Status of cassava mosaic disease and whitefly population in Zambia

P. C. Chikoti¹*, M. Tembo¹, M. Chisola¹, P. Ntawuruhungu² and J. Ndunguru³

¹Zambia Agriculture Research Institute, Mt. Makulu Research Station, P/B 7 Chilanga, Zambia.
²International Institute of Tropical Agriculture, P.O. Box 310142 Chelston, Lusaka, Zambia.
³Mikocheni Agricultural Research Institute (MARI), P.O. Box 6226, Dar es Salaam, Tanzania.

Received 26 May, 2015; Accepted 27 July, 2015

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
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.

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

| Primer      | Sequence (5’ - 3’) | Specificity | Strand | DNA component |
|-------------|--------------------|-------------|--------|---------------|
| JSP001      | ATGTCGAGGCACGAGGAGAT | ACMV        | Sense  | AV1/CP        |
| JSP002      | TGTTTATTAAGCCTGAAATCT | ACMV        | Antisense | AV1/CP |
| EAB555/F   | TACATCGGCTTTGAGTCATGG | EACMV      | Sense  | DNA-B        |
| EAB555/R   | CTATTAAAGCCTATATAGACCC | EACMV      | Antisense | DNA-B   |
| UV-AL1/F   | TGCTCTTGGACTTTGTG | EACMV-UgV  | Sense  | AV1/CP        |
| ACMV-CP/R3 | GCCCTCTGATGATTATATGC | EACMV-UgV  | Antisense | AV1/CP |

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

**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 greenhouse at temperatures between 20 and 30°C. The stem cuttings were planted in insect proof greenhouse 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 thermocycler (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, 1 mM NaEDTA, pH 8.0).

**Data analysis**

Disease incidence was determined as the proportion of diseased
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) | 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.

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...
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...
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 (Muumba-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 co-infections 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.

ACKNOWLEDGEMENTS
Mr. Jackson Mwenya, Mr. Suwilanje Schilima and Ms. Judith Malumo are appreciated for the financial assistance. We acknowledge Mikocheni Agriculture Research Institute for the provision of diagnostic facilities. Africa Development Bank (AfDB) and United States Agency for International Development (USAID) are appreciated for the financial assistance.

