of Gundersen and Lee (1996) with the products of the direct PCR of the P1/P7 primer pair.

For nested PCR using the Gh813f/AwkaSR primer pair, each amplification reaction was performed in a total reaction volume of 12.5 µL. The reaction medium consisted of 6.25 µL of 2X PCR Buffer (GoTaq G2 Green Master Mix, 2X); 1.25 µL of each primer pair Gh813f (10 µM) / AwkaSR (10 µM), R16mF2n (10 µM) /R16mR2 (10 µM) and FU5 (10 µM) /RU3 (10 µM) depending on the primer pair; 1.75 µL of ultrapure water and 2 µL of PCR product amplified with the P1/P7 direct PCR primer. A negative control contained all the components of the reaction medium except the PCR product was replaced by 2 µL of water to reveal the veracity of the diagnosis. The tubes were placed in a thermal cycler (T100m BIORAD) for PCR of each primer pair, according to the program associated with each primer pair GH813f/AwkaSR that was: one initial denaturation cycle at 94°C for 3 min

followed by 35 (reaction) cycles each comprising: denaturation at 94°C for 40 secs, hybridization at 53°C for 40 sec and elongation at 72°C for 100 secs and finally a cycle for final elongation at 72°C for 10 min.

Nested-PCR using the R16mF2n/R16mR2 primer pair for amplification of the 16S region of ribosomal DNA was carried out using the method of (Gundersen et lee, 1996). Each amplification reaction was carried out in a reaction volume of 12.5 µL. the reaction medium consisted of: 6.25 µL of 2X PCR Buffer (GoTaq G2 Green Master Mix, 2X); 1.25 µL of each primer (R16mF2n (10 µM) and R16mR2 (10 µM); 1.75 µL of ultrapure water and 2 µL of PCR product amplified with primer R16mR1/R16mF2 from direct PCR. A negative control containing all the components of the reaction medium in which the PCR product is replaced by water (2 µL of water) was added to the samples in the amplification experiments to reveal the veracity of the diagnostics. The tubes were placed in a thermal cycler (T100m BIORAD) for PCR according to the following programme: 1 initial denaturation cycle at 94°C for 2 min; followed by 35 (reaction) cycles each comprising: denaturation at 94°C for 1 min, hybridization at 55°C for 2 min and elongation at 72°C for 3 min and finally a cycle for final elongation at 72°C for 10 min.

Electrophoresis of PCR products: PCR amplification products were separated according to size by electrophoresis on a 1% agarose gel incorporated with ethidium bromide in 1X TAE buffer (40 Mm Tris, 20 Mm acetate, 1 mM EDTA). For each amplification reaction, 2 µL of PCR products were added to the agarose gel wells. Electrophoretic migration was performed at 90 V for 30 min. A 1 kb plus size marker (ladder 1 kb Invitogen) was used to estimate the size of the various amplified fragments. The gel was then visualized under ultraviolet (UV) light using a gel reader. Amplified DNA stained with ethidium bromide appeared as bands on the agarose gel.

Sequencing and phylogenetic classification of phytoplasma: Amplicons obtained by Nested PCR using the primer pair R16mF2n/R16mR2 were sequenced using the SANGER method by the subcontractor MacroGen Europe (Netherlands). The sequences obtained were aligned and edited using Geneious prime software version 2024.0.5 and subjected to a similarity search using the BLAST algorithm in the NCBI database Altschul *et al.* (1990) in GenBank (<https://blast.ncbi.nlm.nih.gov>). A maximum likelihood (ML) phylogenetic tree was constructed using Geneious prime 2024.0.5 software and edited using Fig Tree V1.3

(<http://tree.bio.ed.ac.uk/software/figtree/>).

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA I) was performed using R software to compare the mean prevalence of the symptoms. Another analysis with one classification criterion was performed to compare the mean severity between symptoms. For any significant difference resulting from the analyses between the means of the prevalence and the severity, at the 5% threshold a Fisher LSD test was performed to constitute the homogeneous groups.

RESULTS

Symptoms observed: A variety of symptoms was observed in symptomatic papaya trees in the visited plantations. These symptoms can be grouped into two main types: changes in leaf color and deformation of plant organs. Changes in leaf color included mosaic

patterns, chlorosis, yellowing, leaf blanching, and chlorotic spots. Regarding organ deformation, it was particularly manifested by leaf crumpling. In addition to these two types, combinations of symptoms (i.e., an association of discoloration and deformation) were observed on the leaves of papaya trees.

The two types of symptoms were observed, namely changes in color and organ deformations, exhibit different forms of symptoms. Changes in leaf color were characterized by alternating yellow and green areas, which are typical of mosaic patterns (Figure 1D); some leaves turned completely yellow (Figure 1A) while others became white (Figure 1B). Some leaves showed yellow spots (Figure 1E). Regarding deformation, the leaves exhibited a crinkled appearance or showed blisters and swellings (Figure 1F). As for the symptom complexes, six (6) forms were observed:

