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*Research article*

# Status of cassava mosaic begomoviruses in farmers' fields in Ghana

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**Abstract:** A survey for cassava mosaic disease (CMD) was carried out in Ghana from 2007−2008 to determine the status of cassava mosaic begomoviruses in farmers' fields. The survey covered cassava growing areas in five major cassava producing regions of Ghana. Out of 136 fields visited, the plants in 5% were not affected by CMD, 18% contained plants with mild symptoms, whereas 77% had cassava with moderately severe or severe symptoms. A total of 412 cassava leaf samples and a symptomatic *Manihot glaziovii* sample were analyzed using polymerase chain reaction. *African cassava mosaic virus* (ACMV) alone was detected in 42.0% of symptomatic cassava leaves with the remaining 58% being mixed infected by ACMV and *East African cassava mosaic virus* (EACMV). Mixed ACMV and EACMV infections were detected in symptomatic *M. glaziovii*, two non-symptomatic cassava samples and in individual whitefly vectors. EACMV was not detected alone in any cassava or whitefly sample. *South African cassava mosaic virus* (SACMV), *Indian cassava mosaic virus* (ICMV), *East African cassava mosaic Zanzibar virus* (EACMZV) and the Uganda strain of EACMV were not detected in any cassava or whitefly sample. The occurrence of high proportion of mixed infections of cassava by cassava mosaic begomoviruses (CMBs), which could lead to emergence of new species or variants in the country, require concerted effort to mitigate the CMD problem.

**Keywords:** cassava mosaic disease; begomovirus; polymerase chain reaction

## 1. Introduction

Cassava is an important staple food for many people in the tropics [1] and is one of the most efficient crops for carbohydrate production. It is an important food security crop. Cassava is cultivated extensively in Ghana, with a total yield of approximately 16 million metric tons [2]. It is the most important staple in the country with per capita daily intake of 642 calories, far exceeding maize and rice with 434 and 217 calories, respectively [3]. However, productivity of cassava in the country is being hindered by several factors, including cassava mosaic disease (CMD). This disease has been known since 1894 and it has long been regarded as the most important disease of cassava in Africa [4]. The disease is prevalent in many parts of Africa [6,7] and was first observed in Ghana in 1926 [5]. Cassava mosaic disease causes severe yield losses in the storage root, ranging from 20–95%, and the effect of the disease is more severe when plants are infected at the early stage of growth than when infected later [7,8]. Annual yield losses due to CMD in Africa is estimated between US$1.9–2.7 billion [9].

CMD has reportedly reached pandemic levels in Africa, a situation where the Ugandan epidemics expanded over substantial areas of Kenya, Tanzania, Sudan, the Democratic Republic of Congo and parts of Burundi [9]. The pandemic was characterized by high incidence of unusually severe CMD and greater abundance of *Bemisia tabaci* vector [9].

Several cassava mosaic begomoviruses (CMBs) have been reported in Sub-Saharan Africa, and include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV) and *South African cassava mosaic virus* (SACMV) [10]. These begomoviruses can be involved in mixed (double) infections, which are usually characterized by severe symptoms [11,12].

Accurate identification of pathogens is indispensable to designing effective disease management strategies. However, with the exception of the first report of EACMV in Ghana [13] and a CMD survey of two major cassava producing regions in the country [14], there is limited information on the begomoviruses associated with the disease in the country. The current study seeks to determine the status of CMD and cassava mosaic begomoviruses associated with cassava and cassava colonizing whiteflies in five major cassava producing regions of Ghana in order to make recommendations for managing CMD in the country.

## 2. Materials and methods

### *2.1. Survey design and sampling*

The survey was undertaken in major cassava producing districts in five regions of Ghana between November 2007 and January 2008. Prior to the Survey, details of cassava and the major crops grown in each district were obtained from the Ministry of Food and Agriculture [2]. The survey routes followed were highways, feeder roads and accessible farm roads. Farms surveyed were separated by a distance of about 10–30 km in the Brong Ahafo, Western, Ashanti, Volta and Northern Regions of the country. Coordinates of farms were taken with the Global Positioning System device, Garmin Geko 301. In each field of three to six months old cassava plants, whenever cassava mixtures (more than one genotype grown on a field) were present, CMD parameters were assessed only on the predominant genotype. Thirty plants were randomly assessed for CMD incidence and severity along two horizontals and a diagonal across each field.

