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Status of cassava mosaic disease and whitefly population in Zambia

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Cassava mosaic disease is the most important disease affecting cassava in Zambia. A study was conducted through a survey to determine the status of cassava mosaic disease incidence, severity and whitefly abundance in farmers' fields in six provinces: Lusaka, Northern, North-Western, Luapula, Eastern and Western between March and May 2014. The study reveals that cassava mosaic disease incidence was highest in Lusaka (70.0%) and Eastern (69.2%) and lowest in Luapula (45.1%) and Northern (48.5%) provinces. Disease symptom severity was moderate to severe in Lusaka (3.48) and Eastern (3.14) and low in the rest of the provinces. Adult whitefly (*Bemisia tabaci*) populations were highest in Western Province (2.71) and lowest in Luapula Province (0.02). Polymerase chain reaction results using specific primers for African cassava mosaic virus and East African cassava mosaic virus detected single infections of African cassava mosaic virus and East African cassava mosaic virus in 67.9 and 6.8% of the positive reactions, respectively. Dual infections of African cassava mosaic virus and East African cassava mosaic virus were detected in 25.6% of the samples tested. Cassava brown streak virus was not detected in any of the samples and no symptoms suggestive of cassava brown streak disease were observed in the surveyed fields.

Key words: Disease, incidence, severity, whitefly.

INTRODUCTION

Cassava is one of the most important root crops in Africa. It is a staple crop in many African countries including Zambia, Mozambique, Tanzania, Malawi, Democratic Republic of Congo (DRC) and Nigeria. The crop is widely grown in the tropical regions in Africa, Asia and Latin America; and cultivated mostly by smallholder farmers. Production of cassava varies from country to country with varying yields. In Zambia, the average yield is around 4.9 t ha^{-1}, which is below that of Africa at 11.1 t ha^{-1} (FAOSTAT, 2015).

Cultivation of cassava in Africa and Zambia in particular, is constrained by a number of biotic factors of which diseases are the most important. The diseases include cassava mosaic disease (CMD), cassava anthracnose disease (CAD), cassava bacterial blight (CBB) and cassava brown streak disease (CBSD). CMD

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Table 1. Primer pairs used for amplification of cassava mosaic begomoviruses.

Primer	Sequence (5' - 3')	Specificity	Strand	DNA component
JSP001[a]	ATGTCGAAGCGACCAGGAGAT	ACMV	Sense	AV1/CP
JSP002[a]	TGTTTATTAATTGCCAATACT	ACMV	Antisense	AV1/CP
EAB555/F[a]	TACATCGGCCTTTGAGTCGCATGG	EACMV	Sense	DNA-B
EAB555/R[a]	CTTATTAACGCCTATATAAACACC	EACMV	Antisense	DNA-B
UV-AL1/F[b]	TGTCTTCTGGGACTTGTGTG	EACMV-UgV	Sense	AV1/CP
ACMV-CP/R3[b]	GCCTCCTGATGATTATATGTC	EACMV-UgV	Antisense	AV1/CP

[a]Primers used for the study are as described by Fondong et al. (1998) and [b]Zhou et al., 1997.

and CBSD are the major diseases of economic importance. CMD is caused by several distinct whitefly transmitted viruses [African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus (EACMCV), East African cassava mosaic Zanzibar virus (EACMZV), East African cassava mosaic Malawi virus (EACMMV), East African cassava mosaic Kenya virus (EACMKV) and South African cassava mosaic virus (SACMV)] (Bull et al., 2006). Two other species have recently been described: African cassava mosaic Burkina Faso virus (ACMBFV) and Cassava mosaic Madagascar virus (CMMGV) (Tiendrébéogo et al., 2012).

The viruses exhibit diverse infection dynamics in terms of symptom expression, progression, recovery, severity, and as well as host range (Bull et al., 2007; Patil and Fauquet, 2009). The genome of each of the viruses consists of two subgenomic DNA components, DNA-A and DNA-B. DNA-A and DNA-B, each of about 2.8 kbp (Stanley et al., 2004), with different roles in the infection process. DNA-A encodes genes responsible for viral replication [AC1 (*Rep*), and AC3 (*Ren*)], regulation of gene expression [AC2 (*Trap*)] and particle encapsidation [AV1 (*CP*)] while DNA-B encodes two proteins, BC1 (*MP*) and BV1 (*NSP*) involved in cell-to-cell movement within the plant, host range and symptom modulation (Stanley et al., 2004).