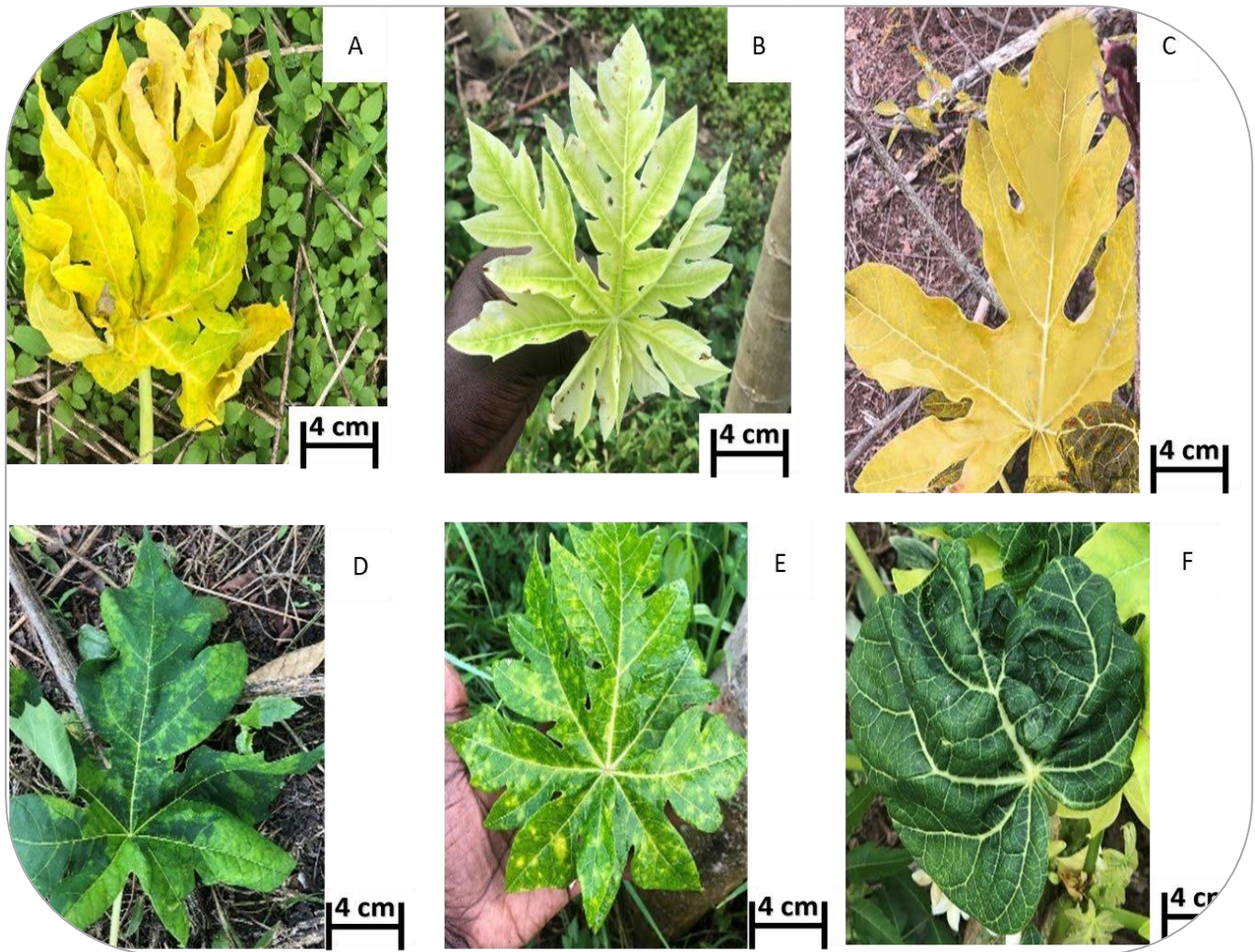


Figure 1. Unique symptoms observed on papaya leaves
 A: Yellowing; B: Blanching; C: Chlorosis; D: Mosaic; E: Chlorotic spots; F: embossing

An association of Shoestring, Embossing, Mosaic, and Curling (SGMR): the leaves had a spoon-like appearance with a reduction in leaf surface area and alternating green variegation (Figure 2G); an association of Embossing, Curling, and Shoestring (GRS): the crinkled leaves had a spoon-like appearance with a reduced leaf surface area (Figure 2K); an association of Shoestring and Curling (SR): the leaves were spoon-shaped and the leaf surface area was

reduced (Figure 2J). An association of Embossing, leaf edge curling, and Shoestring (GAS): the edges of the crinkled leaves were turned downward, in addition to a reduction in leaf surface area (Figure 2I); an association of Embossing and chlorotic spots (GT): presence of yellow spots on crinkled leaves (Figure 2H); an association of Embossing and yellowing: the leaves yellowed while maintaining a crinkled appearance (Figure 2L).

