MAKERERE UNIVERSITY

DISTRIBUTION OF MAIZE LETHAL NECROSIS DISEASE, ITS CAUSAL VIRUSES AND ALTERNATIVE HOSTS IN THE NORTH - CENTRAL REGIONS OF TANZANIA

BY

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DECLARATION

I, Allan Mariki, hereby declare that this dissertation is my original work and has never been submitted to any University or institution for the award of any degree. Furthermore, the dissertation contains no previously published data or material written by another party, unless specifically acknowledged, and the source being detailed in the references sections.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACMV	*African cassava mosaic virus*
AVRDC	Asian Vegetable Research and Development Centre
bp	Base pair
CIMMYT	International Maize and Wheat Improvement Center
CMD	Cassava Mosaic Disease
CP	Coat Protein
cv	Cultivar
DAC-ELISA	Direct Antigen Coating- Enzyme-linked Immunosorbent Assay
DAS-ELISA	Double Antigen Sandwich-Enzyme-linked Immunosorbent Assay
EACMV-UG	East African cassava mosaic virus-Uganda strain
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
GIS	Geographical Information System
GPS	Global Positioning System
ICTV	International Committee on Taxonomy of Viruses
IgG	Immunoglobulin G
IgM	Immuno-globulin M
IITA	International Institute of Tropical Agriculture
kb	Kilo base
kDa	Kilo Dalton
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
Mak	Makerere University Kampala
masl	Meters above sea level
MCDV	*Maize chlorotic dwarf virus*
MCMV	*Maize chlorotic mottle virus*
MDMV	*Maize dwarf mosaic virus*
MLN	Maize Lethal Necrosis
MMV	*Maize mosaic virus*
MRFV	*Maize rayado fino virus*

MSD	Maize streak disease
MSV	*Maize streak virus*
MV	Movement protein
NCBI	National Centre for Biotechnology Information
OMAF	Ontario Ministry of Agriculture and Food
SARI	Selian Agriculture Research Institute
ORF	Open reading frame
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
RdRp	RNA-dependent RNA polymerase
RdRps	RNA dependent RNA polymerases
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SCMV	*Sugarcane mosaic virus*
sgRNA	sub-genomic RNA
ssRNA	single-stranded RNA
TAS-ELISA	Triple-Antibody-Sandwich Enzyme-linked Immunosorbent Assay
USA	United State of America
USAID	United States Agency for International Development
USD	United States Dollars
VPg	Viral protein genome
WCM	Wheat curl mite
WSMV	*Wheat streak mosaic virus*

LIST OF SYMBOLS/UNITS

%	Percent
µl	Microliter
A	Absorbance
cm	Centimeter
g	Gram
h	Hour
k	Kilo
l	Liter
M	Mole
m	Meter
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mM	Millimole
ºC	Degree centigrade
OD	Optical density
pH	Hydrogen ion concentration
rpm	Revolutions per minute
sec	Seconds
t/ha	Tonne per hectare
v	Volume
w	Weight
µ	Micro
t	Tons

ABSTRACT

Maize is an important income generating food crop in Tanzania. However, yields remain low due to several limiting factors including among others diseases caused by fungi and viruses. The threat caused by several biotic factors in the country was further worsened with an outbreak of maize lethal necrosis (MLN) disease in 2012 in Arusha and Mwanza regions. Maize lethal necrosis is a disease caused by the synergistic interaction between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). Of these two, MCMV is a new virus in the African continent, first recognized in an MLN outbreak in Kenya, whereas SCMV is known to be endemic in Africa. This study focused on understanding the extent of MLN spread in north-central regions of Tanzania by conducting surveys between February and June, 2015. A total of 163 farmers' fields were sampled, in 14 districts in Arusha, Dodoma and Manyara regions. Incidence and severity were estimated based on MLN symptoms such as chlorosis, mottling and necrosis. The Enzyme – linked immunosorbent assays (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) methods were used for the detection of MCMV and SCMV in field samples. Disease severity assessed on a 1 to 5 rating scale varied from 2 to 3.6, with an overall mean incidence of 16.1%. Thirty nine percent of the samples tested positive to MCMV, 22% for SCMV and 5.5% for both MCMV and SCMV. A total of 254 non-maize crops and weeds tested for MCMV and SCMV, revealed SCMV in 7 samples (2.8%) in sugarcane (*Saccharum officinarium*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and bristly foxtail (*Setaria verticillata*). The nucleotide sequence of the coat protein region of MCMV showed very high levels of homology (99%) between MCMV from Tanzania and those from Kenya and other countries. However, the SCMV nucleotide sequence of the coat protein region was divergent by up to 11%, compared to other isolates. This study confirmed the occurrence of MLN in 13/14 districts surveyed and also showed that SCMV occurs in maize as well as other cereal hosts. Further study of pathogen diversity and factors contributing towards disease occurrence is recommended as interventions for disease management are developed and deployed at various levels.

CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 Importance, global production and uses of maize

Maize (*Zea mays* L.) is the most important and widely grown cereal in the world after wheat and rice (FAOSTAT, 2010). In Africa, the crop is produced in different parts of the continent under diverse climatic and ecological conditions. The white and yellow maize varieties are preferred by most people depending on the region. In 2014, 1021.6 million tons were harvested worldwide; of this, 77 million tons were harvested in Africa. The top four maize producers in Africa are South Africa (14.9 million tons), Nigeria (10.8 million tons), Ethiopia (7.2 million tons) and Tanzania (6.7 million tons) (FAOSTAT, 2015). By 2025 maize will become the crop with the greatest production globally and by 2050, the demand for maize in developing countries is expected to double (CIMMYT & IITA, 2010).

Maize is a major food crop in Africa. The grains are rich in 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3.0% sugar and 1.7% ash (Chaudhry, 1983). Due to richness in dietary fiber and calories, maize is a good source of energy for more than 1.2 billion people in Sub-Sahara Africa and Latin America. Maize is mainly used for three main purposes: as a staple food crop for human consumption, as a feed for livestock and as raw material for many industrial uses. It is widely prepared as either a solid or soft porridge. In the latter form, maize especially finds utility as baby food, often amended with supplements such as finger millet and groundnuts. All parts of the crop can be used for food and non-food products. In industrialized countries like United State of America, maize is largely used as livestock feed and as a raw material for industrial products like biofuel production (Hay, 2015). Maize grain makes a good biofuel feedstock due to its starch content and its comparatively easy conversion to ethanol. In USA maize-based ethanol production capacity in 2009 was 10.6 billion gallons (Hay, 2015).

1.1.2 Production and constraints to maize production in Tanzania

The crop is the main cereal grown in all regions of Tanzania, generating close to 50% of rural cash income at an average of 100 USD per maize producing household in 2008 (USAID, 2010).

By 2014, the crop was being produced in an area of 4.2 million ha, with a production of 6.73 million tons and productivity of 1.6 t/ha (FAOSTAT, 2015). Most of the maize is produced in the southern highlands (46%), Lake Zone and northern zone (10%).

In the past two decades Tanzania has ranked among the top 25 maize producing countries in the world, dropping out of the list only three times in 1986, 1997 and 2003 (Barreiro, 2012). Compared to other maize producing countries such as South Africa, and some of those in Asia and America, which produce average yields of 4 to 9 tons/ha (FAOSTAT, 2012) productivity in Tanzania is still relatively low. Also there has been notable decrease of maize yield in Manyara, Arusha and Dodoma regions in the past few years (Baha, 2013). The area planted with maize in these regions constitutes 60 to 70 % of the total area planted with annual crops. Maize yield decline has been attributed to many factors which include: poor agronomic practices, abiotic stresses (low soil fertility and drought), inadequate fertilizer use, pests such as the maize stalk borer (*Busseola fusca*), armyworms (*Spodoptera exempta*) and diseases such as maize streak disease (caused by *Maize streak viruses*). More recently, maize lethal necrosis (MLN) disease caused by the synergistic interaction of two co-infecting viruses, MCMV and SCMV is beginning to impact maize production (Wangai *et al.*, 2012).

1.2 Problem statement

The outbreak of MLN disease in East Africa was first reported in Kenya in 2011 where it caused up to 100% yield losses (Wangai *et al.*, 2012). By 2012, the disease had spread to Tanzania and Uganda (MAAIF, 2013 unpublished; Makumbi & Wangai, 2013). In Tanzania, the first reports of disease occurrence emerged from Mwanza and Arusha regions in the north-western and northern parts of the country, respectively. Survey conducted by CIMMYT and IITA in Manyara, Arusha, Dodoma and Mwanza regions revealed 17% incidence of MLN based on field observation, although the extent of disease spread was not clear. Serological tests detected MCMV and SCMV in selected samples from northern and north-western Tanzania, but the identity of causal viruses was not confirmed (Wangai *et al.*, 2012; Kumar *et al.*, 2012 unpublished). Reports of symptoms similar to MLN affecting maize in Mara, Shinyanga and Manyara in north-western and northern parts, respectively; and Singida and Dodoma regions in central Tanzania are also documented (MAFC, 2013). Information is also not available on alternative hosts, including weeds and other crops, harbouring MLN causal agents. Such hosts

may play an important role in off-season survival of the disease. For example in Hawaii MLN causing viruses have been reported to occur in alternate hosts such as *Panicum maximum*, *P. miliaceum, P. ditotomiflorum* and *Bromus molli (*Brunt *et al*., 2010). It is important to identify alternative host of MLN causing viruses for effective management of disease in the region.

1.3 Justification

Since the MLN epidemic was first recognized in 2012 in Tanzania the disease seems to be spreading both in the north-western and southern directions towards central Tanzania. Knowledge on the extent of spread, disease incidence and severity is, however, limited. Factors contributing to disease spread are also not clear. Disease identification, rapid and accurate diagnosis of causal agents is vital in mitigating disease outbreaks and prevalence (Kreuze *et al.*, 2009). Disease spread between 2012 and 2013 was thought to be due to the monocropping system that is predominantly used by maize farmers in Tanzania. Seed companies and farmers in the northern region predominantly use this cropping system in production of seed for distribution to farms throughout Tanzania (CIMMYT & IITA, 2014).

Monocropping is known to support the proliferation of pests, diseases and weeds. Weeds like wild grasses would, thus, act as alternate hosts together with cultivated crops such as sorghum playing a big role as reservoirs of disease between crop cycles since they are often found in maize fields. Confirmation of the alternative hosts for causal agents of MLN would help direct on-going efforts aimed at development of management strategies for the disease in Manyara, Arusha and Dodoma regions.

1.4 Objectives of the study

1.4.1 General objective

This study was done to establish the prevalence of MLN in the maize growing regions of north - central Tanzania.

1.4.2 Specific objectives

1. To establish the distribution of MLN disease and its causal viruses in north central Tanzania

2. To identify the alternative hosts of MLN disease causing viruses in north central Tanzania

1.5 Hypothesis

1. Maize lethal necrosis disease occurs in all districts within Manyara, Arusha and Dodoma regions of Tanzania, although the combination(s) of virus species causing the disease may vary.
2. Maize lethal necrosis disease affects maize and other grass species within Manyara, Arusha and Dodoma regions of Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin, classification and biology of maize

Cultivated maize is assumed to have been derived from teosinte (*Zea mexicana*) (Galinat, 1988). Archaeological records and phylogenetic analysis suggest that domestication began at least 6000 years ago although cultivation of the crop spread around the world after European discovery of the Americas in the 15th Century, particularly in the temperate zones (Matsuoka *et al.*, 2002).

Maize belongs to the family of grasses (*Poaceae*). It is a thick stemmed annual grass, usually with a single stem, one to four meters tall, with one or more tillers. It is monoecious and diclinous, with the male and female inflorescence on different flowers produced separately on the same plant. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 long, on a thickened, almost woody axis (cob). The whole ear is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock & Chase, 1971). Pollen is produced entirely in the staminate inflorescence and eggs entirely in the pistillate inflorescence. Maize is wind pollinated and both self and cross pollination is usually possible. Shed pollen remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe, Nueffer & Hoisington, 1988).

2.2 Ecology and agronomy of maize

Maize is adapted to grow over a wide range of agro climatic zones, it may be grown as a dry land or irrigated crop (Agbonifo & Olufolaji, 2012). It is grown from 58°N to 40°S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Dowswell, Paliwal & Cantrell, 1996). Generally maize is less water stress tolerant than other crops, including sorghum (Beckingham, 2007). The suitability of maize to diverse environments is incomparable to any other crop. Maize is planted when soil temperatures are warm (greater than or equal to 10 ^{0}C) (OMAF, 1994). Temperature of 5^{0} C to 7 ^{0}C may be followed by photo- inhibited physiological damage that may reduce photosynthetic rates for

several days thereafter. Maize plants grow best in well- draining, nutrient-rich soils, with a pH between 5.5 and 7.0.

2.3 Maize lethal necrosis disease

Maize lethal necrosis disease is caused by infection of maize with MCMV in combination with any of the cereal infecting viruses in the family Potyviridae such as SCMV, MDMV or WSM (Niblett & Claflin, 1978; Uyemoto, Bockelman & Clafin, 1980). Viruses from other families, including *Maize rayado fino virus* (MRFV), family Tymoviridae, genus *Marafivirus*, *Maize chlorotic dwarf virus* (MCDV), family Secoviridae, genus *Wakavirus* and *Maize mosaic virus* (MMV), family Rhabdoviridae, genus *Nucleorhabdovirus*, can also cause synergistic reactions in co-infection with MCMV. Abiotic stresses are also reported to exacerbate MCMV infection to cause MLN disease (Redinbaugh & Zambrano, 2014).

2.3.1 History of MLN disease

MCMV belongs to the genus *Machlomovirus* in the family Tombusviridae. The virus was originally identified in maize in Peru (Castillo & Hebert, 1974) and then in the United States in Kansas associated with either MDMV or WSMV causing MLN disease (Niblett & Clafin, 1978; Uyemoto *et al.*, 1980; Jiang, Wilkinson & Berry, 1990). It then spread to Nebraska (Doupnik, 1979). In USA MCMV is not widespread having only been reported in three states of Kansas, Nebraska and in 1992 MCMV was reported in Hawaii (Jiang, Meinke, Wright, Wilkinson & Campbell, 1922). At least two genetically and geographically distinct strains of MCMV have been reported, MCMV- P (Peru) and MCMV- K (Kansas) (Nyvall, 1999). In China it was identified in association with SCMV (Xie *et al*., 2011) and In East Africa MCMV was first identified in Kenya in association with SCMV (Wangai *et al*., 2012) (Table.1).

Table 1: Chronological reports of MCMV and associated potyviruses

Location	Year reported	Potyvirus[a]	Reference
Peru	1973	NR	Castilo & Herbert, 1974
United States, mainland	1976	WSMV/MDMV	Niblett & Claflin, 1978
Argentina	1982	NR	Teyssandier *et al*., 1981
Thailand	1982	NR	Klingkong & subabutra,1982
United States, Hawaii	1992	MDMV	Jiang *et al*., 1992
Colombia	1999	NR	Morales *et al*., 1999
China	2011	SCMV	Xie *at al*., 2011
Kenya	2012	SCMV	Wangai *et al*., 2012
Rwanda	2013	SCMV	Adams *et al*., 2014
DRC	2013	SCMV	Lukanda *et al*., 2014
Taiwan	2014	SCMV	Deng *et al*., 2014
Ethiopia	2015	SCMV	Mahuku *et al*., 2015

[a] potyviruses reported with MCMV: NR=Not reported: DRC= Democratic Republic of Congo; Source Mahuku *et al*., 2015

2.3.2 Genomic organization of causal agents of MLN

MCMV particles are non-enveloped, isometric shaped of about 28 to 34 nm in diameter, with virion composed of 180 polypeptide subunits. The 5' two-thirds of the 4437 nt viral RNA encode proteins in sense strand (Nutter, Scheets, Panganiban, & Lommel, 1989). The first open reading frame (ORF) encodes a 32 kDa protein, p32 which is unique to MCMV, p50 and its read through (rt) protein p111 are related to the highly conserved RNA dependent RNA polymerases (RdRps) encoded by other members of the family Tombusviridae. p50 is similar in size to the ORF1 product of *Panicum mosaic virus*, genus *Panicovirus*, (48 kDa) and two other panicoviruses (41–44 kDa) which are larger than the related ORF1-encoded proteins (23–37 kDa) of other tombusvirids p50 has a unique amino-terminal sequence that coincides with most of the p32 ORF overlap (residues 1-283) (Figure 1).

All ORFs in the 3' third of the genome are expressed from sub genomic RNA1 (sgRNA1) (Scheets, 2000). The first ORF encodes a 7.2 kDa peptide (p7a) and suppression of its stop

codon produces a 31 kDa protein, p31 (Scheets, 2000). The carboxy-terminus of p7a exhibits sequence similarity to movement protein 1 (MP1) found in tombusvirid genera that encode two or more small MPs (Rochon, Rubino, Russo, Martelli & Lommel, 2011). A small ORF with no methionine codons (~8 kDa coding capacity) following the p7a ORF was later identified by comparison to carmovirus sequences (Riviere & Rochon,1990), and the encoded peptide is most closely related to MP2 from panicoviruses (Scheets, Jordan, White & Hernández, 2015). MP1 and MP2 are required for cell to cell movement in viruses from four tombusvirid genera and an unassigned tombusvirid (Scheets *et al*., 2015). The carboxy-terminal extension of the 31 kDa protein is unique. The second AUG of sgRNA1 begins the 25 kDa coat protein (CP) ORF (Scheets, 2000). A 337 nt noncoding RNA (sgRNA2) accumulates in MCMV infected protoplasts and plants (Scheets, 2000), although it is not known whether it is a true sgRNA or a structure-protected degradation product as determined for red clover necrotic mosaic virus (Iwakawa *et al*., 2008).

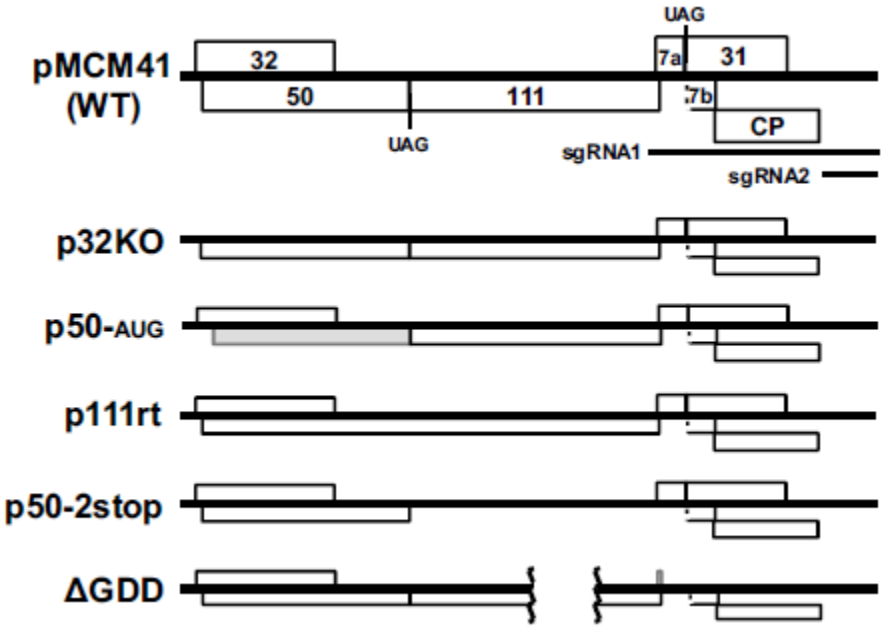

Figure 1: Genome organization of MCMV. Heavy lines represent genomic RNA, and boxes mark the coding regions and their relative reading frames. The locations of sgRNA1 and sgRNA2 are shown for WT. The sizes in kDa of the encoded proteins are shown, and the leaky stop codons are marked. Dashed lines mark a non-AUG start codon. Gray boxes denote smaller

ORFs produced by start or stop codon mutations. The deleted region of GDD is bordered by wavy lines.

