Cassava Mosaic Virus Disease in Ghana: Distribution and Spread

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Research

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Abstract

Background: Cassava is an important staple crop in most of the tropics including Ghana. The productivity of the crop is beset with pest and disease attacks. With the emergence of virulent strains of the cassava mosaic virus (CMV), regular surveys are necessary to ascertain the prevalence of CMV and their whitefly vectors in farmers' fields to help manage CMV disease affecting the crop.

Methods: Field surveys were conducted in September and October of 2015 and December 2016 to January 2017 using a harmonized sampling protocol developed by the West African Virus Epidemiology (WAVE) for root and tuber project. Three hundred and ninety-three fields were visited throughout Ghana and 11,760 cassava leaf samples examined. Whiteflies were counted on 5 plants/field. Diseased samples with varying symptoms collected were assayed using PCR and genomic sequencing.

Results: Cassava mosaic disease (CMD) symptoms were recorded in about ninety-six percent (96.4%) of fields surveyed with varying severity. These symptoms included leaf mosaic, leaf distortion/twisting, malformation, filiform leaves, stunting and chlorosis. Cultivars with red petiole colour were the most prevalent while those with green petiole colour were the least. No whitefly was found on cultivars with purple and Green petioles while cultivars with reddish-green petioles had highest count of whiteflies/plant. The Upper West and Upper East regions had the least number of whiteflies/plant. Six CMV strains were detected: ACMV-Ivory Coast, ACMV-Kenya, EACMV-Cameroun, ACMV-Ghana, EACM-Cameroun virus-Ghana and EACMV-Kenya.

Conclusion: ACMV-Kenya and EACMV-Kenya are being reported for the first time. This indicates that more CMV strains are being detected in the country.

Background

Cassava is the number one root and tuber crop in Ghana (Fig. 1). The crop is cultivated predominantly in the central and southern parts of Ghana with significant production in the middle and northern parts of the country. The only areas of the country which do not have significant production of cassava are the Savannah and Upper East regions (Fig. 1). The estimated total land under cassava production is estimated around 900,000 hectares. In Ghana, over 70% of farmers engage in cassava production, and the sector contributes about 22% of Agricultural GDP (GEPA, 2017). Ghana ranks among the top five cassava producers in Africa with an annual average production of sixteen million metric tons (GEPA, 2017).

Its cultivation and associated businesses along the value chain is a major source of employment for millions of the population. It is a staple food crop in areas where it is cultivated and it is processed into several industrial products such as cassava starch, cassava beer, spray starch, pharmaceutical raw material among several others (Koros *et al*., (2018): https://www.fao.org/3/x5032e/x5032e06.htm). In recent times cassava has become an important crop in the socio-cultural life and the economy of Ghana. However, the production of this crop faces several challenges. These include dependence on low yielding

varieties, poor agronomic practices on the part of farmers and incidence of pests and diseases (Koros *et. al.,* 2018; Thresh and Cooter, 2005). There are several pests and diseases that affect cassava production but cassava mosaic disease (CMD) constitutes the most endemic disease in the country (Torkpo *et al.,* 2017). The disease can cause yield losses of over 90% depending on time of infection and the variety (Bisimwa *et al.,* 2015; Tiendrébéogo *et. al.,* 2012;). The disease was first reported in the country a century ago by Warburg (1894) who described it as “leaf curl” or ‘crinkle’ illness afflicting cassava plants” (Torkpo *et al.,* 2017; Cudjoe *et al.,* 2005). Several strains of the cassava mosaic virus, the causal agent of CMD, have been reported in Ghana and in other parts of Africa and have been shown to be responsible for severe yield losses (Torkpo *et al.,* 2017; Legg *et al.,* 2006; Ogbe *et al.,* 2006, 2003; Owor *et al.,* 2004; Fondong *et al.,* 2000; Fauquet and Fargette, 1990; and).

To ascertain the current distribution of CMD and the strain diversity of the virus in Ghana it is important that periodic surveys and field monitoring are conducted. This is to help develop strategies which can curtail the potential damage that new and more virulent strains of the virus could cause to cassava production in the country and beyond.

To this end, two nationwide surveys were conducted to provide baseline data that can be used by breeders and other stakeholders, including modelers, to identify disease hotspot and cold spots for breeding resistant varieties and the multiplication of disease-free planting materials for farmers. Additionally, this would provide data for predicting the rate of spread of cassava mosaic virus in Ghana and West Africa.

Methods

Field Survey

Surveys were conducted throughout the regions indicated in Figure 1.They were carried out in September and October of 2015 and December 2016 to January 2017, using a harmonized sampling protocol developed by the West African Virus Epidemiology (WAVE) for root and tuber project head quartered at the University of Félix Houphouët-Boigny, Abidjan, Côte d’Ivoire. During each survey, coordinates of fields visited were captured using a GPS device. The minimum distance between two fields where samples were collected was 10 Km and in areas where cassava production was not widespread, the distance between two fields varied depending on how fields were encountered. In each field transect walks along two diagonals were made, and disease incidence and severity were assessed on 15 plants within each transect. Thus, a total of 30 plants were assessed in each field for disease incidence and severity. Disease incidence was measured as a percentage of number of plants infected per field based on the sampled plants while disease severity was assessed based on a scale ranging from 1 to 5 as defined by the International Institute of Tropical Agriculture (IITA). Using this scale, 1 represents absence of infection; 2: mild infection; 3: moderate infection; 4: severe infection and 5 represents very severe infection (IITA 1990). Data were collected via an electronic device application developed by the University of Cambridge, UK. The data were then uploaded into a central repository at the University of Cambridge, UK for analysis.

