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

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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. 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% countrywide, whereas the Coast province recorded the highest incidence (74%). The prevalence of CMD countrywide was 84% with Nyanza province recording the highest (96%) prevalence, whereas Eastern province had the least (67%) prevalence. The spread of CMD through use of infected cuttings accounted for 80% of the infected plants compared to the whitefly-borne infections which only accounted for 19%. 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 of Western and Nyanza provinces. For the first time, ACMV was detected in samples collected from Eastern and Coast provinces. 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 stem cuttings in Coast province. DNA satellites associated with these Begomoviruses were distributed across the areas under survey with 41% of the samples collected tested 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.

Key words: Survey, PCR, detection, whiteflies, *Cassava mosaic virus*

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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 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 10 t/ha against a potential of 32 t/ha [3].

CMD is transmitted by a whitefly vector known as *Bemisia tabacii* 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. 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].

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, *B. tabacii* (Gennadius) (*Aleyrodidae, Hemiptera*) transmits Cassava mosaic geminiviruses (CMGs) from plant to plant [8]. Long-distance spread of CMD occurs by the distribution of infected stem cuttings [9]. 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 [10].

PCR based methods have been used for the sensitive detection and discrimination of the plant viruses [11,12]. Moreover, PCR is more sensitive compared to ELISA for routine detection and discrimination of viruses. Therefore, samples collected from the different field during the survey were confirmed for the infection of CMG and DNA satellites using PCR.

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. Disease incidence and disease prevalence was determined by the percentage of the plants in a field/farm and the percentage of the farms with symptoms of MLND, respectively. Knowledge of the disease incidence and prevalence gave an indication of disease spread in the regions surveyed. The severity on the hand points to the seriousness and economic impact of the disease and the tolerance levels by some varieties.

Material and methods

Sampling sites

The survey was carried out in four distinct regions which are also administrative regions called 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 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 Kenya. 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 in Kenya.

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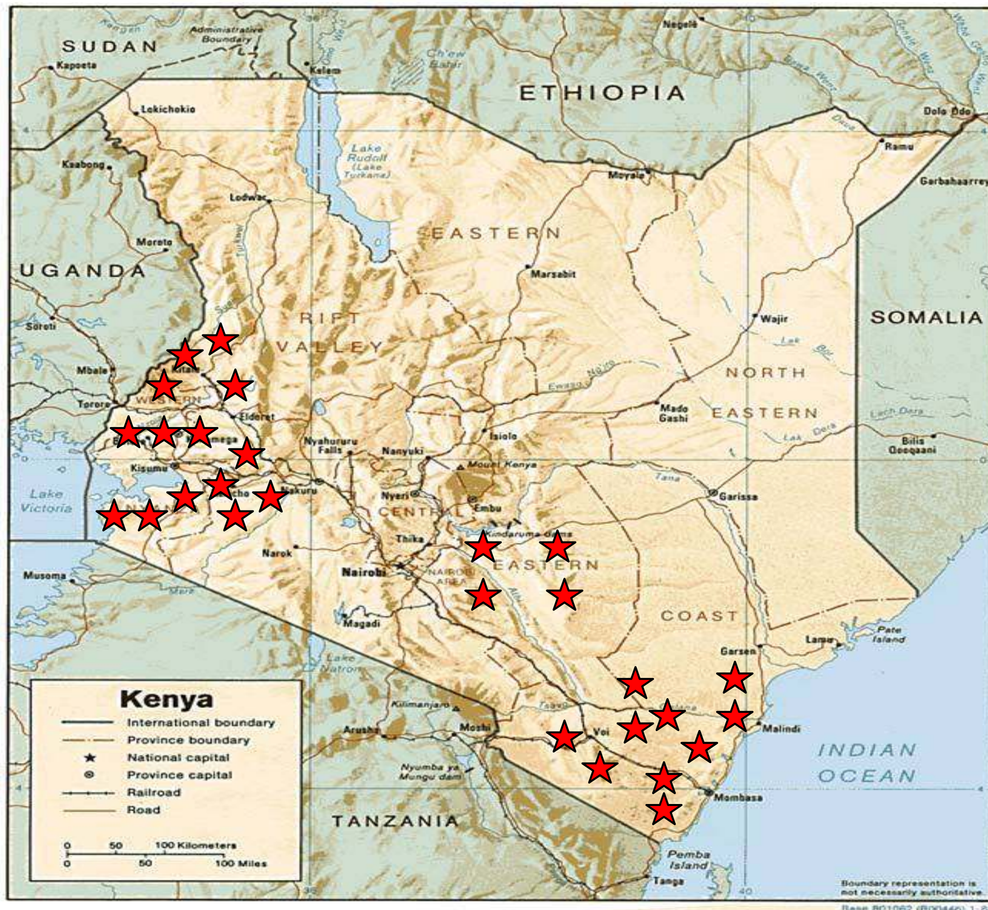