SCMV belongs to the genus *Potyvirus* within the family Potyviridae (Zhu *et al*., 2014). Have a single-stranded positive-sense RNA genome. The genome of SCMV is approximately 9.6 kb long, covalently linked to a virus genome-linked protein at its 5′ terminus and poly (A) at its 3′ terminus (Gell, Sebestyen & Balazs, 2015). The genome encodes a single large polyprotein, which is subsequently cleaved into 10 mature proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb, CP) by 3 self-encoded proteinases (Urcuqui-Inchima, Haenni & Bernardi, 2001). SCMV is easy to mutate because of the weak proofreading activity of RNA-dependent RNA polymerase, short generation time, and large population size (Li, Liu, Zhou & Fan, 2013). As a consequence, the virus exists as numerous strains and replicates as complex and dynamic mutant swarms (Padhi & Ramu, 2011) (Figure 2). In East Africa, SCMV has been identified as the primary potyvirus associated with MLN. SCMV is not new to Africa, having been reported in Kenya (Louie, 1980) and South Africa (Handley, Smith, Dale & Harding, 1998) and perceived to be widely distributed in Africa. SCMV, together with MDMV, causes the most widespread disease of maize worldwide (Redinbaugh & Zambrano, 2014). SCMV is a genetically diverse group for which genome sequences of isolates from maize and sugarcane cluster phylogenetically by host and geographic origin (Li *et al.*, 2013).

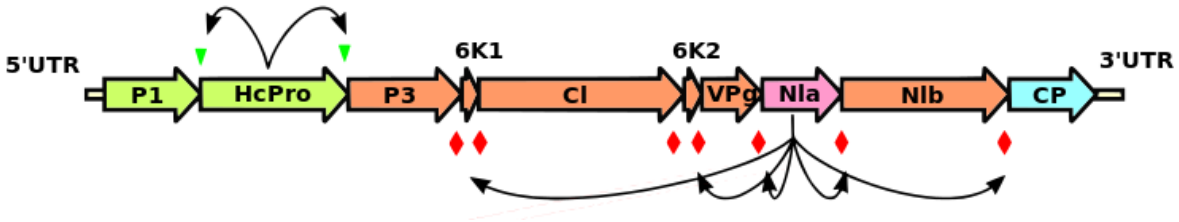

Figure 2: Genome organization of SCMV Retrieved on February, 2017 from https:/ en.wikipedia.org/wiki/File PPV.genome.svg

MDMV is made up of non-enveloped, flexuous filaments of 720 to 850 nm length and 12 to 15 nm diameter. The virus has a capsid protein of 35 kDa and monopartite, linear, ssRNA(+) of 10 kb in size. The 3’ terminus has a poly (A) tract while the 5’ terminus has a genome-linked protein (VPg).

WSMV is a 700 nm flexous rod with a capsid protein of 45 kDa (Brakke, 1971). The virus is non-enveloped, with filaments of 690 to 700 nm length and 12 to 15 nm in diameter. Monopartite or bipartite, linear, ssRNA (+) genome of 9.3 to 10.0 kb in size, 3' terminus has a poly (A) tract, 5' terminus has a genome - linked protein (VPg).

2.3.3 Transmission of MLN viruses

Maize plants are susceptible to MLN viruses at all stages of their growth. A vector transmits the MLN-causing viruses from plant to plant and field to field. Generally for disease to occur three components, the virus, its vector and a susceptible host must come together in a suitable environment (Redinbaugh & Zambrano, 2014).

MCMV is transmitted in a semi-persistent manner by beetles and thrips. The beetles belonging to the family Chrysomelidae include: the cereal leaf beetle *(Oulema melanopa),* the corn flea beetle *(Chaetocnema pulicaria),* the flea beetle *(Systena frontalis),* the Japanese beetle *(Popillia japonica)* and larvae of the southern corn rootworm *(Diabrotica undecimpunctata),* the northern corn rootworm *(D. longicornis),* and the western corn rootworm *(D. virgifer)* (Nault *et al.,* 1978).

Species of corn thrips (*Frankliniella williamsi)* was found to transmit MCMV semi-persistently (Cabanas, Watanabe, Higashi, & Bressan, 2013). The thrips transmit MCMV after acquisition periods of 3h, with no evidence for latent periods; both larvae and adults retain the ability to transmit the virus for up to 6 days after acquisition (Cabanas *et al.,* 2013). The range of vectors for MCMV in Africa is not known, although thrips have been observed in all fields where maize is grown, including in MLN and MCMV- affected farms. It is possible that thrips and other vectors could be playing a major role in MCMV movement within and between fields in the affected countries in Africa. Corn thrips were reported in East Africa in 2009 (Moritz, Brandt, Triapitsyn & Subramanian, 2013), and subsequent surveys detected them in several locations in Kenya, Uganda and DRC. Corn thrips were observed on several other graminaceous crops including baby corn, rice, sorghum and wheat, and were also observed frequently on onions (Moritz *et al.,* 2013).

Transmission from infected maize seed is usually very low (0.04%) (Jensen, Wysong, Ball & Higley, 1991). However, even at low rate, seed transmission is epidemiologically significant as maize is only propagated through seed and it leads to introduction of virus into new areas

through seed (Mahuku *et al.*, 2014). Also, in conjunction with secondary spread by insect vectors, low rates of seed transmission can translate into high numbers of infected plants, resulting in epidemics (Maule & Wang, 1996). It is also possible for MCMV to be transmitted at a very small rate through infested soil, as the virus can survive in corn residue (Nyvall, 1999).

Potyviruses like SCMV, WSMV and MDMV are transmitted by insect vectors in a non-persistent manner where by a virus become attached to the distal tip of the stylet of the insect and on the next plant it feed on; it inoculates it with the virus. SCMV is known to be transmitted by cereal leaf aphids, *Rhopalosiphum maids, R. padi* and *Sitobium avenal* (Pemberton & Charpentier, 1969). SCMV can also be transmitted in maize seed with small rates of 0.4 to 3.9% depending on the genotype (Li, Wang & Zhou, 2011).

WSMV is transmitted by the wheat curl mite (WCM) (*Aceria tosichella*) (Slykhuis, 1955). This virus is acquired by WCM during feeding; WSMV transmission efficiency varies among growth stages of WCM (Orlob, 1966; Siriwetwiwat, 2006; Slykhuis, 1955). Immature stages exhibit a higher ability to transmit WSMV than adult mites. Orlob (1966) showed that the virus can be acquired after only a short feeding period, i.e., after a minimum stay of 15 min on an infected plant.

MDMV is transmitted by a broad range of aphids including *Schizaphis graminum, Aphis maidiradicis, A. craccivora, A. fabae, A. gossypii, Acyrthosiphon pisum, Myzus persicae, Therioaphis maculata, Rhopalosiphum padi, R. poae, R. maidis, R. fitchii, Macrosiphum euphorbiae* and *Brevicoryne brassicae* (Ford, Tosic, & Shukla, 2004). These aphids acquire and transmit the virus in a matter of minutes (Ford *et al.*, 2004). However, under experimental conditions, viruliferous *Schizaphis graminum* were found to retain infectivity for over 20 hours (Berger *et al.*, 1987). Periodically occurrences of MDMV in Southern USA have been reported to be caused by large scale migration of air-borne aphids from Northern states of the USA and Ontario (Zeyen, Berger & Groth, 1987). Apart from aphid transmission, MDMV is also transmitted in corn seed at frequencies from 0.007% to 0.4% (Ford *et al.*, 2004).

2.3.4 MLN Disease symptoms

Characteristic of MLN: chlorotic mottle symptoms on leaves developing from the base of young whorl leaves upward to the leaf tips (Plate 1A); mild to severe leaf mottling and necrosis developing from leaf margins to the mid-rib (Plate 1B); Necrosis of young leaves lead to a "dead heart" symptom, and plant death (Plate 1C); Late infected maize plants have small cobs with little or no grain set (Plate 1D); Plants frequently die before tasseling (Wangai *et al.*, 2012).

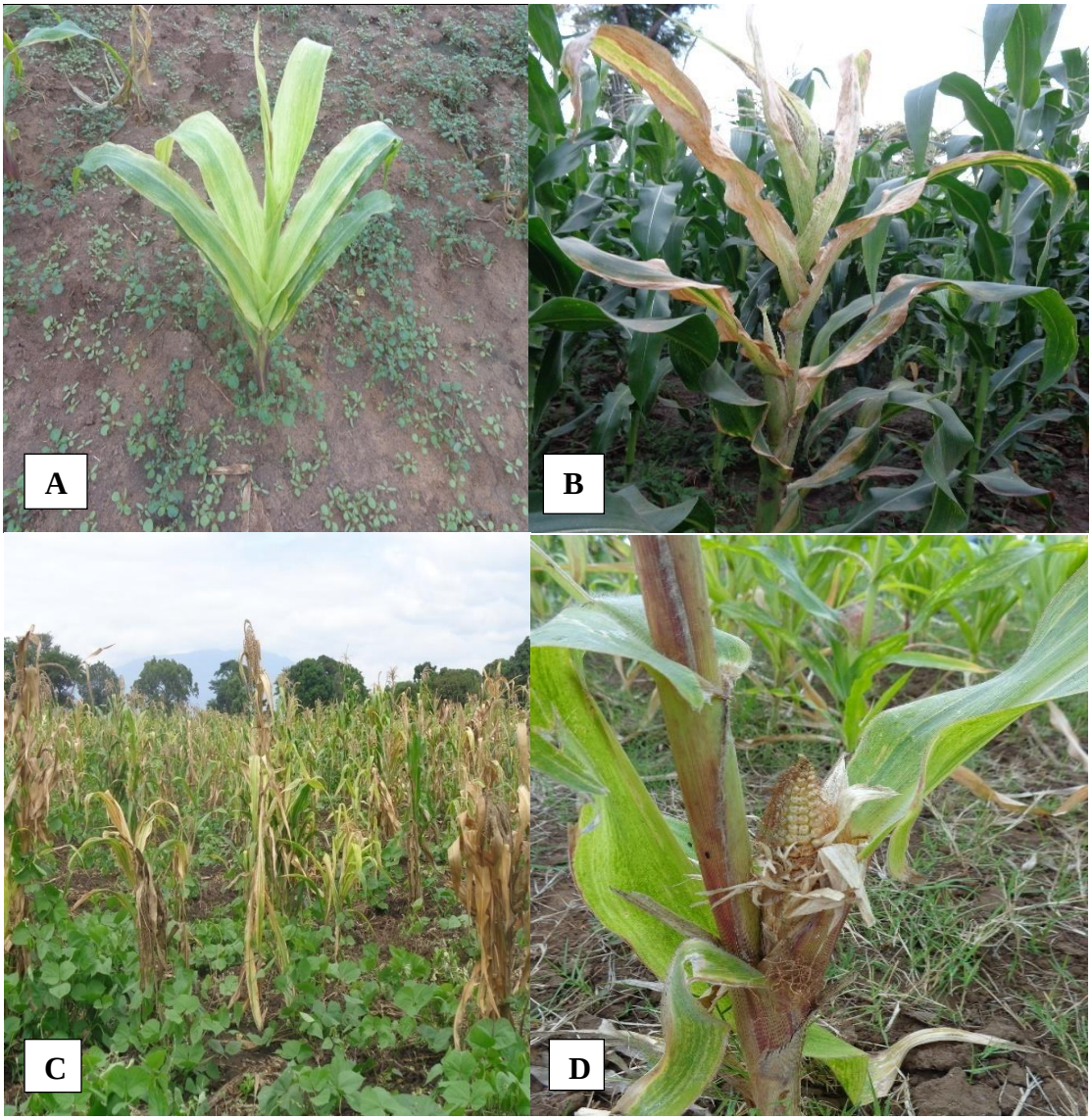

Plate 1: Symptoms of MLN disease in Manyara, Arusha and Dodoma in Tanzania: (Plate 1A) Chlorosis on leaves, (Plate 1B) Mottling and necrosis start from margins to the mid-rib (Plate 1C) Necrosis followed by plant death, (Plate 1D) Small cobs with little or no grains; Photos by Allan Mariki

2.3.5 Host plants of MLN causing viruses

Hosts of MLN causing viruses are limited to the family Poaceae (Scheets, 2004). Maize is known to be the primary host for MCMV, though several other grasses have been identified in Hawaii to host MCMV and include *Bromus mollis, Panicum dichotomiflorum*, *P. maximum* and *P.miliaceum* (Brunt *et al.*, 2010). MCMV has also been reported in Sorghum (Phillips, Uyemoto, & Wilson, 1982). Finger millet in Kenya is reported to host MCMV and SCMV (Kusia *et al*., 2015). In China, sugarcane (*Saccharum officinarum*) was found to host both MCMV and SCMV (Wang, Zhou & Wu, 2014). SCMV was also found to infect and cause mosaic disease in sugarcane, maize and sorghum (Shukla, Ward & Brunt, 1994; Adams, Antoniw & Barker, 1998). Ellis et al., (2003) and Kapooria & Ndunguru (2004) reported wheat as the main host of WSMV but the virus also infects rye, oat, barley, triticale, and some cultivars of maize (S´anchez, Henry C´ardenas-Soriano & Alvizo-Villasana , 2001) although to date no species of dicotyledons have been identified so far. MDMV has been found naturally infecting *Sorghum bicolor*, *S. halepense*, *S. sudanense* and *Zea mays* (Brunt, Crabtree & Gibbs, 1990; Rao, Jain & Varma, 1996; Toler, 1985). Experimentally, susceptible hosts of MDMV are however much broader and include; *Arundo donax*, *Bromus mollis*, *B. tectorum*, *Bromose calinus*, *Chloris gayana*, *Cynodon dactylon*, *Echinochloa crus-galli*, *Eleusine coroacana*, *Lagurus ovatus*, *Oryza sativa*, *Panicum acapillare*, *P. maximum*, *P. miliaceum*, *Paspalum dilatatum*, *Phalaris paradoxa*, *Rotboellia exaltata*, *Saccharum officinarum*, *Sacciolepsis indica*, *Setaria italica*, and *Setaria viridis*, (Brunt *et al.*, 1990).

2.4 Disease management

Effective management should target the three components required for disease to occur which are, the virus, its vector and a susceptible host must come together in a suitable environment. The most effective management of MLN is achieved through the use of integrated approaches combining cultural practices, chemical and biological control of insect vectors, and use of resistant cultivars. In Hawaii, MCMV is managed through the integration of cultural practices with suitable insecticides and host tolerance (Nelson, Brewbaker & Hu, 2011). Cultural methods include crop rotation for at least two seasons, with non-cereal crops like potatoes, cassava, beans, onions and garlic. In the central U.S.A, crop rotation was found effective in reducing the incidence of MCMV (Phillips *et al*., 1982). The use of certified seed, weeding and burning of infected material from the field also reduces pathogen and vector populations. Planting of maize

is recommended on the onset of the main rainy season, with fertilizer application to boost plant vigor.

Intensive integrated vector and cultural management practices could be useful for commercial production of virus-free maize seed. However, this intensive approach could be more difficult to implement for smallholder maize farmers in many areas of eastern Africa, where relay planting of maize is a common practice, and farmers lack awareness of, and resources for, vector control and cultural management practices (Mahuku *et al.*, 2015).

Development of virus-resistant crops is an economically viable and environmentally sustainable approach for disease control, but it requires identification and evaluation of resistant plants, then incorporation of favorable allele into agronomically-desirable genetic backgrounds. Resistant varieties for MLN have been developed in Hawaii and are used with weekly application of appropriate insecticide to control vectors (Nelson *et al.*, 2011). Experiments were conducted in USA to identify sources of tolerance/resistance to MLN in elite maize germplasm. These include: N211 and KS23-6 that developed only mild symptoms late in the rating period. Other lines that had significant delays in symptom development are the inbred line Oh1VI, and six recombinant inbred lines (RIL) derived from a cross of Oh1VI x Oh28 (Redinbaugh & Zambrano, 2014).

2.5 Methods used to identify MLN causing viruses

Under field situations, the most obvious MLN symptoms are mosaic, mottling and necrosis, but for identification of symptom-causing viruses, these features are not very reliable on their own because they are influenced by a number of other factors such as sucking insect pest infestation, plant-water relations, plant genotype and environmental conditions (Green, 1991). Consequently, several laboratory methods have been developed to identify viruses (Albersio *et al.*, 2012). Good diagnostic tools are critical for surveillance, early warning and rapid implementation of prevention strategies. These tools include the use of serology and various molecular tests.

2.5.1 Serological tests

ELISA is a serological test that uses antiserum prepared against a particular virus. The antiserum contains antibodies generated in blood serum of rabbits inoculated with that particular virus antigen (Clark & Adams, 1977). The Double Antigen Sandwich-Enzyme Linked Immunosorbent

Assay (DAS-ELISA) used for immediate serological identification of viruses in a sample, based on viral protein differences. DAS-ELISA is widely used, reagents and chemicals required are readily available, and it gives adequate identification of viruses. Kusia et al. (2015) used DAS-ELISA to detect MCMV and SCMV affecting finger millet in Kenya, Lukanda et al. (2014) used ACP-ELISA to detect MCMV infecting maize in DR Congo. Triple-Antibody-Sandwich (TAS-ELISA) is another form of ELISA that uses monoclonal antibodies to detect viruses such as MLN causing viruses (Thomas, Massaloki & Harrison, 1986; Credi, Betti & Canova, 1989); it is efficient and easy to conduct in conditions of limited time and space.

2.5.2 Molecular tests

Molecular diagnostics detect and measure the presence of genetic material or proteins associated with a specific disease, helping to uncover the underlying mechanisms of disease. Polymerase chain reaction (PCR) is one of these techniques (Duncan & Torrance, 1992). PCR is common and widely used in disease diagnosis; it was developed in 1984 by the American biochemist, Kary Mullis (Bartlett & Stirling, 2003).

Wangai et al. (2012) used RT-PCR to detect MLN causing viruses MCMV and SCMV infecting maize in Kenya; Lukanda et al. (2014) used RT-PCR to detect MCMV infecting maize in Congo; Adam et al. (2014) used real time PCR to detect MLN causing viruses (MCMV and SCMV) in Rwanda; Canabas et al. (2013) used real time RT-PCR to detect MCMV in corn thrips (*Frankliniella williamsi*). Also, Kusia et al. (2015) used RT-PCR to detect MCMV and SCMV in finger millet.

Sequencing is a technique for disease identification, now is most reliable and has led to development of strain - specific probes and primers from extensive sequences available at the NCBI. This technique involves generation of sequences and identification is based on similarity with known viruses in the GenBank (Adams *et al*., 2013). The analysis pipeline as described by Stewart *et al.* (2014) was used to sequence MCMV isolates collected from symptomatic maize in Naivaisha and Bomet, Kenya in 2012 (Wangai *et al.,* 2012). The single full-length MCMV (4452bp: GenBank accession number KP 8519970) obtained was found to be 99% identical to genome sequences of 12 MCMV isolates available in GenBank (Adams *et a.,* 2013) and four from Rwanda (Adams *et al.,* 2014). The sequence was also 99% identical to MCMV isolates from maize and sugarcane collected in Yunnan and Sichnan, China; 98% identical with another

MCMV isolates from Yunnan, China (GU138674) and 96 to 97% identical to genome sequence of MCMV isolate from Kansas and Nebraska in the United States. Sequences of the MCMV coat protein for isolates from Tanzania were 99% identical to those from Kenya, Rwanda and DR Congo (Mahuku *et al*., 2015).

