Received: 13 December 2022 | Accepted: 8 March 2023

DOI: 10.1111/ppa.13723

Plant Pathology WILEY

ORIGINAL ARTICLE

Occurrence of cassava mosaic begomoviruses in national cassava germplasm preserved in two agro-ecological zones of Ivory Coast

William J.-L. Amoakon[1,2] | Aya Ange Naté Yoboué[2,3] | Justin S. Pita[2,3] | J. Musembi Mutuku[2] | Boni N'Zué[4] | Mariam Combala[2,3] | Daniel H. Otron[2,3] | Maïmouna Koné[2,3] | Nazaire K. Kouassi[2,3] | Raoul Sié[1]

[1]UFR Sciences de la Nature, Université Nangui Abrogoua (UNA), 02 BP 801 Abidjan 02, Abidjan, Ivory Coast

[2]The Central and West African Virus Epidemiology (WAVE) for Food Security Program, Pôle Scientifique et d'Innovation, Bingerville, Université Félix Houphouët-Boigny (UFHB), Abidjan, Ivory Coast

[3]UFR Biosciences, Université Félix Houphouët-Boigny (UFHB), 01 BP V34 Abidjan 01, Abidjan, Ivory Coast

[4]Centre National de Recherche Agronomique (CNRA), 01 BP 633 Bouaké 01, Bouake, Ivory Coast

Correspondence
William J.-L. Amoakon, UFR Sciences de la Nature, Université Nangui Abrogoua (UNA) 02 BP 801 Abidjan 02, Abidjan, Ivory Coast.
Email: willamoakon@gmail.com

Funding information
Bill & Melinda Gates Foundation; The UK Foreign, Commonwealth & Development Office, Grant/Award Number: INV-002969

Abstract

Cassava production in Ivory Coast is hampered by cassava mosaic disease (CMD), which is caused by several begomovirus species. To increase cassava production and conserve genetic resources, the Centre National de Recherche Agronomique (CNRA) maintains a germplasm collection of 610 cassava accessions of various origins, which is kept in open fields in Bouaké and Man. We carried out an epidemiological assessment of the collection and a molecular characterization of the viruses infecting the CNRA cassava germplasm. The field in Man was less affected by CMD (incidence 49.78%, severity 2.23, whitefly infection 2.23%), despite the abundant whitefly populations (1.34 per plant), compared to Bouaké (incidence 74.54%, severity 2.92, whitefly infection 8.04% and whitefly population of 0.34 per plant). However, the predominant mode of CMD transmission was through infected cassava cuttings at both sites. PCR analysis showed that the East African cassava mosaic Cameroon virus (EACMCMV) was always found in co-infection with African cassava mosaic virus (ACMV), which also occurred in single infection. Co-infections of EACMCMV and ACMV were more prevalent in Bouaké (52.64%) than Man (13.87%). Eleven complete genome sequences of cassava mosaic begomoviruses infecting the CNRA germplasm were obtained, and phylogenetic analysis showed that they are closely related to ACMV and EACMCMV isolates from Ghana, Burkina Faso and Nigeria. The results of this study will assist breeders to screen accurately for CMD resistance in progeny resulting from parental lines, enabling the strategic distribution of new clean cassava planting materials to reduce the impact of CMD.

KEYWORDS
African cassava mosaic virus (ACMV), cassava germplasm, cassava mosaic disease, East African cassava mosaic Cameroon virus (EACMCMV)

1 | INTRODUCTION

Cassava (*Manihot esculenta*) is the staple food of more than 800 million people in the tropics, including 500 million in Africa (Vernier et al., 2018). It is the basis of a multitude of products, including food, flour, animal feed, alcohol, starches for producing paper, textiles and other biodegradable manufactured products. Ivory Coast is the third largest producer of cassava in West Africa with 6.961 million tonnes (FAOSTAT, 2021).

In Ivory Coast, cassava is consumed in various processed forms, such as *attiéké* (cassava couscous) which is the national dish, *foutou* (pounded cassava alone or mixed with plantain), *placali* (paste) and *gari* (toasted granules). Cassava is both a subsistence and a cash crop for farmers, the demand is increasing, and the production is unable to meet the increasing needs (Mendez del Villar et al., 2018).

Given the importance of this crop and the ever-increasing demand, the Centre National de Recherche Agronomique (CNRA) in Ivory Coast maintains a cassava germplasm of 610 accessions. This collection, kept in open fields on two sites in Ivory Coast (Bouaké and Man) is used for genetic resource preservation, improvement, varietal selection and as planting material for distribution to farmers throughout the country. However, this germplasm is attacked by cassava mosaic disease (CMD) that can cause yield losses of up to 90% (Vernier et al., 2018). Losses due to this disease have an immediate impact on the food supply, threatening food security and the livelihoods of Africa's rapidly growing population (FAO, 2014). This disease is caused by 11 cassava mosaic begomoviruses (CMBs), of which nine occur in Africa and two are found on the Indian subcontinent and in South-east Asia (ICTV, 2019; Minato et al., 2019). In Ivory Coast, CMD is caused by two begomoviruses: African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus (EACMCMV; Pita, Fondong, Sangaré, Kokora, et al., 2001). The CMBs are transmitted by a *Bemisia tabaci* species complex called sub-Saharan Africa (SSA)−5 and spread by cuttings that are routinely used by farmers (Legg et al., 2011; Namuddu et al., 2023).

So far, the 610 CNRA cassava accessions have been used mainly for morphological characterization studies and varietal selection (N'Zué et al., 2009, 2014). The CNRA germplasm has been enriched since 1950 and every 2 years the whole collection is replicated via cuttings in new conservation plots, without any monitoring for viruses and pests. This led to virus evolution, accumulation, recombination and/or reassortment and to the degeneration of the plant material. Therefore, we conducted the first CMD epidemiological study and molecular characterization of CMBs infecting the CNRA cassava collection to know its phytosanitary status and the diversity of CMBs.

2 | MATERIALS AND METHODS

2.1 | Description of the study sites and plant materials assessed

The CNRA cassava conservation plots were evaluated from September to October 2017 in two CNRA research stations located in the cities of Bouaké and Man. The Bouaké site belongs to agro-ecological zone V and Man to agro-ecological zone III (Halle & Bruzon, 2006). The agro-ecological characteristics of these stations are shown in Table 1. The most significant differences between the two sites are the altitude, rainfall, vegetation, temperature and relative humidity. A total of 610 cassava accessions were assessed in this study, with 10 plants evaluated per accession. The 610 accessions were uniformly represented on both sites. These different accessions are from Ivory Coast (465 accessions), International Institute of Tropical Agriculture (IITA)-Nigeria (99 accessions), Central African Republic (CAF;18 accessions), Togo (seven accessions), Kenya (six accessions), Madagascar (eight accessions), Congo (five accessions) and Liberia (two accessions). They are made up of 429 local accessions and 181 improved varieties. Among these accessions, Ivory Coast has 399 local accessions and 67 improved accessions. These local accessions, which are generally appreciated by producers, are used to make many dishes, while improved varieties, which are often little appreciated, have been selected for their yield, taste and suitability for processing into *attiéké*. The accessions obtained from IITA-Nigeria, Kenya and Madagascar are improved varieties presumed to be resistant to CMD.

