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RESEARCH ARTICLE

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# Epidemiological Assessment of Cassava Mosaic Disease (CMD) in Côte D'Ivoire and Benin

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## ABSTRACT

Cassava mosaic disease (CMD) is the primary threat to cassava cultivation throughout Africa. This leads to important losses to farmers. However, environmental conditions and the types of cultivars greatly influence disease manifestation. Hence, this study aimed to evaluate epidemiological parameters in two agricultural systems to determine the key environmental factors influencing CMD. Surveys were conducted conjointly in both Benin and Côte d'Ivoire in 2015 using WAVE harmonized protocol. 134 fields were surveyed throughout Benin, while 160 were surveyed in Côte d'Ivoire. The overall incidence of CMD was higher in Côte d'Ivoire (46.25$\pm$2.20%) than in Benin (22.81$\pm$1.90%). The prevalence of CMD was significantly higher in Côte d'Ivoire. In fact, 96.25% of the plots in Côte d'Ivoire had infected plants, compared to 79.11% in Benin. The percentage of observed healthy plants was higher in Benin (77.18%) than in Côte d'Ivoire (53.74%). The viruses causing CMD in West Africa, the African cassava mosaic virus (ACMV) and the East African cassava mosaic virus (EACMV), were present in both countries. The viruses were mostly found in double infection cases, but EACMV was found as a single infection in Benin. When comparing similar agro-climatic zones in both surveyed countries, we found a higher incidence of CMD in Côte d'Ivoire than in Benin. In all locations, cutting-borne infections were higher. The results of this study will help understand and manage the epidemiology of CMD in West Africa.

**Keywords:** CMD, Epidemiology, ACMV, EACMV, Agro-climatic zone, Benin, Côte d'Ivoire.

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## INTRODUCTION

Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is one of the important crops that provides staple food for several million people in sub-Saharan Africa (Houngue et al., 2018) and has become a good income source to families, and subsistence farmers all year round. It was introduced in West Africa from South America in 1558 (16th century) and into East Africa in the 18th century (Portu et al., 2016; Mendoza et al., 2018). It plays an essential role in food security, job creation, and income generation in West Africa (Houngue et al., 2019). It's a good food security crop in many African countries. Today, cassava has become one of the major staple crops cultivated across sub-Saharan Africa, producing 177 million metric tons (MMT) of the total world production of 284.9 MMT (FAOSTAT, 2022). Although the African continent contains most (62%) of the global cassava growing area, cassava productivity in Africa (8.8 t/ha) is much lower than the world average (10.95t/ha) and the average in Asia (16.8t/ha) (FAOSTAT, 2022). One reason for the low productivity of cassava is the attack by numerous insect pests and diseases (Kalyebi et al., 2018). Like insect pests, whitefly (*Bemisia tabaci*) constitutes the biological vector of virus to the host and feeds voracious

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phloem, honeydew production and subsequent sooty mold growth (Bellotti et al., 1999). It transmits the viruses to cassava plants (Silla et al., 2012), and is found wherever cassava is produced. This biological vector is an important epidemiological factor because its population greatly impacts the spread of the virus. Among the diseases, Cassava Mosaic Disease (CMD) is currently a devastating disease to cassava (Alicai et al., 2007) in West Africa. It is caused by eleven virus species of Begomovirus genus and *Geminiviridae* family. CMD is manifested as leaf chlorosis followed by distortion and plant dieback. It can cause yield losses of 20-90 % (Thresh et al., 1994) and 100 % in susceptible cultivars (Legg et al., 2011). Among the 11 species causing CMD described to date (Legg et al., 2016; Fondong, 2017), the *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) are widely prevalent (Toualy et al., 2014; Houngue et al., 2019). Although CMD caused serious damage to cassava production in West Africa countries (Toualy et al., 2014; Houngue et al., 2019), disease incidence and severity varied among countries according to cultural practices and management strategies developed. Exclusively, in Benin and Cote d'Ivoire which are the focused case of this study, three agro-climatic zones with slight differences in rainfall, temperature, humidity, and soil are existed (MEHU, 2015). In the three agro-climatic zones, namely Sudanian, Sudano-guinean, and Guinean, CMD epidemiological parameters such as whitefly vector populations, CMD incidence and severity, virus strains, and host plants may be influenced by environmental conditions variations. Understanding the epidemiological factors in both countries may explain whether cassava production is varied and what should be urgent action for improving cassava productivity.