Figure1: ★Areas surveyed for CMGs and associated DNA Satellites

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 but 3 – 4 samples were taken from each field giving a total of 350 samples.

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 symptoms severity was established based on the disease severity scale (1-5) [13]. Cassava plants in farmers' fields were observed for virus disease symptoms. Symptomatic plants were picked and preserved in bottles containing silica gel granules. The tender young leaves are the ones that were picked avoiding the old leaves and woody parts. For CMD, the plants were observed for the foliar symptoms and their satellites symptoms. Symptoms of CMD with the associated DNA satellites showed the same symptoms as described but with more leaf distortion assuming a sickle shape and more severe, Fig. 2. DNA satellites are short DNA strands found either in the cell nucleus or in the cytoplasm that interact with viral genomes resulting in modulation of symptom phenotypes. Episomal DNA satellites are found in the cytoplasm while integrated DNA satellites are integrated in the viral genome. DNA satellites have been shown to play a direct role in symptom enhancement with characteristic stem-curling and vein-swelling phenotypes, impacting host-range determination and facilitating accumulation of both the begomovirus molecules and the encoded pathogenicity factors [14,15,16].

Fig. 2: CMD and DNA Satellites symptom and a healthy cassava plant

In each region, a particular representative route that captures the area of interest.Amongst issues considered include the sample area and availability of suitable cassava fields. In marginal and sparsely populated areas like Ukambani districts in Eastern province, a distance interval of 10 km was adopted. In all, 94 fields were visited during the survey. In each field, the coordinates were recorded using a global positioning system (GPS; Magellan GPS 315, San Dimas, CA).

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Whitefly counts and mode of transmission

This study determined whitefly counts and also investigated the method of transmission of the cassava mosaic geminiviruses in all the sites surveyed for CMD. The population of adult whiteflies was determined on the five top-most apical leaves of the tallest shoot of each sampled plant. This was done in early morning hours before 10am. since the flies become active as the day warms up.

Plants exhibiting symptoms on upper leaves indicated inoculation by whiteflies while those showing symptoms in all parts of the plant indicate transmission of CMD through cuttings. As such, scoring for whitefly infected fields was denoted by letter W while those infected by cuttings with CMD by the letter C.

Detection of cassava mosaic geminiviruses in collected samples

Nucleic acid extraction and detection of Cassava mosaic geminivirus

Total nucleic acid (TNA) was extracted from the dry leaf samples using the CTAB based method [17]. The pellet was suspended in sterilized water and stored at -20°C. The PCR mix consisted of GoTaq green (Promega) and the forward and reverse primers. Go Taq green contains Taq polymerase enzyme and dNTPs. The final reaction volume was 20µl. Universal primers were used to detect African Cassava Mosaic Virus (ACMV) with an expected amplicon of 774bp [18]. The Universal primers used for detection of ACMV were JSP001 (5'-ATGTCGAAGCGACCAGGAGAT-3') the forward primer and JSP002 (5'-TGTTTATTAATTGCCAATACT-3') the reverse primer. The PCR detection of EACMV was done using EAB555 F/R primers whose sequences were EAB555/F (5'-TACATCGGCCTTTGAGTCGCATGG-3') EACMV DNA-B and EAB555/R (5'-CTTATTAACGCCTATATAAACACC-3') EACMV DNA-B. These primers are designed to amplify a 556 bp fragment of EACMV DNA B component [18]. The PCR steps were as follows; the first step (initial denaturation) was at 94°C for 3 minutes, 94°C for 1min, 48°C (annealing) for 1min and 72°C extension. The reaction was set for 31 cycles. The PCR steps were the same as those of EACMV detection.

