PREVALENCE OF CASSAVA BROWN STREAK AND CASSAVA MOSAIC DISEASES IN LAMU COUNTY KENYA

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ABSTRACT

Demand for cassava is rapidly increasing due to its resilience and ability to mitigate livelihood insecurities among the smallholder farmers as a result of climate change. Despite its importance, cassava production has been declining in Sub-Saharan Africa compared to other parts globally. This is largely due to surge in cassava brown streak viruses (CBSVs), cassava mosaic geminiviruses (CMGs) and their vector, *Bemisia tabaci.* This has also led to loss of traditional landraces harbouring useful genes vital for future cassava breeding. Of the two diseases, CBSVs is the most devastating causing root necrosis. Emergence of dual infection of CMD and CBSD causes 100% yield losses, economically more than US$1 billion yearly. The overall objective of this study was to determine viral disease incidences among local landraces from farmers' fields in Lamu County (2.2355° S, 40.4720° E) through a multistage sampling technique. The study targeted farmers growing local landraces at regular pre-determined intervals of 30 km between farmer's farms. Fifty-eight plant cuttings and leaf samples were randomly collected. Molecular diagnostics done on the leaf samples detected 39.2% single infection of East Africa cassava mosaic virus (EACMV), 3.6% single infection of Ugandan cassava brown streak virus (UCBSV), 10.7% mixed infection of EACMV, UCBSV and cassava brown streak virus (CBSV). African cassava mosaic virus (ACMV) was not detected in all tested samples. The study identified the need for dual resistant breeding as the only sustainable management strategies of the two major viral diseases of cassava in the county.

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Key words: Cassava Brown Streak Disease, Cassava Mosaic Disease, landraces, UCBSV, EACMV

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a clonally propagated root crop through stem cuttings. It is a drought resistant crop, grown by small-scale farmers with low farm inputs. It has the potential to store food reserve in the roots underground for a long-term, maintaining steady supply of food in times of scarcity (Thresh, 2006). Following climate change, pests and diseases of cassava have rapidly increased especially the cassava viral diseases which have now developed into serious disease pandemic transmitted by use of diseased stem cuttings as planting materials (Legg *et al.,* 2015) thus, limiting cassava productivity. Cassava productivity has been mainly constrained by cassava brown streak disease (CBSD) and cassava mosaic disease (CMD). Additionally, CSBD has been identified to be the most limiting viral disease of cassava (Mohammed *et al*., 2012) in Sub-Saharan Africa. Cassava plants affected by CBSD exhibit brown streak lesions on the stem, root constrictions and necrosis on the starchy edible part of the root rendering it inedible and unmarketable (Winter *et al*., 2010). CMD is found in almost all the cassava growing regions in Sub-Saharan Africa (SSA), and the disease is characterized by chlorosis and wavering leaf margins (Hong *et al*., 1993; Swanson and Harrison, 1994). CMD and CBSD are spread by the whitefly (*Bemisia tabaci*) vector, and planting of diseased cuttings (Storey and Nicholas, 1938; Storey, 1936). Mixed infections of the two diseases often occur in the same plant across farms causing 100% yield losses and economic loss of more than US$1 billion yearly (Elegba *et al.,* 2020). Since the first outbreak of CBSD in 2000 at medium altitudes (>1000m) in Uganda (Alicai *et al*., 2007), western Kenya

(Masinde *et al*., 2016) and northern part of Tanzania (Legg *et al*., 2011), CBSD is now widely spread to other countries in Central and East Africa (Alicai *et al*., 2016; Mulimbi *et al*., 2012 Bigirimana *et al*., 2011;). In those countries where CBSD has established, it has been reported to be the most limiting viral disease of cassava causing high yield losses in cassava production. So far, there are no effective management strategies development against CBSD in SSA. Thus, there is need to improve farmer's knowledge of epidemiology of CBSD and CMD and awareness on CBSD and CMD dual infection alert in areas where cassava is grown. The objective of this study was to quantify incidences and the distribution of both CMD and CBSD in Lamu County in Kenya.