## MATERIALS & METHODS

### Study Areas

This study was carried out in Benin and Côte d'Ivoire, two countries located in West Africa. Both countries are divided to three agro-climatic zones (Sub-equatorial zone (SEZ), Sub-tropical transition zone (STTZ), Sub-tropical zone (STZ)) with slight difference according to country (Fig. 1).

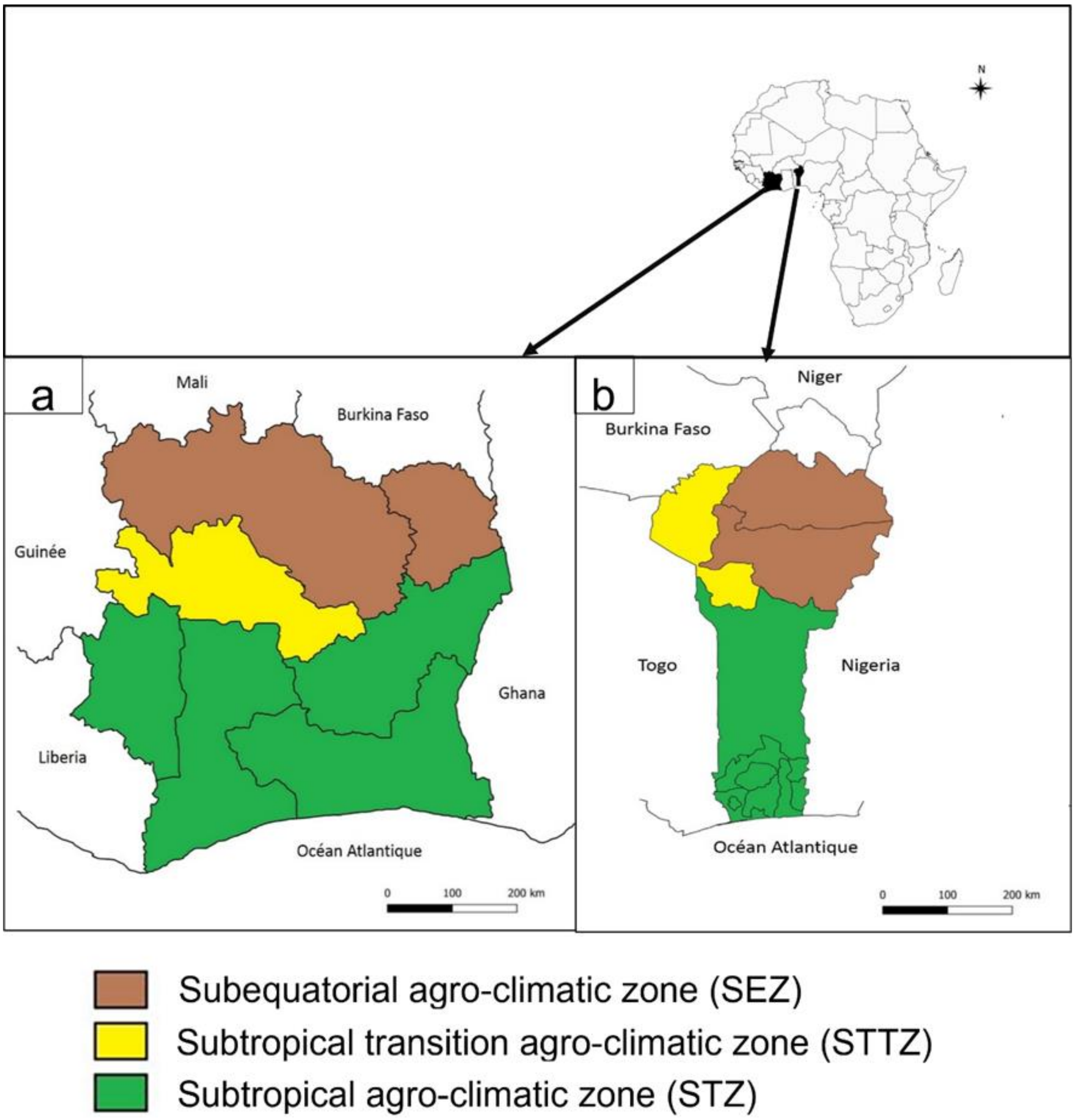


**Fig. 1:** Three similar agro-climatic zones in Côte d'Ivoire (a) and Benin (b). The subequatorial agro-climatic zone (SEZ) in the southern region is represented in green. The subtropical transition agro-climatic zone (STTZ) is colored yellow. The subtropical transition agro-climatic zone (STZ) is colored brown.

### Weather Data

Concerning Benin, SEZ is characterized by two rainy seasons with annual rainfall ranging from 1100 to 1400mm and temperatures between 25 and 35°C whereas STTZ has an average annual rainfall ranging from 800 to 1400mm (MEHU, 2015). The sub-tropical zone STZ receives an average rainfall from 900 to 1300mm and temperatures between 28 and 40°C. Regarding Côte d'Ivoire, SEZ is characterized by an annual rainfall ranging from 1300 to 2500mm wand temperatures ranging from 25 to 30°C whereas STTZ has annual rainfall from 1300 to 1750 mm and temperatures ranging from 20 to 27°C. The sub-tropical zone (STZ) is characterized by annual rainfall ranging from 11,150 to 1,350mm and temperatures between 25 and 28°C (Anonymous, 2016).

### Plots Selection, Survey and Data Collection

Plots of cassava plants between 3 and 6 months old were surveyed in each of the three agro-climatic zones of Côte d'Ivoire and Benin. A total of 134 fields were surveyed in Benin between June and December 2015 and 160 fields were surveyed in