From each field, leaf samples showing symptoms of cassava mosaic virus infection were collected; these were kept in herbarium pressers and labeled. In addition, stem cuttings of infected plants were also collected and labeled. The leaf samples were stored in the laboratory under ambient conditions while the stem cuttings were maintained in insect proof screenhouses prior to their laboratory analysis. Whitefly *Bemisia tabac*i vectors were collected using an aspirator from 5 plants/field and the total number counted and recorded. The whiteflies were preserved in 70% ethanol, labeled and preserved at the laboratory in a freezer. The diversity of cassava genotypes encountered on the fields were differentiated using the leaf petiole colour as most of the farm owners could not readily give the names of the varieties they had planted.

Laboratory Diagnostics

Genomic DNA was extracted from samples based on cetyl trimethylammonium bromide (CTAB) method (Dellaporta *et al.,* 1983).

Polymerase chain reaction (PCR)

PCR was carried out in a 25 µl reaction consisting of the following reagents: PCR-grade water; 5 µl of 5x standard buffer; 2.5 µl 5% Tween-20; 0.25 µl of 10 mM dNTPs; 0.25 µl each of Forward and Reverse primers; 5 U Taq polymerase and 5 µl of DNA template. The list of primers used for the analysis can be found in Table 1.

Thermocycling conditions

Amplification conditions included a first PCR cycle comprising denaturation at 94 ˚C for 5 min, annealing of primers at 52 ^{o}C for 1 min and elongation at 72 ^{o}C for 2 min. This initial amplification cycle was followed by 35 cycles of 1 min at 94 ^{o}C, 1 min at 52 ^{o}C and 2 min at 72 ^{o}C. At the end of the reaction, a final elongation step was achieved at 72 ^{o}C for 10 min and the reaction hold at 4^{o}.

PCR products were separated by electrophoresis on a 1.5 % agarose gel containing ethidium bromide (1 lg⁄$_{10}$ ml) under a constant current of 100 V. Visualization of the amplified bands under UV light was done using a Vilber Lourmat (infinity^{TM}) gel documentation imaging system.

Sequencing

Where the expected bands on the agarose gel were slightly different rom the expected band, recovered PCR products were prepared for sequencing. Sequencing was carried out by GENEWIZ, Inc (South Plainfield, NJ) using Applied Biosystems Big Dye version 3.1. The reactions were then run through an Applied Biosystem 3730xl DNA Analyzer for DNA sequencing.

Data Analysis

For our analysis, we made use of data-analysis software, namely a multi-dimensional data cube referred to, within the WAVE programme, as the WAVE Cube. This tool enabled us to interrogate and view our survey data from different perspectives to present our extracted results as tables and graphics.

Nucleotide sequences obtained from GENEWIZ were used as query and BLAST searched with ACMV and EACMV strains/isolate nucleotide sequences stored in NCBI GenBank database to detect their corresponding strains/isolates. Nucleotide sequence of samples that yielded above 80% identity with those of ACMV and EACMV isolates in the NCBI GenBank database repository were considered similar (Fondong *et al.,* 2000).

Patristic distances between pairs of sequences analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.,* 2004). This analysis involved 56 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

Results

Field Survey

Three hundred and ninety-three (393) cassava farms were surveyed (215 in 2015 and 178 in 2016/17); in total 1,716 plants were examined across the country. Over ninety-six percent (96.44%) of all fields surveyed were diagnosed with CMD (Table 2) showing the endemic nature of CMD in Ghana.

Northern region had the highest incidence of CMD followed by Brong-Ahafo and the Upper West regions respectively (Fig. 2) while Greater Accra had the highest CMD severity followed by Upper West, Ashanti, Eastern, Western and then Brong-Ahafo regions (Fig. 3).

Fields with 50% to 75% disease incidence had the highest number of whitefly populations while those with disease incidence greater than 75% had the least whitefly population (Fig. 7). Ashanti region had the highest whitefly population/plant followed by Central and the Western regions respectively (Table 3).

Dominant varieties encountered were cultivars with red petioles followed by those with yellowish green petiole (Table 4). However, farmers could not identify the varieties by their actual names and most of them classified the plants as local varieties. Varieties with yellowish green petioles had the highest disease severity, followed by varieties with red petioles while plants with yellowish green petioles with pinkish colouration had the lowest severity (Table 4). No whiteflies were collected on plants with purple and Green petioles encountered during the field surveys (Table 4).

Laboratory Diagnostics

The following strains of ACMV and EACMV indicated in the phylogenetic tree (Fig 8) were detected: ACMV-Ivory Coast, ACMV-Kenya, ACMV-Ghana, EACMV-Cameroun, EACM-Cameroon virus-Ghana and

EACMV-Kenya (Oteng-Frimpong *et al.,* 2012; Bull *et al.,* 2006 Fondong *et al.,* 2000). The detection of ACMV-Kenya and EACM-Kenya virus is a first report in Ghana.

Discussion

This survey findings on the incidence and spread of CMD is similar to what have been reported by Fauquet and Fargette (1990) and Torkpo *et al.,* (2017) although their surveys were limited to certain parts of Ghana. Inclusion of the northern parts of the country in current surveys is relevant. Similar surveys done in the past have often focused mainly on the southern parts (Torkpo *et al.,* 2017). In the most recent survey of the country carried out by Torkpo *et al.,* (2017) in 2007 and 2008, they reported the widespread incidence of CMD in mainly southern and central parts of Ghana and they surveyed only 136 farmers' fields, but our work has gone beyond these geographical areas and numbers. The current study represents a large increase in the number of fields surveyed in recent times and gives a better picture of the extent of spread of CMD in Ghana. This result also gives indication as to the levels of productivity lost by farmers, assuming a 10% yield reduction to CMD infection on farmer fields. This finding requires that urgent steps be taken to find a robust remedy to reduce the incidence of CMD in farmers' fields to improve cassava productivity and ensure food security because cassava alone contributes about 22% of Ghana's agricultural GDP.