MATERIALS AND METHODS

Collection of the cassava germplasm

Cassava germplasm was collected from Lamu County situated between latitude 1° 40° 15' and 2° 30' South and longitude 40° 15' and 40° 38' East.. The county is characterized with a flat topography, a condition that makes it susceptible to floods during rainy seasons and high tides. It is endowed with arable land suitable for agriculture. Annual temperatures are high ranging from 24 to 34 °C (MoPD, 2013). This study focused on Lamu West constituency/sub-county which is the major cassava growing region of the county. Fifty-eight stem cuttings and leaves of cassava germplasm were collected from farmers' fields in Lamu County from five major cassava growing Wards namely Witu, Mkunumbi, Hindi, Hongwe and Bahari of Lamu West Sub- County, Lamu County. The sampled leaves were conserved in silica gel carried to Kenya Plant Heath Inspectorate Service (KEPHIS) Muguga for DNA extraction.

DNA Extraction and PCR for the EACMV and ACMV detection

Genomic DNA was extracted according to Lodhi *et al.* (1994), CTAB extraction buffer made up of 2% (w/v) CTAB, 1.4 M NaCl, 0.2% (v/v) 2- mercaptoethanol, 20 mM EDTA, 100 mM Tris - HCl, pH 8.0) was preheated at 65 °C for 10 min.

Using a leaf punch, 100mg of apical leaves were sampled from each of the germplasm and ground into fine powder in liquid nitrogen. Approximately 1ml extraction buffer was added to the ground samples and incubated at 65 °C for 15 minutes (mins.), shaking at an interval of 5 mins. The extract was mixed with 750 ml of chloroform: isoamyl alcohol (24:1); vortexed briefly, centrifugation at 12,000 rpm for 10 mins. at 4 °C. The supernatant solution (500 ml) was transferred into a new micro-centrifuge tube. Thereafter, 0.6 vol. (300 ml) cold Isopropanol was added into a new tube and incubated at -20 °C for at least 10 mins. Centrifugation of the samples was done at 13,000 rpm for 10 mins at 4 ºC. The supernatant was then decanted. The DNA acid pellet was washed in 700 ml of 70% ethanol by vortexing and incubating at -20 °C for at least 10 mins. Finally, centrifugation for 5 mins was conducted at 13,000 rpm and suspended in sterile molecular water and stored at 4 °C.

To detected EACMV and ACMV, extracted cassava leaf DNA was amplified by standard PCR using EACMV -A2469s +EACMV- A391s specific primers for EACMV (Ferguson *et al*. 2020); ACMV- AL1/F+ACMV- ARO/R for ACMV (Zhou *et al.,* 1997). The samples were amplified using the stated primers. The PCR products were resolved on 1 % agarose gel stained with 1µl of GelRed (Biotium, USA) and run at 85 V for I h in 1x Tris-Acetate-EDTA (TAE) buffer (pH: 8). UV light was used to visualize the gel. The gel images were taken using gel documentation system (Azure Biosystem c-200). Amplified band sizes were used for identification of EACMV and ACMV species.

Two - Step PCR

The primers used were UCBSV-CP-F (Shirima *et al.* 2017), UCBSV-CP-R and UCBSV-PROBE (Adams *et al*. 2013); for CBSV CBSVQ-F, CBSVQ-R and CBSVQ-P (Adams *et al*. 2013). The extracted DNA samples were treated with DNase1 enzyme to digest contaminating DNA as per manufacturer's instructions (Sigma). The purity and quality of the extracted RNA was determined by NanoDrop 2000 spectrometer (Thermos scientific) and used for synthesis of complementary DNA (cDNA).

The cDNA was synthesized from 1µg of RNA sample and the remaining RNA samples were stored at -80 °C for future use. Reverse transcription was carried out in 20µL reaction made up of 2µL total RNA,0.5mM each dNTPs (Thermo Scientific), 50mM oligo(dT18), 1x M-MLV buffer (sigma), 200 units M-MLV reverse transcriptase

(RT) (NEB) and molecular water was used to top up to 20 L.