2.2 | Assessment of incidence, severity, mode of infection and number of whiteflies of CMD

Assessment of four CMD epidemiological parameters was carried out on 5-month-old cassava plants: disease incidence, disease severity, mode of infection and whitefly abundance. CMD incidence (I) per accession was calculated using the following formula:

$$I\,(\%) = 100 \times (\text{No. of diseased plants} / \text{Total no. of plants observed}).$$

TABLE 1 Agro-ecological characteristics of cassava field assessment sites in Bouaké and Man, Ivory Coast.

Location	Agro-ecological zone	Vegetation	Soil type	Position	Altitude (m a.s.l.)	Rainfall (mm)	Relative humidity (%)	Mean temp. (°C)
Bouaké	V	Wooded savannah	Ferralitic	07°40′ N, 05°05′ W	399	1060	79	31.7
Man	III	Humid forest	Ferralitic and hydromorph	07°20′ N, 07°36′ W	1050	1636.5	86	26.6

Disease severity was scored by visual assessment using a scale of 1–5 (Hahn et al., 1980) where 1 is absence of disease symptoms and 5 the score attributed to the most severe symptoms, consisting of total leaf distortion and stunting of the entire plant (Table 2). The mean severity (S_m) of each accession was calculated using the following formula:

$$S_m = \text{Total scores of diseased plants} / \text{Total no. of diseased plants.}$$

The mode of infection was determined by the location of symptomatic leaves on the plants. When CMD symptoms were observed only on the lower leaves or on all leaves of the cassava plant, infection was attributed to the use of infected cuttings for planting. When symptoms were observed only on the upper leaves but not on any lower leaves of cassava plants, infection was attributed to whiteflies (Sseruwagi et al., 2004). Only diseased plants were considered to calculate the percentage of infection by cuttings or by whiteflies. Adult whitefly counts were determined on the five topmost developed leaves of each plant by counting all adult whiteflies present on the undersides of these leaves.

2.3 | Cassava leaf sampling

For each cassava accession, fresh leaves with no symptoms, or mild, severe and very severe symptoms were collected for virus detection in the laboratory. Leaves collected were placed in a zip-lock plastic bag and kept in a cool box for transportation to the laboratory where they were stored at −20°C.

2.4 | Molecular characterization of cassava mosaic begomoviruses

Total DNA extraction from cassava leaf samples was performed according to the protocol of Doyle and Doyle (1987). The concentration of each DNA sample was determined using a spectrophotometer (Eppendorf) and adjusted to 50 ng/μL for use in PCR. Specific primers pairs were used for the detection of ACMV (JSP001/JSP002 and ACMVB1/ACMVB2), EACMV (JSP001/JSP002 and EAB555F/EAB555R) and EACMCMV (VNF031/VNF032; Table 3). The PCR mixture consisted of 1 × Colourless GoTaq reaction buffer (Promega), 0.625 U GoTaq polymerase (Promega), 0.4 μM of each primer (Eurogentec), 0.2 mM dNTP (New England Biolabs), 1 mM $MgCl_2$ (Promega). The reaction consisted of an initial denaturation step at 94°C for 4 min; 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min; and a final extension of 72°C for 10 min. Volumes of 10 μL of amplified products were used for electrophoresis in a 1% agarose gel stained with ethidium bromide. The amplified DNA was visualized under UV light using a gel imager.

To generate full-length genomes of the CMBs infecting the CNRA cassava collection, selected DNA samples were amplified by rolling circle amplification using the TempliPhi 100 amplification system (GE Healthcare; Inoue-Nagata et al., 2004). The products were digested with *Bam*HI or *Hin*dIII, *Nde*I and *Eco*RI, to yield the full-length genomes (c.2.8 kb). The full-length genomes of DNA-A, DNA-B, of ACMV and DNA-A, DNA-B of EACMCMV were cloned into the plasmid pGEM-3Zf(+) following the manufacturer's protocol (Promega).

TABLE 2 Description of the symptom severity scores of cassava mosaic disease (CMD; Hahn et al., 1980).

Score	Symptom
1	Plants without symptoms
2	Plants with medium chlorotic spots or some distortion at the base
3	Plants with spots on the whole leaf surface with leaf twisting
4	Plants with distorted or shrunken leaf blades (to 2/3 of the leaf area)
5	Plants with many symptoms of CMD and/or total distortion of 4/5 of the leaf area and stunting of the entire plant

2.5 | Sequencing and phylogenetic analysis

Sequencing of the full-length genomes was carried out to investigate the diversity of begomoviruses infecting the CNRA cassava germplasm. Clones were sequenced by the Sanger method at GENEWWIZ (Germany) by primer walking. Initial sequencing was performed with the universal primers M13-F and M13-R (26). From these initial sequences, internal primers (forward and reverse) were designed using Geneious Prime v. 2022.2.1 software and the complete sequences of the viruses were obtained. Sequences were trimmed and assembled de novo using Geneious Prime v. 2022.2.1. Consensus sequences were then submitted to the BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to check for the identity of viruses. Sequences were aligned separately with representative isolates of CMBs using ClustalW in MEGA X (Kumar et al., 2018). Maximum-likelihood trees were constructed in MEGA X using T92 + G and GTR + G nucleotide substitution models according to MEGA X for the ACMV and EACMV, respectively with a bootstrap of 1000. Phylogenetic trees were visualized and edited using FigTree v. 1.4.3.

2.6 | Statistical analysis

Data were processed using R v. 3.6.1 (R Core Team, 2019). The effect of agro-ecological site was tested on CMD severity mean, incidence, mode of infection and whitefly abundance per plant using *t* tests. We also used a *t* test to compare differences between the sites in the proportion of samples infected by ACMV alone or co-infected with ACMV and EACMCMV. Plots were made with the ggplot2 package (Wickham, 2016).

TABLE 3 PCR primer pairs for detection of African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV) and East African cassava mosaic Cameroon virus (EACMCMV).

Virus	Primer	Sequence (5′–3′)	DNA target	Amplified region	Size (bp)	References
ACMV	JSP001	ATGTCGAAGCGACCAGGAGAT	DNA-A	CP	783	Pita, Fondong, Sangaré, Otim-Nape, et al. (2001)
	JSP002	TGTTTATTAATTGCCAATACT				
	ACMVB1	TCGGGAGTGATACATGCGAAGGC	DNA-B	BV1-BC1	628	Matic et al. (2012)
	ACMVB2	GGCTACACCAGCTACCTGAAGCT				
EACMV	JSP001	ATGTCGAAGCGACCAGGAGAT	DNA-A	CP	780	Pita, Fondong, Sangaré, Otim-Nape, et al. (2001)
	JSP003	CCTTTATTAATTTGTCACTGC				
	EAB555F	TACATCGGCCTTTGAGTCGCATGG	DNA-B	IR-BC1	544–560	Fondong et al. (2000)
	EAB555R	CTTATTAACGCCTATATAAACACC				
EACMCMV	VNF031	GGATACAGATAGGGTTCCCAC	DNA-A	AC2-AC3	560	Fondong et al. (2000)
	VNF032	GACGAGGACAAGAATTCCAAT				

