EPIDEMIOLOGICAL AND MOLECULAR CHARACTERISATION OF CASSAVA MOSAIC GEMINIVIRUSES AND THEIR EFFECT ON YIELD OF CASSAVA IN ZAMBIA

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DECLARATION

I, MATHIAS TEMBO hereby declare that this dissertation is my own original work and that it has not been previously submitted for any degree or examination at this or any other university.

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Signature

_____ Day of _____ 2016

APPROVAL

This dissertation of Mathias Tembo has been approved as partial fulfillment of the requirements for the award of the degree of Master of Science in Agronomy (Plant Science) by the University of Zambia.

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ABSTRACT

An epidemiological and molecular characterization study of cassava mosaic disease was carried out in four cassava growing Provinces of Zambia in 2013. The objectives of the study were to determine the incidence and severity of Cassava mosaic disease (CMD) in the study areas; to determine molecular diversity of Cassava mosaic geminiviruses (CMGs); to evaluate the effect of CMD on yield of selected cassava genotypes. The study involved survey of CMD in four cassava producing provinces, virus isolate characterization using the polymerase chain reaction (PCR) and sequencing of purified PCR products and evaluation of nine landrace and improved cassava genotypes to CMD. The results from the survey showed CMD incidence and severity was 61.5 % and 2.7, respectively. The results also showed that common transmission of the disease was due to cutting infection (57.7 %) compared to whitefly infection (3.8 %). The polymerase chain reaction showed single infections of *African cassava mosaic virus* (ACMV) in 34.7 % of the positive samples while *East African cassava mosaic virus* (EACMV) occurred in 4.5 % of the positive samples. The dual infections of ACMV and EACMV were detected in 60.8 % of the positive samples. Restriction fragment length polymorphisms (RFLP's), complimented by sequence analysis done by multiple sequence analysis established the existence of different CMG isolates in Zambia. The sequence identity analysis and phylogenetic analysis displayed high nucleotide (nt) sequence identities amongst one another with the isolate sequences showing identities of 89 % to 99 % within the coat protein (CP) gene of ACMV. The Zambian isolates in this study showed substantial homology with sequences of ACMV-UGMild Uganda (AF126800.1), ACMV-UGSvr Uganda (AF126802.1), ACMV-[MG:MG310A1] Madagascar, and ACMV-CM39 Cameroon (AY211462.1) with sequence identities of 97 %, 97 %, 97 % and 98 %, respectively. However, the Zambian isolates showed greater variability within the EACMV species nucleotide sequence divergence ranging between 77 % to 99 %. The isolates showed similarity to the Kenyan (EACMV-KE), Malawian (EACMMV) and Tanzanian (EACMV-TZT) with sequence identities of 96 %, 90 % and 96 %, respectively. Among the genotypes evaluated for CMD response, Manyopola and Bangweulu were found to be susceptible (3.5) whilst Kampolombo was resistant (1.7). Mweru had the highest root yield (1.6 kg plant^{-1}) while Kapumba, the second most susceptible genotype, had the lowest root yield (0.2 kg plant^{-1}). The current study showed slight epidemiological variation from previous results in same areas. This underlines the importance of continuous evaluation of the disease in order to develop effective control measures.

DEDICATION

To my late father, Thomas Tembo, whose memories motivated and encouraged me.

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LIST OF ABBREVIATIONS

ACMV	African cassava mosaic virus
CCP	Core coat protein
CMD	Cassava mosaic Disease
CMG's	Cassava mosaic geminiviruses
CMV	Cassava mosaic virus
DNA	Deoxyribonucleic acid
DI	Disease index
DS	Disease severity
dsDNA	Double stranded DNA
EACMV	East African cassava mosaic virus
EACMCV	East African cassava mosaic Cameroon virus
EACMMV	East African cassava mosaic Malawi virus
EACMZV	East African cassava mosaic Zanzibar virus
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immuno-absorbent assay
ICMV	Indian cassava mosaic virus
ICTV	International Committee of the Taxonomy of Viruses
IR	Intergenic region
ORF	Open reading frame
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid

SLCMV	Sri-Lankan cassava mosaic virus
ssDNA	Single stranded DNA
TGMV	Tomato golden mosaic virus
UGV	Uganda variant

CHAPTER 1.0

INTRODUCTION

Cassava (*Manihot esculenta* Crantz), is a perennial plant which is grown mainly for its tuberous roots that are rich in carbohydrate. The plant belongs to the *Euphorbiaceae* family and is the only widely and commercially cultivated member of the genus *Manihot* which is made up of about 100 other species (Alves, 2002). Cassava grows well in warm humid lowland tropics and tolerates mean annual temperature exceeding 20 ^{o}C with annual rainfall that varies between 500 mm and 8000 mm (Puonti-Kaerlas, 1998). Cassava grows best at rainfalls exceeding 1200 mm on many soil types but tolerates drought, acidity and low soil fertility (Kamukondiwa, 1996). The roots can be left in the ground for a long time after maturity before harvesting thereby giving poor farmers security in times of famine. These characteristics make cassava the most cheaply cultivated crop as compared to other major staple crops such as rice, maize, wheat, and sugarcane. Cassava is therefore convenient food source for small-scale farmers in many tropical countries who have limited access to expensive agricultural inputs (Mabasa, 2007).

Worldwide, cassava is consumed by about 600 million people (FAO, 2006). Most of the harvest for human consumption is either as fresh or in various processed forms. Some of the produce is processed as animal feed and industrial products e.g. starch, flour, alcohol and glucose. The leaves, which are rich in proteins and vitamin C and other nutrients such as magnesium, sodium, riboflavin, thiamin, nicotinic acid and citrate (Bradbury and Holloway, 1988), are also consumed in some communities to

supplement the low protein content of the roots (Mabasa, 2007). In Africa, cassava is primarily produced for human consumption, where it is consumed in various processed forms such as cassava flour for the production of biscuits, sausage rolls, meat pies and bread (Ogbe, 2001). In Southern Africa region, the most important growing cassava countries are Mozambique, Angola, Madagascar, Malawi and Zambia respectively. Cassava is thought to have been introduced into Zambia via the Congo basin and via the Portuguese trading routes from Mozambique on the east coast of Africa into Zimbabwe and Malawi (Haggblade and Zulu, 2003).

Cassava is the mainstay for an estimated 30 % of Zambians and is ranked the second most important food crop after maize (Chiona *et al*., 2014). The cassava crop is regarded as a staple in the seven Provinces of Zambia, namely Luapula, Northern, North-Western, Copperbelt, Western, Central and Eastern Provinces where it is mostly grown. There is steady increase of cassava planted in Zambia and production. According to the FAO, production for Zambia in the year 2010 was 1,151,700 mt with total area planted of 198,000 ha (FAO, 2010). In 2013, cassava production was 1,070,700 mt with total area planted of 215,000 ha (FAOSTAT, 2014). This increase in total area planted to cassava could be attributed to deliberate introductions of cassava to the traditional non-cassava growing areas such as Eastern, Southern and Central Provinces by government agencies and non-governmental organizations dealing in agriculture. However, the corresponding cassava yields during the same period shows a decline. Promotion of cassava cultivation in other Provinces other than the traditional cassava-growing areas in Zambia has been necessitated largely by fluctuations in

climatic conditions (erratic rainfall) that make it unfavorable for maize production and increases in fertilizer prices over the years. These factors have made it difficult to grow maize resulting in food shortages in the predominantly maize-growing areas. Cassava, however, requires no such inputs. Therefore, government and organizations involved in agriculture have realised the need to promote cassava, even in areas that do not traditionally grow this crop.

Although there has been efforts in promoting cassava productivity in Zambia, the yields averaging 4.98 t/ha (FAOSTAT, 2014), are still very low when compared to the estimated average yields for Malawi (22.5 t/ha), Angola (17.2 t/ha), Uganda (14 t/ha), Nigeria (13.5 t/ha), Democratic Republic of Congo (DRC) (8.9 t/ha), Mozambique (8.6 t/ha) (FAOSTAT, 2016) as well as that of Africa (10.9 t/ha), South America (13.2 t/ha) and Asia (19.7 t/ha) (Legg *et al.,* 2015). Under optimal conditions, cassava can produce up to 80 t/ha of tuberous roots in a 12-month cultivation period (Legg and Thresh, 2003). Observations from on-farm experiments in Zambia showed yields of 15 t/ha using improved varieties (Ntawuruhunga *et al.,* 2013). Low yields are attributed to a number of factors such as pests and diseases and poor agricultural practices. Among the diseases, cassava mosaic disease (CMD) is a major constraint in many parts of Africa (Fargette *et al*., 1988; Fauquet and Fargette 1990; Legg and Fauquet, 2004). Reports of CMD pandemic affecting at least nine countries in East and Central Africa, covering an area of 2.6 million square kilometres has been estimated to cause annual economic loss of US$1.9–2.7 billion (Patil and Fauquet, 2009).

The need to carry out periodical epidemiological studies of CMD in Zambia is important so as to monitor the spread of CMD and give timely and comprehensive overview of the disease situation in the country. Epidemiology, which is the science that studies the patterns, causes, and effects of health and disease conditions in defined populations or commodities such as cassava, allows for epidemiological studies that provides a confirmation or disapproval of the presence of CMD in an area and also identifies principal modes of cassava mosaic geminivirus (CMG) transmission. Such studies can identify other viruses and their genetic variation. By determining the incidence and severity of CMD in Zambia, a quantitative record and status of CMD and a thorough understanding of the disease situation is possible. This information can be used to mitigate the effects of possible epidemics and other potential arising problems.

The information on the diversity of cassava mosaic geminiviruses (CMGs) infecting cassava in Zambia is limited. Therefore, there is a need to characterize cassava viruses causing CMD in Zambia and establish whether there exist different strains contributing to CMD. This is important in order to complement available scientific information on cassava geminivirus diversity in sub-Saharan Africa, monitor evolutionary trends in cassava geminivirus diversity and monitor developments of new strains of CMGs resulting from recombinations and pseudo-recombinations. Characterisation of CMGs is also important in developing of rapid diagnostic methods for the viruses.

A number of improved, namely Mweru, Chila, Tanganyika, Kampolombo and local, namely Bangweulu, Kapumba, Nalumino, Katobamphunta and Manyopola cassava

genotypes are being grown by local farmers in Zambia. However, many farmers in Zambia continue to use the local materials that are comparatively low yielding due to the fact that most of the improved cassava genotypes lack the consumption attributes that are highly preferred and valued by farmers.

The objectives of the study were:

1. To determine the epidemiology of Cassava mosaic disease and relative abundance of the causative vector in selected cassava producing regions of Zambia,
2. To characterize the Cassava mosaic geminiviruses infecting cassava in Zambia,
3. To evaluate the yield response of improved and local popular cassava genotypes to Cassava mosaic disease in Zambia.

The hypotheses tested in this study were:

1. The prevalence and severity of cassava mosaic disease and the relative abundance of the whitefly vector *Bemisia tabaci* in cassava growing areas of Zambia is high;
2. The Cassava mosaic geminiviruses (CMGs) infecting cassava in Zambia are of the same isolates as those found elsewhere in other countries;
3. The improved and local cassava genotypes in Zambia are tolerant to cassava mosaic disease.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Cassava taxonomy and biology

Cassava (*Manihot esculenta* subspecies *esculenta* Crantz) is a perennial shrub from the family *Euphorbiaceae* cultivated mainly for its starchy roots. It is one of the most important food staples in the tropics, where it is the fourth most important source of energy. On a worldwide basis it is ranked as the sixth most important source of calories in the human diet (Alves, 2002). The origins of cassava have long been obscure (Allem, 2002) but recent evidence based on molecular markers suggests that the plant was domesticated within the southwestern rim of the Amazon basin in modern day Brazil and is derived from its closest wild relative, *M. esculenta* ssp. *flabellifolia* (Pohl) (Legg *et al.*, 2015). Given the crop's tolerance to poor soil and harsh climatic conditions, it is generally cultivated by small farmers as a subsistence crop in a diverse range of agricultural and food systems (Alves, 2002). Cassava is a perennial crop and typically reaches 1–4 m in height at physiological maturity, however, the storage roots can be harvested from 6 to 24 months after planting depending on cultivar and the growing conditions (El-Sharkawy, 1993). In the humid lowland tropics the roots can be harvested after 6–7 months. In regions with prolonged periods of drought or cold, farmers usually harvest after 18–24 months (Cock, 1984). The roots, which have a dry matter content of 30–40 %, provide an important source of starch, and in communities

in South America that have cultivated the crop for many generations, a wide variety of processed products have been developed (Legg *et al.,* 2015). The roots can also be left in the ground without harvesting for a long period of time, making it a very useful crop as a security against famine (Cardoso and Souza, 1999).

Cassava can be propagated from either stem or sexual seed. Propagation from true seed occurs under natural conditions and is widely used in breeding programmes. Plants from true seed take longer to become established, and they are smaller and less vigorous than plants from cuttings (Alves, 2002). The standard cultivation system which makes use of stem cuttings for propagation and establishing a new crop ensures uniformity of a crop variety from season to season and means that planting a new crop is relatively simple; however, this also has the negative consequence of sustaining pathogen populations from one cropping cycle to the next, an attribute which is particularly significant in the epidemiology of viruses that infect the plant (Legg *et al.,* 2015). Despite Latin America being a major producer, more than half of global production is currently in Africa (FAOSTAT, 2014).

2.2 Importance of cassava in Zambia

Cassava, which is the second most important staple crop after maize, is widely grown in seven major cassava-producing Provinces of Zambia namely Luapula, Northwestern, Northern, Lusaka, Western, Eastern and Central (Chikoti *et al*., 2013). Lusaka, Western, Eastern and Central Provinces are located in agro-ecological region II and experience rainfall ranging from 800–1000 mm per year, while Luapula, Northwestern and

Northern Provinces are located in agro-ecological region III, which receives rainfall of between 1000 mm and 1500 mm annually (MAFF, 1997). The broad environmental adaptability of cassava and its tolerance of acid soils and sustained periods of drought are key factors in its widespread adoption (Legg *et al.,* 2015). Cassava leaves are rich in protein and energy hence is consumed as a vegetable in Zambia (Hichaambwa, 2005). Sweet cassava varieties are consumed as fresh raw tubers or boiled. Other people dry the cassava roots, store and eat them as fried and roast chips or they mill it into flour which they mix with maize flour and is prepared into a soft paste which is consumed into what is known as nshima. Dried cassava provides affordable source of calories and it offers an attractive substitute for the wheat and maize products that are common amongst Zambia's food, feed and industrial processors. In Zambia, a number of innovative farmers and feed companies use cassava as stock feed ration as a means of lowering feed cost, which is the major cash expenditure in livestock production (Haggblade and Nyembe, 2008).

2.3 Constraints to cassava production

The production of cassava is constrained by abiotic and biotic factors, which are compounded by sub-optimal management practices (Fermont *et al.,* 2009; Bull *et al.,* 2011). The abiotic factors include inadequate rains and poor soil fertility. The biotic factors are diseases and pests. The pests of cassava include mealy bugs (*Phenacoccus manihoti* Matile-Ferrero), cassava green mites (*Mononychellus tanajoa* Bondar). Diseases such as anthracnose (caused by a fungus *Colletotrichum gloeosporioides* f. sp. *manihotis* Penz.), bacterial blight (*Xanthomonas axonopodis* pv. *manihotis* Berthet &

Bondar) and virus diseases (Hillocks and Jennings, 2003; Owolade 2006; Eke-Okoro *et al.,* 2009; Fermont *et al.,* 2009) affect cassava. The cassava mosaic geminiviruses (CMGs) and Cassava brown streak virus (CBSV), which cause cassava mosaic disease (CMD) and cassava brown streak disease (CBSD), respectively, have been noted to be of social and economic importance (Monger *et al.,* 2001; Hillocks *et al.,* 2001; Hillocks and Jennings, 2003). CMD exists in Zambia, however, CBSD is not present (Chikoti *et al.,* 2013a).

2.4 Cassava mosaic disease

Cassava mosaic disease (CMD) occurs in all cassava growing areas in Africa and it is the most economically important disease of cassava (Thresh *et al.,* 1997). CMD has been reported only on the African continent and Indian subcontinent despite the large-scale cultivation of cassava in Latin America and several South-East Asian countries (Patil *et al*., 2009). CMD was first described in East Africa in the nineteenth century (Warburg, 1894). It was first detected in Uganda in 1928 (Martin, 1928). Storey and Nichols (1938), working at the Amani Research Station in what is now Tanzania, first studied CMD in detail and the viruses assumed to be causing the disease. The disease was later reported in many other countries in east, west and central Africa and it is now known to occur in all the cassava-growing countries of Africa and the adjacent islands, India and Sri Lanka (Mabasa, 2007). The disease was not reported to cause serious damage in East Africa until the 1920s. In West Africa, CMD was first recorded in the coastal areas of Nigeria, Sierra Leone and Ghana in 1929 and then spread northward by 1945 (Fauquet and Fargette, 1990). In Uganda, severe epidemics were reported between

1933 and 1944, though they were successfully controlled by the use of resistant cassava varieties and by sanitation through removal of infected plants (Pita *et al*., 2001a). The situation remained stable until 1988, when an extremely severe epidemic of CMD developed, advancing from the north to the south of Uganda at a rate of approximately 20–25 km per year. The CMD pandemic continues to expand its range to the west, south and east, and the most recent published reports of new occurrences are from Angola (Lava Kumar *et al.*, 2009) and Cameroon (Akinbade *et al.*, 2010).

2.4.1 The causal agent of CMD

There are 16 different viruses isolated from cassava belonging to four families and genera, namely; *Comoviridae*: *Nepovirus.*, *Geminiviridae*: *Begomovirus.*, *Potyviridae*: *Ipomovirus.*, and *Caulimoviridae*: *Caulimovirus* (Legg and Thresh, 2003). However, only two genera are of economic importance in Africa with regard to cassava, namely *Ipomovirus*: *cassava brown streak virus* (CBSV) of the family *Potyviridae* and *Begomovirus*: *cassava mosaic geminiviruses* (CMG's) of the family *Geminiviridae* (Mabasa, 2007). CMG's are the most economically important viruses of cassava in Africa. Geminiviruses are currently divided into four genera on the basis of their genome profile, biological properties and their vectors (Fauquet and Stanley, 2003). Those that have monopartite genomes and are transmitted by leafhopper vectors *Cicadulina mbila* primarily found on monocotyledonous plants, are included in the genus *Mastrevirus* (Group I) of which *Maize streak virus* is the type species. Viruses that have monopartite genomes distinct from those of the mastreviruses and that are transmitted by leafhopper vectors to dicotyledonous plants are included in the genus

Curtovirus (Group II) with *Beet curly top virus* as the type species. The genus *Topocuvirus* (Group III), recognized by the International Committee on Taxonomy of Viruses (ICTV) (Pringle, 1999), has only one member (also the type species), *Tomato pseudo-curly top virus*, which has a monopartite genome and is transmitted by a treehopper vector to dicotyledonous plants. The genus *Begomovirus* (group IV) contains viruses that are transmitted by the whitefly *Bemisia tabaci* (Gennadius) to dicotyledonous plants, with *Bean golden yellow mosaic virus* (originally *Bean golden mosaic virus – Puerto Rico*) as the type species (Legg and Thresh, 2003). Cassava mosaic viruses belongs to this group (i.e. group IV).