Mixture of only dNTPs, oligo dT18and total RNA was initially heated at 70 °C for 5 mins and immediately cooled on ice before addition of 1x M-MLV buffer (sigma) and 200 units of M-MLV reverse transcriptase (RT) (Sigma). This total master mix reaction was incubated at 25 °C for 5 minutes, 42 °C for 60 min followed by deactivation using M-MLVRT at 80 °C for 3 mins. In this reaction, PCR water devoid of RNA and reverse transcriptase was used as the negative control.

The final master mixes comprised of 2 µL cDNA, 1xPCR buffer (sigma), 2mM MgCl2 (Sigma), 200 µM dNTPs (thermos Scientific), 0.04 U/µl Taq DNA Polymerase (Sigma) and either 200 M (each of UCBSV-CP-F, UCBSV-CP-R and UCBSV-Probe) or 250 M (each of CBSVQ-F, CBSVQ-R and CBSVQ-P). PCR was programmed as follows: initial denaturation at 95 °C for 1 mins, 35 PCR cycles of 95 ^{0}C for 30 seconds (s), annealing step at 53 °C for 1 min, elongation 72 °C for 1 min and a final extension step at 72 °C for 10 mins (Shirima *et al*. 2017; Adam *et al*. 2013).

The PCR products were resolved on 1.5% agarose gel stained with 1µl of Gel Red (Biotium) and run at 85 V for I h in 1x Tris-Acetate-EDTA (TAE) buffer (pH: 8). Gels images were visualized under UV light and images captured using gel documentation system (Azure Biosystem c-200) to confirm presence or absence of UCBSVs and CBSVs.

Real Time RT-qPCR for CBSD causing viruses

Primers used for amplification of CBSV and UCBSV are stated above and described by Adam *et al.* (2013) and Shirima *et al.* (2017). Two master mix were made (CBSV and UCBSV) with the final concentrations of 1 x PCR buffer, 5.5 mM MgCl2, 0.5 mM dNTPs, 0.2 µM of each primer, 0.1 µM probe, 0.025 U/µl Taq DNA polymerase and 0.4 U/µl MMLV-reverse transcriptase (NEB). Sterile distilled water was used as negative control while TME 4 as positive control for CBSD causing viruses. RNA from sampled plants were used to confirm the results obtained from two step PCR by generating amplification curves for UCBSV and CBSV. The Real-Time PCR program was set at 42 °C for 30 min for the reverse transcription step followed by 95 °C denaturation for 10min. The cDNA synthesis was followed by 40 cycles of 15 s at 95 °C and a 1 min long combined annealing/elongation step at 60 °C. The reactions were carried out using realplex2 (Eppendorf) (Adam *et al*., 2013).

Data was statistically analysed by analysis of variance (ANOVA) with 5% level of significance, using Genstat (Lawes Agricultural Trust, Rothamsted Experimental Station 2006, Version 15).

RESULTS

DNA was successfully extracted from the leaf materials of all the 58 cassava plants randomly collected from farmers' fields in Lamu County using 2% CTAB protocol as explained above.

Among the 58 tested samples by conventional PCR for cassava mosaic geminiviruses (CMGs) causal viruses, incidences of EACMV were more prevalent at 39.2% with 22 plants testing positive. In all the 58 tested plants, ACMV was not detected (Figure 1a and Figure 1b).

Two step-PCR screening for CBSV and UCBSV in cassava plants across Lamu.

Among the 58 tested samples by RT-PCR, 9 plants were positive for CBSD causal viruses. Incidences of CBSD causal viruses were greater as a single infection of UCBSV than CBSV. Five plants ((9% incidences) that was positive for UCBSV was also positive for EACMV indicating dual infection of CBSVs and CMBs. These results concur with the findings of Koima *et al*. (2018) and Irungu (2011). This dual infection was more prevalent than mixed infections of UCBSV, CBSV and EACMV (2% incidences). One plant was positive for UCBSV, CBSV, and EACMV indicating some possible mixed infections of more than two viruses in a single plant. Figures 2a and 2b show CBSVs and UCBSVs positive and negative samples, respectively.

