CHARACTERIZATION OF MAIZE CHLOROTIC MOTTLE VIRUS AND SUGARCANE MOSAIC VIRUS CAUSING MAIZE LETHAL NECROSIS DISEASE AND SPATIAL DISTRIBUTION OF THEIR ALTERNATIVE HOSTS IN KENYA

ELIZABETH SIAGO KUSIA

MASTER OF SCIENCE

(Molecular biology and biotechnology)

PAN AFRICAN UNIVERSITY INSTITUTE FOR BASIC SCIENCES TECHNOLOGY AND INNOVATION

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Characterization of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* causing maize lethal necrosis disease and spatial distribution of their alternative hosts in Kenya

Elizabeth Siago Kusia

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2014

DECLARATION

This thesis is my original work and has not been submitted to any other university for examination.

Signature:.....…… Date:.....

Elizabeth Siago Kusia

MB300-0006/12

This thesis report has been submitted for examination with our approval as university supervisors.

Signature:..... Date:.....

Prof. Elijah Miinda Ateka

JKUAT, Kenya

Signature:..... Date:.....

Dr. Sevgan Subramanian

ICIPE, Kenya

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DEDICATION

I dedicate this work to my late mother Rosemary Kusa.

ABSTRACT

Maize lethal necrosis (MLN) disease caused by a dual infection of maize with Maize chlorotic mottle virus (MCMV) and any cereal potyvirus such as *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV) or *Wheat streak mosaic virus* (WSMV) was first reported in Kenya in 2011 in Bomet County. This study was aimed at determining the presence and genetic variability of MCMV and SCMV in cereal crops, wild and domesticated grasses and the spatial distribution of alternative hosts in maize production regions of Kenya. Leaf samples of maize, grasses and other cereal crops were collected from fields in Nyamira, Bomet, Vihiga, Makueni and Machakos counties. MCMV and (SCMV) were detected by DAS-ELISA and confirmed by RT-PCR. The PCR products were sequenced in both directions. The resultant sequences were edited and compared with sequences from the Genbank followed by phylogenetic analysis. The distribution of wild grasses harbouring MCMV and SCMV in Kenya was predicted. Six grass weeds tested positive for MCMV and SCMV namely; velvet crabgrass (*Digitaria velutina*), couch grass (*Digitaria abyssinica*), star grass (*Cynodon dactylon*), kikuyu grass (*Pennisetum clandestinum*) and signal grass (*Brachiaria brizantha*). Nut grass (Cyperus rotundus tested positive for MCMV alone. Napier grass (*Pennisetum purpureum*) tested positive for MCMV alone. Sugarcane (Saccharum officinarum L.), finger millet (Eleusine coracana) and sorghum (*Sorghum bicolor*) tested positive for both MCMV and SCMV. The MCMV isolates nucleotide sequences were 98-100% similar and mostly related to the Kenya and Rwanda isolates (99-100%). SCMV isolates were 93-100% and were most related to China isolate (93-99%). Wild grasses

harbouring MCMV and SCMV are spread throughout maize growing regions in Kenya. The results indicate that alternative hosts are important in the epidemiology of MLN-causing viruses and their role should be considered in the development of integrated management strategies for MLN.

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LIST OF ABBREVIATIONS AND ACRONYMS

cDNA	Complementary deoxyribonucleic acid
DAS-ELISA	Double antibody sandwich- enzyme linked immunosorbent assay
DIBA	Dot-immunobinding assay
FAO	Food and Agriculture Organization
MCMV	*Maize chlorotic mottle virus*
MCMV-KS	*Maize chlorotic mottle virus*- Kansas
MCMV-NE	*Maize chlorotic mottle virus*- Nebraska
MCMV-P	*Maize chlorotic mottle virus*- Peru
MCMV-YN	*Maize chlorotic mottle virus*- Yunnan
MDMV	*Maize dwarf mosaic virus*
MDMV-A	*Maize dwarf mosaic virus* strain A
MDMV-B	*Maize dwarf mosaic virus* strain B
MLN	Maize lethal necrosis
MSV	*Maize streak virus*
PBST	Phosphate buffered saline with Tween 20
PNP	p-Nitrophenyl phosphate

PVX	*Potato virus X*
PVY	*Potato virus Y*
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SCMV	*Sugarcane mosaic virus*
SPCSV	*Sweet potato chlorotic stunt virus*
SPFMV	*Sweet potato feathery mottle virus*
SrMV	*Sorghum mosaic virus*
TAS-ELISA	Triple antibody sandwich enzyme linked immunosorbent assay
WSMV	*Wheat streak mosaic virus*

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Maize, *Zea mays* L., is an important staple crop grown widely by smallholder farmers in East Africa. Studies show that about 90% of the Kenyan population depend on the crop for food, income and employment. For instance, in 2012, the area under maize production was 2,266,196 Ha which amounted to 40,037,090 bags of 90 kilograms (Ministry of Agriculture, 2013)

Despite the economic importance of maize, the crop faces a number of production constraints. Yield losses of 30%, for example, have been attributed to biotic factors such as stem borers (Kifr *et al*., 2002), weeds such as *Striga* (Khan *et al.,* 2003) and diseases like maize streak (Martin *et al*., 2001). Abiotic stresses include drought, and poor soils (Ministry of Agriculture 2013). In 2011, a new disease called maize lethal necrosis (MLN) was reported in Bomet County and later spread to Eastern, Western and Central parts of Kenya (Joint Assessment Report, 2012). MLN has also been recently reported in Tanzania, Uganda, Southern Sudan (CIMMYT, 2012; FAO, 2013), Rwanda (Adams *et al*., 2014) and in Congo (Lukanda *et al*., 2014).

Maize lethal necrosis disease is caused by *Maize chlorotic mottle virus* (MCMV) in a synergistic association with any of the cereal viruses in the group potyviridae such as *Sugarcane mosaic virus* (SCMV), *Wheat streak mosaic virus* (WSMV) or *Maize dwarf mosaic virus* (MDMV) (Uyemoto, 1983). In Kenya, the disease is caused by co-infection of maize by MCMV and SCMV (Wangai *et al*., 2012; Adams *et al*., 2012). Maize chlorotic mottle virus was first identified in Peru in 1973 (Castillo and Hebert., 1974; Castillo, 1977), later in Kansas and Nebraska in the USA and China (Uyemoto., 1983; Niblett and Claflin., 1978). SCMV was first reported in Kenya in 1980 (Louie, 1980). MCMV and the resultant MLN disease are new to Africa (Wangai *et al*., 2012).

Characteristic symptoms of MLN-infected plants include a chlorotic mottle on leaves, developing from the base of young leaf whorls upward to the leaf tips, mild to severe leaf mottling, necrosis developing from leaf margins to the mid-rib, necrosis of young leaves leading to a "dead heart" symptom; and eventual plant death. Severely affected plants bear small cobs with little or no grain set. Under severe and early infestation plants frequently die before tasseling (Wangai *et al*, 2012; Nault *et al*., 1978; Castillo and Hebert., 1974).Symptoms of maize chlorotic mottle disease (MCMD) include mild mosaic, severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and shortened, malformed, partially filled ears.

Sugarcane mosaic disease (SCMD) is characterized by a mottled pattern on the leaves produced by contrasting light green to yellow and dark green patches. The patches are irregular in shape and have diffused margins. Infected plants appear paler and more

yellow than healthy plants. The symptoms are most easily seen in young rapidly growing leaves and the symptoms tend to fade as the leaves age (Grisham, 2000).

Maize is usually susceptible to MLN at all stages of its growth with the component viruses being transferred from plant-to-plant and field-to-field by insect vectors (Nault *et al*., 1978). SCMV is spread by aphids (Brunt *et al*, 1996) while corn thrips (Cabanas *et al*., 2013) and chrysomelid beetles (Nault *et al*., 1978) spread MCMV. Seed transmission of MCMV from infected plants ranges from 0.03 to 0.33% (Jensen *et al*., 1991).

1.2 Problem statement and justification

Maize is grown in all counties in Kenya with 90% of the population depending on the crop for food, income and employment. However, since September 2011, maize production has faced a major threat from MLN disease which was first reported in Bomet County and has been spreading rapidly to other parts of the country and the eastern African countries (Wangai *et al*., 2012; FAO, 2013). In some parts of the country, the disease has been very severe leading to 100% loss in yields (Joint Assessment Report, 2012). The yield loss culminates to loss of food and income for smallholder farmers who depend on maize. Therefore, this is a serious threat to maize production in the region and needs to be addressed (CIMMYT, 2012; Joint Assessment

Report, 2012). The disease also affects other cereals and wild grasses that would act as reservoirs for the component viruses causing MLN (Nelson *et al*., 2011).

Suspected viral symptoms on crops such as napier grass and other wild grasses were observed near maize farms (Joint Assessment Report, 2012). Such viral symptoms in wild and domesticated grasses indicate their potential role as alternative hosts. Previously in Hawaii wild grasses have been shown to harbour MCMV (Nelson *et al*., 2011). Therefore, it was necessary to establish whether wild grasses harbour the MCMV and SCMV in Kenya and how they impact on the disease epidemiology in maize fields.

MCMV strains have been found to differ in different parts of the world including Nebraska, Kansas, Peru and China. For instance, at least four genetically and geographically distinct strains of MCMV have been reported. These include, MCMV-P (Peru) and MCMV-K (Kansas) (Nyvall, 1999), MCMV-NE (Nebraska) (Stenger and French, 2008) and MCMV-YN (China) (Xie *et al*., 2011)**.** A study by Adams *et al* (2012) showed that the MCMV found in Kenya is most closely related to the Yunnan isolate from China with more than 96% similarity. The SCMV isolate is also most related to a recently characterized Chinese isolate (Adams *et al*, 2012). However, it was important to establish the diversities and similarities between virus strains from different regions, cereal crops, domesticated and wild grasses in Kenya.

1.3 Research questions

1. Are wild grasses, domesticated grasses and other cereals alternative hosts of MCMV and SCMV?
2. Is there genetic variability between MCMV and SCMV isolates from maize, other cereals, domesticated grasses and wild grasses?
3. How are wild grasses that serve as alternative hosts of MCMV and SCMV distributed in Kenya?
4.

1.4 Objectives

1.4.1 General objective

To determine the presence and genetic variability of MCMV and SCMV in cereals, wild and domesticated grasses and the spatial distribution of the wild grass hosts.

1.4.2 Specific objectives

1. To determine the presence and genetic variability of MCMV and SCMV in cereal crops, wild and domesticated grasses
2. To determine the spatial distribution of wild grasses serving as alternative hosts of MCMV and SCMV

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Production and utilization of maize in Kenya

Maize is widely grown by smallholder farmers in eastern Africa with Kenya having a per capita consumption estimated at 98 kilograms. Maize is wholly produced under rainfed conditions. The crop plays an important role as source of food, income and employment for many Kenyans (Mantel and Van Engelen, 1997). Kenya produces around 3 million tonnes of maize per year; about 15 percent is sold directly to the National Cereals and Produce Board (NCPB) and large millers (FAO, 2013). The remaining maize is sold in markets and used as food. The grains are ground to produce maize flour and it is also consumed as a food grain. It may be consumed fresh, ground, boiled or mixed with other foods. The stalks, leaves, and other remains from the maize cobs are used to feed domestic animals especially dairy cattle. The stalks and cobs are used to provide domestic fuel particularly in the rural areas. They are also used as organic manure.

2.2 Constraints to maize production in Kenya

Maize production in Kenya faces many constraints including diseases like maize streak (Martin *et al*., 2001), pests like stemborers (Kifr *et al*., 2002), drought and other abiotic factors. All these factors greatly reduce maize yield and are a major threat to food

security in Kenya. In addition, a new maize disease referred to as maize lethal necrosis (MLN) was recently reported in Bomet and later spread to other parts of Kenya and eastern Africa (Wangai *et al*, 2012).

2.3 Maize lethal necrosis (MLN)

Maize lethal necrosis (MLN) is a viral disease caused by *Maize chlorotic mottle virus* (MCMV) in combination with either *Sugarcane mosaic virus* (SCMV), *Wheat streak mosaic virus* (WSMV) or *Maize dwarf mosaic virus* (MDMV) (Uyemoto, 1983). In Kenya, MLN is caused by MCMV and SCMV (Wangai *et al*., 2012; Adams *et al*., 2012). The disease has spread to other counties like Nyamira, Trans-nzoia, Embu, Nakuru, Kisii, Uasin Gishu, Busia, Murang'a, Nyeri, Kirinyaga, Meru and Busia (CIMMYT, 2012; FAO, 2013).MLN has also been reported in South Sudan, Tanzania, Uganda and Rwanda (FAO, 2013). The disease causes up to 100% loss in yields in severely affected regions (CIMMYT 2012). Since MLN is new in the East African region, management has also been difficult. This poses a major problem to smallholder farmers who depend on maize as a source of food and income.

2.4 Genome organisation of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

Maize chlorotic mottle virus (MCMV) (Tombusviridae: *Machlomovirus*) is a plant virus with icosahedral virions of 30 nm in diameter containing a single stranded positive-sense RNA of _4.4 kb (Nutter *et al*., 1989; Lommel *et al*., 1991b; Stenger and French, 2008). The RNA is encapsidated in single 25 kDa capsid protein subunit. Translation of the

MCMV genome by a reticulocyte system results in polypeptides of 105, 52, 44, 41, 32 and 25 kDa. A sub-genomic RNA of 1090 nt was identified as the mRNA for the 25kDa coat protein (CP) (Lommel *et al.*, 1991b). The plus-sense single-stranded RNA genome of MCMV contains six open reading frames (ORFs) Figure (2.1) (Nutter *et al*., 1989; Lommel *et al*., 1991b). The genes of interest in this are the polyprotein gene for SCMV and the P111, P31 and P7 genes for MCMV.