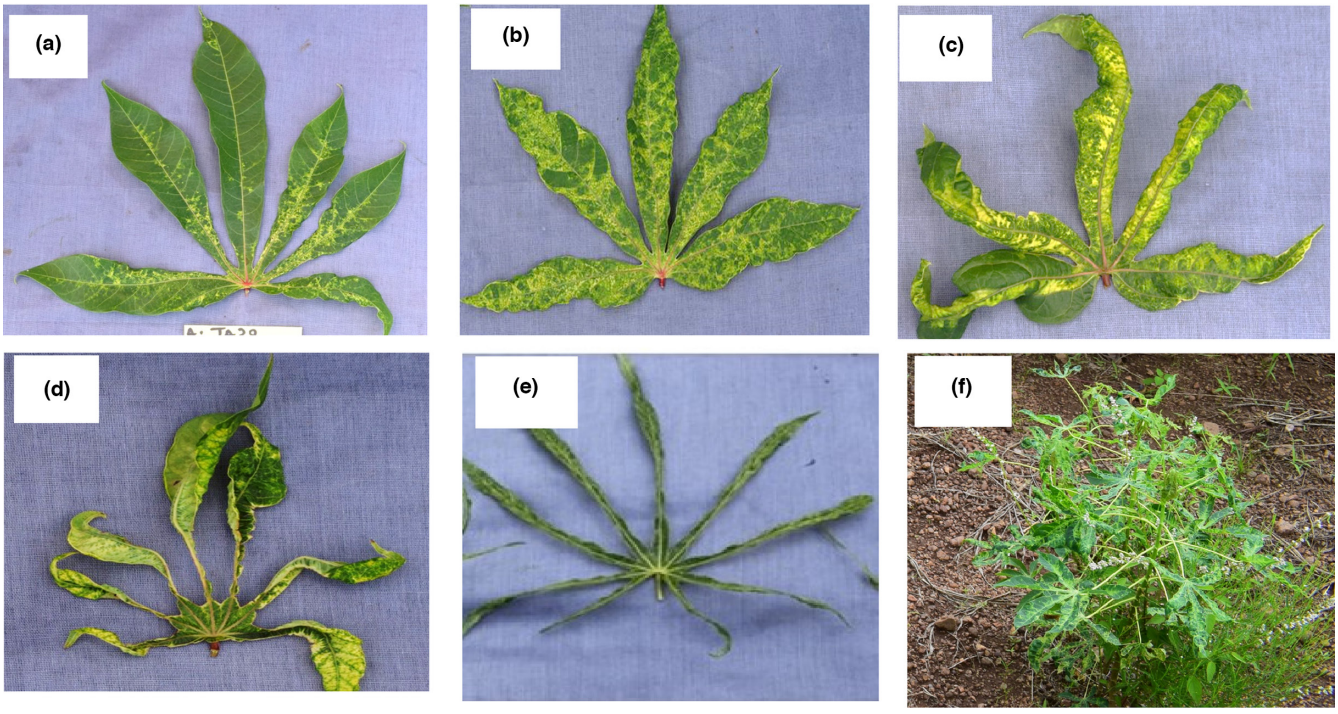

FIGURE 1 Examples of cassava mosaic disease symptoms observed on cassava germplasm in Ivory Coast. (a) Mild mosaic, (b) severe mosaic and mild deformation of the leaf, (c) very severe, deformation and leaf curling, (d) curling and filiform leaf, (e) filiform leaf, (f) stunted plant.

3 | RESULTS

3.1 | CMD incidence and severity

CMD symptoms (mild, severe, very severe mosaic; leaf distortion; leaf curling, filiform leaf and in some cases total stunting of the plant) were observed on both sites, Bouaké and Man (Figure 1). Mean CMD incidence was significantly lower on the Man site (49.78 ± 0.02%) than the Bouaké site (74.54 ± 0.02%; Figure 2a). Similarly, the Man site showed lower mean CMD severity value, 2.23 ± 0.02 compared to 2.92 ± 0.03 in Bouaké (Figure 2b).

Statistical analyses showed a significant difference ($p < 0.05$) in CMD incidence between Bouaké and Man for accessions originated from Congo (98 ± 2% in Bouaké and 52 ± 8% in Man), Ivory Coast (86.06 ± 1.23% in Bouaké and 58.87 ± 1.75% in Man), Madagascar (92.5 ± 7.5% in Bouaké 53.75 ± 13.12% in Man), IITA-Nigeria (16.47 ± 1.75% in Bouaké and 6.05 ± 5.66% in Man), CAF (24.01 ± 8.36% in Bouaké and 35.47 ± 7.14% in

