Original Research Article

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

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 amosaic 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: DNA Satellites and Cassava Mosaic Geminiviruses

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 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).

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.

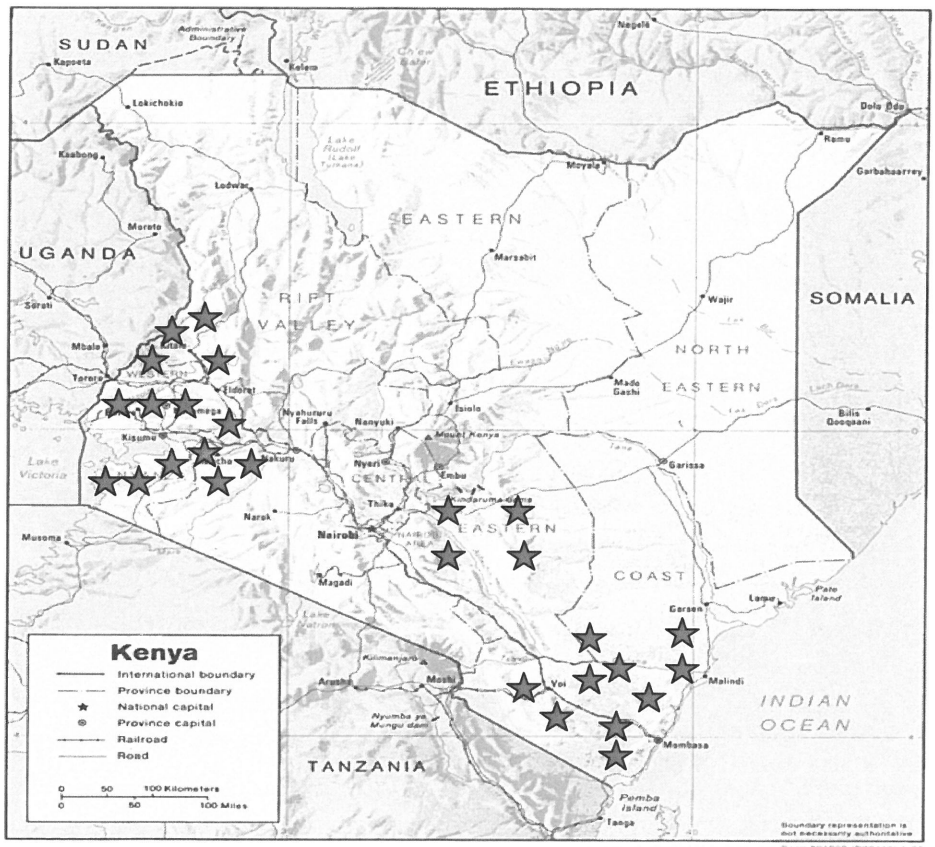

Figure1: ★Areas surveyed for CMGs and associated DNA Satellites

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.

what is satellite symptoms? How do you observe it?

Is it after gDNA isolation and satellite PCR? (See line 307)

? Line 121: 3-4 samples per field vs Line 100 30 plants ?

duplication of line 81-83

Farms or fields having cassava crop as a pure stand or intercropped with other crops were selected and randomly visited along the selected routes within the region. In each region, a particular representative route that captures the area of interest was ~~discussed and agreed upon by the survey team and adopted.~~ selected Amongst issues considered include the sample area and availability of suitable cassava fields. Farmers' fields were selected after every 5 km in densely populated areas due to close proximity of the small scale farms growing cassava such as in Western province and some parts of Coast, Nyanza and Eastern province. 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).

move to line 83-85, it is duplica ion

Cassava plants in farmers' fields were observed for virus disease symptoms. Foliar samples from plants infected by CMD and the DNA satellites 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. In each field, 3 – 4 samples were taken with a total of 350 samples.

move to after line 105

30 ?

Whitefly counts and mode of transmission

On which sample sites were this determined ?

This study determined whitefly counts and also investigated the method of transmission of the cassava mosaic geminiviruses. 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 since the flies become active as the day warms up. This makes it difficult to count the whiteflies in the cassava fields after ~~10 in the morning.~~ 10 am

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 by the letter C.

of CMD

Detection of cassava mosaic geminiviruses in collected samples

Nucleic acid extraction and detection of Cassava mosaic geminivirus

All begomoviruses code for coat protein, which act as the protective coat of the virus particle and determine vector transmitability of the viruses by 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 [14]. Smaller fragments comprising the core coat protein gene (core CP), a partial 575-579 base pair (bp) sequence of the Coat Protein gene [15], 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. Total nucleic acid (TNA) was extracted from the dry leaf samples using the CTAB based method [16]. About 0.03g of the dried leaf samples was ground in 1.5ml of CTAB extraction buffer. About 750µl of the sample was poured into a 1.5ml eppendorf tube and incubated at 65°C for 30min. The samples were then mixed with an equal volume 750µl of chloroform: Isoamyl alcohol (24:1). They were mixed by gentle shaking before being centrifuged at 1200 rpm for 10min. The top aqueous phase was transferred into a new eppendorf tube and an equal volume (750µl) of chloroform:Isoamyl alcohol (24:1) was added, mixed and centrifuged again as in the previous step. 300µl of the top aqueous phase was transferred into a new eppendorf tube and DNA was precipitated by adding

