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Incidence and distribution of cassava mosaic begomoviruses in Côte d'Ivoire

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Abstract

Cassava mosaic disease (CMD) caused by the whitefly-transmitted begomoviruses (family *Geminiviridae*) is a major threat to production of cassava (*Manihot esculenta* Crantz) in Côte d'Ivoire. A survey was conducted in the major production zones in Côte d'Ivoire to assess the incidence, severity, and distribution of cassava viral diseases. At each survey site, up to ten plants were assessed for symptom severity; incidence and samples were taken for virus testing. Techniques based on polymerase chain reaction (PCR) were used for the detection of cassava mosaic begomoviruses (CMBs) in the sampled leaves. Incidence of CMD varied from 0 to 100% and symptom severity from 1 to 5. Incidence differed significantly between the various agro-ecological zones ($P<0.001$), but severity was the same in those zones. Out of the 335 samples tested, *African cassava mosaic virus* (ACMV) was detected in 43.3%, *East African cassava mosaic Cameroon virus* (EACMCV) in 5.7%, and both ACMV and EACMCV in 31.3%; 19.7% of the samples analyzed were negative to all the viruses tested. None of the samples was tested positive to the *East African cassava mosaic virus-Uganda* (EACMV-Ug). These results suggest high incidence of CMD in the cassava production zones in Côte d'lvoire and underscores a need for implementation of control measures including phytosanitary measures with utilization of CMD-free materials for planting and adoption of resistant varieties.

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Introduction

Cassava (*Manihot esculenta* Crantz, family *Euphorbiaceae*) is the third largest source of carbohydrates in the world and an important food staple crop in sub-Saharan Africa (Fargette *et al*., 1994; Legg and Fauquet, 2004). The starchy tuberous roots are a source of food and income for more than 800 million people in Africa, Asia, and Latin America. Africa contributes more than 56% to the world's production (262.6 million tons) (FAO, 2014). Cassava is moving towards an industrialized system in which plant material is used for a variety of products including starch, flour, and animal feed (Thresh, 2006). Côte d'Ivoire is ranked no. 10 in area (360,000 ha) and no. 14 in production (2.4 million tons) among 40 cassava-producing countries in Africa (FAO, 2014). Most families consume cassava in various processed forms, such as attiéké (cassava couscous), foutou (pounded cassava mixed with pounded plantain), placali (paste), and gari (toasted granules). Human consumption of cassava leaves is popular only in the western part of the country. The demand for cassava and cassava-based foods is increasing in the country. However, productivity at 6.7 t/ha is very low compared with the average yield of 9.8 t/ha in Africa. This growing demand is mainly being met by an expansion in the cropping area which has increased by about 25% from 267,616 ha in 2002 to 360,000 ha in 2012 (FAO, 2014). Pests and diseases, especially cassava mosaic disease (CMD) caused by whitefly-transmitted begomoviruses (family *Geminiviridae*), are among the major factors for low yields. CMD is known to seriously decrease yields (Alabi *et al*., 2011), and the effects are further exacerbated by the widespread cultivation of susceptible landraces such as Yacé and Bonoua (N'Zué *et al*., 2005).

Nine different begomovirus species, commonly referred as cassava mosaic begomoviruses (CMBVs), have been identified in the CMD etiology in different regions of Africa (Alabi *et al*., 2011; Harimalala *et al*., 2012; Tiendrébéogo *et al*., 2012). Of the various CMBVs, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), and *East African cassava mosaic Cameroon virus* (EACMCV) are known to be widely prevalent in sub-Saharan Africa (Patil and Fauquet, 2009). Several strains of CMBVs have also been identified; most notable of these is EACMV-Uganda (EACMV-Ug) which was responsible for the devastating pandemic in East Africa in the 1990s (Legg *et al*., 2006). All these viruses are vectored by whitefly, *Bemisia tabaci* Gennadius (Hemiptera: *Aleyrodidae*), and also spread through the cuttings used routinely for vegetative propagation (Legg *et al*., 2011).