Also, to better understand diversity in SCMV from Africa, Illumina HIseq analysis was used to determine the sequences of SCMV isolates collected in Naivasha and Bomet, Kenya in 2012 (Wangai *et al*.,2012) and compare them with other isolates. Using the protocols and software outlined by Stewart et al. (2014) eight contig sequences ranging from 793 to 6321 bp with highest identity to SCMV genome sequence from China (BD8) or Rwanda (Table 2); in the clade containing the BD8 and Rwandan SCMV sequences, the three Rwandan isolates share more than 90% identity with the Ohio SCMV isolates and four isolates have 78 to 79% identity with the BD8 isolates. Two of the eight contigs (contigs 1 and 10) had the highest sequence identity (87 to 99%) with one of the Rwandan isolates. The three partial genome sequences previously reported from Kenya share their highest identity with the BD8 sequences (Adam *et al*., 2013), as do two partial genome sequences previously reported for SCMV isolates from Thailand. Two groups of SCMV sequences were identified, one of which shares a common origin with isolates for Asia (Figure 3).

Table 2: Comparison of partial genome sequence of *Sugarcane mosaic virus* isolates

Sequence[v]	**Accession**[w]	**Length**[x]	**Genome**[z]	**Nucleotides**	**BD8**	**RI**	**R2**	**R3**	**OH**
Contig 1	KP835283	6.321	BD8	416-6,729	98	79	79	79	79
Contig 2	KP835284	959	Rwanda I	4,371-5,328	81	99	98	98	93
Contig 10	KP835285	1835	BD8	6,920-8,754	99	75	75	75	73
Contig 28	KP835286	832	Rwanda I	56-873	69	87	87	86	82
Contig 11.64	KP835287	793	Rwanda I	2,914-3,705	78	94	94	95	93
Contig43.67.30.8.31.8010.74	KP835288	1679	Rwanda 3	7,165-8,846	76	98	97	96	87
Contig 42.114.111.52.101	KP835289	1560	Rwanda I	1,005-2,565	73	90	90	88	86
Contig 62.16.25a.25b	KP835290	959	Rwanda I	5,765-6,722	77	92	92	94	91
SCMV Thailand	AY629310	1144	BD8	8,998-9,339	98	82	82	81	80
SCMV Thailand	AY629310	1150	BD8	8,192-9,339	98	82	82	82	80
SCMV Kenya	JX286706	700	BD8	149-847	98	69	70	70	68
SCMV Kenya	JX286707	730	BD8	6,110-6,835	98	79	79	79	79
SCMV Kenya	JX286708	940	BD8	8,399-9,339	100	80	81	80	79

[v]sequence are contigs or supercontigs obtained from sample collected in western Kenya using RNASeq as previously described (Stewart *et al*., 2014). Supercontigs were assembled from contigs using Sequencher (Stewart *et al.,* 2014).

[w] GenBank accession number for the *Sugarcane mosaic virus* (SCMV) contigs and previously reported partial genome sequences from Thailand and Kenya.

[x]Sequence length in base pairs.

SCMV genome sequence with the highest identity for the contig or partial genome sequence. Genome sequences were from China (BD8: JN021933), Rwanda1 (RI, KF 744392); Rwanda 2 (R2, KF744391), Rwanda 3(R3, KF744390) and Ohio (JX188385).

[z]Nucleotides of the genome sequence corresponding to the contigs or partial genome sequence determined from a pairwise comparison of sequences aligned with Clustal W.

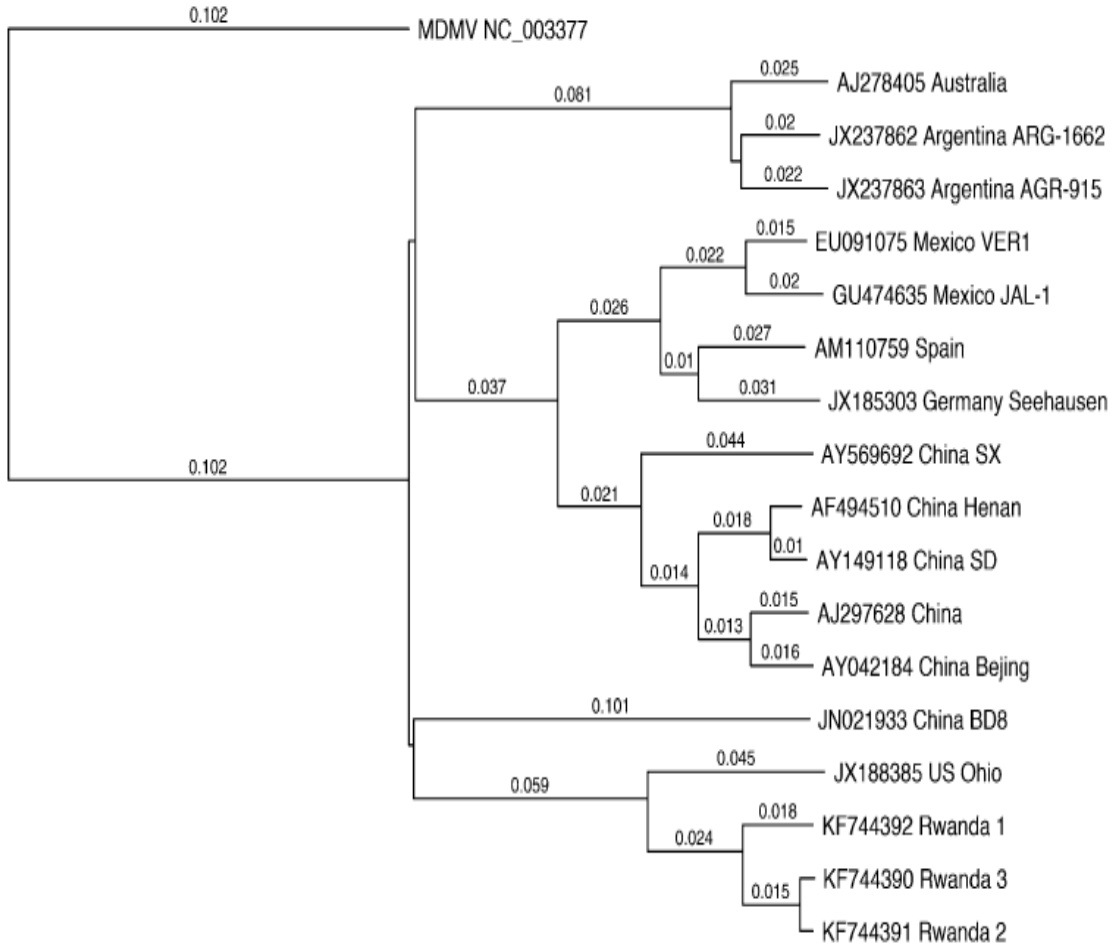

Figure 3: Phylogenetic relationship among SCMV of genome sequence. Genome sequence for SCMV isolate obtained from GenBak were aligned with the Clustal W algorithm in MacVector(v.13.5; MacVector,Inc., Cary,NC). A neighbor-joining analysis using uncorrected P values was used to generate the tree, and MDMV was used as an outgroup. The best tree is shown.GenBank accession numbers and geographic orgin for eaach isolate are shown. Source Mahuku et al. (2015).

2.6 Section summary

Maize varieties grown in Tanzania are mainly due to farmers' choice, the length of seasons, the elevation and the amount of rainfall received in a given area. Commonly grown varieties include: DK 8031, Hybrid 614, Katumani, Kilima, Pan691, SC637, Staha, Stuka and TMVI. Of all these grown varieties only one variety (Staha) is known to be tolerant to *Maize streak virus* (MSV). In 2012, the emergency of MLN disease affecting maize was reported in Tanzania, a new threat to

maize production. The disease was found to affect all varieties of maize including those tolerant to MSV; indicating that no varieties available for famers up to now are resistant. MLN is known to be caused by MCMV in combination with any viruses from Potyviridae family such as SCMV, MDMV and WSMV. This study was conducted to establish the distribution of MLN, to identify the causal agents and to identify the alternative hosts for MLN causing viruses in Tanzania. Diagnosis of disease to ascertain the causal agent is a vital process in any disease management plan. The transmitting vector(s) and alternative host must also be known. Such information is also required by extension staff in order to educate farmers about disease management.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and geographical description of the study area

This study was conducted in three regions: Manyara, Arusha and Dodoma, these regions are in north (Manyara and Arusha) and central (Dodoma) part of Tanzania. Selected regions are hot spot areas for MLN disease (Makumbi & Wangai, 2013) and notable maize growing areas, accounting for 20% of total maize produced in Tanzania. Surveys were conducted in five districts in Manyara region (Babati, Kiteto, Hanang, Simanjiro and Mbulu,) four districts in Arusha region (Meru, Karatu, Monduli and Arusha Urban); and five districts in Dodoma region (Kongwa, Mpwapwa, Chamwino, Kondoa and Chemba) (Table 3).

These districts have been divided into three major agro ecological zones (Nkonya *et al*., 1998) (i) High rainfall zone: in this zone grassland and mountains elevations (1500 to 2200 masl) are the major dominating features and annual rainfall is approximated to be 1200 to 1500 mm. The zone is located in the districts of Babati, Mbulu, Karatu and Hanang. Maize is the main crop with a growing period of 6 to 8 months, followed by other crops like potatoes, beans, wheat, onions and peas (ii) Moderate rainfall zone: in this zone escarpments and; elevated flat areas (900 to 1500 masl) are the major dominating features with annual rainfall of 900 to1100 mm. The zone is located in the districts of Meru, Arusha, Kiteto, Kondoa, Simanjiro, Monduli, Kongwa and Chemba. A wide variety of crops are grown in this zone which include maize, with a growing period of 3 to 6 months. Other crops grown in this zone include beans, sorghum, bananas, coffee intercropped with avocado, sugarcane and rice (iii) Low rainfall zone: in this zone flat areas and small valleys are major dominating features with annual rainfall of 500 to 800 mm. These areas are always in the lowland plains below 900 masl. The zone is located in the districts of Mpwapwa and Chamwino. Sunflower, sorghum, finger millet and Pear millet are major cultivated crops in these districts. Maize is also grown in this zone with the growing periods of 3 to 4 months.

Most of the soils in these regions are of volcanic origin and range from sandy loam to clay alluvial soils. In Manyara and Arusha they have two rain seasons commonly known as Vuli and Mazika period which means short rain and long rain seasons which happens during October to

November and March to June respectively (Thompson, 2009). In Dodoma region there is only one rainy season from November to March.

3.2 Surveys

Two surveys were conducted in two growing seasons: from February to March 2015 after the short rains and from May to June 2015 after the long rains. The surveys covered 37 and 126 fields, respectively (Table 3).

Table 3: Regions and districts surveyed over two growing seasons in 2015

				Sample collected	
Region	**District**	**Seasons**	**Fields**	**Maize**	**Non-Maize**
Manyara	Babati	S.R	11	35	5
	Mbulu		5	19	6
Dodoma	Mpwapwa	S.R	9	16	3
	Kongwa		6	11	3
Arusha	Meru		6	26	12
Sub Total			**37**	**107**	**29**
Manyara	Babati	L.R	28	522	44
	Simanjiro		11	305	-
	Kiteto		11	303	7
	Hanang		10	253	26
	Mbulu		10	269	6
Arusha	Meru	L.R	12	301	14
	Karatu		9	230	-
	Monduli		6	151	2
	Arusha Urban		2	59	-
Dodoma	Kondoa	L.R	10	238	60
	Kongwa		8	209	34
	Chamwino		5	150	-
	Mpwapwa		3	30	32
	Chemba		1	30	-
Sub Total			**126**	**3050**	**225**
Grand Total			**163**	**3157**	**254**

*S.R= Short rain; L.R= Long rain

The number of farms or fields sampled in each district varied depending on the disease symptoms observed and accessibility to the farm. Also, number of samples collected varied from district to district due to differences in level of disease incidence and severity observed in each field; more samples were collected from fields with higher incidences and fewer samples were collected from fields with lower incidences and severities. This procedure was used in order to generate more information on occurrence of MLN causing viruses in surveyed fields. Fields were sampled at 10 to 30 km intervals along the motorable roads. In each field plants were assessed visually for the presence or absence of typical MLN-like symptoms along a 'X' transect by counting 15 randomly selected plants at equal distance in each transect. Disease incidence was determined as the percentage of maize plants with MLN-like symptoms over the total number of maize plants assessed in the field. Disease severity was scored on a rating scale of 1 to 5, where 1 = plant with no visible symptoms on the leaves; 2 = symptoms on 1 to 24% of infected leaves; 3 = symptoms on 25 to 50% of infected leaves; 4 = symptoms on 51 to 75% of infected leaves and 5 = symptoms on 75 to 100% of infected leaves (Appendix 1).

Fields were surveyed at 8 to 16 weeks after sowing, which is the most appropriate for virus disease assessment as plants were in the fields for a sufficiently long time for symptoms to express and they were not affected by senescence. Leaf samples were taken from maize; for the common weeds and non-maize crops samples were taken within a radius of 100m around the infected maize. All samples were placed in labeled paper envelopes and transported to the laboratory for assessing MLN causal agents. Weeds that tested positive for virus occurrence were identified to species level according to Akobundu & Agyakwa (1987). A global positioning system (GPS) (Garmin eTrex® 10, Garmin Ltd) was used to record longitude, latitude and altitude of each sampling site. Other data collected in the survey included; Cropping systems, cultivated varieties

Leaf samples were collected from each field by taking one sample from the apical region of mature symptomatic and asymptomatic maize plants; in so doing, a total of 3,157 maize foliar samples and 254 non-maize were collected (Table 2). Survey data were recorded on a standard survey data collection sheet developed for this study (Appendix 1) and entered into spreadsheets using the Microsoft Excel software, version. 2010, Microsoft) Mean incidence and severity of disease for each region and district were analyzed using the Generalize linear model (GLM) in

the Statistical Package for Social Sciences (SPSS, version 20.0. Armonk, New York: IBM Corporation) to generate Least Significant Differences (LSD) at α= 5%. Maps were also developed, showing incidence of MLN causing viruses in the IITA's Geographical Information Systems (GIS) unit using ArcGIS (version 10.4.1, Environmental Systems Research Institute Redlands, USA).

3.3 Laboratory analyses

Leaf samples collected in the fields were analyzed for detection of SCMV and MCMV by ELISA, RT-PCR and sequencing (IITA, 2009). A hundred and seven (107) maize samples collected from short rains and 254 non - maize samples from the short and long rains were analyzed separately as individual samples; 3,050 maize samples collected during the long rains were pooled and analyzed for each field in RT-PCR (Table 2). Pooling was done by putting together samples collected per field as a single sample. Pooled samples with dual infection of viruses were further analyzed in RT-PCR as individual component samples in order to understand the distribution of MLN causing viruses in surveyed location.

3.3.1 Serological identification in ELISA

Direct antigen coating (DAC) ELISA test was done on the sampled leaves using polyclonal antibodies against MCMV (IITA, Ibadan, Nigeria) and SCMV (DSMZ, Braunschweig, Germany) according to the manufacturers' protocol. In this protocol, 100 mg of leaf tissue was ground in 1 ml of Coating buffer [1.59g Na_2CO_3, 2.93g $NaHCO_3$, 0.1% DIECA, (pH 9.6)]. A 100µl of the resultant plant extract was loaded into one well per sample of a 96 well micro-titre (ELISA) plate and incubated at 37^0C for 1 h. The plate was washed three times with PBS-T buffer [8.0g NaCl, 0.2g KH_2PO_4, 1.1g Na_2HPO_4, 0.2g KCL, 0.05% Tween 20 (pH 7.4)] by flooding for three minutes each time and then dried by tapping on paper towels. 100µl of antibody diluted to 1:5000 (v/v) in Conjugate buffer [0.05% Tween 20, 0.02% w/v egg albumin (Ovalbumin), 0.2% w/v Polyvinylpyrrolidone (PVP)] was loaded to each well for MCMV; and SCMV antibodies were used at 1:1000 (v/v) dilution and incubated at 37^0C for 1 hour. The plate was washed three times with PBS-tween and then dried by tapping on paper towels. 100µl of goat anti-rabbit alkaline phosphatase diluted to 1 µl in 15 ml (1:15,000 (v/v)) in conjugate buffer was added per well and incubated at 37^0C for 1 hr. The plate was washed three times with PBS-Tween by flooding for three minutes each time and then dried by tapping on paper towels. 100µl

of 1 mg/ml (w/v) of p-nitrophenyl phosphate substrate in 10 ml substrate buffer [97ml diethanolamine, 800 ml distilled water **(**pH 9.8)] was added in each well.

The optical densities (OD) of the enzymatic reactions were measured at 405 nm in an ELISA plate reader (Inqaba Biotec Company, Pretoria, South Africa,) with the 405 nm filter after 1 hour of incubation at 37^0C and also after overnight incubation at 4^0C. Leaf sample extracts from non-symptomatic and diseased maize were used as negative and positive controls respectively; the buffer was used as the blank controls. The healthy sample was collected from an asymptomatic leaf then tested negative for both viruses by ELISA then verified on PCR; the diseased sample was collected from a symptomatic leaf then tested positive on ELISA then verified on PCR during this study. A field sample was considered positive in a specific ELISA test if the sample OD at 405 nm was twice the sum of mean and standard deviation absorbance values of healthy maize control at 405 nm in ELISA. MDMV and WSMV were not tested for in this study because these viruses are not widely distributed worldwide and previous reports of occurrence have been in North and South America. In addition, there were limitations in funding.

3.3.2 Virus detection by reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from maize and alternative host leaves using the Cetyltrimethylammonium bromide (CTAB) method (Murray & Thompson, 1980). Briefly, 100 mg of leaf tissue were ground in 1 ml of CTAB buffer [2% CTAB w/v, 1.4 M NaCl, 0.02 M EDTA and 0.1M Trizma base (pH 8.0) (sterilized by autoclaving)] and 5µl of monothioglycerol was added just before use. The content of each sample (about 600 µl of the extract) was transferred into a 1.5 ml sterile eppendorf tube. The mixture was vortexed and incubated at 60°C in a water bath for 10 min. The tubes were cooled to 37^0C and 600 µl of phenol: chloroform: iso-amyl alcohol (25:24:1) were added to the mixture. The tubes were again vortexed and centrifuged at 12,000 rpm for 10 min. The supernatant (about 450 µl) was collected into a separate sterile microfuge tube, and 300 µl of cold iso-propanol was added into the tube and incubated at -20°C for 1 h. The solution was centrifuged at 12,000 rpm for 10 min to precipitate the nucleic acid. The supernatant was carefully removed and the nucleic acid pellet was washed with 500 µl of 70% ethanol and tubes were air dried at 37°C to remove final traces of ethanol. The pellet was dissolved in 50 µl of sterile distilled water and stored at -20°C until further use.

The quality of the nucleic acid was tested by analyzing 6 µl of nucleic acid in 1% TAE agarose gel electrophoresis. Agarose gels were stained with gel red and viewed under Ultra Violet light.

