

Original Research Article**Distribution of cassava mosaic geminiviruses and their associated DNA satellites in Kenya.**

3

Abstract

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

35

Key words: ~~DNA Satellites and Cassava Mosaic Geminiviruses~~

37

Introduction

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

42

43 subsistence where it is an important food security crop. The available information from surveys
44 and yield loss assessments due to CMD is summarized [2], which estimates the losses in Africa
45 to be 15–24%. In Kenya, yields recorded range between 5 and 10t/ha against a potential of 32t/ha
46 [3].

47
48 CMD is transmitted by a whitefly vector known as *Bemisia tabaci* but proof of viral aetiology
49 was not obtained until the 1970s and 1980s, when sap inoculations to herbaceous hosts were
50 successful and virus isolates obtained in this way were purified and characterized [4]. After
51 initial uncertainty, the isolates were shown to cause CMD, Koch's postulates were fulfilled and
52 the various isolates from Africa and India were regarded as strains of a single virus of the
53 geminiviruses group and designated ~~African cassava mosaic virus (ACMV)~~. Subsequent studies
54 have led to the recognition of several distinct but similar viruses namely ~~African cassava mosaic~~
55 ~~virus~~ (ACMV), ~~East African cassava mosaic virus~~ (EACMV), Indian cassava mosaic virus
56 (ICMV) and ~~South African cassava mosaic virus~~ (SACMV) [5].

57
58 In Kenya, Cassava Mosaic Disease (CMD) is caused by begomoviruses in the family
59 Geminiviridae. These include African cassava mosaic virus (ACMV), East African cassava
60 mosaic virus (EACMV), and Uganda variant (EACMV-UG) of the genus begomovirus. Previous
61 studies have shown ACMV, EACMV, EACMV-UG and EACMVZV to be present in Kenya [6]
62 [7]. Earlier reports indicate that EACMV, EACMV-UG and EACMVZV have distinct
63 geographical distributions [7].

64
65 The whitefly vector, *Bemisia tabaci* (Gennadius) (Aleyrodidae, Hemiptera) transmits Cassava
66 mosaic begomoviruses (CMBs) from plant-to-plant. Long-distance spread of CMD occurs by the
67 distribution of infected stem cuttings [8]. Whitefly presence on plants does not necessarily
68 suggest that the disease is spread by the insects. Infected plants are stunted and have greatly
69 diminished tuberous root yield. Cassava is also affected by the DNA satellites associated with
70 Cassava mosaic geminiviruses [9].

71
72 This survey focused on determining the status and distribution of the CMG's and the DNA
73 satellites particularly their incidence, prevalence and severity in all major regions where cassava
74 is grown in the country.

75 **Material and methods**

76 **Sampling sites**

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

86

87 In Nyanza province, the survey and sampling was done in the following districts; Kisii central,
 88 Gucha, Kuria west, Migori, Rongo, Homa Bay, Rachuonyo, Gem, Bondo and Siaya. In Eastern
 89 province, sampling was done in Imenti south, Tharaka south, Maara, Meru south, Embu, Mbeere
 90 north, Mbeere, Kitui, Kitui central, Mwala, Makueni, Kangundo and Kathiani districts,
 91 Western province, survey and sampling was done in the following districts; Kakamega south,
 92 Butere, Mumias, Busia, Bumula, Teso North, Teso South, and Bungoma west. Finally sampling
 93 was done in Coast province in the following districts; Kilifi, Malindi, Kwale, Msambweni and
 94 Taita
 95

