International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 24, No. 1, p. 19-25, 2024

RESEARCH PAPER OPEN ACCESS

Identification of the cassava phytoplasma insect in Côte d'Ivoire

Christine Ahou Kouame*, Daniel Kouamé Kra, Yeyeh Marie Noël Toualy, Hortense Atta Diallo, Arsène Irié Zoro Bi

Plant Health Unit, Plant Production Research Centre, Natural Sciences Training and Research Unit, University Nangui Abrogoua, Abidjan, Côte d'Ivoire

Article published on January 13, 2024

Key words: *Bemisia tabaci*, Cassava, Côte d'Ivoire, Insects, Phytoplasma

Abstract

Disease-causing phytoplasmas are transmitted by insect. Their distribution and transmission of the diseases with which they are associated are strongly linked to the feeding habits and biology of the insect vectors and the conditions of the host plants. This study was carried out in Côte d'Ivoire, specifically in the Grand-Lahou locality (an area endemic to phytoplasma disease), with a view to identifying the insect carriers and vectors of cassava phytoplasma. Insects were collected from the leaves. Total deoxyribonucleic acid (DNA) was extracted from 150 insect specimens and indirect diagnosis was carried out by Polymerase Chain Reaction (PCR) using the universal primer pair P1/P7 followed by AwkaSR/GH813f (specific primers). The molecular tests (PCR) carried out on these insects identified a probable carrier of the specie *Bemisia tabaci*.

* **Corresponding Author:** Christine Ahou Kouame ✉ ahouchristine29@gmail.com

Introduction

Cassava (*Manihot esculenta* Crantz) is an annual plant found in tropical countries. It is grown for its protein- and vitamin-rich leaves (Dostie *et al.*, 1999). Cassava also guarantees food security for small-scale subsistence farmers. It plays an important role in sub-Saharan Africa in efforts to alleviate the food crisis and poverty.

In Côte d'Ivoire, cassava is the second most important food crop after yam, with annual production estimated at 6,961,619 tons in 2021 (FAO, 2022). In addition, tuberous cassava roots are also the raw material for a wide range of transformations, resulting in food, cosmetic and pharmaceutical products (Dixon *et al.*, 2003).

Despite its ease of adaptation to different agro-ecological zones, cassava cultivation is hampered by several constraints that greatly reduce cassava production worldwide. These include fungal, bacterial and especially viral diseases. In addition to the damage caused by pests (Ambang *et al.*, 2007), diseases other than those observed and common infect cassava throughout the world. These include phytoplasma diseases (Alvarez *et al.*, 2013). Phytoplasmas are cell wall-less prokaryotic bacteria associated with disease in many cultivated and non-cultivated plant species worldwide (Lee *et al.*, 2000), living and reproducing in the phloem tissues of plants, as well as in the salivary glands and other tissues of certain hemipteran insects. These insects are mainly phytoplasma-vector leafhoppers in plants (Caasi-Lit *et al.*, 2018).

However, phytoplasma disease remains the least studied infection in cultivated plants in Côte d'Ivoire. Studies conducted on phytoplasma diseases have shown that phytoplasma is present in cassava (Kra *et al.*, 2017). Thus, knowledge of the mode of infection of phytoplasma disease presents an important challenge as it will help to understand the biology of the pathogen and the identification of the vector carrier of cassava phytoplasma. It will also lead to better management of cassava phytoplasma diseases in Côte d'Ivoire. In addition, phytoplasma diseases are mainly transmitted through the use of infected plant material and via vectors. The most important vectors of horizontal transmission are insects. An entomofauna population lives on cassava, in particular sucking biters. Among these insects, the *Bemisia tabaci* species is a major vector of viral diseases and pests of cassava (James *et al.*, 2000). The identification of insect genera capable of carrying the phytoplasma associated with the disease is a key issue in the management of phytoplasma diseases. However, the carrier insect must be able to transmit the phytoplasma to the host plant before it develops symptoms of the disease. Identifying phytoplasma-carrying and transmitting insects in cassava makes it possible to target the insect vector for effective control of the phytoplasma disease. Like virus diseases, phytoplasma is a pathogen that is spread by insect vectors (Weintraub and Beanland, 2006).