Cassava mosaic geminiviruses (CMG) species known to infect cassava reported from Africa and two from the Indian subcontinent include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Kenya virus* (EACMKV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), *South African cassava mosaic virus* (SACMV), *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV) (Fauquet and Stanley, 2003; Fauquet *et al*., 2008). In addition to the above species, several strains have been recognized: *East African cassava mosaic virus-Uganda* (EACMV-UG), -*Kenya* (EACMV-KE), -*Tanzania* (EACMV-TZ); *South African cassava mosaic virus-South Africa* (SACMV-ZA), -*Madagascar* (SACMV-MG); *East African cassava mosaic Cameroon virus-Cameroon* (EACMCV-CM), -*Tanzania* (EACMCV-TZ); *Indian cassava mosaic virus-India* (ICMV-IN), -*Kerala* (ICMV-Ker) and *Sri Lankan*

cassava mosaic virus-India (SLCMV-IN), -*Sri Lanka* (SLCMV-LK) (Patil and Fauquet, 2009).

CMD is caused by cassava mosaic geminiviruses (CMGs) (Family Geminiviridae: Genus Begomovirus) (Bock and Woods, 1983; Swanson and Harrison, 1994; Thresh *et al.*, 1998a). The cassava mosaic geminiviruses (CMGs) are currently one of the most economically important members of the group of geminiviruses, known to pose an alarming threat to world agriculture (Boulton, 2003; Varma and Malathi, 2003). Two of the geminiviruses, East African cassava mosaic virus (EACMV) and African cassava mosaic virus (ACMV), occur in Zambia (Chikoti *et al.*, 2013a). The reports of the presence of CMD in Zambia confirm earlier reports of the occurrence of ACMV and EACMV in north-eastern Zambia where the two viruses occur together (Ogbe *et al.*, 1997). Chikoti (2011) reported the presence of ACMV and EACMV in mixed infections. Although low percentage of mixed infections was reported, this is worrisome since ACMV and EACMV mixed infections produce extremely severe symptoms and cause significant yield losses (Legg and Fauquet, 2004). CMD surveys conducted by Chikoti *et al.* (2013a) noted severe symptoms on some cassava varieties in Zambia. The laboratory analysis of CMD symptomatic plant samples collected from different parts of the country detected also the presence of satellite DNA molecules (Chikoti, 2011). DNA satellites are linear or circular RNA/DNA that require a helper virus to supply proteins for replication, movement and encapsidation but shares little sequence relatedness with the helper virus and is not required for the accumulation of the helper virus. They enhance virus symptoms and break CMD resistance in cassava (Patil and Fauquet, 2010).

The family Geminiviridae is a unique group of viruses characterized by their twinned icosahedral particle morphology. Virions associated with CMD have geminate particle size of 30 nm x 18 nm (Bock, 1975) with a *ca*. 30 kDa protein subunit making up the paired icosahedral coat structure (Bock *et al.*, 1977). Each particle encapsidates a pair of single-stranded circular DNA molecule that make up the virus genome. Geminate virions may contain one (monopartite) or two (bipartite) distinct DNA molecules depending on the genus with a genome size ranging from 2.5 to 5.2 kb. ACMV genome consists of two DNA molecules, DNA-A (or DNA-1) and DNA-B (or DNA-2) of similar size but different nucleotide sequence (Stanley *et. al.,* 2005). DNA-A has 2,776 to 2804 nucleotides while DNA-B ranges from 2,724 to 2,777 nucleotides. Both DNA components contain protein coding nucleotide sequences in the virus strand and in the complementary strand. The DNA-A component which comprises six open reading frames (ORFs) and codes for the functional proteins required for viral DNA replication, transcription enhancement and encapsidation functions, generally contains two genes (AV1 and AV2) in the virus-sense strand and four genes (AC1, AC2, AC3 and AC4) in the complementary strand (Patil and Fauquet, 2009). AV1 codes for the virus coat protein (CP) that is intimately involved in interactions with the *Bemisia tabaci* whitefly insect vector during virus transmission while AV2 codes a precoat protein. AC1 codes for a replication associated protein (Rep) that initiates replication and AC3 codes for the replication-enhancer protein (REn). The protein product of AC2 is a transcriptional activator protein (TrAP) for virus-sense genes that controls gene expression and which is required for initiating transcription of the viral sense-genes, as well as being involved

in the suppression of post-transcriptional gene silencing (Voinnet *et al.,* 1999; Vanitharani *et al.,* 2004). AC4 codes for the RNA-silencing suppressor. The DNA-B component has two ORFs which contain the virus-sense and complementary strands comprising one gene (BV1 and BC1, respectively) and encodes the nuclear shuttle protein (NSP) and movement protein (MP) respectively, that act co-operatively to move the virus both within and between cells in host plants (Hanley-Bowdoin *et al.,* 2004; Sanderfoot and Lazarowitz, 1996; Hull, 2002; Jeske, 2009). Between the initiation codons of AV2 and AC1 in DNA-A lies the intergenic region (IR), also referred to as the common region (CR) and in DNA-B there is an equivalent IR between the initiation codons of BV1 and BC1. This is a conserved intergenic 'common region' and the begomovirus genomic components DNA-A and DNA-B share the common region which is of approximately 200 nucleotides with a high nucleotide sequence identity of more than 80% (Harrison and Robinson, 1999). The common region contains several regulatory elements, including two TATA motifs and also multiple copies of cis-elements known as iterons, which are the binding sites for the replication-associated protein (Rep) (Hanley-Bowdoin *et al*., 1999). Comparisons of nucleotide sequences among bipartite begomoviruses showing the sequences of DNA-B being more diverse than those of DNA-A have been reported (Mabasa, 2007). It is relatively easy to align the DNA-A sequences of diverse begomoviruses, but alignments of DNA-B sequences are less straightforward because there are fewer conserved elements. However, the part of the genome that shows the greatest variation is the IR (Rybicki, 1994; Harrison and Robinson, 1999). Different types of iteron sequences have been identified in CMGs and they can be classified into three groups: the ACMV type with isolates of ACMV,

EACMZV and SLCMV; the EACMV type encompassing all the other EACMV-like viruses and SACMV; and the ICMV type with ICMV isolates alone (Patil and Fauquet, 2009).

2.4.2 Cassava mosaic geminiviruses diversity

The earliest indication of a virus as a causative agent of CMD was proposed by Zimmerman (1906). Storey and Nichols (1938) conducted detailed studies of CMD and the putative causative viral pathogen based on the severity of the disease caused and further separating them into mild and severe strains. Bock (1975) fully described the viral etiology. Further, Bock *et al.* (1977) or as in Padidam *et al.* (1999) or Pita *et al.* (2001 a and b), undertook work to characterize the virus. Bock and Woods (1983) finally determined the etiology of the virus and proved the Koch's postulates for the virus which they named *African cassava mosaic virus* (ACMV). Swanson and Harrison (1994) developed serological techniques that detected distinct serotypes namely; ACMV, EACMV and *Indian cassava mosaic virus* (ICMV). As information about the viruses infecting cassava increased, DNA-based techniques were developed resulting in the discovery of a wider diversity of CMD-causing viruses. Sequence comparisons of a large number of geminivirus species and strains have shown that some of the viruses are the result of recombination, which is a frequent occurrence (Padidam *et al.*, 1999). In the case of ACMV and EACMV, however, there is a high degree of homology in the former and considerable variation and recombination frequency in the latter (Pita *et al*., 2001a).

Recombinations, virus mixtures and pseudo-recombinations are linked to the emergence of strains causing severe CMD symptoms. In Zambia, ACMV and EACMV have been detected in single as well as mixed infections (Chikoti, 2011). In such cases severe CMD symptoms were observed. Similar observations have been reported in South Africa (Berry and Rey, 2001), Cameroon (Fondong *et al.,* 2000), Nigeria (Ogbe *et al.,* 2003) and Ghana (Offei *et al.,* 1999). The inherent ability of geminiviruses to recombine between and among themselves makes them to consistently and continuously evolve, generating new biodiversity. Their ability to act in a synergistic manner, allows them to be highly opportunistic and capable of generating dramatic new epidemics (Zhou *et al*., 1997) such as those significantly impacting cassava farmers in East and Central Africa. The ACMV and EACMV have been implicated in the recombination event that gave rise to the so-called Uganda variant (EACMV-UG) (Zhou *et al.,* 1997). Fondong *et al.* (2000) reported that severe symptom expression is evidence of synergistic interaction between ACMV and EACMV. Recombination and pseudo-recombination have been noted as the major driving forces in the evolution of cassava infecting geminiviruses and these events could result in the evolution of new chimeric viruses having greater virulence and disease capabilities that contribute to epidemics. Patil and Fauquet (2009) reported that recombination and pseudo-recombination between CMGs give rise not only to different strains, but also to members of novel virus species with increased virulence and a new source of biodiversity, causing severe disease epidemics. The recombinant virus EACMV-UG (Uganda variant), which caused a severe form of CMD in Uganda and serious reduction in yields (Zhou *et al.,* 1997), is a very good example. However, though the cassava mosaic geminivirus disease

pandemic continues to expand, CMD has been reported only from the African continent and Indian subcontinent despite the large-scale cultivation of cassava in Latin America and many South-East Asian countries (Patil and Fauquet, 2009). The absence of CMD in South America and several countries in South-East Asia, despite the occurrence of other geminivirus diseases, has been mainly attributed to the inability of the polyphagous *B. tabaci* B-biotype to colonize cassava effectively in this part of the world (Carabali *et al*., 2005).

2.4.3 Transmission of cassava mosaic geminiviruses (CMG's)

Cassava mosaic geminiviruses are transmitted by the whitefly vector *Bemisia tabaci* Gennadius and disseminated in the stem cuttings used for vegetative propagation (Hillocks and Thresh, 2000; Fauquet and Fargette, 1990). Dissemination in stem cuttings can lead to the introduction of CMD to new areas and accounts for the occurrence of the disease in areas where there is little or no spread by the whitefly vector. The vector has been reported to have more than 500 different plant hosts (Legg and Fauquet, 2004). It is the only known whitefly vector of CMG's (Harrison, 1985). Studies to understand transmission of the virus has shown that the coat protein (CP) of the geminivirus is specifically adapted for transmission by the local whitefly population, which explains the antigenic similarity of the CPs of begomoviruses from the same area (Harrison and Robinson, 1999) and co-adaptation between CMG's and their local whitefly populations (Maruthi *et al.,* 2002). Hence, for the efficient transmission of CMGs both genomic components, DNA-A and DNA-B, are required (Patil and Fauquet, 2009). The whitefly B-biotype has been found to be more fecund and has an

extremely broad host range (Colvin *et al.*, 2004), which might have contributed to the transmission of new viruses from weed hosts to cultivated crop plants and hence leading to the emergence of a number of geminivirus diseases in most parts of the world.

2.4.4 The whitefly vector (*Bemisia tabaci*: Gennadius)

The whitefly vector, *Bemisia tabaci*, belongs to the order Homoptera, family *Aleyrodidae* and is primarily a polyphagous insect that colonizes annual herbaceous plants (Brown *et al.*, 1995).

These whiteflies measure 2-3 mm in length and with the wings being present in the adult stages of both sexes. The abdomen has notable missing cornicles with the hind wings being nearly as long as the forewings (Bellows *et al.*, 1994). Generally, homopterans undergo gradual metamorphosis. The metamorphosis of whiteflies, however, shows a pattern more towards complete metamorphosis (Borror *et al.*, 1989) with five instars in the development cycle of *B. tabaci* that includes the adult being noted. The *B. tabaci* has been described as being one of the most important agricultural pests and virus vectors of agricultural and ornamental crops in all tropical, subtropical and some temperate areas (Mabasa, 2007). *B. tabaci* causes significant damage to crops primarily through phloem feeding, phytotoxic disorders and the transmission of plant viruses. The development of insecticide resistance, reduction in natural enemies and monocultural practices have been considered as the main drivers in the emergence of *B. tabaci* as the primary agricultural pest in tropical and subtropical agricultural systems (Brown *et al.*, 1995).

In Uganda, studies have shown an epidemic that was associated with high population density of the whitefly vector, *B. tabaci* (Gibson *et al.,* 1996; Legg and Ogwal, 1998; Colvin *et al.,* 2004). Positive correlations have been observed between populations of *B. tabaci* and the spread of CMD into initially healthy cassava plantings (Fargette *et al.*, 1993; Legg and Raya, 1998). The whitefly population size has also been positively correlated with the spread of the virus from one month after invasion, which has been observed to correspond with the time necessary for symptom development (Fauquet and Fargette, 1990).

2.4.5. Factors influencing the transmission of CMG's

A number of studies have shown the importance of various factors that influence the pattern of spread of virus diseases within and between fields and the factors that inhibit or favour such spread. The abundance of the whitefly vector has been described as an important factor affecting the CMD pandemic in Africa that started with the epidemics in northern Uganda of East Africa in the late 1980's and continued to spread in surrounding countries (Otim-Nape *et al.,* 1997; Legg, 1999). A key feature of the persistent transmission mechanism of CMG's is that these viruses are retained for at least 9 days, and may be retained by adult whiteflies throughout their lives (Dubern, 1994). This has important epidemiological implications, as it appears to provide the mechanism by which CMG's are carried long distances by dispersing *B. tabaci*. Long-term virus retention coupled with long range dispersal has been proposed as the basis for the spread of the CMD pandemic up to 100 km per year along the western side of Lake Victoria (Legg *et al*., 2006; Legg, 2010). Fauquet and Fargette (1990) reported

disease incidence as largely a reflection of the fluctuations in whitefly populations which partly depend on climatic factors that include temperature, rainfall and wind. Ambient temperatures within the range of 20-30 ^{o}C have been cited as the primary factor driving the increase in whitefly populations (Fargette *et al*., 1993). However, inconsistencies are apparent in the association between the epidemiological characteristics of CMD spread and environmental conditions. Higher rainfall and humidity have also been positively correlated with higher disease incidence that results from higher whitefly populations that are supported by vigorous plant growth (Dengel, 1981; Robertson, 1985). Legg and Raya (1998) showed that in Tanzania, regions with the highest incidence were hot and wet coastal areas as well as drier inland areas moderated by neighboring lakes. Wind speed and direction influence the distribution of the whitefly population in a field and it has been shown that the incidence of the disease was higher on the upwind edges than on the downwind edges of the field (Fauquet and Fargette, 1990).

Legg and Fauquet (2004) observed that regardless of the specific agro-ecological or other environmental conditions, rapid spread occurred in areas of Uganda where the epidemic of severe CMD associated with EACMV-UG was present. They attributed the primary reason for this to the fact that in dual ACMV + EACMV-UG infections, synergism leads to 10–50-fold increases in viral DNA accumulation, substantially increasing the potential for a higher efficiency of vector transmission. Their observations are supported by findings carried out by Harrison *et al*. (1997) and those of Pita *et al*. (2001a) which provided the initial indications of the importance of

considering the nature of the virus or virus mixtures causing CMD when making epidemiological assessments. Other factors known to influence disease spread are; cassava varieties used, proximity of other fields or source of inoculum, crop density and virus strains present (Mabasa, 2007). However, it is important to note that the interactions between these factors are complex and should take local conditions into consideration.

2.4.6 Cassava mosaic disease (CMD) symptoms

Storey and Nichols (1938) described the symptoms of CMD occurrence as characteristic leaf mosaic patterns that affect discrete areas and determined at an early stage of leaf development. Leaf chlorosis may be pale yellow or nearly white. The chlorotic areas are usually clearly demarcated and vary in size from the whole leaflet to small flecks or spots. Symptoms are readily distinguished from those of mineral deficiency or cassava green mite damage as the virus-induced chlorosis and malformation of leaflets is asymmetrical about the midrib. Where the virus or virus strain is mild or the cassava variety is tolerant, leaf chlorosis may be patchy and absent on some leaves and there is little or no leaf distortion or malformation and little effect on overall plant vigor (Fig. 2.1). Severely affected leaves are reduced in size, misshapen and twisted with yellow areas separated by areas of normal green colour. The plants are stunted and petioles immediately below the shoot tip may be angled downwards and occasionally may become necrotic, shrivel and absciss (Fig. 2.1).

Figure 2.1: Plants showing: mild symptoms of cassava mosaic disease (left) and severe form of cassava mosaic disease (right), 2013

2.4.7. Epidemiological surveys of CMD

Legg *et al.* (2006) reported the existence of extensive literature describing the epidemiology of CMD and listed the important developments based on the following:

- The early demonstration of the link between seasonal environmental factors and rates of CMD spread at a locality in Tanzania (Storey, 1938).
- Descriptions of the association between vector abundance and rates of CMD spread (Dengel, 1981; Fargette *et al.,* 1993; Fishpool *et al.,* 1995).
- The occurrence of environmental gradients associated with CMD spread and the primary importance of external inoculum sources compared with internal sources (Fargette *et al.,* 1985; 1990).
- The status of temperature and rainfall as key determinants of cassava growth, vector population increase and subsequent virus spread (Fargette *et al.,* 1993; Fishpool *et al.,* 1995).
- The value of resistant varieties in both delaying and reducing rates of virus spread (Hahn *et al.,* 1980; Otim-Nape *et al.,* 1998; Sserubombwe *et al.,* 2001).
- The potential to predict final CMD incidence through a combined assessment of inoculum and the abundance of early vector immigrants (Legg *et al.,* 1997).

A variety of approaches have been used involving the collection of a range of data types such as the series of monitoring surveys done in Uganda, Kenya and Tanzania (Legg *et al*., 1999) in order to monitor the development of the regional pandemic, plot its spread, and develop forecasts to aid the targeting of CMD control measures. Sseruwagi *et al*. (2004) indicated that information from CMD surveys can be used to forecast the future

spread of the severe forms of the disease and thus, is of vital importance in controlling CMD. They further explained that forecasting future spread requires regular diagnostic surveys in key cassava growing areas to establish; the spread of CMD both in space and time, the epidemic characteristics of CMD (i.e. the amount and relative proportion of Whitefly (W) and Cutting (C) infections, the identity and distribution of the different CMG's present, the population and distribution of the whitefly vector (*B. tabaci*) and its occurrence, frequency, amount and type of cassava cultivars (resistant/susceptible) being grown.

Using this information, the rate of spread of the disease can be monitored over time and computer-generated maps produced. Legg (1999) further stressed that by mapping CMD epidemic affected areas or zones and establishing the prevailing epidemic characteristics such as CMG identity and distribution, whitefly populations and the rate of spread of the epidemic, identifying the areas at risk and predicting when they are likely to be affected, is possible. Therefore, by using this information, a model could be produced to forecast future spread of the epidemic and to provide a decision support system for disease management.

2.5 Diagnostic methods of CMG's

There are a number of diagnostic methods used to detect CMGs namely the serological, nucleic acid based tests and the restriction fragment length polymorphism (RFLP) tests. Serological techniques such as the use of the triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) and the double antibody sandwich enzyme-linked

immunosorbent assay (DAS-ELISA) tests, demonstrated earlier evidence of the diversity of cassava-infecting geminiviruses (Sequeira and Harrison, 1982; Swanson and Harrison, 1994). These tests reliably and successfully distinguished the three viruses as being ACMV, EACMV and ICMV using monoclonal antibodies (MAbs) that reacted with all the viruses, another that reacted only with either ACMV or ICMV and one that reacted with ACMV and EACMV but not ICMV (Harrison *et al.,* 1997). However, these tests failed to detect the causal agent of a severe epidemic in Uganda, which was later detected by use of DNA-based techniques (Zhou *et al.,* 1997), as well as failing to distinguishing in one instance between SACMV and EACMV (Berrie *et al.,* 2001).