The RT-PCR for the detection of SCMV and MCV was performed according to Wangai et al.(2012) in a 12.5 µl reaction consisting of 5.5 µl of sterile distilled water, 2.5 µl of 10x PCR reaction buffer supplied by the manufacturer (Promega), 0.25 µl of 10 mM dNTP mixture, 0.75 µl of 25 mM $MgCl_2$, 0.187 µl (10 pMol) each of SCMV and MCMV forward and reverse oligonucleotide primers (Table 4), 0.06 µl of (0.3 U) Taq DNA polymerase (Promega), 0.06 µl of reverse transcriptase (Promega) and 2.0µl of nucleic acid templates. The PCR profile for detection of MCMV and SCMV involved an initial incubation for the RT reaction at 44°C for 30 min, followed by 1 cycle of denaturation at 94°C for 1 min, 54°C for 2min, 72°C for 3min; 35 cycles, of 94°C for 1 min, 54°C for 2 min and 72°C for 1 min; and a final extension at 72°C for 5 min. The RT-PCR product was electrophoresed on 1.5 % agarose gels for 60 min at 120 V in TAE agarose gel, and then visualized under UV light so as to determine successful amplification of the desired fragment. Samples that showed the expected band sizes of 550 bp for MCMV and 900 bp for SCMV were considered positive for MCMV and SCMV, respectively in PCR.

Table 4: Primers used for detection of MCMV and SCMV

Primer code	**Sequence***	**Region**	**Fragment size**	**Reference**
MCMV	F: 5′ATGAGAGCAGTTGGGGAATGCG 3’	CP**	550bp	
	R: 5′CGAATCTACACACACACACTCCAGC3’			Wangai *et al.,* 2012
SCMV	F: 5′GCAATGTCGAAGAAAATGCG3’	Polyprotein	900bp	
	R: 5′GTCTCTCACCAAGAGACTCGCAGC3’			

*F: Forward Primer; R; reverse primer; **Coat protein

3.3.3 Nucleotide sequencing and phylogenetic analysis

A select set of RT-PCR products of MCMV and SCMV were used to determine the nucleotide sequences for phylogenetic analysis (Table 5). Samples were selected to represent at least one isolate for each district and alternative hosts sampled. MCMV coat protein (CP) encoding region was amplified using MCMV CP specific primers (FP- 5’GCG GCA AGT AGC CGG TCT ACC CG 3’; RP- 5’ATG ATT TGC CAG CCC TGG GCC TG3’) using the RT-PCR conditions

described earlier in section 3.3.2. The RT-PCR products of SCMV primer pair (given in Table 4) were used for sequencing the 3' end of the coat protein sequence. The regions targeted for sequencing are commonly used for inferring phylogenetic relationships of MCMV and SCMV (http://www.ictvonline.org/virustaxonomy.asp). The RT-PCR for sequencing was performed as per the procedure given in the section 3.3.2. The amplified products were purified by precipitating with 95% (v/v) cold ethanol and the resultant pellet was dissolved in 40 µl of sterile distilled water, and 5 µl of these were analyzed in the TAE agarose gel to confirm amplification and 1 µl of the same product was used for estimating the nucleic acid concentration using Nanodrop DNA quantification equipment (Kamowa-Mbewe, Kumar, Changadeya, Ntawuruhunga, & Legg, 2015). About 200ng equivalent RT-PCR products were sequenced, in duplicate (four sequence reads per isolate), in forward and reverse orientations using the same primer used for RT-PCR by the Sanger dideoxy sequencing method at the DNA Facility of the Iowa State University Office of Biotechnology, Ames, Iowa, USA.

The nucleotide sequences of each isolate were edited and compared with each other using bioinformatics software the BioEdit Sequence Alignment Editor Ver. 7.0.5.3. A consensus sequence was obtained for each isolate and the sequences were verified by BLAST searches (http://blast.ncbi.nlm.nih.gov/). The 'Translate a DNA Sequence' option of BioEdit was used to ascertain that the sequences were in the correct translation frame prior to using them for further analyses. Alignments of nucleotide sequences were done using CLUSTALW and sequence identities between isolates obtained using the software, MEGA6 (Tamura *et al*., 2013). Phylogenetic relationships among the MCMV and SCMV isolates were inferred from the CLUSTALW alignments of sequences determined in this study using the Neighbor-Joining method (Saitou & Nei, 1987) with 1000 bootstrap replications performed using MEGA6. Estimates of evolutionary divergence between sequences were conducted using Maximum Composite Likelihood model (Tamura, Nei & Kumar, 2004) also available in the MEGA6 program (Tamura, Stecher, Peterson, Filipski & Kumar, 2013). Corresponding sequences of SCMV and MCMV available in the NCBI GenBank database (www.ncbi.nlm.nih.gov) were included for comparative analyses. Sequences included are those reported from Africa and type species from elsewhere for comparison to demonstrate distance between isolates from this study with those reported from other geographies.

Table 5: List of Tanzanian MCMV and SCMV isolates used for sequencing

S/N	District	SAMPLE ID	
		MCMV	SCMV
1	Babati	M4	M56
2	Arusha	M2903	SCMV-1, SCMV-3, SCMV-7*
3	Chamwino	-	M292
4	Chemba	-	-
5	Hanang	M210	M1350
6	Karatu	M2395	M435
7	Kiteto	-	M1224
8	Kondoa	M259	M260
9	Kongwa	M361	M311
10	Mbulu	M442	M388
11	Monduli	-	M445

*SCMV-1, -3 and -7 isolates used were from sorghum; all other isolates sequenced were detected in maize.

CHAPTER FOUR

RESULTS

4.1 Incidence and severity of MLN disease

4.1.1 Based on symptoms and laboratory analysis

Survey results showed presence of MLN in all three regions surveyed over the two maize growing seasons in 2015. Comparing disease incidences between surveyed regions revealed significant differences between Arusha and Dodoma and between Manyara and Dodoma regions. In all cases, Dodoma had a lowest incidence (8.68%), compared to Manyara (28.26%) and Arusha (25.6%) regions. There was no significant difference observed in disease incidences between Arusha and Manyara. The disease severities were also significantly different, at $\alpha = 0.05$, between Arusha and Dodoma and between Manyara and Dodoma regions. Dodoma had a lowest severity of 2, compared to Manyara (2.3) and Arusha (2.5) regions. There was no significant difference in disease severity between Arusha and Manyara regions (Table 6).

Table 6: MLN incidence and severity observed in surveyed regions of Arusha, Dodoma and Manyara

Region	**Means**	
	Incidence*	**Severity***
Dodoma	8.68 [a] (4.8)	2.0 [a] (0.9)
Arusha	25.60 [b] (4.5)	2.5 [b] (0.9)
Manyara	28.26 [b] (3)	2.3 [b] (0.6)
Grand means	**21(4.1)**	**2.3(0.8)**

*Means followed by similar letters are not significantly different; values in brackets refer to standard errors of the means

Disease incidences were significantly difference at ($\alpha = 0.05$) between the districts of Meru and Kiteto, Kongwa; Babati and Chamwino, Hanang, Kiteto, Kondoa, Kongwa, Mpwapwa, Simanjiro; Mbulu and Kiteto, Kongwa. Disease severities were significant different between the districts of Meru and Kiteto, Kondoa; Babati and Kondoa; Kiteto and Monduli; Monduli and Kondoa (Table 6; Appendix 2).

Table 7: MLN severity and incidence mean values from surveyed districts in 2015

	MLN	
District*	**Severity**	**Incidence**
Meru	2.5(0.3)	29.2(6.2)
Arusha Urban	2(0.3)	7(16.3)
Babati	2.4(0.8)	36.82(4)
Chamwino	2(0.3)	9(13.3)
Hanang	2.3(0.2)	14.86(8.7)
Karatu	2.4(0.2)	23.87(8.7)
Kiteto	2(0.2)	6.57(8.7)
Kondoa	2(0.2)	12.44(7.68)
Kongwa	2.11(0.2)	6.11(7.7)
Mbulu	2.2(0.2)	30.10(7.3)
Monduli	2.6(0.2)	25.40(10.3)
Mpwapwa	2(0.2)	5.6(11.5)
Simanjiro	2.3(0.2)	12.3(11.5)
Grand means	**2.2(0.3)**	**16.1(9.4)**

*Chemba district is not in list because it had zero incidences and severity; values in brackets refer to standard errors of the means

The distribution map indicated occurrences of MLN causing viruses in 13 of 14 districts surveyed over the two growing seasons in 2015 (Plate 2). In all districts surveyed symptoms observed were those typical of MLN; the most common symptoms were chlorotic mottle on leaves developing from the base of young whorl leaves upward to the leaf tips; leaf mottling and necrosis developing from leaf margins to the mid-rib and poor or no grains fill on late infected maize. Disease incidence varied from 0% to 100%, with an overall mean incidence from 5.6% to 37% in 13 of 14 districts surveyed (Table 7). Comparing incidence values for the 14 districts surveyed Meru and Babati districts had the highest incidence of 31-37%; Arusha, Monduli, Karatu and Mbulu had incidences of 21-30%; Simanjiro and Kondoa had incidence of 11-20%; Hanang, Kiteto, Kongwa, Mpwapwa and Chamwino had incidence of 1-10%; Chemba recorded 0% incidence.

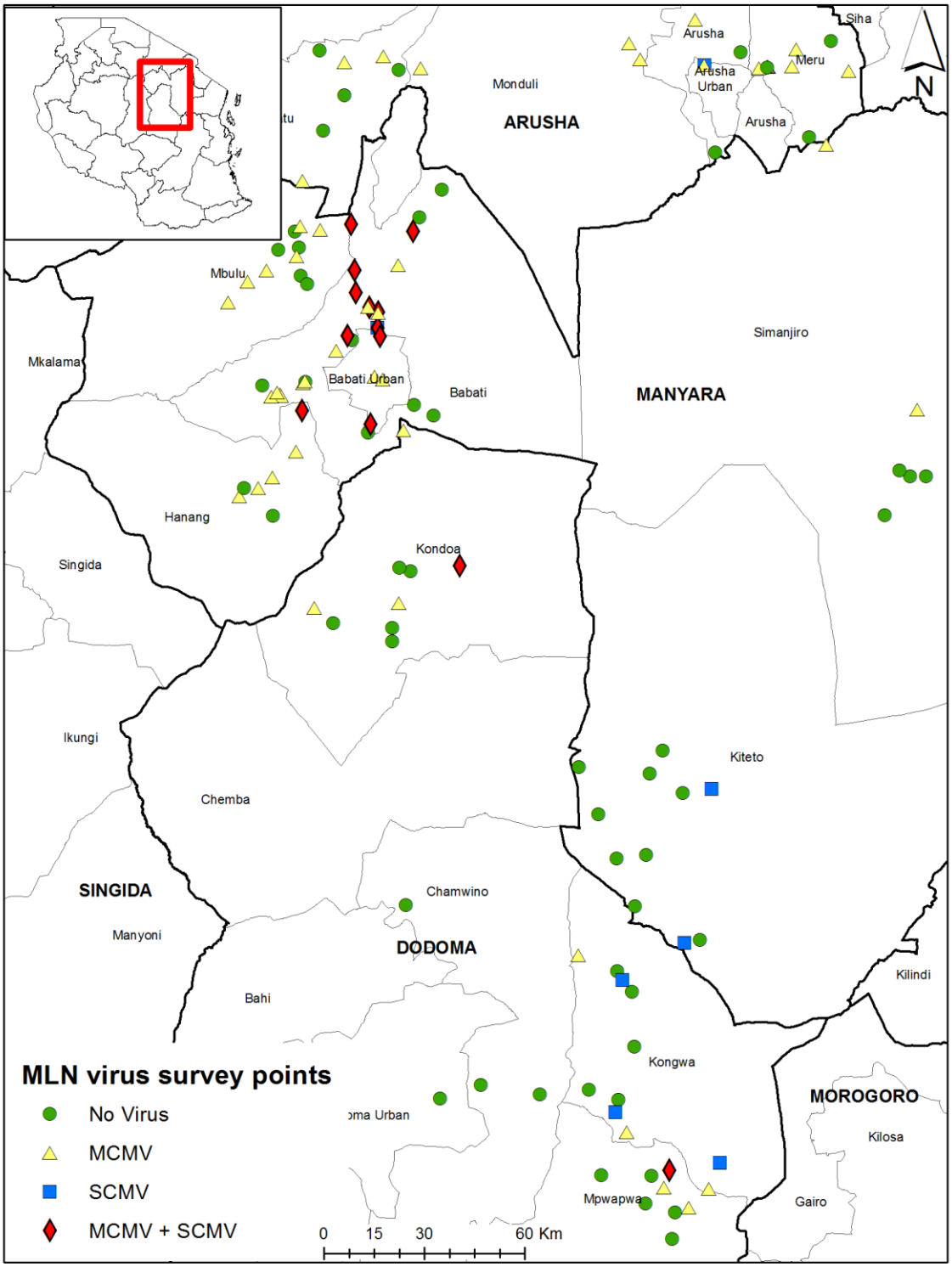

Plate 2: MLN virus distribution map for 14 surveyed districts of Tanzania in 2015

4.1.2 Cropping systems

MLN was present in 61 fields with monocrop and 99 fields with intercropping systems although in intercrop situation the incidence and severity were lower compared to monocrop. Incidence and mean severity observed in intercropping was 22% and 2.3, respectively, compared to monocrop situation in which incidence and mean severity was 25% and 2.4, respectively. There were significant differences in disease incidences between monocropping and intercropping farming systems (Figure 4).

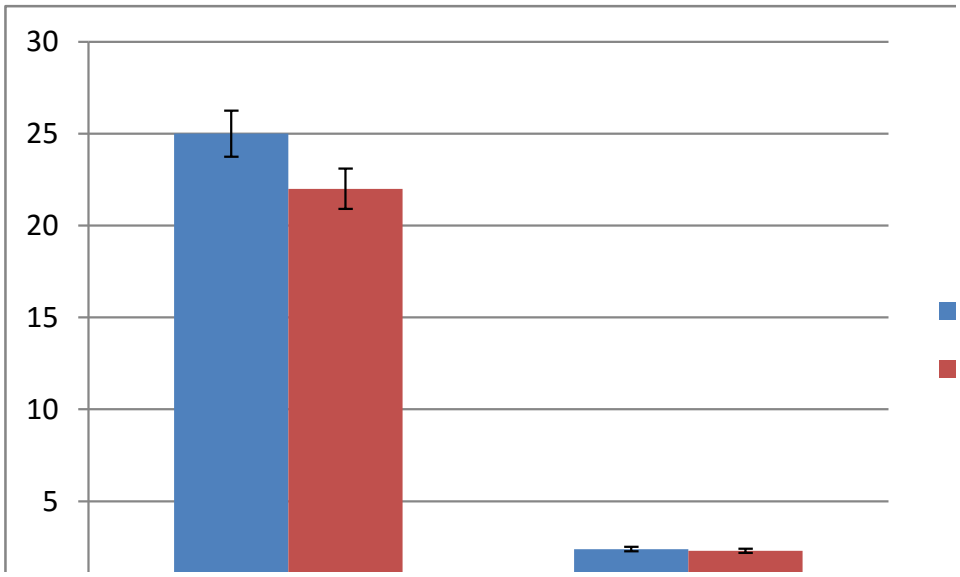

Figure 4: MLN incidence and severity observed in monocrop and intercrops systems

4.1.3 Cultivated varieties

Most cultivars expressed low to moderate incidence. Frequently and widely grown cultivars in the surveyed locations were also susceptible to MLN disease. Local varieties were the most frequently grown and the incidence of disease recorded was 22 %, with a mean symptom severity score of 2.4. The cultivar with the highest MLN incidence was SC 637 (46%), with mean symptom severity of 2.6 (Table 8). Nine cultivars were identified; those that could not be identified were grouped by similarities in the field.

Table 8: MLN incidence and severity of the most frequently grown cultivars in the Manyara, Arusha and Dodoma regions

Cultivar name	No. of fields surveyed	MLN severity	MLN incidence
DK 8031	5	2.3	12.7
Hybrid 614	2	2.13	23.3
Katumani	4	2.3	6.7
Kilima	3	2.5	27.8
Local variety	55	2.4	22
Pan691	5	2.1	3.9
SC637	27	2.6	46
Staha	3	2.2	5.6
Stuka	6	2.3	12.7
TMVI	3	2	3.3
Unknown	21	2.5	36.7

4.1.4 Alternative hosts for MCMV and SCMV

A total of 254 samples from other cereal crops within and around maize crops, and also weeds growing naturally in the maize fields were collected and tested for MCMV and SCMV. Most of the weeds were; *Setaria verticillata* (25), *Galinsuga parviflora* (21), *Pennisetum purpurcum* (10), *Tripacum andersonii* (9), *Cyperus rotundus* (5) and cereals were in the species of S. *bicolor* (112), *Pennisetum glaucum* (50), *S. officinarium* (10), *E. coracana* (9) and *Triticum specie*s (3). Of the samples analyzed, 7 (2.8%) tested positive for SCMV and none of them tested positive for MCMV 0 (0%). Results indicated that among tested samples, sugarcane (*S. officinarium*) (Plate 3A), finger millet (*E. coracana*) (Plate 3B), sorghum (*S. bicolor*) (Plate 3C) and bristly foxtails (*Setaria verticillata*) (Plate 3D) were infected by SCMV.

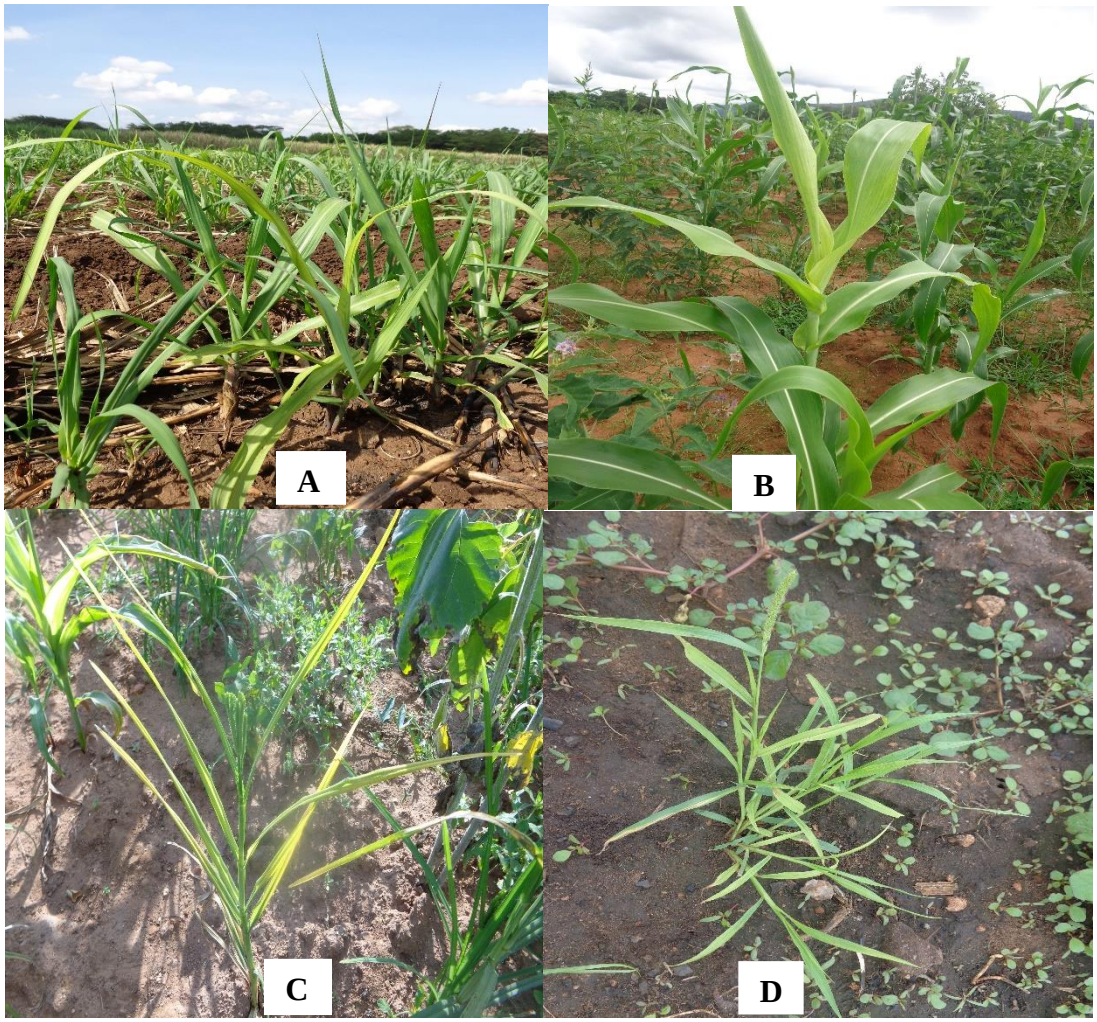

Plate 3: Alternative hosts of SCMV identified in Tanzania: (Plate 3A) *Saccharum officinarium* (Plate 3B) *Sorghum bicolor* (Plate 3A C) *Eleusine coracana* (Plate 3A D) *Setaria verticillata*

4.2 Viruses detected on ELISA

In order to verify the visual estimation of disease, leaf samples were analyzed by both DAC ELISA and RT-PCR. Out of 107 maize samples collected from first survey and tested using DAC ELISA, 24(22.4%) were positive for MCMV, 17(16%) were positive for SCMV and 4(3.7%) were positive for both viruses, none of samples from Kongwa districts was positive (Table 9).