Detection of DNA satellites

Nucleic acid extraction was carried out in a similar method as for the testing for CMD.
The CMD viral DNA was also analyzed for the presence of DNA satellites II and III associated with Cassava mosaic geminiviruses. Specific primers designed for the amplification of the integrated and episomal satellites which amplify the DNA-B with and expected 306bp PCR product.Below are primers for episomal and integrated DNA satellites associated with CMGs used in this study[26]..

SAT II F-5’-GCCGCACCACTGGATCTC-3’
SAT II R-5’-CGTTTACAGCCCACCTCTGT-3

SAT III F-5’-AGGCCTCGTTACTAAAAGTGC-3’
SAT III R-5’-ACCTGACGGCAGAAGGAAT-3’

The mastermix was prepared for 17 samples. PCR cycling conditions were as follows: Initial denaturation 94°C for 3min, denaturation 94°C for 1min, annealing 55°C for 1.5min and extension of 72°C for 1min. The final step in PCR extension was for 4mins at 72°C.

Statistical Analysis

Data on disease prevalence, incidence and severity were subjected to one way Analysis of variance (ANOVA) using Genstat discovery edition software (2005). Mean comparison of the incidence and, severity were done using student t –test at 95% confidence level. ANOVA test was used to determine any significant differences between the means of the three independent variables of CMD, incidence, prevalence and severity.

Results and discussion

CMGs incidence, prevalence and severity based on symptomatology

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 Kenyan 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
	LSD0.05	**6.13**	**34.05**	**0.52**	

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

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

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

CMD was observed in major areas where cassava is grown in Kenya. The disease is widely distributed countrywide with an average incidence of 57% (Table2). Coast province had the highest average CMD incidence (74%) followed by Eastern province recording a mean incidence of 57%. Western and Nyanza province had the lowest CMD incidence of 47% and 51%,

respectively. Overall CMD prevalence was 81% with Nyanza province recording the highest (96%) prevalence followed by coast province with a disease prevalence of 93%. Eastern province had the least disease prevalence of 78.0% and the disease was not detected in Mbeere district (Table 1).

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

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

Means with the same subscripts in the same column denotes no significant differences between the means at p=0.05

Although Coast province had the highest CMD symptom severity (3.4) there was no statistically significant difference between the provinces on disease severity apart from Western province (2.7), Table 2. A mean severity of 3.1 countrywide rather indicates the severe symptoms prevalent in the survey areas. District means were averaged to get the provincial means.

Table 3: Symptom severity of CMD compared to combination of CMD with DNA satellites

District	***CMD Symptom Severity***	***CMD + DNA Satellites Symptom Severity***
Kakamega	2.5	3.1
Teso South	2.8	3.2
Bumula	2.2	2.8
Busia	2.3	3.0
Siaya	2.2	3.1
Rachuonyo	2.4	2.9
Kathiani	2.7	2.8
Makueni	2.6	3.5
Kilifi	2.9	3.2
Kwale	2.8	3.8
Taita	2.6	3.52.

Detection of Cassava mosaic geminiviruses

The PCR product of 556bp was evident as expected for the amplification of the DNA – B with EAB555F/R primers for the detection of EACMV (Fig.3a). For the detection of ACMV, the

expected PCR product of 774bp was realized after amplification of the ACMV coat protein gene by the primer set JSP001/002 (Fig.3b).

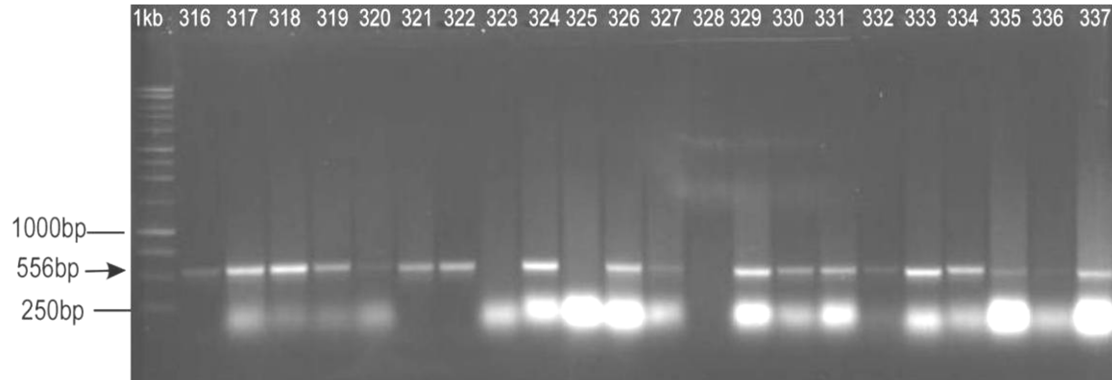

Fig 3a: PCR products (556bp) of East African cassava mosaic virus (EACMV) from infected cassava leaf samples total nucleic acid. The numbers in the gel picture are sample numbers**.**