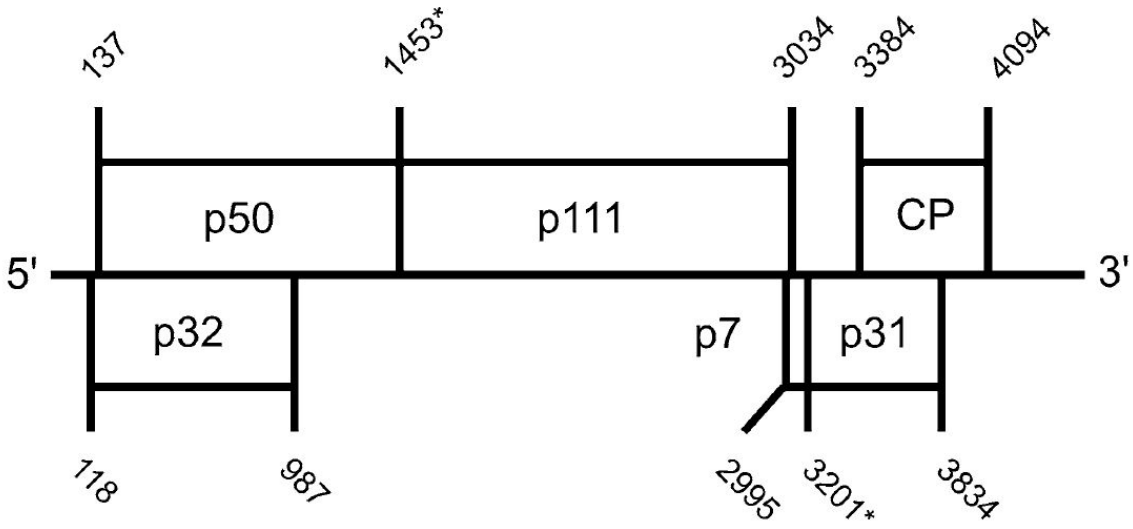

Figure 2.1 Genome map of *Maize chlorotic mottle virus* isolate from Nebraska, USA (MCMV-NE). Open reading frames are indicated by boxes; nucleotide coordinates of start and stop positions are indicated by numbers below and above the boxes. Asterisks denote amber stop codons read through to produce p111 and p31 (Adapted from Stenger and French, 2008)

SCMV belongs to the genus *Potyvirus*, family *Potyviridae*. SCMV is a virus with flexuous, filamentous particles about 750 nm long, which contain a single strand of RNA. SCMV is 750 nm in length, containing a single positive strand RNA which is

about 9.3 kb in size and has a poly (A) tail (Cheng *et al*., 2002). The SCMV genome comprises eight ORFs (Figure 2.2).

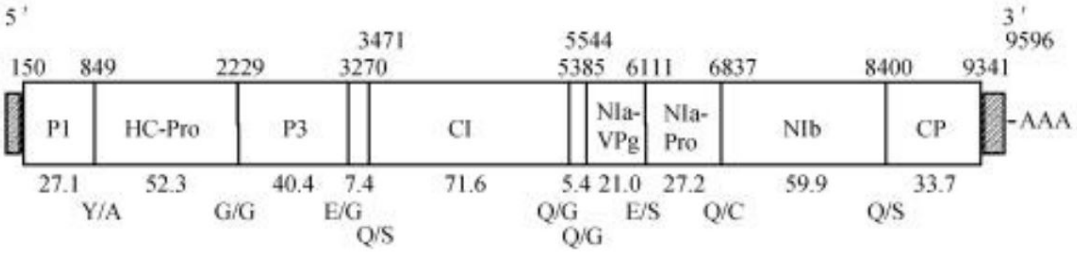

Figure 2.2. Predicted genome organization of the Chinese SCMV. Untranslated regions are shown shaded at the 5′and 3′termini. The open box shows the polyprotein of SCMV with the predicted products. Numbers above the box are the first nucleotides of the different products. Numbers below the box are the predicted sizes (ku) of the proteins. The predicted proteolytic cleavage sites are shown at the bottom of the diagram. (Adapted from Cheng *et al*., 2002).

2.5 Symptomatology of maize lethal necrosis

In Kenya, maize plants infected by maize lethal necrosis have been reported to show symptoms characteristic of virus diseases: a chlorotic mottle on leaves, developing from the base of young whorl leaves upward to the leaf tips; mild to severe leaf mottling; and necrosis developing from leaf margins to the mid-rib. Necrosis of young leaves lead to a "dead heart" symptom, and ultimately leading to plant death (Plate 2.1). Severely affected plants bear small cobs with little or no grain set. Plants frequently die before tasseling (Wangai *et al*., 2012). Apart from the above symptoms severe stunting and shorterned male inflorescences were also observed earlier (Castillo and Hebert, 1977).

Plate 2.1. Typical symptoms of MLN in maize; early leaf necrosis (A), severely affected maize plants showing severe leaf mottling (B), dead heart (C), and poorly filled kernel (D).

In case of infection of maize by either MCMV or SCMV, plants show virus specific symptoms. For instance, typical symptoms of MCMD include mild mosaic, mottling, severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and shortened, malformed, partially filled ears Plate (2.2) (Castillo and Hebert, 1977). On the other hand symptoms of SCMV infection alone is characterized by a mottled pattern on the leaves produced by contrasting light green to yellow and dark green patches Figure 4. The patches are irregular in shape and have diffused

margins. Infected plants appear paler and more yellow than healthy plants. The symptoms are most easily seen in young rapidly growing leaves and the symptoms tend to fade as the leaves age (Grisham, 2000).

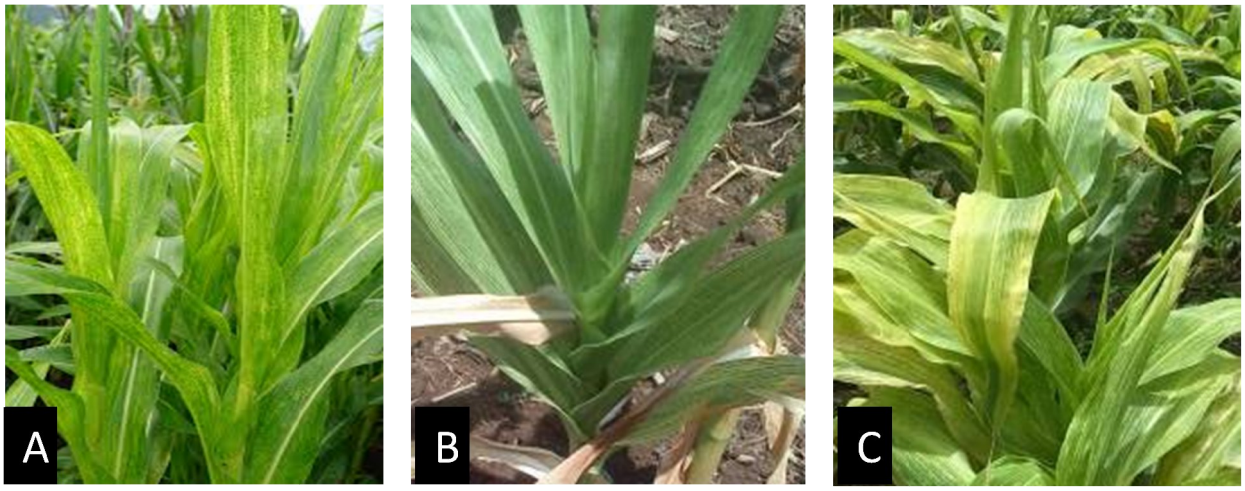

Plate 2.2: A symptom comparison between maize plants infected with MCMV alone (A), SCMV alone (B) and a dual infection with MCMV and SCMV(C).

2.6 Distribution of maize lethal necrosis

Maize chlorotic mottle virus (MCMV) was first described in maize from Peru in 1973 (Castillo and Hebert, 1974) and thereafter was reported on maize plants in United States and Mexico (Niblett and Clafin, 1978; Carrera-Martínez *et al.*, 1989). In China, it was reported first in Yunnan province in 2011 (Xie *et al*., 2011) and in Kenya in the same year (Wangai *et al*., 2012, Adams *et al*., 2012). In 1976, Corn lethal necrosis (CLN) or MLN was an epidemic in Kansas. Assays showed MCMV, *Maize dwarf mosaic virus* A (MDMV-A) and B (MDMV-B), and *Wheat streak mosaic virus* (WSMV), singly or in

combinations, in diseased plants (Uyemoto *et al*., 1980). In the same year, the disease was also reported in Peru (Castillo, 1977) and later in Hawaii and Nebraska, USA (Stenger and French, 2008; Nelson *et al*., 2011).

In September 2011, a high incidence of a MLN was reported in the Longisa division of Bomet County, Southern Rift Valley, Kenya. The disease later spread to the Narok South and North and Naivasha sub-counties and then to Nakuru, Kisii, Nyamira, Trans-Nzoia, Uasin Gishu, Busia, Murang'a, Nyeri, Kirinyaga, Meru and Embu counties (CIMMYT, 2012; Joint Assessment Report, 2012). MLN has also been reported in Tanzania, Uganda, Southern Sudan and Rwanda (CIMMYT, 2012; FAO 2013). Maize chlorotic mottle virus was recently reported in Congo (Lukanda *et al*., 2014).

2.7 Transmission of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

Maize chlorotic mottle virus (MCMV) has been reported to be transmitted by six species of beetles belonging to the family *Chrysomelidae* (Nault *et al*., 1978). The beetles include the cereal leaf beetle (*Oulema melanopa*), the corn flea beetle (*Chaetocnema punlicaria*), the flea beetle (*Systena frontalis*), the southern corn rootworm (*Diabrotica undecimpunctata*), the northern corn rootworm (*D. longicornis*), and the western corn rootworm (*D. virgifera*) (Nault *et al*., 1978). In Hawaii, the corn thrips, *Frankliniella williamsi* Hood (Thysanoptera: Thripidae) has been identified to be the main vector that transmits MCMV (Nelson *et al*., 2011). MCMV transmission by the corn thrips, showed that thrips transmitted the virus with no evidence for latent periods (Cabanas *et al*.,

2013). Both larvae and adults transmitted the virus for up to 6 days after acquisition, with decreasing rates of transmission as time progressed. There was no evidence that adult thrips that acquired the virus as larvae were competent vectors (Jiang *et al*., 1992; Cabanas *et al*., 2013) SCMV is transmitted by a number of aphid species including *Rhopalosiphum maidis, Dactynotus ambrosiae, Hysteroneura setariae*, and *Toxoptera graminum* in a non-persistent manner (Brunt *et al*, 1996).

Transmission of MCMV and SCMV via seed from infected plants normally ranges from 0.003% to 0.3% (Jensen *et al*., 1991) and 0.4% to 7.2% (Li *et al*., 2010), respectively.Transmission of MCMV and SCMV also occurs mechanically (Nyvall, 1999; Bond and Pirone 1969).

2.8 Host range of *Sugarcane mosaic virus* and *Maize chlorotic mottle virus*

The host range for both SCMV and MCMV is limited to members of the *Gramineae* family with maize and sugarcane being the natural hosts of MCMV and SCMV respectively (Scheets, 2004). In Kenya, SCMV was first reported in *Cynodon dactylon, C. nlemfunsis, Digitaria nuda, D. abyssinica, Eragrostis exasperata, Paspalum notatum, P. scrobiculatum, Rhynchelytrym repens*, and an unknown *Tripsacum fasciculatum* cross by Louie (1980).

Maize chlorotic mottle virus has a wide monocot host range with at least 19 grass species including cultivated ones, such as barley (*Hordeum vulgare* L.), proso millet (*Panicum miliaceum* L.), foxtail millet (*Setaria italica* L.), and wheat (*Triticum aestivum* L.) (Bockelman *et al*., 1982). Hosts of MCMV found in Hawaii include soft brome (*Bromus mollis)*, fall panic grass *(Panicum dichotomiflorum* Michx), (Guinea grass *(Panicum maximum*), broomcorn millet *(Panicum miliaceum)*and corn (*Zea mays)*. (Brunt *et al*., 1996).

2.9 Diversity of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

At least four genetically and geographically distinct strains of MCMV have been reported; MCMV-P (Peru) and MCMV-KS (Kansas) (Nyvall, 1999; Uyemoto, 1983), MCMV-NE (Nebraska) (Stenger and French, 2008); and MCMV-YN (China) (Xie *et al*., 2011). The Kenyan isolate has been reported to be more than 96% similar to the Yunnan strain from China (Adam *et al*., 2012). The complete nucleotide sequences of two MCMV isolates (MCMV-KS from Kansas and MCMV-NE from Nebraska) have 99.5% sequence identity with each other (Nutter *et al*., 1989; Stenger and French, 2008).

SCMV isolates are divided into two major geographical groups; the Chinese and the European isolates (Cheng *et al*., 2002; Alegria *et al*., 2003). SCMV strains include strain A, B, D, and E of SCMV and strain Sc, Bc and Sabi of Australian SCMV (Cheng *et al*., 2002).

