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Full Length Research Paper

Pathogenicity of satellite associated with cassava Begomovirus in Côte D'Ivoire

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Abstract

Africa represents more than 50% of global cassava production. A study was carried out in Côte d'Ivoire in Abidjan, Abengourou, Grand-Lahou and Toumodi. In cassava and *Chromolaena odorata*, similar symptoms of virus disease were observed in the fields. Mosaic deformations and discolorations were observed in infected leaves with medium to very high severity. These collected leaves were subjected to total DNA extraction and conventional PCR targeting the Begomoviruses gene. Multiplex PCR was performed with specific primer pairs CMBrep/F, ACMVrep/R and EACMVrep/R for the detection of CMV and ACMV in cassava and *C. odorata* leaves. Additional PCR was performed with the specific primer pair JPS001/JPS002 for detection of ACMV, followed by PCR with primers JPS001/JPS003 for Begomovirus satellite detecting in both leaf samples. PCR confirmed the presence of ACMV, EACMV, Sat II and Sat III satellites, associated with cassava Begomovirus DNA in infected leaves with the highest severity for both cassava and *C.odorata.C. odorata* could ensure the rapid spread of the very serious cassava virus in the fields of farmers in Côte d'Ivoire. It would be important to consider *C. odorata* in the management of cassava viral diseases.

Key words: Begomovirus, Cassava, *Chromolaena odorata*, Satellites, Pathogenicity.

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a crucial staple crop for food security in sub-Saharan Africa (Burns, 2010). Cassava is the major source of food and income that supports the livelihoods of over 500 million smallholder farmers in the African continent.

Cassava grows in acidic soil and under drought and high temperature conditions. Cassava is by far the most important crop, with world production of 315 million tons in 2021 (of which 65% in Africa) (Cirad, 2023). Africa generates more than half of the world's cassava production with Nigeria as the leading African country. In Côte d'Ivoire, smallholder mainly grows cassava farmers who produced more than 5.2 million tons in 2020 (Wacomp, 2022). However, smallholder farmers cannot meet the supply for the ramping growing demand of cassava in Côte d'Ivoire. Over a dozen improved varieties (Yavo, Yacé, Bocou 1, 2, etc) in addition to traditional varieties (Bédépoutou, Bédé mango) (Hédin, 1931) are being grown extensively to try to feed more than 27 480 000 of people in Côte d'Ivoire.

In the past few years, Begomoviruses have become prevalent in the main cassava production areas of Côte d'Ivoire, causing severe losses (Adjata *et al*., 2008) and with not enough available tools to control effectively them. *Cassava mosaic disease* (CMD) is one of the most important viral diseases of cassava and can cause up to 100% yield losses. A complex of at least 11 *cassava mosaic begomoviruses* (CMBs), of which nine occur in

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Africa and two are found on the indian subcontinent, causes CMD. Begomovirus are considered the most important phytovirus group in tropical and subtropical areas causing significant yield losses in many crops (Srivastava *et al*., 2022). The whiteflies vector, *Bemisia tabacci* Gennadius (Xie and Zhou, 2003), transmits these Begomoviruses. This whiteflies feeds on infected cassava or reservoir plants, acquiring the virus and then transmitting them to other healthy plants.

Amoine *et al.,*(2021) showed that many Begomoviruses are associated with satellite DNAs that are packaged into virions and can either increase virulence or alter the plant host range. According to Nawaz-Ul-Rehman and Fauquet (2009), three types of DNA satellite viruses have been described as including Alphasatellites, Betasatellites, and Deltasatellites. Alphasatellites and Betasatellites are approximately 1,300 to 1,400 nucleotides (nt) in size and contain one major open reading frame (ORF), a hair pin structure, and an adenine-rich region. Betasatellites rely on the Rep protein of their helper virus for replication, whereas Alphasatellites encode their own Rep protein and replicate autonomously. Deltasatellites are related to Betasatellites but range from 540 ntto750 nt in size (Hassan *et al*., 2016). Betasatellites encode a single protein, βC1, which induces geminivirus-like symptoms by suppressing posttranscriptional gene silencing (PTGS) and transcriptional silencing (TGS) processes (Prabu *et al*., 2019). Some Alphasatellite viruses show silencing suppression activity and an increase in symptom severity (Nawaz-Ul-Rehmanand Fauquet, 2010). In *Euphorbia heterophylla*, a plant in the same family as cassava, Talita *etal.,*(2017) identified a new Alpha satellite virus associated with high symptom severity as euphorbia yellow mosaic Begomovirus.