Table 9: Maize samples tested by DAC ELISA in short rain seasons in 2015

Region	District	No. of sample	No. of positive samples		
			MCMV	SCMV	MCMV&SCMV
Manyara	Babati	35	6 (17.1)	5 (14.3)	1 (2.9)
	Mbulu	19	8 (42.1)	5 (26.3)	3 (15.8)
Arusha	Meru	26	10 (38.5)	3 (11.5)	-
Dodoma	Mpwapwa	16	-	4 (25)	-
	Kongwa	11	-	-	-
		107	**24 (22.4)**	**17 (16)**	**4 (3.7)**

*Values out of brackets are positive counts, and those in brackets are in percentage; (-) = samples test negative

4.3 Viruses detected on RT-PCR

All 107 samples tested on DAC ELISA were further analyzed in RT-PCR were by 35 (32.7%) tested positive for MCMV, 7 (6.5%) for SCMV and 1(0.93%) for both MCMV and SCMV. Also 93 pooled maize samples from the second survey were tested 43 (46.2%) tested positive for MCMV, 15 (16.1%) to SCMV and 10 (10.7%) to both MCMV and SCMV (Table 10).

Table 10: Maize samples tested by PCR over two growing seasons in 2015

				No. of positive samples		
Region	**District**	**Surveys**	**No. of Sample**	**MCMV**	**SCMV**	**MCMV&SCMV**
Manyara	Babati	S. R	35	7 (20)	4 (11.4)	1 (2.8)
	Mbulu		19	8 (42)	-	-
Arusha	Meru		26	13 (50)	-	-
Dodoma	Mpwapwa		16	4 (25)	3 (18.8)	-
	Kongwa		11	3(27.3)	-	-
Sub total			**107**	**35 (32.7)**	**7 (6.5)**	**1 (0.93)**
Manyara	Babati	L. R	21	17 (80.9)	8 (38.1)	8 (38.1)
	Simanjiro		6	2 (33.3)	-	-
	Kiteto		7	-	2 (28.6)	-
	Hanang		7	5 (71.4)	1 (14.3)	1 (14.3)
	Mbulu		6	4 (66.7)	-	-
Arusha	Meru		10	4 (40)	-	-
	Karatu		9	3 (33.3)	-	-
	Monduli		5	3 (60)	-	-
	Arusha_urban		2	1 (50)	1 (50)	1 (50)
Dodoma	Kondoa		8	3 (37.5)	1 (12.5)	-
	Kongwa		6	1 (16.7)	3 (50)	-
	Chamwino		4	-	-	-
	Mpwapwa		1	-	1 (100)	-
	Chemba		1	-	-	-
Sub total			**93**	**43 (46.2)**	**15 (16.1)**	**10 (10.7)**
Grand total			**200**	**78 (39)**	**22 (11)**	**11 (5.5)**

*Values out of brackets are positive counts, and those in brackets are in percentage; S.R=Short rain and L.R=Long rain; (-) = samples test negative

Tested samples were considered positive for MCMV and SCMV if amplification of PCR products occured at fragment sizes of 550bp in coat protein (Plate 4) and 900bp in polyprotein (Plate 5) for MCMV and SCMV respectively. Mixed infections occurred with MCMVand SCMV where the same sample amplified in both primers, as in the case of sample number six (Plate 4 & 5).

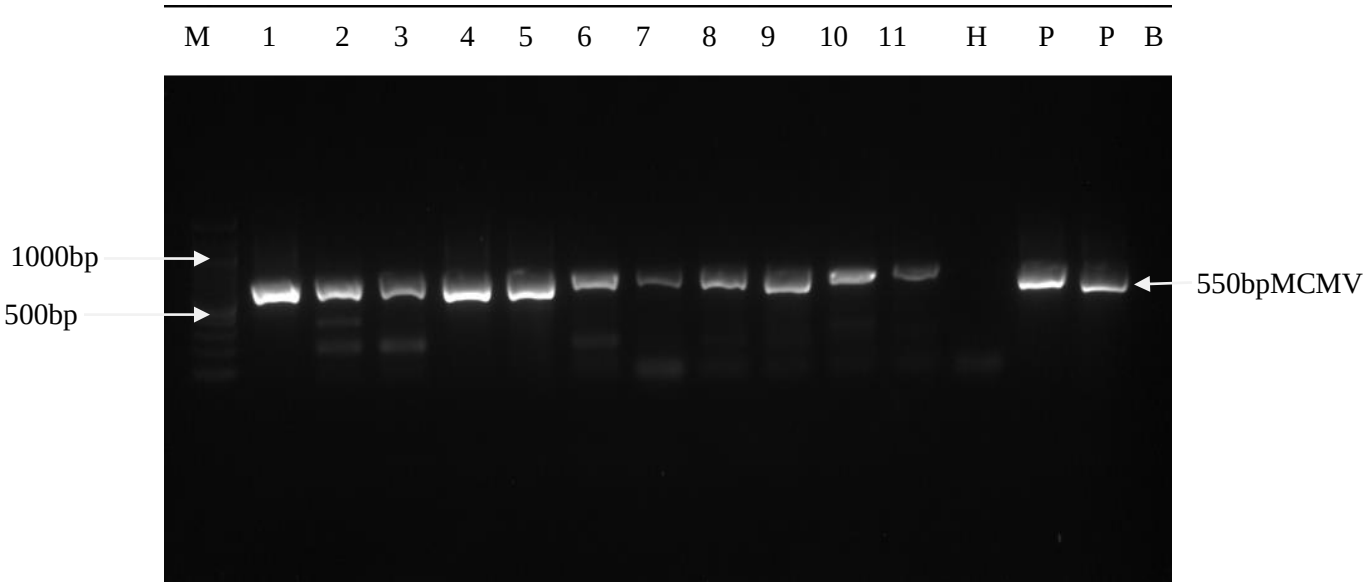

Plate 4: Agarose gel electrophoresis of amplification products derived from analysis of maize samples from 14 districts in Tanzania using MCMV Primers. M-1000 bp Molecular maker, 1-11=Samples, H-Healthy sample, P-Positive Control, B-Buffer

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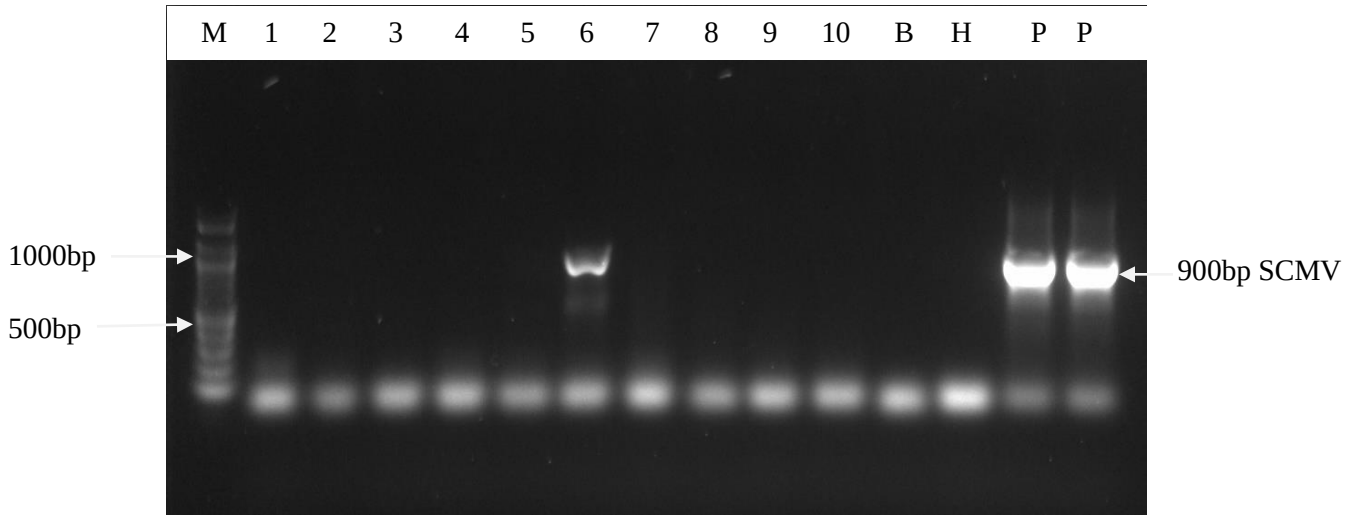

Plate 5: Agarose gel electrophoresis of amplification products derived from analysis of maize samples from 14 districts in Tanzania using SCMV Primers. M-1000bp Molecular maker, 1-11=Samples, H-Healthy sample, P-Positive control, B-Buffer.

4.4 Nucleotide sequencing and BLAST analyses

4.4.1 MCMV

A set of 7 MCMV isolates from maize in Babati, Arumeru, Arusha, Hanang, Kondoa, Mbulu and Kongwa which represent a broad geographic diversity in the surveyed region were sequenced. The sequenced region size was 711 bp, corresponding to the full length coat protein region of MCMV (Figure 5).

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50         60
M4-MCMV-CP       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M562-MCMV-       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M2903-MCMV       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M210-MCMV-       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M259-MCMV-       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M361-MCMV-       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
M442-MCMV-       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
JX286709_M       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
KP798455_M       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
EU358605_M       ATGGCAGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
AY587605_M       ATGGCGGCAA GTAGCCCGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
KT630268_M       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAGGCA
JQ943666_M       ATGGCGGCAA GTAGCCGGTC TACCCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
JF422772_M       ATGGCGGCAA GTAGCCGGTC TACCCGACGT AGAAAGCAGC GCGGATGTAG GGTGGCAGCA
KP203016_M       A--------- ---------- --TGCGAGGT AGAAAGCAGC GCGGACGTAG CGTGGAAGCA
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Figure 5: CLUSTALW alignment of nucleotide sequences for MCMV used in phylogenetic analyses. Text boxes highlight start and end codons.

```
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         70         80         90        100        110        120
M4-MCMV-CP       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M562-MCMV-       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M2903-MCMV       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M210-MCMV-       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M259-MCMV-       AAATCCAGAG CTATTCGAGC CAACCCGCTT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M361-MCMV-       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
M442-MCMV-       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
JX286709_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
KP798455_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
EU358605_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
AY587605_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
KT630268_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
JQ943666_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT
JF422772_M       ACATCCAGAG CTATTCGAGC CAACCCGCCT GTTCCTCGAC CCAACCCGCA GCGAAACCGT
KP203016_M       AAATCCAGAG CTATTCGAGC CAACCCGCCT GTCCCTCGAC CCAACCCGCA GCGAAACCGT


                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        130        140        150        160        170        180
M4-MCMV-CP       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M562-MCMV-       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M2903-MCMV       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M210-MCMV-       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M259-MCMV-       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M361-MCMV-       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
M442-MCMV-       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
JX286709_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
KP798455_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCTGAAATTC TGCTTGCAGT GTCAGCAACA
EU358605_M       CCCCCCCCTG CGGGAACAAC CTGCTCCATG TCCGAAATTC TGCTTGCAGT GTCAGCAACA
AY587605_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCCGAAATTC TGCTTGCAGT GTCAGCAACT
KT630268_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TC-------- ---------- ----------
JQ943666_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCCGAAATTC TGCTTGCAGT GTCAGCAACA
JF422772_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCCGAAATTC TGCTTGCAGT GTCAGCAACA
KP203016_M       CCCCCACCTG CGGGAACAAC CTGCTCCATG TCCGAAATTC TGCTTGCAGT GTCAGCAACA


                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                        190        200        210        220        230        240
M4-MCMV-CP       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M562-MCMV-       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M2903-MCMV       ACTGCTGACC AAATTCTCGA GATTCCTGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M210-MCMV-       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M259-MCMV-       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M361-MCMV-       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
M442-MCMV-       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
JX286709_M       ACTACTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
KP798455_M       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
EU358605_M       ACTGCTGACC AGATTCTCGA GATTCCAGTG TGTGCAGGGA TTGACTTCCC GGCTGGAACG
AY587605_M       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
JF422772_M       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGCGCAGGGA TTGACTTCCC AGCTGGAACG
KP203016_M       ACTGCTGACC AAATTCTCGA GATTCCAGTG TGTGCAGGGA TTGACTTCCC AGCTGGAACG
```

Figure 5 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         250        260        270        280        290        300
M4-MCMV-CP       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M562-MCMV-       CCACCCCGAT ACATTGGGGC AGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M2903-MCMV       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M210-MCMV-       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M259-MCMV-       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M361-MCMV-       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
M442-MCMV-       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
JX286709_M       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
KP798455_M       CCACCCCGAT ACATTGGGGC GGCCAAGTGG CTGGCAGCAC AATCACAGAT GTGGAACACA
EU358605_M       TCGCCCCGAT ACATTGGGGC GGCCAAATGG CTGGCAGCAC AATCACAGAT GTGGAATACA
AY587605_M       CCACCCCGAT ACATCGGGGC GGCCAAATGG CTGGCAGCAC AATCACAGAT GTGGAACACA
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       CCACCCCGAT ACATTGGGGC GGCCAAATGG CTGGCAGCAC AATCACAGAT GTGGAACACA
JF422772_M       CCACCCCGAT ACATTGGGGC GGCTAAATGG CTGGCAGCAC AATCACAGAT GTGGAACACA
KP203016_M       CCACCCCGAT ACATTGGGGC GGCCAAATGG CTGGCAGCAC AATCACAGAT GTGGAACACA

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         310        320        330        340        350        360
M4-MCMV-CP       ATTGTATTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M562-MCMV-       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M2903-MCMV       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M210-MCMV-       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M259-MCMV-       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M361-MCMV-       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
M442-MCMV-       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
JX286709_M       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
KP798455_M       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
EU358605_M       ATTGTGTTCA ACTCTGTGCG TATCACTTGG GAGACATTCA CAGCAGACAC CACTAGCGGA
AY587605_M       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
JF422772_M       ATTGTGTTCA ACTCTGTGCG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA
KP203016_M       ATTGTGTTCA ACTCTGTACG CATCACTTGG GAAACATTCA CAGCAGACAC CACTAGCGGA

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         370        380        390        400        410        420
M4-MCMV-CP       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M562-MCMV-       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M2903-MCMV       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M210-MCMV-       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M259-MCMV-       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M361-MCMV-       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
M442-MCMV-       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
JX286709_M       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
KP798455_M       TACATCTCAA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
EU358605_M       TACATTTCCA TGGCATTCCT CTCCGATTAC ATGTTATCAA TACCCACTGG GGTGGAGGAT
AY587605_M       TACATCTCCA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       TACATCTCCA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
JF422772_M       TACATTTCCA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
KP203016_M       TACATTTCCA TGGCATTCCT CTCTGATTAC ATGCTATCAA TACCCACTGG GGTGGAGGAT
```

Figure 5 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         430        440        450        460        470        480
M4-MCMV-CP       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
M562-MCMV-       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
M2903-MCMV       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
M210-MCMV-       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
M259-MCMV-       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAAA ACAGAGGGCC GTCCATTGTT
M361-MCMV-       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
M442-MCMV-       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
JX286709_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
KP798455_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
EU358605_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC ATCCATTGTC
AY587605_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
JF422772_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT
KP203016_M       GTTGCCAGGA TCGTGCCCTC AGCTACAATA GCTCTGAAGA ACAGAGGGCC GTCCATTGTT


....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         490        500        510        520        530        540
M4-MCMV-CP       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M562-MCMV-       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M2903-MCMV       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M210-MCMV-       ATGCCACAAA ACCGTACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M259-MCMV-       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M361-MCMV-       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
M442-MCMV-       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
JX286709_M       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
KP798455_M       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
EU358605_M       ATGCCACAGA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCATTGGGC
AY587605_M       ATGCCACAAA ACCGCACTGT GTTCAGGTGT ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       ATGCCCCAAA ACCGCACTGT GTTCAGGTGC ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
JF422772_M       ATGCCACAAA ACCGCACTGT GTTCAGGTGC ATACAGGCTG GTCAGTTTGC TGCGTTGGGC
KP203016_M       ATGCCACAAA ACCGCACTGT GTTCAGGTGC ATACAGGCTG GCCAGTTTGC TGCGTTGGGC

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         550        560        570        580        590        600
M4-MCMV-CP       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M562-MCMV-       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M2903-MCMV       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M210-MCMV-       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M259-MCMV-       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M361-MCMV-       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
M442-MCMV-       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
JX286709_M       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
KP798455_M       AGCGCGGCTG ACAAGCAAAT GTATTCTCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
EU358605_M       AGCGCTGCTG ACAAGCAAAT GTATTCCCCA GGACGATTCA TTGTGGCTAT CCCTAAAGCT
AY587605_M       AGCGCAGCTG ACAAGCAAAT GTATTCCCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
KT630268_M       ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M       AGCGCGGCTG ACAAGCAAAT GTATTCCCCC GGACGATTCA TTGTGGCTAT CCCCAAAGCT
JF422772_M       AGCGCGGCTG ACAAGCAAAT GTATTCCCCC GGACGATTCA TCGTGGCTAT CCCCAAAGCT
KP203016_M       AGCGCGGCTG ACAAGCAAAT GTATTCCCCC GGACGATTTA TTGTGGCCAT CCCCAAAGCT
```

Figure 5 Continued

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                    ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                            610        620        630        640        650        660
M4-MCMV-CP          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M562-MCMV-          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M2903-MCMV          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M210-MCMV-          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M259-MCMV-          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M361-MCMV-          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
M442-MCMV-          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
JX286709_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
KP798455_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
EU358605_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTCCTA CCGTGGAGCA
AY587605_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CCGTGTTCTA CCGGGGAGCA
KT630268_M          ---------- ---------- ---------- ---------- ---------- ----------
JQ943666_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGTACT CTGTGTCCTA CCGTGGAGCA
JF422772_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCATACT CCGTGTCCTA CCGTGGAGCA
KP203016_M          AGTGCAACAC AAGCGGTAGG CCAAATCAAA ATCTCGACAC CGACATATTC ACGTGGAGCA


