Journal of Agricultural Science; Vol. 16, No. 7; 2024
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Using PCR and RCA Techniques to Investigate the Variants of Cassava Mosaic Virus and Their Distribution in Ghana

Esther Afoley Annang[1], Allen Oppong[1], Ruth N. A. Prempeh[1], Esther Agyemang Marfo[1], Linda A. Abrokwah[1], Rachel Agyemang[1], Joseph N. L. Lamptey[1,3], Justin Pita[2] & Lord J. J. Gowans[3]

[1] Council for Scientific and Industrial Research, Crops Research Institute, Ghana

[2] University of Felix Houphouet Boigny, Abidjan, Cote d'Ivoire

[3] Kwame Nkrumah University of Science and Technology, Ghana

Correspondence: Esther Afoley Annang. Council for Scientific and Industrial Research, Crops Research Institute, Kumasi, Ghana. E-mail: esther.annang1@gmail.com

Received: March 27, 2024 Accepted: May 5, 2024 Online Published: June 15, 2024

doi:10.5539/jas.v16n7p98 URL: https://doi.org/10.5539/jas.v16n7p98

Abstract

Cassava mosaic disease (CMD) caused by cassava begomoviruses is the major constraint to cassava production in Ghana. The disease is known to cause reduction in root yield. To ascertain the distribution of viruses causing CMD, 95 diseased cassava samples were collected in two agroecological zones of Ghana-Deciduous Forest zone and the Transitional zone. On a scale of 1-5, CMD severity was scored. Mean CMD severity score was 2.9, however there was no significant difference ($p > 0.05$) between the zones. Averagely, CMD score of > 2.8 in 71% of farms visited was recorded. Polymerase chain reaction (PCR) and rolling circle amplification (RCA) were employed for virus identification. PCR revealed that *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and mixed infections were prevalent in both zones. EACMV-Cameroon strain was also identified to be common within these zones. The transitional zone had the highest percentage of CMD infection. Unamplified samples from PCR were amplified using rolling circle amplification (RCA) technique. Amplification and characterisation of complete genome sequences of two isolates were carried out. The complete genome of 2780 nucleotides from samples showed a high similarity to *African cassava mosaic virus*-Ghana (ACMV-GH). Sequences clustered with ACMV-Ivory Coast, ACMV Nigeria-Ogo, ACMV-BF, ACMV-UG having > 96% identity. This shows the close relation that exists amongst the ACMV strains in Africa. These findings highlight the need for a continuous survey of CMD to help manage the disease in the country.

Keywords: ACMV, EACMV, EACMV-CM strain, sequencing, genome

1. Introduction

Cassava (Manihot esculenta Crantz) is an important staple food crop in Africa and Asia, where it is consumed daily by approximately 800 million people and provides food security for over 40% of Africa's population (FAOSTAT, 2017; FAO, 2019). After rice and maize, cassava is the third-largest source of carbohydrates in the tropics and grown by nearly every Ghanaian family involved in farming, *i.e.*, about 70% of Ghanaian farmers (Parkes, 2009). The African continent accounts for approximately 60% of the world's cassava production, with Ghana being the sixth highest producer (FAOSTAT, 2017). Cassava is therefore an important crop for food security in Ghana (Manu-Aduening et al., 2007).

Despite its relevance, cassava production is greatly affected by biotic stresses such as pests and diseases, specifically, cassava mosaic disease (CMD), cassava brown streak disease (CBSD), cassava anthracnose disease (CAD), cassava bacterial blight (CBB) and brown leaf spot (BLS). Among these diseases, CMD and CBSD are the most economically important diseases and a major constraint to cassava production in Africa (Legg & Fauquet, 2004; Legg et al., 2006: Torkpo et al., 2021).

The cassava mosaic disease (CMD) is characterised by symptoms such as chlorosis, mosaic, distortion and crumpling of leaves and stunting of plants severely affecting the roots, the economic part of cassava (Lamptey et al., 1998). CMD is transmitted by the whitefly *Bemisia* sp., which is the only known vector of the disease (Mound & Halsey, 1978; Hillocks & Thresh, 2000; Legg et al., 2002). There are two variants of CMD: Africa cassava mosaic virus (ACMV) and East Africa cassava mosaic virus (EACMV). There have been reported

incidences of both ACMV and EACMV in Ghana (Lamptey et al., 2000; Manu-Aduening et al., 2007; Torkpo, 2009; Oppong et al., 2021). Globally, countries have experienced loss of agricultural produce amounting to billions of US dollars due to plant viral disease spread by *B. tabaci*. In 2002, CMD in Africa caused an economic loss ranging from 19.6% to 27.8% of the annual production of 97 million tonnes, as reported by Zhang et al. (2005). Also, in Central and East Africa, the annual economic loss has been projected at US$ 1.9 to 2.7 billion US dollars (Patil & Fauquet, 2009).

Polymerase Chain Reaction (PCR) and Rolling Circle Amplification (RCA) are established molecular methods for viral detection of both known and novel viruses. Over the years, PCR has become the method of choice for determining the genetic variability within cassava begomoviruses using genome specific primers while RCA is a simple and effective technique used to detect the presence of viral diseases (Zhao et al., 2012; Salki et al., 1988). RCA rapidly synthesizes multiple copies of circular molecules of DNA or RNA, plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids. This enhances virus detection by 10 to 1000 times (Kathurima et al., 2016).

The vegetatively propagated nature of cassava increases the viral load and transmission, leading to a higher number of infected plants, which results in low yields (Thresh & Cooter, 2005). A major step towards the control of this disease is to assess the prevalence or distribution pattern of the various strains/variants of the virus across the important cassava growing ecologies in Ghana. Disease surveys were conducted in the deciduous forest zone and the transitional zone of Ghana to assess the strains of the Cassava mosaic virus in those agro-ecologies. PCR and RCA techniques were both used with the objective of unravelling all the strains of CMV that might be present in those agro-ecologies. It is believed that unravelling all the strains of CMV in those ecologies will boost cassava breeding activities as well as contribute to the control of planting material transfers and thereby improve the productivity of the crop in farmers fields. Achieving such a feat requires effective diagnostic tools for the virus. Thus, the objective of the study was to use PCR and RCA techniques to ascertain CMV variants and their distribution pattern in the transitional and deciduous forest zones of Ghana.

2. Materials and Methods

2.1 Study Area

Samples were collected from two ecological zones: the deciduous forest zone and the transitional zone. The deciduous forest zone comprised parts of the Eastern, Ashanti, Oti, and Volta regions of Ghana. The transitional zone consisted of the Bono, Bono East and Ahafo regions of Ghana. These two zones contain the top five leading producers of cassava (2012-2014) in Ghana. The Eastern region produces 4,307,372.22 metric tonnes (MT), whereas Bono, Bono East and Ahafo regions, which represent the transition zones, together produce 3,460,907.08 MT. The Ashanti region produces 2,435,915.22 MT (SRID-MoFA, 2014).

The deciduous forest zone has average annual rainfall ranging from 1200 to 1600 mm, while the transition zone has average rainfall ranging from 1100 to 1400 mm per year.

The Eastern region has temperatures ranging from 26 °C in August to 30 °C in March. The temperature in the transition zone is generally high, averaging over 23.9 °C throughout the year. The Volta region is also characterised by a moderate temperature range of 21 °C to 32 °C. Ashanti region has an average temperature of 26.3 °C (Adu-Prah et al., 2019; Asare-Nuamah & Botchway, 2019). The two zones together were named Forest-Transition zones for the purpose of this study (Figure 1).