Studies on viral diseases have been carried out in Côte d'Ivoire on cassava. Pita, (2001); and Yao, (2021) results shown that, EACMCMV which is always associated with ACMV was reported on cassava in cassava fields. Toualy *et al.* (2014) identified both viruses ACMV and EACMV infecting cassava in Côte d'Ivoire. The recombinant virus from Uganda, EACMV-UG, is spreading to neighboring countries (Legg, 1998) and has been detected in Burkina Faso (Tiendrebeogo, 2009), a country bordering to Côte d'Ivoire. Despite these studies, knowledge on the epidemiology of cassava mosaic viruses in Côte d'Ivoire and research on the involvement of invasive weeds in cassava viruses' dissemination is scarce.

Therefore, the causes of the increasing severity of symptoms associated with cassava infection by the group of mosaic viruses remain poorly understood.

Currently, smallholders do not usually attempt to control the pest/disease problems of cassava with pesticides because of limited access to such chemicals and because cassava has low value per unit weight in Côte d'Ivoire. In addition, there are interactions between different cassava pests/diseases and environment (Fabres *et al.*, 1994) and hence the need for a holistic approach to the complex. This is consistent with the views of Kesiwani (1987), who states that multiple infections with different pathogens and interactions between them are very common in tropical crops and complicate diagnosis, estimation of crop losses due to diseases and management procedures, especially viral diseases.

This study aims to contribute to the management of Begomoviruses responsible for cassava mosaic in the fields of small farmers in Côte d'Ivoire. The specific objectives of the study were to describe the symptoms of viruses observed, identify the Begomoviruses associated with the symptoms and characterize the satellites associated with the viruses identified.

Study area

This study was carried out in geo-referenced fields of small-scale cassava growers in four main production zones in Côte d'Ivoire: Abidjan, Grand-Lahou, Abengourou and Toumodi (Fig. 1). Abidjan is in the south of Côte d'Ivoire on the Gulf of Guinea, 5° 18' 34.8" North latitude and 04° 01' 36" West longitude. It has a hot and humid equatorial climate with two rainy seasons and two dry seasons. During rainfall, precipitations can accrue more than 1500 mm per year. The average temperature is 26.7 °C. Grand-Lahou is in the south of Côte d'Ivoire, at the edge of the Gulf of Guinea, 5°14'39" North and 5° 00' 11" West. The yearly average temperature is 26.7°C and the average rainfall is 1466.4 mm. Abengourouis in the Indenié-Djuablin region in the east of Côte d'Ivoire, 6° 43' 47" North and 3° 29' 47" West. Its average annual temperature is 26.3°C and the annual average rainfall is 1300 mm. Finally, Toumodi is in the center of Côte d'Ivoire in the areas, 6° 55' north and 5° 03' west. Its climate is humid and tropical. Rainfall accrues around 1200 mm of rain per year. Annual temperatures range from 23 to 33 °C (Bla *et al.,* 2015).

MATERIAL AND METHODS

Epidemiological survey

Symptoms of both plants on leaves were observed and described specifying the different signs and manifestations. Leaf's infection severity parameters were then estimated.

