UNDER PEER REVIEW

1 **Original Research Article**

2 **Distribution of cassava mosaic geminiviruses and their associated DNA satellites in Kenya.**

3

4 **Abstrac**

5 A countrywide survey was conducted to determine the incidence, prevalence and severity of
6 cassava mosaic disease (CMD) and the associated DNA satellites in Kenya. The survey focused
7 on the areas in which cassava is grown as a food crop. Disease incidence, prevalence and
8 severity were assessed in all the selected fields visited. Whitefly counts were done on plants
9 randomly selected in the fields visited. Method of disease transmission either by whitefly or
10 infected cuttings was also determined. PCR detection method was used in the detection of these
11 viruses and the associated DNA satellites using the DNA extracted from the samples collected
12 from the field. CMD was widely distributed in the country with an average incidence of 57.3%
13 countrywide whereas Coast province recorded the highest incidence (73.8%). The prevalence of
14 CMD countrywide was 84.6% with Nyanza province recording the highest (96.2%) prevalence,
15 whereas Eastern province had the least (66.7%) prevalence. The spread of CMD through use of
16 infected cuttings accounted for 80.6% of the infected plants compared to the whitefly-borne
17 infections which only accounted for 19.4%. East African Cassava Mosaic Virus (EACMV) and
18 African Cassava Mosaic Virus (ACMV) accounted for 51% and 20% of samples, respectively.
19 Co-infection of cassava plants with the two viruses was detected in only 9% of the samples.
20 EACMV was detected in samples collected from all the provinces surveyed with nearly all the
21 districts visited recording the presence of EACMV. ACMV on the other hand was mostly
22 prevalent in the districts in Western and Nyanza provinces although for the first time, ACMV
23 was detected in samples collected from Eastern and Coast provinces for the first time. Nyanza
24 province had the highest whitefly count with Western province registering the least whitefly
25 counts per plant. The method of transmission of CMD was mainly through the distribution or
26 use of infected cassava cuttings with 100% transmission by whiteflies in Coast province. DNA
27 satellites associated with these Begomoviruses were distributed across the areas under survey
28 with 41.4% of the samples collected testing positive for the DNA satellites. There was a marked
29 increase in symptom severity in plants infected by Cassava amosaic Geminiviruses (CMGs) and
30 the associated DNA satellites compared to those infected with CMGs only. There is need for the
31 identification of varieties resistant to these viruses and pooling regional efforts in the
32 characterization of the viruses to further understand reasons behind the high disease severities in
33 some areas. The begomovirus symptom modulation by the DNA satellites need to be further
34 investigated to determine any effect on the disease severity and yield of cassava.

35

36 **Key words: DNA Satellites and Cassava Mosaic Geminiviruses**

37

38 **Introduction**

39 Cassava (*Manihot esculenta* Crantz) is a major staple food for many communities in sub-
40 Saharan Africa. In Kenya, cassava is grown on over 90,000 ha with an annual production of
41 about 540 000 tons [1]. Cultivation is concentrated in Nyanza and Western provinces (60%),
42 Eastern (10%), and Coast provinces (30%). The crop is grown by resource poor households for

UNDER PEER REVIEW

subsistence where it is an important food security crop. The available information from surveys and yield loss assessments due to CMD is summarized [2], which estimates the losses in Africa to be 15–24%. In Kenya, yields recorded range between 5 and 10t/ha against a potential of 32t/ha [3].

CMD is transmitted by a whitefly vector known as *Bemisia tabaci* but proof of viral aetiology was not obtained until the 1970s and 1980s, when sap inoculations to herbaceous hosts were successful and virus isolates obtained in this way were purified and characterized [4]. After initial uncertainty, the isolates were shown to cause CMD, Koch's postulates were fulfilled and the various isolates from Africa and India were regarded as strains of a single virus of the geminiviruses group and designated African cassava mosaic virus (ACMV). Subsequent studies have led to the recognition of several distinct but similar viruses namely African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), Indian cassava mosaic virus (ICMV) and South African cassava mosaic virus (SACMV) [5].

In Kenya, Cassava Mosaic Disease (CMD) is caused by begomoviruses in the family Geminiviridae. These include African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), and Uganda variant (EACMV-UG) of the genus begomovirus. Previous studies have shown ACMV, EACMV, EACMV-UG and EACMZV to be present in Kenya [6] [7]. Earlier reports indicate that EACMV, EACMV-UG and EACMZV have distinct geographical distributions [7].

The whitefly vector, *Bemisia tabacii* (Gennadius) (Aleyrodidae, Hemiptera) transmits Cassava mosaic begomoviruses (CMBs) from plant-to-plant. Long-distance spread of CMD occurs by the distribution of infected stem cuttings [8]. Whitefly presence on plants does not necessarily suggest that the disease is spread by the insects. Affected plants are stunted and have greatly diminished tuberous root yield. Cassava is also affected by the DNA satellites associated with Cassava mosaic geminiviruses [9].

This survey focused on determining the status and distribution of the CMG's and the DNA satellites particularly their incidence, prevalence and severity in all major regions where cassava is grown in the country.

## Material and methods

### Sampling sites

The survey was carried out in four distinct regions which are also administrative regions namely provinces. The provinces surveyed were Eastern, Nyanza, Western and Coast provinces. These are the major regions where cassava is grown as one of the major food crops. The districts within these regions where sampling was done were selected according to the importance of cassava as a food crop and where the disease under study has caused serious problems. Fields having a cassava crop as a pure stand or intercropped with other crops were selected and randomly surveyed along selected routes at 5-10 km intervals. A total of 94 cassava fields were surveyed. In each field, the coordinates and altitude were recorded using a global positioning system (GPS; Magellan GPS 315, San Dimas, CA).