2.10 Mixed infections with *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

Severa1 potyvirus-associated synergistic diseases have been examined in detail, and in each, a dramatic increase in host symptoms has been observed in doubly infected plants compared with singly infected plants (Goldberg and Brakke, 1987). The increase in symptoms is correlated with an increase in the accumulation of the non-potyvirus, but there is no corresponding increase or decrease in the level of the potyvirus (Rochow and Ross, 1955; Calvert and Ghabrial, 1983; Goldberg and Brakke, 1987; Vance, 1991).

A study to show synergism between MCMV and *Maize dwarf mosaic virus* strain B (MDMV-B showed the concentration of (MCMV) to be 5.4 times higher in plants infected with both MCMV and MDMV-B than in plants infected with MCMV only (Goldberg and Brakke, 1987). The concentration of MDMV-B was the same in doubly and singly infected plants. Plants infected with both viruses had a reduced level of chlorophyll and a lower than normal ratio of chloroplast to cytoplasmic rRNA (Goldberg and Brake, 1987).

Similar dual infections have also been reported in other crop systems. The classical example is the co-infection of tobacco plants with *Potato virus Y* (PVY), type member of the genus *Potyvirus*, family *Potyviridae*) and *Potato virus X* (PVX), type member of the genus *Potexvirus*). The titres of PVX RNA and coat protein increase and more severe symptoms are induced, but the titre of PVY is not affected (Rochow and Ross,

1955; Vance, 1991). In sweet potato, the s*weet potato feathery mottle virus* (SPFMV, genus *Potyvirus*, family *Potyviridae*) and the whitely-transmitted *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*, family *Closteroviridae*) also infect sweet potato in a synergistic manner (Gibson *et al.,* 1998).

2.11 Detection of Maize chlorotic mottle virus and Sugarcane mosaic virus

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) has been used to detect both MCMV and SCMV (Clark and Adams, 1977) using antisera raised to the same viruses which are commercially available. Appropriate positive control samples are used with each test.

Reverse transcription polymerase chain reaction (RT-PCR) was developed for SCMV detection in 1994 by Smith and Van De Velde, and later improved by Alegria *et al*. (2003) and Xu *et al*. (2008). Additionally, a RT-PCR-restriction fragment length polymorphism (RFLP) for SCMV strain discrimination was reported in 1997 by Yang and Mirkov (1997). Transmission electron microscopy (TEM) has also been used using a leaf-dip preparation method, with uranyl acetate staining and carbon-coated grids to detect MCMV (Hill, 1984). Additionally, 454-sequencing has been used to detect SCMV and MCMV isolates in Kenya (Adams *et al*., 2012). A real-time TaqMan RT-PCR procedure for efficient detection of MCMV has also been developed in China

(Zang *et al*., 2011). RT-PCR detection has also been used by Wangai *et al*. (2012) in Kenya.

2.12 Management of maize lethal necrosis

A variety of management practices have been applied by farmers in MLN affected regions globally. In the U.S. corn-belt, MLN is managed by planting maize varieties resistant to MCMV and other potyvirus diseases and eradication of Johnson grass which serves as an alternative host of SCMV (Uyemoto, 1983). Crop rotation has also been reported as a control measure in Kansas (Uyemoto, 1983). In Hawaii, MCMV and MLN are managed by practising crop rotation and by intensive spray programs to control insect vectors (Nelson *et al*., 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

This study was carried out in five counties namely Bomet, Nyamira, Makueni, Machakos and Vihiga. Vihiga, Machakos, and Makueni counties were selected to represent areas with mild cases of MLN whereas Nyamira and Bomet were selected to represent areas with severe cases of MLN based on an earlier report on the prevalence of MLN in Kenya (Joint Assessment Report, 2012). Laboratory analysis of samples was done at the thrips laboratory, arthropod pathology and molecular biology and bioinformatics units in *icipe.*

3.2 Study design

This study was carried out between March and June 2014. The study was designed to occur in five counties. In each County, six maize fields were selected at random. Ten maize samples were collected from each farm. Any wild grasses growing within and 1-10m around the field were collected. Both symptomatic and asymptomatic grasses were collected. Other cereals and domesticated grasses growing 1-10m from the field were also collected. All these samples were transported to ICIPE for analysis by DAS-ELISA and RT-PCR. The RT-PCR products were sequenced and analysed.

Data on incidence and severity of MLN in maize in the sampled fields in Bomet and Nyamira counties was also collected to establish disease progress in the field.

To establish the spatial distribution of the wild grasses that habour MCMV and SCMV, GPS co-ordinates for all the farms where samples were wild grass collected were recorded. These co-rdinates were then used for species modeling to establish the distribution of the alternative hosts in Kenya.

3.3 Presence and genetic variability of MCMV and SCMV in wild grasses and cereal crops

3.3.1 Sample collection

In each county, six maize fields were randomly selected and sampled for maize, wild grasses, domesticated grasses and other cereals. A rectangular shape was assumed for each maize field and wild grass samples collected within 1-10 metres from the edges of the field in the form of a zigzag walk pattern. Grass samples were also collected from within the maize field. Samples of both symptomatic and asymptomatic grasses were collected in maize fields confirmed to have MLN. Sample collection was done between March and June 2014. A sample for identification was pressed in a herbarium for each grass. Ten maize plant samples were collected randomly from each field where the wild grasses were collected.

Cereals including finger millet and sorghum as well as other domesticated grasses like napier grass and sugarcane growing in the vicinity of MLN-infected fields (1-10m) were also sampled. Sorghurm and finger millet were collected in Nyamira and Bomet counties while napier grass was collected in Nyamira County only. Sugarcane was collected only in Makueni County. Sampling involved cutting the leaves with scissors and placing them in an envelope for DAS ELISA and another portion in a reaction tube for RT-PCR. Each sample was placed in a separate envelope/reaction tube and labelled accordingly. The scissors was cleaned with cotton wool dipped in 70% ethanol for sterilization between samples. A representative of each sample was placed in a portable fridge for DAS-ELISA and another in liquid nitrogen for RT-PCR. The samples were transported to the International Centre for Insect Physiology and Ecology (ICIPE) for laboratory analysis.

To establish disease progress, each field in Bomet and Nyamira counties was divided into four quadrants and incidence and severity of MLN on 10 maize plants per quadrant recorded. A total of four field visits took place. A rectangular shape was assumed for each field after which it was divided into four quadrants. The layout of the farm was drawn in a field notebook to ensure the orientation of the map remained intact for every visit. Incidence and severity was recorded for ten maize samples per quadrant. The data obtained was then used to generate disease progress curves for each farm in the two counties.

This was done biweekly. Disease severity was recorded on a standardized scale of 0-5; where:

0- No mottling and necrosis on leaf, the plant is green.

1- 20% of the leaves show mild mottling and necrosis

2- 40% of the leaves show mottling and necrosis, mild yellowing of the leaves

3 - 60% ofthe leaves show mottling and necrosis, yellow leaves

4 - 80% of the leaves show severe mottling and necrosis, yellow leaves

5 - 100% of the leaves show severe mottling and necrosis, the whole plant is yellow and began drying up from the heart.

The scores were based on the methods outlined in the Joint Assessment Report (2012).

3.3.2 Double antibody sandwich enzyme linked immunosorbent assay (DAS ELISA)

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was performed using standard methods modified from Clark and Adams (1977) using antisera raised against SCMV and MCMV. The antisera were procured from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany. Microtitre plates were coated with the antibody and incubated for three hours at 37˚C. Meanwhile, leaf samples were then crushed in extraction buffer using a micropestle. The reaction tubes

and micropestles and tips used were autoclaved to ensure sterility. A different micropestle was used for each sample and gloves changed regularly to avoid contamination.

The antibody-coated plate was then washed with phosphate buffered saline with Tween 20 (PBST) three times and tapped on paper towel to dry. 100µl of the samples was then added per well. The samples were placed in the wells in duplicate. The positive control, negative control and the extraction buffer were also placed in separate wells in duplicate. The plate was then incubated overnight at 4˚C. The plate was then washed again with PBST three times and tapped on paper towel to dry. 100µl of the enzyme conjugate (alkaline phosphatase) was added to each well and incubated for three hours at 37˚C. The plate was washed again as described earlier after which 100µl p-Nitrophenyl phosphate (PNP) substrate was added. The plate was incubated for one hour at room temperature in the dark for colour development. Optical densities were read at 405nm using an ELISA reader EPOCH™ microplate spectrophotometer. The positive control had a threshold of 3 times the negative control.

Positive and negative controls were raised in separate screen houses at ICIPE. Positive controls included maize inoculated with either MCMV or SCMV singly and raised separately. Negative controls used were maize plants that did not have either MCMV or SCMV also raised in a separate screen house. In addition, the buffer used for extraction was loaded in two wells to serve as a control for the whole process.

A total of 384 samples were tested by DAS-ELISA. Of these samples, there were 145 wild grasses, 239 maize and 54 cereal crops and domesticated grasses.

3.3.3 RNA extraction and reverse transcription polymerase chain reaction (RT-PCR) analysis

The samples that tested positive by DAS-ELISA were subjected to RT-PCR for verification. The samples were dipped in liquid nitrogen and crashed into powder using a micrcopestle. The reaction tubes and micropestles and tips used were autoclaved to ensure sterility. A different micropestle was used for each sample and gloves changed regularly to avoid contamination Total RNA was then extracted using Rneasy plant mini kit (Qiagen, USA) following the manufacturer's instructions. RNA was quantified using a nanodrop (Thermoscientific) to confirm RNA presence. The ratio of the absorbance at 260 and 280nm ($A_{260/280}$) was used to assess the purity of the RNA where pure RNA should have an $A_{260/280}$ ratio of approximately 2.0. The nanodrop was blanked using RNAse free water that was used to elute the RNA.

cDNA was generated using a High capacity cDNA kit (Applied biosystems). The resultant cDNA was used as a template for conventional PCR to amplify a section of the polyprotein gene for SCMV. The 111kDa protein, P31 and P7 genes were amplified for MCMV. PCR was done using a hotstar taq mastermix PCR kit from Qiagen. The primer pairs used for MCMV were 2681F: 5′-ATGAGAGCAGTTGGGGAATGCG and 3226R: 5′-CGAATCTACACACACACACTCCAGC while those used for SCMV were

8679F: 5′-GCAATGTCGAAGAAAATGCG) and 9595R: 5′-GTCTCTCACCAAGAGACTCGCAGC. The PCR thermocycling regimes were as follows: denaturation and enzyme activation at 95˚C for 15 minutes, denaturation at 94˚C for 1 minute, annealing at 56˚C for MCMV and 46˚C for SCMV for 1 minute and elongation at 72˚C for 1 minute. The final extension was for 10 minutes at 72˚C after which the sample was held at 4˚C. The PCR product was then run on 1% agarose gel electrophoresis at 100 volts for 30 minutes.

A total of 384 samples were tested by RT-PCR. Of these samples, there were 36 wild grasses, 157 maize and 20 cereal crops and domesticated grasses.

Selected PCR products were then purified using a Qiaquick PCR purification kit (Qiagen) according to the manufacturer's instructions. The purified PCR products were quantified using a thermoscientific nanodrop. The ratio of the absorbance at 260 and 280nm ($A_{260/280}$) was used to assess the purity of DNA. Pure DNA has an $A_{260/280}$ ratio of approximately 1.8. The purified PCR products were then sent to Macrogen, Netherlands for sequencing. Sequencing was done in both directions using ABI 3700.

3.3.4 Sequence analysis

The obtained sequences were then edited using Geneious (Kearse et al, 2012). The editing entailed alignment of the forward and reverse sequences allowing the ends to slide. A consensus sequence was then generated. The bases were edited by comparing them with the peaks whereby the stronger peak was picked in the event that the base in the forward sequence did not tally with that in the reverse sequence and vice versa. After

editing, multiple sequence alignments were done using Clustal Omega (Sievers *et al*., 2011). The edited sequences together with sequences from the Genbank were aligned and a percentage identity matrix was generated. The GenBank sequences used for comparison included Mexico (Accession number GU474635), Ohio (Accession number JX188385), Rwanda (Accession number KF744391) and China (Accession number JN021933) for SCMV and Nebraska (Accession number EU358605), Rwanda (Accession number KF74439), Kenya (Accession number JX286709), Taiwan (Accession number KJ782300) and China(Accession number JQ982470) for MCMV.

A phylogenetic tree was then generated including selected sequences from the multiple alignments. The neighbour joining method was used to generate a maximum likely hood tree at 500 bootstraps for both MCMV and SCMV isolates using Mega Version 6 (Tamura *et al*., 2013).

3.3.5 Disease severity analysis

Disease severity in maize was recorded in four quadrants per field on a scale of 0-5 as described in section 3.2.1. The average disease severity of each maize field was calculated for each field visit/maize phenological stage. Disease progress curves were then generated by plotting disease severity against maize phenological stages (time).

3.4 Spatial distribution of wild grasses serving as alternative hosts of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

3.4.1 Sample collection

Six maize fields were sampled in Nyamira,Vihiga, Bomet and Makueni counties. Samples of wild grasses were collected within a margin of two metres from the edges of MLN infected maize fields and inside the field. A zig-zag walk pattern was adopted when sampling. Both symptomatic and assymptomatic grasses were collected. Sampling was done between March and June 2014. Disease severity was recorded on a standardized scale of 0-5 as described in section 3.3.1. Any potential vector observed on the wild grasses was also recorded. Global positioning system (GPS) co-ordinates for the locations where samples were collected were also recorded. Once collected, the samples were labelled, placed in a portable refrigerator and transported to the laboratory.