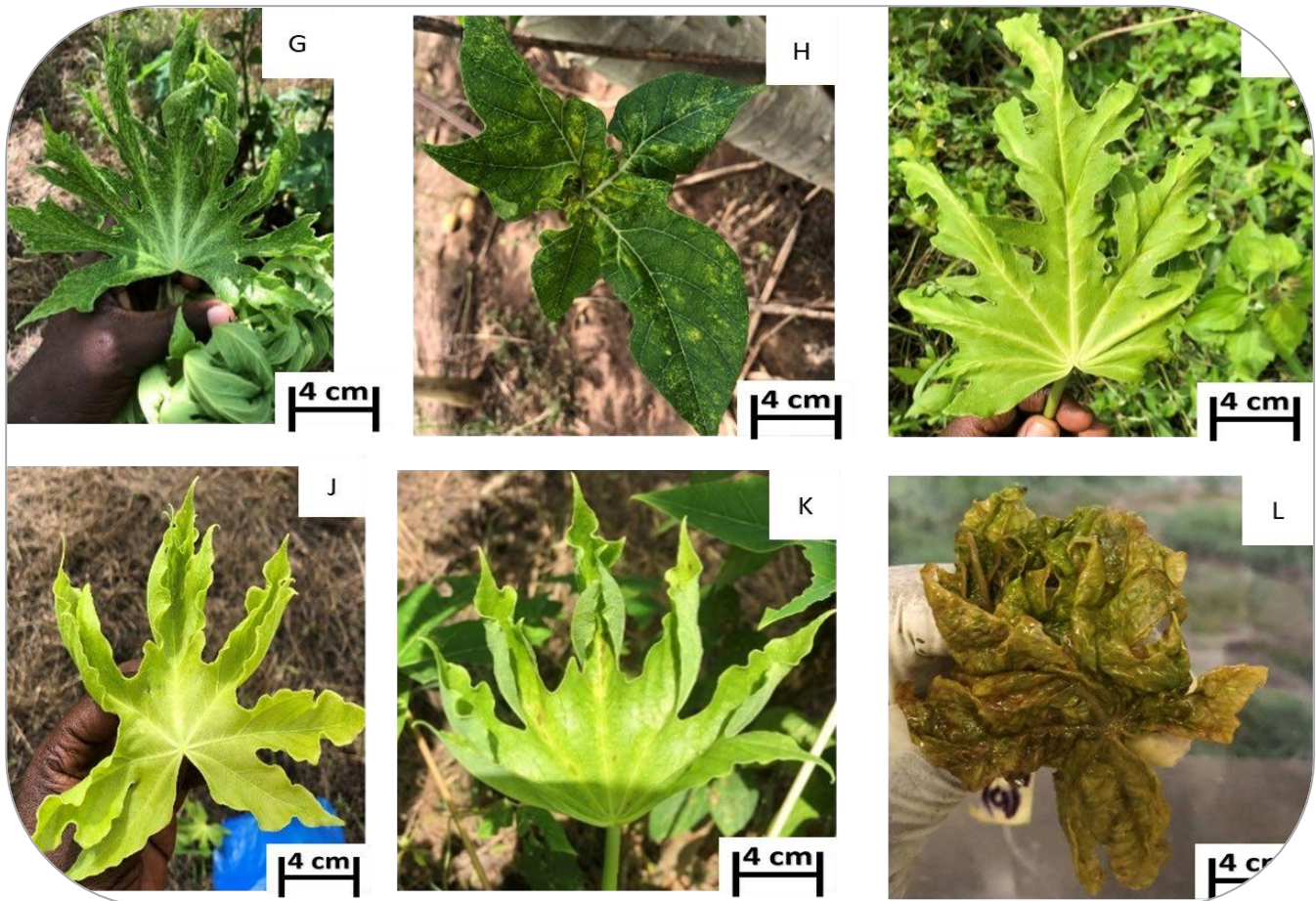


Figure 2. Symptom complexes observed on papaya leaves
 G: Shoestring – Embossing – Mosaic – Curling; H: Embossing – Chlorotic spots
 I: Embossing – Leaf edge curling – Shoestring; J: Shoestring – Curling;
 K: Embossing – Curling – Shoestring; L: Embossing – Yellowing

Geographic distribution of the observed symptoms:

Symptoms observed during this study on papaya leaves were distributed variably across the different locations studied. Regarding individual symptoms: Embossing and yellowing were observed in all four locations. Mosaic was observed in all locations except for the city of Tiassalé. Chlorotic spots were also observed in all cities except Adzopé. Chlorosis was observed only in Yamoussoukro. Finally, leaf blanching was observed in just one location,

which was the city of Adzopé (Table 1).

Symptom complexes were observed in only two out of the four studied locality: Tiassalé and Agboville. All complexes were found in Tiassalé except for the Embossing and yellowing complex (GJ). In Agboville, three out of the six complexes were observed: Embossing, leaf edge curling, and Shoestring (GAS); Embossing and chlorotic spots (GT); and Embossing and yellowing (GJ) (Table 1).

Table 1. Distribution of observed symptoms

Symptoms	Collection areas			
	YAKRO	TIA	AGBO	ADZ
Embossing	+	+	+	+
Mosaic	+	-	+	-
Yellowing	+	+	+	+
Chlorosis	+	-	-	+
Whitening	-	-	-	-
Chlorotic spot	+	+	+	-
SGMR	-	+	-	-
GRS	-	+	-	-
GAS	-	+	+	-
SR	-	+	-	-
GT	-	+	+	-
G.J.	-	+	+	-

a: Values with the same letter are statistically identical at the 5% threshold, according to the Kruskal Wallis test, H: Kruskal Wallis value, P: Probability +: presence of the symptom; -: absence of the symptom; YAKRO: Yamoussoukro; TIA: tiassalé; AGBO: agboville; ADZ: adzope. SGRM: Shoestring – embossing – mosaic – curling; GRS: embossing – curling – shoestring; GAS: embossing – sagging sheet edges – shoestring; SR: shoestring – curling up; GT: embossing - chlorotic stain; GJ embossing – yellowing

Prevalence of papaya leaf symptoms: Statistical analysis revealed variation in symptom prevalence depending on the locations. There was a significant difference ($p < 0.05$) in symptom prevalence across locations. The prevalence of observed symptoms ranged from 1.85% to 60% depending on the location. In Youkro, crinkling and chlorosis were the most

prevalent, with an average of 30%. In Tiassalé, chlorotic spots were the most common unique symptom, with an average of 24.53%. Crinkling was the most prevalent symptom in Agboville, with an average of 20.83%. In Adzopé, yellowing recorded the highest prevalence rate, with an average of 60%, and was observed in all cities (Table 2).