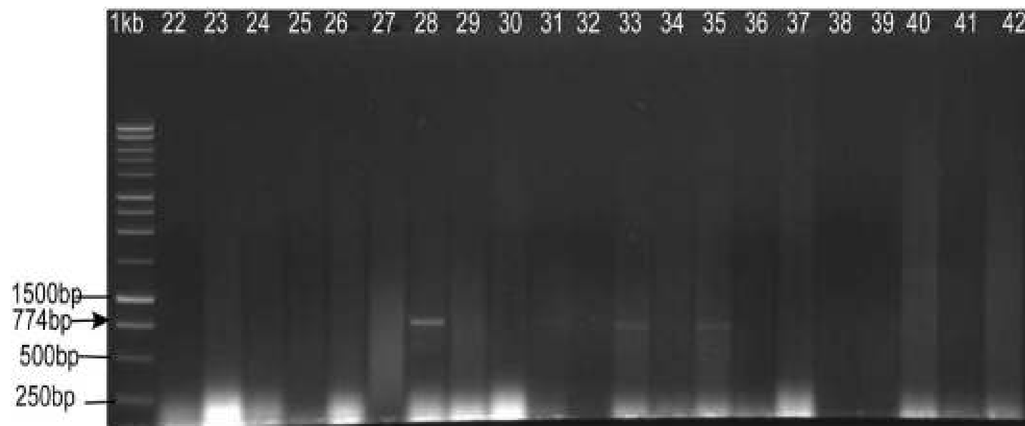

Fig 3b: Amplification of ACMV (coat protein) 774bp expected size. (Annealing temperature of 48°C)

Table 4: Detection for EACMV and ACMV in the four provinces under survey

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

Table 4 summarizes the molecular detection of Cassava mosaic virus amongst the samples collected. ACMV was for the first time detected in Eastern and Coast province. Dual infection of EACMV and ACMV were common in Nyanza, Western and Coast province.
From the PCR-based detection, EACMV was more widespread than ACMV in the country. EACMV occurred in all the provinces surveyed (Table 4). Nearly all the districts under survey showed the presence of EACMV. However, ACMV was mostly prevalent in Western, Nyanza and for the first time in Coast and Eastern province (Table 4). The distribution was not so much intense as EACMV. About 18 out of 61 samples had ACMV constituting 29.5% in Western province. ACMV was recorded only in one sample from Kathiani district in Eastern province. ACMV was detected in leaf samples collected from several fields in Kilifi, Msambweni and Kwale districts of Coast province, an area previously presumed to be ACMV-free. Co-infection of 8% EACMV and ACMV was recorded in field samples collected. Co-infection was more prevalent in Nyanza and Western province and to some extent in Coast province. Teso North, Teso South and Bungoma West districts in Western Kenya had the highest co infection rates of the two viruses.

Survey of CMG's and associated DNA satellites

PCR detection for the CMG DNA Satellites III

The PCR products after the amplification of DNA satellites associated with CMGs and gel electrophoresis were of the expected size of 306 bp (Fig.4). The 1kb molecular marker was used thus perfectly giving the expected PCR product as shown in plate 4. Some samples were negative for the DNA integrated satellites but the majority of the samples collected from the field with typical symptoms of the satellites associating with the CMGs tested positive. The integrated satellites were common amongst the samples collected during the survey. Out the 350 samples collected from the field during the survey, 145 tested positive for the integrated CMD DNA satellites accounting for 41.1%. The episomal DNA satellites for CMD on the other hand were very rare with just a few samples testing positive for the satellites after DNA amplification.