Control of phytoplasma is directed against the insect vector of this agent. To improve management of cassava phytoplasma disease in Côte d'Ivoire, the main objective of this study was to identify the vector associated with the disease.

Materials and methods

Insect collection site

The insects used in this study were collected in the Braffédon area, a village in the locality of Grand-Lahou. This is a forested region characterized by a rural forest zone made up of forest scrub and fallow land. This locality has a lagoon and maritime landscape with a sandy coastline and ferralitic soils that are slightly and/or heavily denatured. The climate is sub-equatorial with an Attienan facies, with a rainy season from March to November, peaking in June and September, and 3 dry months, from December to February. The average annual rainfall is 1,475.07 mm. The average annual temperature varies between 24 and 27°C. Average relative humidity is between 80% and 90% (Kouadio *et al.*, 2009).

Biological material

The biological material consisted of insects on the aerial organs of cassava in (young) plantations. These insects were captured on cassava leaves with or without symptoms.

Description of symptoms

The various symptoms observed on the leaves whose insects were captured in the cassava plantations were described. The description was based on the shape and coloring of the cassava leaves on the plants.

Collection of insects

Insects were collected in three cassava plantations at least one kilometer long. Insects were collected from the cassava leaves using a manual aspirator and then stored in Eppendorf tubes. Samples were collected randomly from the undersides and tops of three- and six-month-old cassava leaves. The insect samples were sent to the laboratory.

Identification of insects carrying the phytoplasma

Extraction of DNA from insects

DNA was extracted from insects using the method of Doyle and Doyle (1990). Five insects of the specie Bemisia tabaci were crushed in 2 ml Eppendorfs tubes with 500 µl of 2% CTAB extraction buffer previously heated in a water bath at 65°C. The tubes were incubated in a water bath for 30 minutes at 65°C. After incubation, the tubes were left to cool for approximately 2 min at room temperature. Next, 500 µl of chloroform-isoamyl alcohol (24:1) was added to the crushed material in each Eppendorf tube, followed by centrifugation at 13,000 rpm for 15 min. After centrifugation, 400 µl of the supernatant was removed from each tube and transferred separately to a new 1.5 mL Eppendorf tube. The DNA was then precipitated in 400 µl of cold isopropanol for 30 min at -20°C in the freezer. After 30 minutes, the tubes were centrifuged at 13,000 rpm for 15 minutes. The supernatant was removed from the tubes and 200 µl of 70% ethanol was added to the tubes. The tubes were centrifuged at 13,000 rpm for 10 min. After centrifugation, the ethanol was removed and the tubes were dried on sterile blotting paper at room temperature to remove all traces of ethanol. The DNA was recovered in 50 µl of TE and the tubes were stored at -20°C in the freezer for future analysis.

Molecular analysis of phytoplasmas in insects

Identification of phytoplasmas in insect samples was carried out using two types of PCR with two pairs of primers. PCR was carried out using the universal primer pair P1/P7 (Deng and Hiruki, 1991, Schneider *et al.*, 1995) in a total reaction volume of 12.5 µl containing: 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 extracted DNA. Amplification was carried out 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, hybridization at 56°C for 40 s and elongation at 72°C for 1 min 40 s. A final elongation cycle at 72°C for 10 min terminated the reaction.

The products of the 1st PCR (P1/P7) were again amplified by Nested PCR with the specific primer pair Awka SR/GH813f (Tymon *et al.*, 1998) in a reaction volume containing 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 final reaction volume was 50 µl. PCR products were amplified 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 seconds, hybridization at 53°C for 40 s and elongation at 72°C for 1 min 40 s and a final elongation cycle at 72°C for 10 min.