The presence of MLN-causing viruses was then confirmed using ELISA-based techniques as described in section 3.3.2. Representative samples of the wild grasses collected were preserved as per standard herbarium procedures for identification (Queensland Herbarium, 2013). The samples were identified by Mr. S. Mathenge, an experienced Botanist of the East African Herbarium, Nairobi, Kenya. This was done by observing the plants morphology including leaves and fluorescence and comparing with photographs and descriptions from taxonomy books. The plant specimen was then assigned a scientific name and a short description of the distribution in the Kenyan agro-ecological zones.

3.4.2 Prediction of spatial distribution of wild grasses in Kenya

Based on the environmental conditions in areas where the grasses harbouring either MCMV or SCMV were found, a geographic distribution map of the grasses was generated by MAXENT version 3.3.3k (Phillips *et al*., 2006) to predict areas highly suitable for the growth of these grasses. This was done to predict the probability of these grasses acting as alternative hosts of MCMV and SCMV in other parts of Kenya.

CHAPTER FOUR

4.0 RESULTS

4.1 Presence and genetic variability of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in cereals, wild and domesticated grasses

All the grasses collected from Bomet, Nyamira Makueni Machakos and Vihiga counties were identified as fourteen different species (Table 4.1). These grasses belonged to nine genera namely: *Digitaria, Cynodon, Pennisetum, Panicum, Cyperus, Brachiaria, Eleusine, Setaria* and *Cenchrus*. All the grasses collected were tested for MCMV and SCMV by DAS-ELISA and verified by RT-PCR. The grasses from different farms in each county were pooled together to represent a county. Of these grasses, six tested positive for MCMV. These included *Digitaria velutina, Digitaria abyssinica, Cynodon dactylon, Pennisetum clandestinum, Cyperus rotundus and Brachiaria brizantha*. Dual infections of both SCMV and MCMV were also detected in *Digitaria velutina, Digitaria abyssinica, Cynodon dactylon, Pennisetum clandestinum,* while *Brachiaria brizantha* harboured either MCMV or SCMV separately. *Cyperus rotundus* tested positive only for MCMV and negative for SCMV. All the other grasses tested negative for both MCMV and SCMV (Table 1).

Table 4.1: RT-PCR and DAS ELISA results for *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) in wild grasses collected in five counties.

County	Plant name	No. of Samples tested (DAS-ELISA)	No. of samples positive for MCMV(RT-PCR and DAS-ELISA)	No. of samples positive for SCMV(RT-PCR and DAS-ELISA)	No. of samples with dual infections (RT-PCR and DAS-ELISA)	No. of negative samples
Nyamira	*Digitaria velutina*	10	4	1	1	5
Nyamira	*Digitaria abyssinica*	6	4	2	2	0
Nyamira	*Cynodon dactylon*	11	4	3	2	4
Nyamira	*Pennisetum clandestinum*	14	5	1	1	8
Nyamira	*Panicum trichocladum*	1	0	0	0	1
Nyamira	*Cyperus rotundus*	3	2	0	0	1
Bomet	*Digitaria velutina*	10	5	1	1	4
Bomet	*Digitaria abyssinica*	10	2	0	0	8
Bomet	*Cynodon dactylon*	10	1	1	0	8
Bomet	*Pennisetum clandestinum*	16	6	1	0	9
Bomet	*Brachiaria brizantha*	7	2	1	0	4
Bomet	*Cyperus rotundus*	1	0	0	0	1
Bomet	*Eleusine indica*	1	0	0	0	1
Vihiga	*Cynodon dactylon*	2	0	0	0	2
Vihiga	*Digitaria abyssinica*	3	0	0	0	3
Makueni	*Pennisetum mezianum*	7	0	0	0	7
Makueni	*Setaria plicatelis*	1	0	0	0	1
Makueni	*Panicum maximum*	6	0	0	0	6
Makueni	*Eleusine indica*	4	0	0	0	4
Makueni	*Digitaria diagonalis*	5	0	0	0	5
Makueni	*Setaria verticillata*	8	0	0	0	8
Makueni	*Brachiaria leersioides*	3	0	0	0	3
Makueni	*Cenchrus ciliaris*	3	0	0	0	3

County	Plant name	No. of Samples tested (DAS-ELISA)	No. of samples positive for MCMV(RT-PCR and DAS-ELISA)	No. of samples positive for SCMV(RT-PCR and DAS-ELISA)	No. of samples with dual infections (RT-PCR and DAS-ELISA)	No. of negative samples
Machakos	*Digitaria diagonalis*	1	0	0	0	1
Machakos	*Brachiaria leersioides*	2	0	0	0	2

Napier grass (*Pennisetum purpureum*) samples collected from Nyamira County tested positive for MCMV alone while those collected from Bomet tested negative for both MCMV and SCMV (Table 4.2). Sugarcane (*Saccharum officinarum L.)* samples from Makueni County tested positive for MCMV and SCMV. Finger millet (*Eleusine coracana*) collected from Bomet and Nyamira counties tested positive for SCMV and MCMV. Some samples contained the viruses singly while others had dual infections (Table 4.2). One Sorghum (*Sorghum bicolor*) sample collected from Bomet County tested positive for SCMV. The other sorghum sample collected from Nyamira County was dually infected with both MCMV and SCMV (Table 4.2). Despite testing positive, sorghum samples collected were asymptomatic.

Overall, a total of 230 maize samples were collected from Nyamira, Bomet, Makueni, Machakos and Vihiga counties. Of these samples, 121 were positive for MCMV, 36 for SCMV and 35 showed dual infection with the two viruses (Table 4.3).

Table 4.2. RT-PCR and DAS-ELISA results for *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) in cereals and domesticated grasses collected from Bomet, Nyamira and Makueni counties

County	Plant name	No. of Samples tested (DAS-ELISA)	No. of Samples positive for MCMV(RT-PCR and DAS-ELISA)	No. of Samples positive for SCMV(RT-PCR and DAS-ELISA)	No. of Samples positive with dual infections(RT-PCR and DAS-ELISA)	No. of negative samples
Nyamira	Napier grass	5	2	0	0	3
Nyamira	Sorghum	12	1	1	1	10
Nyamira	Finger millet	10	3	2	1	5
Bomet	Napier grass	5	0	0	0	5
Bomet	Sorghum	10	0	1	0	9
Bomet	Finger millet	10	5	3	2	2
Makueni	Sugarcane	2	1	1	0	0

Table 4.3. RT-PCR and DAS-ELISA results for *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) in maize samples collected from Bomet, Nyamira, Makueni, Machakos and Vihiga counties

County	Plant name	No. of Samples tested (DAS-ELISA)	No. of Samples positive for MCMV(RT-PCR and DAS-ELISA)	No. of Samples positive for SCMV(RT-PCR and DAS-ELISA)	No. of Samples positive with dual infections(RT-PCR and DAS-ELISA	No. of negative samples
Nyamira	Maize	79	53	19	14	7
Bomet	Maize	74	54	15	20	5
Makueni	Maize	32	9	2	1	21
Machakos	Maize	27	5	0	0	22
Vihiga	Maize	27	0	0	0	27
	Total	239	121	36	35	82

About 99% of the maize cereals, wild and domesticated grass samples showed very strong distinct bands as shown in representative gels for both MCMV and SCMV (Plate 4.3 and Plate 4.4). The band size for SCMV polyprotein gene was 950bp while the fragment containing P111, P7 and P31 genes for MCMV had a band size of 550bp.

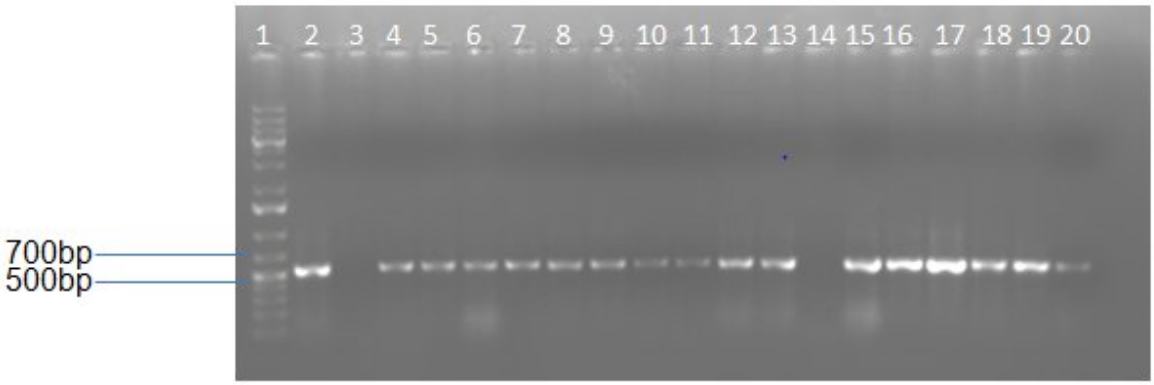

Plate 4.3 A representative gel photograph of grass and maize samples collected from Bomet and Nyamira counties tested for MCMV. Lane 1 represents the ladder, lanes 2 and 3 show the positive and negative controls respectively. Lanes 5,6,9,12,13,14,17 and 18 represent maize samples; Lanes 4 and 7 represent *Pennisetum clandestinum*; lanes ,8,10,11,15,16,19 and 20 represent *Eleusine coracana, Cynodon dactylon, Digitaria velutina, Digitaria abyssinica. Brachiaria brizantha, Cyperus rotundus* and *Sorghum bicolour* in that order.

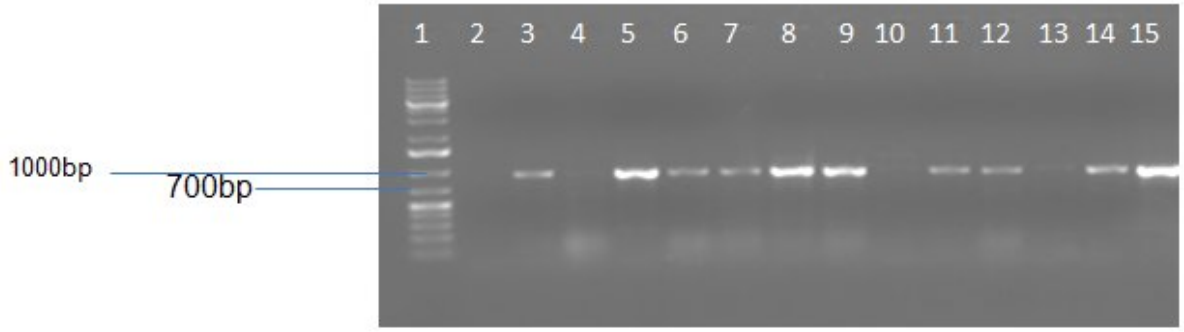

Plate 4.4 A gel representative photograph of RT-PCR products for *Sugarcane mosaic virus* (SCMV) in grass and maize samples collected from Bomet and Nyamira counties. Lane 1 represents the ladder, lanes 2 and 3 show the negative and positive controls respectively. Lanes 4,6 and 8 represent maize samples; Lanes 10 and 14 represent *Eleusine coracana,*; lanes 7,11,12,13 and 15 represent *Digitaria velutina, Brachiaria brizantha, Pennisetum clandestinum, Cynodon dactylon, and Digitaria abyssinica.,* in that order

4.1.1 Maize lethal necrosis disease progress

Disease severity progression for all maize fields exhibited a similar trend. There was a slight increase in disease severity from tasseling to milking stage. In general, all maize fields had an average disease severity score of 3 (Figure 4.3). The increase in disease severity coincided with peak disease symptom expression in wild grasses and increase in the population densities of potential vectors of MCMV and SCMV. After milking stage the maize had reached physiological maturity and leaves were drying up making it difficult to record disease severity.