UNDER PEER REVIEW

In Nyanza province, the survey and sampling was done in the following districts; Kisii central, Gucha, Kuria west, Migori, Rongo, Homa Bay, Rachuonyo, Gem, Bondo and Siaya. In Eastern province, sampling was done in Imenti south, Tharaka south, Maara, Meru south, Embu, Mbeere north, Mbeere, Kitui, Kitui central, Mwala, Makueni, Kangundo and Kathiani districts. In Western province, survey and sampling was done in the following districts; Kakamega south, Butere, Mumias, Busia, Bumula, Teso North, Teso South, and Bungoma west. Finally sampling was done in Coast province in the following districts; Kilifi, Malindi, Kwale, Msambweni and Taita.

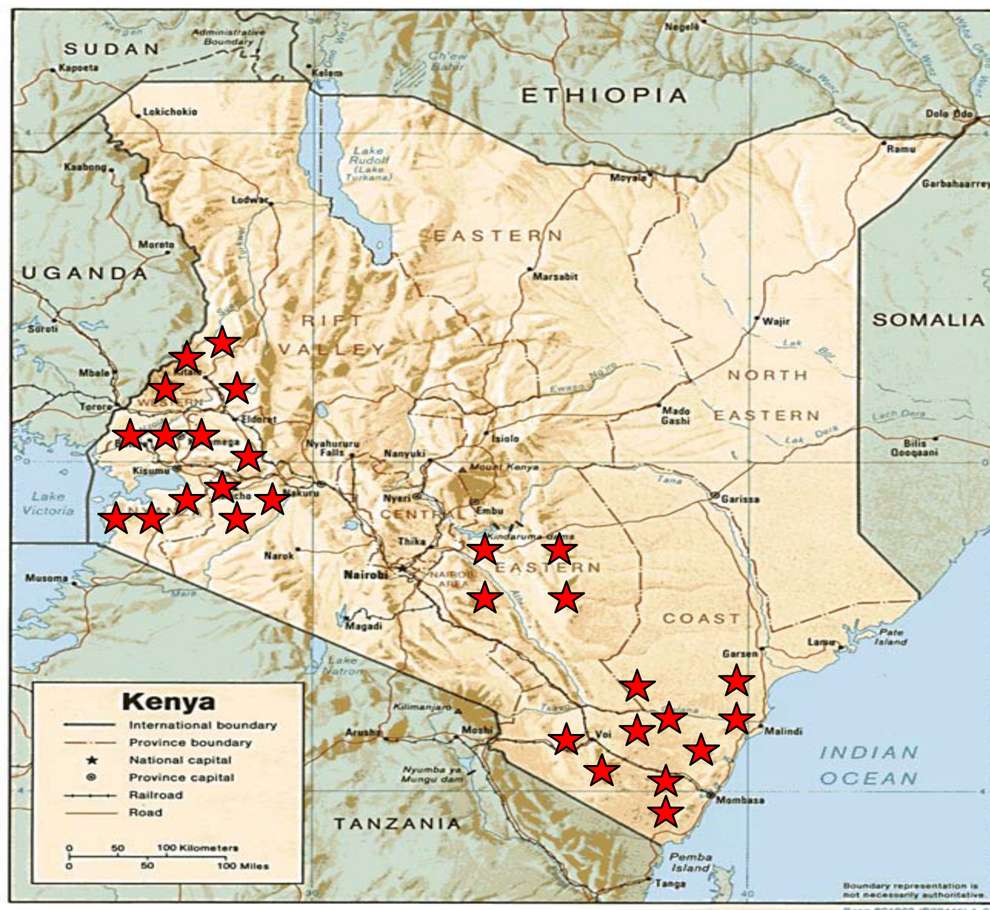



Figure1: ★Areas surveyed for CMGs and associated DNA Satellites

An imaginary line (transect) was drawn diagonally in the field from both directions thus ending up with two transects within one field. A total of 15 plants were examined for the symptoms of both Cassava Mosaic Virus disease (CMD) and the associated DNA satellites on each transect. In total, 30 plants in every field were examined. The prevalence of the viral diseases was evaluated in every region by calculating the number of fields in which at least one cassava plant presented symptoms of viral diseases divided by the total number of fields observed in that region. The disease severity symptoms for both diseases were established with disease severity scale (1-5) [13] which is internationally accepted and adopted. For CMD, the plants were observed for the foliar symptoms and their satellites symptoms.

UNDER PEER REVIEW

107 Farms or fields having cassava crop as a pure stand or intercropped with other crops were
108 selected and randomly visited along the selected routes within the region. In each region, a
109 particular representative route that captures the area of interest was discussed and agreed upon by
110 the survey team and adopted. Amongst issues considered include the sample area and availability
111 of suitable cassava fields. Farmers' fields were selected after every 5 km in densely populated
112 areas due to close proximity of the small scale farms growing cassava such as in Western
113 province and some parts of Coast, Nyanza and Eastern province. In marginal and sparsely
114 populated areas like Ukambani districts in Eastern province, a distance interval of 10 km was
115 adopted. In all, 94 fields were visited during the survey. In each field, the coordinates were
116 recorded using a global positioning system (GPS; Magellan GPS 315, San Dimas, CA).
117
118 Cassava plants in farmers' fields were observed for virus disease symptoms. Foliar samples from
119 plants infected by CMD and the DNA satellites were picked and preserved in bottles containing
120 silica gel granules. The tender young leaves are the ones that were picked avoiding the old leaves
121 and woody parts. In each field, 3 – 4 samples were taken with a total of 350 samples.
122

123 **Whitefly counts and mode of transmission**

124 This study determined whitefly counts and also investigated the method of transmission of the
125 cassava mosaic geminiviruses. The population of adult whiteflies was determined on the five
126 top-most apical leaves of the tallest shoot of each sampled plant. This was done in early morning
127 hours since the flies become active as the day warms up. This makes it difficult to count the
128 whiteflies in the cassava fields after 10 in the morning.
129
130 Plants exhibiting symptoms on upper leaves indicated inoculation by whiteflies while those
131 showing symptoms in all parts of the plant indicate transmission of CMD through cuttings. As
132 such, scoring for whitefly infected fields was denoted by letter W while those infected by
133 cuttings by the letter C.
134