Cote d'Ivoire in the same period. Plots 8–10 km apart were sampled according to the availability of fields on either side of the main roads followed during the survey. In each plot, 30 cassava plants randomly were selected and assessed along two diagonals of the field. The information included cassava plant details as well as the location coordinates (latitude and longitude) and altitude of fields recorded using a global positioning system (Garmin eTrex, Summit HC) was recorded. Cassava Mosaic Disease severity was assessed based on the standard severity scale with a range of 1–5 defined by Hahn et al. (1980) (Table 1). The mode of infection (whitefly-borne or cutting-borne) was determined according to the presence of symptoms on the top of the plant or all over the leaves. Four leaf samples per field were collected and stored in a herbarium press and labeled with an identifier composed of field and plant number as well as the collection date.

**Table 1:** The CMD symptom scale used for plant scoring (Hahn et al., 1980)

| Scale | Symptom description |
|---|---|
| 1 | Unaffected shoots (no symptoms) |
| 2 | Mild chlorosis, mild distortions at the bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy (symptoms on about 25% of the leaves) |
| 3 | Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower third of the leaflets (symptoms on about 50% of the leaves) |
| 4 | Severe mosaic distortion of two-thirds of most leaves, general reduction of leaf size, and stunting of shoots (symptoms on about 75% of the leaves) |
| 5 | Very severe mosaic symptoms in all leaves, distortion, twisting, and severe reduction in most leaves, accompanied by severe stunting of plants (symptoms on about 100% of the leaves) |