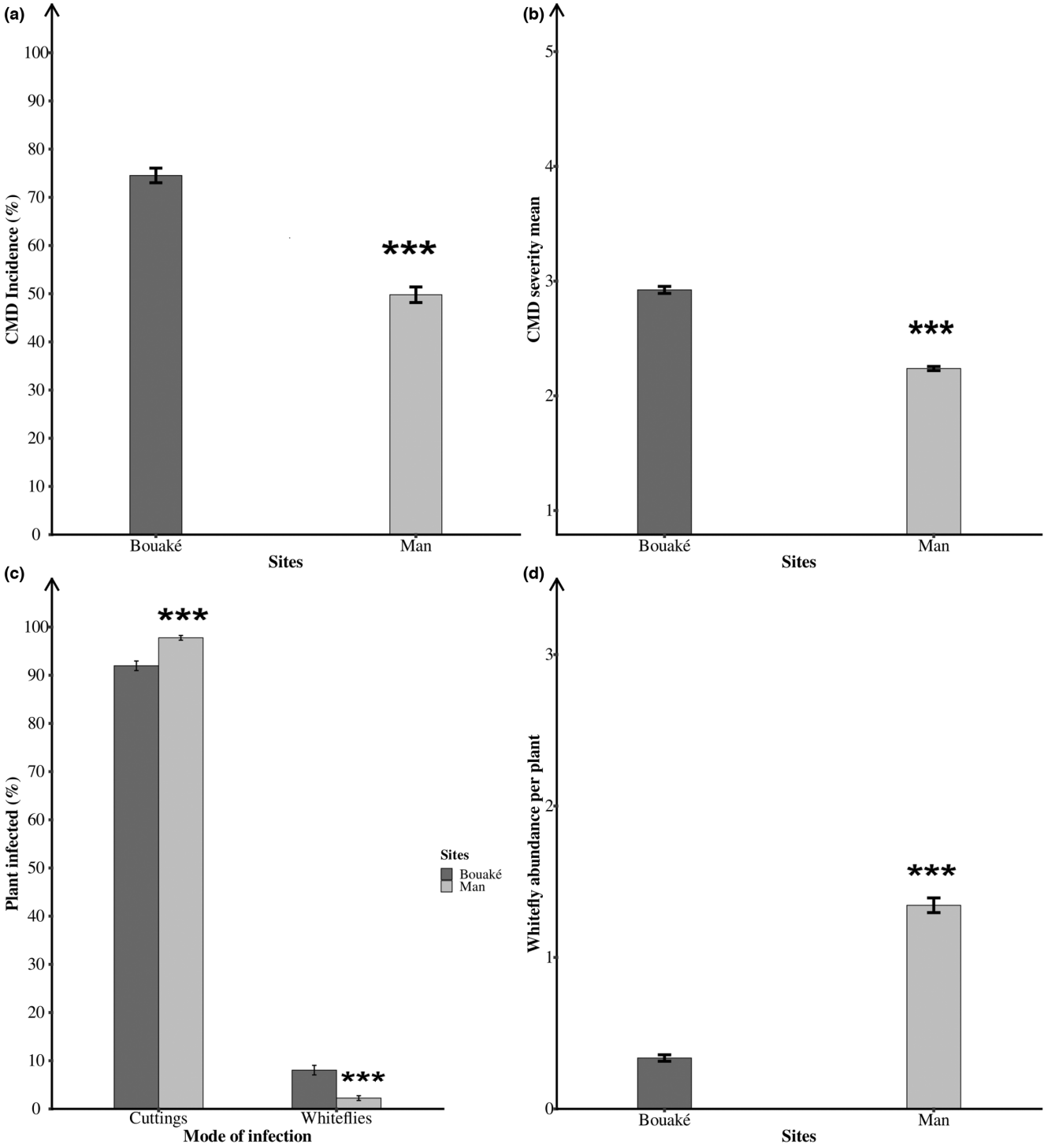

FIGURE 2 Epidemiological assessment of cassava mosaic disease (CMD) at different cassava germplasm conservation sites (Bouaké and Man). (a) Mean incidence of CMD (proportion of infected plants). (b) Mean severity of CMD, assessed visually on a scale of 1–5 of increasing severity. (c) Percentage of plants infected from cuttings or by whiteflies. (d) Whitefly abundance per plant. The error bars represent the standard error (±*SE*). The diagrams marked with *** indicate a highly significant difference ($p < 0.001$) between sites.

Man) and Togo (18.9 ± 7.14% in Bouaké and 28.49 ± 10.77% in Man). There were no significant differences for accessions originated from Kenya and Liberia. Indeed, accessions from Liberia recorded the highest incidence values on both sites (100%) while the lowest incidence values on both sites (0%–25%) were obtained with accessions from Kenya and IITA-Nigeria. A few accessions from IITA-Nigeria had incidence values above 50%. For accessions from the remaining countries, we observed that in most cases, disease incidence was lower in the Man site than the Bouaké site (Figure 3a).

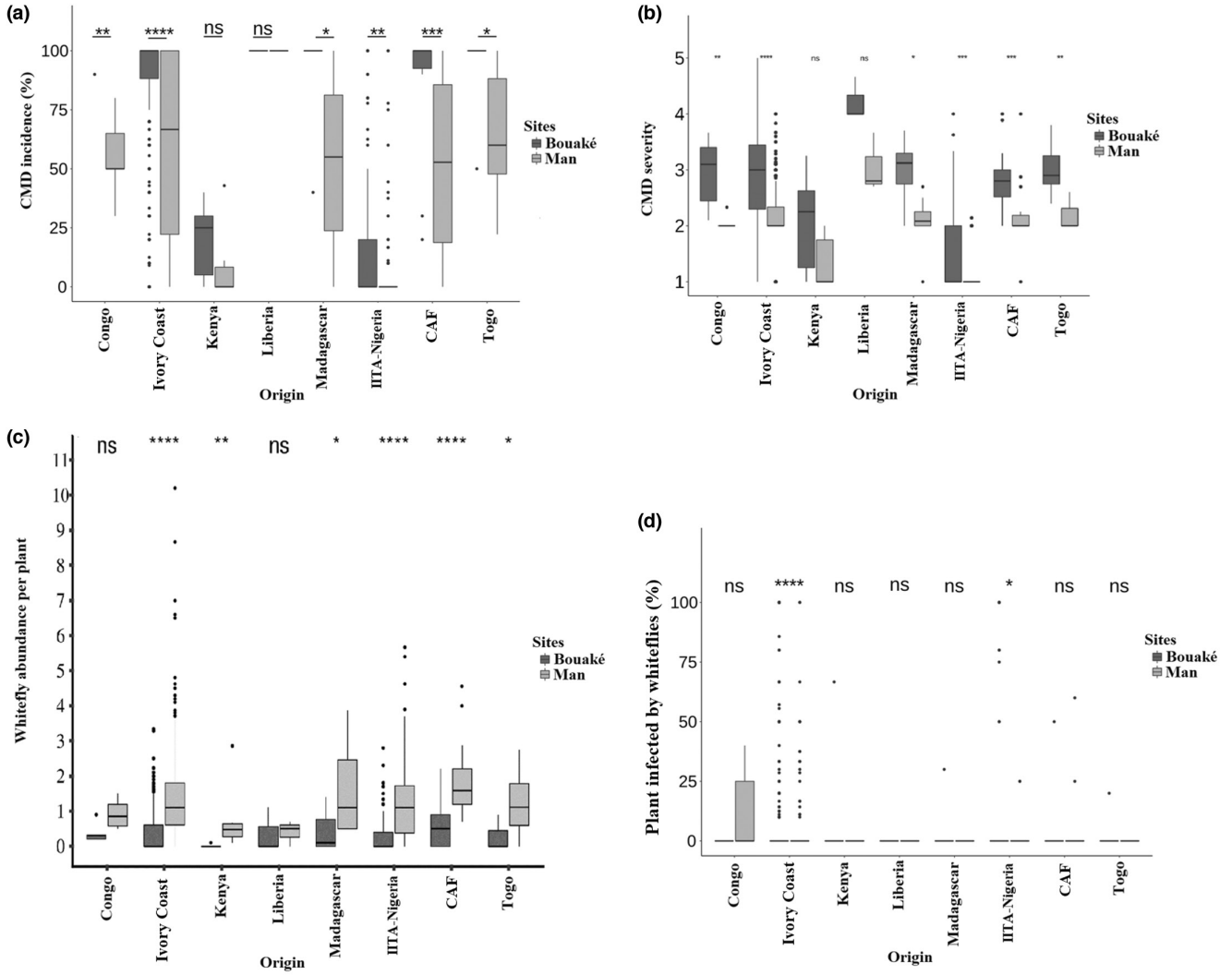

FIGURE 3 Cassava mosaic disease (CMD) incidence (a), severity (b), whitefly abundance (c) and percentage infected by whitefly (d) according to origin of accession and the site where they were tested. CAF, Central African Republic; IITA, International Institute of Tropical Agriculture. Boxes outline lower and upper quartiles; lines within boxes are medians; error bars represent smallest and largest nonoutlier observations; and black points indicate outlier data. Statistical significance was calculated using *t* test; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ns, not significant ($\alpha = 0.05$).

For CMD severity, in most cases, the accessions displayed the lowest severities in the Man site. In addition, most accessions from Kenya and IITA-Nigeria had the lowest severity values at both sites (1–2). The highest severity values were recorded for accessions that originated from Liberia at both the sites (3.18–4). Statistical analyses showed a significant severity difference ($p<0.05$) between Bouaké and Man for accessions originated from Congo (2.94 ± 0.29 in Bouaké and 2.07 ± 0.07 in Man), Ivory Coast (2.98 ± 0.03 in Bouaké and 2.07 ± 0.02 in Man), Madagascar (2.97 ± 0.23 in Bouaké and 2.22 ± 0.1 in Man), IITA-Nigeria (2.26 ± 0.09 in Bouaké and 2.01 ± 0.01 in Man), CAF (2.84 ± 0.13 in Bouaké and 2.24 ± 0.13 in Man) and Togo (3.01 ± 0.18 in Bouaké and 2.17 ± 0.1 in Man; Figure 3b). A breakdown of the data by accession is available in Table S1 for the Bouaké site and in Table S2 for the Man site.