Figure 1. Map showing localities where sample collection occurred

2.2 Collection of Cassava Samples

Cassava cuttings were collected from various towns within the two zones from August to September, 2020 (Figure 1). Cassava cuttings showing symptoms for CMD were collected from Eastern, Ashanti, Bono, Bono East, Ahafo, Oti and Volta regions of Ghana. These regions constitute the Forest- Transition zone of Ghana. Geographic coordinates (latitude and longitude) were recorded using a geographical positioning system (GPS) for each sampled location. A minimum distance between two fields where samples were collected was 10 km and in areas where fields were not close, the distances between two fields were varied. In each field transect walks along two diagonals were made and disease incidence and severity were assessed on 15 plants within each transect.

2.3 CMD Severity Scores on Cassava Plants

To score for the severity of CMD, a scale of 1-5 was used (Houngue et al., 2019). Plants showing no symptoms were scored "1". Plants with moderate chlorotic spots or some distortion at the base were scored "2". Plants with spots on the entire surface of the leaves and twisted leaves were scored "3". Plants whose blades are distorted or shrunk up to 2/3 of leaf area were scored "4". Lastly, plants with severe symptoms of CMD and/or total distortion of 4/5 of the leaf area and stunting of the entire plant were scored 5. Disease Cassava leaves were collected into herbarium pressers labelled and then sent to the lab for analysis.

2.4 Cassava DNA Extraction Using Modified CTAB Extraction Protocol

The DNA extraction protocol was carried out according to the protocol described by J. J. Doyle and J. L. Doyle (1987), with some modifications. Two hundred milligrams (mg) of fresh cassava leaves were weighed into a 2 ml Eppendorf tube, liquid nitrogen was added and was grounded. One millilitre of freshly prepared Cetyl trimethylammonium bromide (CTAB) extraction buffer warmed at 65 °C was added to the ground leaf sample. This procedure was carried out under a fume hood. The content was then mixed by vortexing and was

subsequently incubated in a water bath at 65 °C for 25 minutes, with 5 minutes' interval of gentle mixing. Samples were removed from the water bath and allowed to cool for 3 minutes before centrifuging at 13000 rpm for 10 minutes.

Six hundred microliters (µl) of the aqueous phase were then transferred into a new 2 ml Eppendorf tube and 600 µl of chloroform isoamyl (24:1) was added under a fume hood and mixed gently by inverting the tubes until the mixture turned milky. It was then centrifuged at 13000 rpm for 10 minutes. Five hundred and fifty µl was carefully transferred into a new 2 ml tube without disturbing the middle layer. About 825 µl of cold absolute ethanol and 82.5 µl of 3 M sodium acetate was added to each sample. The resultant solution was mixed 10 times by inverting, followed by centrifugation at 13000 rpm for 10 minutes. The DNA pellet was washed using 1 ml of 70% ethanol and centrifuged at 13000 rpm for 10 minutes. The ethanol was discarded and the pellet dried for 30 minutes.

After drying, the pellet was dissolved in 500 µl of Tris Ethylenediaminetetraacetic acid (TE) buffer and 6 µl RNase A (20mg/ml) was added, followed by incubation at 37 °C for 45 minutes. About 250 µl of ammonium acetate was added to the solution and mixed. The resultant mixture was then incubated on ice for 5 minutes, followed by centrifugation at 13000 rpm for 10 minutes. The supernatant was transferred into a newly labelled 1.5 ml Eppendorf tube. About 700 µl of cold isopropanol was added and mixed by inversion and was incubated in -20 °C for 1 hour, followed by centrifugation at 13000 rpm for 5 minutes. The supernatant was discarded and the nucleic acid pellet washed with 80% ethanol and centrifuged at 13000 rpm for 5 min. The alcohol was discarded and the pellet was dried at room temperature. The dried DNA pellet was then dissolved in 100 µl TE buffer.

2.5 Detection of Cassava Mosaic Begomoviruses (CMBs) Using PCR

Two different sets of primers for CMV (ACMV and EACMV) were used for this study (Table 1). The set for ACMV consisted of two primer sets namely JSP001/JSP002 and ACMV B1/ACMV B2. The primer sets for EACMV were JSP001/JSP003, EAB555F/EAB555R and VNF031/F/VNF032/R.

Table 1. List of primers for CMV detection

Virus Species	Primer name	Primer sequences (5' to 3')	Target region	Size	Reference
ACMV	JSP 001	ATGTCGAAGCGACCAGGAGAT	ACMV-CP **(DNA-A)**	783bp	Pita et al., 2001
	JSP 002	TGTTTATTAATTGCCAATACT			
	ACMVB1	TCGGGAGTGATACATGCGAAGGC	ACMV-BV1/BC1 **(DNA-B)**	628bp	Matic et al., 2012
	ACMVB2	GGCTACACCAGCTACCTGAAGCT			
EACMV	JSP 001	ATGTCGAAGCGACCAGGAGAT	EACMV-CP **(DNA-A)**	780bp	Pita et al., 2001
	JSP 003	CCTTTATTAATTTGTCACTGC			
	VNF031/F	GGATACAGATAGGGTTCCCAC	EACMV-CM AC2/AC3 **(DNA-A)**	≈ 560bp	Fondong et al., 2000
	VNF032/R	GACGAGGACAAGAATTCCAAT			
	EAB555/F	TACATCGGCCTTTGAGTCGCATGG	EACMV-BC1/CR **(DNA-B)**	544-560 bp	Fondong et al., 2000
	EAB555/R	CTTATTAACGCCTATATAAACACC			

2.6 Polymerase Chain Reaction (PCR)

PCR was carried out in a 25 µl reaction consisting of PCR water, 5 µl of 5× standard buffer (New England Biolabs Inc., Massachusetts, USA), 2.5 µl of 5% Tween-20, 0.25 dNTPs, 0.25 µl each of forward and reverse primers, 0.2 Taq polymerase, and 5 µl of DNA template at a concentration of 50 ng.

Amplification conditions included a first PCR cycle comprising denaturation at 94 °C for 5 minutes, annealing at 52 °C for 1 minute, and elongation at 72 °C for 2 minutes, final elongation step was performed at 72 °C for 10 minutes, and the reaction was held at 4 °C. PCR amplicons were separated by electrophoresis on a 1.5% agarose gel containing ethidium bromide (lg/10 ml) under a constant current of 100 mA. This migration was then followed by visualization of the amplified bands under UV light.

2.7 Rolling Circle Amplification (RCA), Cloning and Sequencing

2.7.1 Rolling Circle Amplification (RCA) Technique

To amplify the viral DNA in the plant genome, DNA amplification was conducted using the Templiphi DNA Amplification Kit (Cytiva-formerly GE Healthcare, Life Sciences, Marlborough, USA). Based on phi29 DNA polymerase, Templiphi exponentially amplifies single or double circular DNA by emulating the *in vivo* mechanism of rolling circle DNA replication. In a 200 µl tube, 5 µl of sample buffer was added to 1 µl of DNA template and incubated at 95 °C for 3 minutes. Templiphi premix was prepared by adding 5 µl of reaction buffer and 0.2 µl of enzyme mix was added to the initial solution in the 200 µl tube. The mixture was incubated at 30 °C for 20 hours. The product was then loaded on a 1% agarose gel.