135 **Detection of cassava mosaic geminiviruses in collected samples**

136 **Nucleic acid extraction and detection of Cassava mosaic geminivirus**

137 All begomoviruses code for coat protein which act as the protective coat of the virus particle and
138 determine vector transmitability of the viruses by whitefly vector *B. tabacii*. Thus, the CP gene is
139 highly conserved among begomoviruses originating from the same geographical region and
140 adapted to transmission by local vector populations [14]. Smaller fragments comprising the core
141 coat protein gene (core CP), a partial 575-579 base pair (bp) sequence of the Coat Protein gene
142 [15], or the complete CP sequence have also been used to establish provisional species
143 identification owing to the highly conserved nature of the viral CP sequence. Total nucleic acid
144 (TNA) was extracted from the dry leaf samples using the CTAB based method [16]. About 0.03g
145 of the dried leaf samples was ground in 1.5ml of CTAB extraction buffer. About 750µl of the
146 sample was poured into a 1.5ml eppendorf tube and incubated at 65$^{o}$C for 30min. The samples
147 were then mixed with an equal volume 750µl of chloroform: Isoamyl alcohol (24:1). They were
148 mixed by gentle shaking before being centrifuged at 1200 rpm for 10min. The top aqueous phase
149 was transferred into a new eppendorf tube and an equal volume (750µl) of chloroform:Isoamyl
150 alcohol(24:1) was added, mixed and centrifuged again as in the previous step. 300µl of the top
151 aqueous phase was transferred into a new eppendorf tube and DNA was precipitated by adding

UNDER PEER REVIEW

152 two volumes (600µl) of ice cold isopropanol. The samples were then centrifuged at 8000 rpm for
153 10min and the resulting supernatant discarded. The pellet was then washed in 0.5ml of 70%
154 ethanol by vortexing and then centrifuged at 8000 rpm for 5min. Ethanol was removed gently
155 and the pellet air dried for 30min. The pellet was suspended in sterilized water and stored at -
156 20$^{o}$C.The PCR mix consisted of GoTaq green (Promega), 10µl of each primer (Forward primer
157 EAB555F and reverse primers EAB555R) of the template DNA. Go Taq green contains Taq
158 polymerase enzyme and dNTPs. The final reaction volume was 20µl. Universal primers were
159 used to detect African Cassava Mosaic Virus (ACMV) with an expected amplicon of 774bp [17].
160 The Universal primers used for detection of ACMV were JSP001 (5'-
161 ATGTCGAAGCGACCAGGAGAT-3') ACMV the forward primer and (AV1/CP) JSP002 (5'-
162 TGTTTATTAATTGCCAATACT-3') ACMV (AV1/CP) the reverse primer. The PCR detection
163 of EACMV was done using EAB555 F/R primers whose sequences were EAB555/F (5'-
164 TACATCGGCCTTTGAGTCGCATGG-3') EACMV DNA-B and EAB555/R (5'-
165 CTTATTAACGCCTATATAAACACC-3') EACMV DNA-B.These primers are designed to
166 amplify a 556 bp fragment of EACMV DNA B component [17]. The cycling regimes was as
167 follows; the first step (initial denaturation) was at 94$^{o}$C for 3 minutes, second step was at 94$^{o}$C
168 for 1min, the third cycle at 72$^{o}$C and the final cycle at 48$^{o}$C ( annealing) for 1min. The reaction
169 was set for 31 cycles. After the 31$^{st}$ cycle, the PCR reaction tubes were removed from the
170 thermocycler and stored temporarily at 4$^{o}$C awaiting gel electrophoresis. The PCR cycling
171 regimes were the same as those of EACMV detection. The annealing temperature of 48°C
172 worked perfectly with generation of well amplified DNA bands after agarose gel electrophoresis.
173
174 **Nucleic Acid extraction for detection of DNA satellites**
175 The CMD viral DNA was also analyzed for the detection of DNA satellites associated with
176 Cassava mosaic geminiviruses. Specific primers designed for the amplification of the integrated
177 and episomal satellites were used in the PCR based detection technique. Nucleic acid extraction
178 was carried out in a similar method as for the testing for CMD.
179
180 The primers used for the detection of DNA Integrated satellites which amplify the DNA-B with
181 and expected 306bp PCR product were ;
182 SAT III F-5’-AGGCCTCGTTACTAAAAGTGC-3’
183 SAT III R-5’-ACCTGACGGCAGAAGGAAT-3’
184
185 The mastermix was prepared with one of the set ups for 17 samples. PCR cycling regimes or
186 program was as follows; Initial denaturation 94$^{o}$C for 3min, denaturation 94$^{o}$C for 1min,
187 annealing 55$^{o}$C for 1.5min and extension of 72$^{o}$C for 1min. The final step in PCR extension was
188 for 4mins at 72$^{o}$C.
189
190 **Statistical Analysis**
191 Data on disease prevalence, incidence and severity were subjected to one way Analysis of
192 variance (ANOVA) using Genstat discovery edition software (2005). Mean comparison of the
193 incidence, severity were done using student t –test at 95% confidence level. ANOVA test was
194 used to determine any significant differences between the means of the three independent
195 variables of CMD incidence, prevalence and severity. The t test was used to separate the means.

196

UNDER PEER REVIEW

## Results

### CMGs incidence, prevalence and severity based on symptomatology

A total of 94 fields, 23 in Eastern province, 26 in Nyanza province, 25 in western province and 20 in Coast province were visited during the survey. A total of 350 samples with symptoms of CMGs and the DNA satellites associated with the CMGs were collected from the fields. Table 1 shows the disease incidence, prevalence, symptom severity and types of infection within the districts surveyed in the four provinces surveyed.