Maize phenological stages were classified as follows according to a BBCH scale:

VT = Tasseling stage (lowest branches of tassel visible before silk)

R1 = Silking stage (silks visible outside husk)

R2 = Blister stage (kernels are white and resemble a blister in shape)

R3 = Milking stage (kernels are yellow on the outside with a milky inner fluid) (Ritchie et al., 1992)

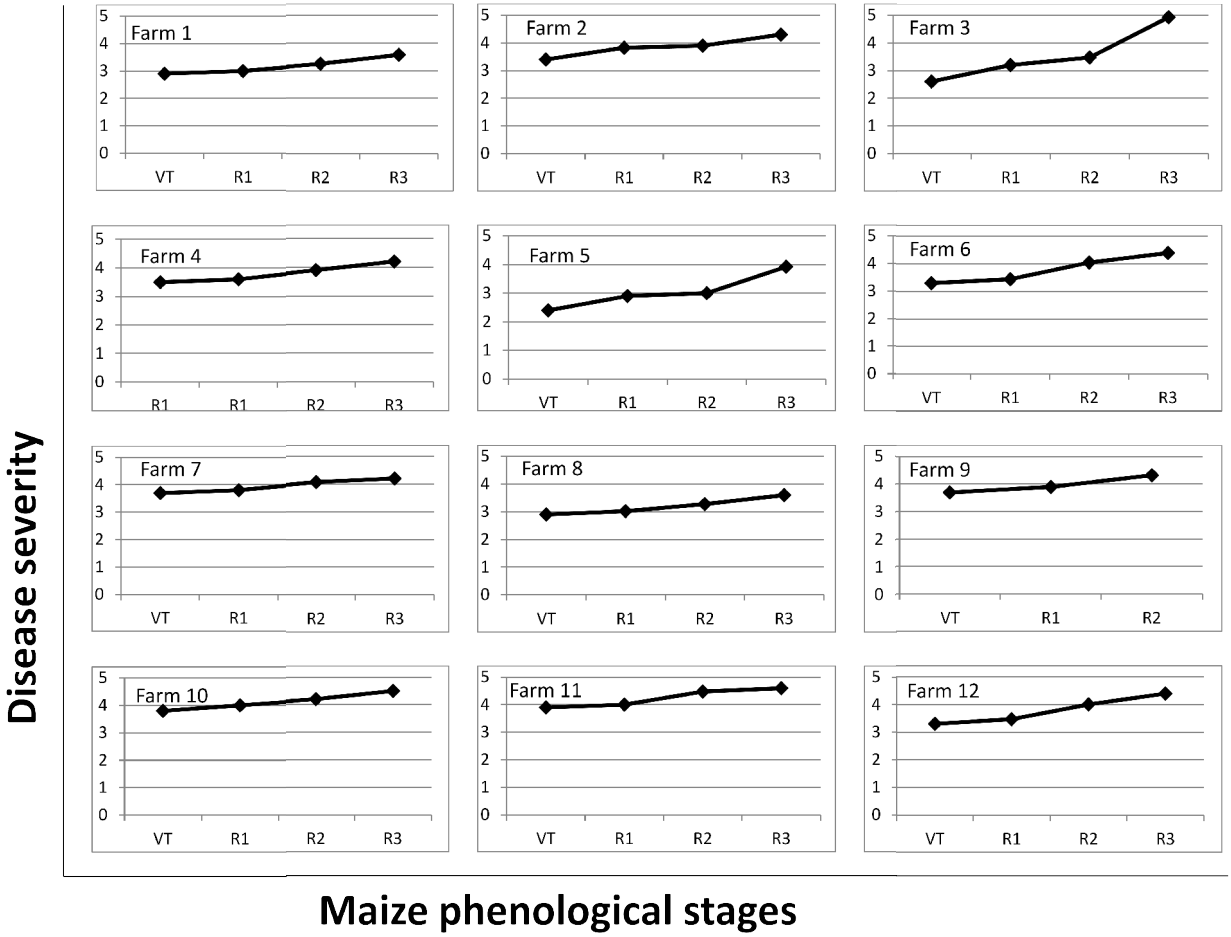

Figure 4.3 Graphs showing MLN disease progress in maize over time in Nyamira (Farm1-6) and Bomet (Farm 7-12) counties. Maize phenological stages were classified as follows: VT = tasseling stage (lowest branches of tassel visible before silk); R1 = silking stage (silks visible outside husk); R2 = Blister stage (kernels are white and resemble a blister in shape); and R3 = milking stage (kernels are yellow on the outside with a milky inner fluid) (Ritchie et al., 1992).

4.1.2 Symptom expression in alternative hosts of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

Grass and cereal samples collected from the field were both symptomatic and asymptomatic. Symptomatic finger millet tested positive for both MCMV and SCMV. The symptoms included The symptoms included chlorotic mottle on leaves developing from the base of young whorl leaves upward to the leaf tips, and necrosis developing from leaf margins to the mid-rib (Plate 4.5). The plant appeared yellow and could easily be distinguished from the asymptomatic plants. *Digitaria abyssinica* expressed symptoms in form of yellowing on the youngest leaves found at the shoot tip (Plate 4.5). *Pennisetum purpureum* and *Pennisetum clandestinum* had mild mottling which could not be observed from a distance. There was mild change in colour which was not obvious (Plate 4.5). *Digitaria velutina* also showed yellowing and mild mottling. *Cynodon dactylon* and *Sorghum bicolor* were asymptomatic but tested positive for both MCMV and SCMV. Sugarcane also showed yellow coloration in form of thin stripes as well as necrosis on the whole leaf.

All wild grasses appeared non-symptomatic at the beginning of the maize season. Disease symptoms were fully expressed as the maize approached the reproductive stage.

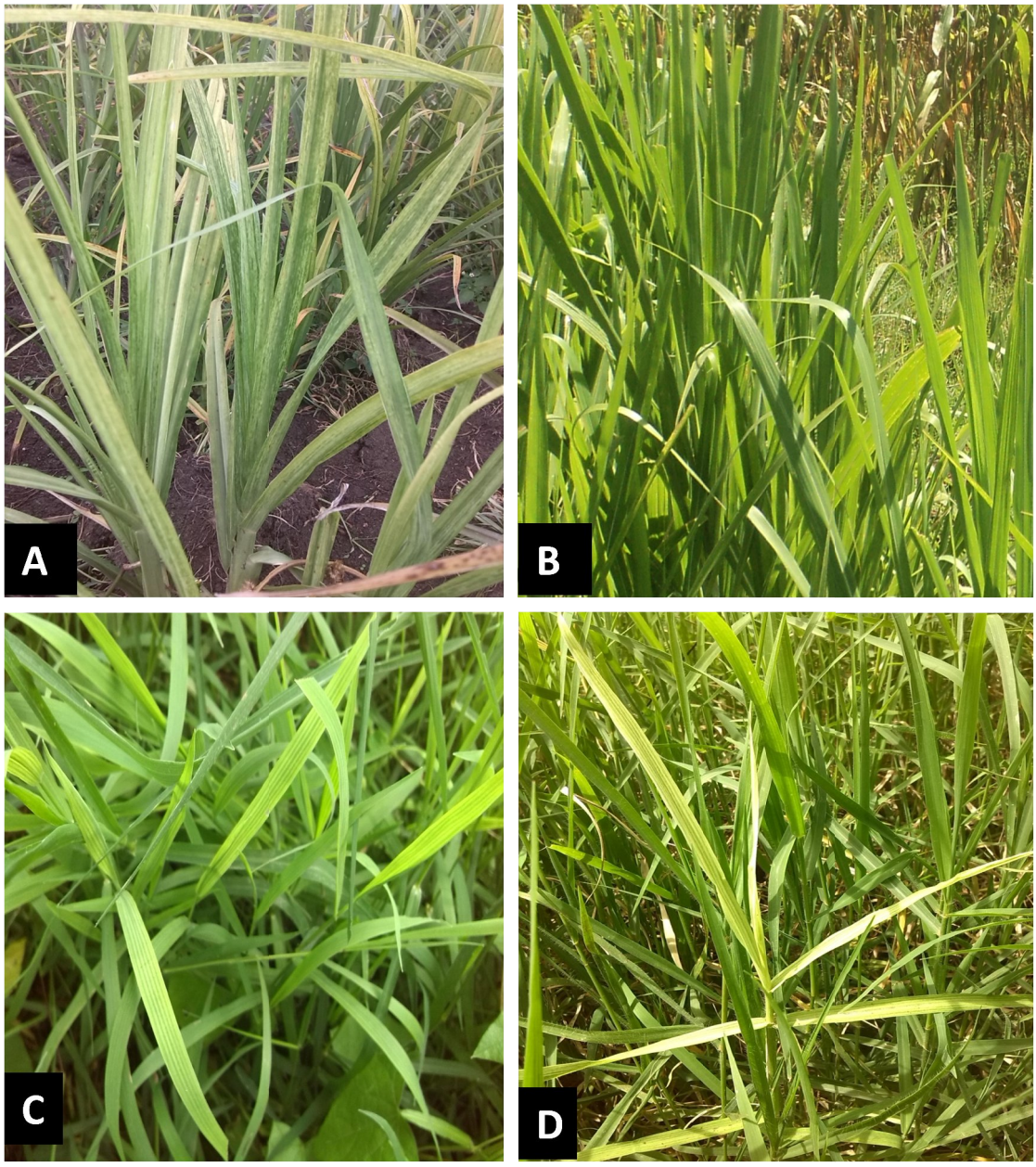

Plate 4.5. Photographs of grasses and cereals showing MLN infected finger millet (A); MCMV infected napier grass (B); MLN infected *Digitaria abyssinica* (C) and MLN infected *Cynodon dactylon* (D).

4.1.3 Genetic variability of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in maize, cereals, wild and domesticated grasses

A total of sixteen SCMV isolates collected in this study were compared with isolates from Mexico, Ohio, Rwanda and China that are deposited in the GenBank. The range in percentage similarity between these isolates was 87-91%, 92-95%, 93-99% and 91-95% with isolates from Mexico (Accession number GU474635), Ohio (Accession number JX188385), Rwanda (Accession number KF744391) and China (Accession number JN021933) respectively (Table 4.4). However, the percentage similarities between the isolates in this study ranged from 93-100% Table 4.5. Phylogenetic analysis grouped the SCMV isolate into two groups; one with a high likeliood to the China isolate and the other to the Rwanda isolate (Figure 4.4).

Table 4.4 Similarity between nucleotide sequences (%) of *Sugarcane mosaic virus (*SCMV) isolates of the study and DNA sequences (polyprotein gene) derived from the GenBank accessions of the virus

			Percentage similarity with Genbank isolates			
Isolate	**Host**	**County**	**Mexico(GU474635)**	**Ohio(JX188385)**	**Rwanda(KF744391)**	**China(JN021933)**
ZMNYR6SCMV2	*Zea mays*	Nyamira	89.77	93.50	97.07	91.32
ZMNYR1SCMV	*Zea mays*	Nyamira	89.29	93.02	98.25	91.493
ECNYRF1SCMV	*Eleusine coracana*	Nyamira	89.40	92.78	98.13	91.25
DVBMT8SCMV	*Digitaria velutina*	Bomet	90.23	94.14	96.82	94.14
DVNYR5SCMV	*Digitaria velutina*	Nyamira	89.39	93.13	98.59	91.64
ZMMKNSCMV	*Zea mays*	Makueni	87.34	91.84	93.18	94.68
ECBMT10SCMV1	*Eleusine coracana*	Bomet	88.93	92.90	98.36	91.43
ECBMT10SCMV2	*Eleusine coracana*	Bomet	90.95	94.74	97.67	92.18
ECBMT10SCMV3	*Eleusine coracana*	Bomet	88.74	93.65	96.76	94.01
ZMBMT11SCMV	*Zea mays*	Bomet	89.05	93.57	95.11	94.76
ZMBMT7SCMV	*Zea mays*	Bomet	90.5	94.76	96.66	91.24
ZMBMT9SCMV	*Zea mays*	Bomet	90.22	94.13	96.82	94.13

Table 4.5. Neucleotide sequence similarity (%)among *Sugarcane mosaic virus* (SCMV) isolates (Polyprotein gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1: GU474635Mexico	100.00	92.88	87.20	90.95	90.50	89.77	90.39	89.39	89.17	89.29	88.93	89.98	89.40	87.34	88.74	89.05	90.23	90.22
2: JX188385Ohio	92.88	100.00	91.27	94.74	94.76	93.50	94.00	93.13	92.90	93.02	92.90	93.64	92.78	91.84	93.05	93.57	94.14	94.13
3: JN021933China	87.20	91.27	100.00	92.18	91.24	91.32	93.01	91.64	91.37	91.49	91.43	92.30	91.25	94.68	94.01	94.76	94.14	94.13
4: ECBMT10SCMV2	90.95	94.74	92.18	100.00	98.53	97.18	97.67	97.67	97.55	97.67	97.31	97.29	97.92	94.12	95.94	96.09	97.17	97.17
5: ZMBMT7SCMV	90.50	94.76	91.24	98.53	100.00	97.89	96.66	96.82	96.26	96.26	96.37	98.17	96.03	94.92	96.41	95.83	97.43	97.43
6: ZMNYR6SCMV2	89.77	93.50	91.32	97.18	97.89	100.00	97.07	96.71	96.48	96.48	96.25	98.04	96.25	94.38	95.81	94.52	96.33	96.33
7: KF744391Rwanda	90.39	94.00	93.01	97.67	96.66	97.07	100.00	98.59	98.37	98.25	98.36	98.78	98.13	93.18	96.76	95.11	96.82	96.82
8: DVBMT5SCMV	89.39	93.13	91.64	97.67	96.82	96.71	98.59	100.00	99.29	99.29	99.06	99.14	99.41	93.41	96.28	95.10	96.82	96.82
9: ZMNYR3SCMV	89.17	92.90	91.37	97.55	96.26	96.48	98.37	99.29	100.00	99.42	99.30	99.02	99.42	93.20	96.40	95.11	96.82	96.82
10: ZMNYR1SCMV	89.29	93.02	91.49	97.67	96.26	96.48	98.25	99.29	99.42	100.00	99.30	99.14	99.30	93.20	96.40	95.11	96.94	96.94
11: ECBMT10SCMV1	88.93	92.90	91.43	97.31	96.37	96.25	98.36	99.06	99.30	99.30	100.00	99.02	99.18	92.97	96.16	94.87	96.58	96.57
12: ZMNYR6SCMV1	89.98	93.64	92.30	97.29	98.17	98.04	98.78	99.14	99.02	99.14	99.02	100.00	99.27	94.87	96.82	95.48	96.58	96.57
13: ECNYR1SCMV	89.40	92.78	91.25	97.92	96.03	96.25	98.13	99.41	99.42	99.30	99.18	99.27	100.00	93.08	96.29	95.11	97.07	97.06
14: ZMMKN6SCMV	87.34	91.84	94.68	94.12	94.92	94.38	93.18	93.41	93.20	93.20	92.97	94.87	93.08	100.00	96.65	97.02	96.33	96.33
15: ECBMT10SCMV3	88.74	93.05	94.01	95.94	96.41	95.81	96.76	96.28	96.40	96.40	96.16	96.82	96.29	96.65	100.00	97.72	96.58	96.70
16: ZMBMT11SCMV	89.05	93.57	94.76	96.09	95.83	94.52	95.11	95.10	95.11	95.11	94.87	95.48	95.11	97.02	97.72	100.00	97.92	97.92
17: DVBMT8SCMV	90.23	94.14	94.14	97.17	97.43	96.33	96.82	96.82	96.82	96.94	96.58	96.58	97.07	96.33	96.58	97.92	100.00	100.00
18: ZMBMT9SCMV1	90.22	94.13	94.13	97.17	97.43	96.33	96.82	96.82	96.82	96.94	96.57	96.57	97.06	96.33	96.70	97.92	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