### Molecular Diagnostic of CMD

DNA was extracted from the collected leaves sample using a CTAB-modified protocol (Doyle and Doyle, 1990). PCR was performed to detect the viruses in the collected leaves sample using several primer pairs: JSP001 (5'ATGTCGAAGCGACCAGGAGAT-3') and JSP002 (3'-TGTTTATTAATTGCCAATACT-5') for *African cassava mosaic virus* (ACMV), and JSP001 and JSP003 (3'-CCTTTATTAATTTGTCACTGC-5') for *East African cassava mosaic virus* (EACMV) (Pita et al., 2001). These couple of primers amplified the coat protein (CP) gene sequence respectively in ACMV and EACMV. The PCR cycling conditions consisted of an initial denaturation step of 94°C for 5min followed by 35 cycles of 45s at 94°C, 45s at 55°C, 55s at 72°C and a final step of 7min at 72°C. PCR was conducted in a total volume of 12.5µL using 1.25µL of $MgCl_2$ (25mM), 2.5µL of 10 × PCR buffer (Qiagen, Hilden, Germany), 0.5µL of each primer (10µmol), 0.25µL of dNTP (10 mM), 0.05µL of Taq Polymerase (Qiagen HotStar Plus TM PCR) and 2µL of DNA template (150 ng/µL). The PCR products were electrophoresed on 1% agarose gels. The gel was then stained in a BET bath and visualized under UV light in a transilluminator.

### Data Processing and Analysis

Data were visualized at Agro-climatic zone and country levels as tables, graphs, or histograms to assist our analysis. CMD incidence (Number of infected plant / Total plant surveyed in the field) has served to build the disease distribution maps using geographic coordinates (longitude and latitude). To evaluate the effect of agro-climatic zones on the incidence of CMD, the t-test was performed. A chi-squared test ($\chi^2$) was run to compare the CMD severity rate, the proportion of cutting-borne infection, whitefly-borne infection, and virus infection rate. Person-correlation test were performed between the CMD incidence and infection mode. All tests were performed using R 3.4.4 (R Core Team, 2018). The comparison was made at the country and agro-climatic zone levels.

## RESULTS

### Observed Symptoms

Similar symptoms of CMD were observed in Côte d'Ivoire and Benin. These include plants with slight or severe mosaic (chlorosis) and leaf deformities. In Côte d'Ivoire, these symptoms were observed in 33.89% of total plants assessed whereas 9.6% is observed in Benin. Leaf deformations were observed in 10% of the plants grown in Côte d'Ivoire compared with 12% in Benin. Curled leaves, stunted plants (reduced vegetative apparatus), and reduction of the leaf blade at the main vein (shoestring) were also observed in 10% of plants in Côte d'Ivoire versus 1% in Benin. (Fig. 2).

### Distribution and Incidence of CMD

CMD symptoms were observed in all agro-climatic zones in both countries, although some fields were symptomless, whereas in others, all plants were infected (Fig. 3). The incidence of CMD was significantly higher in Côte d'Ivoire (46.25±2.2%) than in Benin (22.81±1.9%) ($P<0.0001$) (Table S1). The incidence was significantly different in the agro-climatic zones of the two countries ($P<0.0001$). In fact, the three agro-climatic zones of Côte d'Ivoire recorded higher values than those of Benin. However, while the incidence of Côte d'Ivoire was highest in the Sub-equatorial zone (SEZ), in Benin the Sub-tropical zone (STZ) recorded the highest incidence of CMD (Fig. 4).


**Fig. 2:** Representative similar symptoms of CMD observed during surveys in Côte d'Ivoire and Benin. a: Chlorosis and early leaf deformation; b and c: Curled leaves; d: reduction from the blade to the main rib.