Consider moving to discussion Not M&M

two volumes (600µl) of ice cold isopropanol. The samples were then centrifuged at 8000 rpm for 10min and the resulting supernatant discarded. The pellet was then washed in 0.5ml of 70% ethanol by vortexing and then centrifuged at 8000 rpm for 5min. Ethanol was removed gently and the pellet air dried for 30min. The pellet was suspended in sterilized water and stored at -20°C. The PCR mix consisted of GoTaq green (Promega), 10µl of each primer (Forward primer EAB555F and reverse primers EAB555R) of the template DNA. 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 [17]. The Universal primers used for detection of ACMV were JSP001 (5'-ATGTCGAAGCGACCAGGAGAT-3') ~~ACMV~~ the forward primer and ~~(AV1/CP)~~ JSP002 (5'-TGTTTATTAATTGCCAATACT-3') ~~ACMV (AV1/CP)~~ 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 [17]. The cycling regimes was as follows; the first step (initial denaturation) was at 94°C for 3 minutes, second step was at 94°C for 1min, the third cycle at 72°C and the final cycle at 48°C (annealing) for 1min. The reaction was set for 31 cycles. After the 31st cycle, the PCR reaction tubes were removed from the thermocycler and stored temporarily at 4°C awaiting gel electrophoresis. The PCR cycling regimes were the same as those of EACMV detection. The annealing temperature of 48°C worked perfectly with generation of well amplified DNA bands after agarose gel electrophoresis.

Nucleic Acid extraction for detection of DNA satellites

The CMD viral DNA was also analyzed for the ~~detection~~ of DNA satellites associated with Cassava mosaic geminiviruses. Specific primers designed for the amplification of the integrated and episomal satellites were used in the PCR based detection technique. Nucleic acid extraction was carried out in a similar method as for the testing for CMD.

The primers used for the detection of DNA Integrated satellites which amplify the DNA-B with and expected 306bp PCR product were ;

SAT III F-5'-AGGCCTCGTTACTAAAAGTGC-3'

SAT III R-5'-ACCTGACGGCAGAAGGAAT-3'

The mastermix was prepared with one of the set ups for 17 samples. PCR cycling regimes ~~or program~~ was 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, 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. The t test was used to separate the means.

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 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**	

missing Mean

Table legend

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

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

NB: Why does these value diffe from provnce means in Table 1?

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.

Table 2?

Cassava mosaic disease (CMD) was observed in major areas where cassava is grown in Kenya. The disease is widely distributed countrywide with an average incidence of 57.3% (Table 1). Coast province had the highest average CMD incidence (74.0%) followed by Eastern province recording a mean incidence of 57.6%. Western and Nyanza province had the lowest CMD incidence of 47.0% and 51.0%, respectively. Overall CMD prevalence was 81.6% with Nyanza province recording the highest (96.0%) prevalence followed by Coast province with a disease prevalence of 93.0%. Eastern province had the least disease prevalence of 78.0% and the disease was not detected in Mbeere district (Table 2).

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
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]

Order the same as Table 1

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). 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 2)

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Table 3 summarizes the molecular detection work for 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.

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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**

same order as Table 1 + 2

From the PCR-based detection, EACMV was more widespread than ACMV in the country. EACMV occurred in all the provinces surveyed (Table 3). 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 3). 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. The presence of 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

? 0 → 3 out of 110 samples

line 382 : 21% ?

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

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 (Plate 3). 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.

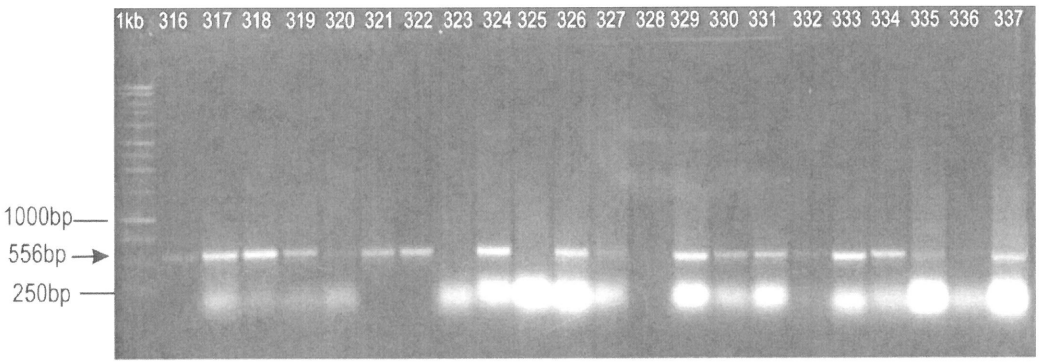

Plate 1:PCR products (556bp) of East African cassava mosaic virus (EACMV) from infected cassava leaf samples total nucleic acid.