2.7.2 Digestion of RCA Product Using Restriction Enzyme

Digestion of the RCA product was carried out to obtain amplicon or insert sizes that meet the carrying capacity of the cloning vector and would facilitate easy cloning of RCA amplicons. The restriction enzyme *Bam*HI (10 U/µl) was used to digest the RCA, and the reaction was incubated at 37 °C for 2 hours.

2.7.3 Digestion of the Vector pGem-3zf (+)

A vector was chosen to serve as a host for the RCA inserts. The pGem-3zf(+) vector was chosen because it has a restriction enzyme site for *Bam*HI, has an antibiotic resistance gene, and also serves as a standard cloning vector. A total of 4 µl of the pGem-3zf (+) vector, 1.5 µl of Buffer E, 0.15 µl of BSA, 0.5 µl of enzyme *Bam*HI (10 U/µl) and 13.85 µl of water were pipetted into a reaction tube and was incubated at 37 °C for 2 hours.

2.7.4 Dephosphorylation of Digested Vector

Dephosphorylation makes use of the thermosensitive alkaline phosphatase (TSAP) enzyme to catalyse the removal of 5' phosphate groups from DNA, thereby preventing the recirculation and re-ligation of the linearised cloning vector. This was carried out in a final volume of 30 µl, consisting of 18 µl of digested pGem-3zf (+) vector, 3 µl of Multicore buffer, 4 µl of TSAP, and 5 µl of water. The reaction was incubated at 37 °C for 30 minutes.

2.7.5 Ligation of pGem-3zf (+) Vector and RCA Digested Product

Ligation of RCA product into pGem-3zf (+) vector was carried out by adding 5 µl of digested RCA product, 0.8 µl of digested vector, 0.7 µl of ligase buffer (10×), 0.5 µl of ligase T4, and 3 µl of water. It was incubated at 4 °C to 8 °C overnight.

2.7.8 Competent Cell Preparation

E. coli strain JM109 (Promega, Madison, USA) was grown in Lysogeny broth (LB) medium and incubated at 37 °C overnight. A total of 2 µl of cultured bacteria was picked and added to 200 ml of LB medium. It was again incubated at 37 °C for 2 to 3 hours. The optical density (DO) at 600 nm was then measured using a spectrophotometer (Mettler Toledo, Zurich, Switzerland). This was done after two hours and checked at 15 minutes intervals to ensure that the product concentration did not exceed 0.6. About 50 µl of the solution was dispensed into a falcon tube and immediately kept on ice. It was centrifuged at 3000 g at 4 °C for 10 minutes. The supernatant was discarded and 5 ml of solution A, consisting of 0.1M $CaCl_2$, was added and mixed gently. It was centrifuged at 4 °C for 10 minutes at 3000 g. The supernatant was discarded, and solution B, comprising 0.1M $CaCl_2$ and 15% glycerol, was added at a volume of 4 ml to the pellet and mixed gently. The resulting reaction was dispensed into 1.5 ml tubes and kept at -80 °C for later use.

2.7.9 Transformation of Competent *E. coli* Cells

A total of 5 µl of ligation product was added to 50 µl of competent cells and mixed gently by pipetting. It was then kept on ice for 20 minutes. A thermal shock was applied to the cells at 42 °C for 45 seconds, and the reaction tube was immediately incubated on ice for 2 minutes. The solution was mixed in a 500 µl Super Optimal broth (SOC) medium which contained 20% glucose. SOC medium is used to grow competent cells to maximize the efficiency of bacterial plasmid transformations.

The reaction was then incubated by shaking on a shaker at 37 °C for 1 hour 30 minutes. The solution was plated on LB solid medium containing ampicillin, Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and Isopropyl β-D-1-thiogalactopyranoside (IPTG). It was incubated at 37 °C overnight. Transformed cells appeared white, whereas untransformed cells appeared blue.

2.7.10 Screening for Positively Transformed *E. coli* Cells

Transformed cells were screened using PCR by adding 5 µl 5× Go Tag buffer (Promega, Madison, USA), 1 µl dNTPs, 0.5 µl of M13 universal primer each of forward and reverse primer and 0.2 µl of Taq polymerase in a total reaction volume of 20 µl. With the aid of a sterilised toothpick, a minute amount of the transformed cells was picked and used as a template for the reaction. This was carried out under certain conditions: hot start at 95 °C for 5 minutes, followed by 30 cycles of 94 °C for 30 seconds of denaturation temperature, 55 °C for 45 seconds of annealing temperature and 72 °C for 3 minutes. A final elongation was carried out at 72 °C for 10 minutes and hold at 4 °C. After the PCR, the product was run on a 1.5% agarose gel electrophoresis at 100 V for 45 minutes.

2.7.11 Plasmid Extraction and Sequencing

Cells were grown again on LB medium and incubated at 37 °C overnight. The recombinant plasmids within the cells were extracted using Qiagen miniprep extraction kit (Germantown, Maryland, USA). A total of 3 µl of cells were pelleted by centrifugation at 10,000 rpm for 3 minutes at room temperature. A total of 250 µl of buffer P1 was added to the pellet, re-suspended and then transferred into a 2.0 ml microcentrifuge tube. Subsequently, 250 µl buffer P2 was added and mixed 4 to 6 times by turning the tube upside now and mixing well until the solution became clear. About 350 µl of buffer N3 was added to the solution and inverted 4 to 6 times, centrifuged for 10 minutes at 13,000 rpm and transferred into a QIAprep 2.0 ml spin column by pipetting. The supernatant of 800 µl was then pipetted into the column and centrifuged for 1 minute at 14, 000 rpm. The QIAprep column was washed by the addition of 0.5 ml buffer PB, centrifuged for 1 minute at 14, 000 rpm and then flow-through was discarded. A second wash was done by adding 0.75 ml buffer PB and centrifuged at 1 minute at 14, 000 rpm and the flow-through was discarded. It was further centrifuged for 1 minute to remove residual wash buffer. The QIAprep column was placed in a clean 1.5 ml microcentrifuge tube and elution of DNA was carried out by adding 50 µl of water to the centre of the column.

To confirm the success of the recombination DNA experiment, PCR amplification was performed on extracted recombinant plasmid using the plasmid as template by adding 5 µl of 5× Go Tag buffer (Promega, Madison, USA), 1 µl dNTPs, 0.5 µl of M13 universal primer each of forward and reverse primer, 0.1 µl of Taq polymerase and 1 µl of the plasmid in a total reaction volume of 20 µl. This was followed by a cycling reaction of 95 °C for 5 minutes, followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 45 seconds and 72 °C for 3 minutes. After the PCR, the product was run on a 1.5% 1 × TAE agarose gel electrophoresis at 100 V for 45 minutes and the amplified products were excised.

The plasmids from the bands were obtained using a GENECLEAN Turbo kit (MP Biomedicals, USA). One hundred microliters of GENECLEAN Turbo salt solution per 100 mg gel slice was added and incubated at 55 °C for 5 minutes. The reaction was then transferred into the GENECLEAN Turbo cartridge and centrifuged at 13,000 × g for 5 seconds. A 500 µl of GENECLEAN Turbo wash solution was added to the cartridge and centrifuged at 13,000 × g for 5 seconds. The cartridge was emptied and span to dry the pellet by centrifuging at 13,000 × g for 4 minutes. A clean 1.5 ml tube was inserted under the cartridge and 30 µl of GENECLEAN Turbo elution solution was added. It was incubated for 5 minutes at room temperature and then centrifuged at 13,000 × g for 1 minute. The obtained plasmid was then sent for sequencing (Genewiz, Takeley, UK) to identify the type of plant virus present.

