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Assessment of cassava mosaic disease and field status of cassava begomoviruses in Kenya

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Abstract

Cassava production in Kenya is affected by cassava mosaic disease caused by cassava mosaic begomoviruses (CMBs). This study was carried out in 2013 to determine the status of cassava mosaic begomoviruses in farmers' fields in Kenya. The survey covered major cassava growing regions of Kenya of Coast, Eastern, Western and Nyanza. The survey covered a total of 88 fields in all the areas with the highest Cassava mosaic disease incidence 60.19% being reported in Western region and the least 34.66% recorded in Eastern Kenya. Using polymerase chain reaction (PCR) 5 begomoviruses were reported being present in Kneya namely *ACMV, EACMV, EACMV-KE*, DNA satellites and *EACMV-UgV*. The highest detections of *ACMV* 41.2% were recorded in Nyanza with no detections in Eastern. The peak 26.5% *EACMV-KE* detections were noted in Nyanza and the least 4.3% in Coast. Nyzanza reported the greatest count 70.6% of EACMV and the lowest count 36.6% noted in Western. The highest *EACMV-UgV* count was in Nyanza 41.2%. Sequencing was used to determine the genetic diversity of the cassava begomoviruses detected by PCR. Wide variability was seen for Kenyan *EACMV* isolates as they formed 2 independent groups. However, with regards to *EACMV-KE,* there was little variability forming a single cluster with bootstrap value of 99%. Of concern in the study was the high incidence of cassava begomoviruses in Nyanza region of Kenya, hence the need for efforts to mitigate the problem.

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Introduction

Cassava, *Manihot esculenta* Crantz, provides carbohydrates to about 800 million people in Africa (FAO, 2013). Its also provides income to farmers in addition to being an industrial raw material. The crop cassava belongs to the family Euphorbiaceae growing in subtropicala nd tropical areas. The crop originated from Brazil and was brought to Africa by the Portuguese in the 16th Century (Owor *et al.,* 2005). In the tropics, cassava is considered a staple crop and supplies plenty of carbohydrates (FAO, 2013). The crop is widely grown in Kenya produces an annual yield of metric tons. Production of cassava is hampered by a number of biotic and abiotic stresses. The biotic stresses of major concern in Kenya include cassava mosaic disease (CMD) caused by cassava begomoviruses (CMBs) and cassava brown streak virus(CBSV) (Patil and Fauquet, 2009). Cassava begomoviruses have been in existence for quite some time in Africa being reported in many countries (Swanson and Harrison, 1994; Thresh *et al.,* 1994).cmBs have got a single tranded, bipartite genome made of DNA-A and DNA-B (Kathurima *et al*., 2016). The virus is propagated via infected cuttings and whitefly vector *Bemisia tabaci.* It was first reported in Kenya in. The yield losses due to cassava begomoviruses range from 20-95% with estimated loss of 2.7 billion US dollars (Thresh et al.,1994;Otim-Nape *et al.,* 1994) in Africa. The effects of the disease have been shown to be more pronounced when the disease affects crops at a younger stage. The disease has reached epidemic level in a number of countries that include Tanzania, Kenya, Uganda (Legg and Fauquet, 2004). The disease is accelerated by the use of cuttings as seed and the whitefly vector *Bemisia tabaci* involved in transmission (9).

The cassava mosaic begomoviruses reported in Africa include; East African cassava mosaic virus. *African cassava mosaic virus, East African cassava mosaic Zanzibar virus(EACMZV), East African cassava mosaic Malawi virus (EACMMV), South African cassava mosaic virus (SACMV)* (Fauquet and Stanley, 2003). Infection of these begomoviruses can act singly or in mixed form (Fondong *et al.,* 2000). Due to the role of cassava as a food security crop in sub-Saharan Africa, efforts to mitigate against crop losses due to diseases need to be undertaken.

Designing effective control strategy for the disease requires accurate diagnosis. This study sought to determine the status of CMD and its associated begomoviruses in major cassava growing areas in Kenya to help guide management strategies.