Cassava mosaic disease incidence was assessed by noting the presence or absence of symptoms on each of the 30 plants. Symptom severity of CMD was assessed on whole plant basis using a scale of 1 to 5, where 1 = no symptoms and 5= very severe mosaic [15]. Overall, CMD symptom severity for a field was mild (when the score was 2), moderately severe (when the score was 3) and severe (when the score was 4 or 5). In each field, cassava leaf samples were collected for each severity level scored on the predominant genotype and from other cassava genotype(s) with characteristic CMD symptoms whenever present. A total of 412 (81 non-symptomtic and 331 symptomatic) cassava leaf samples, and a symptomatic *M. glaziovii* sample were collected from 136 farmers' fields.

In fields where adult whiteflies were present, insects were collected with a pooter, and a total 141 samples were collected into vials. Both the cassava leaf and whitefly samples were kept in a Coleman Thermoelectric cooler (keeps samples at 26 ℃ less than the ambient Temperature) during transit. Cuttings (20−25 cm) were established in a screenhouse and monitored for symptom expression.

### *2.2. DNA extraction from cassava leaves and whitefly samples*

Total DNA (~50 ng $\mu L^{-1}$) was extracted from each of 413 cassava leaf samples using the DNeasy Plant Mini Kit [16] following the manufacturers' protocol while DNA was extracted from 141 individual whiteflies using a modification of the method described by Cenis et al. [17].

### *2.3. Detection of cassava mosaic begomoviruses by polymerase chain reaction (PCR)*

The reaction mixture of 25 µL was made up of 2.0 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, ca 2.5 units puRe Taq DNA Polymerase (New England Biolab), 200 µM of each dNTP, 1.0 µL (5.0 pM) each of forward and reverse primers (Table 1), stabilizers, BSA and 3 µL of template DNA. The reaction was carried out in an Applied Biosystems Thermal Cycler. The reaction cycles were 94 ℃ for 2 minutes followed by 30 cycles of 94 ℃ for 1 minute, 52−55 ℃ for 1 minute, 72 ℃ for 1 minute and a final extension of 72 ℃ for 10 minutes. PCR products were electrophoresed at 100 V for about 1.5 hours on a 1% agarose gel stained with ethidium bromide (10 mg $mL^{-1}$) alongside a 1.0 kb DNA ladder (GIBCO, Life Technologies). Bands were visualized and images saved using Syngene Gel documentation system.

## 3. Results

### *3.1. Status of CMD in farmers' fields and screen house*

Farms with moderately severe CMD symptoms predominated in each of the five regions surveyed in Ghana (Figure 1). In the Ashanti and Northern regions of the country no field was in the severe symptoms category. On the contrary, six fields in the Western, three in the Brong Ahafo and one field in the Volta regions of Ghana were in the severe symptoms category (Figure 1). On the whole, 69% of farms were either moderately severe or severe with farms with moderate CMD symptoms being fairly randomly distributed. A majority of farms with severe symptoms were in the western part of the country whereas farms with mild symptoms were in both the western and eastern parts of the country.

**Table 1.** Primers used for PCR detection of begomoviruses in plants and whiteflies

| Oligoname | PrimerSequence (5'→ 3') | Begomovirus isolate | DNA Component |
|---|---|---|---|
| CP-For | ATGTCGAAGCGACCAGGAGATAT | CMGs | AV1 |
| CP-Rev | CCATATACAGAAGCAAAGCATTCTC | CMGs | AV1 |
| ICMV-F1 | TTCTCTCTCCTCAATCGGTA | ICMV | IR&AV2 |
| ICMV-R1 | ACTCAGGGAACTCGTTTAGT | ICMV | IR&AV2 |
| VNF003 | CCCAAGCTTGGTTAGAGTT | EACMV-CM | DNA-A FL |
| VNF004 | CCCAAGCTTGTTCCTTCATCCCWA | EACMV-CM | DNA-A FL |
| ACMV-AL1/F | GCGGAATCCCTAACATTATC | ACMV | AV2&AC1 |
| ACMV-AR0/R | GCTCGTATGTATCCTCTAAGGCCTG | ACMV | AV2&AC1 |
| UV-AL3/F | TACACATGCCTCRAATCCTG | EACMV | AC1 &AC3 |
| UV-AL1/R2 | CTCCGCCACAAACTTACGTT | EACMV | AC1 &AC3 |
| VSP1 | TCGGGAAGCTTTAAGGACTGGTTCTTTTCC | SACMV | DNA-A FL |
| CSP1 | GGAATAAGCTTGGGCTTTCAAGAATGCAACC | SACMV | DNA-A FL |
| JSP001 | ATGTCGAAGCGACCAGGAGAT | ACMV/EACMV | AV1 |
| JSP002 | TGTTTATTAATTGCCAATACT | ACMV | AVI |
| JSP003 | CCTTTATTAATTTGTCACTGC | EACMV/SACMV | AV1 |
| IC1200R | GACTGACCGTGTTGAGCAGTC | ICMV | AV1 |
| UV-AL1/F1 | TGTCTTCTGGGACTTGTGTG | EACMV-UG2 | AVI & AC1 |
| ACMV-CP/R3 | TGTCTCCTGATGATTATATGT | EACMV-UG2 | AVI & AC1 |
| VSP2 | GGTACCACATGTTGACGC GCTCCACTACTT | EACMZV | DNA-A NFL |
| CSP2 | GGTACCATTGTTAAACGATTTCCCTGAA | EACMZV | DNA-A NFL |