Assessment of symptom severity associated with Begomovirus infection

The severity index of the disease described the damage caused on plants leaves. Surveys were conducted in three to four cassava fields, aged from 4 to 8 months per

ILLUSTRATIONS

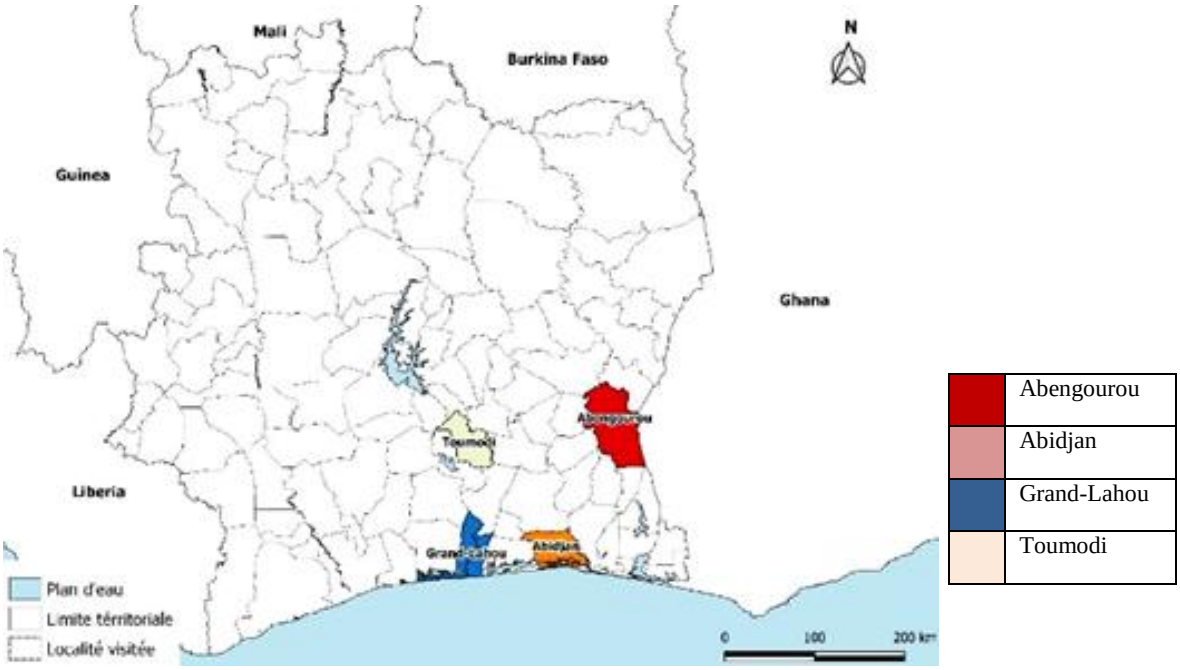

Figure 1: Map showing the location of the four main cassava-growing areas in Côte d'Ivoire.

production zone. Data were collected on the severity of the disease, from around thirty cassava leaves and *Chromoleana odorata* per field. In these different fields, the severity of viral symptoms on cassava and *Chromoleana odorata* leaves was assessed using the Mignouma *et al*. (2001) scale on plants using a scale of 1 to 5:

1: of symptomless leaves,
2: 1-25% of leaves show symptoms,
3: 26-50% of leaves show symptoms,
4: 51-75% of leaves have symptoms and
5: more than 75% of the leaves have symptoms

Disease severity index was then determined for each field using the formula according to Nelson *et al.* (1999) as shown below:

$$\text{Disease Severity Index (DSI)} = \frac{0*P0+1*P1+2*P2+3*P3+4*P4+5*P5}{N(G-1)} X\ 100;$$

Where, P0 to P5: Total number of observed plants in each disease symptom grading per farm site in each state within the agro ecological zone surveyed. G: Number of grading = 6 and N: Total number of observations

Sampling

Samples of infected and symptomless leaves of cassava and *Chromolaena odorata* with the highest severities from infected cassava plots were collected randomly. Three or four leaves per plant from five plants per field, were collected from three fields per locality. Approximately 60 leaves were collected in total. The leaf samples collected were sent to the laboratory and stored at -20°C for DNA extraction and PCR analysis.