Migration of PCR products

The different amplified Nested PCR products were separated by electrophoresis on a 1% agarose gel (Bioshop, Canada) incorporated with ethidium bromide in a 1×TAE buffer solution (400 Mm Tris-Acetate, 10 Mm EDTA, Promega, USA). Two microliters of each Nested PCR product and 3 µl of size marker (BenchTop 100 bp DNA or 1Kb ladder, Promega, USA) were used for electrophoretic migration at 100 volts for one hour. The gel was then visualized under a trans-illuminator (EBOX VX5 Vilbert Lourmat France).

Study of the transmission capacity of the phytoplasma-carrying insect

To perform the transmission ability test, a synthetic nutrient medium was prepared according to the method of Bosco and Tedeschi (2013). For this purpose, 2 ml Eppendorfs tubes were used. The lids of these tubes were filled with 200 ml of a 5%

concentrated TE succrose solution and then these lids were sealed with parafilm. Two insects were introduced per tube. Each tube was maintained at room temperature in the laboratory for 24 to 48 h in an upright position with the cap facing a light source to attract the insects to the feeding medium. After 24 h incubation, the insects and the nutrient medium (TE + sucrose solutions) contained in the tubes were immediately used for molecular analysis in search of phytoplasmas. Our experiment involved a set of 20 insects, two per tube. Molecular analysis was carried out as before.

Results

Symptoms observed

The symptoms observed on the leaves of the cassava plants on which the insects were caught were either single or complex.

These symptoms were mosaic, discoloration in bands of yellow and green between the veins of the leaves (vein banding), chlorosis, shoestring leaves, embossed leaves, reduced leaves and leaves with edges resembling a fan propeller (Fig. 1 and 2).

Phytoplasma(s) present in insect samples and feeding solutions

Molecular identification using the specific AwkaSR/GH813f primer pair by Nested PCR resulted in amplicons of the expected size of 1000 bp (Fig. 3). In DNA samples extracted from captured insects. Out of a total of 30 composite samples, 25 samples (83.33%) reacted positively to the presence of phytoplasma. PCR analyses based on the specific primer revealed the presence of phytoplasmas in the insects captured. These results confirm the presence of *Candidatus* Phytoplasma palmicola, a sub-group XXII-B phytoplasma. Insects of the specie *Bemisia tabaci* are thought to carry the *Candidatus* Phytoplasma palmicola.

Molecular tests using Nested PCR were also used to amplify phytoplasma DNA in feeding solutions (TE + succrose) containing the saliva of insects of the specie *Bemisia tabaci*. The amplicons were deposited at a molecular weight of 1000 bp corresponding to that of the specific primer pair (AwkaSR/GH813f) (Fig. 4) specific to *Candidatus* Phytoplasma palmicola. 12 synthetic nutrient media out of a total of 20 samples, i.e. 60%, contained the phytoplasma.

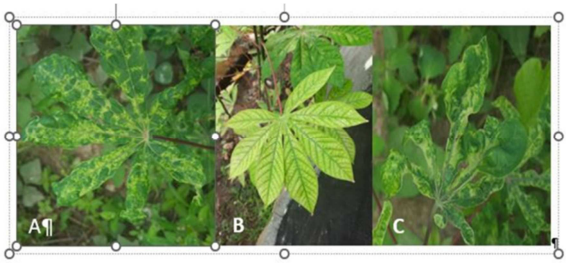

Fig. 1. Some symptoms observed

A: mosaic- embossing, B: vein banding, C: mosaic-deformation

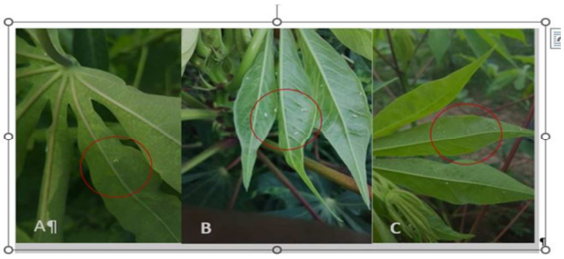

Fig. 2. Insects present on cassava leaves

A: presence of insects on symptomatic leaves, B and C: presence of insects of the specie *Bemisia tabaci* on asymptomatic leaves