CMD in Côte d'Ivoire was first reported by Hedin in 1931. This disease was known to be endemic in the coastal areas and also in the northern parts (Walter, 1980). Past studies have identified the occurrence of ACMV (Walter, 1980) and EACMCV (Pita *et al*., 1999). This study was conducted to provide comprehensive information on the distribution and incidence of CMBVs and the severity of CMD in Côte d'Ivoire so that the complexity of disease situation could be understood and to contribute to the development of appropriate control measures.

Materials and methods

Survey

The survey was conducted in 2009 in 72 localities (farms) covering all the major cassava-growing areas. At each survey site, geo-reference points were taken using a GPS reader and details were recorded of location, varieties grown, and the incidence and severity of disease. Leaf samples for virus testing were taken from a minimum of five plants per field, wrapped in aluminium foil and stored in a cool box, and then transported to the laboratory for virus testing.

Disease incidence per field was calculated using the formula below:

$$\text{Incidence per field (\%)} = \frac{\text{Number of infected (symptomatic) plants} \times 100}{20}$$

The severity of CMD on symptomatic plants was assessed by rating plants on a 1 to 5 scale, as described by Hahn *et al*. (1980), where 1 = unaffected shoots (no symptoms); 2 = mild chlorosis, mild

distortions at 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 one-third of the leaflets (symptoms on about 50% of the leaves); 4 = severe mosaic distortion of two-thirds of most leaves and a general reduction of leaf size and stunting of shoots (symptoms on about 75% of the leaves); and 5 = very severe mosaic symptoms on all leaves, distortion, twisting, and severe reduction of most leaves, accompanied by the severe stunting of plants (symptoms on about 100% of the leaves).

DNA extraction

Total DNA was isolated from the leaf samples according to the protocol described by Dellaporta *et al.* (1983). About 50 to 100 mg of the leaf sample was ground in 500 µL of extraction buffer [100 mM Tris (pH 8.0) 8.5 mM EDTA and 10 mM β-mercaptoethanol]. Each extract was transferred into a 1.5 mL sterile microfuge tube and 33 µL of 20% SDS (Sodium dodecyl sulfate) was added in each tube. The mixture was vortexed briefly and incubated at 65°C in a water bath for 10 min. The tubes were allowed to cool to room temperature, and 160 µL of 5M potassium acetate was added to the mixture. The tubes were vortexed thoroughly and centrifuged at 10,000 g for 10 min. The supernatant was collected into a separate sterile microfuge tube and 200 µL of cold iso-propanol was added to the tube and incubated at 4°C for 20 min. The solution was centrifuged at 10,000 g for 10 min to precipitate DNA. The supernatant was carefully removed and the DNA pellet was washed with 500 µL of 70% ethanol and air dried at room temperature. The DNA pellet was dissolved in 50 µL of TE buffer and stored at -20°C until further use.

Polymerase Chain Reaction (PCR)-based detection of viruses

PCR assays with oligonucleotide primers specific to ACMV, EACMV-like viruses, EACMCV, and EACMV-Ug were used to detect cassava mosaic begomoviruses in the leaf samples collected from the field (Table 1). First, a multiplex-PCR assay developed by Alabi *et al.* (2008a) was used to test all the samples for the detection of ACMV and EACMV-like viruses using the primer CMBrep/F+ACMVrep/R+EACMVrep/R. All the samples that tested positive to EACMV were further analyzed for EACMCV using VNF031+VNF032 (Fondong *et al.*, 2000) and EACMV-Ug using specific primers UV-AL1/F1 and ACMV-CP/R3 (Zhou *et al.*, 1997).

PCR reaction composition was as follows: 2.5 µL of PCR reaction buffer (5x), 0.25 µL of 10 mM dNTPs (Promega, USA), 0.75 µL of 25 mM $MgCl_2$, 0.25 µL of 20 pM of each primer, 1 U *Taq* DNA Polymerase (Promega, USA), 2 µL of 1:50 (v/v) diluted DNA and sterile distilled water to a final volume of 12.5 µL. PCR assays were performed in a GeneAmp PCR System 9700 (Applied Biosystems, CA, USA) using one cycle of 94°C for 1 min; 52°C for 2 min, and 72°C for 3 min, followed by 36 cycles, in which each cycle consisted of 94°C at 1 min, 52°C for 2 min and 72°C for 1.33 min with a final extension at 72°C for 5 min. The PCR amplified products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light using a gel documentation system (Biorad Universal Hood, Biorad Laboratories, Milan, Italy).