Materials and methods

Survey

The survey was done in 4 cassava growing regions of Kenya in 2013 namely; Eastern, Western, Nyanza and Coast. A total of 88 fields were sampled and in each field 30 plants were observed along two diagonals. The location of the farms was taken by global positioning system (GPS). The survey was conducted randomly along motorable roads at 5-10 km intervals. ThecmD severity, incidence and prevalence were assessed in the fields using a severity scale of 1-5 1 = asymptomatic plants, 2 = plants with 25% of leaves showing mild chlorotic pattern or mild distortion, 3 = infected plants with 50% exhibiting moderate mosaic pattern, narrowing and distortion at base of the leaves, 4 = infected plants with 75% exhibiting severe mosaic symptom, leaf distortion and general reduction of leaf size, and 5 = infected plants with 100% of plants exhibiting severe mosaic, leaf distortion, reduced leaf size, vein clearing and in most cases stunted growth (Sseruwagi *et al*., 2004). Infected and healthy leaves were collected each field kept in silica-filled containers lined with cotton wool and taken for further analysis. Incidence was determined by finding the percent between the total infected plants and the total number of infected plants (Mohammed *et al* (24). Disease prevalence was determined by the percentage of infected fields to the total sampled fields in each region. Survey locations are shown in (Fig. 3.1) below.

Detection of cassava begomoviruses

Extraction of DNA from collecetd leaves followed CTAB method with some modifications (Chang *et al*., 1993). The DNA pellet obtained was dissolved in 50 ul TE buffer. Virus amplification from the DNA was done by specific primers (Table 1).

A reaction mix composed of 0.2mm dNTPs, 0.2mmmgCl_2, 0.2 µl taq polymerase, 0.25 µM forward and reverse primers, 10 µl water and 1 µl of template DNA, 2.5 ul 10x reaction buffer was constituted. Then the mixture was placed in a 96 well Thermocycler where virus amplification occurred. The following Thermocycler amplification conditions were used; initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension of 72°C for 10 min. Separation of PCR products was done via gel electrophoresis on 1.2% agarose stained with ethidium bromide. Viewing of the resultant gel was done using UV trans illuminator.