Co-infections of EACMV and ACMV in cassava have been reported in Zambia, Nigeria and Tanzania (Chikoti et al., 2013; Harrison et al., 1997; Ogbe et al., 2003). Of the two viruses, ACMV was reported to be the most predominant and widely distributed wherever cassava is grown in Zambia (Chikoti et al., 2013). With the reemergence of severe form of CMD in East Africa, a new virus strain, the East African cassava mosaic virus Uganda variant (EACMV-UgV) was described (Zhou et al., 1997). Since EACMV-UgV has been reported to occur in DRC, Angola (Kumar et al., 2009), and Tanzania (Ndunguru et al., 2005), the virus is moving southwards towards the border with Zambia. The rapid spread of EACMV-UgV from Uganda to some of the Southern Africa Development Community (SADC) countries such as Tanzania and DRC necessitated this study. Therefore the study was carried out to give an update on the CMD incidence, severity and whitefly population in Zambia.

MATERIALS AND METHODS

Study area

The study was conducted in six provinces: Luapula, North-Western, Northern, Lusaka, Western and Eastern. Luapula, Northern and North-Western are located in agroecological region III, a high rainfall area which experiences rainfall above 1000 mm per year. Eastern, Western, and Lusaka provinces are located in agroecological region II and experiences rainfall between 800 to 1000 mm per year.

Sample collection and propagation

The cassava stem cuttings were collected from plants expressing CMD symptoms from farmers' fields. Plants expressing mild and severe symptoms and from the same variety were targeted. Collection of samples from the field was done through walking in the field using an 'X' pattern. A total of 30 plants were assessed per field, 15 from each diagonal. The whiteflies were also counted from the top most fully expanded leaves from the same plants assessed for disease symptoms. The stem cuttings were labeled and geocoordinates (latitude, longitude and altitude) taken using a global positioning system (Garmin, etrex, summit HC). The collected stem cuttings were transferred to Zambia Agricultural Research Institute (ZARI), Mt. Makulu Research Station and planted in the screenhouse at temperatures between 20 and 30°C. The stem cuttings were planted in insect proof screenhouse in two liter black polythene bags and watering was done when necessary. Monocrotophos insecticide was applied weekly using a knapsack sprayer to control cassava mealybug, cassava green mite and whitefly.

DNA extraction and PCR

Total plant DNA was extracted from cassava leaves expressing CMD symptoms using the Dellaporta method (Dellaporta et al., 1983). Fresh leaf samples were ground with mortar and pestle and virus specific primers (Table 1) were used to detect ACMV, EACMV and EACMV-UgV in 278 virus isolates collected during the survey. Polymerase chain reaction (PCR) was performed using a thermorcycler (Technen 500). PCR conditions were 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and an extension cycle of 10 min at 72°C. The PCR products were visualised by electrophoresis in 1% agarose gel in TAE buffer (10 mM tris-acetate, 1mM NaEDTA, pH 8.0).