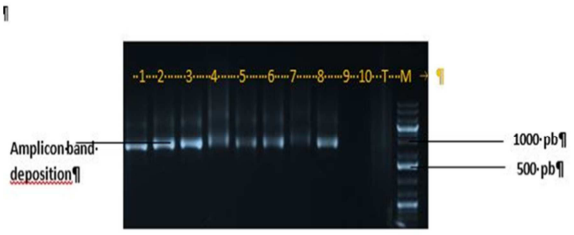

Fig. 3. Agarose gel electrophoresis profile (1%) of Nested PCR products amplified with the AwkaSR/GH813f specific primer pair, M: marker= 1 kb (DNA Ladder); 1-10: Bemisia tabaci DNA samples, T: negative control (water).

The presence of the phytoplasma in the nutrient solutions (TE + succrose) containing saliva would indicate that the insect of the specie *Bemisia tabaci* could be a transmitter of the '*Candidatus* Phytoplasma palmicola' phytoplasma of subgroup XXII-B.

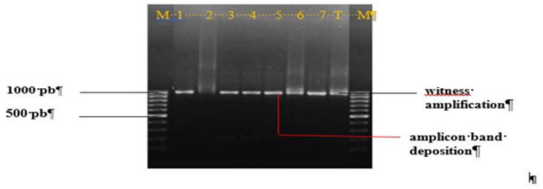

Fig. 1. Some symptoms observed
A: mosaic - embossing, B: vein banding, C: mosaic – deformation

Discussion

Knowledge of the biology of the pathogen and the epidemiology of the disease is very important if we are to improve the control of cassava phytoplasma disease and define strategies to prevent its spread. Control strategies against its spread, knowledge of the biology of the pathogen and the epidemiology of the disease are very important. In addition, identifying the insect(s) that carry(s) and/or vector(s) cassava phytoplasma required insect collections in the disease-endemic area (Grand-Lahou). An inventory of insects on cassava was carried out. A wide variety of arthropod species were observed. The presence of these insects on the plant can be explained by the fact that the plant is both a food resource and a refuge for these insects. The development of white flies on cassava is also explained by the presence of weeds in and near the fields. According to Tiendrébéogo (2010), the abundance of whiteflies in the fields can be explained by the fact that they feed on several species of wild and cultivated plants at the same time. By feeding on leaf sap, these insects secrete large quantities of honeydew, which encourages the development anthrax mold on the plant. These insects can also inoculate the plant with viruses as they feed. Among these, insects of the genus *Bemisia* sp. are a major pest of cassava but also a vector of viral diseases (Homenauth and De Souza, 2011).

In the present study, the cassava leaves on which the insects were captured showed symptoms of discoloration and deformation characterized by mosaic, vein banding, leaf chlorosis, shoestring, waffled leaves and leaf blade reduction. These symptoms are caused by several phytopathogenic agents, notably viruses. Phytoplasmas also have the ability to cause leaf discoloration and deformation in cassava according to Fernandez *et al.* (2018). Also, the work of Kra et al, carried out in 2017 in Côte d'Ivoire on cassava, showed that cassava infected with phytoplasma showed symptoms of leaf discoloration and deformation. These symptoms caused by viruses and phytoplasmas often develop via a vector when they are transmitted. In fact, phytoplasmas are transmitted by vectors that mostly belong to the order Hemiptera, a suborder of Homoptera, when they feed in the phloem of plants (Bosco and Tedeschi, 2013).