REFERENCES
Alabi OJ, Kumar PL, Naidu RA (2011). Cassava mosaic disease: A curse to food security in Sub-Saharan Africa. Online, APSnet Features. doi:10.1094/APSnetFeature-2011-0701. Retrieved on 12 January 2015 from http://www.apsnet.org/publications/apsnetfeatures/Pages/cassava.aspx
Bellotti AC, Arias B (2001). Host plant resistance to whiteflies with emphasis on cassava as a case study. Crop Prot. 20:813-823.
Bull SE, Briddon RW, Sserubombwe WS, Ngugi K, Markham PG, Stanley J (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. J. Gen. Virol. 87:3053-3065.
Bull SE, Bridon RW, Sseubombwe WS, Ngugi K, Markham PG, Stanley J (2007). Infectivity, pseudorecombination and mutagenesis of Kenyan cassava mosaic begomoviruses. J. Gen. Virol. 88:1624-1633.
Bulubulu OF, Diamunii NA, Kakakdimau NF, Mbaya NA, Mutambel H, Lundeke L, Luyindula N, Rufflard G, Lepolvre P (2015). PCR and ELISA detection of cassava mosaic virus in a Congolese cassava landrace. Int. J. Biotechnol. Food Sci. 3: 10-16
Chikoti PC (2011). Development of cassava (Manihot esculenta Crantz) cultivars for resistance to cassava mosaic disease in Zambia. PhD thesis, University of KwaZulu-Natal.
Chikoti FC, Ndunguru J, Melis R, Tairo F, Shanahan P, Sseruwagi P (2013). Cassava mosaic disease and associated viruses in Zambia: occurrence and distribution. Int. J. Pest Manag. 59: 63-72.
Dellaporta SL, Wood J, Higgs JB (1983). A plant DNA minipreparation: version II. Plant Mol. Biol. Rep. 1:19-21.
FAOSTAT (2015). Food and Agriculture Organisation of the United Nations. Retrieved 10 March 2015 from http://faostat3.fao.org/download/Q/QC/E
Fondong VN, Pita JS, Rey C, Fauquet AC (1998). First Report of the Presence of East African Cassava Mosaic Virus in Cameroon. Plant Dis. 82:1172
Fondong VN, Pita JS, Rey ME, de Kochko A, Beachy RN, Fauquet CM (2000). Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. J. Gen. Virol. 81:287-297.
Harrison BD, Zhou X, Otieno-Nape GW, Liu Y, Robinson DJ (1997). Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic virus in Uganda. Ann. Appl. Biol. 131:437-448
Kumar LP, Akinbade SA, Dixon AGO, Mahungu NM, Mutunda MP, Kiala D, Londa L, Legg JP (2009). First report of the occurrence of East African cassava mosaic virus-Uganda (EACMV-UG) in Angola. Plant Pathol. 58:402
Legg J (1994). Bemisia Tabaci: the whitefly vector of cassava mosaic geminiviruses in Africa: an ecological perspective. Afr. Crop Sci. J. 2:437-448
Legg J, James B (2005). Conclusions and recommendations. In: Whitefly and whitefly-borne viruses in the tropics: Building a knowledge base for global action. Edited by Pamela K. Anderson & Francisco J. Morales; with the collaboration of Annie L. Jones and Richard H. Centro Internacional de Agricultura Tropical (CIAT), 2005. 351 p.
Legg JP, Sseruwagi P, Brown J (2004). Bemisia whiteflies cause physical damage and yield loss to cassava in Africa. In: Sixth International Scientific Meeting of the Cassava Biotechnology Network, 8-14 March, 2004, CIAT, Cali, Colombia. p. 78
Muumba-Kankolongo A, Chalwe A, Siyupo P, Kang MS (1997). Distribution, prevalence and outlook for control of cassava mosaic disease in Zambia. Roots 4:2-7.
Ndunguru J, Legg JP, Aveling, TAS, Thompson G, Fauquet CM (2005). Molecular biodiversity of cassava begomoviruses in Tanzania: evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. Virol. J. 2:21.
Ogbe FO, Legg JP, Raya MD, Muumba-Kankolongo A, Theu MP, Kaithsha G, Phiri NA, Chalwe A (1997). Diagnostic survey of cassava mosaic viruses in Tanzania, Malawi and Zambia. Roots 12-15
Ogbe FO, Thottappilly G, Dixon AGO, Atiri GI, Mignouna HD (2003).
Variants of East African cassava mosaic virus and its distribution in double infections with African cassava mosaic virus in Nigeria. Plant Dis. 87:229-232

Omongo CA, Kawuki R, Bellotti AC, Alicai T, Baguma Y, Maruthi MN, Bua A, Colvin J (2012). African Cassava Whitefly, Bemisia tabaci, Resistance in African and South American Cassava Genotypes. Plant Dis. 87:229-232

Patil BL, Fauquet CM (2009). Cassava mosaic geminiviruses: actual knowledge and perspectives. Mol. Plant Pathol. 10:685-701

Sserubombwe W, Thresh M, Legg J, Otim-Nape W (2005). Progress of Cassava Mosaic Disease in Ugandan Cassava Varieties and in Varietal Mixtures. In: Whitefly and whitefly-borne viruses in the tropics: Building a knowledge base for global action. (In: by Pamela, K.A. Francisco, J., Annie, L., Jones & Richard, H.M. Cali, Colombia, Centro Internacional de Agricultura Tropical (CIAT). p. 351.

Stanley J, Bisaro DM, Briddon RW, Brown JK, Fauquet CM, Harrison BD, Rybicki EP, Stenger DC (2004). Geminiviridae. In Virus Taxonomy, VIIth Report of the ICTV. 8th edition. Edited by Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA. Elsevier/Academic Press. 2004:301-326.

Tiendrébéogo F, Lefeuvre P, Hoareaux M, Harimalalala MA, De Bruyn A, Villemot J, Traoré VSE, Konaté G, Traoré AS, Barro N, Reynaud B, Traoré O, Lett JM (2012). Evolution of African cassava mosaic virus by recombination between bipartite and monopartite begomoviruses. Virol. J. 9:67

Were HK, Winter S, Maiss E (2003). Distribution of begomoviruses infecting cassava in Africa. J. Plant Pathol. 85:145-151

Zhou X, Liu Y, Calvert L, Munoz C, Otim-Nape GW, Robinson DJ, Harrison BD (1997). Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. J. Gen. Virol. 78:2101-2111.