Data analysis

Disease incidence was determined as the proportion of diseased

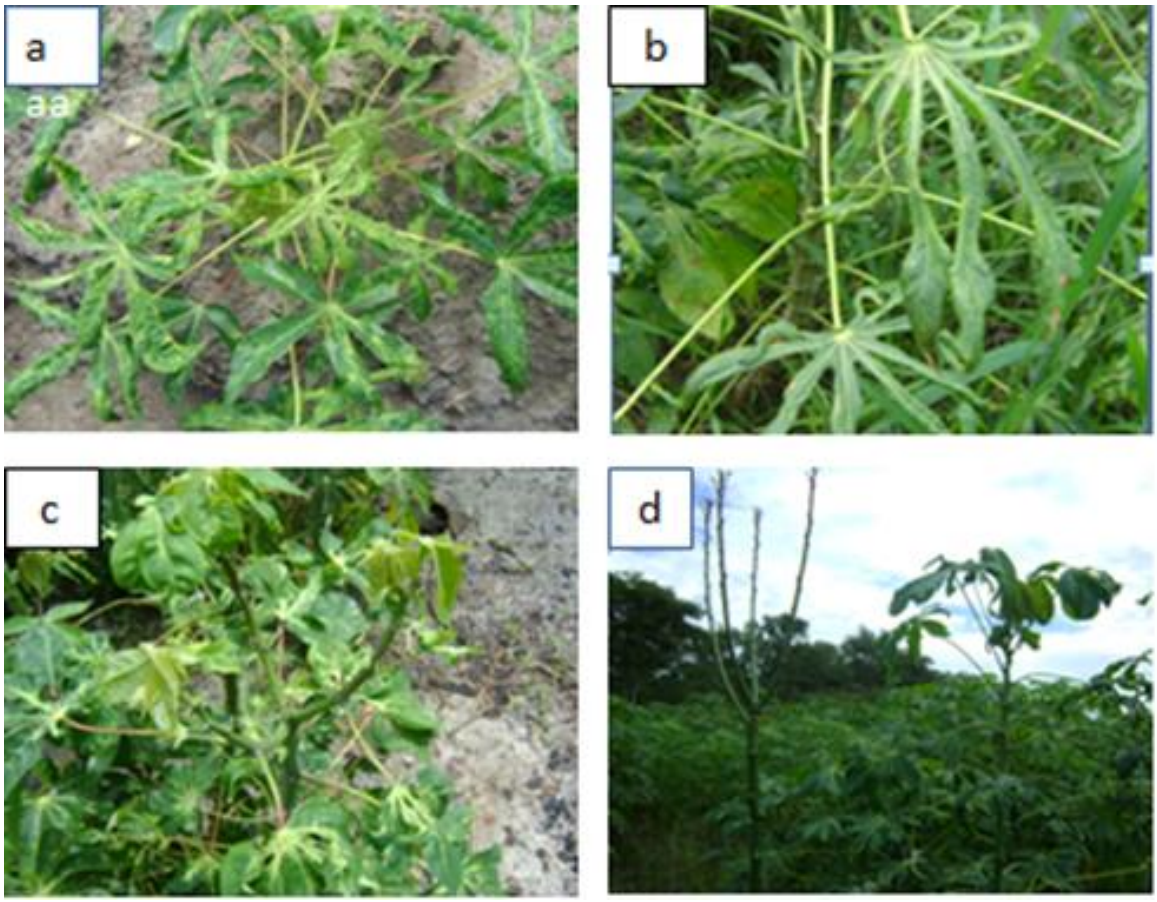

Figure 1. Cassava mosaic disease symptoms. **a)** Light green and yellow patches. **b)** Leaf narrowing. **c)** Leaf curling. **d)** 'Candle stick' symptoms on the left with a normal plant on the right.

plants expressed as a percentage of total number of plants observed per field. Disease symptom severity data were edited to remove plants which showed no symptoms (score 1) and the analysis conducted for the CMD-infected plants (score 2 to 5) per field. Means for incidence, severity and whitefly counts were separated using a one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS). Geo-coordinates (longitude, latitude and altitude) data were used to map the geographical distribution of the cassava viruses using DIVA GIS version 7.5.

RESULTS

Assessment of CMD symptoms

From a total of 245 cuttings collected, 237 were successfully established in the screenhouse. The cassava stem cuttings reproduced similar symptoms observed in the field. Mild and severe CMD symptoms were observed in the surveyed farmers' cassava fields (Figure 1). Mild symptoms comprised patches of yellow and green sectors with less leaf distortion while plants with severe symptoms had deformed leaves and showed stunted growth. Plants with "candle stick" symptoms were also observed (Figure 1).

Cassava mosaic disease severity and incidence

Cassava mosaic disease severity differed significantly ($P<0.044$, $F=2.7$, $df=5$) among the surveyed provinces and averaged 2.95. Lusaka (3.48) and Eastern (3.14) provinces recorded the highest disease severity. The lowest (2.79) was recorded in Northern and Western provinces (Table 2). There were no significant differences for disease incidence among the six provinces surveyed. However, in some fields in Lusaka and Western provinces disease incidence was almost 100%.