NFL (Near full length); FL (Full length); CMBs (cassava mosaic begomoviruses)

### *3.2. Cassava mosaic begomoviruses*

Out of the 331 CMD symptomatic cassava leaf samples, 139 (42%) tested positive for *African cassava mosaic virus* (ACMV) and 192 (58%) for mixed ACMV and EACMV infections in PCR (Figure 2). Amongst the 81 non-symptomatic cassava samples, ACMV alone was detected in 5 (6%) of the samples, whereas mixed ACMV and EACMV infections were detected in two non-symptomatic cassava samples. None of the non-symptomatic plants tested positive for EACMV alone in PCR. The remaining 91% of the asymptomatic samples tested negative for CMBs. Mixed ACMV and EACMV infection was detected in symptomatic *M. glaziovii* collected at the edge of a CMD infected cassava field in the Western region (Figure 3). All EACMV-positive samples were also positive with primer pairs VNF 003/004 (Figure 4), which amplifies the DNA-A component of EACMV-CM. No isolate of EACMV was detected alone in any sample. Farms that had mixed ACMV and EACMV infections, and ACMV alone occurred in each of the five regions, and were widely distributed (Figure 5).

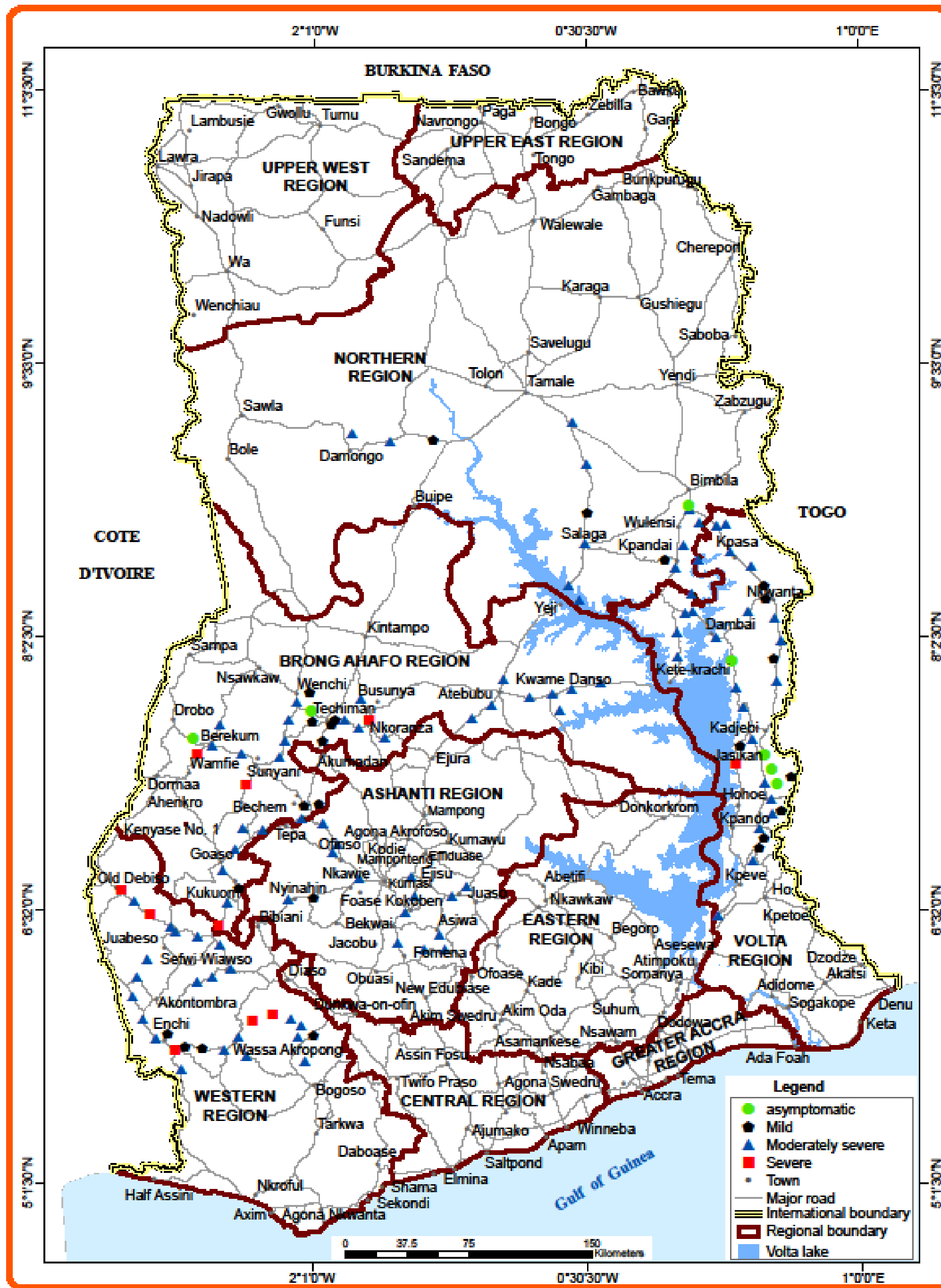



**Figure 1.** Distribution of cassava farms in Ghana showing mean CMD severity levels during CMD survey in 2007–08.

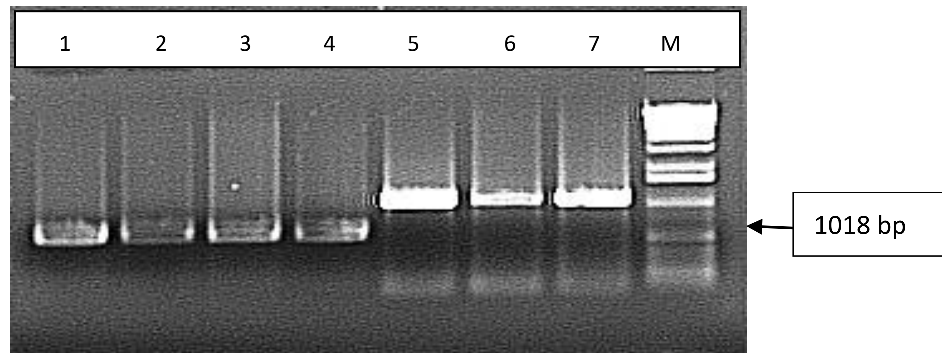



**Figure 2**. Diagnostic PCR for the detection of begomoviruses in cassava. Gel electrophoresis of PCR Products obtained with primers pairs JSP001/002 (lanes 1−4) and UV AL3/F1/UVAL1/R2 (lanes 5−7). The PCR reactions contained DNA extracted from cassava samples. Lanes 1 and 5 contained the same sample, lanes 2 and 6 contained the same sample, lanes 3 and 7 contained the same sample and lane 4 contained another sample. A 1.0 kb ladder was co-electrophoresed to estimate band sizes (lane M).