Molecular characterization of Begomoviruses associated with cassava

The first ten leaves (five for cassava and five for *Chromolaena odorata*) with the highest score obtained from the severity scale were used for DNA extraction and conventional PCR analysis for a quality study. Cassava leaves were subjected to DNA extraction (Doyle and Doyle, 1990) using 100 mg of leaves from each sample. Total DNA was extracted from leaf tissue using a modified CTAB (CetylTrimethyl Ammonium Bromide) method (Permingeat *et al.,* 1998). DNA extracted from leaves samples was quantified by spectrophotometry with a Nanodrop at 260 nm and 280 nm wavelengths. The absorbance OD 260/280 ratio was used to assess the purity of the extracted DNA, considering optimal between the

values 1.8 and 2. DNA extracts showing optimal OD values were used for further PCR testing.

CMV and EACMV detection by Multiplex PCR assays

A multiplex-PCR was performed with specific primer pairs CMBrep/F, ACMVrep/R and EACMVrep/R (Alabi *et al.*, 2008). The primer pair CMBRep/F and ACMVRep/R amplified 368 nt fragment specific to ACMV and the primer pair CMBRep/F and EACMVRep/R amplified 650 nt fragment specific to EACMCV. Each amplification was performed in a final reaction volume of 12.5 µl. The reaction consisted of 6.25 µl GoTaq PCR buffer (2×), 1.25 µl for CMB (10 µM), ACMV (10 µM) and EACMV (10 µM) primers, 0.5 µl ultrapure PCR water, and 2 µl of extracted DNA. 2µl used as negative control. PCR reactions were performed in a T100 m BIORAD thermal cycler with the following program. An initial denaturation cycle at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute and elongation at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

Satellite associated to CMV and EACMV detection by PCR assays

A PCR to amplify specifically DNA-associated satellites was carried out with the specific primer pairs Sat IIF/R and SatIIIF/R (Pita *et al*., 2001) on the same samples (five samples of cassava and five samples of *C. odorata*). Each amplification was performed in a total reaction volume of 25 µl. The reaction contained 12.5 µl of Gotaq reaction buffer (2x), 1.0 µl for each primer, 8.5 µl of PCR ultrapure water and 2 µl of DNA.
For each PCR reaction, a negative control containing PCR grade water instead of DNA was used. The PCR program included an initial denaturation cycle at 94°C for 3 minutes followed by 30 cycles of denaturation at 94°C for one minute, annealing at 56°C for one min 30 s, elongation at 72°C for 1 minute, and a final extension at 72°C for 5 minutes in the T100 m BIORAD thermal cycler.

Visualization of PCR products

All PCR products were subjected to agarose gel electrophoresis in 1X TAE (Tris Acetate- EDTA) buffer for 40 minutes at 70 Volts. To check the PCR products, 2 µl of each sample was loaded on 1% (w/v) agarose gel stained with ethidium bromide (0.5 µg/ml) and visualized under UV trans illuminator. The gels were observed.

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Statistical analysis

In that study all the data obtained were analyzed with the R Studio 4.1.2 software (Rebekka*, et al*., 2023). Analysis of variance with one classification criterion (ANOVA I) was performed to compare the averages of severity of symptoms by location. For any significant differences following the analyses between the severity means at the 5% threshold, a Fisher LSD (Lijing Ma *et al.,* 2023) test was performed to identify homogeneous groups.

RESULTS

Diversity and similarity of symptoms on infected leaves of cassava and *Chromoleana odorata*

Several symptoms were observed on the plants sampled in all the localities of our study. Infected leaves of cassava and *Chromoleana odorata* showed diverse types of symptoms. These included discoloration, distortion, and leaf size reduction. Asymptomatic leaves of cassava were observed (Fig. 2A), mosaic, reduction leaves and distortion (Fig. 2B). Waffling, mosaic, reduction leaf and distortion showed (Fig. 2C), distortion and leaf embossing (Fig. 2D). In contrast to asymptomatic leaves (Fig. 2A), symptomatic leaves of *C.odorata* infected showed similar symptoms (Fig. 2E-2H). Symptoms were similar on infected in cassava field and *C.odorata* plants infected.