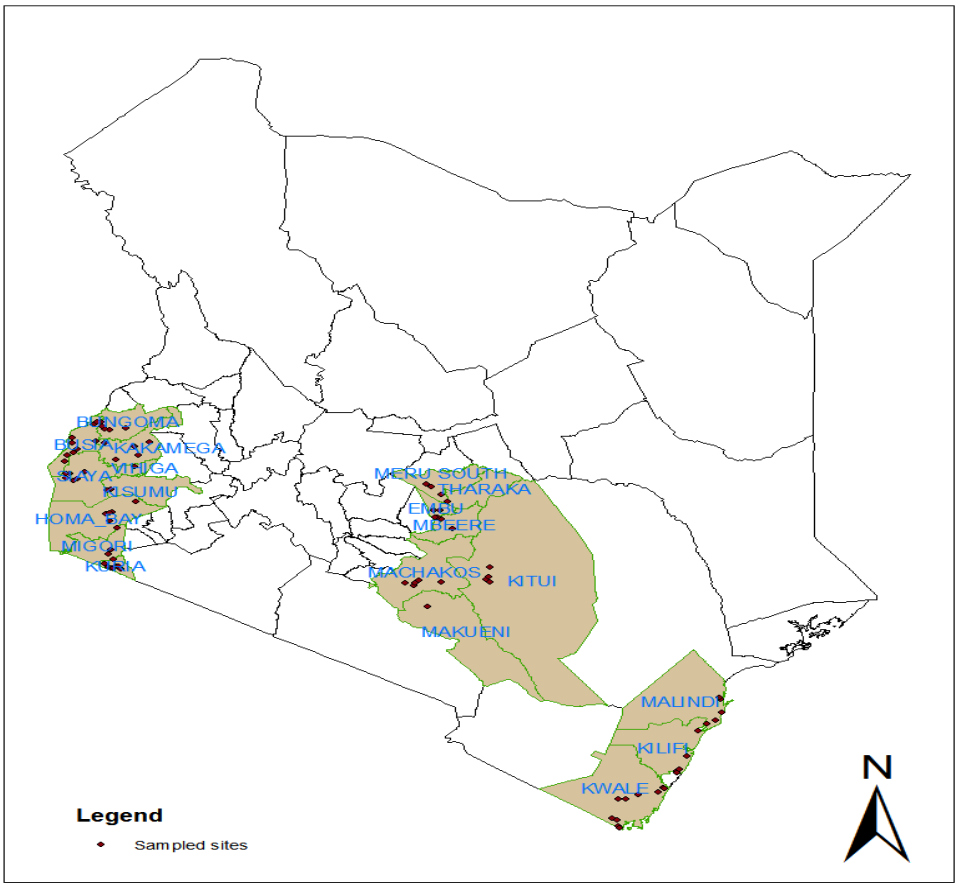

Fig. 3.1 Map of Kenya showing cassava mosaic sampling sites.

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

Primer	Sequence	Virus species	Target
JSP001	ATGTCGAAGCGACCAGGAGAT	ACMV	CP
JSP002	TGTTTATTAATTGCCAATACT	ACMV	CP
EAB555F	TACATCGGCCTTTGAGTCGCATGG	EACMV	DNA-B
EAB555R	CTTATTAACGCCTATATAAACACC	EACMV	DNA-B

(Adapted from Aloyce *et al.,* 2012).

Genetic diversity of cassava mosaic begomoviruses

Positive samples for the different detected begomoviruses; EACMV, EACMV-KE and DNA satellites sequenced. The obtained sequences were then deposited in Gene bank and aligned to available published sequences obtained via the Basic Local Alignment tool (BLAST) search tool in the National Centre for Biotechnology Information database (NCBI

Results

Status of CMD in farmers field

Cassava mosaic disease was assesed using disease incidence and prevalence. The Western region of Kenya was reported to have the highest incidence of 55.5% followed by Coast 47.1%, Nyanza 43.3% and the least 47.1% being reported in Eastern. OOn the other hand the gretaest disease prevalence 90% was seen in

Nyanza folwed by Western 89.5%, Coast 88.9% with the least 58.5% being reported in Eastern.

Record of cassava mosaic begomoviruses

Using primer pair EAB555F/EAB555R (Fig. 3.2), EACMV present in different regions of Kenya were amplified with the highest detection 70.6% occurring in Nyanza, 69.6% Eastern, 67.5% Coast and the least 36.6% in Western Kenya (Table 2). Similarly detection of ACMV was done using primer pair JSP001/002 with Nyanza region reporting highest detection of 29.4% followed by 22.44% Coast (Fig. 3.3), with no detections of ACMV being recorded in Eastern. Primer pair EACMKV1/EACMKV2 was used to detect EACMV-KE begomovirus strains in Kenya(Fig. 3.4). Similar to ACMV, Nyanza region had the highest EACMV-KE detection of 26.5% followed by 5% in Eastern, 4.3% in Coast with no detections being reported from Western region. Also detected in this study were DNA III satellite viruses that were highest in Nyanza at 39.7%, Western 15.9%, Eastern 6% and the least in Coast 1%

Table 2. Cassava begomoviruses detected from four major cassava growing regions of Kenya.

Province	Total	EACMV	EACMV-KE	ACMV	EACMV-UGV
Nyanza	68	*48(70.6%)	18(26.5%)	20(29.4%)	28(41.2%)
Western	82	30(36.6%)	0.0	11(13.4%)	11(13.4%)
Eastern	46	32(69.6%)	2(4.3%)	0.0	11(23.9%)
Coast	80	54(67.5%)	4(5%)	18(22.5%)	12(15%)
Total	276	86(31.2%)	24(8.7%)	49(17.8%)	62(22.5%)

* Numbers outside the brackets represent actual samples testing positive for the viruses.

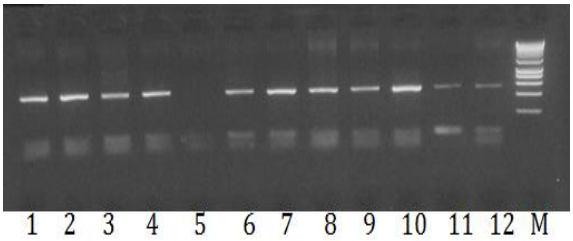

Fig. 3.2. PCR product (556bp) profile showing EACMV amplification using primer pair EABF/R from infected cassava leaves; M is molecular marker of size 1kb.