The advent of the DNA-based diagnostic techniques such as PCR and RFLP analyses provided means for comparison of the DNA of virus isolates collected from different locations, thereby facilitating the detection and differentiation of ACMV, EACMV and EACMV-UG that allowed for the provision of evidence that associated EACMV-UG with the severe epidemic (Harrison *et al.,* 1997; Zhou *et al.,* 1997). These techniques have consequently been used widely to detect ACMV, EACMV, EACMV-UG and other CMG's in virus samples collected during diagnostic surveys and mapping the distribution and spread of EACMV-UG and mixtures of CMG's in East and Central Africa (Legg *et al.,* 1999, 2001; Legg and Okao-Okuja, 1999; Markham *et al.,* 2004; Neuenschwander *et al.,* 2002), in the Democratic Republic of Congo (DRC) (Ntawuruhunga *et al.,* 2007) and in Nigeria (Ogbe *et al.,* 2003). Similar diagnostic techniques have been used to detect CMGs from surveys carried out in Zambia by

Chikoti *et al.* (2013a). However, the study by Chikoti *et al.* (2013a) did not characterize the viruses detected from the survey. Therefore, this study will provide the molecular diversity of the viruses.

The DNA-based diagnostic techniques have proved to be reliable means of virus diagnosis based on their increased specificity and sensitivity of the polymerase chain reaction (PCR). It is however important to note that when using DNA-based tests, different geminiviruses tend to share nucleotide sequences in various parts of their DNA-A molecules and that this tendency is much less in DNA-B (Harrison *et al*., 1997), therefore, in the differentiation of CMG's, PCR analysis can be based on shared or unique sequences. Two PCR-based approaches have proved valuable in differentiating a wide range of begomoviruses. One approach relies on selecting primers based on nucleotide sequences that do not occur in other CMG's with only the target virus being detected (Deng *et al*., 1994). The use of primers ACMV-AL1/F and ACMV-CP/R3 to detect ACMV only, UV-AL1/F1 and ACMV-CP/R3 to detect ACMV-UG only, UV-AL1/F1 and EACMV-CP/R to detect EACMV only and UV-AL1/F1 and UV-AL1/R1 to detect either EACMV-UG or EACMV are examples (Zhou *et al*., 1997).

The other approach involves the use of degenerate primers based on sequences occurring in several CMG's being used in PCR and the viruses being eventually distinguished by the pattern of restriction fragment length polymorphism (RFLP)

fragments obtained through restriction endonuclease digestion of the amplified DNA (Rojas *et al*., 1993).

2.5.1 Effects of CMD on growth and yield of cassava

Extensive literature exists on the effect of CMD on growth and yield of cassava. Studies investigating yield loss have been conducted in many locations under diverse conditions of cultivar susceptibility and inoculum pressure conditions and these have consequently provided a wide range of loss estimates from 20 % to 95 % (Fauquet and Fargette, 1990). In Kenya, losses of 86 % have been reported for the susceptible cultivar F279 (Bock & Guthrie, 1978) while in Ivory Coast, losses of 37 % in cultivar CB have reported (Fargette *et al.*, 1988) and between 20-90 % for susceptible cultivars (Beck and Chant, 1958; Thresh *et al.*, 1994a). In Zambia, studies by Muimba-Kankolongo *et al.* (1997) showed that cassava plants with increased CMD severity always yielded less in number and weight of the tuberous roots than CMD-free plants. They found the average yield reduction in sampled cassava fields ranging between 50-70 % depending on the location and cultivar. More than 50 % reduction in yield of tuberous roots was observed in 78.9% of the cassava fields sampled. Coupled with such similar studies, molecular evidence demonstrates clearly that different viruses and virus mixtures have strongly contrasting effects on the symptom expression and growth of cassava plants (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001b). Thresh *et al.* (1994a) indicated that cassava varieties differ in their response to CMD. In the field based trials set up to evaluate the response and inherent resistance mechanisms to CMD of four improved varieties, Adriko *et al.* (2011) found that growth and yield parameters depended on the

variety, growth stage at infection and health status of the cuttings used, with improved varieties showing lower yielding losses than the susceptible varieties. Plants infected earlier in the growth period also suffered higher yield losses. They further found that in plots planted with stems from diseased cuttings, there were higher yield losses recorded as compared to those planted from healthy cuttings.

In a review of studies done on the effects of ACMV on yield, Thresh *et al.* (1994a) showed that the effects of ACMV on yield depend on variety and stage of infection and that plants grown from infected cuttings are much more seriously affected than those infected later by the whitefly vector (*Bemisia tabaci*) and also that plants infected at a later stage of crop growth are almost unaffected. They concluded that positive relationships exist between the extent and severity of the leaf symptoms and yield loss but also that losses can be considerable even in varieties designated as resistant. Fauquet and Fargette (1990), reported yield losses with individual cultivars from different countries ranging from 20 to 95 % with yields of cassava declining dramatically as intensity of symptoms increased. This was observed in the mean yield of tubers in Ivory Coast which showed 29 t/ha and 9 t/ha from those with mild and severe symptoms respectively. They also found that yield loss is greater at between 55-77 % in cassava plants grown from infected cuttings as compared to 35-60 % yield loss in plants infected later by whitefly. In a study carried out to assess the effect of different CMGs occurring in Uganda on the growth and yield of a susceptible cultivar, Owor *et al.* (2004a) indicated that CMGs whether in single or mixed infections, reduce root yield and numbers of tuberous roots produced and that the losses are substantially

increased following mixed infection. They found that plants infected with mild EACMV-UG2 yielded the largest number and heaviest tuberous roots, followed by ACMV and then by severe EACMV-UG2 respectively whilst plants dually infected with ACMV and EACMV-UG2 yielded the least with losses in tuberous root weight attributed to ACMV alone, EACMV-UG2 mild alone and EACMV-UG severe alone, being 42 %, 12 % and 68 %, respectively.

2.5.2. Management of CMD

A number of approaches to controlling CMD exists (Thresh and Otim-Nape, 1994). The use of virus-resistant cultivars is considered to be the most effective and convenient (Storey and Nichols, 1938; Cours, 1951; Hahn *et al.*, 1989). The search for cassava genotypes with resistance to CMD started in 1920 and led to the several trials of cultivars and selections done in Kenya, Tanzania, Uganda, Madagascar, Democratic Republic of Congo, Nigeria and many other countries (Jennings, 1994). The studies revealed significant variations in the incidences of CMD between genotypes and between locations (Storey and Nichols, 1938; Jennings, 1957; Bock and Guthrie, 1978; Fauquet *et al.*, 1987, 1988; Hahn *et al.*, 1989; Fargette *et al.*, 1996; Legg *et al.*, 1997). Several genotypes resistant to CMD were eventually identified with many of them being widely adopted and grown in several African countries (Mahungu *et al.*, 1994). These genotypes were known as Tropical Manihot Series (TMS) and had inherited resistance genes derived from the Amani (north-eastern Tanzania) breeding programme of the 1950s through inter-specific crosses which were made between cassava and its wild relative – *Manihot glaziovii* Müll. Arg. (Jennings, 1957). Thresh and Otim-Nape

(1994) described two major approaches that have been used in attempts to control CMD in Africa namely, the maintenance of a CMD-free crop through phytosanitation and the development and deployment of host-plant resistance.

Phytosanitation involves the removal (roguing) of diseased plants from the field to prevent further spread and/or the selection of CMD-free stems at the end of each growing cycle in order to plant new fields with "clean" material. Sseruwagi *et al*. (2004) reported on a number of CMD control measures that are available including the use of phytosanitation involving selection of cuttings for propagation solely from symptomless plants, the removal ('roguing') of diseased cassava from partially infected stands and proper disposal and burning of crop debris to decrease the risk of infection. Possibilities equally exist to adjust the disposition of crops and cropping practices to decrease the risk of infection (Fargette *et al*., 1985; Otim-Nape, 1987; Thresh and Otim-Nape, 1994) and to use virus-resistant cultivars (Nichols, 1947; Jennings, 1957, 1994; Otim-Nape, 1993; Mahungu *et al*., 1994; Thresh and Otim-Nape, 1994; Thresh *et al*., 1998). They further reported that although these methods are available for use either singly or in combination, host plant resistance is the most widely used approach in both national and regional CMD management programmes. Data obtained from CMD field surveys is also vital in devising appropriate control measures to manage the disease in severely affected areas and to make adequate preparations in threatened areas (Sseruwagi *et al.*, 2004). Most of the cassava planting materials used by Zambian farmers are obtained locally. The recycling of the diseased planting material could result in the deterioration of crop quality through decreased growth and yield of cassava. Alene *et al.* (2013) reported a

total of four out of the seven or 57 % of the released varieties had IITA parent material crossed with best local varieties in order to enhance local adaptation and variety attributes. These are materials that were bred for resistance to viruses in their countries of origin. An evaluation of the capacity of the cassava varieties for CMD resistance in Zambia is of importance due to the devastating impact of CMD on cassava production. There is inadequate information on the susceptibility, resistance and the yield loss due to CMD of the improved and local cassava genotypes in Zambia. Therefore, understanding the resistance and integrating it into a holistic strategy for the management of CMD is of significant importance.

CHAPTER 3.0

MATERIALS AND METHODS

3.1 Determination of incidence and severity of CMD

3.1.1 Survey area

A survey was conducted in four cassava producing Provinces of Zambia namely Western (Kaoma, Luampa, Mongu and Senanga districts), Central (Mkushi, Serenje and Kapiri Mposhi districts), Eastern (Petauke, Lundazi and Mambwe districts) and Lusaka (Lusaka and Rufunsa districts) during the rainy season of February to March, 2013. The Provinces were selected for the survey as cassava is regarded as an important crop, in addition the CMD is prevalent (Chikoti *et al*., 2013a). The Provinces were spread out across the different agro-ecological regions of Zambia. Rufunsa is allocated in Agro-ecological Region I. The agro-ecological Region I which lies between 300 and 900 metres above sea level (m.a.s.l) has a mean annual rainfall of 800 mm (MAFF, 1997). Kaoma, Luampa, Mongu, Senanga, Kapiri Mposhi, Petauke, Lundazi and Lusaka districts are located in agro-ecological region II and have altitudes between 900 to 1300 m.a.s.l with the length of the growing season ranging from 100 to 140 days. The region experiences annual rainfall of between 800 and 1000 mm. Some parts of Serenje and Mkushi are located within agro-ecological region III with altitude ranging between 1100 to 1700 m.a.s.l. The length of the growing season varies between 120 days to 150 days. (MAFF, 1997).

3.1.2 Sample collection

A total number of 186 fresh leaf samples from 88 cassava fields were collected from symptomatic young shoots of CMD-affected plants in a survey carried out in February-March 2013 according to the method described by Sseruwagi *et al.* (2004). Cassava fields aged between 3 to 6 months after planting (MAP) were sampled at intervals of 10 to 50 km in Eastern, Central and Lusaka Provinces where cassava growing is less intense while in areas with intense cassava cultivation such as Western Province, fields were sampled at intervals of ≤ 10 km along main motorable roads. Sampling was done at 3-6 months growth stage, in order to distinguish between cutting-derived (C) and whitefly-derived (W) infections. Infections due to whitefly express disease symptoms on only the upper-most leaves, whereas 'C' infections express symptoms on all the leaves. A plant was considered as having been infected through whitefly if at least the first six leaves at the base of its primary stem were symptomless (Sseruwagi *et al.*, 2004). Similarly, a plant was considered to have been infected through cutting, i.e. infected planting material, if at least the first six leaves at the base of the primary stem were diseased.

In each field 30 plants were assessed for presence or absence of CMD, symptom severity (appendix 1), infection type and adult whitefly population using an 'X' configuration. Therefore, the 30 plants of the predominant cassava genotype were counted along two diagonals allowing for 15 plants per diagonal to be assessed. Two leaf samples were collected from a plant with mild CMD symptoms and another from a severely diseased plant of the sampled genotype. The samples were kept in ice in a cool

box until delivery to the Plant Virology Laboratory at the Zambia Agriculture Research Institute (ZARI) based at Mount Makulu Central Research Station in Chilanga. The leaf samples were kept at -20 ^{o}C until analysis. Cassava cuttings were also collected in the field from plants showing unique CMD symptoms and were planted in the screen house at 20-30 ^{o}C at Mount Makulu Research Station. The plants were monitored weekly and scored for disease symptoms in order to confirm reproducibility of the field-symptoms.

3.1.3 DNA extraction and PCR analysis

Genomic DNA was extracted from cassava leaf samples using the method as described by Dellaporta *et al.* (1983). Lysis of plant cells to release DNA was carried out by grinding 150-200 mg of plant leaf tissue in 700 µl of Dellaporta extraction buffer [100 mM Tris-HCL (pH 8); 50 mM EDTA (pH 8); 500 mM NaCl] with freshly prepared 10 mM Mercaptoethanol using a pestle and mortar. The extract was transferred to 1.5 ml microfuge tubes and 42 µl of 20 % SDS added and mixed gently. Thereafter the extracts were incubated in a water bath at 65 ^{o}C for 10 minutes and mixed by inverting five times at five minute interval. A volume of 160 µl of 5 M potassium acetate (pH 5.2) was added and the contents mixed thoroughly and incubated on ice for 10 minutes. To recover the DNA the tubes were centrifuged at 13,000 rpm for 10 minutes and 600 µl of supernatant transferred to new tubes, where 600 µl of equal volume isopropanol was added. The precipitated DNA was centrifuged at 13,000 rpm for 10 minutes and the isopropanol decanted. The DNA pellet was then cleaned and washed by adding 500 µl of 70 % ethanol and the contents centrifuged at 13,000 rpm for 5 minutes. The supernatant was then discarded and the DNA pellet air-dried for 1 hour. The dried DNA

pellet was resuspended in 200 µl of 1xTE buffer. 10 µl of 10 mg/ml RNase A was added and mixed gently by inverting the tube five times in order to dissolve any contaminating RNA. The DNA was then incubated in a water bath at 37 °C for 1 hour and stored at 4 °C until subsequent use.

3.1.4 Detection of CMGs

Virus specific primers (Table 3.1) were used to detect ACMV and EACMV in infected cassava tissues collected during the survey. Polymerase chain reaction (PCR) was performed using a thermal cycler (Technen 500). To detect ACMV, the following conditions were used; first cycle at 94 ^{o}C denaturation for 2 minutes followed by 35 cycles of denaturation for 1 minute at 94 ^{o}C, annealing at 47 ^{o}C for 1 minute, extension at 72 ^{o}C for 2 minutes and a final extension cycle of 10 minutes at 72 ^{o}C. The DNA-B partial fragment of 556 bp of EACMV was amplified with the following PCR conditions; first cycle at 94 ^{o}C denaturation for 2 minutes followed by 35 cycles of denaturation for 1 minute at 94 ^{o}C, annealing at 56 ^{o}C for 1 minute, extension at 72 ^{o}C for 2 minutes and a final extension cycle of 10 minutes at 72 ^{o}C. The PCR reaction mixture with a total volume of 50 µl comprised of 42.5 µl PCR water, 2.5 µl PCR Buffer (10X), 0.5 µl of 10 mM dNTP mix, 1 µl each of 10 mM reverse and forward primers respectively, 0.5 µl Taq DNA Polymerase and 2 µl DNA template. The ACMV and EACMV positive controls were included from previously characterised CMG DNA by Chikoti *et al.* (2013), while the negative control comprised the PCR reaction mixture without the DNA template. Electrophoresis was performed in 1 % agarose gel, stained in Gelred, at 100 V for 60 min in gels buffered with 1X TAE buffer using a Bio-Rad gel

apparatus. The gel was visualized and photographed using a gel documentation system (Bio-Rad Gel Doc XR+ system).

3.1.5 Data analysis

The mean CMD incidence, severity scores and whitefly population for different Provinces and districts were compared using One-way ANOVA (Non- parametric tests). Disease incidence was calculated as the percentage of CMD-symptomatic plants per field. Disease symptom severity data were edited to remove the symptom less (healthy) counts (score 1) and the analysis conducted for the CMD-affected plants (score 2 to 5) per field. To allow for the effect of multiple infection, values for whitefly-borne CMD were transformed using the formula for "change in CMD caused by whiteflies (y), for any given locality" as follows:

$$y = [\log_e(1/(1-C-W)) - \log_e(1/(1-C))]$$

Where W = proportion of plants with whitefly-borne CMD (where 1 is equivalent to 100 % infected) and C = proportion of plants with cutting-borne CMD. Transformed values were then multiplied by 100 to convert them to multiple infection units (Gregory, 1948). Adult whitefly population data were determined at plant level. The analysis was then performed using Genstat 16th Edition (VSN International, 2013). Geo-coordinates (latitude and longitude) were recorded using a geographical positioning system (GPS) handset (Garmin, etrex summit HC) for each sampled site and data points were used to

map the distribution of the CMG's using ArcView software (Environmental Systems Research Institute, Inc., Redlands, CA).

3.2 Molecular characterisation of the cassava mosaic geminiviruses (CMG's)

3.2.1 Purification of PCR products for sequencing and sequence analysis

Sixty-one samples of virus DNA-A's near full length (c. 2760-2780 bp) were PCR-amplified using the degenerate (universal) primer pair Uni/F and Uni/R (designed to amplify all but 17 nt of the DNA-A component of begomoviruses) (Table 3.1) (Briddon and Markham, 1994). The PCR conditions were; first cycle of denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1.5 min, extension at 72 °C for 2 min and a final extension cycle at 72 °C for 10 min. The amplified near full-length DNA-A fragments were excised from the gels and purified using the GeneJET Gel Extraction and DNA Cleanup Micro Kit (www.thermoscientific.com/onebio). The samples were selected based on the location to ensure representation of all the areas surveyed. The purified PCR products were sequenced by the dideoxynucleotide chain termination method using an ABI automatic sequencer on both orientations at the Macrogen Company, USA. The phylogenetic diversity of the different CMG isolates were analyzed using a partial DNA-A genomic region spanning AV1 and AV2 of the ORFs and the partial DNA-B genomic region spanning the N-terminal region of BC1 to the intergenic region (IR), respectively. The virus sequences were edited to obtain a consensus sequence for each using the editing and alignment software of CLC Main Workbench (CLC Bio, 2014). The sequences

were analysed by comparing to sequences available in the global National Center for Biotechnology Information (NCBI) GenBank (Table 3.2) using the basic alignment search tool (BLASTX) (Altschul *et al.*, 1997).