**Table 1:CMD incidence, prevalence,symptom severity and type of infection in sampled Kenyandistricts in 2009.**

| Province | District | Disease incidence (%) | Prevalence (%) | Severity(1-5 scale) | Type of infection |
|---|---|---|---|---|---|
| **Western** | Kakamega | 73±1.15 | 50±0.33 | 3.1±0.11 | C |
| | Butere | 66±1.15 | 100±0.00 | 2.8±0.11 | C |
| | Mumias | 75±0.57 | 100±0.00 | 2.3±0.05 | C and W |
| | Busia | 22±1.15 | 75±1.15 | 2.1±0.05 | C |
| | Teso South | 31±0.57 | 80±0.57 | 2.4±0.11 | C and W |
| | Teso North | 25±1.15 | 66±1.15 | 2.8±0.11 | C |
| | Bumula | 26±1.73 | 85±2.3 | 2.1±0.05 | C and W |
| | Bungoma W. | 63±1.73 | 100±0.00 | 3.9±0.05 | C and W |
| | **Mean** | **47.6** | **82** | **2.7** | |
| **Nyanza** | Siaya | 58±1.73 | 100±0.00 | 3.7±0.17 | C |
| | Bondo | 62±1.15 | 100±0.00 | 3.1±0.05 | C |
| | Rachuonyo | 36±1.15 | 100±0.00 | 3.2±0.11 | C |
| | Homa Bay | 55±1.15 | 60±0.57 | 3.3±0.11 | C |
| | Rongo | 46±0.57 | 100±0.00 | 2.8±0.11 | C |
| | Migori | 6±0.57 | 100±0.00 | 3.0±0.11 | C |
| | Kuria West | 54±1.73 | 100±0.00 | 3.8±0.17 | C |
| | Gucha | 13±1.15 | 100±0.33 | 2.0±0.12 | W |
| | Kisii Central | 70±1.73 | 100±0.00 | 3.5±0.11 | C |
| | **Mean** | **44.4** | **95.5** | **3.2** | |
| **Eastern** | Kathiani | 53±0.33 | 50±2.98 | 3.4±0.11 | C and W |
| | Kangundo | 30±1.73 | 100±0.00 | 2.3±0.11 | C and W |
| | Makueni | 68±1.15 | 90±2.3 | 3.3±0,11 | C and W |

UNDER PEER REVIEW

| | | | | | |
|---|---|---|---|---|---|
| | Mwala | 16±0,57 | 50±0.57 | 2.5±0.12 | W |
| | Kitui Central | 100±0.00 | 100±0.00 | 4.3±0.08 | C |
| | Mbeere South | 0 | 0 | - | - |
| | Mbeere Notht | 0 | 0 | - | - |
| | Embu | 33±0.11 | 33±0.57 | 2.3±0.05 | C |
| | Meru South | 6.6±1.15 | 45±0.57 | 2.1±0,12 | C |
| | Maara | 81.6±0,3 | 100±0.33 | 4.1±0.55 | C |
| | Tharaka South | 96±1.15 | 100±0.00 | 3.8±0.11 | C |
| | Imenti South | 76±0.57 | 100±0.00 | 2.1±0.05 | C and W |
| | **Mean** | **46.7** | **64** | **2,8** | |
| **Coast** | Kilifi | 80±1.73 | 100±0.00 | 3.5±0,11 | C |
| | Malindi | 98±0.57 | 100±0.00 | 4±0.55 | C |
| | Msambweni | 68±1.15 | 100±0.00 | 3.3±0.12 | C |
| | Kwale | 52±2,3 | 100±0.00 | 2.8±0.11 | C |
| | Taita | 71±1.15 | 66±0.57 | 3.2±0.11 | C |
| | **LSD0.05** | **6.13** | **34.05** | **0.52** | |

207 **C-Infection caused by cuttings W-Infection caused by whiteflies**

208 Incidence and prevalence is expressed in percentages while severity in the scale 1 – 5.
209

210 Table 2 depicts the analyzed data for disease incidence, prevalence, severity and whitefly
211 counts at the provinces level. There was a significant difference in CMD prevalence between all
212 the provinces where the survey was done. The same trend was evident with the disease
213 incidences in the four provinces under study. However, there was no significant difference of
214 CMD severity in all the provinces surveyed apart from Western province.
215

216 Cassava mosaic disease was observed in major areas where cassava is grown in Kenya. The
217 disease is widely distributed countrywide with an average incidence of 57.3% (Table1). Coast
218 province had the highest average CMD incidence (74.0%) followed by Eastern province
219 recording a mean incidence of 57.0%. Western and Nyanza province had the lowest CMD
220 incidence of 47.0% and 51.0%, respectively. Overall CMD prevalence was 81.6% with Nyanza
221 province recording the highest (96.0 %) prevalence followed by coast province with a disease
222 prevalence of 93.0%. Eastern province had the least disease prevalence of 78.0% and the disease
223 was not detected in Mbeere district (Table 2).

UNDER PEER REVIEW

224 **Table 2: Incidence, Prevalence, Severity of cassava mosaic disease and the whitefly counts**
225 **in the four major cassava growing provinces in Kenya(2009)**

| Province | No.of fields | CMD incidence | CMD prevalence (%) | Whitefly counts | CMD severity |
|---|---|---|---|---|---|
| Eastern | 23 | 57.4±0. 3[b] | 78.0±2.0[d] | 1.86±0.16[b] | 3.1±0.3[a] |
| Nyanza | 26 | 51.0±0.4[d] | 96.0±2.0[a] | 3.18±0.17[a] | 3.2±0.2[a] |
| Western | 25 | 47.0±0.3[c] | 82.0±3.0[c] | 1.16±0.07[c] | 2.7±0.2[b] |
| Coast | 20 | 74.0±2.0[a] | 93.0±2.0[b] | 2.99±0.21[a] | 3.4±0.1[a] |