Lane 1 is the 1kb DNA marker. The numbers in the gel picture are sample numbers.

PCR detection for the CMD DNA Satellites III

The PCR products after the amplification and gel electrophoresis were of the expected size of 306 base pairs (Plate 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. The interaction of the DNA satellites with begomoviruses leads to different symptoms expression of CMD with a likelihood of increasing the disease severity. This was evident with the same scoring slightly severe symptoms when infected with CMD and the associated DNA satellites.

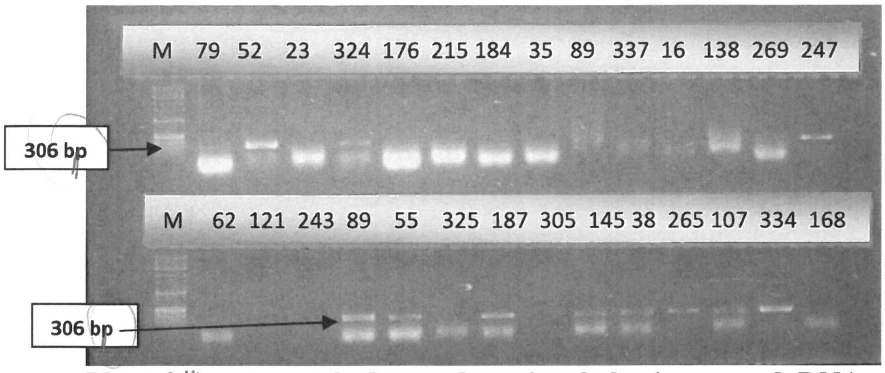

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Plate 2: Agarose gel electrophoresis of the integrated DNA satellites specific PCR products of 30bp

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

Require a heading of whitefly counts / methods of transmission

Nyanza region had the highest (3.2) adult whiteflies per plant 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 4). There was no significant difference in whitefly infestation in coast and Nyanza provinces. Likewise, there was no significant difference in cuttings and whitefly method of transmission in eastern and western provinces.

Table 4: CMD severity, whitefly count, cuttings infection and whitefly count per plant in 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**

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 80.6% compared to the whitefly infection of 19.5%.

Discussion

This survey of viruses infecting cassava in Kenya was the most comprehensive covering the entire country including Eastern province and the Mt. Kenya region which has not been studied. 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. Symptoms of CMD with the associated DNA satellites showed the same symptoms as described but with more leaf distortion assuming a sickle shape.

Cassava mosaic disease 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. 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 KARI 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. This was followed by Eastern province with Western province registering the least whitefly counts per plant. 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 ~~2.99~~ but has 0% infection due to whiteflies. All the plants sampled in coast showed that the method of CMD infection is purely ~~(100%)~~ due use of infected cuttings for planting. The same ~~replicates for~~ 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 there at 66.6%.

The PCR detection 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 CMD. Previous studies show indeed that EAMCV is the most common species of CMV in Kenya than ACMV [6]. However, in this study, ACMV was only detected in western and Nyanza provinces but none in Eastern and Coast provinces.

The DNA satellites associated with CMGs in this study were common across the country amongst the samples collected during the survey. A total of 145 from the 350 samples collected during the survey tested positive for the integrated Begomoviruses DNA satellites accounting for 41.1%. The episomal DNA satellites for CMD on the other hand were very rare with just a few samples showing positive for the satellites after DNA amplification. The interaction of the DNA satellites with Begomoviruses leads to different symptoms expression of Cassava mosaic Begomoviruses 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. It is likely that 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 [20]. 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 [21].

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 [22]. These DNA molecules can either enhance symptoms severity or even ameliorate the symptoms in some cases [23]. 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 .

CONCLUSIONS

EACMV is more prevalent than ACMV and the two viral species of the cassava Mosaic virus disease 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. 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 distribution or use of infected cuttings is widespread. Even in the provinces where the whitefly infestation is high like in Coast, 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.

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.

field → field ?

These field observations of the symptom severity could be extrapolated to field situations in order to hypothesize about the possibility of acquisition of such DNA satellites currently associated with other begomoviruses. 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.

CMG/cmv

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