Table 3.1: List of the oligonucleotide primers used in this study for amplification of cassava mosaic geminiviruses from cassava samples collected during the 2013 survey in Zambia

Primer*	Nucleotide sequences (5'→3')	Virus species	Target region	Expected size (nt)
JSP001	(5'-ATGTCGAAGCGACCAGGAGAT-3')	ACMV	AV1/CP	774
JSP002	(5'-TGTTTATTAATTGCCAATACT-3')			
EAB555/F	(5'-TACATCGGCCTTTGAGTCGCATGG-3')	CMGs	DNA B (BCI/CR)	556
EAB555/R	(5'-CTTATTAACGCCTATATAAACACC-3')			
UNIF	(5'-KSGGGTCGACGTCATCAATGACGTTRTAC-3')	CMGs	DNA-A nfl[a]	2700 - 2800
UNIR	(5'-AARGAATTCATKGGGGCCCARARRGACTGGC-3')			

UNIF and UNIR: K = G + T, R = A + G, S = G + C.
*Cassava mosaic begomoviruses (CMB) specific primers used for the study (Fondong *et al.*, 1998; Briddon and Markham, 1994)
([a]nfl = near full-length)

Table 3.2: Geminivirus sequences used for comparisons and phylogenetic analysis, their genomic sequence accession numbers and assigned abbreviations from genebank

Virus	Accession number	Acronym
African cassava mosaic virus - [Cameroon]	AF112352	ACMV-[CM]
African cassava mosaic virus - [Cameroon-39]	AY211462	ACMV-[CM39]
African cassava mosaic virus - [Cameroon-DO2]	AF366902	ACMV-[CM/DO2]
African cassava mosaic virus - [Cameroon-Mg]	AY211884	ACMV-[CM/Mg]
African cassava mosaic virus - [Cameroon-DO3]	AY211885	ACMV-[CM/DO3]
African cassava mosaic virus - Uganda Mild	AF126800	ACMV-UGMld
African cassava mosaic virus - Uganda Mild 2	AY562429	ACMV-UGMld 2
African cassava mosaic virus - Uganda Severe	AF126802	ACMV-UGSvr
African cassava mosaic virus - Uganda [Namulonge]	AF423177	ACMV-[Nam]
African cassava mosaic virus - Madagascar	KJ887885	ACMV-[MG/MG310A1]
East African cassava mosaic Cameroon virus	KJ887667	EACMCV
East African cassava mosaic virus - Cameroon [Ivory Coast]	AF259897	EACMV-CM[CI]
East African cassava mosaic virus - Cameroon [Ghana]	JN165087	EACMV-CM[GH]
East African cassava mosaic Malawi virus - Malawi [MA]	JX658684	EACMMV-MW[MA:S8:07]
East African cassava mosaic virus - [Malawi]	AJ006461	EACMV-[MW]
East African cassava mosaic virus - Uganda [Kenya-K72]	AJ704974	EACMV-UG[KE-K72]
East African cassava mosaic virus - [Kenya-K35]	AJ704934	EACMV-[KE-K35]
East African cassava mosaic virus - [Kenya-KE2-K197]	AJ704973	EACMV-[KE2-K197]
East African cassava mosaic Kenya virus	NC_011584	EACMKV
East African cassava mosaic virus - Tanzania	AY795986	EACMV-[TZ]
East African cassava mosaic virus - Tanzania	AY800262	EACMV-[TZ]
East African cassava mosaic virus - Uganda2 Mild	AF126804	EACMV-UG2Mld
East African cassava mosaic virus - Uganda2 Severe	AF126806	EACMV-UG2Svr
East African cassava mosaic virus - Uganda3 Mild	AF126805	EACMV-UG3Mld
East African cassava mosaic virus - Uganda3 Severe	AF126807	EACMV-UG3Svr
East African cassava mosaic virus - [Zanzibar-K270]	AJ704947	EACMV-[ZB-K270]
South African cassava mosaic virus	AF155807	SACMV
South African cassava mosaic virus - [South Africa]	AF012824	SACMV-[SA]
South African cassava mosaic virus - [Zimbabwe]	AJ575560	SACMV-[ZW]
Indian cassava mosaic virus	AJ314740	ICMV

The begomovirus sequences from the database that had the highest similarity to each BLAST query sequence were selected for subsequent sequence similarity and phylogenetic analysis. Multiple alignments of the sequences was performed using MEGA version 5 (Tamura *et al.*, 2013).

3.2.2. Restriction fragment length polymorphisms (RFLP's)

The variability of CMG's in the purified PCR-amplified products was investigated by analyzing the RFLPs using *EcoR*V and *Mlu*I as described by Sseruwagi *et al*. (2004). The 61 near full length fragments of DNA-A PCR amplified products were digested with restriction enzymes *EcoR*V and *Mlu*I for 3 hours at 37 ºC and visualized on a 1.0 % gel-red-stained agarose gels by using the Biorad Gel Documentation System UV transilluminator.

Using predicted restriction patterns for these enzymes on CMG's in the GenBank (Table 3.3), the band pattern and fragments produced after digestion were then compared.

Table 3.3: Predicted RFLP's for published cassava mosaic geminivirus sequences after computer-based digestion of near full-length DNA-A with the restriction enzymes *Eco*RV and *Mlu*I (Sseruwagi *et al.*, 2004)

Enzyme	Fragment	ACMV-[NG]/[CI]/[CM]/[CM/DO2]	ACMV-UGMild/UGSvr/[KE]	SACMV	EACMV-UG2/UG2Svr	EACMV-UG2Mild	EACMV-[KE-K2B]/[MW]/[TZ]/EACMCV/[CI]
*Eco*RV	1[a]	1.48[b]	1.48	2.19	2.19	2.19	2.19
	2	1.28	1.28	0.59	0.59	0.59	0.59
*Mlu*I	1	2.76	1.55	1.21	1.21	1.21	1.21
	2	-	1.21	0.67	0.67	0.67	1.06
	3	-	-	0.52	0.52	0.46	0.52
	4	-	-	0.39	0.39	0.39	-
	5	-	-	-	-	0.06	-

3.3 Evaluating the effect of cassava mosaic geminiviruses (CMG's) on improved and local popular cassava genotypes

3.3.1 Experimental site, design and layout

The evaluation of cassava genotypes was conducted in Rufunsa district of Lusaka Province from December, 2012 to November, 2013, where high numbers of adult whiteflies were observed during surveys carried out by Chikoti *et al.* (2013a). Rufunsa has a large number of cassava farmers in Lusaka Province. It was therefore suitable for evaluation of CMD since it also had high CMD incidence and pressure as well a high number of whiteflies as reported for Lusaka Province by Chikoti *et al.* (2013a). The number of whiteflies present in an area has been used and considered as a proxy for the incidence and severity of cassava mosaic disease (Legg and Ogwal, 1998; Legg, 1999). The geographical position of Rufunsa district is $15^{o}5'0"$ south and $29^{o}38'0"$ east. It is located in Agro-ecological region I which lies between 300 and 900 metres above sea level (m.a.s.l) with the length of the growing season varying from 80 to 120 days. The mean annual rainfall does not exceed 800 mm (MAFF, 1997). Fewer parts in this region can reach up to 1200 m.a.s.l. The soils are loamy and clayey with course to fine loam top soils ranging from slightly acid to alkaline.

The treatments consisted of nine cassava genotypes from the Root and Tuber Improvement Programme at Mansa Research Station and included four improved genotypes (Mweru, Chila, Tanganyika and Kampolombo), four local landrace genotypes (Nalumino, Kapumba, Bangweulu and Katobamphunta) and a local popular landrace genotype (Manyopola).

The planting materials were healthy symptomless cuttings and were laid out in an experiment following the Randomised complete block design (RCBD) with four replications. The stem cuttings, each 30 cm long and having at least four nodes were planted in plots measuring 11 m x 7 m at a spacing of 1 m between plants and 1 m between rows. Out of the 77 plants, 44 plants were the healthy treatment plants consisting of 4 rows in each plot whilst 33 plants were CMD infected plants of the highly susceptible genotype (Bangweulu) used as a spreader to ensure that high CMD pressure prevailed in the evaluation plots and was therefore planted every after 2 row treatments in each plot. The experiment was carried out under rain fed conditions without applying pesticides and fertilisers and was kept weed free by regular hand-hoe weeding.

3.3.2 Data collection and analysis

Data on CMD foliar symptoms and whitefly abundance was collected at 2 months after planting (MAP). The experiment was left to grow in the field until harvest at 12 MAP. CMD severity was scored for each plant in the plot as described by Hahn *et al*. (1980). The rationale for categorizing resistance or susceptibility of the cassava genotypes was based on the % infection of the degree of susceptibility assessment using scoring systems described by Prasangika *et al.* (2008) as follows: 0 = highly resistant; 1 – 10 = resistant; 11 – 20 = less susceptible; 21 – 50 = moderately susceptible and > 50 = highly susceptible. The number of whiteflies was counted on the five fully expanded leaves. The leaves were held gently and turned over to count the whiteflies on the underside of the leaf. Counting of whiteflies was done from the same plants that were examined for

CMD incidence and severity. A total of 20 plants out of the 44 plants in each plot were sampled for each parameter measured. At 12 MAP, the height of each plant was measured from ground level to the highest shoot tip. The number of stems per genotype was also counted. Plants were harvested at 12 MAP and yield data were determined by harvesting each plant individually and taking records of the number of marketable and non-marketable tuberous roots and their weights. Marketable and non-marketable tuberous roots were separated based on size. Tubers weighing more than 100 g were considered marketable and those less than this weight non-marketable as described by Owor *et al.* (2004a). The total weight and mean number of tuberous roots for each treatment were determined and computed. CMD symptom severity, height of plants, number of stems and yield data were subjected to ANOVA to separate means using GenStat, 16th edition computer package (VSN International, 2013).

CHAPTER 4.0

RESULTS

4.1 Cassava mosaic disease incidence, severity and whitefly abundance

4.1.1 Symptom description

Cassava mosaic disease was detected in all the four Provinces surveyed in Zambia. The CMD symptoms varied widely among the infected plants in the field. The symptoms generally consisted of reduced plant height and leaf size, leaf narrowing, distorted leaf margins and patches of pronounced yellow and light green mosaic in severely diseased plants. Mildly infected plants showed sections of normal green and yellow mosaic pattern but without or with moderate leaf distortion.

4.1.2 Cassava mosaic disease incidence

Disease incidence was significantly lower ($P < 0.001$, $F = 9.07$, $df = 3$) in Eastern Province (43 %) compared to Western (73 %) and Lusaka (87.5 %) Provinces (Fig 4.1). There were no significant differences in the CMD incidences between Eastern (43 %) and Central (59.6 %), Central (59.6 %) and Western (73 %), and between Western (73 %) and Lusaka (87.5 %) Provinces. The average CMD incidence for all Provinces was 61.5 %. There were more cutting-borne infections than whitefly-borne infections in all the Provinces surveyed (Table 4.1). Lusaka Province had higher whitefly-borne CMD incidence (11.7 %, $P < 0.0001$) compared to Central (2.8 %), Eastern (6.3 %) and Western (2.5 %) Provinces. There were no significant differences in whitefly-borne CMD incidences between Central, Eastern and Western Provinces. Cutting-borne CMD

incidences were not significantly different between Central (56.8 %), Lusaka (75.8 %) and Western (70.5 %) Provinces.

Similarly, there were no significant differences in cutting-borne CMD incidence between Central (56.8 %) and Eastern (36.7 %) Provinces. However, in Eastern Province, cutting-borne CMD incidence was significantly lower than the incidences in both Lusaka (75.8 %) and Western (70.5 %) Provinces (Table 4.2). The results of the regression analysis tests describing the relationship between CMD incidence in the surveyed Provinces and the prevalent whitefly population showed no linear relationship between either total CMD incidence or incidence of whitefly-borne CMD and the adult *B. tabaci* whitefly abundance observed in the surveyed Provinces (Table 4.3).

4.1.3 Cassava mosaic disease severity

CMD severity did not vary significantly among the Provinces surveyed ($P < 0.001$, $F = 6.09$, $df = 3$). The average CMD severity across all the surveyed Provinces was 2.7. The mean CMD severity ranged between 2.57 and 2.88. The lowest severity (2.57) was recorded in Central Province and the highest (2.88) in Western Province (Figure 4.2)

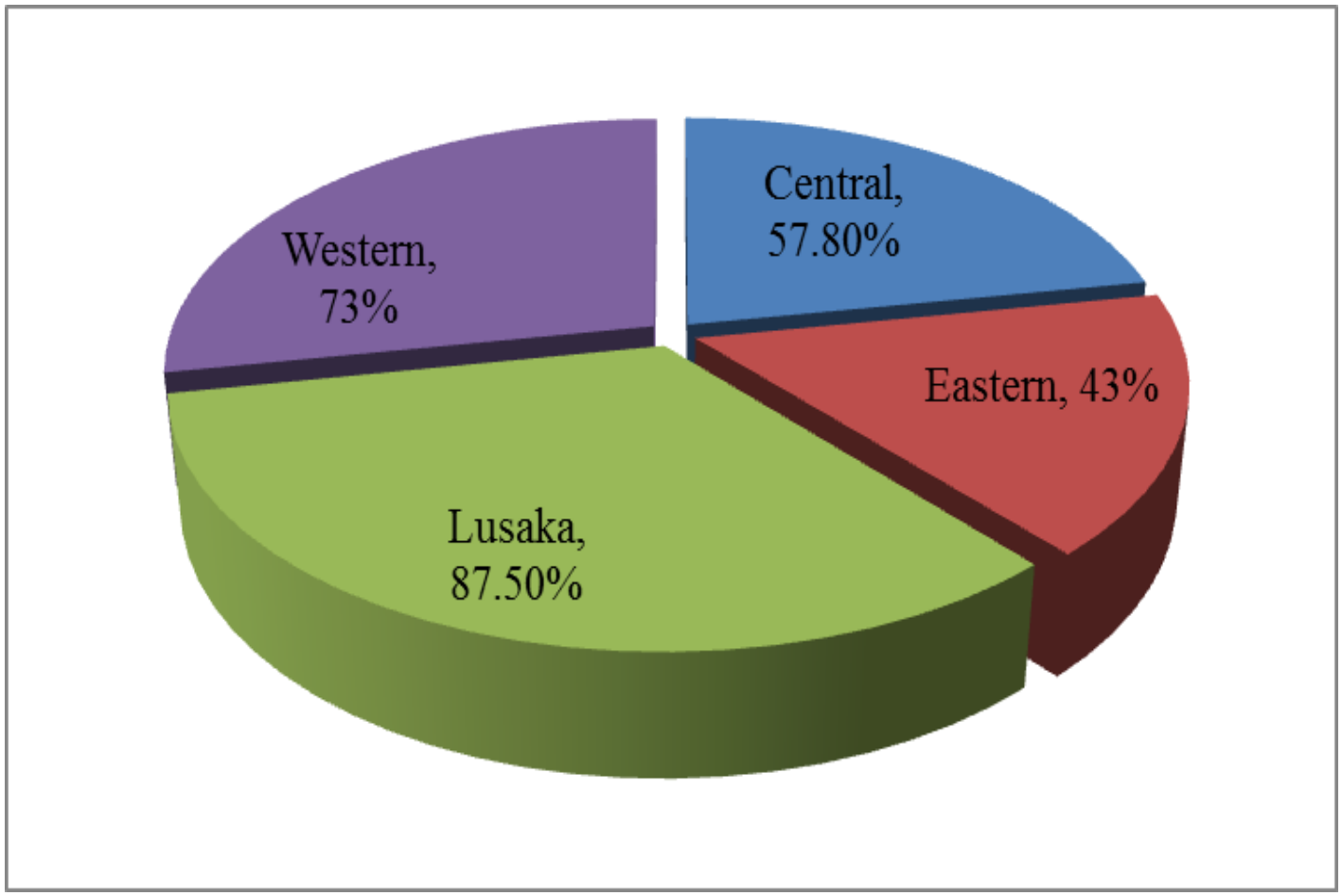

Figure 4.1: Proportion of incidence of cassava mosaic disease (CMD) in Central, Eastern, Lusaka and Western Provinces of Zambia (n = 88), February-March 2013. n = the total number of fields surveyed.

Table 4.1: Cassava infection type expressed as either whitefly (W) or cutting (C) on cassava (*Manihot esculenta*) plants sampled in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013.

Region	Cassava Mosaic Disease Infection Type	
	Mean whitefly infection (%)	Mean cutting infection (%)
Central	2.8	56.8
Eastern	6.3	36.7
Lusaka	11.7	75.8
Western	2.5	70.5
Mean	3.8	57.7
$LSD_{(0.05)}$	6.2	23.9
CV %	91.5	24.7
S.E.	2.2	4.6

Table 4.2: Average incidence of cassava mosaic disease and adult *Bemisia tabaci* abundance in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013.

	Cassava Mosaic Disease incidence			
Region	Total [a] (%)	Cutting (%)	Whitefly-borne (%) (transformed[b])	*B. tabaci* (max top 5 leaves)
Central	59.6	56.8	2.8 (6.6)	0.3
Eastern	43	36.7	6.3 (15.8)	1
Lusaka	87.5	75.8	11.7 (84.5)	5.6
Western	73	70.5	2.5 (8.7)	10.5
Mean	77	59.95	5.9	4.35
$LSD_{(0.05)}$	23.4	23.9	6.2	9.3

[a] Total Cassava Mosaic Disease incidence
[b] Percentage expressed as multiple infection units (Gregory, 1948)

Table 4.3: Correlation of cassava mosaic disease incidence and adult whitefly abundance in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013.

Parameter 1	Parameter 2	Coefficient	*P* value	Significance
CMD incidence (total)	*B. tabaci number*	0.32	0.239	ns
CMD whitefly-borne (transformed)	*B. tabaci number*	0.365	0.253	ns

ns = not significant at 5% level

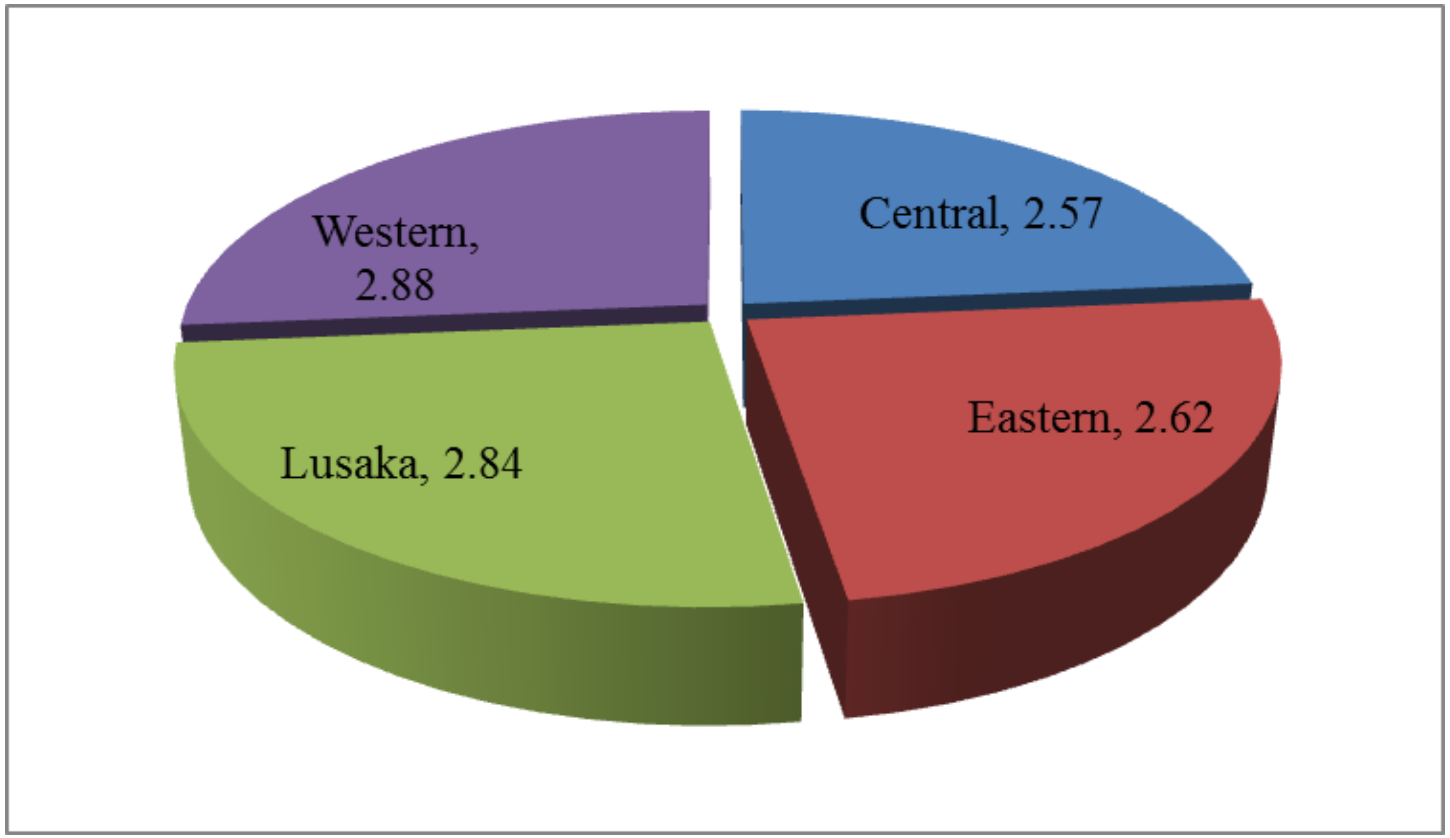

Figure 4.2: Proportion of cassava mosaic disease (CMD) severity on a scale of 1 - 5 in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013

The range of the average CMD severity from the sampled fields showed the highest and lowest CMD severity scores of 3.3 and 2.0 respectively with 25 % of the sampled fields having high severity score of above 3 (Table 4.4). The majority of the fields representing 48.9 % of the sampled fields had moderate severity scores ranging between 2.5 and 3. The low category severity scores where below 2.5 and accounted for 26.1 % of the sampled fields.