2.8 Data Analysis

Descriptive and quantitative statistics were utilized in the data presentation. Descriptive statistics included bar charts, dendrograms, and maps. PCR results for CMD were scored as 1 and 0 for the presence and absence of a virus respectively. Reference sequence retrieval for the two sequences obtained from GENEWIZ (105C1 and 110C11) from the National Center for Biotechnology Information (NCBI) was conducted using Geneious Prime software with the following parameters: Megablast, E value of 0.05, and maximum hit of 100. The metadata for the sequences retrieved was exported as an Excel file and selection of 25 sequences based on unique sequences was conducted for the generation of the phylogenetic tree along with the two sequences. The 27 sequences were imported and aligned using the ClustalW method in BioEdit software Version 7.2.5 (Hall, 1999). The aligned file was exported as a fasta file and imported into Geneious Prime software for the phylogenetic analysis. The Geneious Tree Builder interface was used to generate a Neighbour-Joining tree using Tamura-Nei genetic distance model and 1000 bootstraps. ArcGIS software was used to map out CMD distribution in the study areas.

3. Results

3.1 Cassava Mosaic Disease (CMD) Diagnostics

The severity of CMD varied between the transition and deciduous zones of the seven regions, though this was not significant (Figure 2). CMD severity was high in the transitional zone as compared to the deciduous forest zone. The transitional zone recorded a mean CMD score of 3.0 for both the Bono and Bono East regions, with the Ahafo region recording 2.9. Scores were also moderate to high in the deciduous zone ranging from 2.4 to 3.0, with the Eastern region recording the lowest severity. These results suggest that the transitional zone is likely a hotspot for CMD. There was no significant difference ($p > 0.05$) with regards to severity amongst the zones and no significant difference ($P > 0.05$) within zones. In general, the trend in symptom severity was consistent throughout the seven regions.

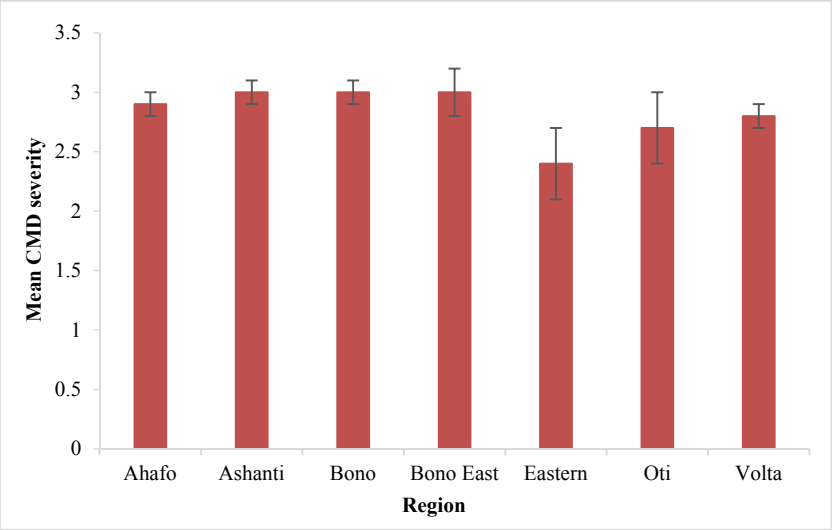

Figure 2. Average CMD severity scores

3.2 Identification of CMV Using Molecular Markers and PCR

The percentage of CMD scores for ninety-five samples were also analysed (Figure 3). The transitional zone showed the highest percentage of samples infected with ACMV (100%), EACMV (92.59%) and mixed infection (92.59%). Mixed infection means that the sample was positive for ACMV as well as EACMV disease. The percentage of samples with the virus in the deciduous forest zone for ACMV, EACMV and mixed infection was 83.82%, 88.24% and 73.53% respectively. About 1.47% of CMD free samples were observed in the deciduous forest zone. However, CMD scores showed no significant differences ($p > 0.05$) for ACMV, EACMV and mixed infections (Appendix A).

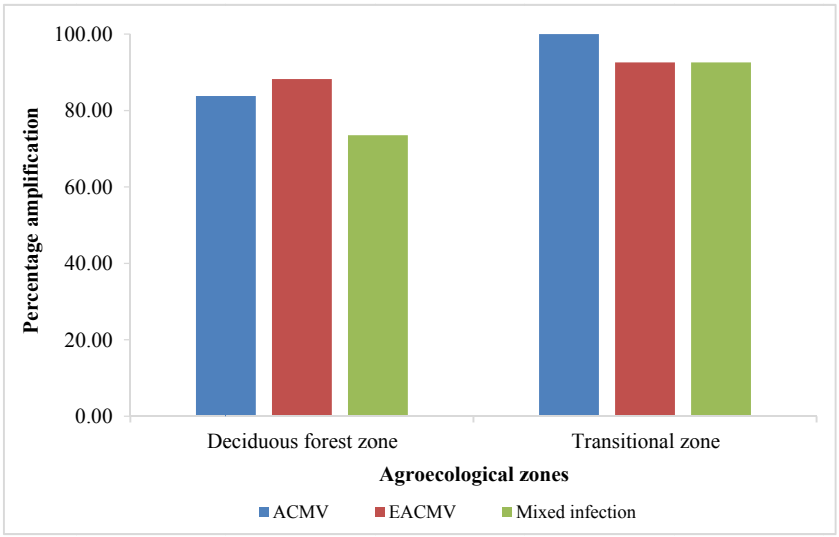

Figure 3. Ecological zones and the type of virus present

Almost all plants collected from the transition zone showed the presence of ACMV and EACMV. However, it is worth noting that the Bono East region recorded the highest disease prevalence within the transition zone compared to the Ahafo and Bono regions. This seems to suggest that Bono East is a hot spot for ACMV and EACMV in the Transitional zone (Figure 3).

Regions within the deciduous forest zone also showed varied prevalence for CMD, with the Volta region recording the highest infected samples. The Eastern region recorded the lowest percentage of infection for ACMV, as well as mixed infections; the Eastern region, however, recorded high numbers of EACMV.