The CMD symptoms observed is consistent with CMD symptoms reported by several authors including Koros *et al.,* (2018); Torkpo *et al.,* (2017); Fauquet and Fargette (1990).

The low incidence of whiteflies on plants showing very high incidence of CMD (75% to 100%) suggests that the highly infected fields become unattractive to the insects and therefore the insects move on to fields that are fresher and healthier or possibly younger (Fauquet and Fargette 1990). The studies by Fargette *et al.,* (1990) have reported that the disease incidence on fields planted with infected cuttings and the relative abundance of whiteflies contribute differently to the incidence of the disease. Analysis of symptoms observed on infected plants and fields during our survey indicated that infections could be from infected cuttings used as planting materials as well as from whiteflies. These two sources have been established as key sources of infections and it is believed that both infected cuttings and whiteflies affected disease incidence and severity in the fields surveyed (Fargette *et al.,* 1990).

Generally, the whitefly populations encountered in the fields were low and varied among the regions; this is similar to findings by Ntawuruhunga *et al.,* (2007) in Congo Brazzaville and Torkpo *et al*., (2017) in Ghana. The northern parts of Ghana which have a savannah agro-ecology with unimodal rainfall had the lowest incidence of whiteflies which is similar to observations reported by Fargette *et al.,* (1994). The northern parts of Ghana can be proposed for the establishment and maintenance of clean planting materials production fields. This is because with low incidence of whiteflies it is expected that fields planted with disease-free cuttings can maintain their health status for some time before they become infected (Bock, 1994; Fauquet and Fargette, 1990).

Laboratory Diagnostics

The detection of ACMV-Kenya isolate and EACM-Kenya virus is a first report in Ghana. Previous reports have detected ACMV-Ghana and EACMV-Cameroun and others (Torkpo *et al.,* 2017). The detection of ACMV-Kenya isolate and EACMV-Kenya for instance is a clear indication that more strains of CMV could be present already in Ghana or new strains of the CMV could be entering the country. It is therefore important that regular monitoring and surveillance are conducted to identify any potential new strains to assist the management of CMD including breeding for robust CMV-resistant cultivars thereby improving cassava productivity. The possible detection of new strains from regular monitoring and surveillance can also help modelers to predict the spread of CMD in the country and possibly in the West African sub region.

Conclusion

The study has shown the spread and distribution of CMD in Ghana. Disease symptoms regularly encountered in farmers field included leaf mosaic, leaf distortion/twisting, malformation, filiform leaves, stunting and chlorosis. Cultivars with red petiole colour were the most prevalent while those with green petiole colour were the least. No whitefly was found on cultivars with purple and green petioles while cultivars with reddish-green petioles had highest count of whiteflies/plant. The Upper West and Upper East regions had the least number of whiteflies/plant and could be good locations for healthy planting material production. Six CMV strains were detected: ACMV-Ivory Coast, ACMV-Kenya, EACMV-Cameroun, ACMV-Ghana, EACM-Cameroun virus-Ghana and EACMV-Kenya. ACMV-Kenya isolate and EACMV-Kenya are being reported for the first time in Ghana. This indicates that more CMV strains are being detected in the country. Areas with high incidence and severity of CMD were all identified which are good for breeding for disease resistant cultivars in the country. The data that have been generated by this work could be used by modelers to help predict the spread of CMD in Ghana and the West African sub region.

Abbreviations

ACMV – African cassava mosaic virus

CMD – Cassava mosaic disease

CMV – Cassava mosaic virus

CTAB - Cetyl trimethylammonium bromide

DNA - Deoxyribonucleic acid

EACMV – East cassava mosaic virus

EACM – East African cassava mosaic

GDP – Gross domestic product

GEPA – Ghana export promotion council

IITA – International Institute of Tropical Agriculture

NCBI – National center for biotechnology information,

PCR – Polymerase chain reaction

WAVE - West African Virus Epidemiology for root and tuber crops

UK – United Kingdom

UV – Ultraviolet

Declarations

Ethics approval and consent to participate

Not applicable

Consent of Publication

Not applicable

Availability of data and material

Field and laboratory data are stored at the repository of Scriptoria Agshare, Today, UK and is readily available to interested parties

Competing interests

The authors declare that “they have no competing interests”.

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Authors' contributions

Allen Oppong: Lead investigator and lead author of this article

Ruth N.A Prempeh, Linda A. Abrokwah, Supported data entry, laboratory analysis and writing of article

Esther Afoley Annang, Esther Agyeman Marfo, Zipporah Appiah Kubi, Supported laboratory analysis and proof reading of article

Nana A. O. Danquah Supported in laboratory analysis

Augustine Agyekum Assisted in field survey and collection of data

Benedicta Nsiah Frimpong, Joseph N.L. Lamptey, Moses Brandford Mochiah, Assisted in manuscript review

Justin S. Pita Conceptualization of project idea and main recipient of project grant; review of manuscript

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Tables

Table 1. Primers used in this study to amplify DNA from cassava samples collected during disease survey