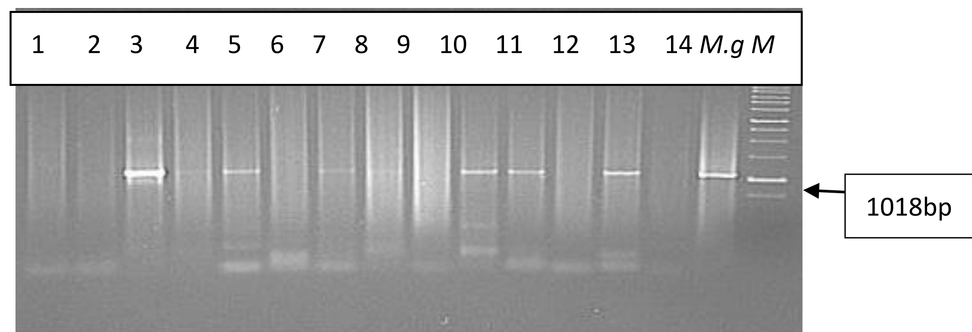



**Figure 3.** Diagnostic PCR for the detection of begomoviruses in cassava. Gel electrophoresis of EACMV in CMB infected cassava and *M. glaziovii* leaf samples using primer pair UV-AL3/F1/UV-AL1/R2. Lanes 1−14 represent separate cassava samples; M.g (Manihot glaziovii); A 1.0 kb ladder was co-electrophoresed to estimate band sizes (lane M).

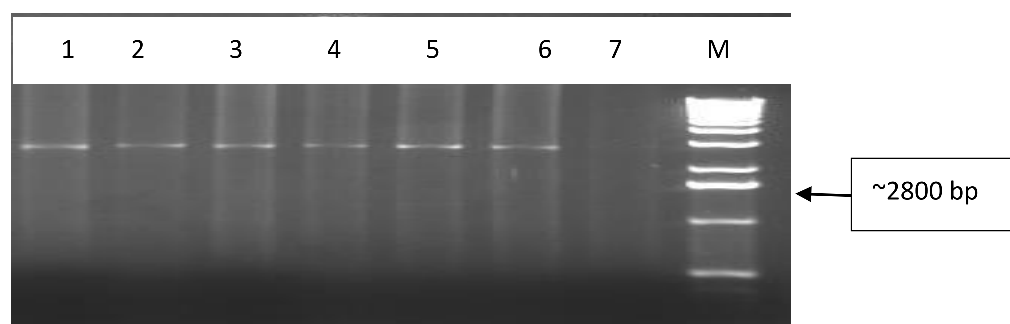
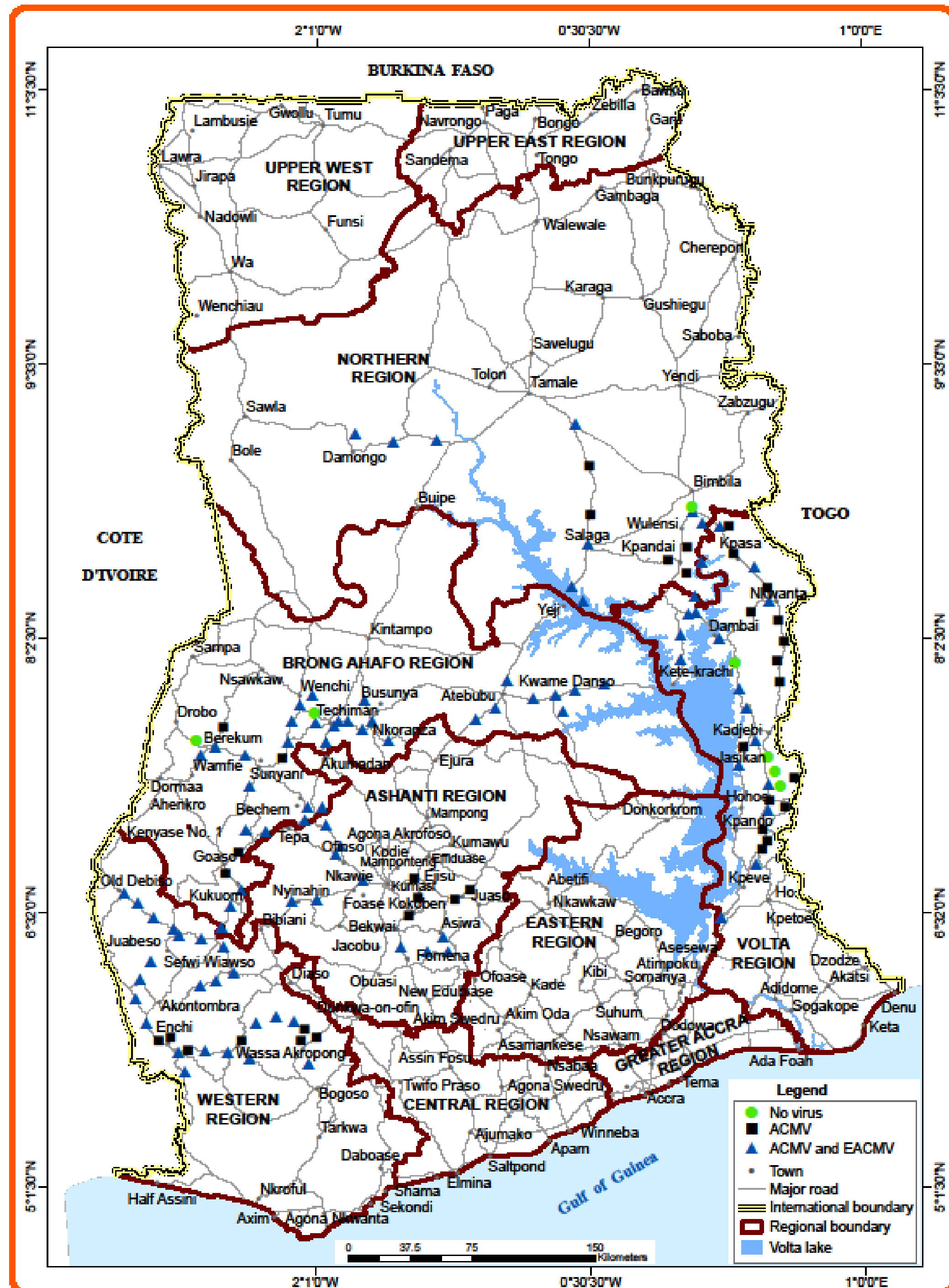