                    ....|....| ....|....| ....|....| ....|....| ....|....| .
                            670        680        690        700        710
M4-MCMV-CP          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M562-MCMV-          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M2903-MCMV          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M210-MCMV-          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M259-MCMV-          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M361-MCMV-          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
M442-MCMV-          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
JX286709_M          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
KP798455_M          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
EU358605_M          GCGATCCTAC AACCCGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
AY587605_M          ACAATCCTAC AAACTGGCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
KT630268_M          ---------- ---------- ---------- ---------- ---------- -
JQ943666_M          GCAATCCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
JF422772_M          ACAAACCTAC AACCTGCCCT GGTTCCAGGC CCAGGGCTGG CAAATCATTG A
KP203016_M          ACAATCCTAA AAACTGGCCT A--------- ---------- ---------- -
```

Figure 5 Continued

Although, there is no criterion for demarcating species in the genus *Machlomovirus* as MCMV is the only member in this genus, comparisons of sequences generated in this study with others using the Basic Local Alignment Search Tool, an on-line algorithm for comparing primary biological sequence information revealed the greatest sequence identities (99%) with MCMV isolates from Kenya, Ethiopia, Rwanda, China, Thailand, USA and Ecuador that are available in the NCBI GenBank Database. The same level of homology was observed between the MCMV isolates of Tanzania and also those that have been reported in East Africa (Kenya, Ethiopia, Rwanda) from maize and finger millet.

4.4.2 SCMV

A set of 12 SCMV isolates were sequenced, 9 were maize isolates and 3 were from sorghum and they represent broad geographic diversity in the surveyed region in this study. Isolate were from districts of Karatu, Kiteto, Kongwa, Mbulu, Simanjiro, Chamwino, Hanang, Babati and Kondoa. Comparisons of these sequences using the BLAST algorithm revealed greatest sequence identities (87 to 99%) with SCMV isolates available in the NCBI GenBank Database. The region used for sequence analysis corresponded to 785 bp to 808 bp of the 3' end of the coat protein region (Figure 6), which is commonly used for phylogenetic analysis (Mahuku *et al*., 2015).

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                          10         20         30         40         50         60
M435-SCMV_       CTTAGATTTC TTGTTAACAT ATAAACCACA ACAGCAAGAC ATATCAAACA CTAGAGCAAC
M1224-SCMV       TTTGGATTTC CTGTTAACAT ATAAACCACA ACAGCAAGAC ATATCAAACA CTAGAGCAAC
M260-SCMV_       CTTAGATTTC TTGTTAACAT ATAAACCACA ACAGCAAGAC ATATCAAACA CTAGAGCAAC
M311-SCMV_       CTTAGATTTC TTGTTAACAT ATAAACCACA ACAGCAAGAC ATATCAAACA CTAGAGCAAC
M388-SCMV_       CTTAGATTTT CTGTTAACAT ATAAACCACA ACAGCAAGAC ATATCAAACA CTAGAGCAAC
S77088_(Ma       CTTAGATTTC CTGTTAACAT ATAAACCACA ACAGCAAGAT ATATCAAATA CTAGAGCAAC
M445-SCMV_       TTTGGACTTT TTGTTGACGT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
M292-SCMV_       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAGCAAGAC ATATCAAACA CAAGAGCAAC
M1350-SCMV       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAGCAAGAC ATATCAAACA CAAGAGCAAC
M56-SCMV_B       TTTGGACTTT TTGTTGACAT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
SCMV-7-Sor       TTTGGACTTT TTGTTGACGT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
SCMV-3-Sor       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAGCAAGAC ATATCAAACA CAAGAGCAAC
KP86093_SC       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAACAAGAC ATATCAAACA CAAGAGCAAC
SCMV-1-Sor       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAGCAAGAC ATATCAAACA CAAGAGCAAC
KM926614|S       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAACAAGAC ATATCAAACA CAAGAGCAAC
KM926615|S       TCTGGACTTC TTGCTTACAT ACAAACCACA GCAGCAAGAC ATATCGAACA CAAGAGCAAC
KF744391-S       TCTGGACTTC TTGCTTACAT ACAAGCCACA GCAGCAAGAC ATATCAAACA CAAGAGCAAC
JX188385-S       TTTGGATTTT CTATTGACAT ATAAACCACA ACAACAAGAC ATATCAAACA CTAGAGCAAC
KT630803_S       TTTGGACTTT TTGTTGACAT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
JX286708-S       TTTGGACTTT TTGTTGACAT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
JX286708-S       TTTGGACTTT TTGTTGACAT ACAAGCCACA ACAGCAGGAC ATATCGAACA CAAGAGCAAC
```

Figure 6: CLUSTALW alignment of SCMV sequences used for phylogenetic analyses

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         70         80         90        100        110        120
M435-SCMV_       TAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAG GAGTACGAAA TTGATGACAC
M1224-SCMV       CAAGGAAGAG TTTGATAGAT GGTATGACGC CATAAAGAAG GAGTACGAAA TCGACGACAC
M260-SCMV_       TAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAG GAGTACGAAA TTGATGACAC
M311-SCMV_       TAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAG GAGTACGAAA TTGATGACAC
M388-SCMV_       CAAGGAGGAG TTTGATAGAT GGTATGACGC CATAAAGAAG GAGTATGAAA TCGATGACAC
S77088_(Ma       TAAGGAAGAG TTTGATAGAT GGTATGATGC CATAAAGAAG GAGTACGAAA TTGATGACAC
M445-SCMV_       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
M292-SCMV_       TAAGGAGGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
M1350-SCMV       CAAGGAAGAG TTTGATAGAT GGTATGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
M56-SCMV_B       TAAGGAAGAG TTCGATAGAT GGTATGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
SCMV-7-Sor       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
SCMV-3-Sor       CAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
KP86093_SC       TAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
SCMV-1-Sor       CAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
KM926614|S       CAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGAAAC
KM926615|S       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
KF744391-S       TAAGGAAGAG TTTGATAGAT GGTACGATGC CATAAAGAAA GAATACGAGA TTGATGACAC
JX188385-S       CAAAGAAGAG TTTGATAGAT GGTATGATGC CATAAAGAAG GAATATGAAA TTGATGACAC
KT630803_S       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
JX286708-S       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
JX286708-S       TAAGGAAGAG TTCGATAGAT GGTACGACGC CATAAAGAAG GAGTACGAGA TCGATGATAC
                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|

                        130        140        150        160        170        180
M435-SCMV_       ACAAATGACA GTTGTCATGA GTGGTCTCAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
M1224-SCMV       ACAAATGACA GTTGTCATGA GTGGTCTAAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
M260-SCMV_       ACAAATGACA GTTGTCATGA GTGGTCTCAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
M311-SCMV_       ACAAATGACA GTTGTCATGA GTGGTCTCAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
M388-SCMV_       ACAAATGACA ATCGTCATGA GTGGTCTAAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
S77088_(Ma       ACAAATGACA GTTGTCATGA GTGGTCTCAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
M445-SCMV_       ACAAATGACA GTCGTCATGA GTGGTCTGAT GGTCTGGTGC ATCGAAAATG GTTGCTCACC
M292-SCMV_       ACAAATGACA GTTGTCATGA GCGGCCTTAT GGTGTGGTGC ATTGAGAACG GTTGCTCACC
M1350-SCMV       ACAAATGACA GTTGTCATGA GCGGTCTTAT GGTATGGTGC ATTGAGAACG GTTGCTCACC
M56-SCMV_B       ACAAATGACA GTCGTCATGA GTGGTTTGAT GGTGTGGTGC ATCGAAAATG GTTGCTCACC
SCMV-7-Sor       ACAAATGACA GTCGTCATGA GTGGTCTGAT GGTCTGGTGC ATCGAAAATG GTTGCTCACC
SCMV-3-Sor       ACAAATGACA GTTGTCATGA GCGGTCTTAT GGTGTGGTGC ATTGAAAACG GTTGCTCACC
KP86093_SC       ACAAATGACA GTCGTCATGA GCGGTCTTAT GGTGTGGTGC ATTGAGAATG GTTGCTCACC
SCMV-1-Sor       ACAAATGACA GTTGTCATGA GCGGTCTTAT GGTATGGTGC ATTGAGAACG GTTGCTCACC
KM926614|S       ACAAATGACA GTTGTCATGA GTGGTCTTAT GGTATGGTGC ATTGAGAACG GTTGCTCACC
KM926615|S       ACAAATGACA GTCGTCATGA GTGGTCTGAT GGTCTGGTGC ATCGAAAATG GTTGCTCACC
KF744391-S       ACAAATGACA GTTGTCATGA GCGGTCTTAT GGTGTGGTGC ATTGAGAACG GTTGCTCACC
JX188385-S       ACAAATGACA GTTGTCATGA GTGGTCTTAT GGTATGGTGC ATCGAAAATG GTTGCTCACC
KT630803_S       ACAAATGACA GTTGTCATGA GCGGTCTCAT GGTATGGTGC ATTGAGAACG GTTGCTCACC
JX286708-S       ACAAATGACA GTCGTCATGA GTGGTCTGAT GGTCTGGTGC ATTGAAAATG GTTGCTCACC
JX286708-S       ACAAATGACA GTCGTCATGA GTGGTCTGAT GGTCTGGTGC ATTGAAAATG GTTGCTCACC
```

Figure 6 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         190        200        210        220        230        240
M435-SCMV_       AAACATAAAC GGAAATTGGA CAATGATGGA CGGGGACGAA CAAAGGGTTT TTCCATTAAA
M1224-SCMV       AAACATAAAC GGAAATTGGA CGATGATGGA CGGGGATGAA CAAAGGGTTT TTCCATTGAA
M260-SCMV_       AAACATAAAC GGAAATTGGA CAATGATGGA CGGGGACGAA CAAAGGGTTT TTCCATTAAA
M311-SCMV_       AAACATAAAC GGAAATTGGA CAATGATGGA CGGGGATGAA CAAAGGGTTT TTCCATTAAA
M388-SCMV_       AAACATAAAC GGAAATTGGA CGATGATGGA CGGGGATGAA CAAAGGGTTT TTCCACTGAA
S77088_(Ma       AAACATAAAC GGAAATTGGA CGATGATGGA CGGAGACGAA CAAAGGGTTT TTCCATTAAA
M445-SCMV_       AAACATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TCCCACTAAA
M292-SCMV_       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTCT TTCCACTCAA
M1350-SCMV       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTTT TTCCACTCAA
M56-SCMV_B       AAATATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TTCCACTAAA
SCMV-7-Sor       AAATATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TCCCACTAAA
SCMV-3-Sor       AAACATAAAC GGAARTTGGA CAATGATGGA TGGAGATGAA CARAGAGTCT TTYCACTCAA
KP86093_SC       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTCT TTCCACTCAA
SCMV-1-Sor       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTTT TTCCACTCAA
KM926614|S       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTTT TTCCACTCAA
KM926615|S       AAACATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TCCCACTAAA
KF744391-S       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGAGTTT TTCCACTCAA
JX188385-S       AAACATAAAC GGAAATTGGA CAATGATGGA TGGAGATGAA CAAAGGGTTT TTCCACTCAA
KT630803_S       AAACATAAAC GGAAATTGGA CAATGATGGA TGAAGATGAA CAAAGAGTTT TTCCACTCAA
JX286708-S       AAACATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TCCCACTAAA
JX286708-S       AAACATAAAC GGAAATTGGA CGATGATGGA TGGGGATGAA CAAAGAGTTT TCCCACTAAA


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                         250        260        270        280        290        300
M435-SCMV_       GCCAGTTATT GAAAACGCAT CTCCAACTTT CCGACAAATC ATGCATCATT TCAGTGATGC
M1224-SCMV       ACCAGTTATT GAAAATGCAT CTCCAACTTT CCGACAAATC ATGCATCATT TCAGTGATGC
M260-SCMV_       GCCAGTTATT GAAAACGCAT CTCCAACTTT CCGACAAATC ATGCATCATT TCAGTGATGC
M311-SCMV_       GCCAGTTATT GAAAACGCAT CTCCAACTTT CCGACAAATC ATGCATCATT TCAGTGATGC
M388-SCMV_       ACCAGTCATT GAAAATGCAT CTCCAACTTT TCGACAAATT ATGCATCATT TCAGTGATGC
S77088_(Ma       GCCAGTTATT GAGAACGCAT CTCCAACTTT CCGACAGATA ATGCATCATT TTAGTGATGC
M445-SCMV_       ACCAGTTATT GAAAACGCAT CTCCAACTTT TCGACAAGTT ATGCATCATT TCAGTGATGC
M292-SCMV_       ACCAGTCATT GAAAACGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
M1350-SCMV       ACCAGTTATT GAAAATGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
M56-SCMV_B       ACCAGTTATT GAAAACGCAT CTCCAACTTT TCGACAAGTT ATGCATCATT TCAGTGATGC
SCMV-7-Sor       ACCAGTTATT GAAAACGCAT CTCCAACTTT TCGACAAGTT ATGCATCATT TCAGTGATGC
SCMV-3-Sor       ACCAGTTATT GAAAACGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
KP86093_SC       ACCAGTCATT GAAAACGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
SCMV-1-Sor       ACCAGTTATT GAAAATGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
KM926614|S       ACCAGTTATT GAAAATGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
KM926615|S       ACCAGTCATT GAAAACGCAT CCCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
KF744391-S       ACCAGTCATT GAAAACGCAT CTCCAACTTT TCGACAAATT ATGCATCATT TTAGTGATGC
JX188385-S       ACCGGTCATT GAGAATGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
KT630803_S       ACCAGTTATT GAAAATGCAT CTCCAACTTT CCGACAAATT ATGCATCATT TTAGTGATGC
JX286708-S       ACCAGTTATT GAAAACGCAT CTCCAACTTT TCGACAAGTT ATGCATCATT TCAGTGATGC
JX286708-S       ACCAGTTATT GAAAACGCAT CTCCAACTTT TCGACAAGTT ATGCATCATT TCAGTGATGC
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Figure 6 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         310        320        330        340        350        360
M435-SCMV_       AGCTGAAGCG TATATAGAGT ACCGAAATTC TACAGAGCGA TACATGCCAA GATACGGTCT
M1224-SCMV       AGCTGAAGCG TATATAGAGT ACCGAAACTC TACAGAGCGA TACATGCCAA GATACGGTCT
M260-SCMV_       AGCTGAAGCG TATATAGAGT ACCGAAATTC TACAGAGCGA TACATGCCAA GATACGGTCT
M311-SCMV_       AGCTGAAGCG TATATAGAGT ACCGAAATTC TACAGAGCGA TACATGCCAA GATACGGTCT
M388-SCMV_       AGCTGAAGCG TATATAGAGT ACCGAAATTC TACAGAGCGA TACATGCCAA GATACGGTCT
S77088_(Ma       AGCTGAAGCG TATATAGAGT ACCGAAACTC TACAGAGCGA TACATGCCAA GATACGGTCT
M445-SCMV_       AGCTGAAGCG TATATAGAAT ACAGAAATTC TACTGAGCGA TACATGCCAA GATATGGACT
M292-SCMV_       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
M1350-SCMV       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
M56-SCMV_B       AGCTGAAGCG TATATAGAAT ACAGAAATTC TACTGAGCGA TACATGCCAA GATATGGACT
SCMV-7-Sor       AGCTGAAGCG TATATAGAAT ACAGAAATTC TACTGAGCGA TACATGCCAA GATATGGACT
SCMV-3-Sor       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
KP86093_SC       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
SCMV-1-Sor       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
KM926614|S       AGCTGAAGCG TACATAGAGT ATAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
KM926615|S       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
KF744391-S       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
JX188385-S       AGCTGAAGCG TATATAGAGT ACAGAAACTC TACTGAGCGA TATATGCCAA GATACGGACT
KT630803_S       AGCTGAAGCG TACATAGAGT ACAGAAACTC TACTGAGCGA TACATGCCAA GATACGGACT
JX286708-S       AGCTGAAGCG TATATAGAAT ACAGAAATTC TACTGAGCGA TACATGCCAA GATATGGACT
JX286708-S       AGCTGAAGCG TATATAGAAT ACAGAAATTC TACTGAGCGA TACATGCCAA GATATGGACT

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                         370        380        390        400        410        420
M435-SCMV_       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ATGAAATGAC
M1224-SCMV       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGACTTCT ATGAAATGAC
M260-SCMV_       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ATGAAATGAC
M311-SCMV_       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ATGAAATGAC
M388-SCMV_       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ACGAAATGAC
S77088_(Ma       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTCGATTTCT ATGAAATGAC
M445-SCMV_       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ACGAAATGAC
M292-SCMV_       TCAGCGCAAT CTTACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
M1350-SCMV       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
M56-SCMV_B       TCAGCGAAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
SCMV-7-Sor       TCAGCGAAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
SCMV-3-Sor       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
KP86093_SC       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
SCMV-1-Sor       TCAGCGCAAT CTCACCGACT ATAGCTTAGC GCGGTATGCA TTTGATTTCT ACGAAATGAC
KM926614|S       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
KM926615|S       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
KF744391-S       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
JX188385-S       TCAGCGCAAT CTCACCGACT ACAGCTTAGC ACGGTATGCA TTTGACTTCT ACGAAATGAC
KT630803_S       TCAGCGCAAT CTCACCGACT ATAGCTTAGC ACGGTATGCA TTTGATTTCT ATGAAATGAC
JX286708-S       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ATGAAATGAC
JX286708-S       TCAGCGAAAT CTCACCGACT ATAGCTTAGC GCGGTATGCT TTTGATTTCT ATGAAATGAC
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Figure 6 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         430        440        450        460        470        480
M435-SCMV_       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M1224-SCMV       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M260-SCMV_       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M311-SCMV_       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M388-SCMV_       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
S77088_(Ma       TTCGCGGACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M445-SCMV_       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M292-SCMV_       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M1350-SCMV       TTCACGCACA CCTGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
M56-SCMV_B       TTCACGCACA CCCGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
SCMV-7-Sor       TTCACGCACA CCCGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
SCMV-3-Sor       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
KP86093_SC       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
SCMV-1-Sor       TTCACGCACA CCAGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
KM926614|S       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
KM926615|S       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
KF744391-S       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
JX188385-S       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCTG CAGCAGTTCG
KT630803_S       TTCACGCACA CCTGCTAGAG CTAAAGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
JX286708-S       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG
JX286708-S       TTCACGCACA CCAGCTAGAG CTAAGGAAGC CCACATGCAG ATGAAAGCCG CAGCAGTTCG


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                         490        500        510        520        530        540
M435-SCMV_       TGGTTCAAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGTGAGACTC AGGAGAATAC
M1224-SCMV       TGGTTCAAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
M260-SCMV_       TGGTTCAAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGTGAGACTC AGGAGAATAC
M311-SCMV_       TGGTTCAAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGTGAGACTC AGGAGAATAC
M388-SCMV_       TGGTTCAAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
S77088_(Ma       TGGTTCGAAC ACACGTCTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
M445-SCMV_       TGGTTCAAAC ACACGACTGT TCGGTTTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
M292-SCMV_       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
M1350-SCMV       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
M56-SCMV_B       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
SCMV-7-Sor       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
SCMV-3-Sor       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
KP86093_SC       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
SCMV-1-Sor       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
KM926614|S       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
KM926615|S       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
KF744391-S       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
JX188385-S       TGGTTCAAAC ACACGACTGT TCGGCTTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
KT630803_S       TGGTTCAAAC ACACGACTGT TCGGTCTGGA CGGAAATGTC GGCGAGACCC AGGAGAATAC
JX286708-S       TGGCTCAAAC ACACGACTGT TCGGCTTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
JX286708-S       TGGCTCAAAC ACACGACTGT TCGGCTTGGA CGGAAATGTC GGCGAGACTC AGGAGAATAC
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Figure 6 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         550        560        570        580        590        600
M435-SCMV_       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAACATGCAC TCTCTGTTGG GAGTGCAGCA
M1224-SCMV       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAACATGCAC TCTCTGTTGG GAGTGCAGCA
M260-SCMV_       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAACATGCAC TCTCTGTTGG GAGTGCAGCA
M311-SCMV_       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAACATGCAC TCTCTGTTGG GAGTGCAGCA
M388-SCMV_       AGAGAGACAC ACAGCCGGCG ACGTTAGTCG CAACATGCAC TCTCTGTTGG GAGTGCAGCA
S77088_(Ma       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAACATGCAC TCTTTGTTGG GAGTGCAGCA
M445-SCMV_       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
M292-SCMV_       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
M1350-SCMV       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
M56-SCMV_B       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
SCMV-7-Sor       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
SCMV-3-Sor       AGAGAGACAC ACAGCTGGCG ATGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
KP86093_SC       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
SCMV-1-Sor       AGAGAGACAC ACAGCTGGCG ATGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
KM926614|S       AGAGAGACAC ACAGCTGGCG ATGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
KM926615|S       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
KF744391-S       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
JX188385-S       AGAGAGACAC ACAGCTGGCG ATGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
KT630803_S       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
JX286708-S       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA
JX286708-S       AGAGAGACAC ACAGCTGGCG ACGTTAGTCG CAATATGCAC TCTCTGTTGG GAGTGCAGCA

                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         610        620        630        640        650        660
M435-SCMV_       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AGTATATTAC TAAATAGTAC
M1224-SCMV       GCACCACTAG TCTCCTGGAA ACCCTGTTAG CAGTACCTAT AGTATATTAC TAAATAGTAC
M260-SCMV_       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AGTATATTAC TAAATAGTAC
M311-SCMV_       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AGTATATTAC TAAATAGTAC
M388-SCMV_       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AGTATATTAC TAAATAGTAT
S77088_(Ma       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AGTATATTAC TAAATAGTAC
M445-SCMV_       ACACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATAT-AC TAA-------
M292-SCMV_       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
M1350-SCMV       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
M56-SCMV_B       GCACCACTAG TTTCCTGGAA ACCCTGTTTG CAGTACCTAC AATATGT-AC TAA-------
SCMV-7-Sor       GCACCACTAG TTTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
SCMV-3-Sor       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATAT-AC TAA-------
KP86093_SC       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
SCMV-1-Sor       GCACCACTAG TCTCTTGGAA ACCCTGTTTG CAGTACCTAT AATATAT-AC TAA-------
KM926614|S       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATAT-AC TAA-------
KM926615|S       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
KF744391-S       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
JX188385-S       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCAAT AATATAT-AC TAA-------
KT630803_S       GCACCACTAG TCTCCTGGAA ACCCTGTTTG CAGTACCTAT AATATGT-AC TAA-------
JX286708-S       ACATCAC--- ---------- ---------- ---------- ---------- ----------
JX286708-S       ACATCAC--- ---------- ---------- ---------- ---------- ----------
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Figure 6 Continued

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                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|

                         670        680        690        700        710        720
M435-SCMV_       GTTCGTGAGG CCTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
M1224-SCMV       GTTCGTGAGG CCTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
M260-SCMV_       GTTCGTGAGG CCTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
M311-SCMV_       GTTCGTGAGG CCTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
M388-SCMV_       GTTCGTGAGG CTTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
S77088_(Ma       GTTCGCGAGG CCTTGCCTCG TGTGTATGTG AGGTTCTACC TCGTATTTAC TATTTCAGTA
M445-SCMV_       ---------- ---TATATAG TACGTTGGTG AGGCTTTGCC TCGGTTTTAC TATCTTATTA
M292-SCMV_       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTCTTCAC TATTT-GTTA
M1350-SCMV       ---------- ---TATATAG TATGTCAGTG AGGTCTTACC TCGTCTTTAC TATTT-GTTA
M56-SCMV_B       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTCTTTAC TAT-TTGTTA
SCMV-7-Sor       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTCTTTAC TAT-TTGTTA
SCMV-3-Sor       ---------- ---TATATAG TATCTCAGTG AGGTTTTACC TCGATTTTAC TATTTTATTA
KP86093_SC       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTCTTTAC TATTT-GTTA
SCMV-1-Sor       ---------- ---TATATAG TATCTCAGTG AGGTTTTACC TCGACTTTAC TATTTTATTA
KM926614|S       ---------- ---TATCTAG TATCTCAGTG AGGTTTTACC TCGACTTTAC TATTTTATTA
KM926615|S       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTCTTTAC TATTT-GTTA
KF744391-S       ---------- ---TATATAG TATGTCAGTG AGGTTTTACC TCGTTTTTAC TATTT-GTTA
JX188385-S       ---------- ---TATATAG TACTTTAGTG AGGTTTTACC TCGTCTTTAC TATTTTATTA
KT630803_S       ---------- ---TATATAG TATCTCAGTG AGGTTTTACC TCGACTCTAC TATTTTATTA
JX286708-S       ---------- ---------- ---------- ---------- ---------- ----------
JX286708-S       ---------- ---------- ---------- ---------- ---------- ----------


                 ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                         730        740        750        760        770        780
M435-SCMV_       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
M1224-SCMV       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
M260-SCMV_       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
M311-SCMV_       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
M388-SCMV_       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
S77088_(Ma       --TATGTACT TTTAGCGTGA ACCAGTCTGC AGGACACAGG GTTGGACCCA GTGTCTTCTG
M445-SCMV_       TGTATGTATT TACAGCGTGA ACCAGTCTGC AGCATGCAGG GTTGGACCCA GCGTGTTCTG
M292-SCMV_       TGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
M1350-SCMV       TGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
M56-SCMV_B       TGTATGTATT TAA-GCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
SCMV-7-Sor       TGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
SCMV-3-Sor       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
KP86093_SC       TGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
SCMV-1-Sor       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
KM926614|S       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
KM926615|S       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
KF744391-S       TGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
JX188385-S       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
KT630803_S       CGTATGTATT TAAAGCGTGA ACCAGTCTGC AGCATACAGG GTTGGACCCA GTGTGTTCTG
JX286708-S       ---------- ---------- ---------- ---------- ---------- ----------
JX286708-S       ---------- ---------- ---------- ---------- ---------- ----------
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Figure 6 Continued

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                ....|....| ....|....| ....|...
                        790        800
M435-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCAAT
M1224-SCMV      GTGTAGCGTG TACTAGCGTC GAGCCAAT
M260-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCAAT
M311-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCAAT
M388-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCAAT
S77088_(Ma      GTGTAGCGTG TACTAGCGTC GAGCCAAT
M445-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCATG
M292-SCMV_      GTGTAGCGTG TACTAGCGTC GAGCCATG
M1350-SCMV      GTGTAGCGTG TACTAGCGTC GAGCCATG
M56-SCMV_B      GTGTAGCGTG TACTAGCGTC GAGCCATG
SCMV-7-Sor      GTGTAGCGTG TAC-AGCGTC GAGCCATG
SCMV-3-Sor      GTGTAGCGTG TACTAGCGTC GAGCCATG
KP86093_SC      GTGTAGCGTG TACTAGCGTC GAGCCATG
SCMV-1-Sor      GTGTAGCGTG TAC-AGCGTC GAGCCATG
KM926614|S      GTGTAGCGTG TACTAGCGTC GAGCCATG
KM926615|S      GTGTAGCGTG TACTAGCGTC GAGCCATG
KF744391-S      GTGTAGCGTG TACTAGCGTC GAGCCATG
JX188385-S      GTGTAGCGTG TACTAGCGTC GAGCCATG
KT630803_S      GTGTAGCGTG TACTAGCGTC GAGCCATG
JX286708-S      ---------- ---------- --------
JX286708-S      ---------- ---------- --------
```