226 Means with the same subscripts in the same column denotes no significant differences between
227 the means at p═0.05
228
229 Although Coast province had the highest CMD symptom severity (3.4) there was no statistically
230 significant difference between the provinces on disease severity apart from Western province
231 (2.7). A mean severity of 3.1 countrywide rather indicates the severe symptoms prevalent in the
232 survey areas. District means were averaged to get the provincial means.
233
234 Table 3 summarizes the molecular detection work for Cassava mosaic virus amongst the samples
235 collected. ACMV was for the first time detected in Eastern and Coast province. Dual infection of
236 EACMV and ACMV were common in Nyanza, Western and Coast province.
237
238
239 **Table 3: Detection for EACMV and ACMV in the four provinces under survey**

| Province | No. of Samples tested | Positive for EACMV | Positive for ACMV | Dual infections |
|---|---|---|---|---|
| **Nyanza** | 97 | 21 | 4 | 4 |
| **Western** | 110 | 11 | 3 | 2 |
| **Coast** | 62 | 11 | 3 | 3 |
| **Eastern** | 78 | 11 | 1 | 0 |
| **Total samples** | **350** | **51** | **11** | **9** |

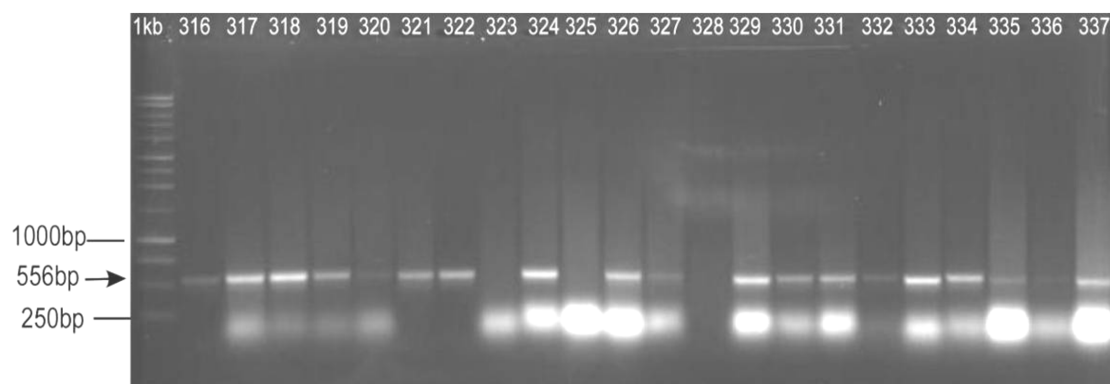
240
241
242 From the PCR-based detection, EACMV was more widespread than ACMV in the country.
243 EACMV occurred in all the provinces surveyed (Table 3). Nearly all the districts under survey
244 showed the presence of EACMV. However, ACMV was mostly prevalent in Western, Nyanza
245 and for the first time in Coast and Eastern province (Table 3). The distribution was not so much
246 intense as EACMV. About 18 out of 61 samples had ACMV constituting 29.5% in Western
247 province. ACMV was recorded only in one sample from Kathiani district in Eastern province.
248 The presence of ACMV was detected in leaf samples collected from several fields in Kilifi,
249 Msambweni and Kwale districts of Coast province, an area previously presumed to be ACMV-
250 free. Co-infection of 8% EACMV and ACMV was recorded in field samples collected. Co-
251 infection was more prevalent in Nyanza and Western province and to some extent in Coast

UNDER PEER REVIEW

252 province. Teso North, Teso South and Bungoma West districts in Western Kenya had the highest
253 co infection rates of the two viruses.
254

255 **Survey of CMG's and associated DNA satellites**
256 **Detection of Cassava mosaic geminiviruses**

257 The PCR product of 556bp was evident as expected for the amplification of the DNA – B with
258 EAB555F/R primers for the detection of EACMV (Plate 3). For the detection of ACMV, the
259 expected PCR product of 774bp was realized after amplification of the ACMV coat protein gene
260 by the primer set JSP001/002.
261




262

263 **Plate 1:PCR products (556bp) of East African cassava mosaic virus (EACMV) from**
264 **infected cassava leaf samples total nucleic acid.**
265 Lane 1 is the 1kb DNA marker. The numbers in the gel picture are sample numbers.

266

267 **PCR detection for the CMD DNA Satellites III**
268 The PCR products after the amplification and gel electrophoresis were of the expected size of
269 306 base pairs (Plate 4). The 1kb molecular marker was used thus perfectly giving the expected
270 PCR product as shown in plate 4. Some samples were negative for the DNA integrated satellites
271 but the majority of the samples collected from the field with typical symptoms of the satellites
272 associating with the CMGs tested positive. The integrated satellites were common amongst the
273 samples collected during the survey. Out the 350 samples collected from the field during the
274 survey, 145 tested positive for the integrated CMD DNA satellites accounting for 41.1%. The
275 episomal DNA satellites for CMD on the other hand were very rare with just a few samples
276 testing positive for thesatellites after DNA amplification. The interaction of the DNA satellites
277 with begomoviruses leads to different symptoms expression of CMD with a likelihood of
278 increasing the disease severity. This was evident with the same scoring slightly severe symptoms
279 when infected with CMD and the associated DNA satellites.
280
281
282
283
284

UNDER PEER REVIEW

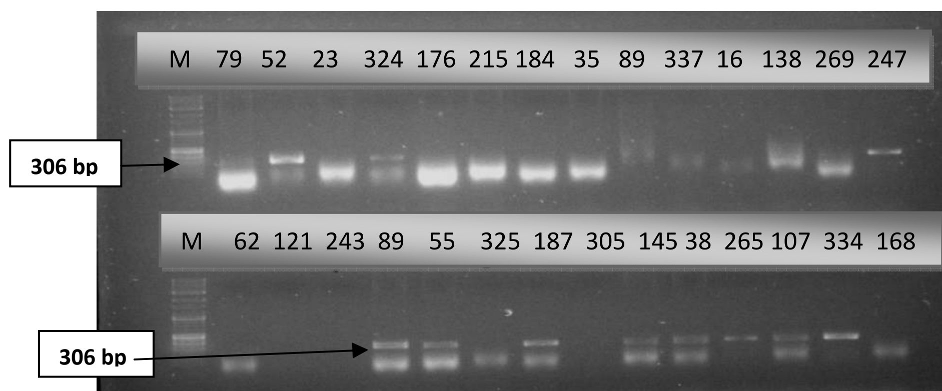



**Plate 2:Agarose gel electrophoresis of the integrated DNA satellites specific PCR products of 30bp**

Primers used Sat III F/R. The numbers in the plate are sample numbers.