**Figure 4.** Diagnostic PCR for the detection of begomoviruses in cassava. Gel electrophoresis of full length Ghanaian East African cassava mosaic virus isolates amplified with the EACMV-CM primer pair VNF 003/004. Lanes 1−7 represent different isolates. A 1.0 kb ladder was co-electrophoresed to estimate band sizes (lane M).




Distribution of cassava begomoviruses in farmers' fields in Ghana during CMD survey in 2007 - 08

**Figure 5.** Distribution of cassava begomoviruses in farmers' fields in Ghana during CMD survey in 2007−08

*African cassava mosaic virus* was detected alone, and in double infections with EACMV in adult whiteflies in all the five regions of the country. Twenty-seven, representing 22% out of 141 individual whitefly vector samples tested positive with the degenerate primer pair CP-For/Rev which detects ACMV, EACMV, SACMV, ICMV and SLCMV. Using species and strain specific primers, all

27 samples were positive with the primer pair JSP001/002 which amplifies AV1 of ACMV, and ACMV AL1/F/ACMV ARO/R2 which is specific for the AV2 and AC1 genes of ACMV. Eleven (representing 41%) of the ACMV positive samples tested positive with primer pairs JSP001/003, which is specific for AV1 of EACMV (Figure 6). No cassava or whitefly sample tested positive for EACMV-UG, EACMZV, SACMV or ICMV.