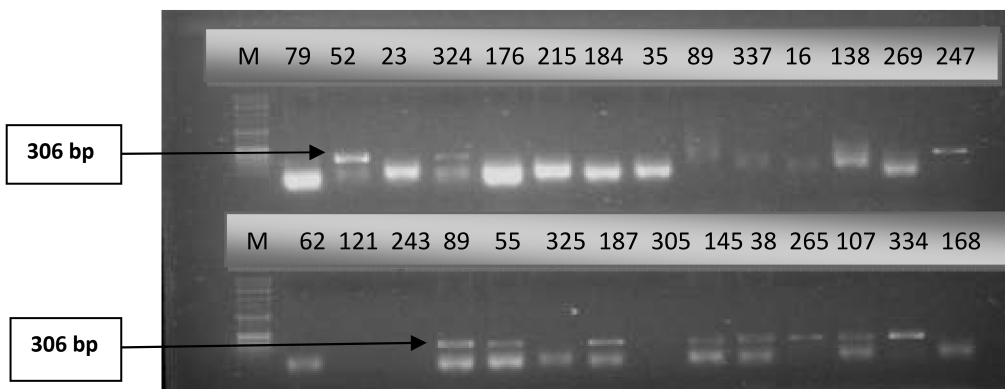

Fig.4: Agarose gel electrophoresis of the integrated DNA satellites specific PCR products of 306bp

Primers used were Sat III F and R. The numbers in the figure are sample numbers.

Whitefly counts and mode of transmission of CMGs

Nyanza region had the highest number of adult whiteflies per plant (3.2) which was not significantly (P=0.05) higher than the population recorded in Coast region (2.9). The lowest whitely population was recorded in Western province (Table 5). There was no significant difference in cuttings and whitefly method of transmission in Eastern and Western provinces.

Table 5: CMD severity, mode of infection and whitefly count per plant in the four provinces

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

The infection due to cuttings is correlated to the high severity symptoms. There is significant difference in white fly infection across the provinces. However, there is no significant difference in cuttings borne infections in Eastern and western provinces. Cutting-borne infection of CMD accounted for 80% compared to the whitefly infection of 19%.

This survey of viruses infecting cassava in Kenya was the most comprehensive to date covering the entire country including Eastern province and the Mt. Kenya region which has not been studied. Yet the plants showing symptoms of cassava mosaic disease were easily identified due to the symptoms they exhibited. Typical symptoms of CMD observed were leaf chlorosis which ranged from pale yellow to white and others were paler that the normal leaf colour. Defined mosaic patterns, leaf malformation and distortion were associated with more severe symptoms of the disease.

CMD was reported in all the major areas where cassava is grown in Kenya. CMD incidence was observed to be highest in Coast province compared to other provinces. Western and Eastern provinces had the least CMD incidence. On the other hand, Nyanza province had the highest CMD prevalence followed by Coast province with Eastern province registering the lowest disease prevalence. A mean severity of 3.1 countrywide indicates the severity of CMD in the surveyed areas is high. However, Coast province had the highest CMD severity (3.4). Farmers in this province indeed expressed the fear that the symptoms are nowadays more severe compared to the recent years. The high CMD severity is due to cuttings infection since the young plants sprout already infected with the virus and therefore they developed the disease earlier than in the whitefly infected plants. The presence of dual infection of ACMV and EACMV together with DNA satellites also contributed to the high severity levels of the disease in Coast. Nyanza province recorded the second most CMD severe symptoms of 3.2 with Western province posting the least severity symptoms of 2.7. CMD was very severe in the late 1980's to early 1990's but the disease severity was greatly reduced due to the introduction of resistant and tolerant varieties by KALRO and the Ministry of Agriculture [18]. The same measures were not taken in Coast and Nyanza districts at that time.

Nyanza province had the highest whitefly count in the country followed by Coast province. It is vividly clear that infection by cuttings is more rampant than that caused by whiteflies. Though whiteflies carry the CMD viruses, the method of transmission through distribution or use of infected cuttings is widespread. This phenomenon has also been observed in Togo [19]. It is quite contrasting for Coast,where the average whitefly count per plant is 2.9 but has 0% infection due to whiteflies. All the plant sampled in coast showed that the method of CMD infection is mainly due to the use of infected cuttings for planting. The same applies to Nyanza province, where the whitefly infection accounts for 11.1% and through infected cuttings accounting for 88.8%. Eastern province had the highest whitefly method of infection at 33.3% but still infection by cuttings is more prevalent at 66.6%.