285 Nyanza region had the highest (3.2) adult whiteflies per plant which was not significantly
286 (P=0.05) higher than the population recorded in Coast region (2.9). The lowest whitely
287 population was recorded in Western province (Table 4). There was no significant difference in
288 whitefly infestation in coast and Nyannza provinces. Likewise, there was no significant
289 difference in cuttings and whitefly method of transmission in eastern and western provinces.
290

291
292 **Table 4: CMD severity, whitefly count, cuttings infection and whitefly count per plant in**
293 **the sampled areas**

| Province | CMD Severity(1-5) | Whitefly Infection (%) | Cuttings infection (%) | Whitefly counts |
|---|---|---|---|---|
| **Eastern** | 3.1$^{b}$ | 33.3$^{c}$ | 66.6$^{a}$ | 1.9±0.16$^{b}$ |
| **Nyanza** | 3.2$^{d}$ | 11.1$^{b}$ | 88.8$^{b}$ | 3.2±0.17$^{a}$ |
| **Western** | 2.7$^{c}$ | 33.3$^{c}$ | 66.6$^{a}$ | 1.2±0.07$^{c}$ |
| **Coast** | 3.4$^{a}$ | 0$^{a}$ | 100$^{c}$ | 2.9±0.21$^{a}$ |
| **Mean** | **57.4** | **19.6** | **80.5** | **2.3** |

294
295 The infection due to cuttings is correlated to the high severity symptoms. There is significant
296 difference in white fly infection across the provinces. However, there is no significant difference
297 in cuttings borne infections in Eastern and western provinces. Cutting-borne infection of CMD
298 accounted for 80.6% compared to the whitefly infection of 19.5%.

UNDER PEER REVIEW

299 **Discussion**
300 This survey of viruses infecting cassava in Kenya was the most comprehensive covering the
301 entire country including Eastern province and the Mt. Kenya region which has not been studied.
302 The plants showing symptoms of cassava mosaic disease were easily identified due to the
303 symptoms they exhibited. Typical symptoms of CMD observed were leaf chlorosis which ranged
304 from pale yellow to white and others were paler that the normal leaf colour. Defined mosaic
305 patterns, leaf malformation and distortion were associated with more severe symptoms of the
306 disease. Symptoms of CMD with the associated DNA satellites showed the same symptoms as
307 described but with more leaf distortion assuming a sickle shape.
308
309 Cassava mosaic disease was reported in all the major areas where cassava is grown in Kenya.
310 CMD incidence was observed to be highest in Coast province compared to other provinces.
311 Western and Eastern provinces had the least CMD incidence. On the other hand, Nyanza
312 province had the highest CMD prevalence followed by Coast province with Eastern province
313 registering the lowest disease prevalence. A mean severity of 3.1 countrywide indicates the
314 severity of CMD in the surveyed areas is high. However, Coast province had the highest CMD
315 severity (3.4). Farmers in this province indeed expressed the fear that the symptoms are
316 nowadays more severe compared to the recent years. Nyanza province recorded the second most
317 CMD severe symptoms of 3.2 with Western province posting the least severity symptoms of 2.7.
318 CMD was very severe in the late 1980's to early 1990's but the disease severity was greatly
319 reduced due to the introduction of resistant and tolerant varieties by KARI and the Ministry of
320 Agriculture [18]. The same measures were not taken in Coast and Nyanza districts at that time.
321
322 Nyanza province had the highest whitefly count in the country followed by Coast province. This
323 was followed by Eastern province with Western province registering the least whitefly counts
324 per plant. It is vividly clear that infection by cuttings is more rampant than that caused by
325 whiteflies. Though whiteflies carry the CMD viruses, the method of transmission through
326 distribution or use of infected cuttings is widespread. This phenomenon has also been observed
327 in Togo [19] It is quite contrasting for Coast where the average whitefly count per plant is 2.99
328 but has 0% infection due to whiteflies. All the plant sampled in coast showed that the method of
329 CMD infection is purely (100%) due use of infected cuttings for planting. The same replicates
330 for Nyanza province where the whitefly infection accounts for 11.1% and through infected
331 cuttings accounting for 88.8%. Eastern province had the highest whitefly method of infection at
332 33.3% but still infection by cuttings is more prevalent there at 66.6%.
333
334 The PCR detection from the samples collected in the nationwide survey showed that EACMV is
335 more widespread than ACMV in the country. EACMV occurs in all the provinces and was
336 distributed across the country. Nearly all the districts under survey showed the presence of
337 EACMV. ACMV was recorded only in Kathiani district in Eastern province signalling the first
338 recorded occurrence of ACMV species in this region. In Coast province, an area presumed to be
339 free of ACMV reported the presence of ACMV also for the first time. ACMV was detected in
340 several farmers' fields in Kilifi, Msambweni and Kwale districts which had been presumably
341 been thought to be free from this species of CMD. Previous studies show indeed that EAMCV is
342 the most common species of CMV in Kenya than ACMV [6]. However, in this study, ACMV
343 was only detected in western and Nyanza provinces but none in Eastern and Coast provinces.
344