Lanes 1-12: DNA extracts from field samples collected in Kenya.

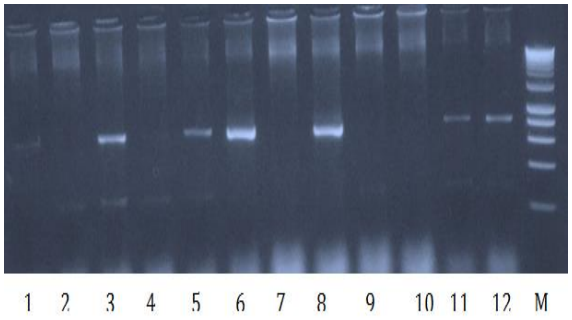

Fig. 3.3. PCR product (774bp) profile showing ACMV amplification using primer pair JSP001/JSP002 from infected cassava leaves; M is molecular marker of size 1kb.

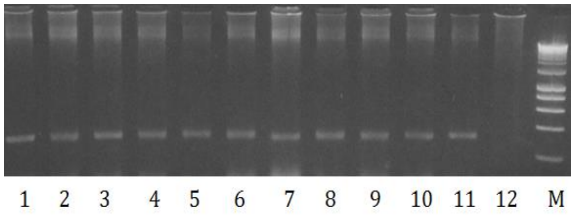

Fig. 3.4. PCR product (306bp) profile showing DNA satellite amplification from symptomatic cassava leaves; M is molecular marker of size 1kb.

Genetic diversity of cassava begomoviruses

The EACMV isolates from Kenya clustered in 2 groups; minor and major clades. The clustering was not based on region of isolate origin with siolates from similar regions clusering differently in the phylogenetic tree(Fugure 3.5). Homology studies of the Kneyan isolates with those present in NCBI database showed that the Kenyan isolates found in the major clade were related to sequences from Uganda (HE979780.1, AJ704958.1 and AF126805.1).

An independent minor clade was formed by 2 isolates named Odiado 1 and 2. The EACMV-KE isolates did not show high genetic variability and formed an independent clade with a boostrap value of 99% (Fig. 3.6). On comparison to isolates from NCBI database from Uganda, Ghana, Cameroon and Ivory-Coast they

showed no correlation. With regards to DNA III satellite genetic diversity, three independent clades for the Kenyan isolates were identified. Isolates from different locations such as Tagari in Nyanza, Kibaoni in Coast, Luriba in Western and Lwala from Nyanza formed a similar cluster (Fig. 3.7).