PRIMER NAME	SEQUENCES (5’ to 3’)	VIRUS SPECIES	Product size (bp)
JSP001F/	ATGTCGAAGCGACCAGGAGAT	ACMV	≈ 770
JSP002R	TGTTTATTAATTGCCAATACT		
JSP001F	ATGTCGGAAGCGACCAGGAGAT	EACMV	≈ 770
JSP003R	CCTTTATTAATTTGTCACTGC	EACMV	
CMBRep/F	CRTCAATGACGTTGTACCA	ACMV	368
ACMVRep/R	CAGCGGMAGTAAGTCMGA	ACMV	
CMBCP/F	GKCGAAGCGACCAGGAGAT	ACMV	650
ACMVCP/R	CCCTGYCTCCTGATGATTATA	ACMV	
ACMV-AL1/F	GCGGAATCCCTAACATTATC	ACMV	1000
ACMV-ARO/R	GCTCGTATGTATCCTCTAAGGCCTG	ACMV	
UV-AL1/F1	TGTCTTCTGGGACTTGTGTG	EACMV	1600
UV-AL1/R1	AACCTATCCCCGATGCTCAT	EACMV	
UV-AL1/F2	GTAATTGGGAAAGGGCCTCT	EACMV	1000
UV-CP/R	GTTACGGAGCAACATGCAAT	EACMV	
UV-AL1/F1	TGTCTTCTGGGACTTGTGTG	EACMV-UG	1600
ACMV-CP/R3	TGCCTCCTGATGATTATATGTC	EACMV-UG	
CMBCP/F	GKCGAAGCGACCAGGAGAT	EACMV-UG	1000
EACMV-UG/R	CGCCTAAGCAAGGAATGGCGT	EACMV-UG	
JSP 001	ATGTCGAAGCGACCAGGAGAT	ACMV	783
JSP 002	TGTTTATTAATTGCCAATACT	ACMV	
ACMVB1F	TCGGGAGTGATACATGCGAAGGC	ACMV	628
ACMVB2R	GGCTACACCAGCTACCTGAAGCT	ACMV	
JSP 001F	ATGTCGAAGCGACCAGGAGAT	EACMV	780
JSP 003R	CCTTTATTAATTTGTCACTGC	EACMV	
VNF031/F	GGATACAGATAGGGTTCCCAC	EACMV	≈ 560

VNF032/R	GACGAGGACAAGAATTCCAAT	EACMV	
EAB555/F	TACATCGGCCTTTGAGTCGCATGG	EACMV	544-560
EAB555/R	CTTATTAACGCCTATATAAACACC	EACMV	

Table 2. Percentage of cassava fields showing CMD-infected and non-infected

Year of Survey	CMD-Infected Fields %	Healthy Fields %
2015 (Year 1)	95.35%	4.65%
2016/17 (Year 2)	97.75%	2.25%
Grand Total	**96.44%**	**3.56%**

Table 3. Mean Whitefly population per plant across the regions encountered during the 2-year survey

Region	Whitefly - Mean population/plant
Ashanti	2.11
Brong-Ahafo	0.85
Central	1.38
Eastern	0.85
Greater Accra	0.72
Northern	0.12
Upper East	0.05
Upper West	0.07
Volta	0.75
Western	1.66
Grand Mean	**1.03**

Table 4. Cultivar petiole colour, CMD severity and whitefly population for the combined survey periods

Cultivar type (Petiole colour)	No. of samples	CMD Severity Mean	Whitefly count	Whitefly count/plant
Reddish green	30	2.67	29.00 (0.5392)	0.86
Pinkish petiole	330	2.82	15.00 (0.0084)	0.04
Purple petiole	30	2.33	0.00 (0.0000)	0
Red petiole	8,250	2.98	1,974.00 (0.0398)	0.34
Yellowish green petiole	3,030	2.99	729.00 (0.0523)	0.24
Greenish red Petiole	30	2.00	6.00 (0.1390)	0.2
Green petiole	60	2.78	0.00 (0.0000)	0
Total	**11,760**	**2.98**	**2,753.00 (0.0266)**	