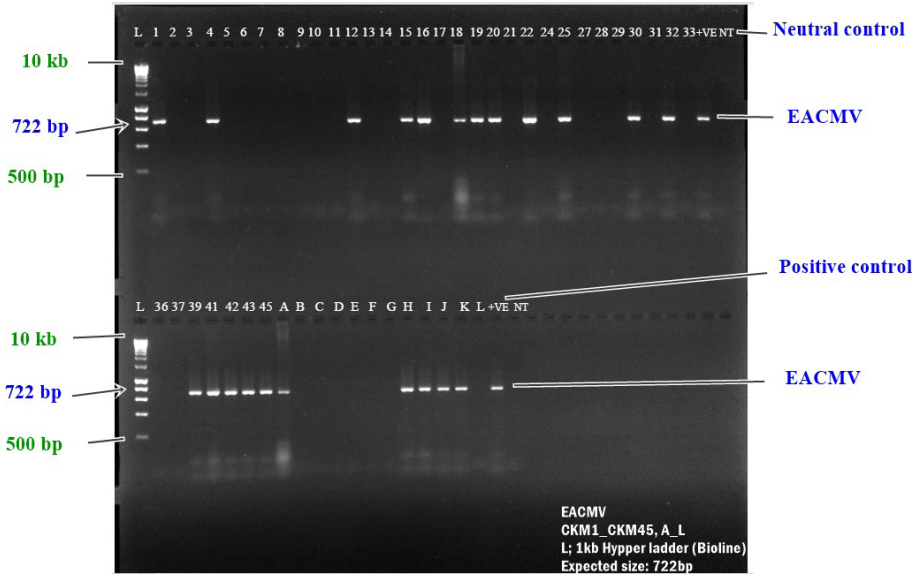

Figure 1a. PCR amplicons from the 58 leaf samples showing positive and negative samples amplified with EACMV primer, L, Molecular weight ladder (Hyper ladder 1 Bioline ranging from 50 base pairs (bp) to show the band of interest. Lane1 to lane L are the samples from farmers' fields in Lamu County.

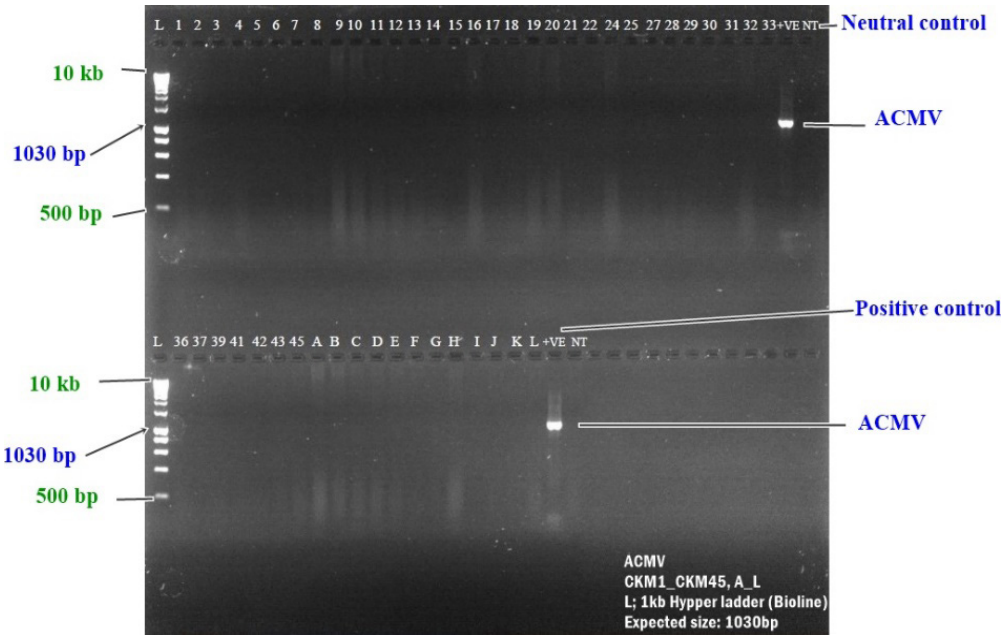

Figure 1b. PCR amplicons from the 58 samples showing positive and negative samples amplified with ACMV primer, L, Molecular weight ladder (Hyper ladder 1 bioline ranging from 50 base pairs (bp) to show the band of interest. Lane1 to lane L are the collected samples from farmers' fields in Lamu County.