Table 2. Prevalence of symptoms observed in localities

Symptoms	Collection areas			
	YAKRO	TIA	AGBO	ADZ
Embossing	30 ± 3.12a	23.15 ± 2.23a	20.83 ± 2.06a	15 ± 1.37a
Mosaic	3.33 ± 1.07a	-	16.66 ± 1.89a	-
Yellowing	13.33 ± 1.23a	1.85 ± 0.20a	16.66 ± 0.66a	60 ± 0.1a
Chlorosis	30 ± 2.16a	-	-	-
Whitening	-	-	-	20 ± 3.14a
Chlorotic spot	6.66 ± 1.70a	24.53 ± 2.14a	12.5 ± 1;60a	-
SGMR	-	13.33 ± 2.12a	-	-
GRS	-	6.66 ± 1.56a	-	-
GAS	-	16.38 ± 2.60a	8.33 ± 1.80a	-
SR	-	10.37 ± 1.90a	-	-
GT	-	3.7 ± 0.29a	16.66 ± 2.12a	-
G.J.	-	-	8.33 ± 2.45a	-
H	4	7	6	2
P	> 0.05	> 0.05	> 0.05	> 0.05

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

-: absence of symptom; YAKRO: Yamoussoukro; TIA: tiassalé; AGBO: agboville; ADZ: adzope. SGRM: Shoestring – embossing – mosaic – curling; GRS: embossing – curling – shoestring; GAS: embossing – sagging sheet edges – shoestring; SR: shoestring – curling up; GT: embossing - chlorotic stain; GJ embossing – yellowing

Severity of observed leaf symptoms: Various levels of severity were observed on papaya leaves in the collection

areas. These severities ranged from 1 to 4 for individual symptoms and from 1 to 3 for symptom complexes. In

Yamoussoukro, chlorosis was the most severe symptom, with an average severity of 3. The Shoestring – Crinkling – Curling – Mosaic complex was the most severe in Tiassalé, with an average severity of 4. In Agboville, the

Crinkling – Chlorotic spots complex was the most severe, with an average severity of 4. In Adzopé, yellowing was the most severe symptom, with an average severity of 4 (table 3).

Table 3. Severity of symptoms observed in each locality

Symptoms	Collection areas			
	YAKRO	TIA	AGBO	ADZ
Embossing	2 ± 0.29a	2 ± 0.28a	2 ± 0.37a	2 ± 0.11a
Mosaic	2 ± 0.14a	-	2 ± 0.33a	-
Yellowing	3 ± 0.2a	2 ± 0.1a	2 ± 0.66a	4 ± 0.1a
Chlorosis	3 ± 0.39a	-	-	-
Whitening	-	-	-	3 ± 0.43a
Chlorotic spot	2 ± 0.46a	3 ± 0.25a	2 ± 0.25a	-
SGMR	-	4 ± 0.13a	-	-
GRS	-	4 ± 0.15a	-	-
GAS	-	4 ± 0.26a	2 ± 0.25a	-
SR	-	3 ± 0.19a	-	-
GT	-	3 ± 0.06a	4 ± 0.17a	-
G.J.	-	-	2 ± 0.16a	-
H	4	7	7	2
P	> 0.05	> 0.05	> 0.05	> 0.05

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

-: absence of symptom; YAKRO: yamoussoukro; TIA: tiassalé; AGBO: agboville; ADZ: adzope. SGRM: Shoestring – embossing – mosaic – curling; GRS: embossing – curling – shoestring; GAS: embossing – sagging sheet edges – shoestring; SR: shoestring – curling up; GT: embossing - chlorotic stain; GJ embossing – yellowing.

Identification of phytoplasmas associated with observed symptoms: Characterization of phytoplasmas using the primer pairs R16F2n/R16R2 allowed the amplification of DNA fragments approximately 1200 bp in size (Figure 3) in 11 out of 38 symptomatic and asymptomatic samples tested, representing 28,94%. This included both individual

symptoms and complexes tested. Only the complexes Embossing – Curling – Shoestring, Embossing – Leaf edge curling – Shoestring, and Shoestring – Embossing – Mosaic – Curling reacted positively to the Nested PCR performed with the R16F2n/R16R2 primer pair, based on PCR products from the P1/P7 primer pair (Figure 3).

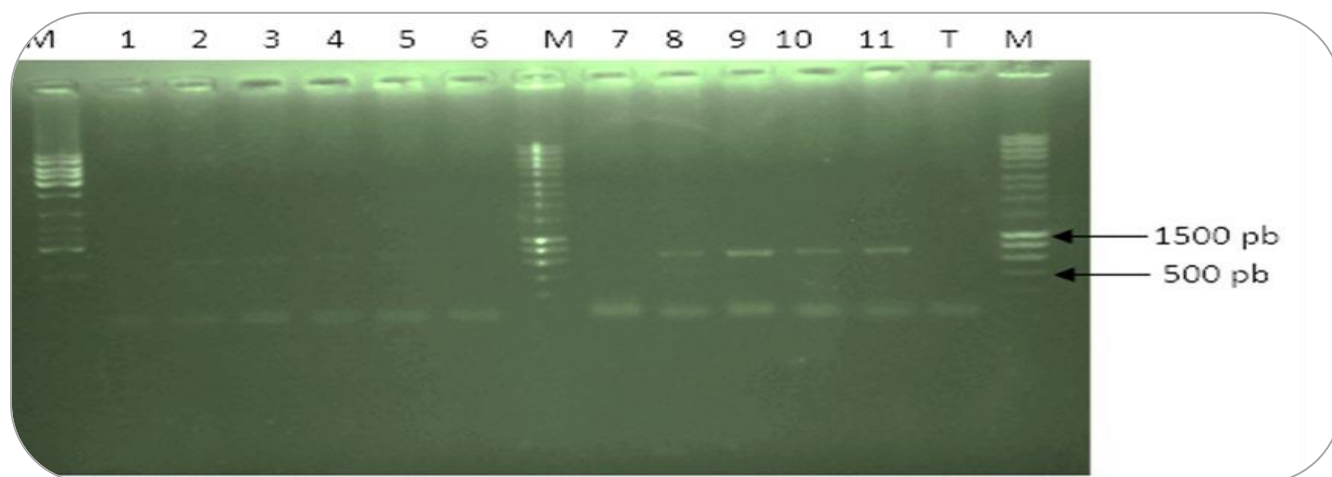


Figure 3. Agarose gel (1%) electrophoresis of PCR products using the R16F2n/R16R2 primer pair from DNA of papaya leaves M: 1Kb Plus Marker; 1 – 11: Samples; T: Negative Control (water); P b : Base Pair

Nested PCR conducted with the AkwaSR/GH813f primer pair did not generate any DNA fragments, demonstrating the absence of the phytoplasma *Candidatus Phytoplasma palmicola*. This phytoplasma

has been identified as responsible for lethal yellowing of coconut palms in Ivory Coast, as well as phytoplasma infections in cassava in Ivory Coast (Figure 4).