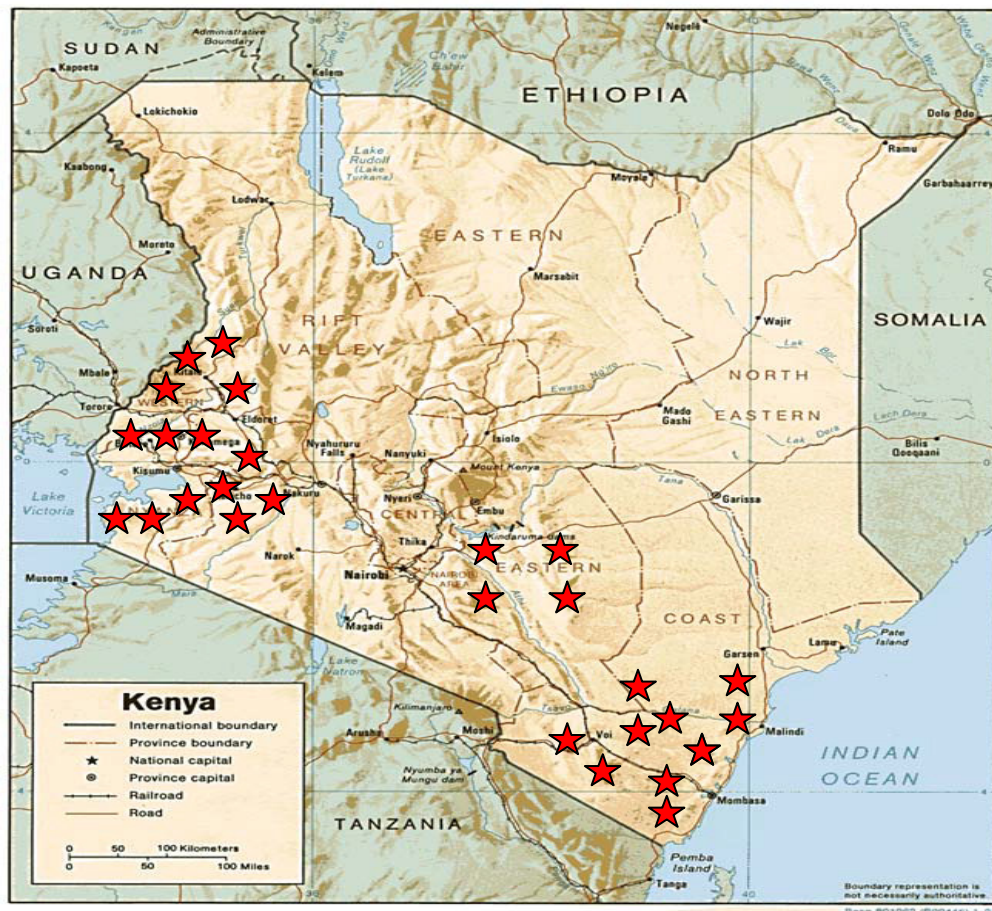


Figure1: ★ Areas surveyed for CMGs and associated DNA Satellites

96
 97 An imaginary line (transect) was drawn diagonally in the field from both directions thus ending
 98 up with two transects within one field. A total of 15 plants were examined for the symptoms of
 99 both Cassava Mosaic Virus disease (CMD) and the associated DNA satellites on each transect.
 100 In total, 30 plants in every field were examined. The prevalence of the viral diseases was
 101 evaluated in every region by calculating the number of fields in which at least one cassava plant
 102 presented symptoms of viral diseases divided by the total number of fields observed in that
 103 region. The disease severity symptoms for both diseases were established with disease severity
 104 scale (1-5) [13] which is internationally accepted and adopted. For CMD, the plants were
 105 observed for the foliar symptoms and their satellites symptoms.
 106

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 **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.

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

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

137 All begomoviruses coat or coat protein which act as the protective coat of the virus particle and
138 determine vector transmissibility of the viruses by whitefly vector *B. tabaci*. 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°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

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°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°C for 3 minutes, second step was at 94°C
 168 for 1min, the third cycle at 72°C and the final cycle at 48°C (annealing) for 1min. ~~The reaction
 169 was set for 31 cycles. After the 31st cycle, the PCR reaction tubes were removed from the
 170 thermocycler and stored temporarily at 4°C awaiting gel electrophoresis. The PCR cycling
 171 regimes were the same as those of EACMV detection. The annealing temperature 48°C
 172 worked perfectly with generation of well amplified DNA bands after agarose gel electrophoresis.~~

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.

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
 183 SAT III R-5'-ACCTGACGGCAGAAGGAAT-3'

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°C for 3min, denaturation 94°C for 1min,
 187 annealing 55°C for 1.5min and extension of 72°C for 1min. The final step in PCR extension was
 188 for 4mins at 72°C.

189 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.

197 **Results**198 **CMGs incidence, prevalence and severity based on symptomatology**

199 A total of 94 fields, 23 in Eastern province, 26 in Nyanza province, 25 in western province and
 200 20 in Coast province were visited during the survey. A total of 350 samples with symptoms of
 201 CMGs and the DNA satellites associated with the CMGs were collected from the fields. Table 1
 202 shows the disease incidence, prevalence, symptom severity and types of infection within the
 203 districts surveyed in the four provinces surveyed.

204

205 **Table 1:CMD incidence, prevalence,symptom severity and type of infection in sampled**
 206 **Kenya districts 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

	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
	LSD_{0,05}	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).