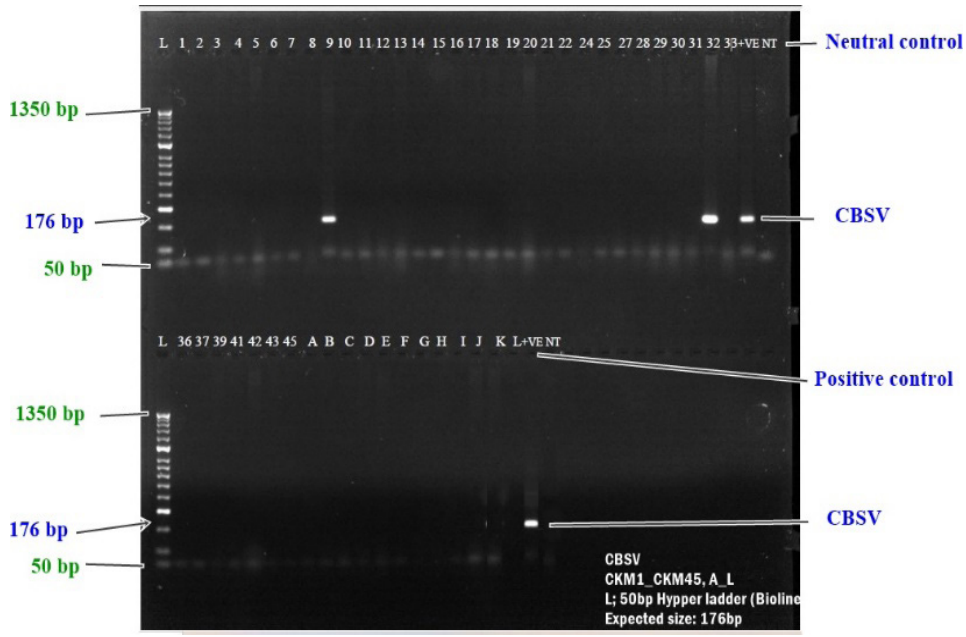

Figure 2a. Diagnostic RT-PCR for CBSV results from the 58 leaf samples showing positive and negative samples amplified with CBSV primer, L, Molecular weight ladder (Hyper ladder 1 bioline ranging from 50 base pairs (bp) to show the band of interest. Lane1 to lane L are the collected samples from farmers' fields in Lamu County.