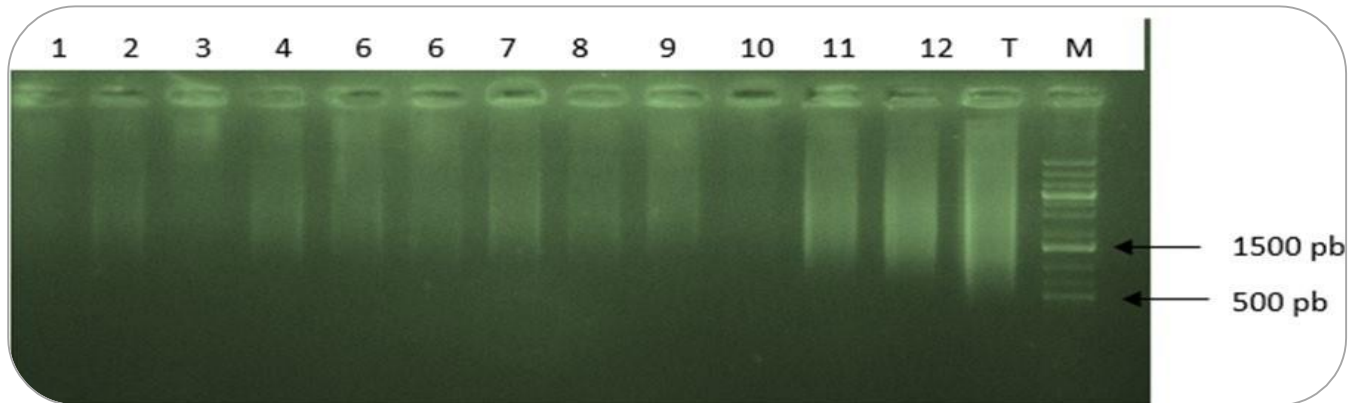


Figure 4. Agarose gel (1%) electrophoresis of PCR products using the GH813f/AwkaSR primer pair from DNA of papaya leaves

BLAST results revealed that the 16S rRNA gene sequences of the phytoplasmas detected (PP820865; PP820866; PP880867) by the Nested PCR with the R16F2n/R16R2 primer pair shared over 99%

sequence identity with **Candidatus Phytoplasma asteris** (OL625608; PP754500), which has been identified in papaya in China and coconut palms in India (Figure 5).

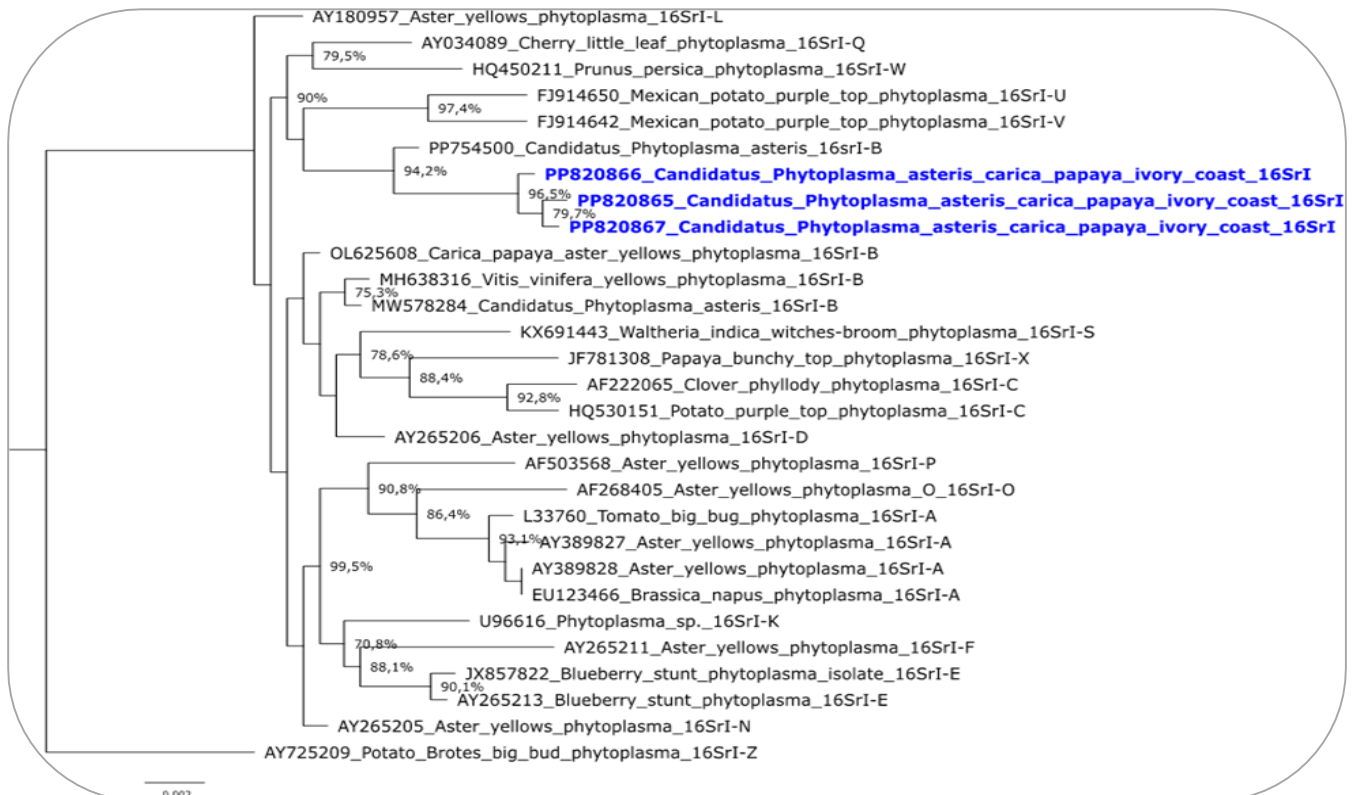


Figure 5. Phylogenetic tree based on 16S rRNA gene sequences showing the genetic relationships between phytoplasmas of subgroups 16SrI-A, B, C, D, E, F, K, L, N, O, P, Q, U, V, W, and Z associated with phytoplasma infections in various crops around the world.