Figure 6 Continued

About 23 bp variations in the sequences were due to the nucleotide insertions and deletions. The SCMV isolates M388, M311, M260, M1224 and M435 had a 20 bp insertion "ATAGTAC/TGTTCGTGAGGC/TCT", which was also detected in the GenBank Acc. No S77088. These isolates were selected to represent SCMV sequences reported from maize and other cereals in African countries where MLN was reported so as to provide diverse representation for comparative analysis with the SCMV isolates sequenced in this study.

4.5 Phylogenetic analysis

Pairwise and divergence analysis revealed very high sequence homology (99% to 100%) among the 7 MCMV isolates from Tanzania (Table 11). Of the SCMV isolates, only M260 and M435 were 100% identical. Other isolates differed by 2 to 11% as revealed by the pairwise percent homolog analysis and the divergence analysis (Table 12).

Table 11: Estimates of evolutionary divergence between the nucleotide sequences of coat protein encoding gene of MCMV

Sl. No.	Sl. No -->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	M4-MCMV-CP_Baba	0														
2	M562-MCMV-CP_Arum	0.000	0													
3	M2903-MCMV-CP_Arus	0.000	0.000	0												
4	M210-MCMV-CP_Hana	0.000	0.000	0.000	0											
5	M259-MCMV-CP-Kond	0.008	0.008	0.008	0.008	0										
6	M361-MCMV-CP_Kong	0.000	0.000	0.000	0.000	0.008	0									
7	M442-MCMV-CP_Mbul	0.000	0.000	0.000	0.000	0.008	0.000	0								
8	JX286709_MCMV_CP_Kenya	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0							
9	KP798455_MCMV-CP_Ethiopia	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0						
10	EU358605_MCMV-CP_USA	0.008	0.008	0.008	0.008	0.015	0.008	0.008	0.008	0.008	0					
11	AY587605_MCMV-CP_Thailand	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.008	0				
12	KT630268_MCMV-CP_Ecuador	0.008	0.008	0.008	0.008	0.015	0.008	0.008	0.008	0.008	0.016	0.008	0			
13	JQ943666_MCMV-CP_China	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.008	0.000	0.008	0		
14	JF422772_MCMV-CP_China	0.048	0.048	0.048	0.048	0.056	0.048	0.048	0.048	0.048	0.057	0.048	0.056	0.048	0	
15	KP203016_MCMV-CP_China_Sorghum	0.015	0.015	0.015	0.015	0.023	0.015	0.015	0.015	0.015	0.023	0.015	0.023	0.015	0.065	0

The number of base substitutions per site between the nucleotide sequences of coat protein encoding gene of MCMV analyzed using the Maximum Composite Likelihood model (Tamura *et al*., 2004) in MEGA6 program (Tamura *et al*., 2013). The analysis involved 15 nucleotide sequences and codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 131 positions in the final dataset.

Table 12: Estimates of evolutionary divergence between the nucleotide sequences of coat protein encoding gene of SCMV

Sl. No.	Sl. No. -->	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	M435-SCMV_Kara	0																			
2	M1224-SCMV_Kite	0.029	0																		
3	M260-SCMV_Kond	0.000	0.029	0																	
4	M311-SCMV_Kong	0.002	0.027	0.002	0																
5	M388-SCMV_Mbul	0.039	0.027	0.039	0.038	0															
6	S77088_(Maize dwarf mosaic	0.029	0.041	0.029	0.031	0.054	0														
7	M445-SCMV_Sima	0.071	0.071	0.071	0.069	0.067	0.095	0													
8	M292-SCMV_Cham	0.083	0.089	0.083	0.081	0.096	0.099	0.081	0												
9	M1350-SCMV_Hana	0.077	0.071	0.077	0.075	0.087	0.089	0.077	0.018	0											
10	M56-SCMV_Bab	0.077	0.073	0.077	0.075	0.073	0.097	0.024	0.070	0.065	0										
11	SCMV-7-Sorghum-Tz	0.077	0.077	0.077	0.075	0.077	0.101	0.015	0.073	0.069	0.008	0									
12	SCMV-3-Sorghum-Tz	0.079	0.081	0.079	0.077	0.096	0.095	0.077	0.013	0.012	0.063	0.065	0								
13	KP86093_SCMV-Ethiopia-finger_millet	0.079	0.085	0.079	0.077	0.093	0.095	0.073	0.010	0.018	0.063	0.065	0.013	0							
14	SCMV-1-Sorghum-Tz	0.077	0.075	0.077	0.075	0.087	0.093	0.073	0.022	0.010	0.071	0.071	0.012	0.022	0						
15	KM926614|SCMV-Kenya-finger_millet	0.083	0.081	0.083	0.081	0.097	0.099	0.081	0.024	0.012	0.069	0.069	0.013	0.020	0.012	0					
16	KM926615|SCMV-Kenya-finger_millet	0.075	0.071	0.075	0.073	0.079	0.091	0.043	0.041	0.045	0.041	0.036	0.041	0.034	0.049	0.045	0				
17	KF744391-SCMV-Rwanda-Maize	0.077	0.083	0.077	0.075	0.091	0.093	0.071	0.008	0.013	0.061	0.063	0.012	0.008	0.017	0.018	0.036	0			
18	JX188385-SCMV-Maize-Ohio	0.082	0.069	0.082	0.080	0.073	0.087	0.092	0.072	0.059	0.084	0.088	0.061	0.065	0.059	0.057	0.078	0.067	0		
19	KT630803_SCMV-Maize-Kenya	0.079	0.075	0.079	0.077	0.087	0.095	0.052	0.039	0.031	0.041	0.041	0.036	0.039	0.034	0.034	0.040	0.034	0.066	0	
20	JX286708-SCMV-Maize-Kenya(2)	0.075	0.074	0.075	0.073	0.075	0.098	0.010	0.081	0.077	0.027	0.022	0.077	0.073	0.076	0.081	0.047	0.071	0.096	0.052	0
21	JX286708-SCMV-Maize-Kenya	0.075	0.074	0.075	0.073	0.075	0.098	0.010	0.081	0.077	0.027	0.022	0.077	0.073	0.076	0.081	0.047	0.071	0.096	0.052	0.000

The number of base substitutions per site between the nucleotide sequences of coat protein encoding gene of SCMV analyzed using the Maximum Composite Likelihood model (Tamura *et al*., 2004) in MEGA6 program (Tamura *et al*., 2013). The analysis involved 21 nucleotide sequences and codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 604 positions in the final dataset.

Phylogenetic analysis performed together with the corresponding nucleotide sequences of 8 MCMV isolates available in the NCBI GenBank database revealed a single grouping without any noticeable divergence (Table 10; Figure 7) indicating a high degree of sequence homology between the various geographic isolates of MCMV reported globally. Phylogenetic for SCMV reconstruction arranged 21 sequences used for the analysis into two groups (Table 11; Figure 8). Group 1 consisted of SCMV isolates SCMV-1, SCMV-3, SCMV-7, M56, M445, M1350 and M292; whereas the Group 2 consisted of M388, M311, M260, M1224 and M435. All the SCMV isolates reported previously from East Africa were aligned in Group 1. Nucleotide sequence identities for isolates in Group 1 were 94% to 99%, while isolates in group 2 were 96% to 100% identical. The nucleotide sequences between the isolates in the two groups were about 89% identical.

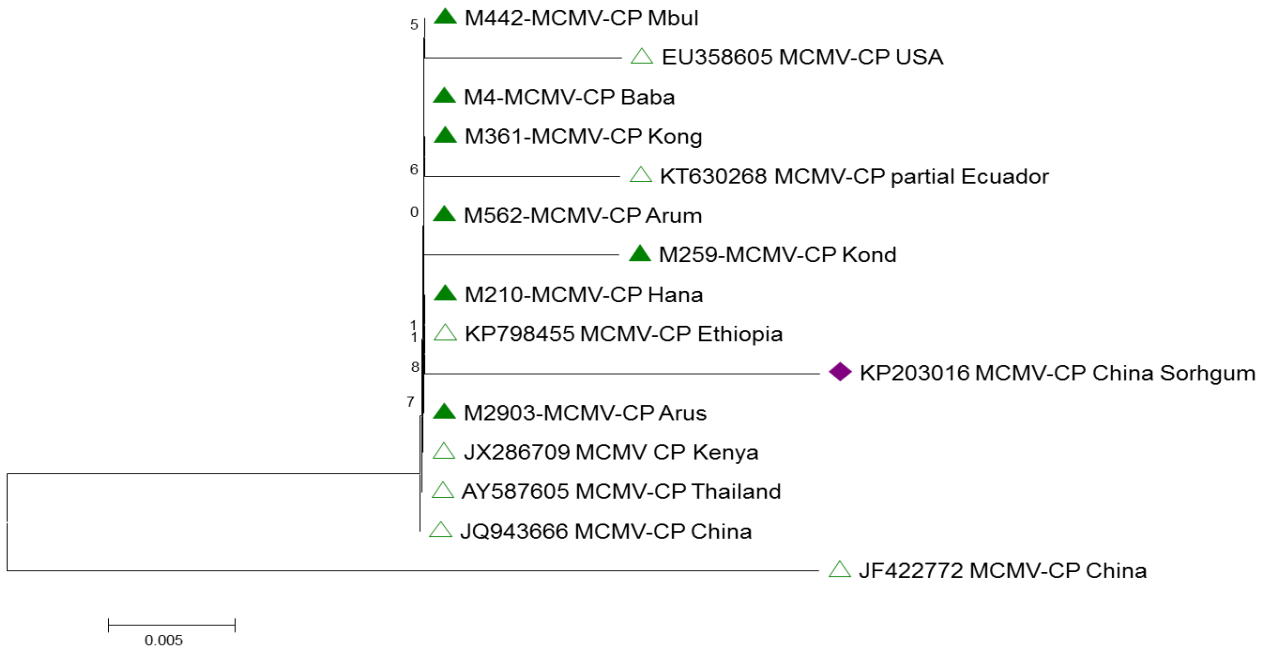

Figure 7: The phylogenetic relationship of MCMV isolates of Tanzania inferred from the CLUSTALW aligned coat protein encoding gene sequence using the Neighbor - Joining method (Saitou and Nei, 1987) in MEGA6 program (Tamura *et al*., 2013). The optimal tree with the sum of branch length = 0.08655555 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Tanzania isolates

sequenced in this study are indicated with a solid triangle and the accession numbers are indicated for those downloaded from the NCBI GenBank. First letter representing Maize, a numeral represent sampling number and last four letters represent Districts (Mbul = Mbulu, Baba = Babati, Kong = Kongwa, Arum = Arumeru, Kond = Kondoa, Hana = Hanang and Arus = Arusha)