3.2 | Mode of infection and whitefly abundance

Based on the location of symptomatic leaves on cassava plants, we concluded that CMD propagation at both sites was mostly through the use of infected cuttings. The Bouaké site recorded 91.96 ± 0.99% cutting infection, which was lower than that recorded at the Man site (97.76 ± 0.49%; Figure 2c). Although the percentage of whitefly transmission was low in both sites (Figure 2c), it was significantly lower in Man (2.24 ± 0.10%) compared to Bouaké (8.04 ± 0.23%). Similarly, a small number of whiteflies was observed on both sites (Figure 2d) and the mean number of whiteflies per plant was significantly higher in Man (1.34 ± 0.05) than in Bouaké (0.34 ± 0.02; $p<0.001$). Statistical analyses showed a significant difference in whitefly abundance ($p<0.05$) between Bouaké and Man for accessions originated from Ivory Coast, Kenya, Madagascar, IITA-Nigeria,

CAR and Togo, whereas no significant difference was observed for accessions from Liberia and Congo (Figure 3c). The number of plants infected by whitefly varied and was higher in Bouaké than Man. However, the highest values of whitefly infection were obtained for plant accessions from Congo (14.67 ± 9.04), Kenya (11.11 ± 9.09) Nigeria (08.43 ± 9.04) and Ivory Coast (7.06 ± 0.98; Figure 3d). A breakdown of the data by accession is available in the Table S1 for Bouaké and in Table S2 for Man.

3.3 | Detection of CMBs by PCR

African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) were detected in cassava leaves from both sites using PCR. The subsequent use of the specific pair of primers VNF031/VNF032 confirmed that most of the amplified EACMV fragments were East African cassava mosaic Cameroon virus (EACMCMV). A total of 1041 samples from the Bouaké site and 894 samples from the Man site were analysed. The proportion of ACMV was 90.20% (939/1041) in Bouaké and 81.54% (729/894) in Man. A significant difference was observed between sites in the proportion of plants with EACMCMV with 52.64% (548/1041) in Bouaké and 13.41% in Man (124/894). Our results also showed the presence of uninfected cassava plants in Bouaké (9.79%: 102/1041) and in Man (18.46%: 165/894). We detected single infections of ACMV as well as co-infections of ACMV with EACMCMV. Surprisingly, EACMCMV was never detected alone, but was always detected in co-infection with ACMV in the samples tested. In Bouaké, 391 samples (37.56 ± 0.02%) were infected with ACMV (single infection) and 548 (52.64 ± 0.02%) were co-infected by ACMV and EACMCMV. In Man, we detected ACMV as single infections in 605 samples (67.67 ± 0.02%), and ACMV and EACMCMV in co-infection in 124 samples (13.87 ± 0.01%; Figure 4). Statistical analyses indicated a highly significant difference between the proportion of infection type per site. The proportion of co-infection (ACMV + EACMCMV) recorded in Bouaké was significantly higher ($p < 0.001$) than in Man (Figure 4). In contrast, single ACMV infection was higher in Man compared to the value observed in Bouaké.

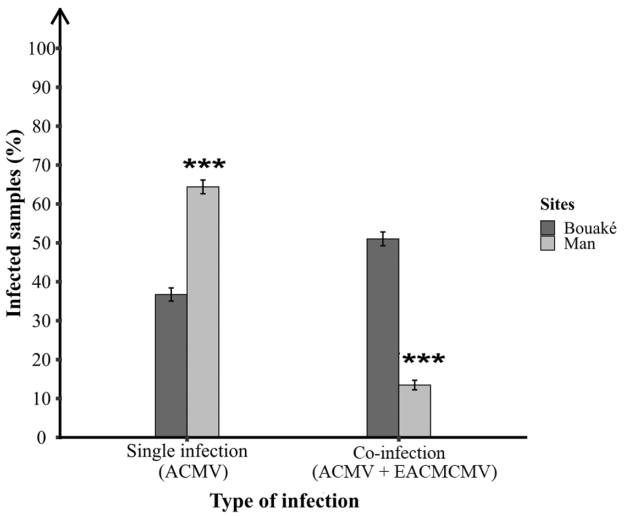

FIGURE 4 Proportion of cassava plants per site infected by African cassava mosaic virus (ACMV) alone or co-infected with ACMV and East African cassava mosaic Cameroon virus (EACMCMV). The error bars represent the standard error (±*SE*). The asterisks *** indicate a highly significant difference between sites according to *t* test ($p < 0.001$).

3.4 | Types of infection and symptom severity

A total of 1935 samples were tested for ACMV and EACMCMV in Bouaké and Man. Table 4 shows the number of single and co-infections in correlation with symptom severity. Symptom severity varied according to the type of infection, single infection of ACMV or co-infection of ACMV and EACMCMV. For single infection with ACMV, 61.8% of positive samples presented mild symptoms, 30.4% severe symptoms and 19.6% very severe symptoms. Thus, as the proportion of single ACMV infection instead of co-infection decreased, the symptoms become more severe (Table 4). In contrast, for plants with co-infection with ACMV + EACMCV, 38.2% exhibited mild symptoms, 69.6% severe symptoms and 80.4% very severe symptoms. This increase in severe symptoms with co-infection (Table 4) indicates a positive correlation between co-infection (ACMV + EACMCMV) and symptom severity.

3.5 | Analysis of full-length genome and phylogenetic relationships

After sequencing the cloned products, sequence trimming and assembly of the contigs, 11 complete sequences with sizes ranging from 2737 to 2802 bp were obtained. Sequence annotation showed that eight of them have genomic organization typical of that of old world bipartite begomovirus DNA-A. The other three have genomic organization typical of the bipartite begomovirus DNA-B. For the sequences with genomic organization similar to that of DNA-A, two open reading frames (ORFs) were found in the viral strand. These are the AV1 ORF, which encodes the capsid protein (CP), and the AV2 ORF, which encodes the movement protein (MP). In the complementary strand, four ORFs were found: AC1 that encodes the replication protein (Rep), AC2 that encodes the transcriptional activator protein (TrAP), AC3 that encodes the replication enhancer (REn) protein and AC4 that encodes the C4 protein. Two ORFs were found on each of the three sequences whose genomic organization is typical of that of DNA-B. These are the ORF BV1 in the direction of the viral strand, which codes for the nuclear shuttle protein (NSP) and the ORF BC1 in the complementary direction, which codes for the MP.

All sequences (DNA-A and DNA-B) have the nanonucleotide sequence TAATATTAC, which is in the loop stem and is a conserved area in the *Geminiviridae* family. BLASTn search in GenBank database (NCBI, BLASTN) for related sequences showed that five sequences are closely related to ACMV (four DNA-A and one DNA-B) and six

Symptom severity	No. of samples	Virus detected		
		ACMV (%)	ACMV + EACMCMV (%)	None (%)
Healthy	665	333 (50.1)	75 (11.3)	257 (38.6)
Mild	928	574 (61.8)	354 (38.2)	0 (0)
Severe	296	90 (30.4)	206 (69.6)	0 (0)
Very severe	46	9 (19.6)	37 (80.4)	0 (0)
Total	1935	1006 (52.0)	672 (34.7)	257 (13.3)

TABLE 4 Symptom severities and viruses infecting cassava plants from the germplasm collection of the Centre National de Recherche Agronomique, Ivory Coast.