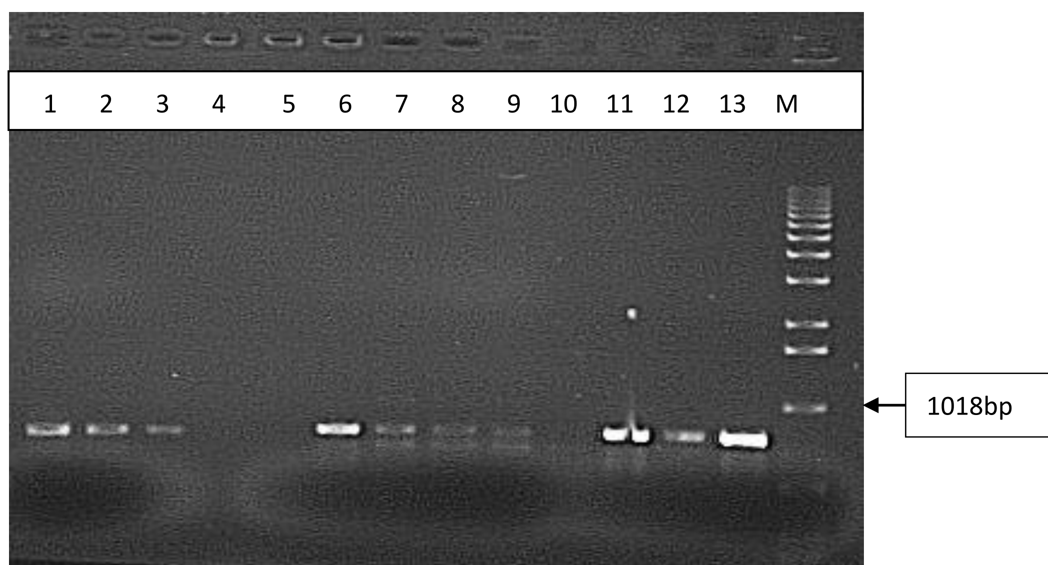



**Figure 6.** Diagnostic PCR for the detection of begomoviruses in whiteflies. Gel electrophoresis of cassava mosaic begomovirus from individual whiteflies samples. Lanes 1−13 contained PCR products of individual adult whiteflies amplified using primer pair JSP001/003, which amplifies AV1 of EACMV; A 1.0 kb ladder was co-electrophoresed to estimate band sizes (lane M).

## 4. Discussion

Several species of CMBs have been affecting cassava either singly or in mixed infections in Africa. The predominant species of CMB identified in the study here was ACMV occurring either alone or in mixed infections with EACMV. The predominance of ACMV infections in the country is consistent with Offei et al. [13]. EACMV was only detected in mixed infections with ACMV, and was widely distributed across the entire country, usually characterized by severe symptoms. This agrees with earlier studies [11,18]. Also, some singly ACMV infected plants expressed severe mosaic symptoms. Both types of severe infections could be implicated in substantially increased yield losses [19]. Both mild and severe strains of CMBs have been reported by Pita et al. [12], with mild strains causing less yield losses than severe strains [20]. Hence, mildly diseased plants could be used as planting materials in whitefly-free areas of the country such as Nanumba North when virus-free planting material is not available.

Mixed infections involving ACMV and EACMV have been described in neighbouring African countries of Ivory Coast [18], Nigeria [21-23], Cameroon [11], and other African countries including Democratic Republic of Congo and Congo Republic [24], and Kenya [25,26]. In all locations in

Central and West Africa, EACMV-CM rarely occurred in single infections, even where CMD incidence was relatively low [9]. This concurs with the results of the present study in Ghana and earlier report in country [13] as well as Cameroon [11], Ivory Coast [18], Nigeria [22,23]. However, in the CMD pandemic area, EACMV-UG or EACMZV, *East African cassava mosaic Kenya virus* (EACMKV) predominated, occurring alone or in mixed infections with ACMV [9,27,28]. Extent of mixed ACMV and EACMV-CMD infections detected in the present study was consistent with Torkpo and Offei [14], and can be attributed to the high levels of local dissemination of infected cuttings but contradicted other reports in Nigeria [21,23,29], Ivory Coast [18], Cameroon [11], and other African countries [24,28,30,31] where less than 50% mixed infections by ACMV and EACMV were reported.

Detection of single ACMV and mixed ACMV and EACMV-CM infections in non-symptomatic samples contradicts Torkpo and Offei [14] but concurs with Ogbe et al. [23]. These could undermine the effectiveness of using non-symptomatic cassava landraces in phytosanitation management options as they may contain the virus (es). Detection of ACMV and EACMV-CM together in adult whiteflies was consistent with other reports [14,23]. Identification of mixed ACMV and EACMV-CM infections in *M. glaziovii* concurs with earlier reports in West Africa [23,29] and East Africa [32].

## 5. Conclusion and recommendation

The present study revealed that in most of the farms surveyed, the symptoms of CMD were moderately severe. The majority of farms where severe CMD was detected were in the western part of the country. ACMV was the predominant CMB detected. Mixed infections of isolates of EACMV-CM and ACMV were widespread and were detected both in cassava plants and adult whiteflies. In order to reduce the possible reservoirs for cassava mosaic begomoviruses, all symptomatic *M. glaziovii* bordering cassava fields should be removed. Occurrence of high proportions of mixed infections by ACMV and EACMV-CM in the country require regular diagnostic surveys and concerted efforts to minimize the impact of these species of cassava begomoviruses on the crop and to safeguard its cassava cultivation. There is also the need to develop infectious clones of Ghanaian isolates to be used for screening of cassava genotypes as was done by Ariyo et al [33].

## Conflicts of interest

The authors hereby declare that this manuscript is not under consideration elsewhere, and that the funding agencies did not influence the outcome of the research and are not opposed to it publication.

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