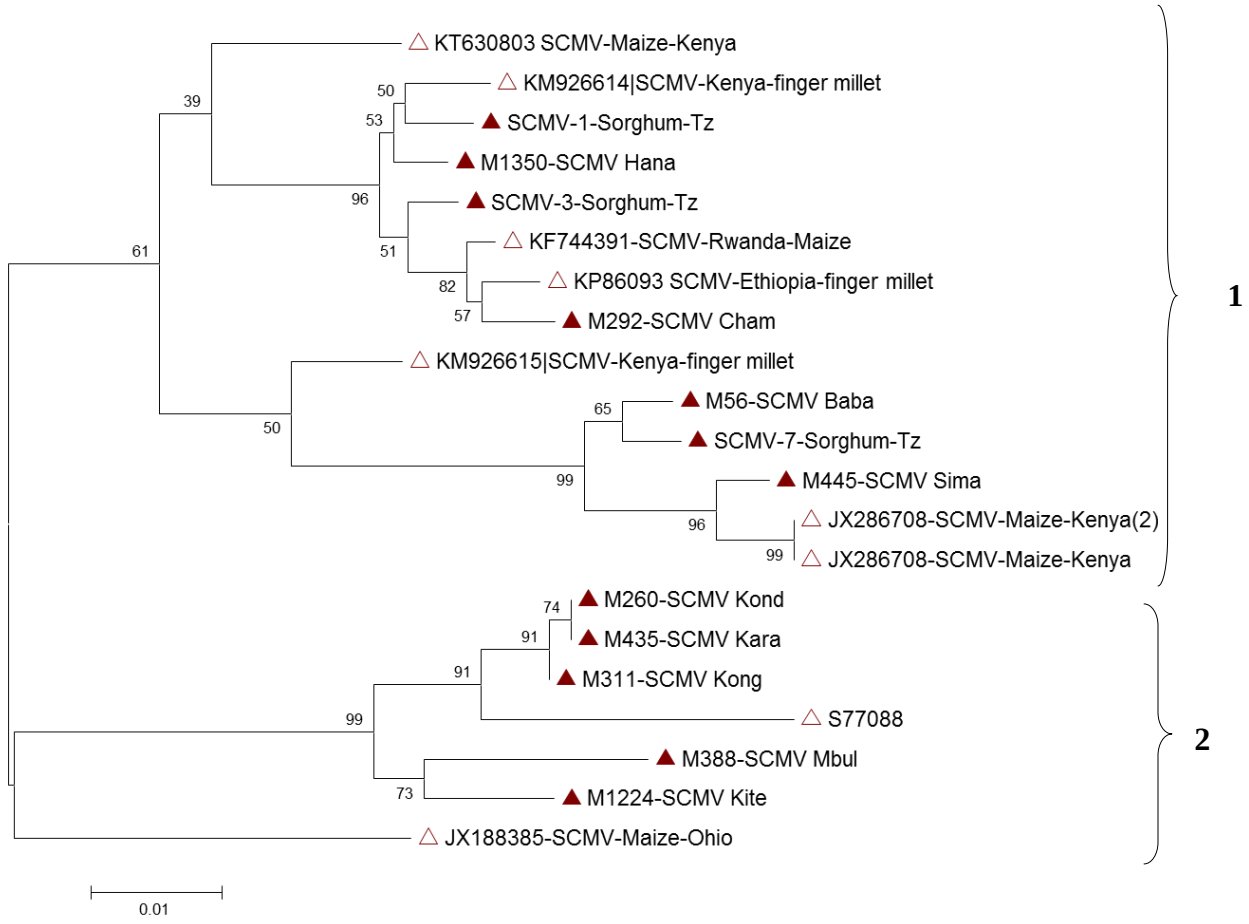

Figure 8: The phylogenetic relationship of SCMV isolates of Tanzania inferred from the CLUSTALW aligned 3' end of the coat protein encoding gene sequence using the Neighbor-Joining method (Saitou and Nei, 1987) in MEGA6 program (Tamura *et al*., 2013). The optimal tree with the sum of branch length = 0.28384360 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Tanzanian isolates

sequenced in this study are indicated with solid triangle and the accession numbers are indicated for those downloaded from the NCBI GenBank. First letter representing Maize, a numeral represent sampling number and last four letters represent Districts (Mbul = Mbulu, Baba = Babati, Kong = Kongwa, Kite = Kiteto, Kara = Karatu, Cham = Chamwino, Kond = Kondoa, Hana = Hanang and Sima = Simanjiro)

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussions

The objectives of this study were to establish the distribution of MLN disease and its causal viruses; and identify the alternative hosts for the disease. Incidence of MLN disease varied from region to region and from district to district, with high rates in regions of Manyara in Babati districts and Arusha in Meru district and low incidences observed in Dodoma in Mpwapwa districts (Table 6, 7). High incidence could be due to the cropping system in these regions, which contributes to vector population growth and virus spread. Specifically, farmers in Meru are known to grow maize throughout the year. For the farmers around the AVRDC - Madira area this is due to availability of water for irrigation and good market for roasted maize during periods of maize scarcity. In Babati, maize seed farming and sugarcane farming are practised throughout the year, for example at the Krishna Seed Farm and Mara Estate for maize seed and sugar processing, respectively.

Continuous presence of the hosts of these vector(s) provides conducive conditions for vector population build up and virus spread to new fields. Viruses such as MCMV and SCMV would also be harbored, contributing towards high incidence of MLN causing viruses in Babati district, compared to other districts surveyed. In fact, in a place where maize production is continuous all year round with no maize-free periods, early planted maize will serve as a reservoir of both viruses and vectors for a newly planted crop as well as a number of wild grass species (Konate & Traore, 1992; Shepherd *et al.,* 2010). This also explains the high incidence observed during the long rains compared to the short rains during the survey period (Table 10); the maize planted during short rains served as the reservoir for both viruses and insect vectors for maize planted during the long rains.

Low incidences observed in Dodoma region is mainly due to climatic conditions and cropping systems in this region. Dodoma is a dry region, which receives about 570mm annual rainfall. Farmers in this region, therefore, prefer to grow crops other than maize which are drought tolerant; these crops include: sorghum, pear millet and groundnut. These crops are not highly

preferred by MLN causing viruses compared to maize and sugarcane, which are natural hosts for MCMV and SCMV, respectively. Also, in this region there is only one maize cropping season start from December to March followed by long periods of up to 7 months with no maize in the field (URT, 2006); such conditions reduce inoculum potential for both viruses and vectors.

In all districts surveyed disease severity was moderate, ranging from 2 to 2.6 (Table 7). Generally, incidence and severity of a disease could be attributed to a number of factors which includes; cultivar, nature of infection, virus strain/species, vector density, initial inoculum, time of infection, rainfall, quality of soil, and crop management. In this study the moderate level of severity observed is mainly attributed to single infections of either MCMV or SCMV. For severe occurrence of disease symptoms mixed infection with both viruses is required. Symptom severities were high in a few fields in Monduli, Meru, Karatu and in Babati district, specifically where double infection of MCMV and SCMV was observed (Table 10). Mixed infections have also been reported in previous studies of MLN in Kenya and Rwanda (Wangai *et al*., 2012; Adams *et al*., 2014). Co-infection is possible since causal viruses are transmitted by both mechanical means and vector transmission. Synergistic interaction between co-infecting viruses has been shown to result in severe symptoms. This type of interaction is reported to occur with MLN and is comparable to the increased severity of cassava mosaic disease realized in Uganda with mixed infection of ACMV and EACMV-UG in the late 90's (Pita *et al*., 2001). Mixed infections have epidemiological implications because synergism between viruses increases virus titer, disease incidence, severity and associated yield losses (Zhang, Holt & Colvin, 2001).

The high incidence of 100% found in some surveyed locations shows that maize is greatly affected by MLN causing viruses. Most infection was attributed to insect vector transmission and cropping systems since the incidence was lower for maize planted during short rains and higher for that maize after long rains (Table 10). The possible role of seed in transmission of MLN causing viruses can, however, not be ignored. Jensen at al. (1991) reported that out of 42,000 maize seeds planted from different seed lots, 17 transmit MCMV to the seedlings; this is equal to 0.04% transmission of MCMV through seed. Seed transmission only occurs during germination when the seed coat mechanically injures the germinating plumule (Hohmann, Fuchs, Gruntzig, & Oertel, 1999). Epidemiologically, this small rate of transmission is significant since a virus can be introduced to a new area through seed and subsequently be spread by insect vectors, leading

to a high number of infected plants (Maule & Wang, 1996). However, the situation may not be the same in Tanzania since MLN seems to be highly concentrated in the northern part of the country bordering Kenya with low incidence reports from the western parts that border Uganda (Makumbi & Wangai, 2013). No reports of maize with MLN symptoms have been documented from the southern part of Tanzania, which is the major maize producing region that contributes more than 46% of maize in Tanzania (Nkonya *et al*., 1998). Unfortunately, most of the maize seed used for commercial production comes from northern Tanzania and Kenya, which are hot spots for MLN (Makumbi & Wangai, 2013). The Selian Agriculture Research Institutes (SARI) uses the same seed and registered no infection in the short rains although the subsequent (long) rains showed positive infection with MCMV.

All maize varieties grown by farmers were infected by MLN causing viruses (Table 8). While this may indicate that the maize varieties lack resistance to these viruses, the fact that the disease surveys were conducted on farmers' fields with different inoculum levels negates such a conclusion. Further testing of maize varieties under known inoculum pressures would be required. However, of all grown varieties, only TMV1, Pan691 and Katumani had low disease incidences (Table 8). Differential response of varieties to virus disease infection is not new and has been reported with other virus diseases such as CMD with cassava (Ntawuruhunga *et al*., 2007).

The symptoms of MLN disease observed in farmers' fields in Tanzania included chlorotic mottling on leaves developing from the base of young whorl leaves upward to the leaf tips; leaf mottling and necrosis developing from leaf margins to the mid-rib, infected crops had no or small cobs and plants frequently die before tasseling. Similar symptoms have been associated with MLN in Hawaii (Nelson *et al.,* 2011), Kenya (Wangai *et al.,* 2012), Rwanda (Adam *et al.,* 2014) and Congo (Lukanda *et al.,* 2014). Identification of the causal agents of virus diseases based on visual examination is not reliable since symptom development is dependent on several factors, including: time of infection, plant variety and growth stage, virus strain and environmental conditions. Disease symptoms may also be observed after major damage has been done (Agrios, 2005; Batool, Khan, Farooq, Mughal & Iftikhar, 2011). Early diagnosis is, therefore, essential for disease management.

Studying the host range of MLN in the surveyed area established the SCMV hosts are sugarcane (*Saccharum officinarium*), finger millet (*Eleusine coracana*), sorghum (*Sorghum bicolor*) and bristly foxtail (*Setaria verticillata)* (Plate 3). This is not surprising since MLN causing viruses are known to have alternative hosts belonging to the plant genera *Bromus, Panicum, Saccharum, Eleusine* and *Sorghum.* MCMV was not detected in all 254 weeds and non-maize samples tested, this could be due to availability of maize in fields throughout the year so MCMV tends to survive in the primary host; a similar finding was reported by Nelson et al. (2011). This is the first report of SCMV infecting sugarcane, finger millet, sorghum and bristly foxtail in Tanzania. Alternative crop hosts and weeds were commonly found around maize fields and may explain early infection of maize plants with SCMV. It is interesting to note that in certain districts such as Babati, maize and sugarcane are grown as monocrop but within the same farm. In other districts such as Hanang, finger millet was intercropped with maize. On the other hand, sorghum was a widely distributed crop in all districts surveyed. This information on cropping systems provides a foundation for the development of Integrated Pest Management (IPM) options for MLN in Tanzania. It also raises some questions on the nature these options may take. For example, while bristly foxtail could be regularly removed from within and around maize fields, the same cannot always be said for sorghum and sugarcane that are crops of interest except where they occur as volunteers.

In this study, MLN causing viruses were detected using serological tests with results being confirmed in RT-PCR and sequencing. Analysis of the infected maize leaves confirmed the presence of MCMV and SCMV in 13 of 14 districts surveyed. These viruses have also been identified in maize from East and Central Africa (Kenya, Rwanda, Congo and Ethiopia). MCMV was the most prevalent virus infecting maize [78 out of 200 (39%)] tested positive for MCMV (Table 10). Fewer maize samples [22 out of 200 (11 %)] tested positive for SCMV (Plate 5). This observation has also been reported by Mahuku *et al*. (2015) whereby few maize samples tested positive for SCMV compared to MCMV. However, it is possible that SCMV was not detected in some samples because of low concentration of viral nucleic acid in sampled plants (Nono-Womdim *et al.,* 1996).

This is the first study to analyze sequences of MCMV and SCMV in Tanzania (at the time of writing, there were no sequences of MCMV or SCMV from Tanzania in the NCBI GenBank

database). Sequence analysis indicated very high levels (99 to 100%) of homology among MCMV isolates so far identified in Africa (Mahuku *et al*., 2015). Greater genetic variability was, however, observed amongst the SCMV isolates. The main difference was a 20 bp insertion [ATAGTAC/TGTTCGTGAGGC/TCT] which was also detected in the SCMV sequence isolate ID# S77088 (MDMV) reported from maize in USA. Although this isolate in the NCBI database is named as the MDMV isolates (GenBank Acc. No. S77088), it is likely to be misnomer based on the close alignment of this sequence with the SCMV isolates.

MCMV isolates from maize in Tanzania, Kenya, Rwanda and Ethiopia were identical (Figure 7: Table 11). These isolates were also identical to MCMV isolates from sorghum in China and finger millet in Kenya indicating that the same virus is likely spreading between different hosts and in different countries this could be due to movement of material between and within countries. The high degree of homology within the coat protein region also indicates that, currently, MCMV isolates in Africa have a common origin or don't differ in coat protein region sequenced. In contrast to MCMV, the SCMV isolates sequenced were divergent by up to 11% (Figure 8; Table 12). They are also widely distributed compared to MCMV and seems to have wider host range. According to International Committee on Taxonomy of Viruses (ICTV), <76% identity in the nucleotide sequence of the complete coat protein gene is needed to define a new potyvirus species (Adams *et al*., 2011). Greater SCMV diversity suggests possible presence of virus isolates in the region for much longer period. Analyzed sequences did not reveal any host specific isolates indicating the movement of isolates between the hosts most likely by insect vectors. This study also indicates that diverse SCMV isolates in nature are already widely distributed in the surveyed regions of Tanzania.

5.2 Conclusions

These results of this study present a good starting point for further diagnosis of MLN disease and its management in Tanzania. The viruses MCMV and SCMV were identified to cause MLN disease in 13 of 14 districts surveyed in these studies. MCMV was most prevalent (39%), SCMV (11%) and MCMV & SCMV (5.5%) and are characterized by symptoms such chlorosis, leaf mottling, necrosis and poor grain fill for late infected maize. These viruses are already reported to occur in Kenya, Uganda, Rwanda, Democratic Republic of Congo and Ethiopia (Wangai *et al.*, 2012, MAAIF, 2013, Adams *et al.*, 2014, Lukanda *et al*., 2014, Mahuku *et al*., 2015).

Sugarcane, finger millet, sorghum and bristly foxtail were reported to be alternative hosts of SCMV for the first time in Tanzania; these alternative hosts were found to occur around maize crops or in sole sugarcane farms. MCMV was not detected to occur in other crops or weeds; these indicate MCMV is not overwintering between seasons in other crops apart from maize as reported early in Hawaii maize to be the natural host for MCMV (Nelson *et al.,* 2011). Generated sequences have confirmed occurrence of SCMV as the only potyvirus associated with cereals in East Africa.

5.3 Recommendations

Countries which reported MLN diseases also report almost the same symptom characteristics. For researchers and extension workers these similar reports on symptoms may be very useful in disease identification, but for farmers symptomatology may not be an appropriate way to identify MLN disease. It is recommended that the Government of Tanzania establishes a diagnostics laboratory in the northern part of the country, which will serve as a national laboratory for MLN disease. This would provide information on MLN disease occurrence in other parts of Tanzania and support early identification of the causing agents for early intervention. This proposal is made in the context of SARI in Arusha having the mandate for research and development in maize, wheat and beans. More work could also be done in indexing of seed and development of farmer-friendly techniques for virus elimination from seed.

Findings of this study lead to a clear understanding of the necessity for management approaches especially in those districts which report high incidences like Babati, Mbulu, Meru and Monduli which include; Intensive education of the farmers on the economic importance of maize viruses, Promotion of TMVI, Staha, Pan691 and Katumani maize varieties to all areas under MLN infection, breeding of resistant varieties and encouraging farmers to manage weeds and plant maize at the onset of rains. Other options for further consideration are: evaluation of pesticides for management of MLN (Gururani *et al*., 2012). Measures such as planting of legumes crops during the short rains would also reduce the level of inoculum of the causal pathogens during long rains. In addition, rotation with non-susceptible alternative crops, like tomatoes, onions and legumes especially during periods of poor returns from the maize crop and in the absence of susceptible weeds, breaks the disease cycle (Wisler *et al.,* 1997).

More work is required to identify the alternative hosts of MCMV in Tanzania. The hosts identified with SCMV should also be confirmed in controlled experiments.

Full sequencing of SCMV genomes will perhaps be required for better understanding of the virus' taxonomy. It is also worth investigating if the various SCMV isolates interact similarly with MCMV in causing MLN.

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APPENDICES

Appendix1: Disease and Insect Pest Survey: Data Collection Sheet

Sheet #	
Date/Time	
Location name	
District/LGA	
State	
Agro-ecology	
Latitude	
Longitude	
Altitude (m)	
Summary	Severity range:

Plant (#)	Symptoms	Severity score	Details of sampled plant				
			P#	Photo ID	Symptoms	Sev. score	Variety
1			1				
2			2				
3			3				
4			4				
5			5				
6							
7							
8							
9							
10							
11							
12							
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30							

Abbreviations for symptom description:
Prefix: m – mild; o – moderate; s – severe; Suffix: m – mosaic; mo – mottling; puc – puckering; st – stunting; d – deformation; de – death

Severity rating criteria:

1. No visible symptoms; plants apparently healthy
2. Mild mosaic/mild mottling on few leaves/branches of a plant (symptoms on 25% of the plant)
3. Mosaic/puckering/mottling/necrosis/vein clearing symptoms cover 50% of the plant
4. Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant) but no stunting of deformation
5. Severe mosaic/mottling/yellowing/necrosis and severe stunting (entire plant) deformation and death of the infected plants

Appendix 2: Comparison of mean severity and incidence of MLN in district surveyed in 2015

District		MLN Severity	MLN Incidence
Meru		2.5±0.3	29.2±6.2
	Arusha_urban	2±0.3	7±16.3
	Babati	2.4±0.8	36.82±4
	Chamwino	2±0.3	9±13.3
	Hanang	2.3±0.2	14.86±8.7
	Karatu	2.4±0.2	23.87±8.7
	Kiteto	2±0.2*	6.57±8.7*
	Kondoa	2±0.2*	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7*
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Arusha_Urban	Babati	2.4±0.8	36.82±4
	Chamwino	2±0.3	9±13.3
	Hanang	2.3±0.2	14.86±8.7
	Karatu	2.4±0.2	23.87±8.7
	Kiteto	2±0.2	6.57±8.7
	Kondoa	2±0.2	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Babati	Chamwino	2±0.3	9±13.3*
	Hanang	2.3±0.2	14.86±8.7*
	Karatu	2.4±0.2	23.87±8.7
	Kiteto	2±0.2	6.57±8.7*
	Kondoa	2±0.2*	12.44±7.68*
	Kongwa	2.11±0.2	6.11±7.7*
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5*
	Simanjiro	2.3±0.2	12.3±11.5*

Chamwino	Hanang	2.3±0.2	14.86±8.7
	Karatu	2.4±0.2	23.87±8.7
	Kiteto	2±0.2	6.57±8.7
	Kondoa	2±0.2	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Hanang	Karatu	2.4±0.2	23.87±8.7
	Kiteto	2±0.2	6.57±8.7
	Kondoa	2±0.2	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Karatu	Kiteto	2±0.2	6.57±8.7
	Kondoa	2±0.2	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Kiteto	Kondoa	2±0.2	12.44±7.68
	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3*
	Monduli	2.6±0.2*	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Kondoa	Kongwa	2.11±0.2	6.11±7.7
	Mbulu	2.2±0.2	30.10±7.3
	Monduli	2.6±0.2*	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Kongwa	Mbulu	2.2±0.2	30.10±7.3*
	Monduli	2.6±0.2	25.40±10.3
	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Mbulu	Monduli	2.6±0.2	25.40±10.3

	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Monduli	Mpwapwa	2±0.2	5.6±11.5
	Simanjiro	2.3±0.2	12.3±11.5
Mpwapwa	Simanjiro	2.3±0.2	12.3±11.5