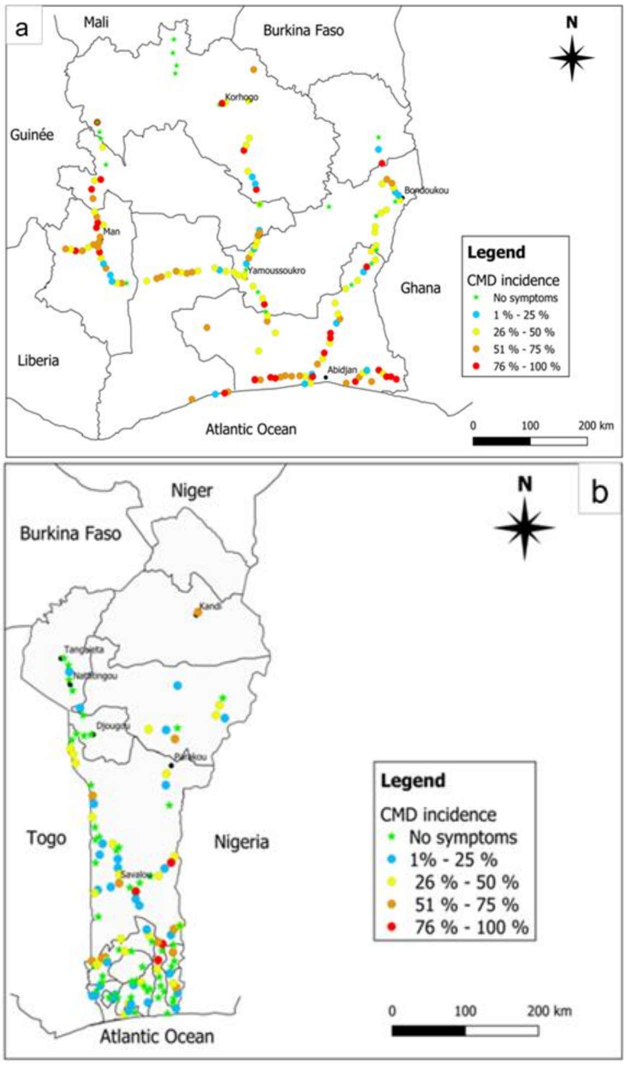


**Fig. 3:** Distribution of CMD following its incidence in Côte d'Ivoire (a) and in Benin (b).CMD was regularly distributed in both countries, with incidence varying from 1% for uninfected plots to 100% for all infected plots.

## Severity of CMD in Benin and Côte d'Ivoire

The overall severity of CMD did not differ significantly between Benin and Côte d'Ivoire, with recorded average severity levels of 2.16 and 2.24, respectively. Statistical analyses showed a significant difference (P<0.001) in the proportion of plants with severity 1 and plant severity 2 between Côte d'Ivoire and Benin. Indeed, the proportion of plants with the severity1 was higher in Benin (77.18%) than in Côte d'Ivoire (53.74%), whereas the proportion of severity 2 was higher in Côte d'Ivoire (33.89%) than in Benin (9.6%) (Table S1). The proportions of severity levels 3, 4, and 5 were relatively equal in both countries (Fig. 5).

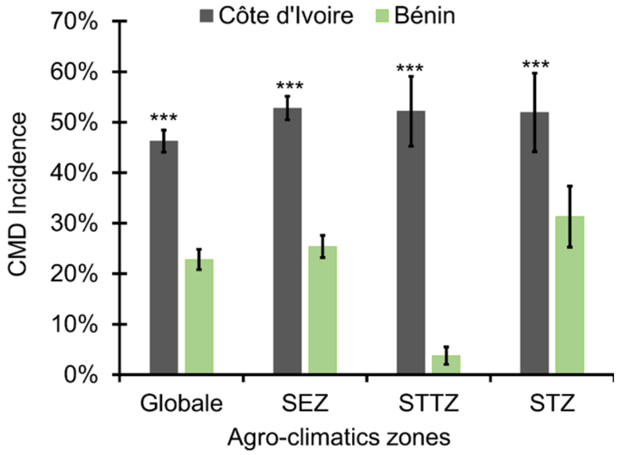


**Fig. 4:** Incidence of CMD in Côte d'Ivoire and Benin. Data are mean±SE (standard error). The global incidence of CMD was significantly higher in the Côte d'Ivoire as-well-as in all three agro-climatic zones. SEZ: subequatorial agro-climatic zone; STTZ: subtropical transition agro-climatic zone; STZ: subtropical agro-climatic zone. *** represents significant difference at P<0.001 according to the t-test.