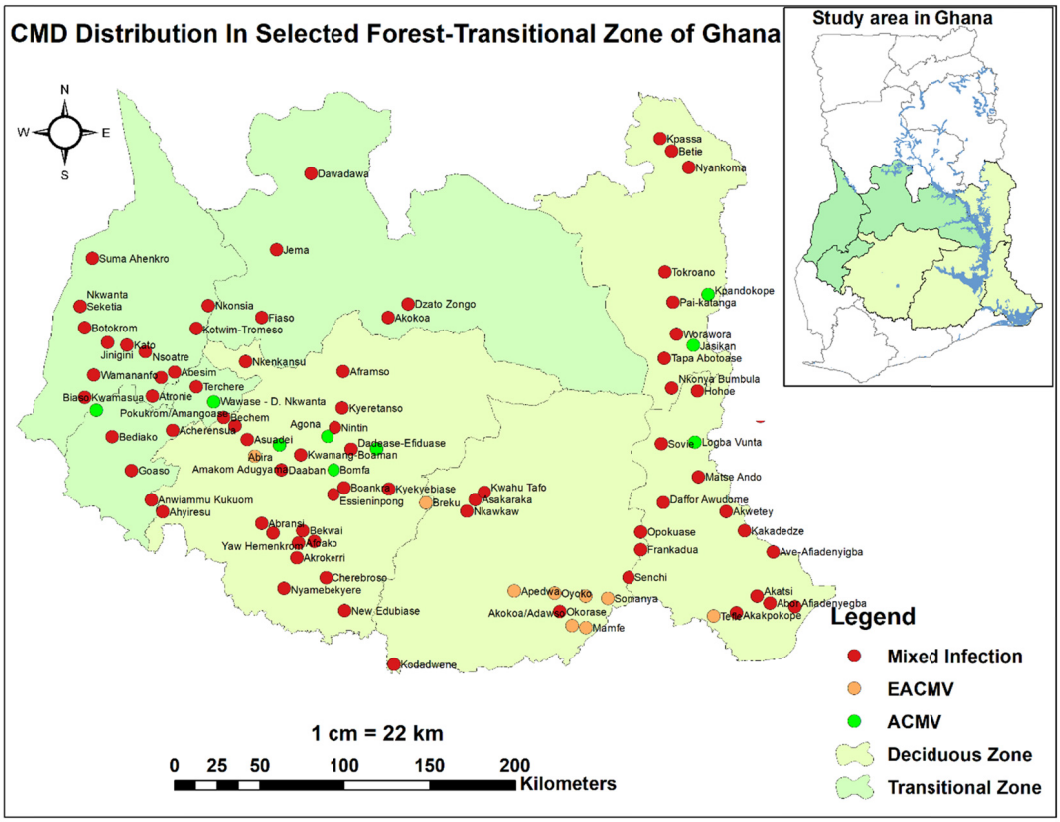

Figure 4. A map showing Cassava mosaic disease distribution in the Forest Transition zone

All selected study sites showed evidence of CMD infections, with both ACMV and EACMV being sparsely distributed across the study areas (Figure 4). Cassava infected with only ACMV was seen to cluster more in the deciduous forest zone, specifically in the Ashanti region. The Ashanti region recorded single infections in some communities. One town (Adugyama) recorded only EACMV infection, and four other communities also recorded only ACMV infection. The Eastern region recorded high numbers of EACMV compared to ACMV and also had few mixed infections. Even though the surveyed communities were few for the Eastern region as compared to the Ashanti region, it was evident that there was a high incidence of EACMV in areas close to the Greater Accra region. This presupposes that the Eastern region could be a hot spot for EACMV in the deciduous forest zone. In the eastern part of the deciduous zone (Volta and Oti regions), mixed infections were largely observed, with three communities demonstrating evidence of only ACMV infection; however, the town (Tefle) recorded only EACMV infection. The transitional zone did not record any area with a single EACMV infection but had an abundance of mixed infections. Bechem was the only community with a single ACMV infection (Figure 4).

3.3 Diagnosis of CMV Using Rolling Circle Amplification (RCA)

Over the years, PCR has been the method of choice for determining the genetic variability within cassava begomoviruses using genome specific primers (Salki et al., 1988). According to Fargette et al. (1996), Bull et al. (2006), and Tiendrébéogo et al. (2012), nine strains of these viruses have been linked with cassava in Africa (Fargette et al., 1996; Bull et al., 2006; Tiendrébéogo et al., 2012). In Ghana, ACMV, EACMV, ACMV-GH, and EACMV-GH have been associated with CMD (Lamptey et al., 1998, 2000; Offei et al., 1999; Manu-Aduening et al., 2007; Torkpo, 2009; Oteng- Frimpong et al., 2012). In order to detect viral strains that could not be amplified using the conventional PCR method, RCA was employed in this regard. Double stranded DNA was used, and this generally yielded high molecular weight products. As a result, these products are digested into smaller lengths fragments, used as inserts for a plasmid vector and then cloned. Recombinant plasmids obtained from these transformed cells were then verified using Sanger sequencing as described by Inoue-Nagata et al. (2004).

In this experiment, of the ninety-five (95) samples utilized, some samples did not show any evidence of viral infection because the primers employed could not amplify any target sequence. RCA has become an important technique in disease diagnostics. For this work, plasmids obtained from the transformed cells were sent for sequencing (Genewiz, Takeley, UK). The presence or otherwise of viruses on samples were ascertained by aligning the sequence result to reference sequences employing the basic local alignment search tool (BLASTn) from the National Center for Biotechnology Information (NBCI) website. A total of twelve plasmids were sequenced; however, these plasmids were obtained from two main samples labelled 105c1 and 110c11.

The results after blastn showed that the plasmids sequence aligned to ACMV-GH and showed > 97% identity to the ACMV GH strain (Appendix B). Sequences obtained had a query length of 2780 nucleotides for both sequences.

When the sequences were compared to other known sequences of ACMV using Geneious prime, two main clusters were observed. Samples 105c1and 110c11 grouped in the same cluster with ACMV Nigeria, ACMV Uganda, ACMV DR Congo, ACMV Burkina and ACMV Ivory Coast (Figure 5). This, however, sub-grouped as subcluster (ACMV-GH and 110c11) and subcluster (ACMV Uganda, ACMV Kenya, ACMV Burkina Faso and ACMV Nigeria). The results obtained showed that there is a relationship between the Ghanaian isolates and other geminiviruses in the NCBI database.