4.1.4 Cassava varietal effect

The local genotypes Nalumino and Manyopola had 72.9 % and 50.6 % mean CMD incidences with mean CMD severity scores of 2.9 and 2.7, respectively (Table 4.5). In Central Province, 88.6 % of the cassava genotypes sampled were local cultivars with the most predominant being Mwakamoya having 61 % CMD incidence and severity score of 2.6. Bangweulu, with CMD incidence of 69.2 % and severity score 2.6, was sampled in 11.4 % of the fields surveyed in Central Province. The most predominant and only genotype sampled in Lusaka and Eastern Provinces was Manyopola (Table 4.6). Nalumino, the most predominant genotype in Western Province, was grown in 74.2 % of the cassava fields sampled. The frequency of occurrences of the cassava genotypes from the sampled fields in the survey are presented in Table 4.6.

Table 4.4: CMD severity scores sampled from cassava fields in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March, 2013 (Score class adapted from Legg, 1999).

Mean CMD severity score range[a]	Designation of severity	Number of samples
> 3	High	22 (25)
2.5 - 3	Moderate	43 (48.9)
< 2.5	Low	23 (26.1)

[a] Severity scores ranged from 2 - 5 after editing to remove the symptomless (healthy) counts (score 1) (Hahn *et al.*, 1980). Numbers in parenthesis were percentage of samples for each severity score range out of the total number of samples

Table 4.5: Cassava mosaic disease incidence and severity on selected cassava genotypes grown in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March, 2013.

Genotype	Designation	Number of fields*	CMD Incidence (%)	CMD Severity
Nalumino	Landrace	23	72.9	2.9
Manyopola	Landrace	23	50.6	2.7
Mwakamoya	Landrace	21	61.0	2.6
Lingoma	Landrace	7	70.0	2.7
Bangweulu	Landrace	4	69.2	2.6
Bunganabutu	Landrace	1	90.0	2.8
Baba	Landrace	1	56.7	2.2
Masengu	Landrace	1	3.3	2.0
Tana	Landrace	1	60.0	2.4
Chalata	Landrace	1	3.3	2.0
Kalaba	Landrace	1	33.3	3.0
Kasomoloshi	Landrace	1	80.0	3.0
Unknown	-	3	66.7	2.4
Mean			55.2	2.6
SE			26.8	0.3

* Total number of fields sampled was 88

Table 4.6: The frequency of occurrence of different genotypes in Central, Eastern, Lusaka and Western Provinces, February-March, 2013.

Landrace	Occurrence in each region				Total
	Central	Eastern	Lusaka	Western	
Nalumino	-	-	-	23	23
Manyopola	1	18	4	-	23
Mwakamoya	21	-	-	-	21
Lingoma	-	-	-	7	7
Bangweulu	4	-	-	-	4
Bunganabutu	1	-	-	-	1
Baba	1	-	-	-	1
Masengu	1	-	-	-	1
Tana	1	-	-	-	1
Chalata	1	-	-	-	1
Kalaba	1	-	-	-	1
Kasomoloshi	1	-	-	-	1
Unknown	2	-	-	1	3
Total	**35**	**18**	**4**	**31**	**88**

4.1.5 Cassava mosaic geminiviruses (CMGs) detection using polymerase chain reaction (PCR)

Ninety-four percent (176/186) of the virus samples produced partial fragments of 774bp (DNA-A AV1/CP) and 556 bp (DNA-B), respectively (Fig. 4.3). ACMV occurred in 34.7 % (61/176) of the positive samples, while EACMV occurred in 4.5 % (8/176) of the positive samples. Co-infections of ACMV and EACMV were detected in 60.8 % (107/176) of the positive samples. Single infections of ACMV were high in Central Province (43.7 %) and low in Lusaka Province (11.1 %). Single infections of EACMV occurred in Central (5.6 %), Eastern (3.2 %), and Western Provinces (4 %). However, mixed infections of ACMV and EACMV varied among the Provinces with Central (36.6 %), Eastern (87.1 %), Lusaka (88.8 %) and Western Provinces (61.3 %) (Table 4.7). ACMV and EACMV were detected in all the Provinces (Central, Eastern, Lusaka and Western Provinces) and the geographical distribution of the respective viruses is shown in Fig. 4.4.

4.1.6 Whitefly abundance

Adult whitefly numbers ranged from 0.2 to 10.5 per plant. Western Province had the highest (10.5) and Central Province had the lowest (0.2) (Fig. 4.5) ($P < 0.001$, $F = 8.34$, $df = 3$). However, there were no significant differences between Central and Eastern Provinces. The average whitefly abundance across Provinces was 4.3.

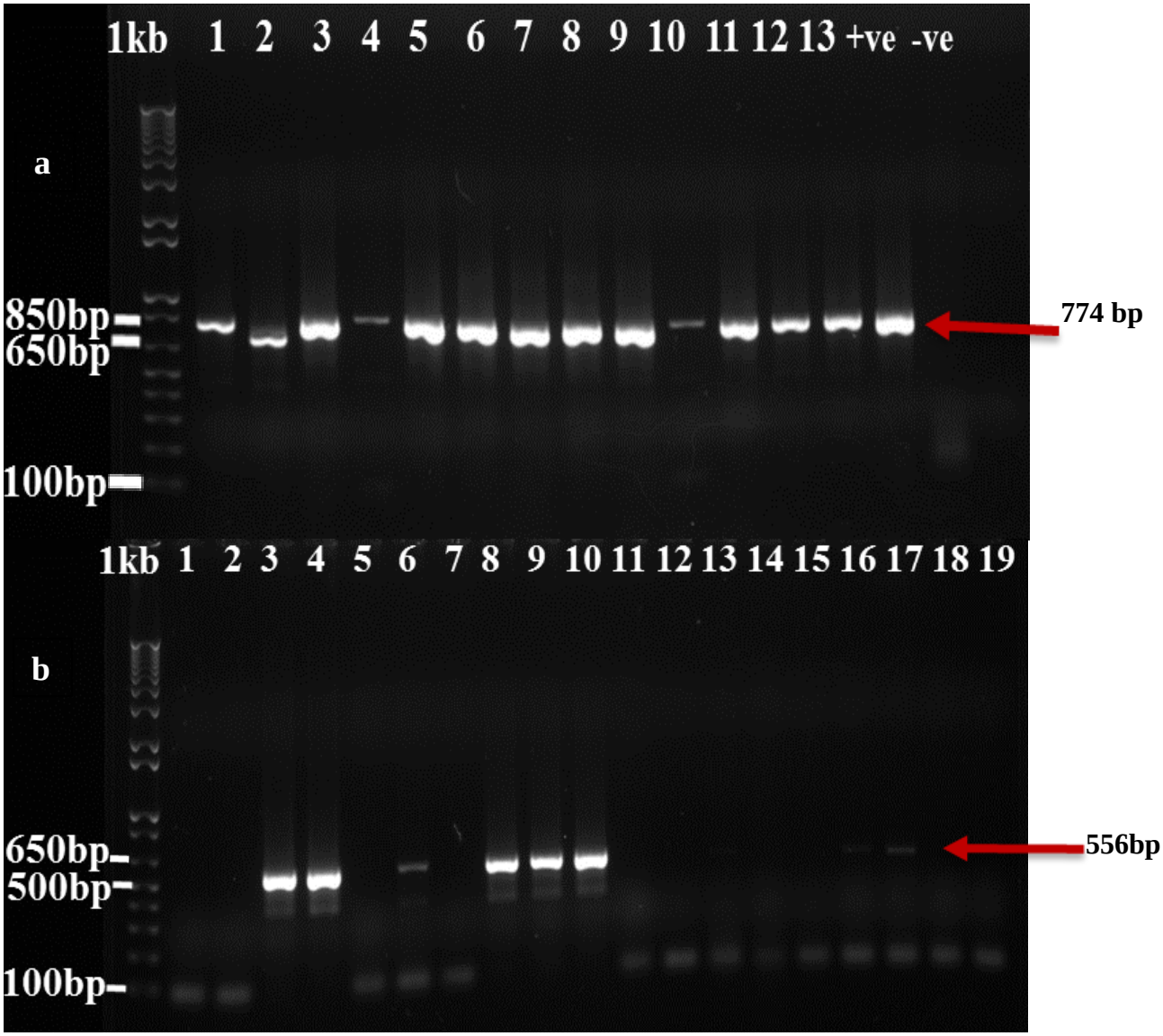

Figure 4.3: Gel electrophoresis of DNA fragments of representative Zambian isolates of (a) *African cassava mosaic virus* (774bp) and (b) *East African cassava mosaic virus* (556bp) using the specific primers JSP001/002 and EAB555F/R, respectively (Samples were collected from Central, Eastern, Lusaka and Western Provinces of Zambia, February-March, 2013).

Table 4.7: Cassava mosaic geminiviruses detected in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March, 2013.

Region	Cassava mosaic geminiviruses			Total
	ACMV	EACMV	ACMV + EACMV	
Central	31 (43.7)	4 (5.6)	26 (36.6)	61
Eastern	3 (9.7)	1 (3.2)	27 (87.1)	31
Lusaka	1 (11.1)	0 (0)	8 (88.8)	9
Western	26 (34.7)	3 (4)	46 (61.3)	75
Total	**61 (34.7)**	**8 (4.5)**	**107 (60.8)**	**176**

ACMV = *African cassava mosaic virus*; EACMV = *East African cassava mosaic virus*; ACMV + EACMV = both ACMV and EACMV present in the same plant.

Numbers in parenthesis represents the percentage of samples testing positive to the particular type of virus in each Province.

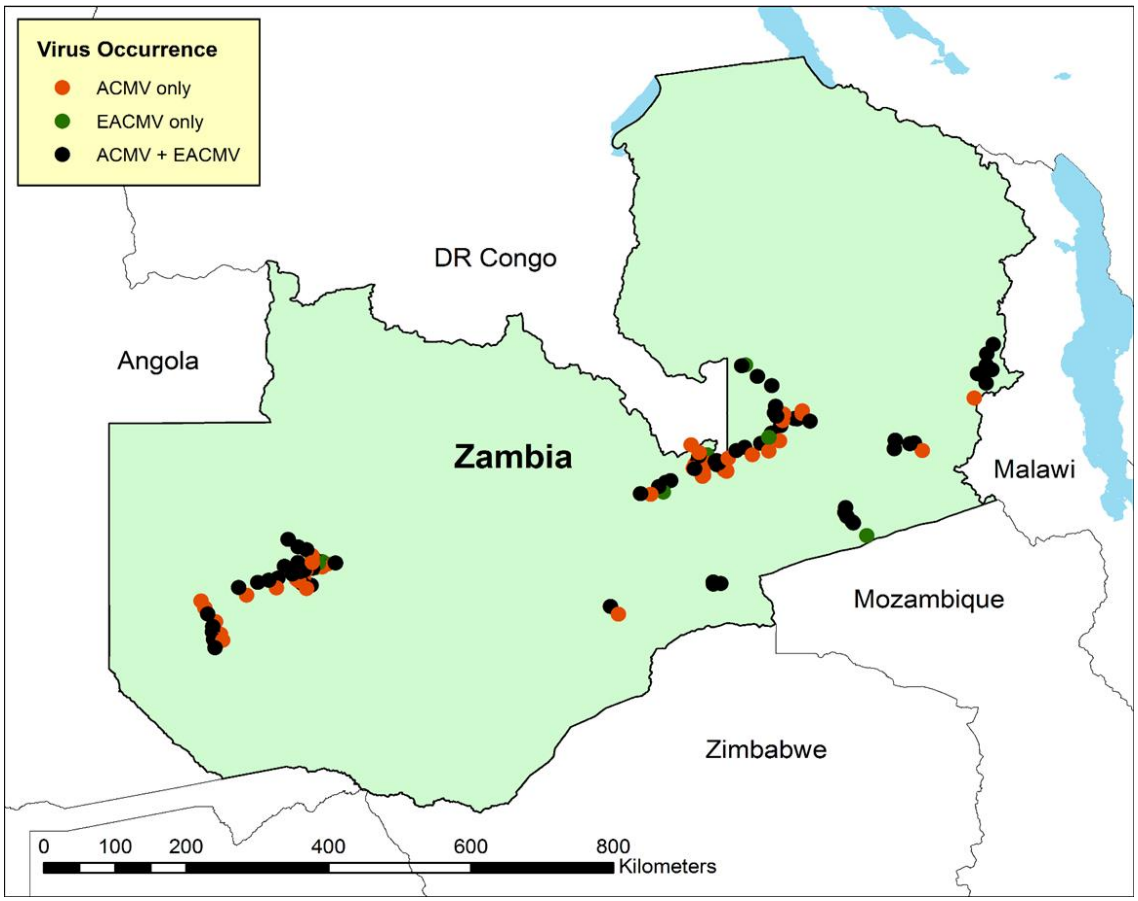

Figure 4.4: The geographical distribution of *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV) in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013.

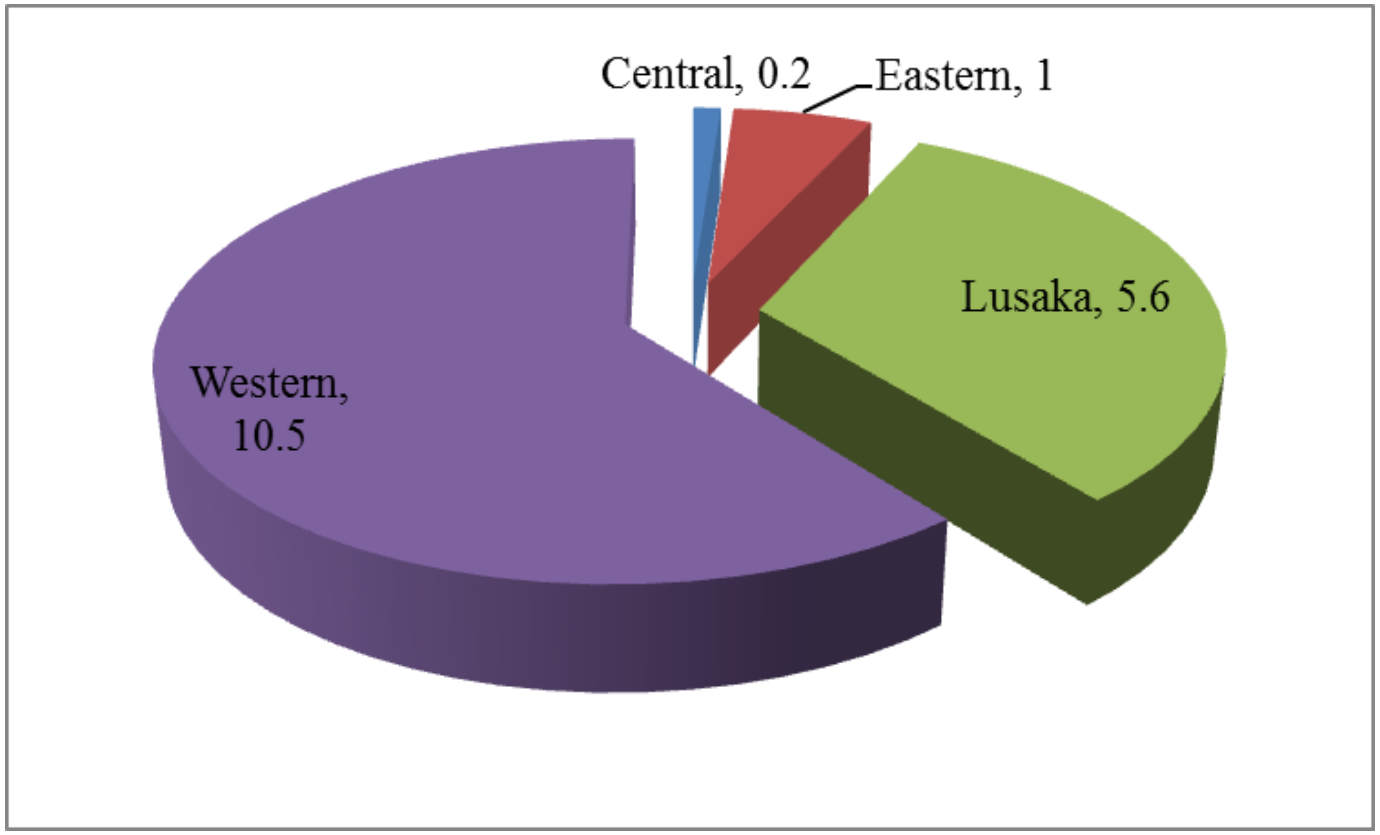

Figure 4.5: Proportion of average whitefly abundance observed during surveys in in Central, Eastern, Lusaka and Western Provinces of Zambia, February-March 2013.

4.2 Molecular diversity of cassava mosaic geminiviruses

4.2.1 PCR amplification and RFLP analysis of CMG variability in Zambia

The preliminary identification of the CMG's using differential primers JSP001/2 and EAB555F/R to distinguish between ACMV and EACMV, produced the amplification products as shown above in Figure 4.3. Ninety-four percent (176/186) of the samples tested positive for either ACMV or EACMV. Of the samples that tested positive, 60.8 % were dual infections (ACMV and EACMV). Single infections of ACMV and EACMV accounted for 34.7 % and 4.5 %, respectively. A total of 61 samples were randomly selected from the ninety-four percent that tested positive to CMG's from all the Provinces surveyed for RFLP. The selected samples where those that produced near full-length (c. 2760 - 2780 bp) DNA-A fragment after PCR amplification of CMG genomic DNA using the universal primers Uni/F and Uni/R (Fig. 4.6).