UNDER PEER REVIEW

345 The DNA satellites associated with CMGs in this study were common across the country
346 amongst the samples collected during the survey. A total of 145 from the 350 samples collected
347 during the survey tested positive for the integrated Begomoviruses DNA satellites accounting for
348 41.1%. The episomal DNA satellites for CMD on the other hand were very rare with just a few
349 samples showing positive for the satellites after DNA amplification. The interaction of the DNA
350 satellites with Begomoviruses leads to different symptoms expression of Cassava mosaic
351 Begomoviruses with a likelihood of increasing the disease severity [9]. The leaves exhibiting
352 these symptoms were definitely also having typical symptoms of the cassava mosaic
353 Begomoviruses. It is likely that ACMV and EACMV are synergistically interacting leading to
354 severe symptoms as reported by farmers. The study shows that the method of infection is
355 predominantly due to use of infected cuttings with farmers almost not utilizing any management
356 practices [20]. The same trend was noted with CMD symptom severity where again Coast
357 province recorded the highest symptom severity of 3.36. This observation was amplified by the
358 respondents' interviewed during the survey. The farmers whose fields were sampled expressed
359 that they have known the disease symptoms of the disease and still were able to get some yields.
360 They have also noted that the disease symptoms are now quite severe and that the yields have
361 greatly reduced.The detection of ACMV in Kathiani district and several districts in Coast
362 province present challenges in the management of CMD in these regions. Dual infections of
363 EACMV and ACMV in these regions point to a possibility of more severe forms of CMD due to
364 synergism and genetic recombination between EACMV and ACMV [21].
365
366 The interaction of the DNA satellites with Begomoviruses leads to enhanced symptom severity
367 of Cassava mosaic Begomoviruses [7]. In this study, the symptom phenotypes modulation by the
368 DNA satellites on the CMGs symptoms was quite evident. DNA satellites species are often
369 associated with geminivirus infection [22].These DNA molecules can either enhance symptoms
370 severity or even ameliorate the symptoms in some cases [23]. The leaves of the plants infected
371 assumed a sickle shape thus distinguishing them from other CMGs infected leaves. It was also
372 established that varieties infected with CMGs and DNA satellites exhibited more severe
373 symptoms compared to the same varieties infected only with CMGs. The effect of the DNA
374 satellites on the quality and yield of cassava is not known. Studies in Sri Lanka show that each of
375 the cassava-infecting geminiviruses showed a contrasting and differential interaction with the
376 DNA satellites, not only in the capacity to interact with these molecules but also in the
377 modulation of symptom phenotypes by the satellites .
378

379 **CONCLUSIONS**

380 EACMV is more prevalent than ACMV and the two viral species of the cassava Mosaic virus
381 disease are now well mapped in the country. The study has revealed cases of dual infection
382 accounting for 21% of all the samples analyzed for the presence of the virus. The increased
383 symptom severity is attributed to the dual infections of the two CMV species and the combined
384 infection of CMD and the associated DNA satellites. It is vividly clear that infection by cuttings
385 is more rampant than that caused by whiteflies. Though whiteflies carry the CMD viruses, the
386 mode of transmission distribution or use of infected cuttings is widespread. Even in the
387 provinces where the whitefly infestation is high like in Coast, the dominant mode of transmission
388 of the virus is by infected cuttings. There exist DNA satellite molecules which associate with the
389 viral DNA of Cassava mosaic virus. The symptoms severity score correlated well with the

UNDER PEER REVIEW

molecular detection of the DNA satellite molecules. The DNA integrated satellites were far more prevalent and are distributed across the county than the episomal satellites as determined from this study.

This study has revealed that Cassava mosaic geminiviruses in Kenya are caused by the two species of CMD namely EACMV and ACMV. Kenyan EACMV strains have a high homology to the EACMV – Ug strains. The high sequence identity of 96% to the ACMV –Uganda severe isolate points at the possibility of these Kenyan isolates to cause a severe form of the disease as witnessed in the field during the survey.

The DNA satellites obtained from this study exhibited low sequence identity with the begomoviruses associated DNA III satellites East African region and India. There is a large genetic variability amongst the DNA III satellites characterized in this study. This study has therefore clearly demonstrated that there are four distinct groups of begomovirus associated DNA satellites with two groups being predominant in Kenya, one in Eastern Africa and the other one in Southern Africa The DNA satellites identified in this study are distantly related to those from other parts of east Africa, South Africa and India.

## RECOMMENDATIONS

The detections of ACMV in Kathiani district of Eastern province and several districts in Coast province in this study present challenges in the management of CMD in these regions and the county at large. Dual infections of EACMV and ACMV in these regions point to a possibility of more severe forms of CMD due to synergism and genetic recombination between EACMV and ACMV. As such there is need to continue evaluating varieties resistant or tolerant to these viruses and pooling regional efforts in the characterization of the viruses. The existing varieties that are resistant or tolerant to CMD can now be deployed in areas where the disease severity, prevalence and incidence have been determined to be high. This will lead to reduced severity levels hence increased yields.

Breeders can now target resistance to the two main species of CMD i.e. ACMV and EACMV since the two species are now characterized, Genetic modification techniques or conventional breeding techniques can now be tailored to coming up with resistant and tolerant varieties to mitigate this situation. Further characterization studies are therefore required to ascertain the isolates from Coast and Nyanza where exceptionally high severity symptoms were recorded in the study.

The DNA satellites associated with the CMD virus had a strong correlation between the symptoms expression and the molecular detection especially the DNA integrated satellites for the plant viruses under study. There is need to evaluate the Integrated DNA satellites associated with CMGs to determine their modulation of symptom expression of the CMGs and the possibility of causing more severe symptoms of the disease. The effect on the yield of cassava also needs to be evaluated.