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

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

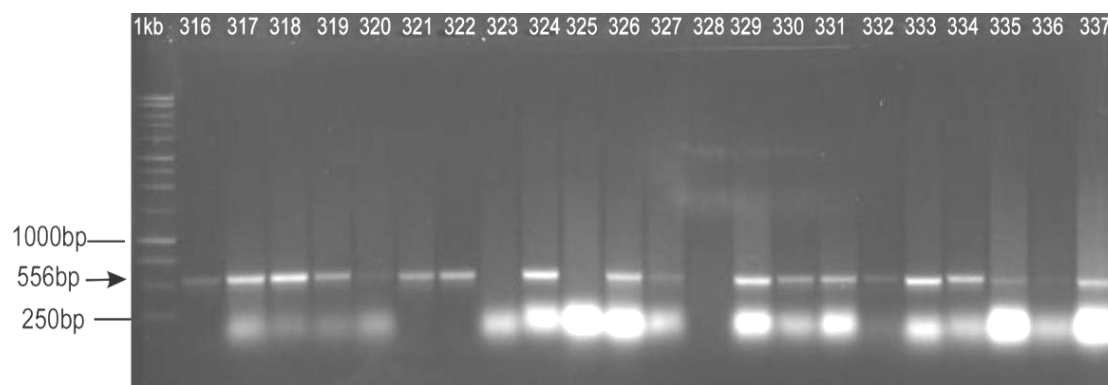
254

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 the satellites 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.

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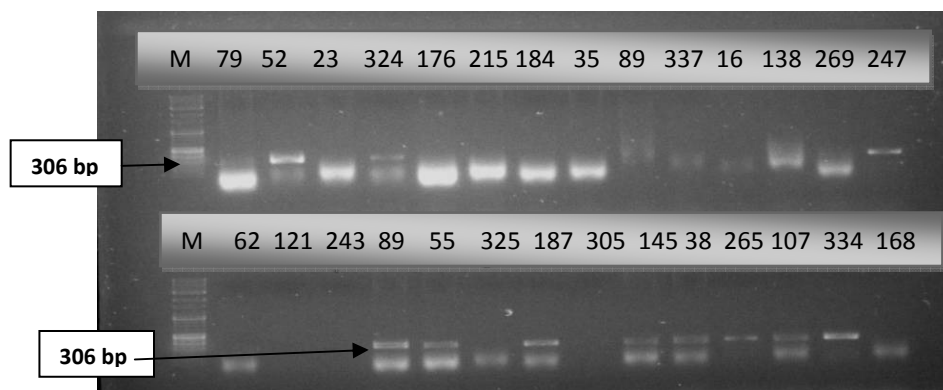


Plate 2:Agarose gel electrophoresis of the integrated DNA satellites specific PCR products of 306bp

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 whitefly
 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%.

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 than 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

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

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

393
394 This study has revealed that Cassava mosaic geminiviruses in Kenya are caused by the two
395 species of CMD namely EACMV and ACMV. Kenyan EACMV strains have a high homology
396 to the EACMV – Ug strains. The high sequence identity of 96% to the ACMV –Uganda severe
397 isolate points at the possibility of these Kenyan isolates to cause a severe form of the disease as
398 witnessed in the field during the survey.

399
400 The DNA satellites obtained from this study exhibited low sequence identity with the
401 begomoviruses associated DNA III satellites East African region and India. There is a large
402 genetic variability amongst the DNA III satellites characterized in this study. This study has
403 therefore clearly demonstrated that there are four distinct groups of begomovirus associated
404 DNA satellites with two groups being predominant in Kenya, one in Eastern Africa and the other
405 one in Southern Africa The DNA satellites identified in this study are distantly related to those
406 from other parts of east Africa, South Africa and India.

407

408 **RECOMMENDATIONS**

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

418
419 Breeders can now target resistance to the two main species of CMD i.e. ACMV and EACMV
420 since the two species are now characterized, Genetic modification techniques or conventional
421 breeding techniques can now be tailored to coming up with resistant and tolerant varieties to
422 mitigate this situation. Further characterization studies are therefore required to ascertain the
423 isolates from Coast and Nyanza where exceptionally high severity symptoms were recorded in
424 the study.

425
426
427 The DNA satellites associated with the CMD virus had a strong correlation between the
428 symptoms expression and the molecular detection especially the DNA integrated satellites for the
429 plant viruses under study. There is need to evaluate the Integrated DNA satellites associated with
430 CMGs to determine their modulation of symptom expression of the CMGs and the possibility of
431 causing more severe symptoms of the disease. The effect on the yield of cassava also needs to be
432 evaluated.

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 **Referen** 

- 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 ecological regions 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

- 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 in Tanzania: 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 Magut T.
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 ARI 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 β C1 protein associated
506 with Bendi yellow vein mosaic disease. *Virus Res* 122, 127–136.