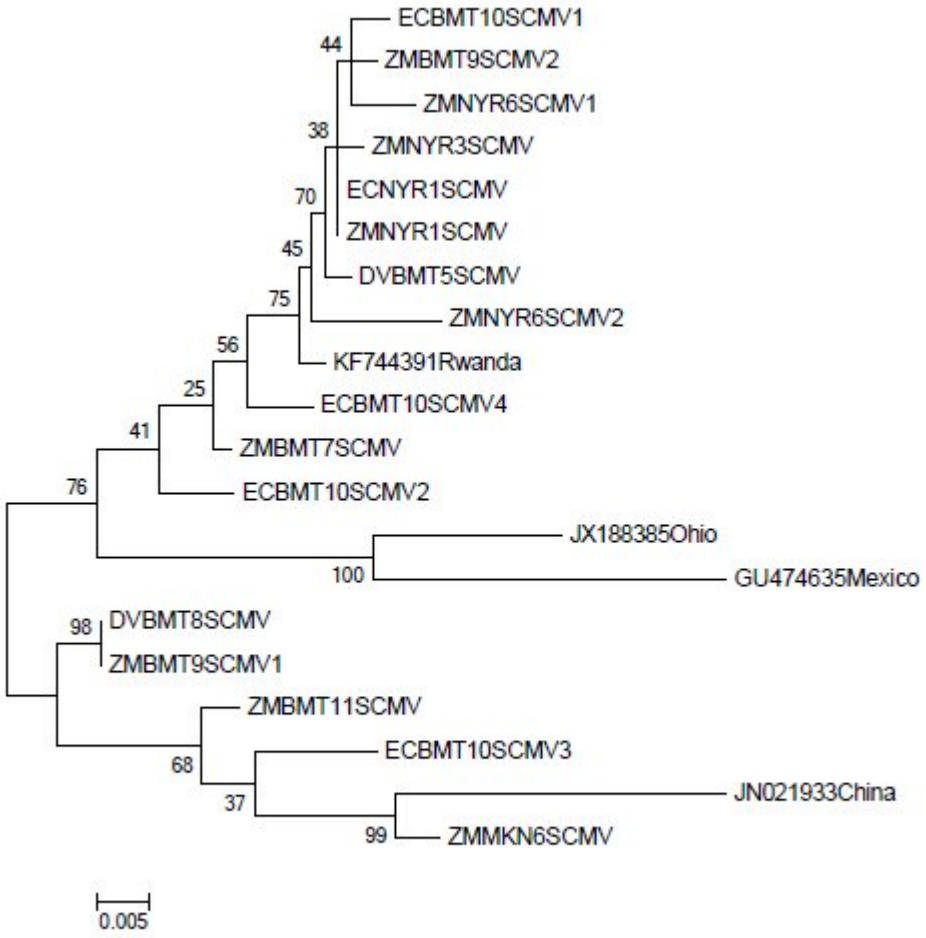

Figure 4.4 A neighbour-joining tree constructed with MEGA 6 using 500 bootstrap replicates for polyprotein gene of *Sugarcane mosaic virus* (SCMV). The tree includes selected SCMV isolates from maize, crop cereals, domesticated grasses and wild grasses collected from Nyamira, Bomet, Makueni and Machakos counties.

The MCMV isolates sequences were compared with isolates from Nebraska (Accession number EU358605), Rwanda (Accession number KF74439), Kenya (Accession number JX286709), China (Accession number JQ982470) and Taiwan (Accession number KJ782300) that are deposited in the Genbank. Nineteen selected isolates were used in this comparison and to generate a phylogenetic tree. The selected isolates were representative of maize, cereals, wild and domesticated grasses collected from different counties. The range in percentage similarity between these isolates was 95-97%, 99-100%, 99-100%, 98-99% and 97-98% with isolates from Nebraska, Rwanda, Kenya China and Taiwan respectively Table 4.6. The percentage similarities between the isolates in this study range from 98-100% Table 4.7- 4.11. Phylogenetic analysis showed that there was no big variation between the isolates and that they were most related to the Kenya and Rwanda isolates (Figure 4.5).

Table 4.6 Nucleotide sequence similarity between selected MCMV isolates of the study and DNA sequences (111kDA protein, P31 and P7 genes) derived from GenBank accessions of the virus.

			Percentage similarity with Genbank isolates				
			Nebraska EU358605	**Rwanda KF74439**	**Kenya JX286709**	**China JQ982470**	**Taiwan KJ782300**
Isolate	**Host**	**County**					
ZMNYR4MCMV	*Zea mays*	Nyamira	96.48	99.41	99.41	98.63	97.85
PCNYR3MCMV	*Pennisetum clandestinum*	Nyamira	95.90	100	100	99.14	98.27
ZMNYR1MCMV2	*Zea mays*	Nyamira	96.15	100	100	99.14	98.38
DVNYR4MCMV	*Digitaria velutina*	Nyamira	96.29	100	100	99.22	98.44
ZMNYR3MCMV	*Zea mays*	Nyamira	95.68	99.78	99.78	98.92	98.06
ZMNYR6MCMV	*Zea mays*	Nyamira	96.09	99.80	99.80	99.02	98.24
CDBMT7MCMV	*Cynodon dactylon*	Bomet	96.29	100	100	99.22	98.44
BBBMT9MCMV1	*Brachiaria brizantha*	Bomet	95.90	100	100	99.14	98.27
ECBMT10MCMV1	*Eleusine coracana*	Bomet	96.29	100	100	99.22	98.44
DVBMT7MCMV1	*Digitaria velutina*	Bomet	96.48	99.80	99.80	99.02	98.24
SBBMT8MCMV	*Sorghum bicolor*	Bomet	95.90	99.57	99.57	98.70	98.27
ZMMKS1MCMV1	*Zea mays*	Machakos	96.33	99.57	99.57	99.14	98.27
ZMMKN2MCMV	*Zea mays*	Makueni	95.69	99.78	99.78	98.92	98.06
PPNYR4MCMV	*Pennisetum purpureum*	Nyamira	96.29	100	100	99.22	98.44
ZMBMT11MCMV	*Zea mays*	Bomet	95.68	99.78	99.78	98.92	98.06
SOMKN1MCMV	*Sacharum officarum*	Makueni	95.27	99.02	99.02	98.24	97.45
DANYR2MCMV	*Digitaria abyssinica*	Nyamira	96.42	100	100	99.10	98.21
CRNYR4MCMV	*Cyperus rotundus*	Nyamira	96.29	100	100	99.22	98.44

			Percentage similarity with Genbank isolates				
			Nebraska EU358605	**Rwanda KF74439**	**Kenya JX286709**	**China JQ982470**	**Taiwan KJ782300**
Isolate	**Host**	**County**					
BBBMT9MCMV2	*Brachiaria brizantha*	Bomet	96.30	100	100	99.22	98.44
SBNYR2MCMV	*Sorghum bicolor*	Nyamira	95.46	99.46	99.46	98.70	97.84

Table 4.7 Nucleotide sequence similarity (%) among MCMV isolates (111kDa protein, P31 and P7 gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1: EU358605Nebraska	100.00	96.52	97.07	96.48	96.28	96.27	95.90	96.15	95.90	95.90	95.90	96.29	96.02	96.29	95.68	96.09	96.09
2: KJ782300Taiwan	96.52	100.00	98.90	97.85	98.51	98.51	98.27	98.38	98.27	98.27	98.27	98.44	98.45	98.44	98.06	98.24	98.24
3: JQ982470China	97.07	98.90	100.00	98.63	99.26	99.25	99.14	99.19	99.14	99.14	99.14	99.22	99.12	99.22	98.92	99.02	99.02
4: ZMNYR4MCMV	96.48	97.85	98.63	100.00	99.41	99.41	99.35	99.39	99.35	99.35	99.35	99.41	99.34	99.41	99.14	99.22	99.22
5: JX286709Kenya	96.28	98.51	99.26	99.41	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
6: KF744394Rwanda	96.27	98.51	99.25	99.41	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
7: ZMNYR1MCMV1	95.90	98.27	99.14	99.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
8: ZMNYR1MCMV2	96.15	98.38	99.19	99.39	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
9: ZMNYR2MCMV	95.90	98.27	99.14	99.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
10: ZMTTAMCMV	95.90	98.27	99.14	99.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
11: PCNYR3MCMV	95.90	98.27	99.14	99.35	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
12: DVNYR4MCMV	96.29	98.44	99.22	99.41	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
13: ZMNYR5MCMV1	96.02	98.45	99.12	99.34	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
14: PCNYR6MCMV1	96.29	98.44	99.22	99.41	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
15: ZMNYR3MCMV	95.68	98.06	98.92	99.14	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	100.00	100.00	100.00
16: ZMNYR5MCMV2	96.09	98.24	99.02	99.22	99.80	99.80	99.78	99.80	99.78	99.78	99.78	99.80	99.78	99.80	100.00	100.00	100.00
17: ZMNYR6MCMV	96.09	98.24	99.02	99.22	99.80	99.80	99.78	99.80	99.78	99.78	99.78	99.80	99.78	99.80	100.00	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

Table 4.8 Nucleotide sequence similarity (%) among MCMV isolates (111kDa protein, P31 and P7 gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1: EU358605Nebraska	100.00	96.52	97.07	95.90	95.68	96.28	96.27	95.91	96.29	96.29	95.90	95.90	95.90	96.29	96.11	96.48	96.48
2: KJ782300Taiwan	96.52	100.00	98.90	98.27	98.06	98.51	98.51	98.28	98.44	98.44	98.27	98.27	98.27	98.44	98.06	98.24	98.24
3: JQ982470China	97.07	98.90	100.00	98.70	98.92	99.26	99.25	99.14	99.22	99.22	99.14	99.14	99.14	99.22	98.92	99.02	99.02
4: SBBMT8MCMV	95.90	98.27	98.70	100.00	99.35	99.57	99.57	99.57	99.57	99.57	99.57	99.57	99.57	99.57	99.35	99.35	99.35
5: ZMBMT9MCMV	95.68	98.06	98.92	99.35	100.00	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.57	99.57	99.57
6: JX286709Kenya	96.28	98.51	99.26	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
7: KF744394Rwanda	96.27	98.51	99.25	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
8: ZMBMT7MCMV1	95.91	98.28	99.14	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
9: CDBMT7MCMV	96.29	98.44	99.22	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
10: PCBMT7MCMV1	96.29	98.44	99.22	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
11: ZMBMT8MCMV	95.90	98.27	99.14	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
12: BBBMT9MCMV1	95.90	98.27	99.14	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
13: ZMBMT10MCMV	95.90	98.27	99.14	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.78	99.78
14: ECBMT10MCMV1	96.29	98.44	99.22	99.57	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	99.78	99.80	99.80
15: ZMBMT7MCMV2	96.11	98.06	98.92	99.35	99.57	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	100.00	100.00	100.00
16: DVBMT7MCMV1	96.48	98.24	99.02	99.35	99.57	99.80	99.80	99.78	99.80	99.80	99.78	99.78	99.78	99.80	100.00	100.00	100.00
17: ECBMT10MCMV2	96.48	98.24	99.02	99.35	99.57	99.80	99.80	99.78	99.80	99.80	99.78	99.78	99.78	99.80	100.00	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

Table 4.9. Nucleotide sequence similarity (%) among MCMV isolates (111kDa protein, P31 and P7 gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1: EU358605Nebraska	100.00	96.52	97.07	96.33	95.68	95.69	96.09	96.30	96.28	96.27	96.29	95.90	95.90	95.90	95.90	95.90	96.29
2: KJ782300Taiwan	96.52	100.00	98.90	98.27	98.06	98.06	98.24	98.47	98.51	98.51	98.44	98.27	98.27	98.27	98.27	98.27	98.44
3: JQ982470China	97.07	98.90	100.00	99.14	98.92	98.92	99.02	99.35	99.26	99.25	99.22	99.14	99.14	99.14	99.14	99.14	99.22
4: ZMMKS1MCMV1	96.33	98.27	99.14	100.00	99.35	99.35	99.57	99.76	99.57	99.57	99.57	99.57	99.57	99.57	99.57	99.57	99.57
5: ZMBMT11MCMV	95.68	98.06	98.92	99.35	100.00	99.57	99.78	99.76	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78
6: ZMMKN2MCMV	95.69	98.06	98.92	99.35	99.57	100.00	99.78	99.76	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78	99.78
7: ZMMKN4MCMV1	96.09	98.24	99.02	99.57	99.78	99.78	100.00	99.78	99.80	99.80	99.80	100.00	100.00	100.00	100.00	100.00	99.80
8: ZMMKS1MCMV2	96.30	98.47	99.35	99.76	99.76	99.76	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
9: JX286709Kenya	96.28	98.51	99.26	99.57	99.78	99.78	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
10: KF744394Rwanda	96.27	98.51	99.25	99.57	99.78	99.78	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
11: PCBMT11MCMV	96.29	98.44	99.22	99.57	99.78	99.78	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
12: ZMBMT12MCMV	95.90	98.27	99.14	99.57	99.78	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
13: ZMICPMCMV	95.90	98.27	99.14	99.57	99.78	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
14: PPICPMCMV	95.90	98.27	99.14	99.57	99.78	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15: ZMMKS2MCMV	95.90	98.27	99.14	99.57	99.78	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
16: PCBMT8MCMV	95.90	98.27	99.14	99.57	99.78	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
17: PPNYR4MCMV	96.29	98.44	99.22	99.57	99.78	99.78	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