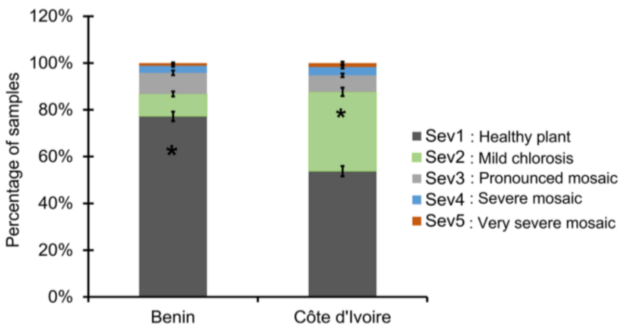


**Fig. 5:** Severity of CMD in Côte d'Ivoire and Benin. Data are average ± SE. The Sev1: rate of healthy plants was significantly higher in Benin. The rate of Sev2, which is the rate of plants with mild chlorosis, was significantly higher in Côte d'Ivoire. The rates of Sev3, Sev4, and Sev5, which are plants with pronounced mosaic, severe mosaic, and very severe mosaic, respectively, were not significantly different between both countries. *: significant difference at P<0.05 according to Chi-squared test ($\chi^2$).

## Rate of Infection Plots (Prevalence) and Mode of Infection

The Chi$^2$ test showed that the proportion of infected fields was significantly higher (P<0.0001) in Côte d'Ivoire than Benin. Indeed, 96.25% of the plots in Côte d'Ivoire had infected plants, compared to 79.11% in Benin (Table 2) (Table S1). Cutting-borne infection was higher than whitefly-borne infection in both countries (Fig. 6). A strong positive correlation (P<0.05; r=0.96) between cutting-borne infection and disease incidence was observed in both countries.

**Table 2:** Rates of infection in Côte d'Ivoire and Benin

| | Côte d'Ivoire | Benin |
|---|---|---|
| Number of visited plots | 160 | 134 |
| Number of plots attacked by CMD | 154 (96.25%) *** | 106 (79.11%) |
| Number of CMD-free plots | 6 (3.75%) | 28 (20.89%) *** |

***: P<0.001

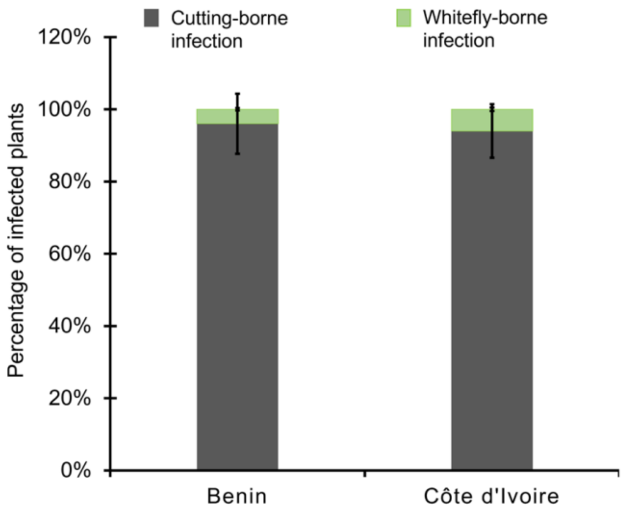


**Fig. 6:** Rate of the modes of infection in Côte d'Ivoire and Benin**.** The percentage of cutting- and whitefly-borne infections was higher in both Benin and Côte d'Ivoire. The rates of cutting-borne infection were not significantly different between both countries as well as whitefly-borne infection (P>0.05, Chi-squared test ($\chi^2$)).

### Cassava Mosaic Begomovirus (CMBs) Species Identified in Samples Collected in Côte d'Ivoire and Benin

Next, we investigated the viruses causing CMD. ACMV and EACMV were detected in both Benin and Côte d'Ivoire (Fig. 7 and 8). In Cote d'Ivoire, 68.51% of tested sample were positive ACMV whereas 98.16% were positive in Benin. The viruses were found as a single infection or double infection in 31.49% in Côte d'Ivoire and 0.92% in Benin. Surprisingly, EACMV was found as a single infection in Benin but not in Côte d'Ivoire. According to the Chi$^2$ test, the rate of ACMV detected in samples from Benin was significantly higher than that detected in samples from Côte d'Ivoire (P<0.001). In contrast to Côte d'Ivoire, where EACMV was not detected in a single infection, it was detected in two samples from Benin. Very low levels of ACMV and EACMV in sample with double infection were observed in Benin (Table 3) (Table S1).