DISCUSSION

The diversity of symptoms observed on papaya leaves at the different collection sites could be due to the presence of one or more pathogens acting alone or in co-infection in infected papayas in Ivory Coast. It should also be noted that these symptoms are often associated with viruses, which makes their distinction difficult. Unique symptoms have also been observed by several authors who have conducted research on phytoplasma diseases in papayas (Duduk 2004 Arocha 2009). Recent work by Mejia de Los (2014) on cassava has linked phytoplasmas to the presence of mosaic, chlorosis, and the mosaic and deformation complex. Additionally, some of these symptoms have also been observed in papayas co-infected with viruses and phytoplasmas in Cuba, as reported by (Acosta-Pérez *et al.*, 2017). Some studies have demonstrated that when phytoplasmas enter the plant, they localize in the phloem and multiply rapidly within the conductive vessels, disrupting the normal flow of sap (Cousin and Boudon 2002). This also affects the photosynthetic mechanism by utilizing chlorophyll pigments, which leads to leaf discoloration.

Statistical analysis of prevalence showed no significant difference between symptoms across cities. The appearance of symptoms varied from one locality to another, with prevalence rates ranging from 1.83% to 60%. These prevalence rates could be explained by the presence of one or more phytoplasmas in the leaf samples, as Ventura *et al.* (2004) reported that phytoplasma attacks in papaya orchards can lead to incidence levels as high as 75%. Some symptoms were observed only in specific locations: blanching was noted exclusively in Adzopé, and the complexes Shoestring – Crinkling – Mosaic – Curling; Crinkling – Curling – Shoestring; Crinkling – Leaf edge curling – Shoestring; and Shoestring – Curling were observed only in Tiassalé. The uneven distribution of symptoms may be attributed to the presence of different pathogen strains depending on the collection areas. These symptoms have been associated with the presence of phytoplasmas in various crops, including coconut palms, vines, and papayas, as reported by several authors (Yaima *et al.*, 2009; Chrystel *et al.*, 2011; Ouattara *et al.*, 2022; Atiq *et al.*, 2022).

Additionally, statistical analysis of the average severity of symptoms in each city showed no significant difference between the mean severities. However, severities varied from 2 to 4. This variation in symptom expression could be attributed to the nature of the host-pathogen

relationship. Differences may also be linked to factors such as the virulence of pathogen strains, environmental conditions, and the genetic variability of the host. Symptom complexes were the most severe, with average severities ranging from 3 to 4 in each locality where they were observed. The high severity of these symptom complexes may be due to the virulence of the pathogen strain responsible for these symptoms (Owor *et al.*, 2004). This could also be explained by the presence of multiple pathogens or strains of phytoplasmas acting in co-infection, or by a high presence of vector insects in localities exhibiting symptom complexes.

The characterization of phytoplasmas using the GH813f/AwkaSR primer pair, specific to « *Candidatus Phytoplasma palmicola* », did not detect its presence in the tested leaf samples. It should be noted that « *Candidatus Phytoplasma palmicola* », responsible for lethal yellowing of coconut palms, is the only phytoplasma identified so far in Ivory Coast in coconut palms and cassava, which are two different crop families (Ouattara *et al.*, 2022; Acosta-Pérez *et al.*, 2017). The absence of « *Candidatus Phytoplasma palmicola* » in the tested leaf samples could further confirm the presence of another phytoplasma or multiple phytoplasmas in papayas in Ivory Coast.

The primer pair R16F2n/R16R2 is specific for detecting phytoplasmas. Using these primers allows for the amplification of a conserved region of the 16S rRNA gene of phytoplasmas. Characterization of phytoplasmas with the R16F2n/R16R2 primer pair confirmed the presence of phytoplasmas in the collected leaf samples. Sequencing and BLAST analysis of the obtained sequences identified the presence of « *Candidatus phytoplasma asteris* », a phytoplasma of the 16SrI-B subgroup, associated with the observed symptoms in papaya (Shad *et al.*, 2023; Atiq *et al.*, 2023). This phytoplasma was also identified in papaya orchards in China with similar symptoms (Yu *et al.*, 2021; Shad *et al.*, 2024). The same symptoms observed in papaya orchards in Ivory Coast have always been associated with the presence of viruses (Diallo *et al.*, 2008). This is therefore the first report that phytoplasmas of the 16SrI-B subgroup infect papaya trees in Ivory Coast. This result could likely be due to a high concentration of phytoplasmas in these samples, which aligns with the hypothesis of a high presence of the pathogen indicated by elevated severity averages. The absence of amplicons in other samples showing symptoms associated with phytoplasmas, as reported by

several authors, might be due to the pathogen being present in the plant at low concentrations (Marcone 2010; Usman *et al.*, 2024). Additionally, phytoplasmas are mobile, and their localization within the plant can vary depending on the season (Lherminier *et al.*, 1994). The failure of PCR is not an indication of the absence of other fastidious prokaryotes and there may still be a latent infection in low titers (De Silva *et al.*, 2023). Phytoplasmas are mobile within the plant and can concentrate at varying levels depending on the seasons. This study was conducted over a limited period, which did not allow for the observation of this seasonal variability. A longitudinal study would provide a better understanding of the dynamics of the infection.