A wide range of symptom severity in cassava and *Chromoleana odorata*

A wide range of symptom severities was observed on both plants from 2.8 to 3.8.
Distortion was observed with the highest mean severity of 3.8 on leaves of cassava and *Chromoleana odorata* regardless of the location. Leaf embossing, mosaic and leaf reduction were the most severe symptoms in Abidjan with an average value of 3.8 in cassava. In samples from the other localities, mosaic was predominant in Abengourou with mean values of 3.8 and 2.8, respectively. However, statistical analysis showed no significant difference ($p > 0.05$) between the mean severities of symptoms according to locality.
C.odorata plants showed infected leaves with higher mean of symptom severities in Abidjan than the other localities with a mean value of 3.8. However, no significant difference ($p > 0.05$) between the mean severities of these symptoms was noted. Cassava and *C. odorata* developed leaf distortion and discoloration with very high mean levels of severities in the different localities, particularly in Abidjan.

Co-infection of *Chromoleana odorata* and cassava with ACMV and EACMV

Using DNA extracts from the various leaf samples, multiplex-PCR was performed with primer pairs CMB- R / ACMV- F / EACMV-F. Migration of the amplification products confirmed the presence of amplicons from the

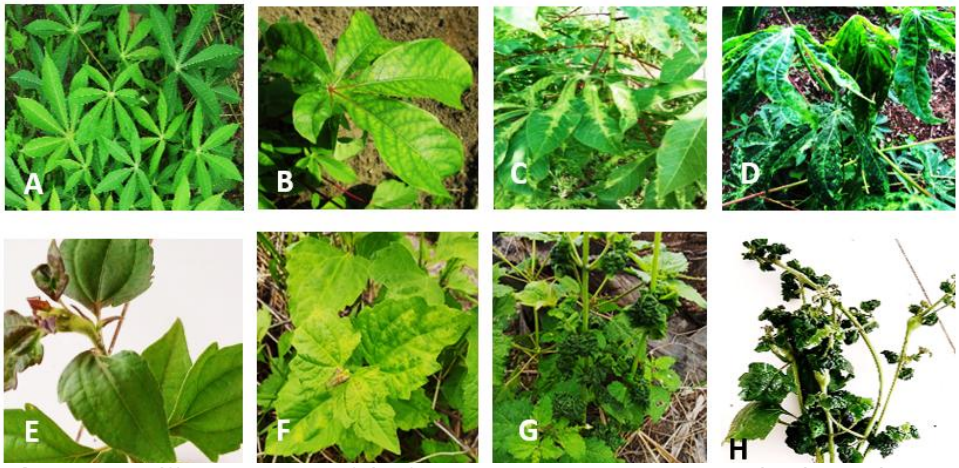

Figure 2: Different types of leaf symptoms on cassava and *Chromolaena odorata*. **A:** Cassava asymptomatic leaf, **B:** Mosaic plus reduction plus leaf deformation of cassava, **C:** Cassava waffling and leaf size reduction, **D:** Cassava leaf size reduction, **E:** *C. odorata* asymptomatic leaf, **F:** Mosaic plus reduction plus leaf deformation of *C. odorata*, **G:***C. odorata* waffling and leaf size reduction, **H:** Cassava leaf size reduction.

primer pairs used. Two amplification bands with expected sizes were obtained from the mixture of PCR products. The size of the amplicons was 368 bp and 650 bp respectively (Fig.3), corresponding to the molecular weights of the Begomoviruses ACMV and EACMV respectively.