Restriction digestions with *EcoR*V produced a single banding pattern, characteristic of ACMV for the ACMV infected plants, for all digested samples, namely, two polymorphic fragments of approximately 1.48 and 1.28 kbp (Samples 27, 38, 41, 50, 51, 52, 53, 55, 57, 90, 95, 102, 109, 111, 126, 136, 149, 154, 167, 182, 187, 188 and 190). Restriction digestions with *Mlu*I produced two patterns which were distinct. One pattern, comprising a single uncut fragment of DNA-A (approximately 2.76 kbp), the same as that predicted for ACMV-NG, ACMV-IC, ACMV-CM and ACMV-DO2 (Fig. 4.7), was recorded for 29.5 % (18/61) of digested samples (designated as samples 27, 38, 41, 51, 52, 53, 55, 57, 90, 95, 109, 111, 136, 167, 182, 187, 188 and 190), mostly originating from Western and Central Provinces. A sample originating from Western

Province gave a banding pattern comprising two polymorphic fragment bands of approximately 1.55 and 1.2 kbp, as predicted for ACMV-KE, ACMV-UGSvr and ACMV-UGMld (designated as sample 50) (Fig. 4.7). Several samples produced distinct polymorphic fragments that did not correlate with any of the published CMGs and included the following: sample 102 from Central Province with two distinct polymorphic fragments (2.1 and 1.06 kbp), samples 126, 149 and 154 from Central and Western Provinces with two distinct polymorphic fragments (1.9 and 1.21 kbp). Samples 63, 65 and 77 from Eastern and Western Provinces were not cut by *EcoR*V while *Mlu*I did not cut samples 63 and 65, all from Western Province.

Similarly, restriction digestions with *EcoR*V produced a single banding pattern, characteristic of EACMV for the EACMV infected plants, for all digested samples, namely, two polymorphic fragments of approximately 2.19 and 0.59 kbp (Samples 16, 36, 78, 88, 91, 94, 131, 139, 144 and 160), mostly from Central and Eastern Provinces, denoting 16.4% (10/61) of digested samples. Additionally, there were several distinct polymorphic variants established for EACMV after restriction digestion with *Mlu*I. These were samples 94, 144 and 160 from Eastern and Lusaka Provinces, producing three polymorphic fragments (1.21, 1.06, 0.52 kbp). However, several samples produced distinct polymorphic fragments and did not correlate with any of the published CMGs and included: sample 16 from Central Province (1.8 and 1.21 kbp), sample 78 from Eastern Province with one distinct polymorphic fragment (2.1 kbp), samples 88, 131 and 139 from Central and Eastern Provinces with two distinct polymorphic fragments (2.1 and 1.06 kbp) and sample 91 from Eastern Province that

were not cut. Some samples consistently produced three polymorphic fragments (2.19, 0.59 and 0.50 kbp) with *Eco*RV digestion and (2.1 and 1.06 kbp) after *Mlu*I digestion that did not correlate with any of the published CMGs. The samples were designated as 87, 88, 89, 155, 158, 162, 169, 171 and 172 from Eastern, Luapula, Lusaka, Northern and Western Provinces. Mixed infections of ACMV and EACMV occurred in 19.7 % (12/61) of digested samples (20, 68, 71, 74, 75, 79, 113, 143, 146, 159, 163 and 184) from Eastern, Luapula, Lusaka and Northern Provinces, producing four distinct polymorphic fragments (2.19, 1.48, 1.28, 0.59 kbp) with the *Eco*RV digestion.

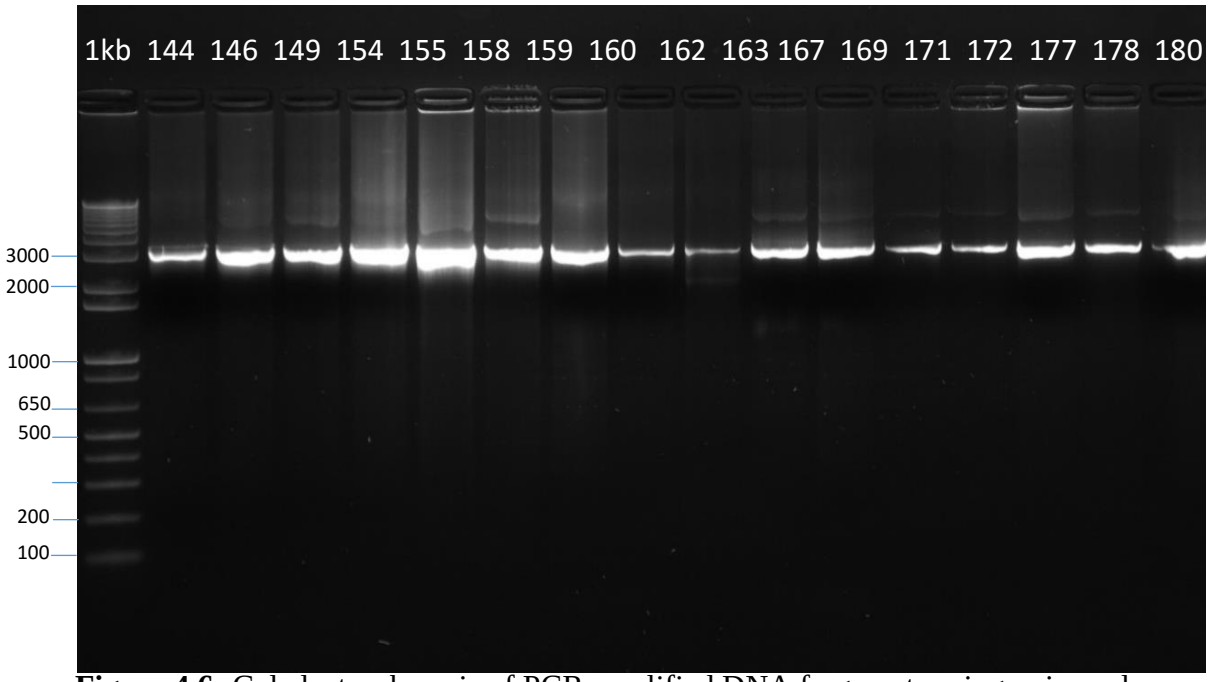

Figure 4.6: Gel electrophoresis of PCR amplified DNA fragments using universal oligonucleotide primers UNI/F and UNI/R for near full-length DNA-A after purification with the GeneJET Gel Extraction and DNA Cleanup Micro Kit (Samples are representatives collected from Central, Eastern, Lusaka and Western Provinces of Zambia, February-March, 2013).

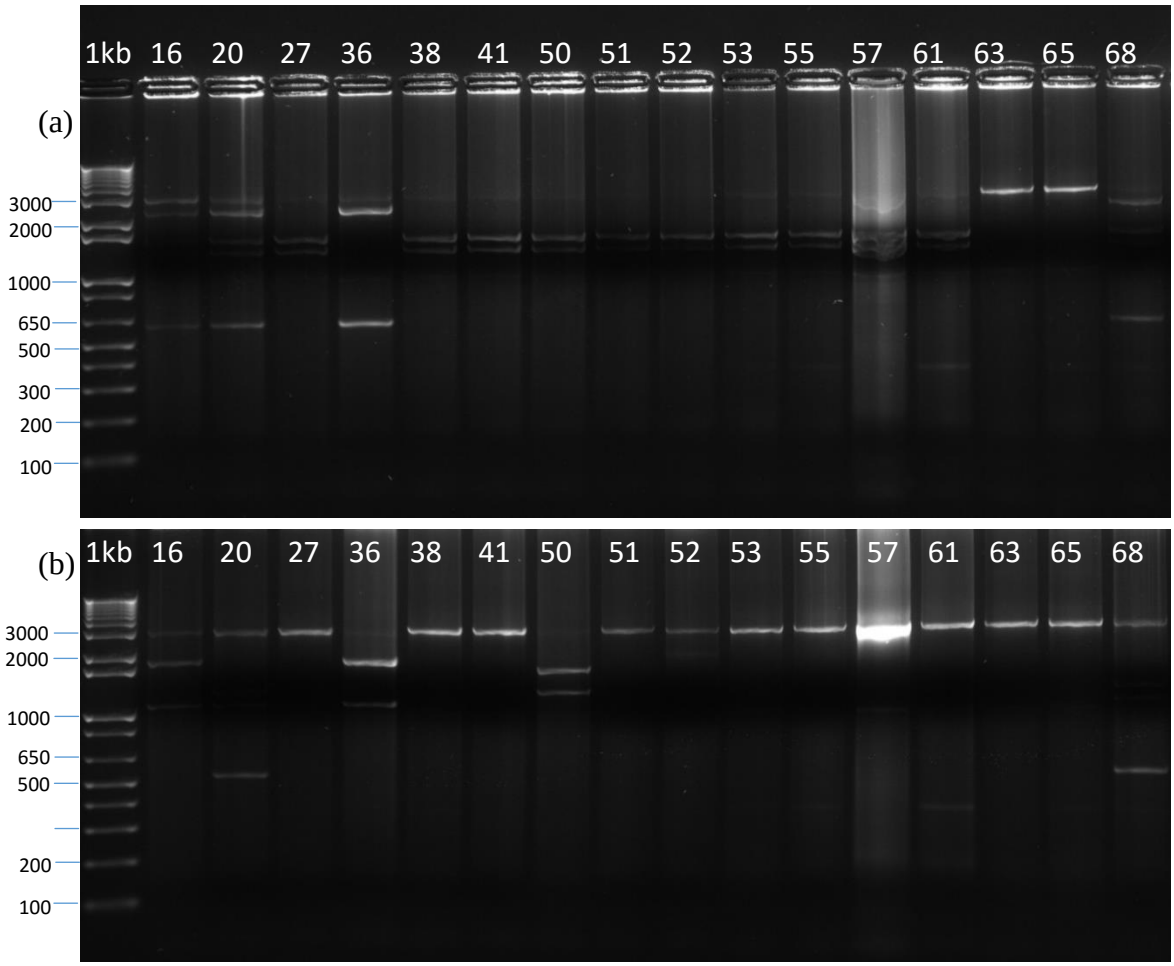

Figure 4.7: Restriction digestion of the PCR amplified products using (a) *EcoR*V and (b) *Mlu*I

4.2.2 Phylogenetic relationships and CMG sequences analysis

4.2.2.1 The phylogenetic diversity of ACMV DNA-A using partial sequences

The identities of the sequences of ACMV isolates with those of the corresponding DNA-A genomic regions of other CMGs in GenBank based on sequence alignment (BLAST) results, pairwise nucleotide identities and phylogenetic analysis, generally showed lower genetic divergence (Figure 4.8 and Table 4.8). The isolates collected from the different areas displayed high nucleotide (nt) sequence identities amongst one another with the isolate sequences showing identities of 89 % to 99 % (Figure 4.8 and Table 4.8). However, only one isolate, ACMV-MAT-19 dispalyed low nt sequence identity with the rest of the isolate sequences of between 70 % to 72 %. The isolate was distinct showing a close relationship with a South African isolate of 83 % nt sequence identity. This isolate appears different from the reported sequences in the global gene bank (NCBI) and therefore would require a full genome sequence that would reveal additional novel features to allow for its suitable classification either as ACMV or as a unique species. Further comparison with the sequences in the NCBI genebank by multiple alignment of the coat protein gene, the Zambian isolates used in this study showed substantial homology with sequences of ACMV-UGMild Uganda (AF126800.1), ACMV-UGSvr Uganda (AF126802.1), ACMV-[MG:MG310A1] Madagascar, and ACMV-CM39 Cameroon (AY211462.1) with sequence identities of 97 %, 97 %, 97 % and 98 %, respectively. The same observation is also depicted in the phylogenetic tree of ACMV with the Zambian isolates in the same branch and the others in the other branch on the phylogenetic tree generated (Fig. 4.8).

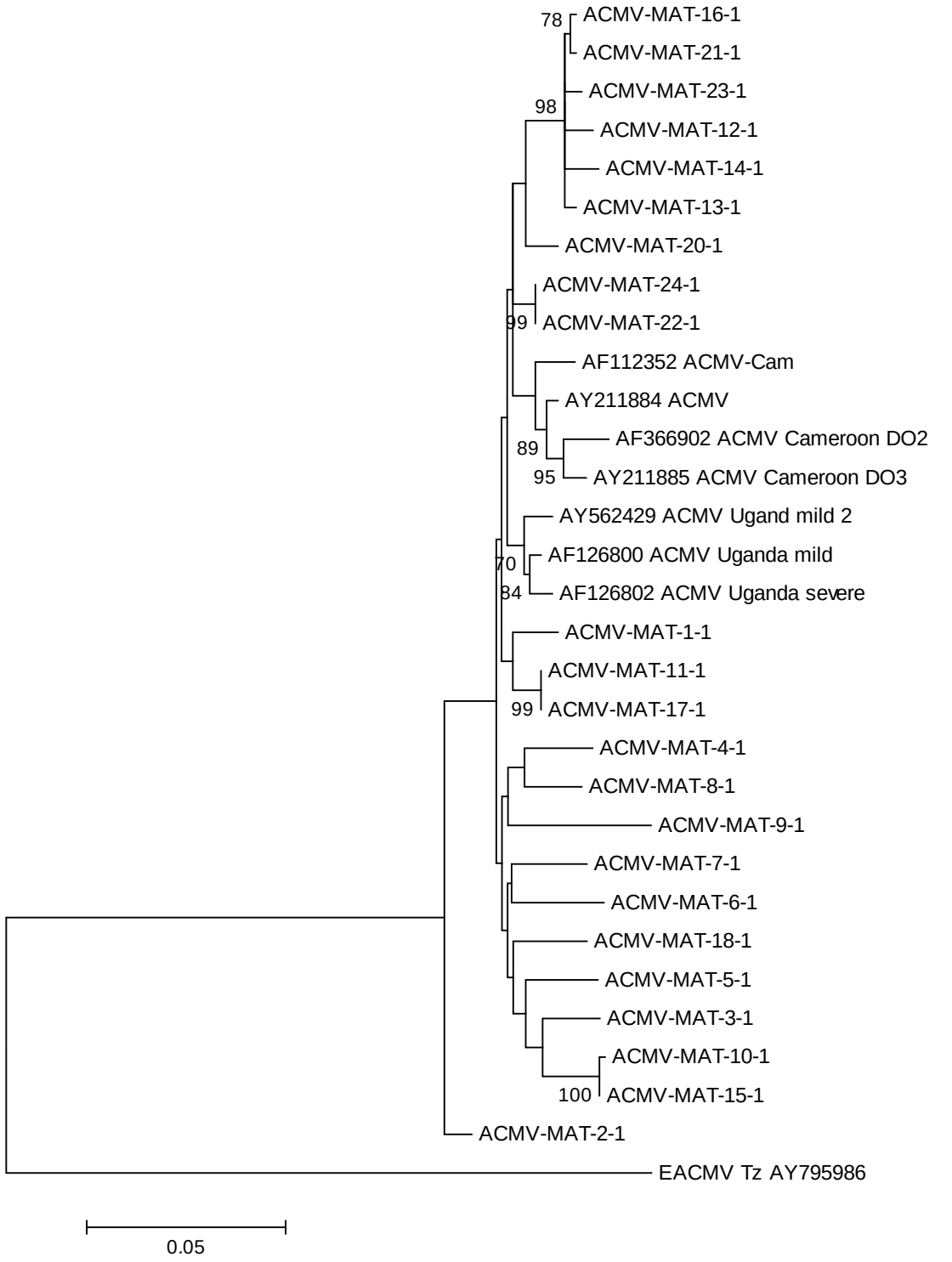

Figure 4.8: Phylogenetic tree (1000 boot strap replications) obtained from comparisons of the partial A component sequences from Zambia and available cassava mosaic geminivirus DNA-A component sequences based on the coat protein gene.

Table 4.8: Pairwise DNA-A nucleotide sequence homology matrix of 20 isolate sequences using the CLC Bio software

	Isolate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	ACMV-[CM/DO3]	100																			
2	ACMV-[CM/DO2]	97.7	100																		
3	ACMV-[CM]	96.4	97.1	100																	
4	ACMV-UGMld	94.8	95.8	95.8	100																
5	ACMV-UGSvr	95.1	96.0	95.8	97.3	100															
6	ACMV-UGMld	44.4	45.0	44.8	45.8	45.7	100														
7	ACMV-[Nam]	26.9	27.1	27.3	27.8	27.7	59.2	100													
8	ACMV-24-Petauke	26.2	26.4	26.6	26.8	26.7	57.2	90.8	100												
9	ACMV-11-Mambwe	26.3	26.6	26.7	26.9	26.8	57.7	91.4	96.6	100											
10	ACMV-13-Rufunsa	25.6	25.8	26.0	26.3	26.2	55.9	90.2	96.0	95.2	100										
11	ACMV-16-Lundazi	26.1	26.4	26.4	27.0	26.9	56.6	89.1	94.3	94.7	96.3	100									
12	ACMV-23-Rufunsa	26.3	26.3	26.6	27.0	27.0	56.6	89.4	94.9	94.3	96.3	97.7	100								
13	ACMV-2-Kaoma	26.0	26.2	26.4	26.6	26.5	56.7	89.1	95.6	95.0	94.1	92.4	93.1	100							
14	ACMV-3-Mongu	25.8	26.0	26.2	26.4	26.3	66.1	89.6	94.7	93.4	93.5	91.1	91.6	92.8	100						
15	ACMV-7-Senanga	25.8	26.0	26.3	26.8	26.3	58.2	87.3	92.8	94.0	92.0	94.3	93.5	92.3	91.0	100					
16	ACMV-6-Kaoma	25.7	26.0	26.2	26.4	26.5	56.2	89.6	95.5	96.1	94.3	93.6	93.1	94.4	93.7	93.4	100				
17	ACMV-9-Mongu	25.8	26.0	26.2	26.3	26.2	56.2	89.0	92.4	92.0	92.1	90.2	90.4	90.7	92.8	89.3	91.5	100			
18	ACMV-UGSvr	38.7	38.7	38.4	39.3	39.2	20.3	13.9	14.2	14.3	14.0	14.6	14.4	14.2	14.3	14.7	14.3	13.8	100		
19	ICMV	35.2	35.0	35.2	35.3	36.3	18.2	12.1	12.3	12.4	11.9	12.5	12.4	12.3	12.5	12.4	12.4	12.5	27.5	100	
20	ACMV-19-Lundazi	20.9	21.1	21.1	21.2	21.1	44.5	69.9	70.8	71.0	71.7	69.7	70.4	69.9	70.3	69.4	69.9	70.3	17.4	16.7	100

4.2.2.2 The phylogenetic diversity of EACMV DNA-B using partial sequences

The identities of the 31 sequences of EACMV isolates from this study with those of the corresponding DNA-B genomic regions of other CMGs in GenBank generally showed greater genetic divergence and isolates collected from the different areas (Fig. 4.9). Multiple sequence alignment and pairwise nucleotide sequence identity showed that the Zambian isolates have greater variability within the EACMV species (Fig. 4.9 and Table 4.9).

The phylogenetic analysis of the sequences from this study together with selected reference sequences revealed a clear partitioning into 10 clusters (Fig. 4.9) with a nucleotide sequence divergence ranging between 77 % to 99 % (Table 4.9). However, by comparing with the sequences in the NCBI genebank by multiple alignment of the coat protein gene, the Zambian isolates showed similarity to the Kenyan isolates (EACMV-KE), Malawian isolates (EACMMV) and Tanzanian isolates (EACMV-TZT) with sequence identities of 96 %, 90 % and 96 %, respectively. However, there was sequence identity to the isolates of EACMV-UG3-Mld at 84 %, EACMV-UG3-Svr at 86 %, EACMZV at 84 %, EACMCV at 80 % and SACMV at 89 %. The different clusters of the Zambian EACMV isolates obtained from this study (Fig. 4.9) appears different from the reported sequences in the NCBI and therefore, a full genome sequences would reveal additional novel features and allow the proper classification of these isolates to either EACMV or as unique species of EACMV.