*Numbers in brackets are standard error of mean (SEM) values

Figures

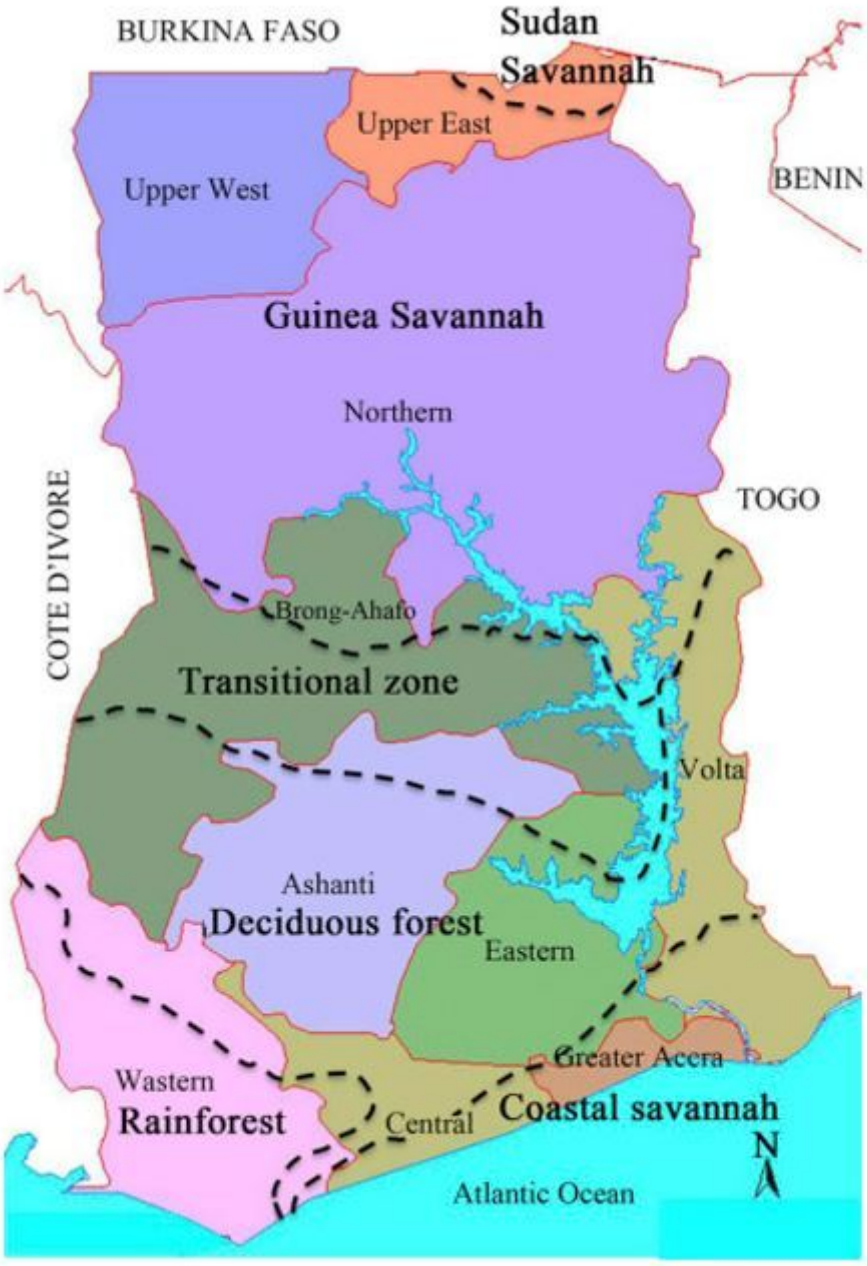

Figure 1

Map of Ghana showing regional boundaries and the agro-ecologies (Source: Kemausuor et al 2013). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