Adult whitefly population

Although there were no significant differences in the mean adult whitefly population, Western (2.71) and North-Western (1.16) provinces recorded the highest whitefly population. The lowest (0.02) was recorded in Luapula Province. In the two provinces which recorded high whitefly population, sooty mould was also observed on the middle and lower leaves of healthy and CMD infected plants (Figure 2).

Table 2. Incidence, symptom severity and whitefly populations on cassava crop in six provinces (March/May 2014).

Province	Incidence (%)	Mean of symptom severity (scale 1-5)[a]	Mean of adult whitefly population
Lusaka	70.0	3.48	0.26
Luapula	45.1	2.88	0.02
Northern	48.5	2.79	0.04
North-Western	58.4	2.87	1.16
Western	62.7	2.79	2.71
Eastern	69.2	3.14	0.67
Mean	57.4	2.95	0.89
P-value (5%)	0.487	0.044	0.092
F-statistic	0.995	2.700	1.336

[a]Scale (1-5) 1, no symptoms observed; 2, mild chlorotic pattern over entire leaflets or mild distortion at the base of leaflets only with the remainder of the leaflets appearing green and healthy; 3, moderate mosaic pattern throughout the leaf, narrowing and distortion of the lower one-third of leaflets; 4, severe mosaic, distortion of two thirds of the leaflets and general reduction of leaf size; 5, severe mosaic distortion of the entire leaf.

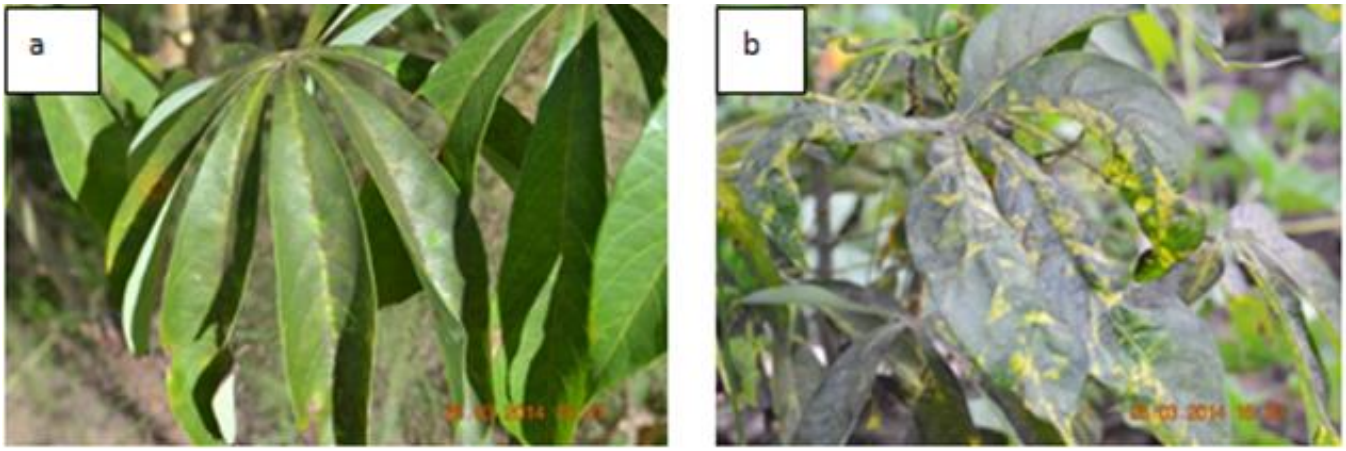

Figure 2. Sooty mould on (a) healthy CMD free plant and (b) CMD infected plant.

Detection of cassava mosaic begomoviruses

PCR amplification products were observed for all the cassava mosaic geminivirus isolates tested using JSP001/2 and EAB555F/R primers. DNA-A (744 kbp) and DNA-B (544 to 560 kbp) partial fragments were amplified by PCR using primers JSP001/2 and EAB555-F/R (Figure 3). There was no amplification for EACMV-UgV, CBSV and UCBSV isolates.

Distribution of virus species in Zambia

A total of 237 samples were analyzed by PCR and of these 219 (92.4%) partial fragments of 774 bp (DNA-A AV1/CP) for ACMV and 556 bp (DNA-B) for EACMV were amplified. Single infections of ACMV occurred in 67.6% (148/219) of the samples, while EACMV occurred in 6.8% (15/219). Mixed infections of ACMV+EACMV were detected in only 25.6% (56/219) of the positive samples (Table 3).