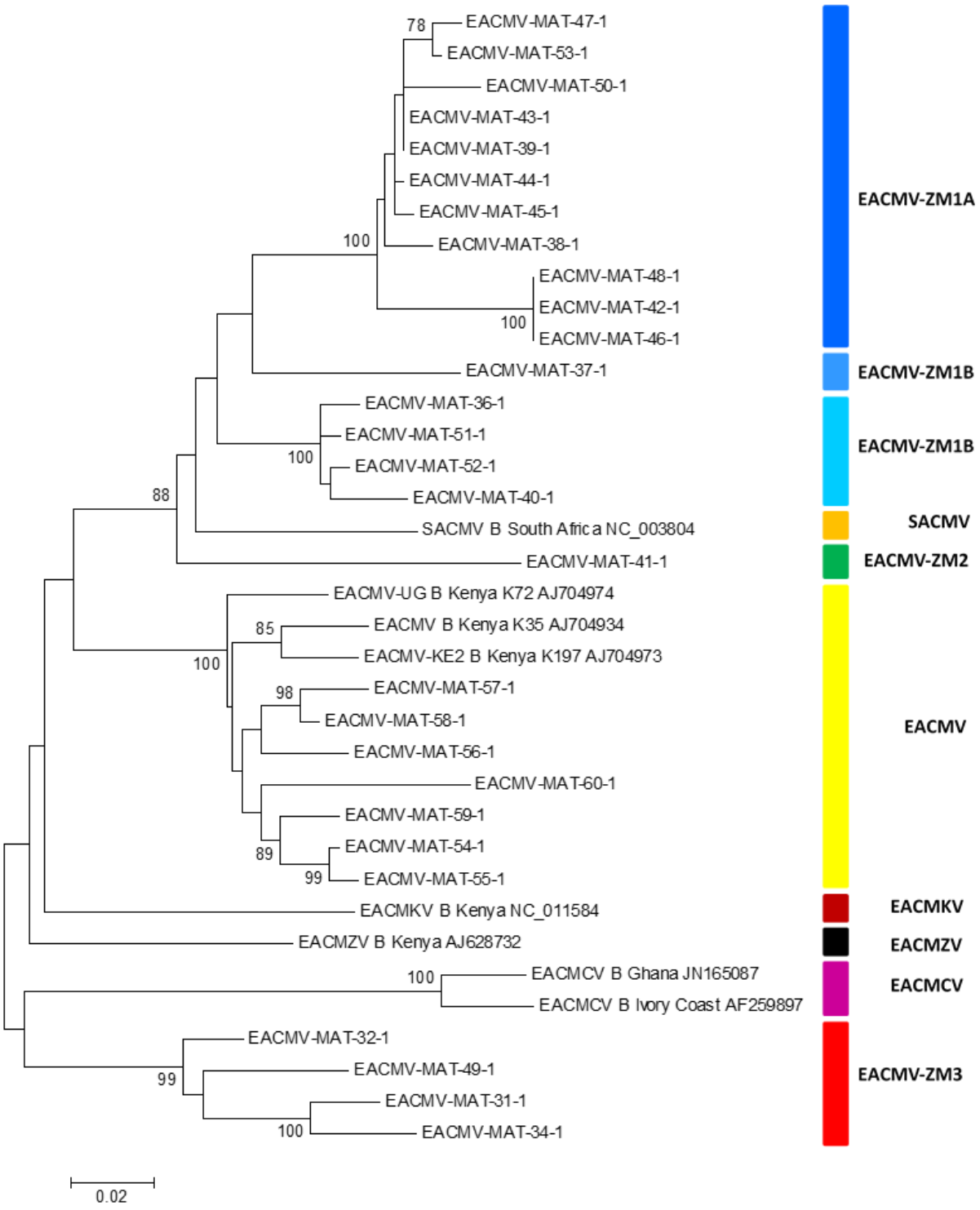

Figure 4.9: Phylogenetic tree (1000 boot strap replications) obtained from comparison of the partial B component sequences from Zambia and available NCBI genebank cassava mosaic geminivirus DNA-B component sequences.

Table 4.9: Pairwise DNA-B nucleotide sequence homology matrix of 16 isolate sequences using the CLC Bio software

	Isolate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	EACMV-42-Kaoma	100															
2	EACMV-44-Lundazi	93.7	100														
3	EACMV-52-Lundazi	87.5	90.7	100													
4	EACMV-37-Mwense	88.6	90.3	89.8	100												
5	EACMV-KE2[K208]	15.6	16.3	16.4	16.0	100											
6	EACMV-TZT	77.3	80.8	81.1	79.4	18.8	100										
7	EACMV-56-Mwense	81.7	83.6	85.3	82.8	17.7	87.9	100									
8	EACMV-57-Rufunsa	81.8	84.5	84.4	82.7	17.6	87.8	91.1	100								
9	EACMV-41-Petauke	86.0	86.2	87.1	86.2	16.1	78.4	81.5	82.0	100							
10	EACMV-34-Lundazi	78.8	82.0	83.3	81.4	16.0	79.1	81.6	83.6	79.7	100						
11	EACMV-UG2-Svr	9.3	9.5	9.7	9.5	17.8	9.2	9.2	9.6	9.8	9.3	100					
12	EACMV-UG2-Mld	9.4	9.6	9.7	9.6	17.7	9.2	9.3	9.7	9.8	9.2	99.3	100				
13	SACMV-[ZW]	9.0	9.1	9.3	9.2	17.6	8.7	8.9	9.3	9.4	9.0	79.2	79.2	100			
14	EACMMV	1.4	1.8	1.9	1.8	17.0	1.4	1.6	1.9	2.1	1.6	31.3	31.3	28.7	100		
15	ICMV	8.4	8.4	8.8	8.5	30.9	8.8	8.5	8.6	8.8	8.2	16.3	16.2	16.0	16.2	100	
16	SACMV-[ZA]	17.8	18.4	19.0	17.8	3.7	17.8	18.9	18.6	17.8	18.6	3.0	3.1	2.7	0.3	3.0	100

4.3 The effect of cassava mosaic disease on improved and local popular cassava genotypes in Zambia

4.3.1 CMD symptom severity of cassava genotypes

CMD symptoms were observed on both the local and the improved genotypes. Highly significant differences ($P < 0.001$) in symptom severity was recorded among the cassava genotypes in the field experiment and ranged from low to high (Table 4.10). The highest CMD mean severity was recorded on Manyopola and Bangweulu (3.5), while the lowest was recorded on Kampolombo (1.7) (Table 4.10). However, there were plants that showed severe symptoms above 4 on a scale of 1-5. In general, the trend in symptom severity was consistent throughout the growing period with no significant reduction in disease severities observed in all of the varieties except for Kampolombo and Kapumba over the entire period of the experiment (Table 4.10).

4.3.2 Incidence of CMD on cassava genotypes

Analysis of variance (ANOVA) showed significant differences ($P < 0.001$) in the incidence of CMD among genotypes. (Table 4.11). The lowest CMD incidence (0.7 %) was recorded on Kampolombo at 6 MAP while the highest (97.5 %) was recorded in Manyopola. There was a progressive increase in incidence of CMD in the cassava genotypes followed by a decline occurring after 5 MAP in varieties Kapumba and Nalumino, indicating symptom recovery (Table 4.11). Similarly, some recovery was observed in plots planted with cassava genotypes: Chila, Mweru and Tanganyika. This, however, occurred only after 4 MAP. In Kampolombo, there was complete recovery at

6 MAP, whereas in Bangweulu, Katobamphunta and Manyopola, spread was continuous (Table 4.11).

4.3.3 Whitefly abundance on cassava genotypes

The average number of adult whiteflies recorded differed among genotypes ($P < 0.001$) (Table 4.12). The numbers were generally high in all the genotypes at 2 MAP and progressively declined except for Chila and Katobamphunta (4 MAP). The highest average number of adult whiteflies was recorded on Mweru (32.1) and the lowest on Tanganyika (12). No correlation was found between either total CMD incidence or incidence of whitefly-borne CMD and the numbers of adult *B. tabaci* whitefly populations in the field experiment (Table 4.15). There was neither correlation found between above-ground biomass and CMD severity scores nor any relationship between the above-ground biomass and the numbers of the adult whitefly *B. tabaci* (Figure 4.10; Tables 4.13 and 4.15).

4.3.4 Above-ground biomass

The above-ground biomass was observed on both the local and the improved genotypes. Analysis of variance (ANOVA) showed significant differences ($P < 0.001$) in the above-ground biomass among genotypes ranging from 1.4 – 6.3 kg (Table 4.13).

Table 4.10: Disease severity scores on different cassava (*Manihot esculenta*) genotypes determined at different ages of growth infected by CMD in field experiment at Rufunsa, Lusaka district for the 2012/2013 cropping season.

Genotypes	Age (months)					
	2	3	4	5	6	Mean
Bangweulu	3.3	4	3.3	3.5	3.7	3.5
Chila	2.2	2	2.1	2.2	2.5	2.2
Kampolombo	1.0	3	2.0	2.0	1.0	1.7
Kapumba	2.4	3	2.6	2.8	2.4	2.6
Katobampunta	2.8	3	3.1	3.2	3.3	3.1
Manyopola	3.0	4	3.5	3.7	3.7	3.5
Mweru	2.2	2	2.1	2.2	2.4	2.2
Nalumino	2.0	2	2.0	2.1	2.3	2.1
Tanganyika	2.4	2	2.1	2.2	2.4	2.3
Mean	2.4	3	2.5	2.6	2.6	2.6
$LSD_{(0.05)}$	0.3	0	0.3	0.3	0.3	
CV %	5.5	7	5.3	7.5	8.0	

Values are mean scores on a scale of 1- 5 where 1 = no symptoms and 5 = severe mosaic, distortion of entire leaf (Hahn *et al.*, 1980).

Table 4.11: Cassava mosaic disease (CMD) incidence on different cassava (*Manihot esculenta*) genotypes at different ages of growth at Rufunsa, Lusaka district during the 2012/2013 cropping season.

	CMD Incidence (%)					
Genotypes	Age (months)					
	2	3	4	5	6	Mean
Bangweulu	55	72.5	80	81.2	85	74.7
Chila	31.2	55	48.8	41.2	25	40.2
Kampolombo	0	1.2	1.2	1.2	0	0.7
Kapumba	51.2	87.5	93.8	87.5	71.2	78.2
Katobampunta	70	83.8	90	90	91.2	85.0
Manyopola	88.8	98.8	100	100	100	97.5
Mweru	41.2	68.8	66.2	65	40	56.2
Nalumino	21.2	36.2	56.2	38.8	15	33.5
Tanganyika	75	86.2	78.8	81.2	71.2	78.5
Mean	48.2	65.6	68.3	65.1	55.4	60.5
$LSD_{(0.05)}$	20.99	24.19	13.53	22.98	26.96	
CV %	6.2	6	9.1	9.5	7.3	

Values are average percentage incidence of CMD on a plant for each genotype

Table 4.12: Adult whitefly (*Bemisia tabaci*) abundance at different ages of growth in field experiment at Rufunsa, Lusaka district for the 2012/2013 cropping season.

Genotypes	Age (months)				
	2	3	4	5	Mean
Bangweulu	24.4	21.8	16.6	7.5	17.6
Chila	30.3	25.5	30.7	23.5	27.5
Kampolombo	36.9	20.2	18.2	16.8	23.0
Kapumba	26.5	21.8	4.6	2.8	13.9
Katobampunta	31.8	16.5	19.6	14.8	20.7
Manyopola	25.6	19.7	15.3	11.9	18.1
Mweru	39.6	35.3	28.5	24.9	32.1
Nalumino	28.7	40.5	17.5	14	25.2
Tanganyika	17.7	13.7	10.6	6	12.0
Mean	29.1	23.9	18	13.7	21.2
$LSD_{(0.05)}$	8.36	9.09	7.21	6.1	7.7
CV %	44.5	49.4	11.5	48	38.4

Values are average numbers of whiteflies per plant on each genotype

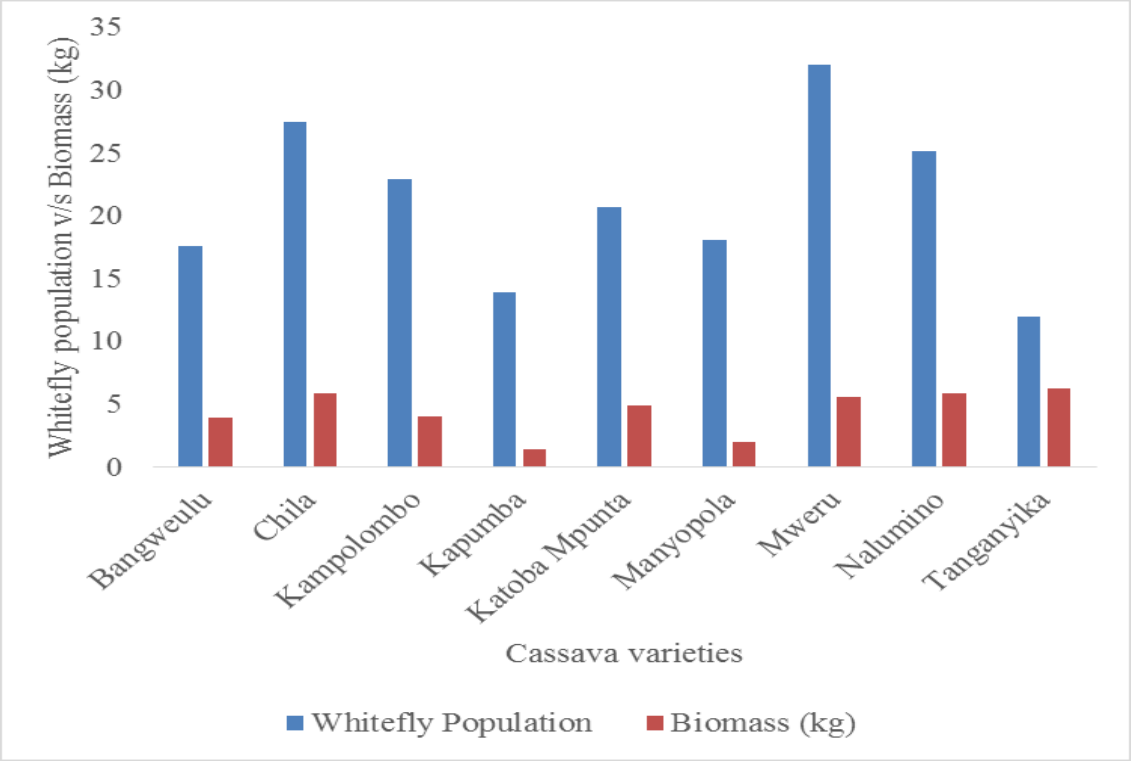

Figure 4.10: Relationship of the whitefly abundance against biomass of the different cassava genotypes at Rufunsa, Lusaka district during the 2012/2013 cropping season.

Table 4.13: Above-ground biomass, mean CMD severity and mean numbers of adult whitefly (*Bemisia tabaci*) abundance in field experiment at Rufunsa, Lusaka district for the 2012/2013 cropping season

Genotypes	Average attributes per genotype		
	Above-ground biomass (kg)	Mean CMD severity	Mean numbers of whitefly abundance
Bangweulu	4	3.7	17.6
Chila	5.9	2.5	27.5
Kampolombo	4.1	1	23
Kapumba	1.4	2.4	13.9
Katobamphunta	4.9	3.3	20.7
Manyopola	2	3.7	18.1
Mweru	5.6	2.4	32.1
Nalumino	5.9	2.3	25.2
Tanganyika	6.3	2.4	12
Mean	4.5	2.6	21.2
$LSD_{(0.05)}$	1.16	0.3	7.7
CV %	14.5	8	38.4

4.3.5 Yields of the cassava genotypes

There were significant differences ($P < 0.001$) in tuberous root weights produced for the different cassava genotypes in the trial. Mweru recorded the highest root yield (1.6 kg plant^{-1}) while Kapumba had the lowest (0.2 kg plant^{-1}) average root yield. In general, lower root yields were recorded in most of the CMD susceptible plants when compared to the less CMD susceptible varieties (Table 4.14). Only Mweru (1.6 kg), Chila (1.2 kg) and Kampolombo (1.1 kg) produced significantly ($P < 0.001$) higher root yields above the overall average root yield of 0.8 kg plant^{-1} across the cassava genotypes observed (Table 4.14). Mweru produced a significantly higher percentage of marketable roots at 36.7 % whilst the lowest percentage of marketable roots was recorded on Tanganyika with 12.5 % (Table 4.14). There was however, a significant negative correlation between the root weights and CMD symptom severity (Table 4.15).

Table 4.14: Average yields per plant for the different cassava (*Manihot esculenta*) genotypes 12 months after planting in field experiment at Rufunsa, Lusaka district for the 2012/2013 cropping season

Genotypes	Average numbers and weights of root tubers			
	Total yield (kg)	Weight of marketable yield (kg)	Number of tuberous roots	Percentage number of marketable roots
Bangweulu	0.9	0.6	5.5	25.5%
Chila	1.2	0.8	8.6	31.4%
Kampolombo	1.1	0.8	6.2	33.9%
Kapumba	0.2	0.1	1.7	17.6%
Katobampunta	0.6	0.3	5.9	16.9%
Manyopola	0.3	0.2	2.6	23.1%
Mweru	1.6	1.2	9	36.7%
Nalumino	0.7	0.3	6.8	14.7%
Tanganyika	0.6	0.2	7.2	12.5%
Mean	0.8	0.5	5.9	
$LSD_{(0.05)}$	0.27	0.2	1.4	
CV %	11.5	18.1	4.8	

Table 4.15: Correlation of Root weight, CMD incidence, above-ground biomass and CMD severity and numbers of adult whitefly (*B. tabaci*)

Parameter 1	Parameter 2	Coefficient	*P* value	Significance
Root weight	CMD severity	-0.16	<0.001	*
Above-ground biomass	CMD severity	-0.508	0.528	ns
CMD incidence (total)	*B. tabaci number*	-0.036	0.897	ns
CMD incidence (transformed)	*B. tabaci number*	-0.105	0.885	ns
Above-ground biomass	*B. tabaci number*	0.124	0.226	ns

ns = not significant at 5% level

* = Significant at P < 0.05 level

CHAPTER 5.0

DISCUSSION

Based on the results of this study, cassava mosaic disease was widely spread with incidences ranging from moderate to high in the surveyed Provinces. The average CMD incidence of 61.5 % recorded in the surveyed Provinces is an increase from the 52 % previously reported in Zambia (Chikoti *et al*., 2013a) and the 45 % reported in 1995/1996 (Muimba-Kankolongo *et al*., 1997; 1999). The increase in the average adult whitefly abundance levels of 4.3 per plant obtained from the surveyed Provinces compared to the 0.64 recorded by Chikoti *et al.* (2013a) may have contributed to the increase in CMD incidence. The super-abundant *B. tabaci* whiteflies reported in Zambia (Chikoti *et al.,* 2013b) and which have been associated with the spread of the severe CMD pandemic in East and Central Africa (Legg *et al.,* 2011) could have contributed to the increased CMD incidence. Other factors that could have contributed to the increased CMD incidence are the use of susceptible landraces and the recycling of planting materials. Similar observations were noted elsewhere (Mallowa *et al.,* 2006; Mabasa, 2007).