Statistical analysis

Statistical analysis of the incidence and symptoms severity by zone were conducted with the analysis of variance (ANOVA) with one criteria of classification. In the case of difference between the means, they were compared using the LSD test at 5%. The software used was Statistica 7.1.

Results

Symptomatology

A total of 335 leaf samples were collected in the 72 farms surveyed. Different types of symptom phenotypes occurred in the different locations in all the surveyed fields. Severe or mild mosaic symptoms were observed on 14.9% of the symptomatic plants; coiling, shoe-string, leaf distortion, stunting, and leaf

reduction were also observed on 78.9% of the plants (Fig. 1)

Fig. 1. Different types of virus symptoms on cassava leaves. (A) Asymptomatic leaves, (B) mild mosaic, (C) severe mosaic, (D) severe mosaic, distortion, leaf curl, (E) severe mosaic, severe distortion, severe reduction of leaf area, stunting.

Detection of viruses

In multiplex-PCR, CMBrep/ F+ACMVrep/ R+EACMVrep/ R primers amplified expected DNA fragments of 400 bp corresponding to ACMV in 74.6% of the leaf samples and 650 bp corresponding to EACMV in 37.01%. Mixed infection of ACMV and EACMV was detected in 31.34% of the samples. All the 124 samples that tested positive to EACMV in multiplex-PCR were re-tested with specific primers for EACMCV and EACMV-Ug. EACMCV was detected in 121 samples (97.58%), indicating that EACMCV is the prevailing EACMV-type of virus in the country. None of the samples was tested positive to EACMV-Ug. Altogether, 80.3% of the leaf samples were tested positive to viruses (ACMV, EACMCV or both). Out of the 335 leaf samples 43.3% were found to be infected by ACMV alone; 5.7% by EACMCV alone, and 31.3% with both ACMV and EACMCV; 19.7% of samples analyzed were negative to all the viruses tested. ACMV was detected in 71 and EACMCV in 49 of the 72 locations surveyed (Fig. 2). Only 6 of the 24 asymptomatic samples were tested positive to ACMV or EACMCV.

Table 1. Primers used for the detection of cassava mosaic begomoviruses.

Target virus	***Primer***	***Primer sequence (5'→3')***	***Reference***
ACMV+EACMV	CMB Rep-F ACMV Rep-R EACMV Rep-R	CRTCAATGACGTTGTACCA CAGCGGMAGTAAGTCMGA GGTTTGCAGAGAACTACATC	Alabi *et al.*, 2008a
EACMCV	VNF031 VNF032	GGATACAGATAGGGTTCCCAC GACGAGGACAAGAATTCCAAT	Fondong *et al.*, 2000
EACMV-Ug	UV-AL1/F1 ACMV-CP/R3	TGTCTTCTGGGACTTGTGTG TGCCTCCTGATGATTATATGTC	Zhou *et al.*,1997

Table 2. Incidence and distribution of begomoviruses infecting cassava in Côte d'Ivoire.

Zones	***Viruses (%)***				
	ACMV	**EACMV**	**ACMV+ EACMV**	**EACMCV**	**EACMV-Ug**
Central	28.8	8.47	23.7	32.2	0
Central-West	33.3	9.1	27.3	30.3	0
East	22.2	5.6	69.4	75	0
North-East	69.2	0	19.2	19.2	0
North	47.4	7.7	17.9	24.3	0
South	52.4	2.9	36.9	39.8	0

NB: EACMCV percentage was calculated after detection by multiplex-PCR for ACMV and EACMV; all samples which were positive for EACMV were used for EACMCV and EACMV-Ug detection (124 tested samples after multiplex-PCR were positives for EACMV)

Distribution of cassava begomoviruses across zones

The incidence of ACMV was highest in the North-East (69.2%), North (47.4%) and South (52.4%) followed by Central-West (33.3%), Central (28.85%) and East (22.2%). Incidence of EACMV was low and varied from 2.9 to 9%; the virus was not detected in single infection in the North-East. Mixed infections of ACMV and EACMCV were found to be more common in all zones than the single infection of EACMCV (Table 2). EACMV-Ug was not detected in this survey.