ACMV was widely distributed in all the provinces in which the survey was conducted. Co-infections of EACMV and ACMV occurred in all the provinces except for North-Western Provinces (Figure 4). Single infection of EACMV was detected in Lusaka, Northern and Eastern provinces.

DISCUSSION

This study constitutes the most current survey of cassava mosaic disease in Zambia. The survey was carried out between March and May 2014 in six provinces (Lusaka, Northern, North-Western, Luapula, Eastern and

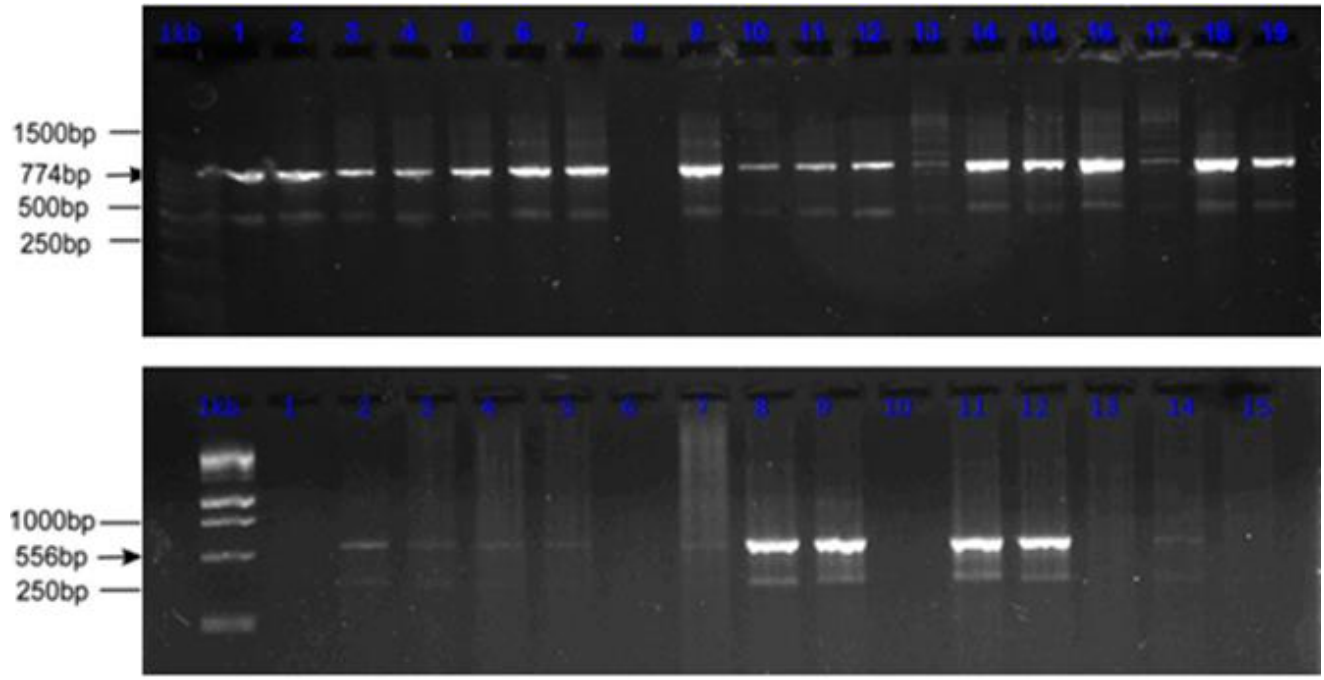

Figure 3. PCR amplification of coat protein of ACMV (774 bp) and EACMV (556 bp) from cassava samples using specific primers JSP001/2 and EAB555F/R, respectively and a DNA 1Kb Ladder

Table 3. Distribution of cassava virus isolates in the six provinces of Zambia.