Abbreviations: ACMV, African cassava mosaic virus; EACMCMV, East African cassava mosaic Cameroon virus.

to EACMCMV (four DNA-A and two DNA-B). The ACMV DNA-A, corresponding with accession numbers LC721738, LC721739, LC721740 and LC722230, had between 96.79% and 98.46% nucleotide identity with isolates from Nigeria (MN809986.1), Ghana (MG250146, MG250143) and Ivory Coast (AF259894). The ACMV DNA-B (LC722237) had between 92.45% and 94.44% nucleotide identity with isolates from Ghana (MH646721) and Ivory Coast (AF259895). For EACMCMV, the DNA-A sequences (accession numbers LC722232, LC722233, LC722234 and LC722231) share between 96.64% and 97.75% nucleotide identity with isolates from Ghana (MG250164), Burkina Faso (LC659083) and Ivory Coast (AF259896). The remaining two DNA-B sequences of EACMCMV (LC722235 and LC722236) share between 97.07% and 97.18% nucleotide identity with an isolate from Ivory Coast (AF259897). No significant variation was observed in the amino acid sequences. The percentage amino acid identity ranged between 96% and 100%, except for an ACMV DNA-A sequence that had 77.96% identity for AC4 and 89.23% for AC1. The phylogenetic tree constructed using the maximum-likelihood model from the full nucleotide sequence alignment of the Ivory Coast CMBs obtained in this study show that they are most closely related to isolates from Ghana, Burkina Faso and Nigeria (Figure 5a,b).

4 | DISCUSSION

The epidemiological assessment of CNRA's cassava germplasm for CMD was carried out on 610 accessions maintained in Bouaké and Man. Our results showed that the incidence of the disease and the severity of symptoms were significantly lower in Man than in Bouaké. This indicates that conditions at the Man site are not as conducive to disease dissemination and symptom development. As described by Bisimwa et al. (2015), such observations can be attributed to the difference in altitude between these two agrosystems (1050 m a.s.l. for Man and 399 m a.s.l. for Bouaké). Indeed, it has been shown that in a low altitude environment, CMD spreads rapidly and the severity of symptoms is high. Thus, environmental conditions could be contributing factors influencing the spread of the CMD. Similar findings have been observed in Madagascar (Harimalala et al., 2015) and Tanzania (Legg & Raya, 1998). According to these authors, altitude as an indicator of temperature would be the main factor explaining these results. On relatively cool sites (high altitude), the activity of the whitefly is reduced compared to that observed at low altitude (Legg & Fauquet, 2004) and *B. tabaci* does not adapt well to altitudes above 1000 m (Morales & Jones, 2004). This is in agreement with our observations of a higher percentage whitefly infection of plants at Bouaké, with its lower altitude, than at Man. The whitefly population in Bouaké would be more active than that in Man. In addition, Bouaké had a higher diversity of *B. tabaci* (SSA1-SG3, SSA1-SG1, SSA2, MED-ASL and MED-Q1) than Man (SSA1-SG3; authors' unpublished data). Whitefly transmission of CMD is biotype dependent; studies have shown that some whitefly biotypes are attracted by the yellow mosaic patterns of CMD-infected cassava plants while others are not (Manani et al., 2017; Omongo et al., 2012). According to Ning et al. (2015), the ability of virus transmission by whiteflies could vary from one biotype to another. Although the number of whiteflies per plant was significantly higher in Man compared to Bouaké, the low number of whiteflies on the Bouaké site could be explained by the rainfall recorded during the assessment period (200.2 mm of rain). In fact, other environmental factors such as rainfall considerably limit the presence and development of *B. tabaci* populations, as suggested by Harimalala et al. (2015) and demonstrated by Selvaraj and Ramesh (2012).

The poor quality of planting materials used to conserve the CNRA cassava germplasm has been demonstrated by our finding that more than 90% of the cuttings used for the renewal of the germplasm plots were initially infected by CMD. Thus, CMD infection of the CNRA cassava germplasm collection is mainly associated with infected planting material. Our results are in accordance with the observations made in 2017 for cassava field surveys conducted throughout Ivory Coast (authors' unpublished data), Burkina Faso (Soro et al., 2021) and Nigeria (Eni et al., 2021). These observations highlight the significance and urgency of raising awareness among cassava farmers and other stakeholders on the importance of CMD, its impact on yield and control strategies based on the use of resistant varieties and virus-free planting materials.

Our study reveals that ACMV and EACMCMV infect the CNRA cassava germplasm preserved at Bouaké and Man. ACMV was the predominant CMB found in both locations. The predominance of infections by ACMV observed during our study is similar to the finding of a previous country-wide survey conducted in Ivory Coast (Toualy