## DISCUSSION

The CMD was observed in all agro-climatic zones in Benin and Côte d'Ivoire. Indeed, several symptoms, reported in previous work (N'zué et al., 2013; Toualy et al., 2014) as being caused by viruses associated with CMD were identified in the fields surveyed in these two countries. Although the incidence of CMD varied from 0% to 100% in both countries, its overall incidence was higher in Côte d'Ivoire than in Benin. This may be due to the high rate of adoption of improved varieties in Benin. Three improved cassava varieties, RB 89509; BEN 86052, and TMS 30572, have been introduced by the Beninese Agricultural Research Centers and disseminated by the Regional Action Center for Rural Development (CARDER). The variety BEN 86052 is recommended in Benin for its resistance to diseases and insect pests (Biaou et al., 2004; Cacaï, 2013). A previous study conducted in Benin showed that these three improved varieties are more commonly found in fields than local varieties (Djinadou et al., 2018). Unlike in Benin, improved varieties in Côte d'Ivoire have a lower adoption rate (Kouassi et al., 2018). In fact, producers prefer local varieties such as the variety Yacé, which is found in all Ivorian cassava production zones (Kouassi et al., 2018) but is very sensitive to CMD (N'zué et al., 2013). Mondé et al. (2013) showed that the incidence of CMD varies according to the cultivar and is higher in local cultivars. Together, these observations explain the higher number of healthy plants in Benin (77.18% in Benin and 53.74% in Côte d'Ivoire).

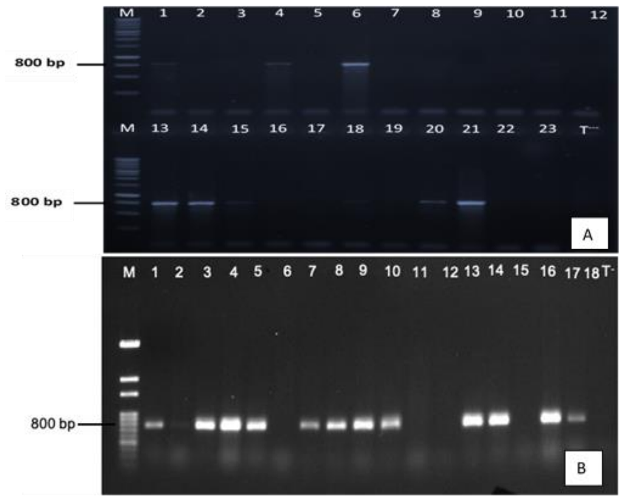


**Fig. 7:** Electrophoretic gel showing a part of tested samples positive to ACMV using the couple of primers JSP001/JSP002 specific to ACMV DNA-A (coat protein). The expected band size is 783 base pairs (bp). M = loader, T$^-$ = negative control (H2O). A: gel from Benin; B: gel from Côte d'Ivoire.

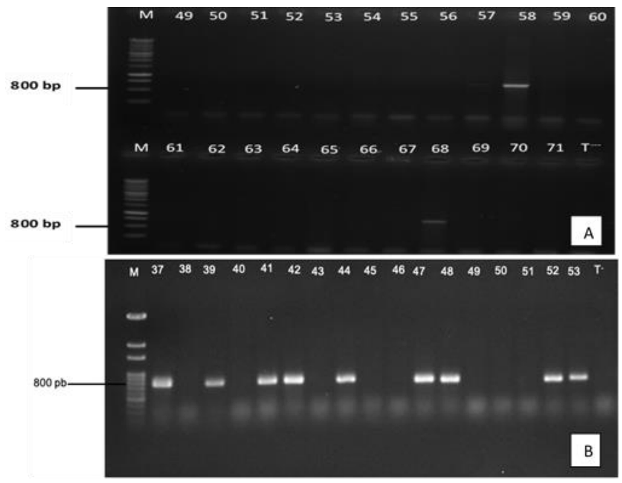


**Fig. 8:** Electrophoretic gel showing a part of tested samples positive to EACMV using a couple of primers JSP001/JSP003 specific to EACMV DNA-A (coat protein). The expected band size is 780bp. M = loader, T$^-$ = negative control (H2O). A: Gel from Benin; B: Gel from Côte d'Ivoire.