Satellites of ACMV and EACMV in cassava leaves

PCR of DNA from the various leaf samples was carried out in the presence of the Sat II F /R and Sat III F /R primer pairs. Migration of the amplification products confirmed the presence of amplicons from the primer pair used at 895 bp and 306 bp on the agarose gel respectively (Fig.4B and 4C). Molecular methods were used to identify the Begomoviruses and their associated satellites present in the leaves collected. These cassava begomoviruses and their satellites were identified in the leaves presenting the two types of symptoms: discoloration and organ deformation, as well as in the different forms of symptoms associated with them. Of all these agents, ACMV and Sat II were identified in both cassava and *Chromolaena odorata* leaves in all localities. Moreover, ACMV was the more prevalent Begomovirus than EACMV in cassava. EACMV and satellites Sat II and Sat III were associated with at least two forms of symptoms.

The Begomoviruses tested were associated with both types of symptoms, with the exception of Sat III in cassava. All the Begomoviruses and their satellites were associated with the waffling observed on *C.odorata* plants and with the mosaic observed on cassava.

DISCUSSION

Chromolaena odorata (L.) King and Robinson (Asteraceae) also named *Eupatorium odoratum* or Siam weed is one of the world's most invasive plant species, with an area of distribution extending over Central and Western Africa in Côte d'Ivoire (Gautam *et al*., 2014).The ecological factors of development and optimal growth of this weed seem to be present in Côte d'Ivoire. However, that study revealed infected cassava and *C.odorata* plants, showed similar symptoms on leaves, including leaf discoloration and deformation (waffling, mosaic, size reduction). These symptoms have been associated with plant susceptibility to Begomoviruses, particularly ACMV and EACMV, and have been previously observed not only on cassava but also yam and papaya (Toualy *et al*., 2014) and on *C. odorata* (Alabi *et al.,* 2008). Symptoms specific to Begomovirus infections in cassava such as waffle and mosaic disease, which are typically associated with CMV vary with the level of disease severity and cultivar (CMD resistant or susceptible) (Hillocks and Thresh, 2002).

On the other hand, the exacerbation of symptoms by synergistic phenomena between several viruses species has been documented (Ariyo *et al*., 2005). The main consequences of these symptoms that develop on the leaves are the loss of yield of cassava tubers. Since these symptoms have been also observed on the leaves of *C. odorata*, they could be related to the polyphagous nature of the whiteflies specific for cassava.

Other polyphagous vectors for instance those for the *tobacco* mosaic virus could also infect the leaves of C. odorata.

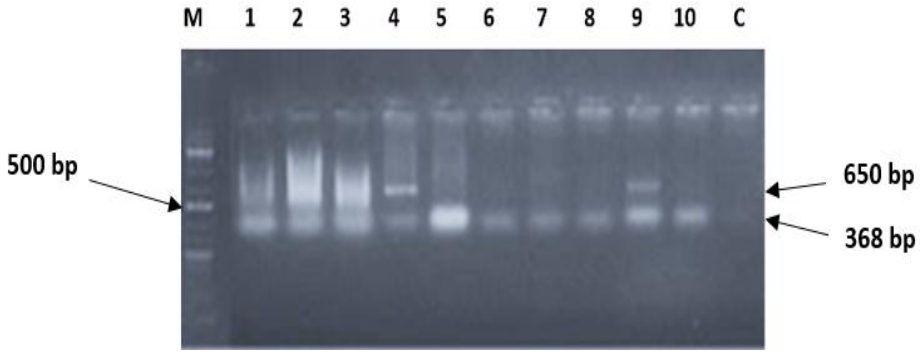

Figure 3: Electrophoresis gel on agarose (1%) of multiplex-PCR products with primers CMBrep/F, ACMVrep/R and EACMVrep/R for ACMV and EACMV detectionin cassava and *Chromolaenaodorata*leaves. **M:** molecular weight marker of size 1 Kb. **1-5:** *C. odorata* samples tested.**6-10:**cassava samples tested. **C:** control (water).