Table 4.10. Nucleotide sequence similarity (%) among MCMV isolates (111kDa protein, P31 and P7 gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1: EU358605Nebraska	100.00	96.52	95.12	95.29	96.42	97.07	96.48	96.09	96.23	96.28	96.27	95.92	96.29	96.29	96.29	96.29	96.29
2: KJ782300Taiwan	96.52	100.00	97.27	97.45	98.21	98.90	98.24	98.24	98.45	98.51	98.51	98.28	98.44	98.44	98.44	98.44	98.44
3: PCBMT10MCMV	95.12	97.27	100.00	99.41	100.00	98.05	98.63	98.83	99.11	98.83	98.83	98.71	98.83	98.83	98.83	98.83	98.83
4: SOMKN1MCMV	95.29	97.45	99.41	100.00	100.00	98.24	98.82	99.02	99.33	99.02	99.02	98.92	99.02	99.02	99.02	99.02	99.02
5: DANYR2MCMV	96.42	98.21	100.00	100.00	100.00	99.10	99.70	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
6: JQ982470China	97.07	98.90	98.05	98.24	99.10	100.00	99.02	99.02	99.33	99.26	99.25	99.14	99.22	99.22	99.22	99.22	99.22
7: ZMMKN5MCMV	96.48	98.24	98.63	98.82	99.70	99.02	100.00	99.61	99.78	99.80	99.80	99.79	99.80	99.80	99.80	99.80	99.80
8: PCNYR5MCMV	96.09	98.24	98.83	99.02	100.00	99.02	99.61	100.00	100.00	99.80	99.80	99.79	99.80	99.80	99.80	99.80	99.80
9: DANYR1MCMV	96.23	98.45	99.11	99.33	100.00	99.33	99.78	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
10: JX286709Kenya	96.28	98.51	98.83	99.02	100.00	99.26	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
11: KF744394Rwanda	96.27	98.51	98.83	99.02	100.00	99.25	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
12: ZMMKN4MCMV2	95.92	98.28	98.71	98.92	100.00	99.14	99.79	99.79	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
13: DANYR3MCMV	96.29	98.44	98.83	99.02	100.00	99.22	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
14: CDNYR4MCMV	96.29	98.44	98.83	99.02	100.00	99.22	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
15: CRNYR4MCMV	96.29	98.44	98.83	99.02	100.00	99.22	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
16: DANYR5MCMV	96.29	98.44	98.83	99.02	100.00	99.22	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
17: PCBMT7MCMV2	96.29	98.44	98.83	99.02	100.00	99.22	99.80	99.80	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

Table 4.11. Nucleotide sequence similarity (%) among MCMV isolates (111kDa protein, P31 and P7 gene) in this study and DNA sequences derived from GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10
1: EU358605Nebraska	100.00	96.52	97.07	96.28	96.27	96.30	96.29	96.29	95.90	95.46
2: KJ782300Taiwan	96.52	100.00	98.90	98.51	98.51	98.44	98.44	98.44	98.05	97.84
3: JQ982470China	97.07	98.90	100.00	99.26	99.25	99.22	99.22	99.22	98.83	98.70
4: JX286709Kenya	96.28	98.51	99.26	100.00	100.00	100.00	100.00	100.00	99.61	99.57
5: KF744394Rwanda	96.27	98.51	99.25	100.00	100.00	100.00	100.00	100.00	99.61	99.57
6: BBBMT9MCMV2	96.30	98.44	99.22	100.00	100.00	100.00	100.00	100.00	99.61	99.57
7: PCNYR6MCMV2	96.29	98.44	99.22	100.00	100.00	100.00	100.00	100.00	99.61	99.57
8: ECBMT10MCMV3	96.29	98.44	99.22	100.00	100.00	100.00	100.00	100.00	99.61	99.57
9: DVBMT7MCMV2	95.90	98.05	98.83	99.61	99.61	99.61	99.61	99.61	100.00	100.00
10: SBNYR2MCMV	95.46	97.84	98.70	99.57	99.57	99.57	99.57	99.57	100.00	100.00

Matrix generated using Clustal Omega version 12.1.

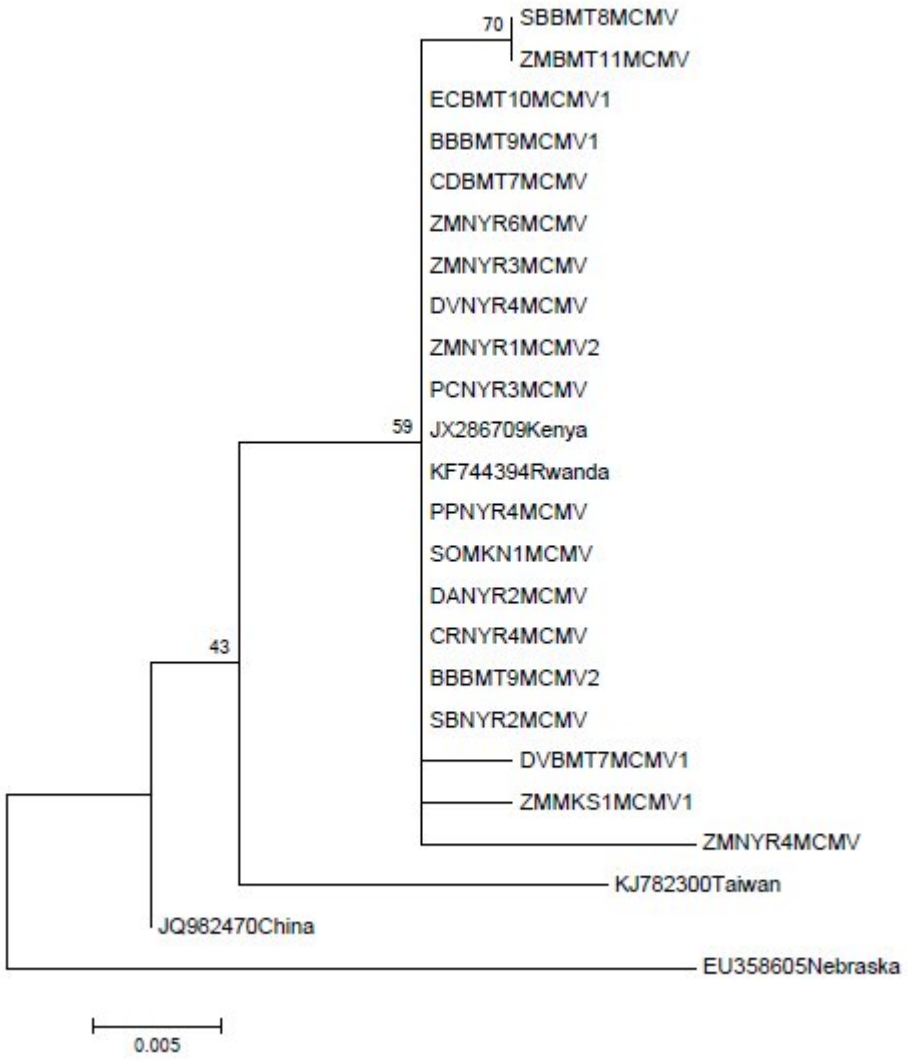

Figure 4.5. A neighbour-joining tree constructed with MEGA 6 using 500 bootstrap replicates for 111kDa protein, P31 and P7 genes of MCMV. The tree includes selected MCMV isolates from maize, crop cereals, domesticated grasses and wild grasses collected from Nyamira, Bomet, Makueni and Machakos counties.

4.2 Spatial distribution of wild grasses serving as alternative hosts of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

In this study, MCMV and SCMV were detected in either grass species or cereal crops in Nyamira, Bomet, Machakos, and Makueni (Figure 4.6). Conversely, MCMV and SCMV were not detected in either grass species or cereal crops in Vihiga County (Figure 4.6). Wild grasses that tested positive for MCMV and SCMV were collected from Nyamira and Bomet counties. Contrastingly, grasses collected from Makueni, Machakos and Vihiga counties tested negative for both viruses. However, maize samples collected from Makueni and Machakos counties tested positive for both MCMV and SCMV while those collected from Vihiga County tested negative for both viruses.

In this study, six grasses tested positive for MCMV and SCMV as illustrated in (Table 4.1.) They included *Brachiaria Digitaria velutina, Digitaria abyssinica, Cynodon dactylon, Pennisetum clandestinum* and *Cyperus rotundus.* All these grasses were found in both Bomet and Nyamira counties. All the grasses that habour MCMV and SCMV are found in zones 2, 3, 4 and 5 of the Kenyan agroecological zones except Pennisetum clandestinum which is only found in zones 2,3 and 4. Based on the results obtained in this study, a table that depicts the distribution of these grasses in the Kenyan agroecological zones was developed (Table 4.12):

Zone 1 – Humid, more than 80% moisture, 1100mm to 2700mm annual rainfall.

Zone 2 – Sub-humid, 65-80% moisture, 1000mm to 1600mm annual rainfall

Zone 3 – Semi-humid, 50-65% moisture, 800mm to1400mm annual rainfall

Zone 4 – Semi-humid to Semi-arid, 40-50% moisture, 600mm to 1100mm annual rainfall

Zone 5 – Semi-arid, 25-40% moisture, 450mm to 900mm annual rainfall (FAO, 1997)

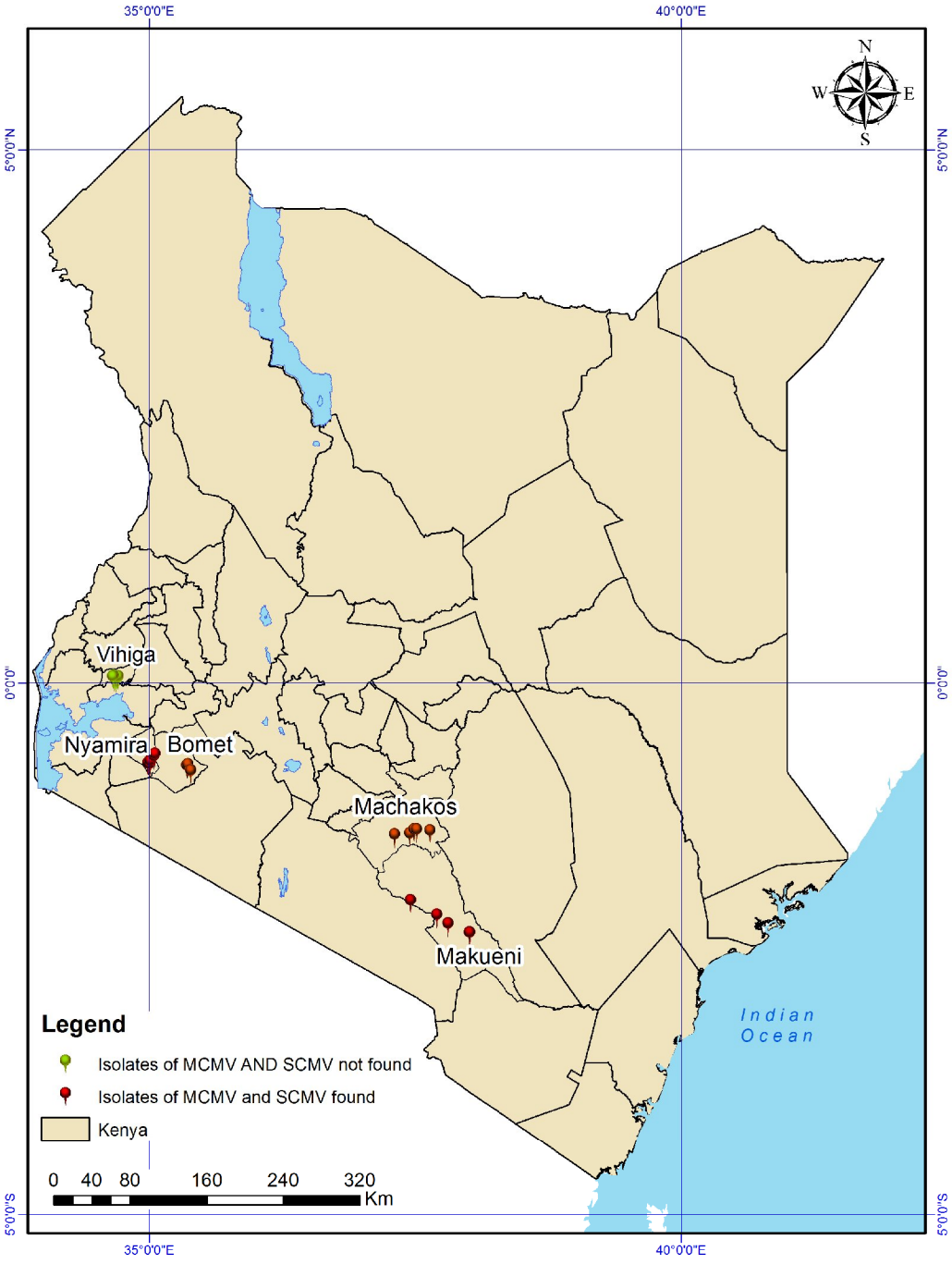

Figure 4.6 A map of Kenya showing occurrence of *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) in plant samples in Vihiga, Nyamira, Bomet, Machakos, and Makueni counties