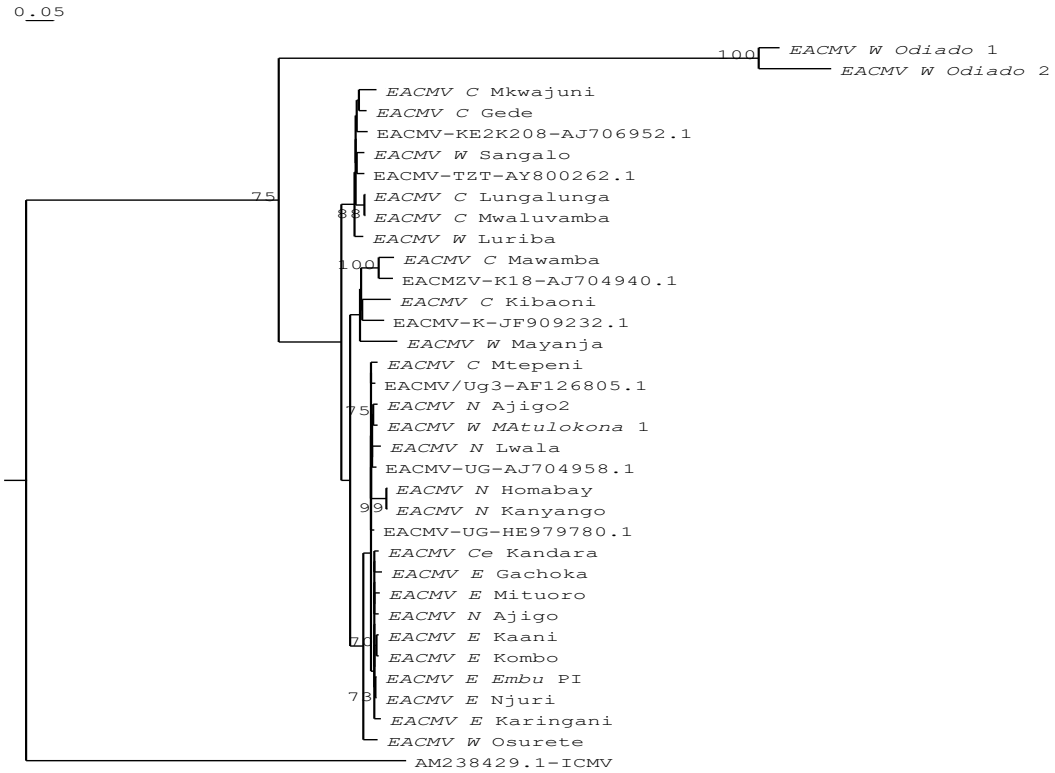

Fig. 3.5. Phylogenetic tree based on EACMV partial CP nucleotide sequence of isolates collected from Nyanza, Coast, Western and Eastern regions of Kenya obtained by MEGA 5 software program.

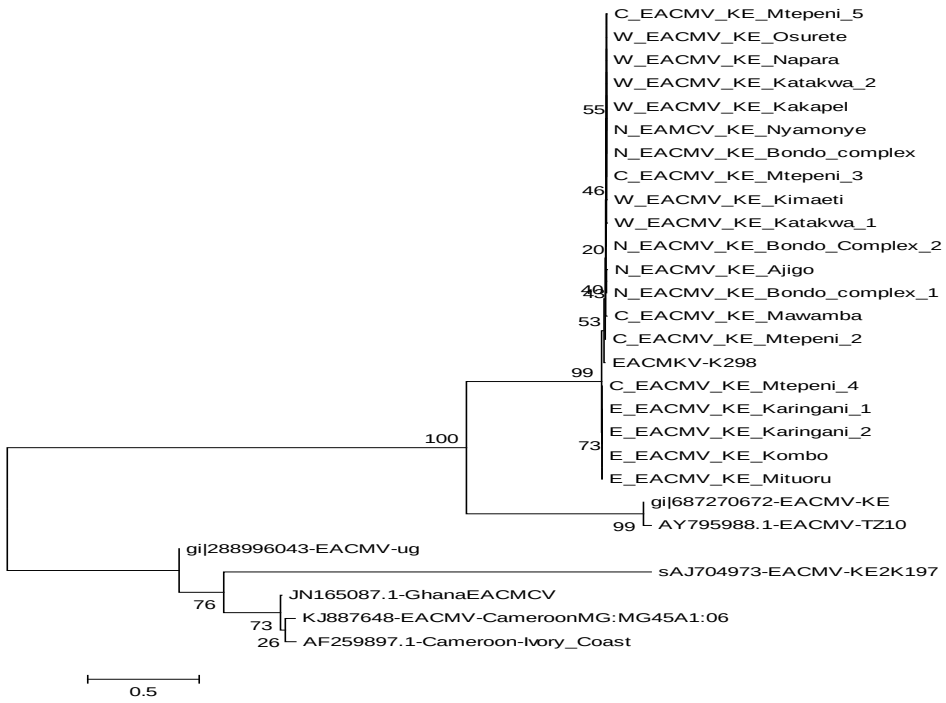

Fig. 3.6. Maximum likelihood tree of base alignment of a partial CP of isolates of *East African Cassava mosaic Kenyan virus* (EACM-KE) using MEGA 5 software.