Although they are vectors of viral diseases of cassava, the molecular tests carried out revealed the presence of phytoplasma in insects of the specie *Bemisia tabaci* captured on cassava leaves. The phytoplasma identified was *Candidatus* Phytoplasma palmicola of subgroup XXII-B. The presence of the phytoplasma in these insects would mean that the insect of the specie *Bemisia tabaci* could be a probable carrier of the '*Candidatus* Phytoplasma palmicola' phytoplasma. The presence of phytoplasma in these insects can be explained by the fact that it multiplies and perpetuates itself on different hosts, namely crops of interest and neighboring weeds, which are sources of food for these insects. By feeding on these plants, the insects pick up the phytoplasmas present, which are then stored in their vectors (insects). In fact, once these insects come into contact with the leaves on a plant, they prick the vessels to feed. As they do so, they inject or remove the phytoplasmas. Once inside the insect, the phytoplasma multiplies in the cells of the intestinal wall, emerging in the hemolymph and reaching the insect's salivary glands, making them infectious after a more or less long incubation period (Hogenhout *et al.*, 2008). Our results could confirm those of Chavarriaga-aguirre *et al.*, 2016. According to these authors, *Bemisia tabaci* and *Aleurotrachelus socialis* are two insect species that could be involved in the transmission of a still unidentified agent (probably a phytoplasma) causing cassava frog skin disease (CFSD) in cassava in Colombia. Instead, Caasi *et al.*, 2018 reported the presence of phytoplasma in mealybugs in their study on the arthropod profile of witches' broom infected cassava in cassava fields in Mindoro, Leyte and Bohol (Phillipine).

Molecular tests also revealed the presence of phytoplasma in feeding solutions (TE + succrose) containing the saliva of insects of the specie *Bemisia tabaci*. This can be explained by the fact that phytoplasmas are transported from the insect to the plant via the salivary canal and from the plant to the insect via the alimentary canal. The successive mechanisms of endo and exocytosis transport them from the light to the cells of the digestive tract and then into the hemolymph, salivary glands and saliva, from where they are transmitted to the plant via the insect's salivary duct. According to Huet *et al*., 2020, when phytoplasmas reach the salivary gland cells of the insect vector, the latter becomes capable of inoculating the phytoplasma into the plant by secreting them with saliva in the phloem of a feeding puncture. From these results, it can be deduced that the insect of the specie *Bemisia tabaci* could be a transmitter of the "*Candidatus* Phytoplasma palmicola" phytoplasma of subgroup XXII-B in cassava. The *Bemisia tabaci* insect would have the capacity to transmit the phytoplasma to cassava and induce symptoms in the latter.

Conclusion

Cassava is a crop subject to attack by numerous insect pests. This study was initiated to identify the insect carrier and/or vector of the phytoplasma in cassava. Molecular analyses were used to identify the presence of phytoplasma in insects of the specie *Bemisia tabaci* and also in nutrition solutions (succrose +TE) containing the saliva of these insects. The phytoplasma present in the DNA of the insects and the solutions is *Candidatus* Phytoplasma palmicola of subgroup XXII-B. This result shows that the insect of the specie *Bemisia tabaci* could be a probable carrier of cassava phytoplasmas. The results of this study open up new avenues of research into cassava phytoplasmas, and further work should be undertaken. Horizontal transmission tests will be continued on test plants with other insects, and vertical transmission tests will also be carried out in order to propose appropriate control methods against cassava phytoplasma diseases.

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.

References

Alvarez E, Pardo JM, Mejia JF, Bertaccini A, Thanh ND, Hoat TX. 2013. Detection and identification of 'Candidatus Phytoplasma asteris related phytoplasmas associated with a witches' broom disease of cassava in Vietnam. Phytopathogenic Mollicutes **3** (2), 78-81.

Ambang Z, Akoa A, Nantia J, Nyobe L, Ongono BYS. 2007. Tolerance of some cassava cultivars (*Manihot esculenta* Crantz) and the wild species (*Manihot glaziovii*) to African viral mosaic and cassava cercosporium blight. Tropicultura **25** (3), 140-145.

Bosco RE, Tedeschi R. 2013. Insect vector transmission tests. In Dickinson M Hodgetts J (eds), Phytoplasma: Methods and Protocols. Methods in Molecular Biology. Humana (Spring), New York, NY, 73-85p.