Province	Number of samples	ACMV[a]	Cassava mosaic begomoviruses	
			EACMV[b]	ACMV + EACMV
Lusaka	15 (6.8%)	7 (4.7%)	2 (13.3%)	6 (10.7%)
Luapula	14 (6.4%)	12 (8.1%)	0 (0.0%)	2 (3.6%)
Northern	52 (23.7%)	25 (16.9%)	3 (20.0%)	24 (42.9%)
North-Western	10 (4.6%)	10 (6.8%)	0 (0.0%)	0 (0.0%)
Western	71 (32.4%)	69 (46.6%)	0 (0.0%)	2 (3.6%)
Eastern	57 (26.0%)	25 (16.9%)	10 (66.7%)	22 (39.3%)
Total	219	148 (67.6%)	15 (6.8%)	56 (25.6%)

[a]Africa cassava mosaic virus. [b]East Africa cassava mosaic virus.

Western). Severe and mild symptoms were observed in all the areas visited. The presence of severe symptoms could be as a result of planting susceptible materials by the farmers. Few farmers have adopted improved varieties bred by the Zambia Agriculture Research Institute which are resistant to CMD. Other reasons could be recycling of seed by the farmers and as a result of virus co-infections. Co-infection with ACMV and EACMV has been reported to cause severe symptoms due to synergistic interactions (Fondong et al., 2000). In Lusaka and Eastern provinces, incidence and severity were high as evidenced from the results obtained. 'Candle stick' symptoms which are characteristic of plants infected with EACMV-UgV were observed. What caused the candle stick appearance in the field calls for further investigations.

The adult whitefly population in the current survey averaged 0.89 slightly higher than what was reported by Chikoti et al. (2013) which reported 0.64 whiteflies per shoot in a survey of 2009. However, the whitefly numbers are lower compared to what has been reported in East and West Africa (Legg and James, 2005). This could be attributed to the long dry climatic conditions which Zambia experiences for most of the year unlike in East and West Africa which experience bimodal rainfall pattern. Climatic factors have been reported to have significant effects on whitefly population (Legg, 1994).

In the current survey, Luapula and Northern provinces

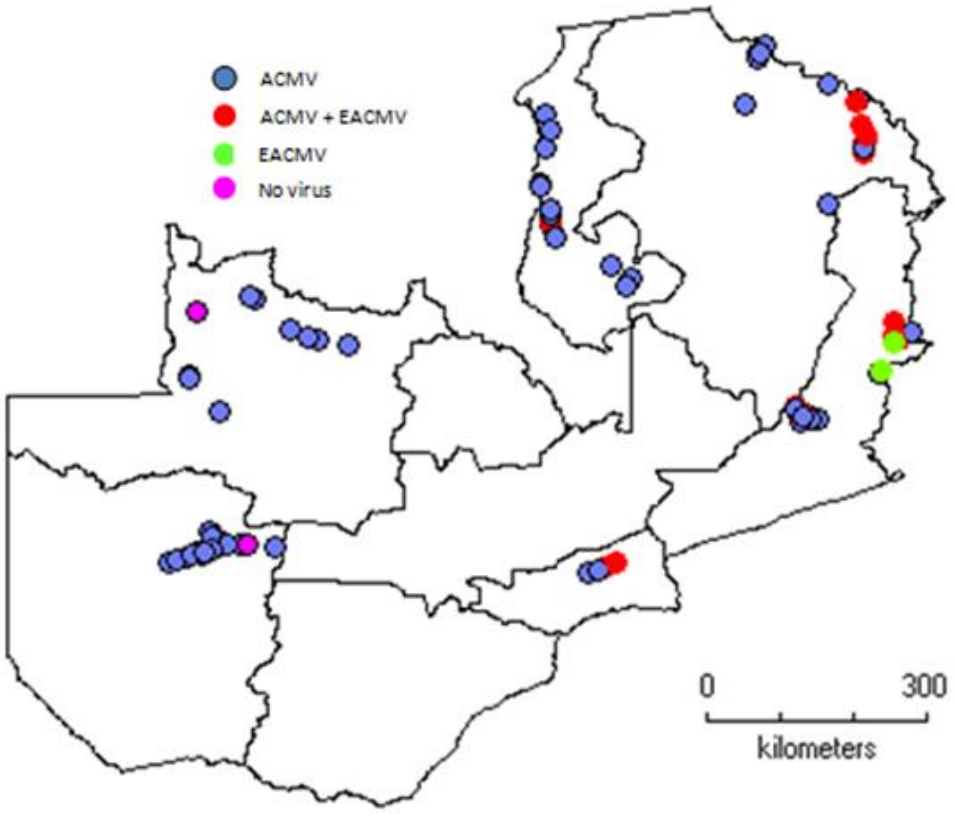

Figure 4. Distribution of cassava mosaic begomoviruses isolates in the surveyed provinces.

recorded the lowest whitefly population compared to what has been observed in previous studies (Chikoti, 2011). In Western and North-Western provinces, high whitefly populations observed in some fields is similar to earlier surveys by Muimba-kankolongo (1997). The high whitefly population in these provinces is not clearly understood. However, we think that it could be due to different whitefly biotypes that exist in these ecologies.