All begomoviruses code for coat protein which act as the protective coat of the virus particle and determine vector transmutability of the viruses by the whitefly vector *B. tabacii*. Thus, the CP gene is highly conserved among begomoviruses originating from the same geographical region and adapted to transmission by local vector populations [19]. Smaller fragments comprising the core coat protein gene (core CP), a partial 575-579 base pair (bp) sequence of the Coat Protein gene [20], or the complete CP sequence have also been used to establish provisional species identification owing to the highly conserved nature of the viral CP sequence.

The PCR detection of viruses from the samples collected in the nationwide survey showed that EACMV is more widespread than ACMV in the country. EACMV occurs in all the provinces and was distributed across the country. Nearly all the districts under survey showed the presence of EACMV. ACMV was recorded only in Kathiani district in Eastern province, signalling the first recorded occurrence of ACMV species in this region. In Coast province, an area presumed to be free of ACMV reported the presence of ACMV, also for the first time. ACMV was detected in several farmers' fields in Kilifi, Msambweni and Kwale districts which had been presumably been thought to be free from this species of CMV. Previous studies show indeed that EAMCV is the most common species of CMV in Kenya more than ACMV [6]. However, in this study, ACMV was only detected in western and Nyanza provinces, but none in Eastern and Coast provinces.

ACMV and EACMV are synergistically interacting leading to severe symptoms as reported by farmers. The study shows that the method of infection is predominantly due to use of infected cuttings with farmers almost not utilizing any management practices [21]. The same trend was noted with CMD symptom severity where again Coast province recorded the highest symptom severity of 3.36. This observation was amplified by the respondents' interviewed during the survey. The farmers whose fields were sampled expressed that they have known the disease symptoms of the disease and still were able to get some yields. They have also noted that the disease symptoms are now quite severe and that the yields have greatly reduced.The detection of ACMV in Kathiani district and several districts in Coast province present challenges in the management of CMD in these regions. 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 [22].

The DNA satellites associated with CMGs in this study were common across the country in the samples collected during the survey. The integrated DNA satellites were common while the

episomal DNA satellites for on the other hand were very rare with just four samples testing positive for the satellites after DNA amplification. The interaction of the DNA satellites with Begomoviruses leads to different symptoms expression of Cassava mosaic Disease with a likelihood of increasing the disease severity [9]. The leaves exhibiting these symptoms were definitely also having typical symptoms of the cassava mosaic Begomoviruses.

The interaction of the DNA satellites with Begomoviruses leads to enhanced symptom severity of Cassava mosaic Begomoviruses [7]. In this study, the symptom phenotypes modulation by the DNA satellites on the CMGs symptoms was quite evident. DNA satellites species are often associated with geminivirus infection [23].These DNA molecules can either enhance symptoms severity or even ameliorate the symptoms in some cases [24]. The leaves of the plants infected assumed a sickle shape thus distinguishing them from other CMGs infected leaves. It was also established that varieties infected with CMGs and DNA satellites exhibited more severe symptoms compared to the same varieties infected only with CMGs. The effect of the DNA satellites on the quality and yield of cassava is not known. Studies in Sri Lanka show that each of the cassava-infecting geminiviruses showed a contrasting and differential interaction with the DNA satellites, not only in the capacity to interact with these molecules but also in the modulation of symptom phenotypes by the satellites [27].

Conclusions and recommendations

EACMV is more prevalent than ACMV and the two viral species of the CMD are now well mapped in the country. The study has revealed cases of dual infection accounting for 21% of all the samples analyzed for the presence of the virus. The increased symptom severity is attributed to the dual infections of the two CMV species and the combined infection of CMD and the associated DNA satellites as shown in table 3. It is vividly clear that infection by cuttings is more rampant than that caused by whiteflies. Though whiteflies carry the CMD viruses, the mode of transmission through distribution or use of infected cuttings is widespread. Even in the provinces where the whitefly infestation is high, like in Coast province, the dominant mode of transmission of the virus is by infected cuttings. There exist DNA satellite molecules which associate with the viral DNA of Cassava mosaic virus. The symptoms severity score correlated well with the 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.

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 the 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 CMV i.e. ACMV and EACMV since the two species are now characterized. Genetic modification techniques or conventional

breeding techniques can now be tailored to come 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.

There is the 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.

These results call for more detailed analyses of these sub viral components and an investigation of their possible interaction with the cassava mosaic disease complex. There is need to investigate the above mentioned phenomenon with special interest on interaction of the DNA satellites with plants having dual infection of the two species of CMD, ACMV and EACMV.

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