CONCLUSION

This study revealed that symptoms associated with phytoplasma diseases were observed on papayas in the surveyed plantations. These symptoms include organ deformation and discoloration, expressed in various

forms such as mosaic, chlorosis, yellowing, leaf whitening, and chlorotic spots for discoloration, and crinkling for foliar deformation. Additionally, symptom complexes were also observed. Molecular analyses confirmed the presence of phytoplasmas in the collected leaf samples, with this presence associated with symptom complexes. Various molecular tests conducted showed that the symptoms observed in the different sampled papaya plantations are associated with the presence of *Candidatus Phytoplasma asteris*, a phytoplasma of the 16SrI-B subgroup. The phytoplasma *Candidatus Phytoplasma palmicola*, responsible for phytoplasma diseases in coconut palms and cassava in Ivory Coast, does not infect papayas. Finally, this study lays the groundwork for diagnosing phytoplasma diseases in Ivorian papaya orchards. To identify the vectors of the identified phytoplasma as well as on the development of integrated vector control methods.

REFERENCE

- Acosta-Pérez K. I., B. E. Piñol-Pérez, L. Zamora-Gutierrez, M. L. Quiñones-Pantoja, I. Miranda-Cabrera, N. E. Leyva-López, and Y. Arocha-Rosete. (2017). A phytoplasma representative of a new subgroup 16SrI-Z associated with Bunchy top symptoms (BTS) on papaya in Cuba.
- Ahmad, H., N.A. Rajput, M. Atiq, G.A. Kachelo, M. Usman, H. Tariq and M. Wahab. 2024. Detection of *Phytophthora nicotiana* induced citrus gummosis by the loop mediated isothermal amplification. Pakistan Journal of Botany, 56(5): 1-11.
- Altschul S., W. Gish, W. Miller, E. Meyers and D. Lipman. 1990. Basic local alignment search tool. Molecular Biology. 215:430-410
- Arocha Y., B. Pinol, K. Acosta, R. Almeida, J. Devonshire, A. Van de meene, E. Boa and J. Lucas. 2009. Detection of phytoplasma and potyvirus pathogens in papaya (*Carica papaya* L.) affected with 'Bunchy Top Symptom' (BTS) in eastern Cuba. Crop Protection 28:640- 646
- Atiq, M., M.Z. Talib, N.A. Rajput, S.T. Sahi, M.A. Khan, M. Usman, A. Jabar, M.J. Arif, M.D. Gogi, M.A. Khan and A. Akram. 2023. New-fangled tactics towards cotton leaf curl virus disease A review. Journal of Natural Fibers, 20 (2): 2217364.
- Atiq, M., N.A. Rajput, S.T. Sahi, A. Akram, M. Usman, G.A. Kachelo, H. Ahmad, A.Q. Khan, H. Tariq, S. Ramzan, Z.B. Tahir and M.J. Matloob. 2022. A way forward towards the management of chilli anthracnose -a review. Agricultural Science Journal, 4(1): 1-10.
- Bertaccini A. and B. Duduk. 2009. Phytoplasma and phytoplasma diseases: A review of recent research. Phytopathologia Mediterranea. 48. 355-378
- Chrystel O. 2012. Complexe scientifique du Québec, 2700 Einstein, SteFoy, Qc. G1P 3W8.
- Chrystel O., B. Galka, J. Saguez, C. Vincent, T. Lowery, L. Stobbs, K. Whybourne, L. Bittner and et C. Xiansheng. 2011. Phytoplasma yellows in Canadian Vineyards Past, present and future research projects. Faunistic Entomology 63(3):87-90
- Cousin M. T. and E.B. Padiou. 2002. Phytoplasma and phytoplasma diseases: vectors, control, and research topics. French-speaking/agriculture study and research notebook, 11:26-115
- De Silva P.R., C.N. Perera, B.W. Bahder and R.N. Attanayake. 2023. Nested PCR-Based Rapid Detection of Phytoplasma Leaf Wilt Disease of Coconut in Sri Lanka and Systemic Movement of the pathogen. Pathogens 12(2):294.
- Diallo A.H., W. Monger, K.N. Kouassi, D.T. Yoro and P. Jones. 2008. Occurrence of *Papaya ringspot virus* Infecting Papaya in Ivory Coast Plant Viruses 2 (1), 52-57
- Doyle J.J. and et J.L. Doyle 1990. Plant DNA isolation from fresh tissue. Focus 12:1-13
- Duduk B., B. M. Ivanovic, B. Krstic, N. Dukić and A. Bertaccini. 2004. Identification of Phytoplasmas Associated with Grapevine Yellows in Serbia. Journal Phytopathology 152:575-579
- Fuentes G., and J. M. Santamaría. (2013). Papaya (*Carica papaya* L.): origin, domestication, and production. In Genetics and genomics of papaya (pp. 3-15). New York, NY: Springer New York.