The presence of sooty mould was indicative of high whitefly populations particularly in Western, North-Western and some parts of Eastern Province. Sooty mould is not common in Zambia unlike in East Africa (Omongo et al., 2012). The large whitefly populations has effects on cassava plants; direct feeding damage by whiteflies and the production of honey dew, which falls onto the lower leaves. This is subsequently colonised by black sooty mould (Bellotti and Arias, 2001; Legg et al., 2004), which reduces the ability of the leaves to photosynthesize and contributes to yield losses.

It is apparent from the results of this study that single infections of ACMV were more common compared to those of EACMV. Previous studies (Ogbe et al., 1997) have also reported high frequency of single infections of ACMV compared to EACMV. Similarly, co-infections were also lower than infections of ACMV alone. This could be due to farmers leaving out severely infected plants when selecting cuttings for planting. The occurrence of co-infections in all the provinces surveyed except North-Western Province could be fueled by movement of infected cassava planting materials which are not tested for virus presence in the country.

Our study confirms the occurrence of EACMV as single infections in Western and Eastern provinces as previously reported (Chikoti et al., 2013). However, absence of single infection of EACMV in Luapula Province could be that the fields which had the virus could have been missed when the study was conducted. Detection of ACMV and EACMV in dual infected plants and single infection (EACMV) in Northern Province suggests that the occurrence of the two viruses (ACMV and EACMV) is wide spread than previously thought. These findings agree with previous studies (Were et al., 2003) which reported the presence of the viruses in the province.

Candle stick symptoms synonymous with EACMV-UgV (Alabi, 2011) were observed in the fields. However, the Ugandan variant was not detected in all the samples collected from the surveyed areas. The absence of EACMV-UgV from the current and earlier surveys confirms that Zambia is still free of the Ugandan variant. The fact that EACMV-UgV was not detected in samples from the surveyed areas is not surprising given the long

distance between Zambia and the Democratic Republic of the Congo (northern neighbour) where the virus has been reported to occur (Bulubulu et al., 2015). Most of the land between the common borders of the two countries is covered by forests and is sparsely populated.

It was observed from this study that disease incidence has increased to 57.4% from the previously reported 41.0% in the 1996 study (Muimba-Kankolongo et al., 1997). This could be that farmers are still recycling infected planting materials. The increase in whitefly population in Western and North-Western province could have contributed to the increase in the levels of disease incidence. In the 1990s during the pandemic in East Africa, high whitefly populations were seen as the driver of high CMD incidence. In Uganda, high whitefly population has been observed to increase CMD incidence (Sserubombwe et al., 2005). The decrease of the severity disease scores in Luapula, Northern, North-Western, and Western provinces in this study compared to previous survey (Chikoti et al., 2013) could be attributed to the farmers selecting health cassava planting materials. There have also been campaigns by agriculture extension agents, non- governmental organizations (NGOs), and Research Institutes sensitizing and encouraging farmers to use disease free planting materials.

In conclusion, our study has shown that CMD is still widely distributed in most of the cassava growing provinces. We have established the existence of ACMV and EACMV in single and coinfections in Northern Province. This study demonstrates that ACMV is still wide spread and it also highlights the urgent need for more information. The high incidence recorded in this survey is also of concern given that farmers mostly obtain cassava cuttings for planting from own fields or their neighbours which are not tested. Therefore, there is need to sensitize institutions involved in distribution of cassava planting materials to test them before selling them to the farmers. Future efforts should also aim at characterizing the cassava mosaic begomoviruses in the country in order to have a clear understanding of the existing types of strains.

Conflict of interests

The authors did not declare any conflict of interest.

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