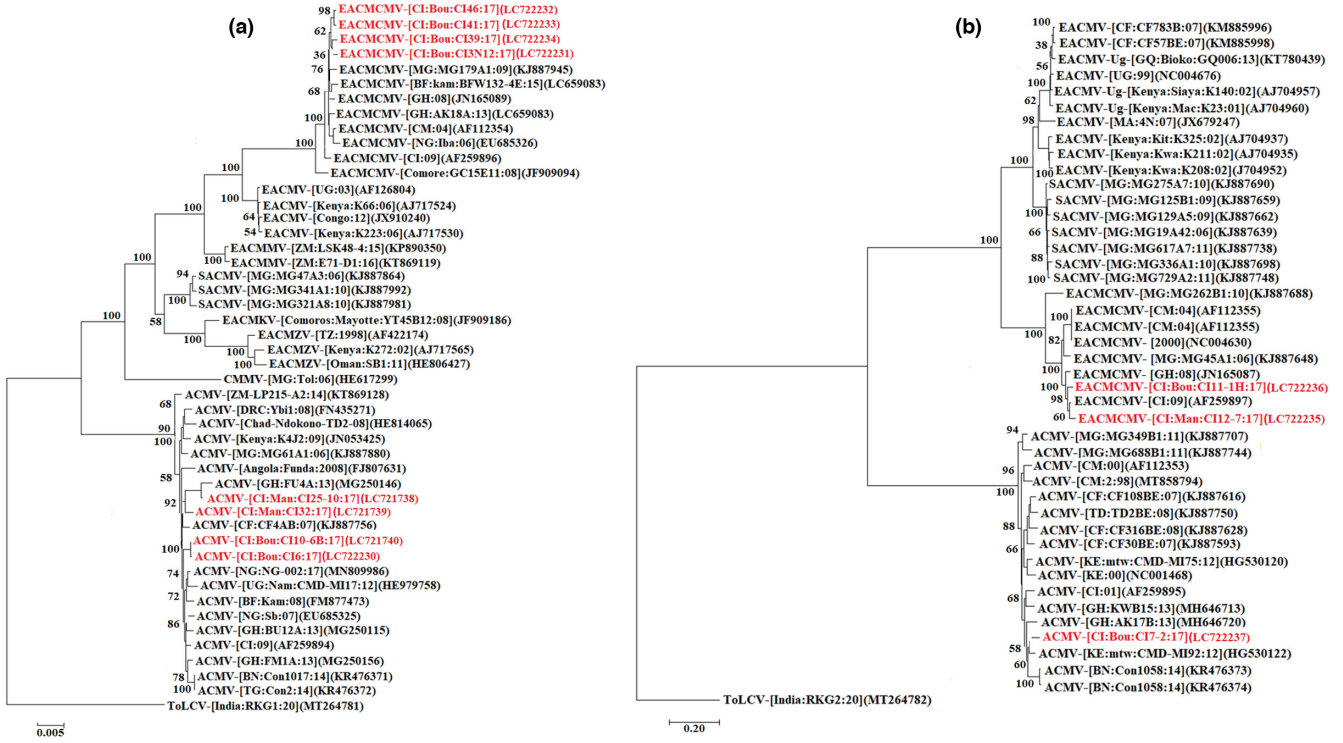

FIGURE 5 Maximum-likelihood phylogenetic tree indicating the relationships between Ivory Coast isolates of East African cassava mosaic Cameroon virus (EACMCMV; six isolates), African cassava mosaic virus (ACMV; five isolates) and diverse representative isolates of cassava mosaic begomoviruses. (a) The tree is based on full-length sequences for EACMCMV (DNA-A), ACMV (DNA-A) and rooted using tomato leaf curl virus (GenBank accession, DNA-A: MT264781) as an outgroup. (b) The tree is based on full-length sequences for EACMCMV (DNA-B), ACMV (DNA-B) and rooted using tomato leaf curl virus (GenBank accession, DNA-B: MT264782) as an outgroup. The sequences obtained in this study are in red while those in black were taken from GenBank. Bootstrap analysis was performed with 1000 replicates.

et al., 2014). In addition, EACMCMV was always detected in co-infection with ACMV as shown by Pita and collaborators 21 years ago (Pita, Fondong, Sangaré, Kokora, et al., 2001). The occurrence of co-infection by ACMV and EACMCMV has been reported in other African studies in Cameroon and Nigeria (Ariyo et al., 2005; Fondong et al., 2000). These authors suggested a synergistic interaction between these two viruses. However, further research would be necessary to understand the mechanisms responsible for such synergy.

The percentage of co-infection by ACMV + EACMCMV in Bouaké (399 m a.s.l.) was higher than in Man (1050 m a.s.l.). This suggests the existence of a negative correlation between high altitude and the presence of EACMCMV. These results are consistent with those of Bisimwa et al. (2015) who showed that in Democratic Republic of Congo, at low altitude, co-infection between ACMV and EACMV is higher while at high altitude, single ACMV infection is predominant. According to Harimalala et al. (2015), in Madagascar, ACMV is more prevalent in the Central Highlands, whereas EACMCV is found throughout the country with a high prevalence in the northern region. Indeed, interactions between the host plant, the environment, the type of vector (whitefly species) and virus strains could explain our findings. Also, in this study, symptom severity increased as the proportion of co-infection increased. This is consistent with observations made by Fondong et al. (2000) in Cameroon.

Approximately 60% of the asymptomatic cassava samples analysed were infected by ACMV and EACMCMV, indicating latent infections. As suggested by Soko et al. (2015), the expression of symptoms depends on the accumulation of virus in the plant; below a certain virus accumulation threshold, plants show no disease symptoms. Therefore, the use of asymptomatic cassava planting material as a control option for CMD could inadvertently lead to increased propagation of the disease as these asymptomatic plants could be virus reservoirs. This highlights the importance of laboratory diagnosis or the use of suitable kits for field diagnosis when selecting cassava planting material.

The virus sequences obtained from our study and the classification of begomoviruses according to Brown et al. (2015) show that the cassava viruses infecting the CNRA germplasm (ACMV and EACMCMV) are very similar to those previously described by Pita, Fondong, Sangaré, Kokora, et al. (2001) in Ivory Coast. Phylogenetic analysis also revealed that most of the isolates in this study are more closely related to isolates from Ghana, Burkina Faso and Nigeria. This is probably due to the exchange of plant material between Ivory Coast and these different countries. Although the nucleotide sequences we obtained for ACMV and EACMCMV varied slightly from those of isolates from other countries, most of the observed nucleotide variations resulted in silent mutations (substitution); thus, their amino acids did not vary significantly. However, lower amino acid percentage identities of 77.96% and 89.23% were observed for the two

genes AC4 and AC1, respectively, in the ACMV-DNA. This variation is probably a direct result of the vegetative mode of transmission in cassava cultivation. Indeed, according to Aimone et al. (2021), vegetative propagation is the main factor leading to recombination events and the generation of new geminivirus species. This reinforces the need to avoid using infected plants for the establishment of new fields.

This study has unveiled the phytosanitary status of the CNRA cassava germplasm preserved in open fields in Bouaké and Man. The contrast observed in the CMD manifestation uncovered between Bouaké and Man offers research opportunities to study the molecular mechanisms governing CMBs evolution and accumulation in plants, as well as cassava resistance to CMD.

The CNRA cassava collection is very important not only for Ivory Coast, but also for the cassava community, because it is used for preservation of genetic resources, improvement, varietal selection and the generation of planting materials for distribution throughout the country. However, we have shown that the collection is highly infected by begomoviruses and this highlights the necessity for a change of practices and protocols used for cassava germplasm conservation. The population diversity of CMBs increases over the course of serial vegetative propagation (Aimone et al., 2021); thus, high variability of begomoviruses could develop through the serial vegetative propagation/passages of the viruses in CNRA's collection via infected cuttings used for duplicating the collection in new conservation plots. New devastating strains or species could emerge from recombination or reassortment and could hinder the sustainable production of cassava by their propagation in Ivory Coast and abroad. Therefore, this work serves as a starting point in the development of a standard operating procedure for cassava germplasm preservation in developing countries.

ACKNOWLEDGEMENTS

The authors thank the CNRA for providing its germplasm for this study and Scriptoria for their contribution. This work was supported by the Central and West African Virus Epidemiology (WAVE) program for root and tuber crops through Bill & Melinda Gates Foundation; The UK Foreign, Commonwealth & Development Office, Grant/Award Number: INV-002969 (formerly OPP1212988).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets supporting findings and the results for each cassava accession of this study are provided in the manuscript and supporting information.

ORCID

William J.-L. Amoakon https://orcid.org/0000-0003-2575-7408

REFERENCES

Aimone, C.D., Lavington, E., Hoyer, J.S., Deppong, D.O., Mickelson-Young, L., Jacobson, A. et al. (2021) Population diversity of cassava mosaic begomoviruses increases over the course of serial vegetative propagation. *Journal of General Virology*, 102, 001622.

Ariyo, O.A., Koerbler, M., Dixon, A.G.O., Atiri, G.I. & Winter, S. (2005) Molecular variability and distribution of cassava mosaic begomoviruses in Nigeria. *Journal of Plant Pathology*, 153, 226–231.

Bisimwa, E., Walangululu, J. & Bragard, C. (2015) Cassava mosaic disease yield loss assessment under various altitude agroecosystems in the SudKivu region, Democratic Republic of Congo. *Tropicultura*, 33, 101–110.

Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Romos-Sobrinho, R., Silva, J.C. et al. (2015) Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. *Archives of Virology*, 160, 1593–1619.

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.

Eni, A.O., Efekemo, O.P., Onile-ere, O.A. & Pita, J.S. (2021) South west and north Central Nigeria: assessment of cassava mosaic disease and field status of African cassava mosaic virus and east African cassava mosaic virus. *Annals of Applied Biology*, 178, 466–479.

FAO. (2014) *FAO statistical yearbook 2014. Africa food and agriculture*. Rome: FAO.

FAOSTAT. (2021) *Food and agriculture organization of the united nations statistics*. Available from: https://www.fao.org/faostat/fr/#data/QCL/visualize [Accessed 30th January 2023].