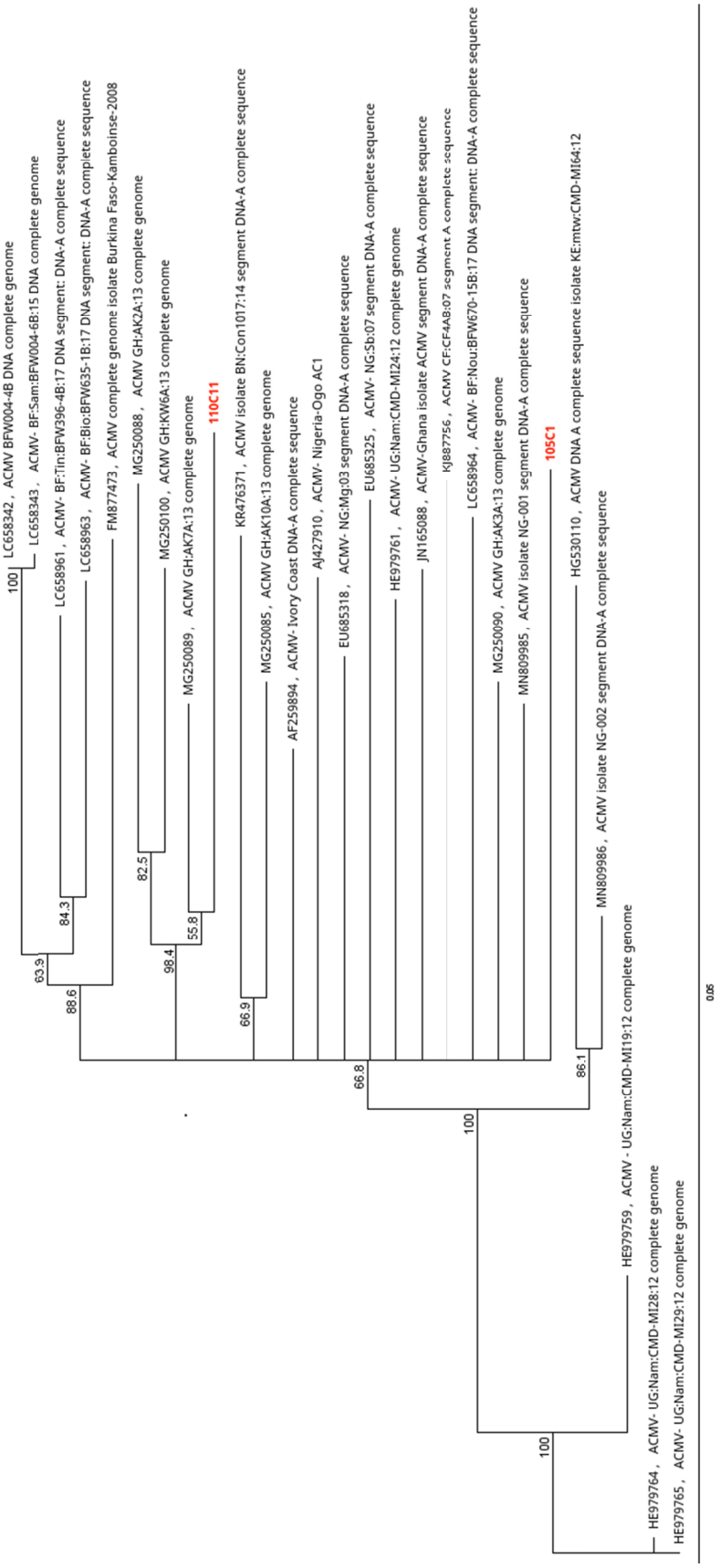

Figure 5. A phylogenetic tree depicting the probable identity of the viral isolates

4. Discussion

4.1 CMD Diagnostics

4.1.1 CMD Severity Score

The main disease symptoms observed were mosaic, chlorosis, leaf distortion, stunted growth and spindle leaf for some areas. The mean severity score in the transition zone was 3.0, which was not significantly ($p > 0.05$) higher than that observed for the deciduous zone with a score of 2.7. Both zones had a mean viral score of 2.9. Averagely, a CMD score of > 2.8 in 71% of farms visited was recorded. This observation largely confirms the findings of Torkpo et al. (2017) who recorded an average CMD score of 3.0 in 69% of farms surveyed in Ghana. Oppong et al. (2021) also recorded a severity score of 2.98 on a nationwide CMD field survey in Ghana, confirming our current findings. This gives an indication of the need for CMD to be effectively managed in the country to reduce disease prevalence thereby maximising yield considering the fact that these two zones are the main cassava growing zones in the country.

4.1.2 CMD Diagnostics Using Molecular Markers

Cassava as a staple crop in Ghana is consumed in almost every part of the country. However, disease affecting this crop has reduced the expected yield of the crop (Elegba et al., 2013). This study used molecular markers to identify viral strains that cause cassava mosaic disease in some parts of the country. It was demonstrated by this study that almost all cassava samples collected from the survey were diseased.

Out of the five primers used, ACMVB1/B2 was able to amplify most of the samples indicating that the target sequence for this primer is a reliable marker for diagnosis of CMD. Surprisingly, VNF031F/VNF032R which is a primer for EACMV-CM also amplified for most of the samples implying that the Cameroon viral strain has found its way into Ghana. Torkpo et al. (2017): Oppong et al. (2021) also observed widespread of EACMV-CM strain in Ghana. Since there were amplification for both ACMV and EACMV, mixed infection then existed for most of the sampled sites, with only a few samples being virus free. Those that were virus free did not mean they are not infected with the virus but could be because the primers used could not amplify that strain of the virus and therefore may be infected by other strains of the virus which the primers used could not amplify. This gives the need for more primers specific to other CMV strains to be used in diagnostics; this will give a broader view of the different types of CMD present. All primers used proved to be effective in the identification of both ACMV and EACMV. Elegba et al. (2013) reliably used JSP001/JSP002 and EAB555F/EAB555R to detect ACMV and EACMV, respectively, in Ghana; this confirms the findings of the current study. However, the low percentage of amplification of JSP001/JSP002 and JSP001/JSP003 in amplifying for the virus in the zones gives an indication that these two primers cannot be completely relied on for ACMV and EACMV detection in the deciduous zone and the transitional zone.

Overall, this study demonstrated that both ACMV and EACMV occur in the country, an observation that is in harmony with those observed elsewhere (Lamptey et al., 2000; Manu-Aduening et al., 2007; Torkpo, 2009; Oppong et al., 2021). EACMV was restricted to eastern Africa but Fondong et al. (2000) showed that the virus has migrated to Western Kenya, Western Tanzania, Zambia, Nigeria, Togo, Guinea, Ivory Coast and Cameroon. Further studies on EACMV shows it is gradually spreading from East Africa to West Africa (Ndunguru et al., 2005; Legg et al., 2006; Patil & Fauquet, 2009); this study confirms these earlier findings.

Mixed infections observed in both the deciduous forest zone and transitional zone were alarming and should be a national concern. Mixed infections can ultimately result in the formation of highly virulent strains like EACMV-Uganda which reduces yield drastically (Pita et al., 2001). Also, the incidence of both ACMV and EACMV in Ghana can be one of the many reasons why the country is recording lower root yields (Elegba et al., 2013).

Eastern region had the lowest percentage of CMD, which is currently the largest cassava producer in Ghana and may explain some of the reasons why the region recorded a three-year average production from 2012 to 2014 of 4.3 million tonnes of cassava (SRID-MoFA, 2014). High incidence of EACMV in the Eastern region also makes it a hot spot for the virus. This could be as a result of introduction of a diseased stem cutting which was cultivated in one part and gradually most farmers around the locality also started growing it as it is the norm for farmers to exchange cuttings of preferred varieties. This may explain why the disease was centred around Oyoko, Apedwa, Amanfrom, Akokoa and Mamfe, all in the southern part of the region. The Eastern region is still able to produce high cassava yield probably because of low numbers of mixed infections.

Overall, the transitional zone had the highest percentage of CMD infection even though it is the second-highest cassava producing region in the country. This may be due to the fact that though the disease is present, the large

area of cultivated land is able to compensate for the yield losses. Moreover, not every part of the region was surveyed and sampled, suggesting that the sampled areas may probably be more CMD-prone than the un-sampled localities.

These two zones are the main cassava growing zones in the country and this study gives an indication of the need to better manage CMD in Ghana, justifying the relevance of this work. The use of molecular tools is therefore very important in plant disease diagnostics. It also helps to give prompt information on the disease to farmers.

4.2 Identification of Unamplified Viral Strains Using RCA

Polymerase Chain Reaction (PCR) has been the widely used method of amplification for which many protocols have been developed. PCR also has its limitations as it requires sequence-specific primers. RCA is gradually becoming a preferred choice in identifying unknown viral strains. This is because specific primer sequences are not required for this technique and as a result, novel viruses have been discovered (Haible et al., 2006). Also, RCA technique is better as one does not need to have prior knowledge of viral genomic sequence.