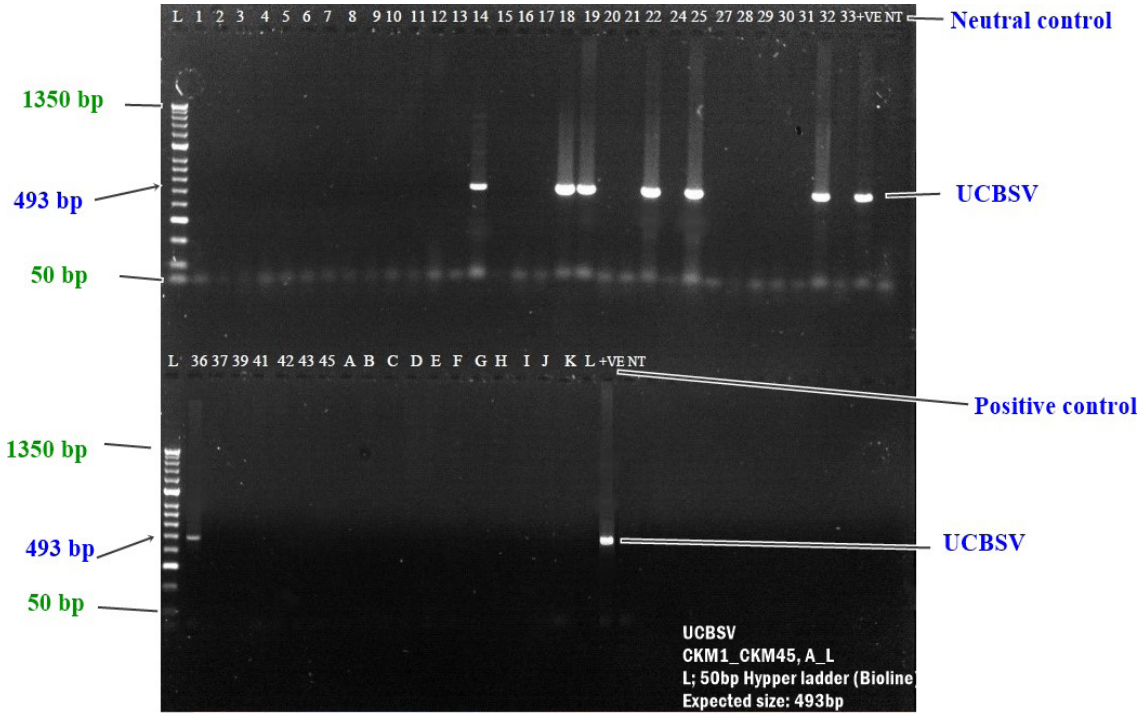

Figure 2b. Diagnostic RT-PCR for UCBSV results from the 58 leaf samples showing positive and negative samples amplified with UCBSV primer, L, Molecular weight ladder (Hyper ladder 1 bioline ranging from 50 base pairs (bp) to show the band of interest. Lane1 to lane L are the collected samples from farmers' fields in Lamu County.