CMD incidence in the six production zones varied from 41.9% to 59.4%. Central (58.4 %) and South (59.4%) zones have the highest incidence, North-East has intermediate incidence (54.8 %) and Central-West, East (41.9 %), and North zones (49.4%) have the lowest. Incidence was particularly higher in the southern part of Côte d'Ivoire than in the North. However there was no variation in CMD severity (Table 3).

Table 3. Incidence and severity of virus infection in different cassava-growing zones in Côte d'Ivoire.

Zones	Mean severity	Mean incidence (%)
Central	3.67 ± 0.16**a**	58.42 ± 1.93**a**
Central-West	3.78 ± 0.21**a**	43.38 ± 3.30**b**
East	3.56 ± 0.17**a**	41.94 ± 6.07**b**
North	3.24 ± 0.14**a**	49.48 ± 3.12**b**
North-East	3.58 ± 0.19**a**	54.81 ± 5.63**ab**
South	3.66 ± 0.11**a**	59.42 ± 2.61**a**
F	1.71	4.39
P	0.13	<0.001

Means followed by the same letter are not significantly different from one another at LSD (α = 0.05)

Incidence of cassava virus disease inside the fields

Disease incidence varied from 0 to 100% with a mean incidence of 52% for the 72 fields surveyed. Incidence exceeded 50% in 67% of the fields (Fig. 3). Highest incidence (100%) was recorded in two locations; Balamilido in the North-East, and Savane-recherche in the South; the lowest (0%) was recorded in one location, Tômougou in the North. Symptom severity ranged from 1 to 5 with a global mean of 3.5 (data not shown). Eighty-three percent of the sample leaves analyzed had severity scores of 3 to 5 (Fig. 4). Seven percent of all samples were asymptomatic (score = 1) and 10% had mild symptoms (score = 2).

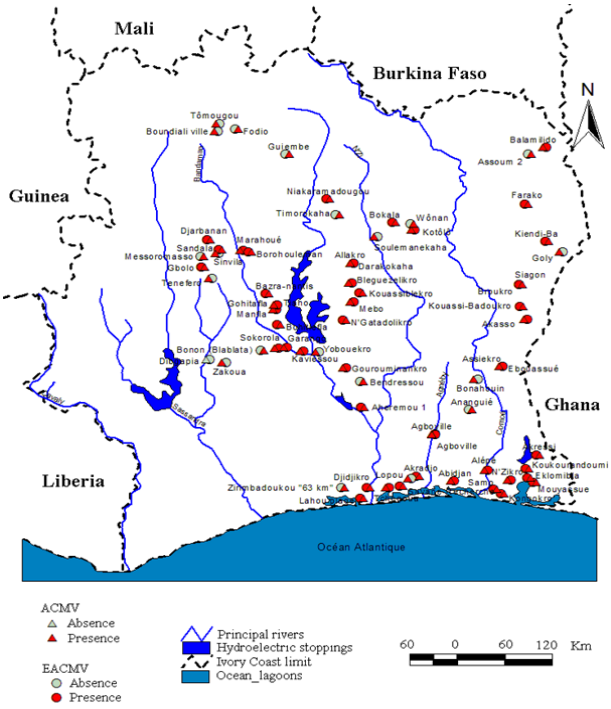

Fig. 2. Distribution of ACMV and EACMV in cassava-growing zones in Côte d'Ivoire.

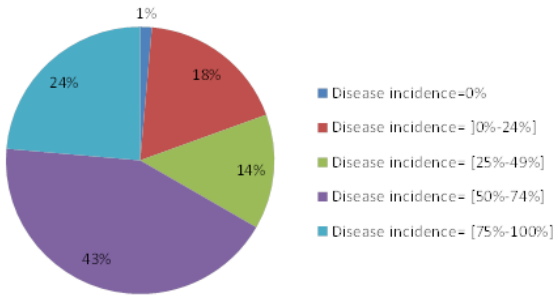

Fig. 3. Frequency (%) of fields by CMD incidence.