mosaic virus could also infect the leaves of *C. odorata*. This weed could be a natural reservoir of viruses responsible for infection of other crops by showing The presence of *C.odorata* in cassava fields could pose a threat of virus infection for cassava. Indeed, insect vectors can acquire the virus while feeding on *C.odorata* leaves and then transmit it to cassava by inoculation during a second feeding phase. The permanent presence of this virus reservoir plant, *C. odorata*, may result in a very high probability of virus incidence in crops (Traoré *et al.,* 2013). Although weeds can serve as sources of inoculum for viruses (Gong *et al.,* 2013).These results are confirmed by the work of Traoré in 2013, which showed that *C.odorata* harbored viruses including CMV. The interaction between plant viruses, potential reservoirs, and insect vectors for spraying has poorly studied *C. odorata* in Côted'Ivoire (Kouadio *et al.,* 2015).The development of similar symptoms in both cassava and *C. odorata* on the same geographic location could explain a possible inter-infection phase that may keep a pathogenic pool leading to the development of new genotypes and consequently increasing the severity of the symptoms.

In cassava, ACMV and EACMV are responsible for natural mixed infections that result in severe disease symptoms (Ogbe *et al*., 1999; Angela *et al.,* 2021). The severity of symptoms on cassava leaves and *C.odorata* seen in both infected plants was also observed in sweetpotato associated with co-infection of the chlorotic stunt virus (SPCSV) with the sweetpotato feathery mottle virus (SPFMV) that resulted in an increased symptom severity and large yield loss in sweetpotato (Angela *et al.,*2021). PCR analyses confirmed co-infection of cassava and *C.odorata* with ACMV and EACMV. Work conducted by Monde *et al.,* (2010); Alabi *et al.,* (2008) deformations and discoloration of cassava leaves (Ezeji*et al.,* 2023). This may be related to the cultivation zone as shown in our study.

and Ogbe *et al*., (2006) on *Ageratum conyzoides*, *Boerhavia diffusa*, *Croton hirtus*, *Euphorbia heterophylla*, and *C. odorata*in Nigeria showed that these weeds served as alternative hosts for plant pathogenic viruses. Thus, *C.odorata* leaf's samples were also infected with ACMV and EACMV as in cassava.

Virus development in cassava is most likely maintained by the presence of *C.odorata* plants that harbor the same viruses responsible f or the infection. The two plants exchange viral inoculum through whiteflies vectors that feed on them. Cassava mosaic virus is the common name for one of eleven different species of plant pathogenic viruses in the genus Begomovirus. *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *South African cassava mosaic virus* (SACMV) are distinct species of circular, single-stranded DNA viruses that are transmitted by whiteflies and primarily infect cassava plants.

When the Begomoviruses ACMV and EACMV infect cassava, the plant defense system is triggered following the viral infection. Cassava uses gene silencing to suppress viral replication to mitigate viral pathogenic activity. However, Begomoviruses generate a suppressor protein that acts against this natural defense of cassava. Dual infections of EACMV and ACMV or EACMV-UG and ACMV commonly lead to synergistic interactions resulting in more severe symptoms (Harrison *et al.,* 1997; Fondong *et al.,* 2000). The diversity and severity of symptoms observed in cassava is a result of the weakening of the plant's defense system. Begomovirus-associated Betasatellites, as well as at least one Alphasate-