Caasi-Lit MT, Dolores LM, DelRosario EE, Pinili MS. 2018. Arthopod profile of witches broom infected cassava in farmer's fields in Mindoro, Leyte and Bohol. Philippine Entomologist (Philippines) **32** (2), 165-166.

Chavarriaga-Aguirre P, Brand A, Medina A, Prías M, Escobar R, Martinez J, Díaz P, López C, Roca MW, Tohme J. 2016. The potential of using biotechnology to improve cassava: A review, In Vitro Cellular & Developmental Biology-Plant. *In vitro* Cellular & Developmental Biology-Plant. **45**, 461-478.

Deng S, Hiruki C. 1991. Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes. Journal of Microbiological Methods, **14**, 53-61.

Dixon AGO., Bandyopadhyay R, Coyne D, Ferguson M, Ferris S, Hanna R, Hughes J, Ingelbrecht, Whyte J, Hartmann P, Ortiz R. 2003. Cassava: From poor farmers' crop to pacesetter of African rural development. Chronica Horticulta **43**, 8-15.

Dostie B, Josée Randriamamonjy Lala Rabenasolo. 1999. The cassava sector: The forgotten shock absorber for the vulnerable, 21-29p.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. Focus **12**, 13-15.

FAO. 2021. Statistic of production (FAOSTAT). Food and Agriculture Organization of the United Nations https://www.faostat.fao.org, accessed 26 June 2021.

Fernández F, Uset A, Baumgratz G, Conci Luis. 2018. Detection and identification of a 16SrIII-J. phytoplasma affecting cassava (*Manihot esculenta* Crantz) in Argentina. Australasian Plant Disease **13** (1), 24.

Hogenhout SA, Oshima K, Ammar E, Kakizawa S, Kingdom HN, Namba S. 2008. Phytoplasmas: Bacteria that manipulate plants and insects. Molecular Plant Pathology **9**, 403-423.

Homenauth O, DeSouza SP. 2011. Pests and diseases of cassava in Guyana. Ministry of Agriculture, Guyana, 14p.

Huet, Constant, Carrot, Mallié. 2020. Everything you need to know about Flavescence Dorée. [On-line]. 2020. [Accessed on 10 May 2020]. Available at: https://occitanie.chambreagriculture.fr/fileadmin/user_upload/National/FAL_commun/publications/Occitanie/Productions_techniques/ToutSavoirSurLaFD-CRAO2020.pdf

James B, Yaninek J, Neuenschwander P, Cudjoe A, Modder W, Echendu N, Toko M. 2000. Controlling cassava pests: an integrated pest management guide for extension workers. IITA, Cotonou, Benin, 37p.

Kouadio EM, Ouattara S, Tahoux TM. 2009. Influence of diet on parturition interval the parturition interval of captive aulacodes in the Grand-Lahou region (Côte d'Ivoire, West Africa). Journal of Animal & Plant Sciences **4**(1), 311-319.

Kra KD, Toualy YMN, Kouamé AC, DialloA H, Arocha RY. 2017. First report of a phytoplasma affecting cassava orchards in Cote d'Ivoire. New Disease Reports **35**, 21.

Lee IM, Davis RE, Gundersen-Rindal DE. 2000. Phytoplasma: Phytopathogenic mollicutes. Annual Review of Microbiology **54** (1), 221-255.

Schneider B, Seemüller E, Smart CD, Kirkpatrick BC. 1995. Phylogenetic classification of plant pathogenic mycoplasmalike organisms or phytoplasmas. In Molecular and Diagnostic Procedures in Mycoplasmology **1**, 369-380.

Tiendrebeogo F. 2010. Characterisation and epidemiological aspects of Begomoviruses infecting market garden plants and cassava in Burkina Faso. PhD thesis. University of Ouagadougou, Burkina Faso, 154p.

Tymon AM, Jones P, Harrison NA. 1998. Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. Annals of Applied Biology **132**, 437-452.

Weintraub PG, Beanland L. 2006. Insect vectors of phytoplasmas. Annual. Review of Entomology **51**, 91-111.