In this study, circular DNA containing begomoviruses were enriched by RCA. The enriched DNA was digested with restriction enzymes, cloned into a plasmid vector which was then used to transform *E. coli* strain JM109 (Promega, Madison, USA). The transformed bacteria were subsequently cultured to obtain large amount of the recombinant DNA. The recombinant plasmid was extracted from the bacteria containing the begomovirus and subjected to Sanger sequencing. The sequences obtained were subjected to BLAST analysis and were compared to genome sequences already deposited at the Genebank. The virus obtained after blasting was ACMV-GH virus complete genome. It clustered more closely to ACMV-Nigeria (NG:Mg:03). This result agrees with findings from Oteng-Frimpong et al. (2012) who employed RCA to detect a new viral strain which is currently known as the ACMV-GH strain. It is evident from this study that ACMV is prevalent and widespread in Ghana. The ACMV strain observed in this study has high percentage similarity to the ones found in other African countries. The clustering of RCA sequence genome gave ACMV-GH, ACMV- Ivory Coast, ACMV Nigeria-Ogo and these viral strains had > 96% identity. This demonstrates that ACMV strains in Africa are closely related (Ndunguru et al., 2005). The similarity may also be as a result of geographical closeness between the countries.

The confirmation of ACMV-GH sheds light on the viral strains present in the study sites. This study is a preliminary work to carefully study the unique symptoms associated with ACMV-GH, which may enhance the development of unique primers for its detection. In conclusion, mean CMD severity score was 2.9 which means the disease is of economic importance and measures should be taken to reduce disease prevalence in these ecological zones. The use of virus-free planting materials via tissue technique are recommended as it takes sometime for plants to be infected with the virus to the levels of having severe effect on the crop yield.

Our findings showed that there was high prevalence of ACMV, EACMV and mixed infections in the forest-transition zones of Ghana. This result is alarming since these areas are important cassava producers in Ghana. This therefore calls for effective disease control measures. RCA technique has also proved to be efficient and was able to identify the specific viral strain which was revealed as ACMV-GH. This finding highlights the need for continuous survey of CMD to help manage the disease in the country.

References

Adu-Prah, S., Appiah-Opoku, S., & Aboagye, D. (2019). Spatiotemporal evidence of recent climate variability in Ghana. *African Geographical Review, 38*(2), 172-190. https://doi.org/10.1080/19376812.2017.1404923

Asare-Nuamah, P., & Botchway, E. (2019). Understanding climate variability and change: Analysis of temperature and rainfall across agroecological zones in Ghana. *Heliyon, 5*(10), e02654. https://doi.org/10.1016/j.heliyon.2019.e02654

Bull, S. E., Briddon, R. W., Sserubombwe, W. S., Ngugi, K., Markham, P. G., & Stanley, J. (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. *Journal of General Virology, 87*(10), 3053-3065. https://doi.org/10.1099/vir.0.82013-0

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

Elegba, W., Appiah, A. S., Azu, E., Afful, N., Agbemavor, W. K., Agyei-Amponsah, J., ... Danso, K. E. (2013). Effect of mosaic virus diseases on dry matter content and starch yield of five cassava (*Manihot esculenta* Crantz) accessions in Ghana. *African Journal of Biotechnology, 12*(27). https://doi.org/10.5897/AJB12.2958

FAO. (2019). Food and Agriculture Organization of the United Nations, Statistic Division. Retrieved from https://www.fao.org/faost at/en/# data

FAOSTAT. (2017). Food and Agriculture Organization of the United Nations, Corporate Statistical Database. Retrieved June 24, 2019, from http://www.fao.org/faostat/en/#data/QC/visualize

Fargette, D., Colon, L. T., Bouveau, R., & Fauquet, C. (1996). Components of resistance of cassava to African cassava mosaic virus. *European Journal of Plant Pathology, 102*(7), 645-654. https://doi.org/10.1007/BF01877245

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*(1), 287-297. https://doi.org/10.1099/0022-1317-81-1-287

Haible, D., Kober, S., & Jeske, H. (2006). Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *Journal of Virological Methods, 135*(1), 9-16. https://doi.org/10.1016/j.jviromet.2006.01.017

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*(41), 95-98.

Hillocks, R. J., & Thresh, J. M. (2000) Cassava Mosaic and Cassava Brown Streak Virus diseases in Africa: a comparative guide to symptoms and aetiologies. *Roots, 7*(1), 1-8.

Houngue, J. A., Zandjanakou-Tachin, M., Ngalle, H. B., Pita, J. S., Cacaï, G. H. T., Ngatat, S. E., ... Ahanhanzo, C. (2019). Evaluation of resistance to cassava mosaic disease in selected African cassava cultivars using combined molecular and greenhouse grafting tools. *Physiological and Molecular Plant Pathology, 105*, 47-53. https://doi.org/10.1016/j.pmpp.2018.07.003

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*(2), 209-211. https://doi.org/10.1016/j.jviromet.2003.11.015

Kathurima, T. M., Ateka, E. M., Nyende, A. B., & Holton, T. A. (2016). The rolling circle amplification and next generation sequencing approaches reveal genome wide diversity of Kenyan cassava mosaic geminivirus. *African Journal of Biotechnology, 15*(37), 2045-2052. https://doi.org/10.5897/AJB2016.15357

Lamptey, J. N. L., Okoli, O. O., & Frimpong-Manso, P. P. (1998). Incidence and severity of African cassava mosaic disease (ACMD) and cassava bacterial blight (CBB) on some local and exotic cassava varieties in different ecological zones of Ghana. *Ghana Journal of Agricultural Science, 31*(1), 35-43. https://doi.org/10.4314/gjas.v31i1.1943

Lamptey, J. N. L., Okoli, O. O., Rossel, H. W., & Frimpong-Manso, P. P. (2000). A method for determining tolerance of cassava genotypes to African cassava mosaic disease in the screenhouse. *Ghana Journal of Agricultural Science, 33*(1), 29-32. https://doi.org/10.4314/gjas.v33i1.1881

Legg, J. P., & Fauquet, C. M. (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology, 56*(4), 585-599. https://doi.org/10.1007/s11103-004-1651-7

Legg, J. P., French, R., Rogan, D., Okao‐Okuja, G., & Brown, J. K. (2002). A distinct Bemisia tabaci (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. *Molecular Ecology, 11*(7), 1219-1229. https://doi.org/10.1046/j.1365-294X.2002.01514.x

Legg, J. P., Owor, B., Sseruwagi, P., & Ndunguru, J. (2006). Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of a regional pandemic. *Advances in Virus Research, 67*, 355-418. https://doi.org/10.1016/S0065-3527(06)67010-3

Manu-Aduening, J. A., Lamboll, R. I., Mensah, G. A., & Gibson, R. W. (2007). Farmers' perceptions and knowledge of cassava pests and diseases and their approach to germplasm selection for resistance in Ghana. *Annals of Applied Biology, 151*(2), 189-198. https://doi.org/10.1111/j.1744-7348.2007.00156.x

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*, 443-450.