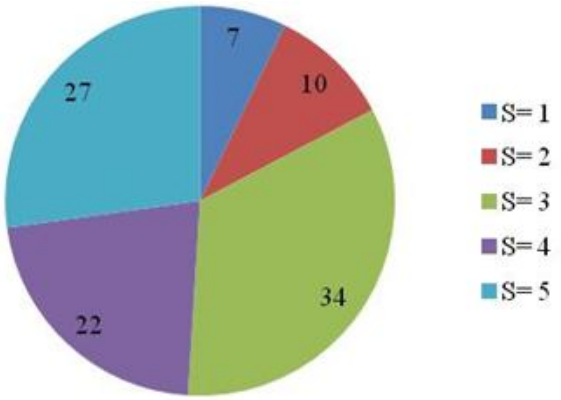

Fig. 4. Frequency (%) of leaf samples by CMD severity.

Discussion

Findings of this study confirm that CMD is an endemic problem in all agro-ecological zones of Côte d'Ivoire. The incidence of disease varied from one zone to another but severity was similar in all zones. The incidence was high in the southern part of the country and relatively low in the North. The high incidence rates observed in various fields suggests that stem cuttings are the likely origin of the virus. Traditionally, farmers reuse as planting materials stems from their own farms which are often infected by viruses. This explains why CMD is widely disseminated and may be prevalent in areas where disease spread by vectors is limited. In addition, the two widely grown cultivars, Yacé and Bonoua, were found to be highly susceptible to the viruses that cause CMD. Most of the planting materials in the fields are already infected thus creating a dearth of CMD-free material. This leads to the perpetuation of viruses through infected stems (N'zué *et al.*, 2005). Previous studies have reported a similar situation with regard to CMD in several countries in sub-Saharan Africa and suggest that symptoms depend on the virus species, strains, and mixed infections (Fauquet and Fargette, 1990; Harrison *et al.*, 1997; Otim-Nape *et al.*, 1997; Fondong *et al.*, 2000; Pita *et al.*, 2001a, b; Ogbe *et al.*, 2003; Alabi *et al.*, 2008b).

Analysis of infected cassava leaf samples confirmed the presence of ACMV and EACMCV but not EACMV-Ug. ACMV was the most prevalent begomovirus infecting cassava in all the zones of Côte d'Ivoire. Similar observations were reported by Harrison *et al.* (1997) in Uganda and Karakacha (2000) in Kenya. ACMV and EACMV occur in infected plants in Africa either alone or as mixed infections of different combinations (Fondong *et al.*, 2000; Berry and Rey, 2001; Ogbe *et al.*, 2003; Were *et al.*, 2004; Bull *et al.*, 2006). The proportion of single infections by EACMCV was lower (5.67%) than co-infections with ACMV (31%). Sources of inoculum are naturally infected plants when used as planting materials in successive years and also other herbaceous hosts of begomoviruses (Alabi *et al.*, 2008b). Together this may explain the severity observed on cassava in the survey. The majority of the new fields were planted with cuttings of plants harvested in previous fields, and probably infected. Also, the activities of insect vectors have an effect on CMD incidence and the transmission of begomoviruses (Patil and Fauquet, 2009). Most infections of EACMCV in Côte d'Ivoire were observed in the South.

Ogbe in his studies on begomoviruses on cassava in Nigeria (Ogbe *et al.*, 2001) observed that EACMCV was found in the humid forest, derived/coastal and southern Guinea savannas. EACMV-Ug was not detected in this study. However, occurrence of this strain in Burkina Faso underscores the need for vigilance against its spread in the country (Tiendrébéogo *et al.*, 2009).

Forty-eight (14.32%) samples from some symptomatic leaves were tested negative. No further investigations were made to determine the reasons for this negative reaction in PCR. Some samples from asymptomatic leaves were tested positive for viruses. This indicates that the absence of virus infection

cannot be assumed from the absence of visual symptoms on leaves.

Conclusion

The study confirms the occurrence of two cassava mosaic begomoviruses, ACMV and EACMV, causing CMD infection in Côte d'Ivoire. ACMV and mixed infections of ACMV and EACMV were the most frequently occurring viruses in the plants infected by CMD. Characterization of EACMV using species specific primers indicated that EACMV species prevalent in the country is EACMCV. The high levels of disease incidence and severity found in the surveyed fields warrant the wider introduction of CMD resistant varieties.

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