UNDER PEER REVIEW

433 These field observations of the symptom severity could be extrapolated to field situations in
434 order to hypothesize about the possibility of acquisition of such DNA satellites currently
435 associated with other begomoviruses. These results call for more detailed analyses of these sub
436 viral components and an investigation of their possible interaction with the cassava mosaic
437 disease complex. There is need to investigate the above mentioned phenomenon with special
438 interest on interaction of the DNA satellites with plants having dual infection of the two species
439 of CMD, ACMV and EACMV.
440

441 **Reference**


442 1. Thresh, J. M., Otim-Nape, G. W., Legg, J. P. and FaRgette, D. 1997. African cassava
443 mosaic virus disease: the magnitude of the problem. *African Journal of Root and Tuber*
444 *Crops* 2(1):13-19.
445 2. Munga, T.L. (2000). Root and Tuber crops. In: Annual Report (2000), Regional Research
446 Centre, KARI-Mtwapa. *Internal report*.
447 3. Monger, W.A., SEAL, S., ISAAC, A.M. and FOSTER, G.D. (2001a). Molecular
448 Characterization of Cassava Brown Streak virus coat protein. Plant Pathology 50, 527-
449 534.
450 4. Briddon, R. W., Robertson, I., Markham, P. G. & Stanley, J. (2004). Occurrence of South
451 African cassava mosaic virus (SACMV) in Zimbabwe. *Plant Pathol* 53, 233.
452 5. Were, H. K., Winter, S. &Maiss, E. (2004a).Occurrence and distribution of cassava
453 begomoviruses in Kenya. *Ann Appl. Biol* 145, 175–184.
454 6. Bull,S.E., Briddon, r.w., 14 Sserubombwe, W.S., Ngugi K., MarkhaM, P.G., and
455 Stanley, J.(2006) Genetic diversity and phylogeography of cassava mosaic viruses in
456 Kenya *.Journal of General Virology (2006), 87, 3053–3065*
457 7. Olufemi J. A., Francis O. Ogbe., Ranajit Bandyopadhyay, Kumar L., Alfred G. O.,
458 Jaqueline d'A.H. and Rayapati A. N. (2008). Alternate hosts of African cassava mosaic
459 virus and East African cassava mosaic Cameroon virus in Nigeria. *Arch. Virol*. Impact
460 factor: 2.11). 01/2008; 153(9):1743-7
461 8. Ndunguru J., Kapinga R. (2007). Viruses and virus-like diseases affecting sweetpotato
462 subsistence farming in southern Tanzania. *Afr. J. Agric. Res*. 5: 232-239.
463 9. Otim-Nape, G.W. (1993). The epidemiology of the African cassava mosaic geminivirus
464 disease in Uganda. *PhD Thesis University of Redding* 252 pp
465 10. Otim-Nape, G.W., Thresh, J.M., Bua, A., Baguma, Y., Shaw, M.W. (1998a). Temporal
466 spread of cassava mosaic disease in a range of cassava cultivars in different agro-
467 ecologicalregions of Uganda. *Annals of Applied Biology,* 133, 415 - 430.
468 11. Sseruwagi P, Otim-Nape GW, Osiru DSO, Thresh JM (2003). Influence of NPK fertiliser
469 on populations of the whitefly vector and incidence of cassava mosaic virus disease. *Afr.*
470 *Crop Sci. J*. II: 171–179
471 12. James V.E.(1974). Crop losses caused by viruses. Science direct journal Vol.1:3 pp 263 –
472 282
473 13. Maruthi MN, Colvin J, Seal S, Gibson G, Cooper J (2002) Co-adaptation between
474 cassava mosaic geminiviruses and their local vector populations. *Virus Research* 86:71-
475 85.
476 14. Brown, J. K. Idris, A. M., Torres-Jerez, I., Banks, G. K., and Wyatt, S. D. 2001. The
477 provisional identification of begomoviruses. *Arch. Virol.* 146: 1581-1598

UNDER PEER REVIEW

478 15. Lodhi, M.A., Ye, G.N., Weeden, N.F., and Reisch, B. (1994). A simple and efficient
479 method for DNA extraction from grapevine cultivars and Vitis species. *Plant Molecular*
480 *Biology Report.* 12, 6-13.
481 16. Ndunguru, J., Legg, J. P., Aveling, T. A. S., Thompson, G. and Fauquet, C. M. (2005).
482 Molecular biodiversity of cassava begomoviruses inTanzania: evolution of cassava
483 geminiviruses in Africa and evidence East Africa being a center of diversity of cassava
484 geminiviruses. *Virology Journal.* 2, 21.
485 17. Obiero H.M., Whyte J.A.B., Legg J.P., Akhwale M.S., Malinga J. and MagutT.
486 Proceedings of the 13th ISTRC Symposium, 2007 pp. 682 – 685
487 18. Adjata K.D., T. Tchacondo, K. Tchansi, E. Banla and Y.M.D. Gumedzoe (2008).
488 Cassava Mosaic Disease Transmission by Whiteflies (*Bemisia tabaci* Genn.) and its
489 Development on Some Plots of Cassava (*Manihot esculenta* Crantz) Clones Planted at
490 Different Dates in Togo. *Plant Virology*. 126:845-853
491 *19.* Njenga P.W., Njeru R.W., Mukunya D., Ngure G.K, Muinga R. And Ateka E.M. (2005)
492 Farmers' knowledge on virus diseases of cassava in coastal Kenya. *African Crop Science*
493 *Conference Proceedings, Vol. 7.* pp. 1449-1451
494 20. Pita, J. S., Fondong, V. N., Sangaré, A., Otim-Nape, G. W., Ogwal, S., and Fauquet, C.
495 M. (2001). Recombination, pseudo recombination and synergism of geminiviruses are
496 determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of*
497 *General Virology*.82: 655-665
498 21. Sunter, G. & Bisaro, D. M. (1992). Transactivation of geminivirus ARl and BR1 gene
499 expression by the viral AL2 gene product occurs at the level of transcription. *Plant Cell*
500 4, 1321-1331.
501 22. Ian B Dry, Leslie R. Krake, Justin E. Ringden M. Ali razan(1997). A novel subviral agent
502 associated with geminiviruses: The first report of a DNA satellite. *Journal of plant*
503 *biology* 94,pp7088 – 7093
504 23. Kumar, P. P., Usha, R., Zrachya, A., Levy, Y., Spanov, H. & Gafni, Y. (2007). Protein–
505 protein interactions and nuclear trafficking of coat protein and $\beta$C1 protein associated
506 with Bhendi yellow vein mosaic disease. *Virus Res* 122, 127–136.