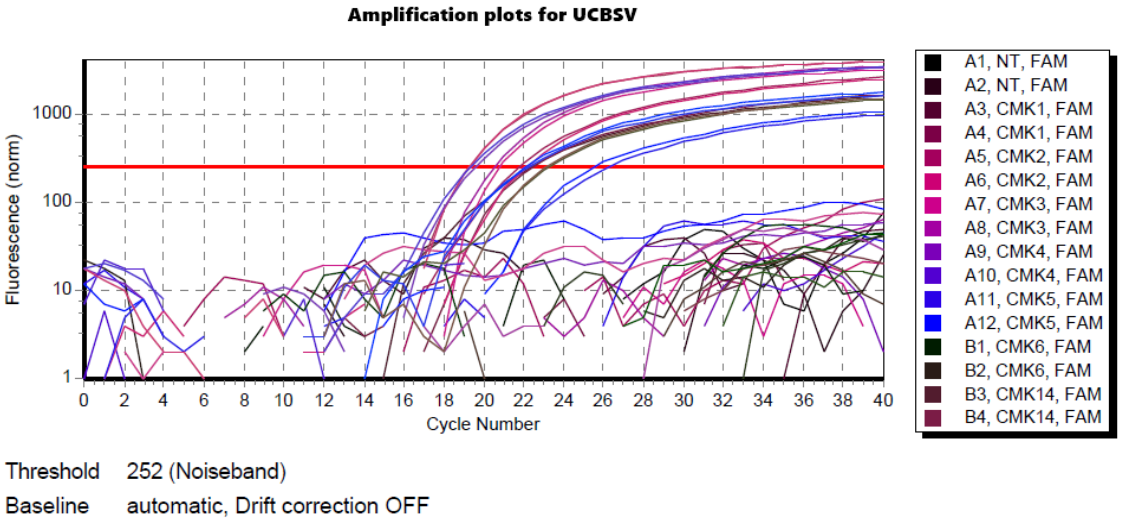

Figure 2c: RT-PCR amplification plots for CBSV

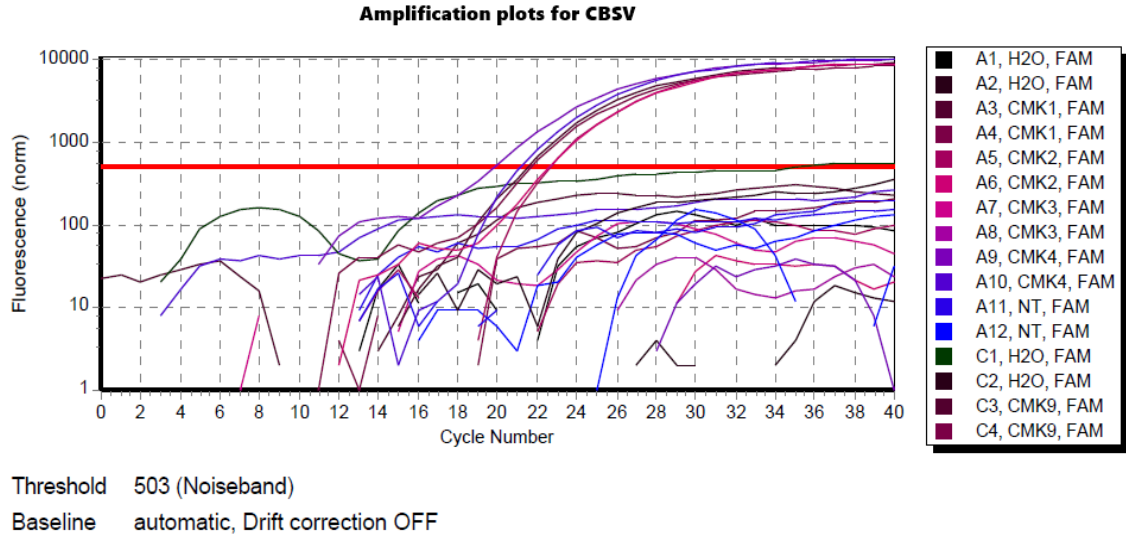

Figure 2d: RT-PCR amplification plot for UCBSV

Statistically, incidences of EACMV were more significant at a p-value of 0.009, followed by UCBSV at a p-value of 0.026. CBSV was not significant.

DISCUSSION

Molecular analysis of leaf samples detected high prevalence of EACMV causing CMD than CBSV and UCBSV causing CBSD. From the results, ACMV was not amplified. The results showed incidences of CBSD causal virus was greater as single infections of UCBSV which were prevalent than single infections of CBSV in Lamu county. Dual infection incidences of UCBSV with CBSV were lowest. The findings of this study are in agreement with the findings in Muhindo *et al.* (2020) and Casinga *et al.* (2019). However, these results are contrary to the previous findings by Hillocks *et al*. (2002) which showed CBSV to be restricted to lowlands areas below 300m above sea level. The reason for wide distribution of

UCBSV relative to CBSV still remains unclear.

In general, CMD is widespread in all cassava growing farms in Lamu County owing to high prevalence of EACMV. This agrees with various studies which have reported CMD to be widely spread in all cassava growing areas in Africa, Kenya included. Incidences of CMD and CBSD causal viruses from the farmers' fields in Lamu County can be attributed to the high exchange rate of diseased cassava cuttings as planting materials from neighbours and other farmers, which concurs with the findings of Maruthi *et al.* (2017); Legg *et al.* (2014); and Jeremiah (2014).

CONCLUSION

Following single and mixed infections of EACMV, UCBSV and CBSD detected from the screened samples, this study suggests urgent need to develop dual resistance cultivars as the only sustainable strategy to control the spread, severity of CBSD and CMD in farmers' fields (Legg *et al.,* 2014) and ultimately improve cassava production.

Although dissemination of quality planting material is key to success of cassava production, sustainable cassava seed systems must be restructured, set in place to ensure rapid multiplication and disseminate of quality planting materials including the developed improved resistant varieties to farmers. This will prevent the spread of cassava viruses in planting materials and reduce food insecurity but also create livelihood resilient from effects of climate change. There is great need for CMD and CBSD control strategies in Lamu County. The results from this study could be incorporated in future of virus resistance cassava breeding.

ACKNOWLEDGEMENTS

The authors thank World Bank through the Kenya Climate Smart Agriculture Project for funding this research and the Lamu West Sub County staff/authority for identifying the farmers and the cassava farmers for allowing the researcher to collect the cassava materials from their farms.

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