- Gundersen D.E. and et IM Lee. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterranea*, 35:144-151
- Iqbal, S., M.A. Khan, M. Atiq, N.A. Rajput, M. Usman, A. Nawaz, G.A. Kacheo, A. Akram and H. Ahmad. 2022. Mango anthracnose: Global status and the way forward for disease management. *Journal of Innovative Sciences*, 8(2): 222-235.
- Kouame C.A., K.D. Kra, Y.M.N. Toualy, H.A. Diallo and B.A.I. Zoro. 2022. Characterization of Phytoplasmas associated with cassava (*Manihot esculenta crantz*) infection in Southern and Southeastern Ivory Coast. *International Journal of Current Research in Biosciences and Plant Biology* 9(6):17-29
- Lherminier J., M. Courtois and A. Caudwell. 1994. Determination of the distribution and multiplication sites of flavescence dorée mycoplasma-like organisms in the host plant *Vicia faba* by ELISA and immunocytochemistry. *Physiological and Molecular Plant Pathology*.45: 125-138
- Marcone C. 2010. Movement of Phytoplasmas and Disease Development. In: Weintraub.G. and Jones P. (Ed *Phytoplasmas: Genomes*).Pl. Host. Vectors, pp:114-131
- Mejia de Los Rios, J. F. (2014). Identification and genetic diversity in phytoplasmas associated with diseases of cassava and other agronomic relevant crops in south-east Asia and Latin America.
- Mignouna H.D., P. Njukeng, M.M. Abang and R. Asiedu. 2001. Inheritance of resistance to Yam mosaic virus genus Potyvirus in white yam (*Dioscorea rotundata*). *Theoretical and Applied Genetics*, 103: 1196-1200. National of Senegal Fruit Arboriculture Section. Papaya cultivation in Senegal
- N'Da A. A., A. N. Guessan, A. Djaha, N. Hala, K.N. Kouassi, F. Coulibaly, K. Edo and E.Zongo. 2008. Cultivate papaya properly in Ivory Coast. CNRA. Technical sheet on papaya
- Ogara I.M and R.N.D. Bina. 2010. Prevalence and persistence of galls and the galling nematodes *Meloidogyne* spp on yams in Nasarawa state, Nigeria, *acta SATECH* 3: 10-13
- Ouattara B.W.M., K.D. Kra, Y.M.N. Toualy, Y.Y.F.R. Kouakou and H.A. Diallo. 2022. Detection of a new strain of phytoplasma associated with lethal yellowing disease of coconut (*Cocos nucifera*) in Ivory Coast. *International journal of agriculture and biology* 28:193-200
- Owor B., P.P. Legg, O. Okao, R. Obonyo, M.W.O. Latigo. 2004. The effect of *cassava mosaic geminivirus* on symptom severity, growth and root yield of a *cassava mosaic virus* disease susceptible cultivar in Uganda. *Annals of Applied Biology*, 145:331-337 pp: 9-11
- PP:12-38
- Schneider B., E. Seemüller, C.D. SMART, B.C. Kirkpatrick. 1995. Phylogenetic classification of plant pathogenetic mycoplasma-like organisms or phytoplasmas. In *Molecular and Diagnostic Procedures in Mycoplasmaology*. (Ed S. Razin and J.F. Tully. San Diego, CA: Academic Press. Volume I, 369-379
- Séka K., A.J. N'cho, F.K. Yao and H.A. Diallo. 2023. Viral Diseases of Papaya in Ivory Coast: Distribution and Molecular Characterization of *Ringspot virus* and Other Viruses. *American Journal of Microbiological Research*. Vol. 11, No. 3, 2023, pp 83-87.
- Shad, M., A. Nazir, M. Usman, M.W. Akhtar and M. Sajjad. 2024. Investigating the effect of SUMO fusion on solubility and stability of amylase-catalytic domain from *Pyrococcus abyssi*. *International Journal of Biological Macromolecules*, 266: 131310.
- Shad, M., N. Hussain, M. Usman, M.W. Akhtar and M. Sajjad. 2023. Exploration of computational approaches to predict the structural features and recent trends in α -amylase production for industrial production. *Biotechnology and Bioengineering*, 120: 2092-2116.
- Tahir, Z.B. M. Atiq, N.A. Rajput, A. Akram, A.M. Arif, S. Iqbal, S. Ali, A. Nawaz, M. Usman and A. Husnain. 2023. Determination of Biochemical base line of resistance against bacterial leaf spot of chilli after application of plant defense activators. *Journal of Global Innovations in Agricultural Sciences*, 11(1): 61-67.
- Toure M., E. Koffi, E. Kwadjo, M. Doumbia, S. Kreiter et and K. Kra. 2020. Diversity of predatory ladybugs (Coleoptera: Coccinellidae) in papaya orchards (*Carica papaya* L.) in Ivory Coast. *Africa development*. Afrique et développement. 271-278.
- Tymon A.M., P. Jones and N.A. Harrison. 1998. Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. *Annals of Applied Biology*, 132: 437- 452
- Usman, M., M. Atiq, N.A. Rajput, S.T Sahi, M. Shad, N. Lili, S. Iqbal, A.M Arif, U. Ahmad, K.S. Khan, M. Asif and F.U. Haider. 2024. Efficacy of green synthesized silver-based nanomaterials against early blight of tomato caused by *Alternaria solani*. *Gesunde Pflanzen*, 76(1): 105-115.
- Valadez R.P., D.O.L. Martínez, V.G. Ponce and E.B. Rodríguez. 2024. Identification of phytoplasmas associated with Bunchy Top disease of papaya in Colima, Mexico. *Mexican Journal of Phytopathology*, 42(3): 24
- Ventura J.A., H. Costa and T.J. Da-Silva 2004. Papaya diseases and integrated control. In: Naqvi SAMH (Ed) *Diseases of Fruits and Vegetables (Vol II)*,

Kluwer Academic Publishers, The Netherlands, pp:
201–268

Yu S.S., A. Zhu and W.S. Wei, 2021. *Carica papaya* represents

a new host of 16SrI-B associated with yellow
symptoms in China. Aps publication. Plant Disease
2023 107:1, 211

Contribution of Authors:

- | | | |
|------------------------|---|---|
| Néhémie P. A. Lobognon | : | Initiated the study, carried out the sampling, data collection, statistical analysis of the data and wrote the article. |
| Kouamé D. Kra | : | Supervised the study and reviewed the manuscript. |
| Marie-Noel Y. Toualy | : | Participating in the various PCR tests carried out during the study and revising the manuscript. |