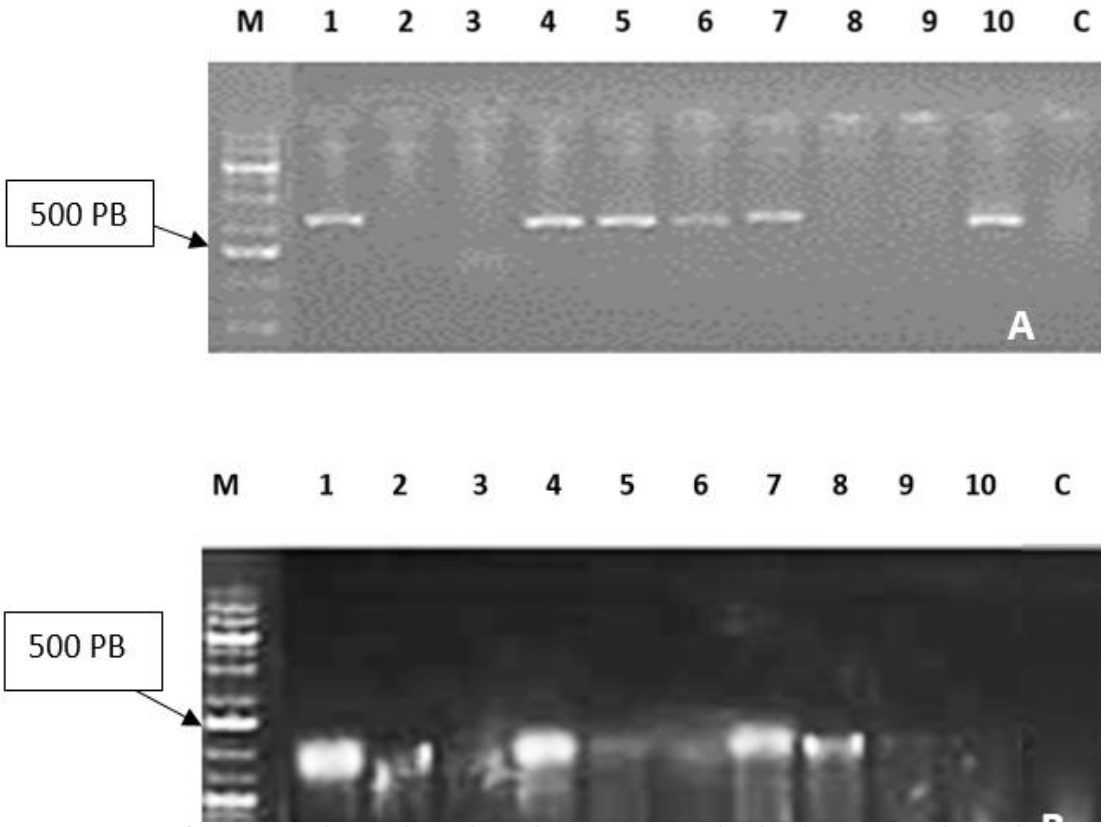

Figure 4: Electrophoresis gel on agarose (1%) of PCR products for satellites Sat II and Sat III detection in cassava and *Chromolaenaodorata* leaves. **A:** with primersSat II F/R.**B:**with primer Sat III F/R.**M:**molecular weight marker of size 1 Kb. **1-5:***C.odorata*samples tested. **6-10:** cassava samples tested.**C:** control (water).

llite, enhance symptom severity and viral DNA accumulation (Ali *et al.,* 2011).

CONCLUSION

Cassava cultivation in Côte d'Ivoire is threatened by a viral co-infection in the areas of Abidjan, Grand-Lahou, Abengourou, and Toumodi. The virus infection of cassava in these major growing areas is associated with a diversity of symptoms characterized by leaf waffling, mosaic, size reduction, and deformation. A similar symptom complex was observed also in *Chromoleana odorata* with the same levels of severity.

The similarity of the symptoms on these two plants grown at the same geographic area may be explained by the co-infection of ACMV and EACMV with their associated satellites. The satellites would be involved in the expression of virulence of the assisting viruses, hence the very high prevalence and severity of symptoms observed in cassava.

The weed *C. odorata* present in infected cassava fields could be a natural reservoir for Begomoviruses and their satellites. A viral co-infection of ACMV and EACMV, their satellites, and their spread from the weed *C. odorata* in Côte d'Ivoire therefore threaten cassava cultivation and production.

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