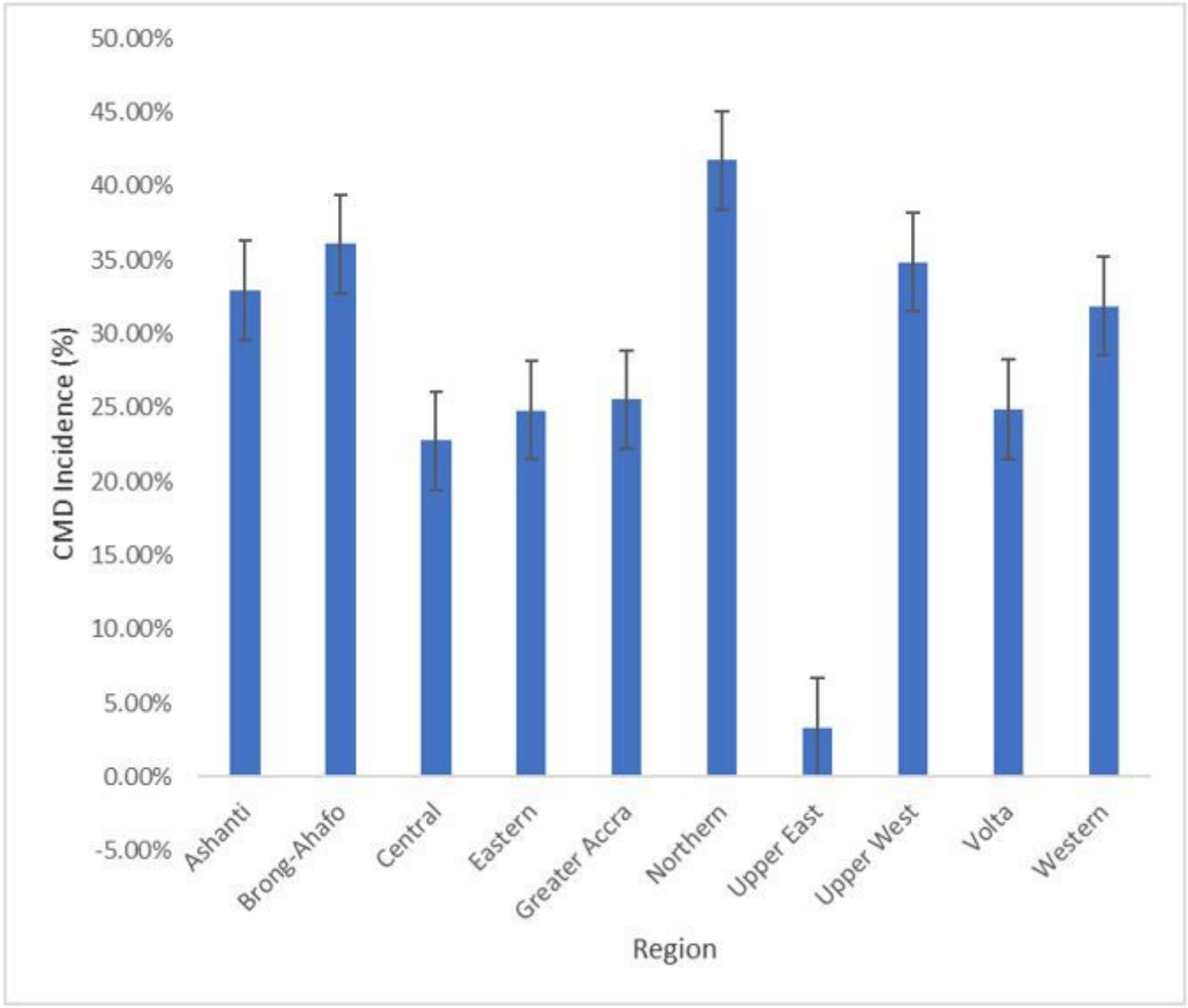

Figure 2

Percentage mean incidence of CMD across the regions surveyed in 2015 and 2016/17

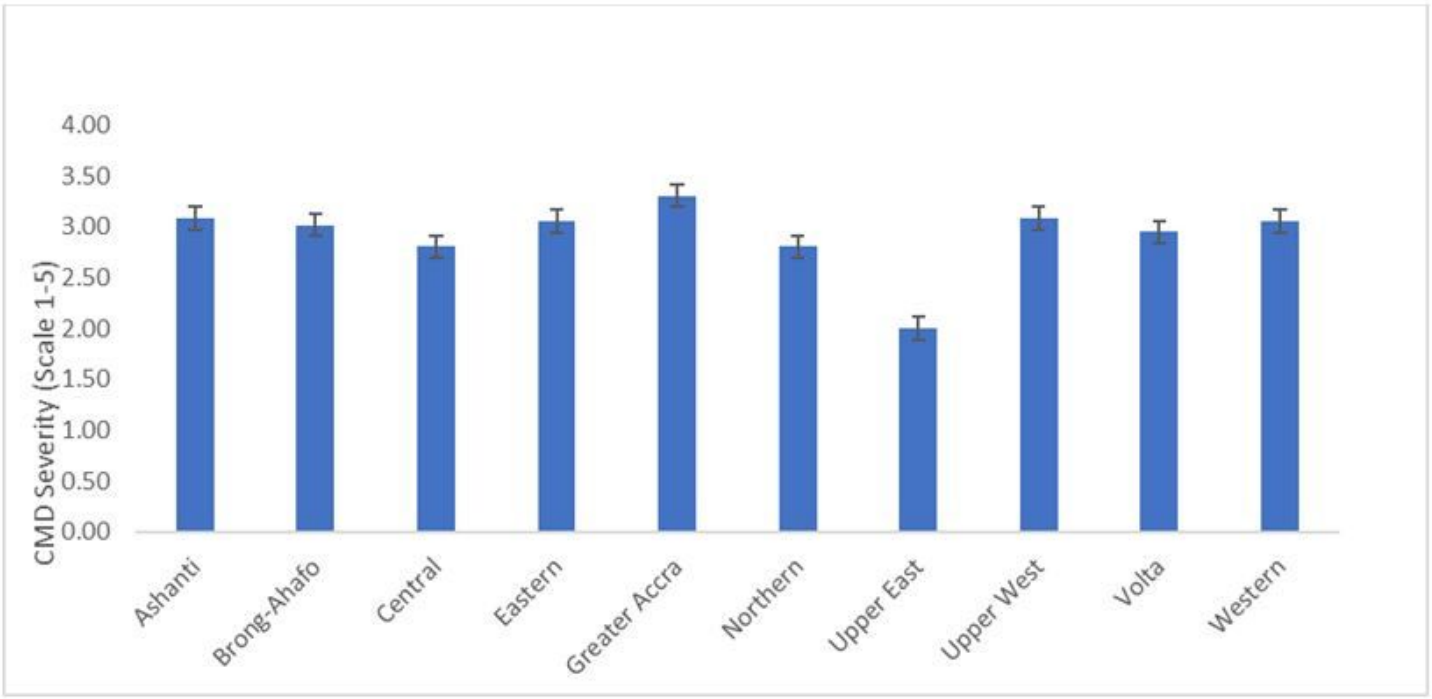

Figure 3

Mean severity of CMD disease across the regions during the 2015 and 2016/17 surveys

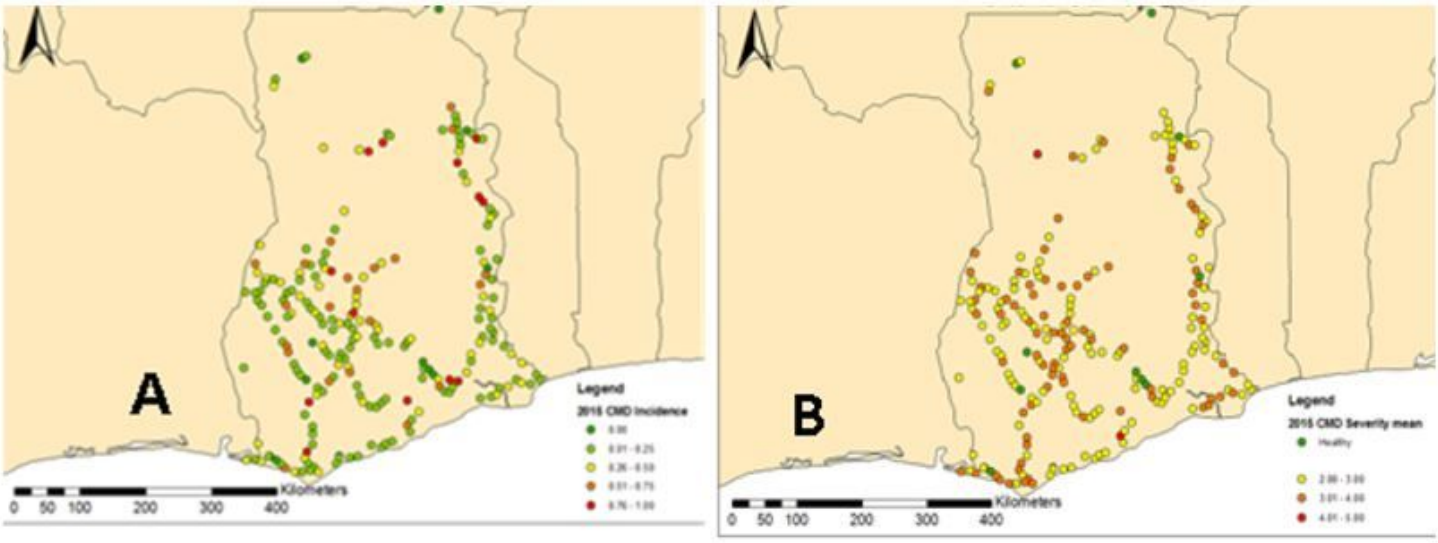

Figure 4

Map showing the incidence (A) and severity (B) of CMD in Ghana for 2015 Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