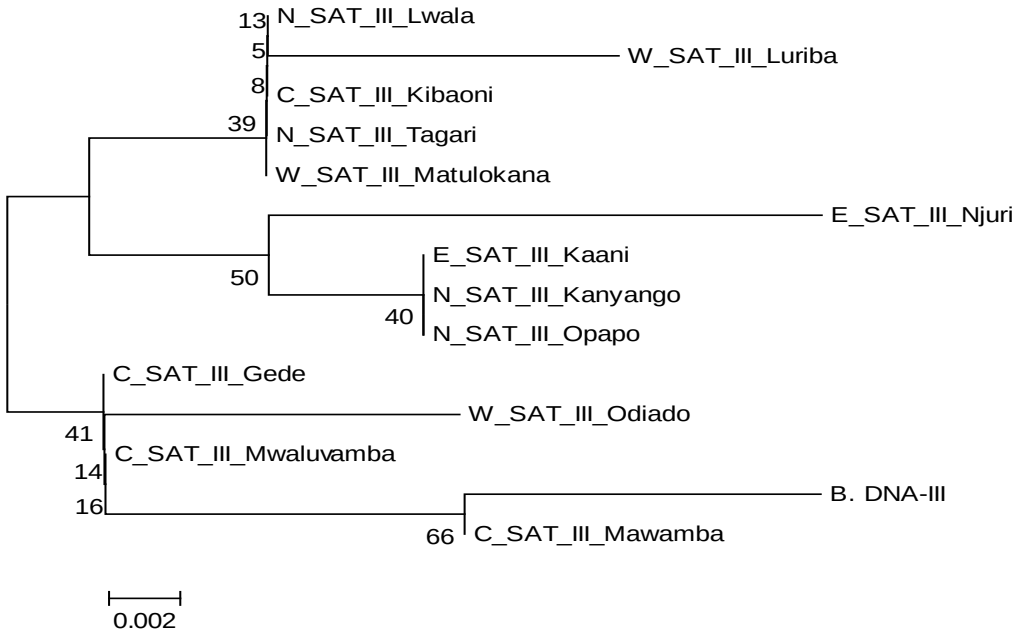

Fig. 3.7. Phylogenetic tree obtained from the alignment of DNA satellites sequences from major cassava growing regions of Kenya with those available in the NCBI database using MEGA 5 software.

Discussion

The African continent is prone to attack by different begomoviruses. Nyanza and Western regions reported the greatest disease prevalence. These findings agree with Mwaituni *et al.* (2015) who reported high prevalence of the disease in Nyanza region. 5 begomoviruses were prevalent in major cassava growing regions of Kenya namely; ACMV, EACMV, EACMV-UV, EACMV-KE and DNA satellites. This is in agreement with previous studies that have shown presence of the begomoviruses in different locations in Kenya (Mwaituni *et al.*, 2015). Two of the CMBs EACMV and EACMV-UG were found located in all cassava growing regions. However the highest detection of these 2 begomoviruses were in Western and Nyanza region. These areas have different climatic conditions and altitude. It has been reported that there is a high incidence of EACMV in East Africa (Tembo and Sseruwagi, 2019). In Madagascar, ACMV has been shown to be high in Central highlands whereas EACMV was seen to be high in lowlands (Harimalala *et al.*, 2015). Coat protein sequences for EACMV analysis showed that they cluster in to 2 groups with those from Odiado forming a sole cluster. Previous similar studies in Togo revealed that the EACMV isolates clustered into two groups with one cluster having isolates similar to those from Uganda and another group of isolates similar to the Kenyan isolates (Adjata *et al.*, 2008b). With regards to EACMV-KE, there was no genetic variability amongst the Kenyan isolates as they grouped together with bootstrap values of 99%. According to the international committee of virus taxonomy rules, virus isolates with 90-99% homology are similar (Bull *et al.*, 2006). The EACMV-KE isolates were similar to those from Tanzania as compared to those from Ghana, Cameroon and Uganda.

Conclusion

Cassava mosaic begomoviruses have been reported to infect all cassava growing regions of Kenya. The greatest effect was found to be in the Nyanza where severe infections with all the begomoviruses were reported. This is a concern for policymakers on how to reverse the effects of the disease in the regions. Management options such as use of resistant varieties, use of clean seed, elimination of whitefly vectors can be adopted to reduce the impact of the disease. Amongst the begomoviruses the EACMV-KE had the lowest detections among the regions. With regards to diversity, begomoviruses did not cluster according to region of origin with isolates from the same region being found in different clusters in the phylogenetic tree.

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