Fondong, V.N., Pita, J.S., Rey, M.E.C., De Kochko, A., Beachy, R.N. & Fauquet, C.M. (2000) Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology*, 81, 287–297.

Hahn, S.K., Terry, E.R. & Leuschner, K. (1980) Breeding cassava for resistance to cassava mosaic disease. *Euphytica*, 29, 673–683.

Halle, B. & Bruzon, V. (2006) Profil environnemental de la Côte d'Ivoire. *Rapport final de la Commission Européenne*.

Harimalala, M., Chiroleu, F., Giraud-Carrier, C., Hoareau, M. & Zinga, I. (2015) Molecular epidemiology of cassava mosaic disease in Madagascar. *Plant Pathology*, 64, 501–507.

ICTV. (2019) *Virus taxonomy: 2019 release*. Berlin: ICTV.

Inoue-Nagata, A.K., Albuquerque, L.C., Rocha, W.B. & Nagata, T. (2004) A simple method for cloning the complete begomovirus genome using the bacteriophage φ29 DNA polymerase. *Journal of Virological Methods*, 116, 209–211.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.

Legg, J.P., Jeremiah, S.C., Obiero, H.M., Maruthi, M.N., Ndyetabula, I., Okao-Okuya, G. et al. (2011) Comparing the regional epidemiology of the cassava mosaic and cassava brown streak virus pandemics in Africa. *Virus Research*, 159, 161–170.

Legg, J.P. & Raya, M.D. (1998) International Journal of Pest Management survey of cassava virus diseases in Tanzania. *International Journal of Pest Management*, 44, 17–23.

Legg, J.Q. & Fauquet, C.M. (2004) Cassava mosaic geminiviruses. *Plant Molecular Biology*, 56, 585–599.

Manani, D.M., Ateka, E.M., Nyanjom, S.R.G. & Boykin, L.M. (2017) Phylogenetic relationships among whiteflies in the *Bemisia tabaci* (Gennadius) species complex from major cassava growing areas in Kenya. *Insects*, 8, 1–14.

Matic, S., da Cunha, A.P., Thompson, J.R. & Tepfer, M. (2012) An analysis of viruses associated with cassava mosaic disease in three Angolan provinces. *Journal of Plant Pathology*, 94, 443–450.

Mendez del Villar, P., Tran, T., Adaye, A., Bancal, V. & Allagba, K. (2018) Analyse de la chaîne de valeur du manioc en Côte d'Ivoire. Rapport pour l'Union Européenne, DG-DEVCO. Final report 2017.

Minato, N., Sok, S., Chen, S., Delaquis, E., Phirun, I., Le, V.X. et al. (2019) Surveillance for Sri Lankan cassava mosaic virus (SLCMV) in

Cambodia and Vietnam one year after its initial detection in a single plantation in 2015. *PLoS One*, 14, e0212780.
Morales, F.J. & Jones, P.G. (2004) The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Research*, 100, 57–65.
Namuddu, A., Seal, S., van Brunschot, S., Malka, O., Kabaalu, R., Morin, S. et al. (2023) Distribution of *Bemisia tabaci* in different agro-ecological regions in Uganda and the threat of vector-borne pandemics into new cassava growing areas. *Frontiers in Sustainable Food Systems*, 7, 35.
Ning, W., Shi, X., Liu, B., Pan, H., Wei, W., Zeng, Y. et al. (2015) Transmission of tomato yellow leaf curl virus by *Bemisia tabaci* as affected by whitefly sex and biotype. *Scientific Reports*, 5, 10744.
N'Zué, B., Okoma, M.P., Kouakou, A.M., Dibi, K.E.B., Zohourou, G.P., Essis, B.S. et al. (2014) Morphological characterization of cassava (*Manihot esculenta* Crantz) accessions collected in the centre-west, south-west and west of Côte d'Ivoire. *Greener Journal of Agricultural Sciences*, 4, 220–231.
N'Zué, B., Sangare, A., Letourmy, P., Zohouri, G.P. & Vernier, P. (2009) Morphological characterization of the national cassava (*Manihot esculenta* Crantz) collection in Côte d'Ivoire. *African Journal of Root and Tuber Crops*, 7, 32–38.
Omongo, C.A., Kawuki, R., Bellotti, A.C., Alicai, T., Baguma, Y., Maruthi, M.N. et al. (2012) African cassava whitefly, *Bemisia tabaci*, resistance in African and south American cassava genotypes. *Journal of Integrative Agriculture*, 11, 327–336.
Pita, J.S., Fondong, V.N., Sangaré, A., Kokora, R.N.N. & Fauquet, C.M. (2001) Genomic and biological diversity of the African cassava geminiviruses. *Euphytica*, 120, 115–125.
Pita, J.S., Fondong, V.N., Sangaré, A., Otim-Nape, G.W., Ogwal, S. & Fauquet, C.M. (2001) Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology*, 82, 655–665.
R Core Team. (2019) *R: a language and environment for statistical computing, version 3.0.2*. Austria: R Foundation for Statistical Computing.
Selvaraj, S. & Ramesh, V. (2012) Seasonal abundance of whitefly, *Bemisia tabaci* Gennadius and their relation to weather parameters. *International Journal of Food, Agriculture and Veterinary Sciences*, 2, 57–63.
Soko, D.F., Siene, L.A.C., Kotchi, V., Gogbeu, S.J., Sere, Y. & Ake, S. (2015) Évaluation du niveau de sensibilité et de résistance des variétés de riz différentielles d'AfricaRice à huit isolats de la panachure jaune du riz (RYMV) de Gagnoa (Côte d'Ivoire). *Journal of Animal & Plant Sciences*, 26, 4138–4149.
Soro, M., Tiendrébéogo, F., Pita, J.S., Traoré, E.T., Somé, K., Tibiri, E.B. et al. (2021) Epidemiological assessment of cassava mosaic disease in Burkina Faso. *Plant Pathology*, 70, 2207–2216.
Sseruwagi, P., Sserubombwe, W.S., Legg, J.P., Ndunguru, J. & Thresh, J.M. (2004) Methods of surveying the incidence and severity of cassava mosaic disease and whitefly vector populations on cassava in Africa: a review. *Virus Research*, 100, 129–142.
Toualy, M.N.Y., Akinbade, S.A., Koutoua, S., Diallo, H.A. & Lava, P. (2014) Incidence and distribution of cassava mosaic begomoviruses in Côte d'Ivoire. International Institute of Tropical Agriculture (IITA), PMB 5320, Ibadan, Nigeria. 4, 131–139.
Vernier, P., N'Zué, B. & Zakhia-Rozis, N. (2018) Le manioc, entre culture alimentaire et filière agro-industrielle. Quæ, CTA, Presses. Agronomiques de Gembloux, pp. 1–208.
Wickham, H. (2016) *ggplot2: elegant graphics for data analysis*. Available at: http://ggplot2.org [Accessed 5th September 2022].

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Amoakon, W.J.-L., Yoboué, A.A.N., Pita, J.S., Mutuku, J.M., N'Zué, B., Combala, M. et al. (2023) Occurrence of cassava mosaic begomoviruses in national cassava germplasm preserved in two agro-ecological zones of Ivory Coast. *Plant Pathology*, 72, 1011–1021. Available from: https://doi.org/10.1111/ppa.13723