This study showed that there was no linear relationship between CMD incidence or incidence of whitefly-borne CMD and the numbers of adult *B. tabaci* whitefly abundance in the surveyed Provinces. Comparable results of cutting-borne CMD or whitefly numbers relating less well to changes in CMD caused by whiteflies have been obtained (Legg and Ogwal, 1998). A plausible explanation to this effect has been the non-direct correspondence between adult whiteflies numbers and whitefly-borne CMD

latent period (3-5 weeks) between inoculation of virus by whiteflies and symptom expression (Fauquet and Fargette, 1990). The cutting-borne infection (57.7 %) was predominant throughout the surveyed areas. This highlights the fact that the primary source of infection of CMD in farmers' fields is through the use of CMD-infected cuttings resulting from farmers recycling and exchanging infected cuttings. The findings are similar with other workers who reported cutting-borne infection as the primary source of cassava mosaic disease (Legg and Ogwal, 1998; Legg and Raya, 1998; Sseruwagi *et al.*, 2005; Thresh and Cooter, 2005; Prasangika *et al.*, 2008). Countrywide surveys in Tanzania indicated whitefly-borne incidence of 3.6 % compared to 27 % cutting-borne incidence (Legg and Raya, 1998) while in Kenya, 61 % CMD incidence was as a result of infection through planting diseased cuttings and 10 % as a result of whitefly infection (Mallowa *et al.*, 2006). In the Republic of Congo, whitefly-borne infections were 6.2 % compared to 74.2 % cutting-borne infection in 2002 while in 2003, 4.2 % incidence was attributed to whitefly-borne infection and 81.8 % due to cutting-borne infection (Ntawuruhunga *et al.*, 2007). In Uganda, CMD incidence due to whitefly-borne infections at the epidemic front was 80 % (Legg and Ogwal, 1998). The findings of this study shows that at low whitefly populations, CMD is primarily through farmers recycling infected material, although this may change if whiteflies become more abundant, as what happened in Uganda (Legg and Ogwal, 1998).

The regression analysis in this study indicate a non-linear relation between either the total CMD incidence or incidence of whitefly-borne CMD and the low adult *B. tabaci*

whitefly population. This implies that the variation in either total CMD incidence or incidence of whitefly-borne CMD was not attributed to the whitefly population. Therefore, the 68 % and 63 % variation in CMD incidence was due to other factors such as mainly the use of infected cuttings.

Disease severity was moderate with an average severity score of 2.7 in this study compared to the severity score of 3.41 obtained by Chikoti *et al.* (2013a). Elsewhere, especially in the CMD epidemic areas of East Africa, frequent severity scores of 3.0 and above have been recorded (Legg *et al.,* 2001). Several factors have been attributed to play a major role in disease severity. These include cultivar, virus strain/species, mixed infections, rainfall and the quality of soil (Fauquet and Fargette, 1990; Harrison *et al.,* 1997; Otim-Nape *et al.,* 1997; Fondong *et al.,* 2000; Pita *et al.,* 2001a, b; Ogbe *et al.,* 2003; Alabi *et al.,* 2008).

ACMV, which is known to induce mild symptoms compared to EACMV (Fondong *et al.,* 2000; Maruthi *et al.,* 2005), was the most occurring begomovirus infecting cassava in all the Provinces surveyed. However, there was a reduction from the 65.4 % ACMV of the positive samples recorded by Chikoti *et al.* (2013a) to the current 34.7 % recorded in this study. Similar observations have been reported in Uganda, Kenya, Republic of Congo and Madagascar (Harrison *et al.,* 1997; Colvin *et al.,* 2004; Karakacha, 2001; Ntawuruhunga *et al.,* 2007; Harimalala *et al.,* 2014). A high proportion of dual infections of ACMV and EACMV observed in this study compared to the study by Chikoti *et al.* (2013a) could have been as a result of a large number of

fields that were sampled. Secondly, the increased average whitefly abundance compared to the one recorded by Chikoti *et al.* (2013a) may have contributed to the significant increase of dual infections of ACMV and EACMV. Mixed infections have been implicated in viral DNA recombination that gave rise to EACMV-Ug (Zhou *et al.*, 1997; Pita *et al.*, 2001a). However, no sample tested positive for EACMV-Ug from the surveyed Provinces.

In Southern Africa, a number of cassava mosaic geminiviruses that include ACMV-UG, ACMV, EACMV and SACMV, have reportedly been in existence (Berry and Rey, 2001). In Zambia, both ACMV and EACMV have been reported to exist in both single and dual infections (Chikoti *et al.,* 2013a). PCR-RFLP analysis of the 61 begomovirus isolates revealed a greater diversity of restriction patterns than previously anticipated. There were 16 different RFLP classes identified of which most of them did not correspond to the predicted RFLP patterns of previously identified virus sequences available in Genbank. This level of diversity is also extremely higher than was found in a similar study carried out in Kenya (Bull *et al.,* 2006). However, the RFLP results from this study consistently showed ACMV as the predominant virus species in the four sampled Provinces. The ACMV from the surveyed Provinces in Zambia had high sequence relationships to all the other isolates of ACMV sequenced thus far from this study and were closely associated with sequences of ACMV isolates from Uganda (AF126800 and AF126802.1), Cameroon (AY211462.1) and Madagascar (KJ887885) with sequence identities of 97 %, 98 %, and 97 %, respectively. However, one isolate ACMV-MAT-19 from Lundazi, Eastern Province had low nt sequence identity when

compared with the rest of the ACMV isolate sequences used in this study ranging from 70 % to 72 %. The International Committee of Taxonomy on Viruses (ICTV) stipulates that geminiviruses of 89 % and below are of different strains and that those from 90 % to 99% are strains of the same virus. However, most of the Zambian ACMV isolates studied are strains of ACMV since they showed sequence identities of greater than 90 %. Previous studies in Kenya have found less genetic variability amongst viruses of this species (Were *et al.,* 2004; Bull *et al.,* 2006). The diversity of DNA-B components of EACMV from Zambia was also studied using partial DNA-B nt sequences (BC1-CR) of ~560 bp. Generally, there was distinct genetic divergence among the compared isolates represented by unique clustering that could reflect an even greater molecular diversity in the B components of CMGs in Zambia than we currently understand and know. The Zambian isolates showed similarity to Kenyan isolates (EACMV-KE), Malawian isolates (EACMMV) and Tanzanian isolates (EACMV-TZT) with sequence identities of 96 %, 90 % and 96 %, respectively. However, there was a sequence identity to the isolates of EACMV-UG3-Mld at 84 %, EACMV-UG3-Svr at 86 %, EACMZV at 84 %, EACMCV at 80 % and SACMV at 89 %. The different clusters of the Zambian EACMV isolates obtained from this study appears novel and therefore, full genome sequences would reveal additional novel features and allow for the suitable classification of these isolates to either EACMV or as unique species of EACMV. In this study partial sequence analysis was carried out. However, a full genome sequencing would allow for sequence analysis of nearly all the coat proteins that codes for all the functional characteristics of the virus.

From the results of CMD incidence and disease progress (Tables 10 and 11), there were significant differences in reaction to CMD. Manyopola variety showed high susceptibility and also confirms Rufunsa as a hot spot for CMD. The reaction of Kampolombo variety to CMD infection was much less, indicating its resistance under high disease pressure conditions. These results indicate the differential response to CMD infection as also reported by Lapidot and Friedmann (2002) and Ogbe *et al.* (2002). Despite the substantial inoculum pressure in the susceptible genotypes, the resistance in Kampolombo was apparent. The results are consistent with other observations of TMS genotypes from IITA showing considerable resistance to infection with CMD (Hahn *et al.,* 1989; Otim-Nape *et al.,* 1998; Ogbe *et al.,* 2003). Chila, another improved variety crossed with IITA parent material was moderately susceptible. The other varieties derived from IITA parent materials, Mweru and Tanganyika, were all susceptible. These observations are consistent with similar observations attributing tolerance to CMD infection (Ayeh and Ramsell, 2008).

The findings of this study showed no correlation between total CMD incidence or incidence of whitefly-borne CMD and the numbers of adult *B. tabaci* whitefly populations among the cassava varieties. The cassava varieties also differed in whitefly infestation, confirming previous findings elsewhere (Otim-Nape *et al.,* 1998; Omongo, 2003). Mweru had the highest whitefly populations which was not associated with the CMD spread in the variety. The lowest whitefly populations were found in the variety Tanganyika and yet had a significantly higher CMD incidence which was not significantly different to the overall highest CMD incidence obtained in the susceptible

variety Manyopola. These findings equally indicate varietal preferences by whiteflies as reported previously (Fauquet *et al.,* 1987; Otim-Nape, 1993; Otim-Nape *et al.,* 1996).

The results of this study provide evidence as reported by other workers suggesting that the resistance to CMD as was observed in the variety Kampolombo, is not due to resistance, but could be due to the ability of the varieties to withstand inoculation/infection or suppression of the virus soon after its establishment (Hahn *et al.,* 1980; Fargette *et al.,* 1996). Further evidence has been provided suggesting that plant vigour plays a key role in the colonization of the plant by whiteflies (Adipala *et al.,* 1998; Byabakama *et al.,* 1997, 1999, Sserubombwe *et al.,* 2001). These findings are consistent with the present experiment based on the relationship of the amount of aboveground biomass which is associated with plant vigour and the corresponding whitefly population for each variety. Therefore, the results showed Mweru with high populations of whiteflies which could at least in some part be explained by the vigour of this variety that maintained a significantly high amount of aboveground biomass even though it was classified as being highly susceptible to CMD. However, results obtained elsewhere on spread of CMD in improved varieties found a lack of association between whitefly populations and CMD spread (Legg *et al.,* 2003; Sserubombwe *et al.,* 2001).

In this study, as observed previously (Otim-Nape et al. 1994; Thresh et al. 1994b; Owor et al. 2004a), there was a linear and negative relationship between CMD symptom severity and tuberous root weight. The cassava varieties gave moderate yields of between 0.2 and 1.6 kg per plant (approximately equivalent to 2-16 t/ha). The improved

varieties generally had higher yields than the CMD susceptible variety, Manyopola. Results indicated the negative effect of CMD infection on yield for all the tested varieties. However, the findings also revealed that high yields can be obtained from improved varieties even though the plants get infected with CMD. Many CMD-resistant varieties are known to become infected by CMD yet express mild symptoms that have little significant impact on yield (Cours 1951, Thresh et al. 1994b). This observation has been attributed to the possibility that slightly or mildly CMD symptomatic plants improve partitioning of assimilates between roots and aerial parts of the plant (Cours 1951). Mildly diseased plants are characterized by chlorotic areas that are smaller, less intensely yellow and distributed more sparsely than the mostly conspicuous symptoms of severely CMD diseased plants (Storey and Nichols 1938; Fargette et al. 1987). Associated with this, differing degrees of yield loss resulting from varied CMD severities have been attributed to the degree to which the metabolic and photosynthetic processes are affected (Chant et al. 1971; Cock 1978; Otim-Nape et al. 1994). It is this effect on photosynthesis and growth of the plant that has a detrimental effect on tuberisation (Owor et al. 2004a).

CHAPTER 6.0

CONCLUSION AND RECOMMENDATIONS

The high incidence of CMD and wide variation in distribution in the surveyed Provinces when compared to past surveys can be mainly attributed due to farmers recycling and exchanging of infected plant cuttings and increased whitefly abundance in Zambia. The CMD severity of 2.7 can be designated as moderate but increasing. The increased average severity can be due to different virus strains and the use of susceptible cassava genotypes.

The study established the existence of different CMG isolates in Zambia with some isolates resembling those reported previously in other countries. The CMG's from the surveyed Provinces in Zambia are similar to ACMV, ACMV-UG, ACMV-CM, ACMV-MG and EACMV, EACMKV, EACMMV and EACMV-TZT reported elsewhere. The ACMV isolates from the surveyed Provinces showed less variability between them but having a high sequence identity of 96% to the ACMV-Uganda mild and severe isolates, hence signifying the potential of these Zambian isolates to cause severe forms of the CMD in farmers' fields. However, the Zambian isolates of EACMV species showed greater variability within the species with a high homology to Kenyan, Tanzanian and Madagascar EACMV strains.

The study showed different reaction of the genotypes to CMD in Zambia. Contrally to previous findings that cassava varieties are resistant, the conclusion from this evaluation indicate that most landraces and improved genotypes (released for cultivation) are

susceptible to CMD and hence highlighting the differential response of cassava genotypes' genetic ability to resist CMD infection and the negative effect of CMD infection on yield for all the tested varieties.

Most of the genotypes showed varying levels of recovery with progression of the season. The occurrence of such a phenomenon among the varieties tested offer options for their deployment in different epidemiological backgrounds.

Additionally, the development of resistant varieties should be complemented with an efficient seed system that include production and distribution of high quality and disease-free planting material from established disease free propagation and multiplication blocks. Further studies on the diversity of virus strains causing CMD in Zambia should continue to be exploited.

There is a need to understand and characterize the whitefly *Bemisia tabaci* observed in this study in order to determine any relationship to the ones that are associated with the cassava epidemics encountered in East and West Africa.

In addition to cassava genotypes evaluated in the present study, there is a need for more cassava genotypes to be included for evaluation across the three agro-ecological regions in Zambia over two or more seasons in order to confirm the CMD resistant cassava genotypes. This is because expression of CMD in different cassava genotypes is known to be dependent on the environment, host and the virus species.

CHAPTER 7.0

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CHAPTER 8.0

APPENDICES

Appendix 1: The cassava mosaic disease symptom scale of 1 - 5[a]

Scale	Symptom description
1	Unaffected shoots, no symptoms
2	Mild chlorosis, mild distortions at bases of most leaves, while the remaining parts of the leaves and leaflets appear green and healthy
3	Pronounced mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets
4	Severe mosaic distortion of two thirds of most leaves and general reduction of leaf size and stunting of shoots
5	Very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reductions of most leaves accompanied by severe stunting of plants

[a] **From Hahn *et al.* (1980).**

Appendix 2: UNIF-R PCR product order of sample loading on the plate for RFLP analysis

SERIAL NO.	DISTRICT	PROVINCE
1	Mwense	Luapula
2	Kasama	Northern
3	Senanga	Western
4	Serenje	Central
5	Serenje	Central
6	Serenje	Central
7	Kaoma	Western
8	Mongu	Western
9	Kaoma	Western
10	Kaoma	Western
11	Kaoma	Western
12	Senanga	Western
13	Senanga	Western
14	Mongu	Western
15	Kaoma-Luampa	Western
16	Mambwe	Eastern
17	Rufunsa	Lusaka

18	Rufunsa	Lusaka
19	Rufunsa	Lusaka
20	Mambwe	Eastern
21	Lundazi	Eastern
22	Mambwe	Eastern
23	Lundazi	Eastern
24	Lundazi	Eastern
25	Lundazi	Eastern
26	Lusaka	Lusaka
27	Petauke	Eastern
28	Rufunsa	Lusaka
29	Petauke	Eastern
30	Serenje	Central
31	Kaoma	Western
32	Kaoma	Western
33	Unknown	Unknown
34	Serenje	Central
35	Serenje	Central
36	Serenje	Central
37	Serenje	Central
38	Samfya	Luapula
39	Mwense	Luapula
40	Mwense	Luapula
41	Kaoma	Western
42	Kaoma-Luampa	Western
43	Lundazi	Eastern
44	Lundazi	Eastern
45	Rufunsa	Lusaka
46	Petauke	Eastern
47	Lundazi	Eastern
48	Mambwe	Eastern
49	Kaoma	Western
50	Lundazi	Eastern
51	Lundazi	Eastern
52	Lundazi	Eastern
53	Lundazi	Eastern
54	Rufunsa	Lusaka
55	Lundazi	Eastern
56	Rufunsa	Lusaka

57	Kaoma	Western
58	Kaoma	Western
59	Senanga	Western
60	Kaoma	Western
61	Kaoma	Western

Appendix 3: Partial PCR product order of sample loading on the plate for ACMV and EACMV sequencing

SERIAL NO.		SAMPLE IDENTITY	DISTRICT	PROVINCE
ACMV	1	ZAM 1	Senanga	Western
	2	ZAM 2	Kaoma	Western
	3	ZAM 3	Mongu	Western
	4	ZAM 4	Kaoma	Western
	5	ZAM 5	Kaoma	Western
	6	ZAM 6	Kaoma	Western
	7	ZAM 7	Senanga	Western
	8	ZAM 8	Senanga	Western
	9	ZAM 9	Mongu	Western
	10	ZAM 10	Kaoma-Luampa	Western
	11	ZAM 11	Mambwe	Eastern
	12	ZAM 12	Rufunsa	Lusaka
	13	ZAM 13	Rufunsa	Lusaka
	14	ZAM 14	Rufunsa	Lusaka
	15	ZAM 15	Mambwe	Eastern
	16	ZAM 16	Lundazi	Eastern
	17	ZAM 17	Mambwe	Eastern
	18	ZAM 18	Lundazi	Eastern
	19	ZAM 19	Lundazi	Eastern
	20	ZAM 20	Lundazi	Eastern
	21	ZAM 21	Lusaka	Lusaka
	22	ZAM 22	Petauke	Eastern
	23	ZAM 23	Rufunsa	Lusaka
	24	ZAM 24	Petauke	Eastern
EACMV	25	ZAM 31	Kaoma	Western
	26	ZAM 32	Kaoma	Western
	27	ZAM 33	Kaoma-Luampa	Western
	28	ZAM 34	Lundazi	Eastern

29	ZAM 35	Senanga	Western
30	ZAM 36	Kaoma	Western
31	ZAM 37	Mwense	Luapula
32	ZAM 38	Kasama	Northern
33	ZAM 39	Unknown	Unknown
34	ZAM 40	Samfya	Luapula
35	ZAM 41	Mwense	Luapula
36	ZAM 42	Kaoma	Western
37	ZAM 43	Serenje	Central
38	ZAM 44	Lundazi	Eastern
39	ZAM 45	Lundazi	Eastern
40	ZAM 46	Rufunsa	Eastern
41	ZAM 47	Petauke	Eastern
42	ZAM 48	Kaoma	Western
43	ZAM 49	Lundazi	Eastern
44	ZAM 50	Lundazi	Eastern
45	ZAM 51	Lundazi	Eastern
46	ZAM 52	Lundazi	Eastern
47	ZAM 53	Lundazi	Eastern
48	ZAM 54	Rufunsa	Lusaka
49	ZAM 55	Mwense	Luapula
50	ZAM 56	Lundazi	Eastern
51	ZAM 57	Rufunsa	Lusaka
52	ZAM 58	Rufunsa	Lusaka
53	ZAM 59	Lundazi	Eastern

Appendix 4: UNIF-R PCR product order of sample loading on the plate for full length sequencing

SERIAL NO.	IDENTITY	DISTRICT	PROVINCE
1	ZAM 31	Mwense	Luapula
2	ZAM 1	Senanga	Western
3	ZAM 2	Kaoma	Western
4	ZAM 3	Mongu	Western
5	ZAM 4	Kaoma	Western
6	ZAM 6	Kaoma	Western
7	ZAM 7	Senanga	Western
8	ZAM 9	Mongu	Western
9	ZAM 10	Kaoma-Luampa	Western
10	ZAM 11	Mambwe	Eastern
11	ZAM 12	Rufunsa	Lusaka
12	ZAM 13	Rufunsa	Lusaka
13	ZAM 14	Rufunsa	Lusaka
14	ZAM 20	Lundazi	Eastern
15	ZAM 22	Petauke	Eastern
16	ZAM 26	Kaoma	Western
17	ZAM 49	Mwense	Luapula
18	ZAM 27	Kaoma-Luampa	Western
19	ZAM 38	Lundazi	Eastern
20	ZAM 40	Rufunsa	Eastern
21	ZAM 41	Petauke	Eastern
22	ZAM 42	Kaoma	Western
23	ZAM 44	Lundazi	Eastern
24	ZAM 50	Lundazi	Eastern
25	ZAM 51	Rufunsa	Lusaka