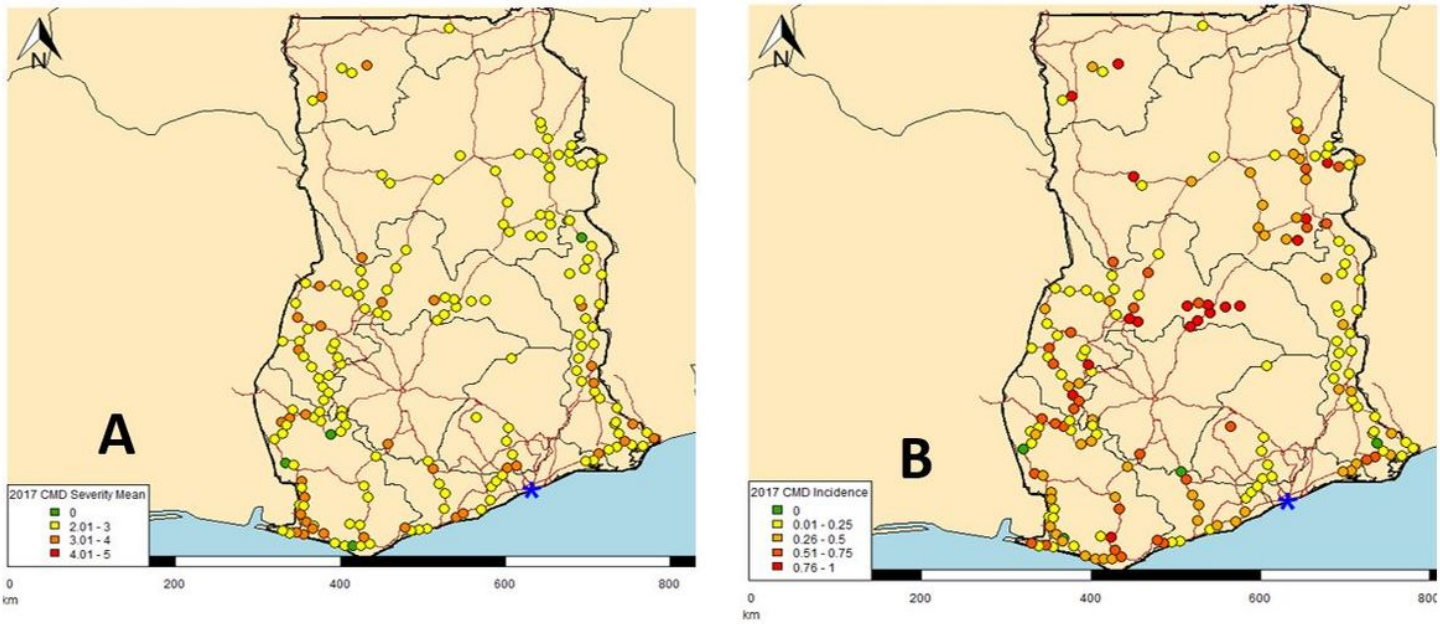

Figure 5

Map showing CMD severity (A) and incidence (B) of areas surveyed in 2016/17. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 6

CMD symptoms on infected plants encountered during the survey. The arrows point to A; leaf mosaic/distortion, B; Filiforms and C; Stunting/leaf mosaic/distortion.