**Table 3:** Cassava Mosaic Geminiviruses (CMGs) detected in Côte d'Ivoire and Benin

| Countries | Number of PCR-positive samples | ACMV | EACMV | ACMV/EACMV |
|---|---|---|---|---|
| Côte d'Ivoire | 308 | 211 (68.51%) | 0 (0%) | 97 (31.49%) *** |
| Benin | 218 | 214 (98.16%) *** | 2 (0.92%) *** | 2 (0.92%) |

***: P<0.001.

When we compared similar agro-climatic zones in both countries, Côte d'Ivoire recorded higher CMD incidence rates than Benin. The climatic conditions in Côte d'Ivoire could be more favorable for the evolution of CMD. Indeed, temperatures are lower in Côte d'Ivoire than in Benin (MEHU, 2015; Anonymous, 2016; Bonou-gbo et al., 2017). Previous studies have shown that high temperatures are one of the main factors affecting the mortality of *B. tabaci* larvae (Ohnesorge et al., 1981; N'zi et al., 2019), which directly influences the dynamics of *B. tabaci* populations that transmit CMD. In Benin, the subtropical agro-climatic zone in the North, which is typically drier than the South, had the highest CMD incidence. In contrast, we expected the South to have a higher incidence due to the wetter climate there. This unusually pronounced disease occurrence could be due to the unusually high rainfall in northern Benin during the survey year. This observation is an example of the effects of climatic conditions on parasites involved in expressing plant diseases. This study showed that the prevalence of the disease expressed by the proportion of fields infected with CMD significantly differs between the two countries and is lower in Benin. The distribution of healthy planting material can contribute to reducing viral pressure. In Benin, sanitation programs for cassava varieties have provided healthy planting material to producers (Cacaï, 2013). Cutting-borne infection was higher than whitefly-borne infection in Benin and Côte d'Ivoire. Suggesting that producers used infected cuttings from previous harvests or from neighboring fields (Houngue et al., 2019; Djaha et al., 2018). This practice might lead to the re-infection of new plots because few farmers remove infected plants. Teaching farmers to recognize CMD symptoms will allow them to take appropriate measures to control the disease in their own field and minimize its spread to neighboring fields (Houngue et al., 2019). Samples collected from both countries were analyzed to identify the viruses responsible for CMD. We found that the rate of double infection in Côte d'Ivoire was higher than that in Benin, confirming the higher severity of CMD in Côte d'Ivoire. Fondong et al. (2000) showed that double infection increased the severity of CMD symptoms. In fact, these authors showed that when N. *benthamiana* plants were doubly inoculated with ACMV and EACMV (ACMV/CM, EACMV/CM) using sap from cassava plants or infectious clones, the symptoms were more severe than those observed on plants inoculated with either virus alone.

## Conclusion

This study showed that the CMD is widespread in all Agro-climatic zones of Côte d'Ivoire and Benin that it continues to spread to new cassava-growing areas of both countries. Plants showing severe mosaic symptoms were observed in both countries, with the risk of yield losses. The incidence of CMD is 46.25% Côte d'Ivoire and 22.81% in Benin. ACMV is detected in most of sample in Benin and Côte d'Ivoire. Co-infection ACMV and EACMV is detected in Côte d'Ivoire. Cutting-borne infection constituting the rapid method of virus propagation was dominant in both countries. It is therefore necessary that awareness-raising and training campaigns are also carried out by agricultural extension agents, non-governmental organizations, and research institutions on the use of healthy planting material and the adoption of varieties or resistant cultivars. We propose that future research efforts should be aimed at the sequencing of ACMV/EACMV associated with CMD in both countries to facilitate the management strategies.

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**Competing Interests:** The authors declare that they have no conflicts of interest.

**Authors' Contribution:** JSP initiated and designed the study. JSP mobilized funds for the research. EFY, JAH, SMBK, YAAN, and NKK collected samples. The EFY, OHD, NKK, and JAH data were analyzed. EFY, SMBK, YAAN, and OHD wrote the manuscript. JSP and CA reviewed the manuscript. All authors have read, corrected, and approved the manuscript.

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