Mound, L. A., & Halsey, S. H. (1978). *Whitefly of the world. A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data*. John Wiley and Sons. https://doi.org/10.5962/bhl.title.118687

Ndunguru, J., Legg, J. P., Aveling, T. A. S., Thompson, G., & Fauquet, C. M. (2005). Molecular biodiversity of cassava begomoviruses in Tanzania: evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. *Virology Journal, 2*(1), 21. https://doi.org/10.1186/1743-422X-2-21

Offei, S. K., Owuna-Kwakye, M., & Thottappilly, G. (1999). First report of East African cassava mosaic begomovirus in Ghana. *Plant Disease, 83*(9), 877-877. https://doi.org/10.1094/PDIS.1999.83.9.877C

Oppong, A., Prempeh, R. N. A., Abrokwah, L. A., Annang, E. A., Marfo, E. A., Kubi, Z. A., ... Pita, J. S. (2021). Cassava Mosaic Virus Disease in Ghana: Distribution and Spread. *Journal of Plant Physiology & Pathology, 9*(8), 258. https://doi.org/10.21203/rs.3.rs-118262/v1

Oteng-Frimpong, R., Levy, Y., Torkpo, S. K., Danquah, E. Y., Offei, S. K., & Gafni, Y. (2012). Complete genome sequencing of two causative viruses of cassava mosaic disease in Ghana. *Acta Virologica, 56*(4), 305-314. https://doi.org/10.4149/av_2012_04_305

Parkes, E. (2009). *Using molecular markers to identify new sources of CMD resistant genes for cassava improvement*. 2nd GSS Workshop, CIAT, Cali, Colombia.

Patil, B. L., & Fauquet, C. M. (2009). Cassava mosaic geminiviruses: Actual knowledge and perspectives. *Molecular Plant Pathology, 10*(5), 685-701. https://doi.org/10.1111/j.1364-3703.2009.00559.x

Pita, J. S., Fondong, V. N., Sangare, 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*(3), 655-665. https://doi.org/10.1099/0022-1317-82-3-655

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., ... Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science, 239*(4839), 487-491. https://doi.org/10.1126/science.239.4839.487

SRID-MoFA. (2014). *Agriculture in Ghana (Facts and Figures)*. Ministry of Food and Agriculture. Retrieved from http://facts/mofa.gov.gh/site/wp-content/uploads/.../mofa_ facts_and_figures.pdf

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*(1), 129-142. https://doi.org/10.1016/j.virusres.2003.12.021

Thresh, J. M., & Cooter, R. J. (2005). Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology, 54*, 587-614. https://doi.org/10.1111/j.1365-3059.2005.01282.x

Tiendrébéogo, F., Lefeuvre, P., Hoareau, M., Harimalala, M. A., De Bruyn, A., Villemot, J., ... Reynaud, B. (2012). Evolution of African cassava mosaic virus by recombination between bipartite and monopartite begomoviruses. *Virology Journal, 9*(1), 67. https://doi.org/10.1186/1743-422X-9-67

Torkpo, S. K. (2009). *Analysis of cassava mosaic begomoviruses associated with cassava mosaic disease in Ghana*. University of Ghana, Ghana.

Torkpo, S. K., Amponsah, E., Arhin, C. D., Offei, S. K. (2021). Occurrence of cassava mosaic begomovirus-associated satellites on cassava in Ghana. *Cogent Food & Agriculture, 7*(1), 196392. https://doi.org/10.1080/23311932.2021.1963929

Torkpo, S. K., Offei, K., Danquah, E. Y., & Gafni, Y. (2017). Status of cassava mosaic begomoviruses in farmers' fields in Ghana. *AIMS Agriculture and Food, 2*(3), 279. https://doi.org/10.3934/agrfood.2017.3.279

Zhang, P., Vanderschuren, H., Futterer, J., & Gruissem, W. (2005). Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. Plant Biotechnology Journal, 3(4), 385–397. https://doi.org/10.1111/j.1467-7652.2005.00132.x

Zhao, Y., Qi, L., Chen, F., Dong, Y., Kong, Y., Wu, Y., & Fan, C. (2012). Ultrasensitive and selective detection of nicotinamide adenine dinucleotide by target-triggered ligation-rolling circle amplification. *Chemical Communications, 48*(27), 3354-3356. https://doi.org/10.1039/c2cc17422g

Appendix A

Table A1. Table of means

Virus/Primer	Grand mean	Deciduous mean	Transitional mean	*P* value
ACMV	89.14	81	100	NS
EACMV	90.53	88.66	93.02	NS
Mixed Infection	80.18	70.56	93.02	NS

Appendix B

Table B1. Hit table derived sequences producing significant alignments for 105c1 and 110c11

Description for 105c1	Percentage identity	Accession
African cassava mosaic virus isolate ACMV_GH:BU13A:13, complete genome	97.52	MG250117.1
African cassava mosaic virus isolate ACMV_GH:AK7A:13, complete genome	97.48	MG250089.1
African cassava mosaic virus isolate ACMV_GH:BU12A:13, complete genome	97.48	MG250115.1
African cassava mosaic virus isolate ACMV_GH:BU6A:13, complete genome	97.48	MG250107.1
African cassava mosaic virus isolate ACMV_GH:AK3A:13, complete genome	97.48	MG250090.1
African cassava mosaic virus isolate ACMV_GH:FM5A:13, complete genome	97.45	MG250150.1
African cassava mosaic virus isolate ACMV_GH:BU14A:13, complete genome	97.45	MG250118.1
African cassava mosaic virus isolate ACMV_GH:BU1A:13, complete genome	97.45	MG250112.1
African cassava mosaic virus isolate ACMV_GH:BU2A:13, complete genome	97.45	MG250108.1
African cassava mosaic virus isolate ACMV_GH:BU10A:13, complete genome	97.41	MG250114.1
Description for 110c11	Percentage identity	Accession
African cassava mosaic virus isolate ACMV_GH:AK7A:13, complete genome	98.20	MG250089.1
African cassava mosaic virus isolate ACMV_GH:FM14A:13, complete genome	98.06	MG250159.1
African cassava mosaic virus isolate ACMV_GH:BU4A:13, complete genome	98.02	MG250119.1
African cassava mosaic virus isolate ACMV_GH:KW6A:13, complete genome	97.95	MG250100.1
African cassava mosaic virus isolate ACMV_GH:KW4A:13, complete genome	97.92	MG250099.1
African cassava mosaic virus isolate ACMV_GH:BW4A:13, complete genome	97.74	MG250128.1
African cassava mosaic virus isolate ACMV_GH:BW2A:13, complete genome	97.56	MG250127.1
African cassava mosaic virus isolate ACMV_GH:KW1A:13, complete genome	97.56	MG250094.1
African cassava mosaic virus isolate ACMV_GH:AK2A:13, complete genome	97.56	MG250088.1
African cassava mosaic virus isolate ACMV_GH:KW8A:13, complete genome	97.52	MG250097.1

Acknowledgments

The authors are very grateful to the Gates Foundation and UK Department for International Development (DFID) through University of Félix Houphouët-Boigny and the WAVE project (OPP1082413) for providing funds for this work. We also thank all staff of the plant health division, biotechnology section of CSIR- Crops Research Institute for their contribution towards this work. The authors are also grateful to the various Agric Extension Officers and drivers, who assisted during the field surveys. We also thank Dr. Fidèle Tiendrébéogo and his lab technicians for the RCA training.

Authors Contributions

JP and AO conceived, designed and managed the research. JP was project manager for Central and West African Virus Epidemiology for Roots and Tuber Crops (WAVE) facilitated the study through financial and logistical support. AO and EAA collected samples. EAA, RNAP, RA, and EAM carried out the laboratory analysis. EAA carried out the data analysis. JLLG, AO, and JNLL provided academic, scientific and bioinformatics support. EAA drafted the manuscript with scientific guidance from AO and JLLG. All authors read and approved the article.

Funding

The study was funded by the Bill and Melinda Gates Foundation and UK Department for International Development (DFID) through University of Félix Houphouët-Boigny and the WAVE project (OPP1082413).

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.