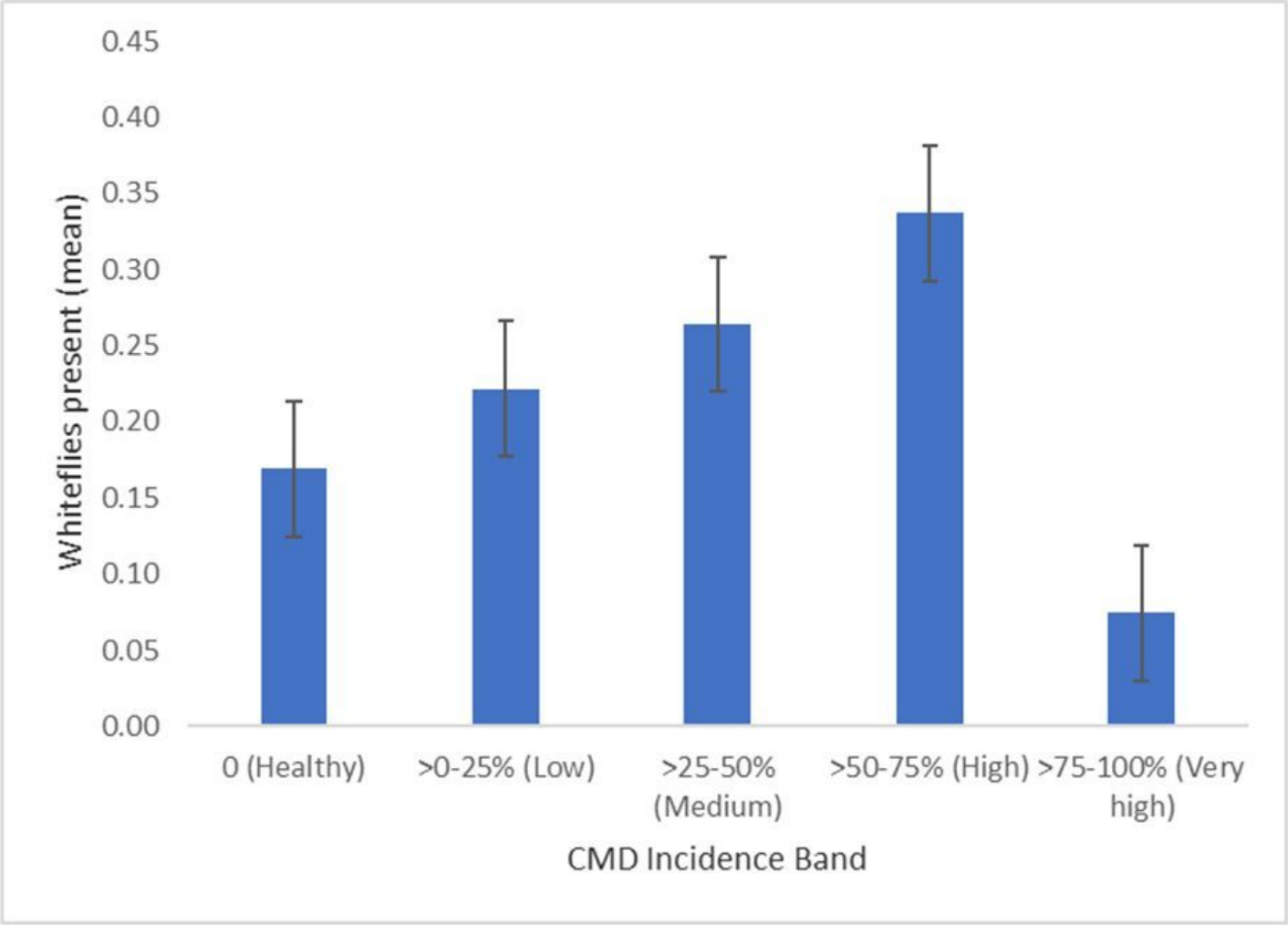

Figure 7

Mean CMD percentage incidence and mean whitefly number in fields surveyed over the two-year period

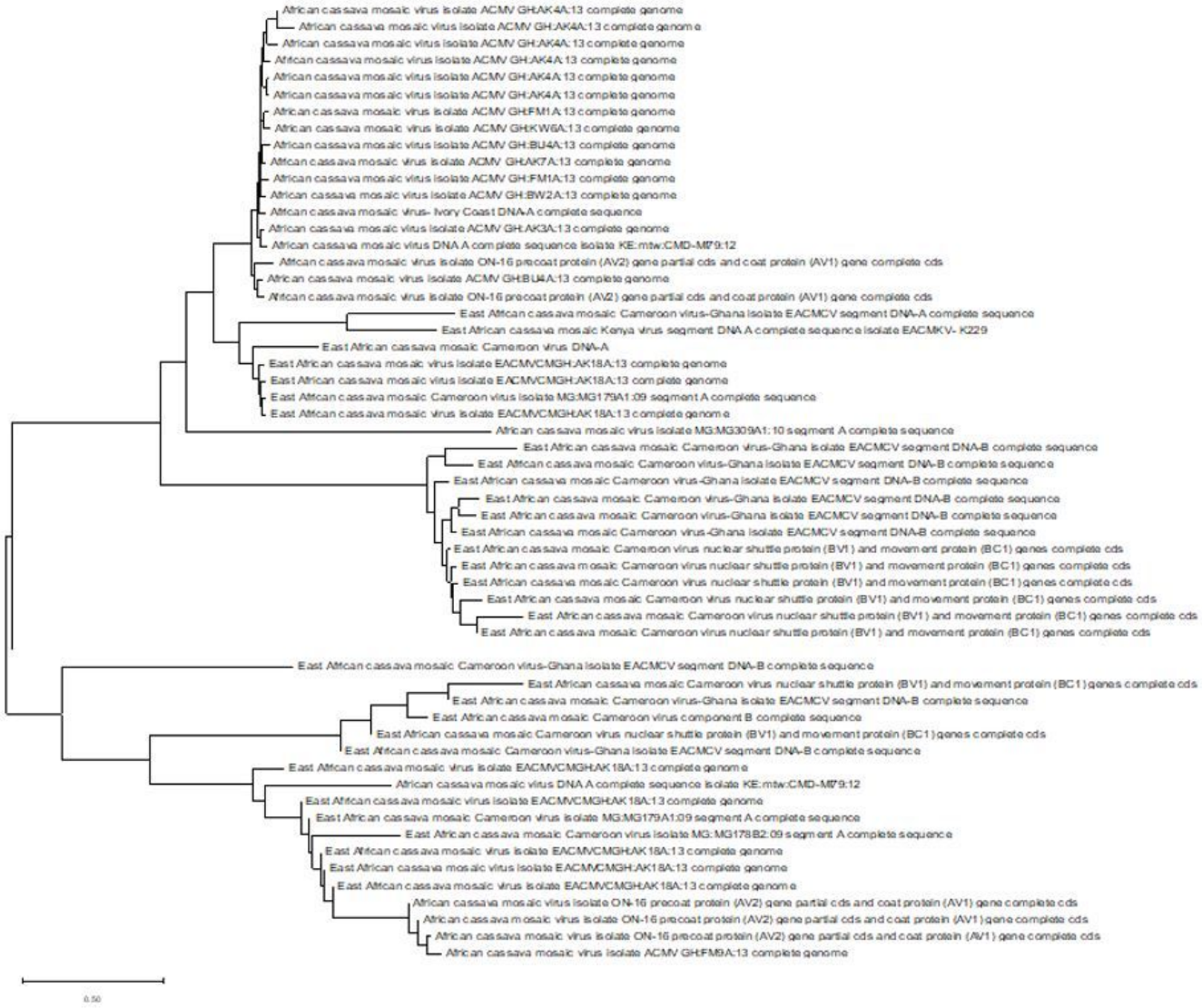

Figure 8

Phylogenetic tree indicating the placement of cassava mosaic viral strains sequenced from CMD-infected cassava samples.