Table 4.12: The occurrence of alternative hosts harbouring *Maize chlorotic mottle* (MCMV) and *Sugarcane mosaic virus* (SCMV) in Kenyan agro-ecological zones

Weed species	**Family**	**Occurrence**	**Presence**	**Virus detected**
Cynodon dactylon	Poaceae	Zone 2,3,4,5	Perennial	MCMV and SCMV
Digitaria abyssinica	Poaceae	Zone 1,2,3,4,5	Perennial	MCMV and SCMV
Pennisetum clandestinum	Poaceae	Zone 2,3,4	Perennial	MCMV and SCMV
Digitaria velutina	Poaceae	Zone 2,3,4,5	Annual	MCMV and SCMV
Brachiaria brizantha	Poaceae	Zone 2,3,4,5	Perennial	MCMV and SCMV
Cyperus rotundus	Cyperaceae	Zone 2,3,4,5	Perennial	MCMV
Panicum trichocladium	Poaceae	Zone 2	Perennial	None
Pennisetum mezianum	Poaceae	Zone 4	Perennial	None
Setaria plicatilis	Poaceae	Zone 2, 3,4	Perennial	None
Panicum maximum	Poaceae	Zone 2,3,4,5	Perennial	None
Eleusine indica	Poaceae	Zone 2,3,4,5	Annual	None
Setaria verticillata	Poaceae	Zone 2,3,4,5	Annual	None
Digitaria diagonalis	Poaceae	Zone 4	Perennial	None
Brachiaria leersioides	Poaceae	Zone 3,4,5,6	Annual	None
Cenchrus ciliaris	Poaceae	Zone 3,4,5	Perennial	None

Based on the environmental conditions in areas where the grasses harbouring either MCMV or SCMV were found, a geographic distribution map of the grasses was generated by MAXENT version 3.3.3k (Phillips *et al*., 2006) to predict areas most suitable for these grasses to grow and serve as a source of innoculum of MCMV and SCMV The GPS co-ordinates of the sampling regions were used to develop the map putting in consideration the presence and absence of the wild grass host and the Kenyan agro-ecological zones. Highest precipitation of the wettest and the driest months had the most weight when generating the distribution map (Figure 4.7). The map shows that humid regions where maize is grown (parts of Western, Nyanza, North and South Rift, Upper and Lower Eastern, Central and Taita Taveta) are the most suitable areas for wild grasses that harbour MCMV and SCMV (Figure 4.7).

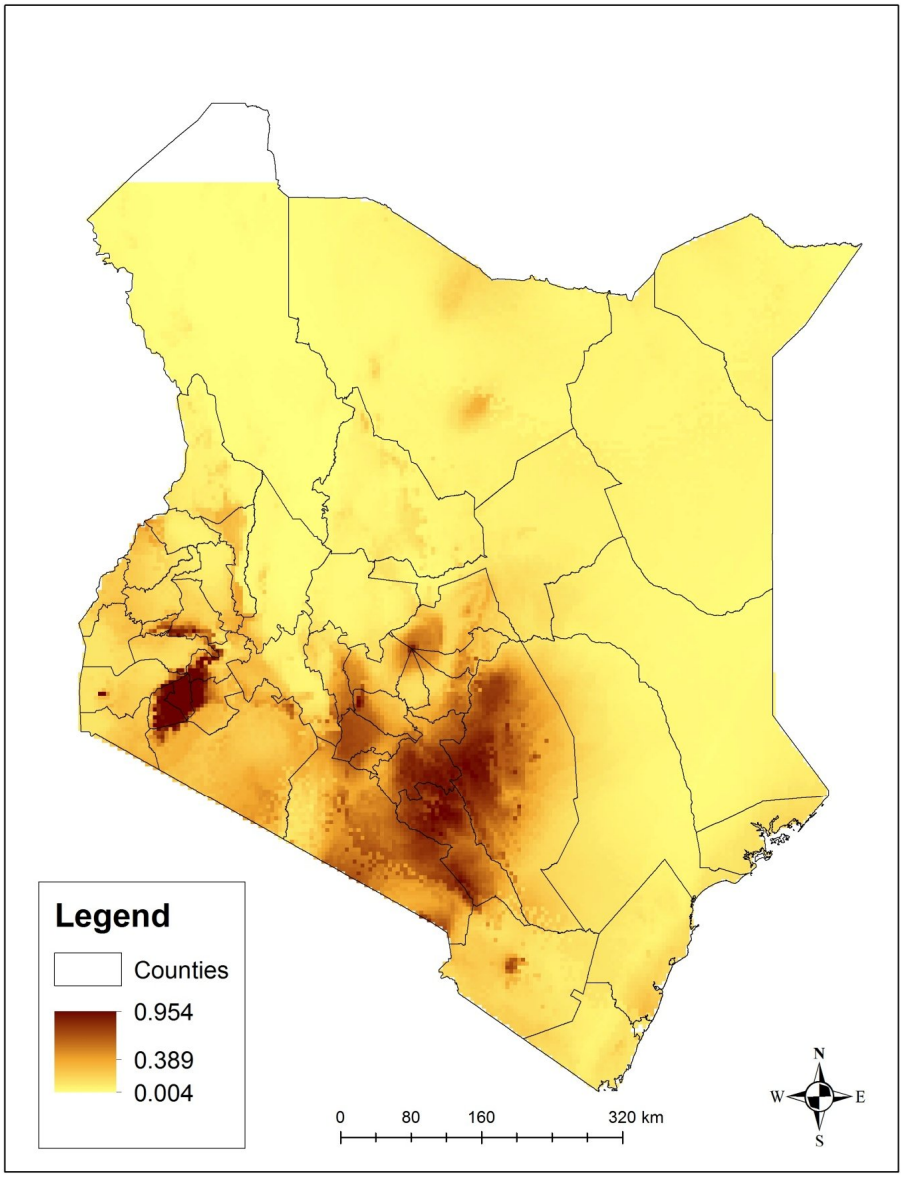

Figure 4.7 A map of Kenya showing areas suitable for growth of wild grasses that serve as alternative hosts of *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). Dark brown depicts most suitable areas, a lighter brown, mild suitability and a very light brown, low suitability areas for the growth of the wild grasses.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Presence of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in cereals, wild and domesticated grasses

In this study maize, wild grasses, domesticated grasses and crop cereals were found to host MCMV and SCMV either singly or dually. Wild grasses that tested positive for MCMV and SCMV dually include: *Digitaria velutina, Digitaria abyssinica, Cynodon dactylon, and Pennisetum clandestinum. Maize chlorotic mottle virus* is known to be restricted to the Poaceae family with maize as the main natural host (Gordon *et al*., 1984). However, in the present study, MCMV was found in *Cyperus rotundus* which belongs to the *Cyperaceae* family. *Brachiaria brizantha* harboured either MCMV or SCMV separately. This is the first report of the occurrence of MCMV and SCMV in these grasses in the field. *Digitaria abyssinica* expressed symptoms in form of yellowing on the youngest leaves found at the shoot tip. *Pennisetum clandestinum Brachiaria brizantha* and *Digitaria velutina* had mild mottling which could not be observed from a distance. *Cynodon dactylon* and *Cyperus rotundus* were asymptomatic despite testing positive for both SCMV and MCMV and MCMV alone respectively.

There are no published reports on infection of wild grasses by MCMV under field conditions. Nevertheless, grass species that have been reported to habour MCMV when mechanically inoculated include

Bromus spp., *Digitaria sanguinalis, Eragrostis trichodes, Hordeum* spp., *Panicum* spp., *Setaria* spp., *Eleusine indica*, *Sorghum* spp. and *Triticum aestivum* (Castillo and Hebert, 1974; Niblett and Claflin, 1978; Bockelman*et al*., 1982). On inoculation, *Cynodon dactylon* was immune to MCMV-P but susceptible to MCMV-K (Bockleman 1982). SCMV is also restricted to the Poaceae family. Louie (1980), found SCMV in *Digitaria abyssinica, Digitaria velutina, Cynodon dactylon and Setaria verticillata* However, samples of *Setaria verticillata* collected in Makueni County tested negative for both MCMV and SCMV.

Panicum maximum tested positive for MCMV in Hawaii (Nelson *et al*., 2011.), however samples of *Panicum maximum* and *Setaria verticillata* collected from Makueni County tested negative for both MCMV and SCMV. The results can be attributed to the low disease prevalence and severity recorded in Makueni. *Eleusine indica* collected from Bomet County also tested negative for both viruses.

Other domesticated grasses that were tested included napier grass (*Pennisetum purpureum*) which harboured MCMV singly and Sugarcane. (*Saccharum officinarum L*) which was found to habour both MCMV and SCMV. Both napier grass and sugar cane showed MLN symptoms but the necrosis in napier grass was mild and not obvious. Recently, sugarcane has been found to host MCMV naturally (Wang *et al*., 2014). SCMV has also been reported in Napier grass in Kenya (Louie, 1980)

Sorghum (*Sorghum bicolor*) and finger millet (*Eleusine coracana*) constitute cereal crops that serve as alternative hosts of both MCMV and SCMV either dually or singly.

Despite testing positive, sorghum samples collected were asymptomatic unlike finger millet which was symptomatic. *Sorghum bicolor* was susceptible to MCMV-K by mechanical inoculation (Bockleman, 1982). Sorghum is also a host of SCMV (Louie, 1980). However this is the first report of SCMV and MCMV in finger millet.

The results indicated thatMLN disease severity increases gradually from tasseling to milking stage. The increase in disease severity may be attributed to the increase in population densities of potential vectors of MCMV and SCMV during the reproductive stage of maize. Disease symptom expression in wild grasses also coincides with the reproductive stage of maize and therefore, the abundant sources of virus inoculum for vectors of MCMV and SCMV may contribute to increase in disease severity. Cereals and domesticated grasses are planted in the same fields as maize and may serve as virus inoculum for transmission to healthy maize and susceptible non-maize plants by insect vectors. Farmers may get rid of wild grasses during weeding but they retain the cereal crops and therefore maintaining the sources of virus inoculum. Some crops like sorghum are asymptomatic and can therefore contribute to the spread of the MLN 'silently'.

It is evident that both MCMV and SCMV have a wide range of alternative hosts. Wild grasses grow as weeds inside and around maize farms and since they harbour the viruses, they serve as a continuous source of inoculum of MCMV and SCMV. Some grasses are asymptomatic and therefore cannot be noticed by farmers. Therefore, the wild grasses might have greatly contributed to the rapid spread of MLN.

5.2 Genetic diversity of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in wild grasses and cereal crops

MCMV was never reported in Kenya until 2011 (Wangai *et al*., 2012) unlike SCMV which has been in Kenya since 1980 (Louie, 1980). The isolates of MCMV and SCMV collected in this study are most closely related to the isolates from China, Congo and Rwanda with reference to the 111kDa protein, P31 and P7 open reading frames (ORFs) for MCMV and a section of the polyprotein gene for SCMV. Such relationships have been established earlier (Adams *et al*., 2012; Adams *et al*., 2014; Lukanda *et al*., 2014). MLN was first reported in China in 2011 (Xie *et al* 2011). The close relatedness (91-99%) of the Kenyan MCMV isolates with the China isolates suggests that they have a common origin.

5.3 Spatial distribution of wild grasses serving as alternative hosts *Maize chlorotic mottle virus* and *Sugarcane mosaic virus*

The wild grasses that habour both MCMV and SCMV are widely distributed in the Kenyan agro-ecological zones. Digitaria abyssinica, Brachiaria brizantha, Pennisetum clandestinum, Cyperus rotundus and Cynodon dactylon are all perennial grasses while Digitaria velutina is an annual grass. Perennial grasses persist across seasons and can therefore carry the virus from the previous maize planting season. MLN is spreading rapidly in Kenya and East Africa with the newest report of MCMV in Congo (Lukanda et al., 2014) and the availability of grass weeds in most parts of Kenya where maize is

grown is likely to boost their potential to host the viruses and spread them to other susceptible crops.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1Conclusions

This study has clearly shown that MCMV and SCMV which cause MLN in Kenya have a wide range of alternative hosts including wild grasses, domesticated grasses and crop cereals. Wild grasses that serve as alternative hosts of MCMV and SCMV are widely spread across the Kenyan agro-ecological zones. The genetic variability among MCMV and SCMV isolates from different parts of Kenya has also been established. It is also evident that DAS-ELISA is an efficient method for the detection of MCMV and SCMV.

6.2 Recommendations

This study has revealed that wild grasses and cereal crops are vital in the spread of MLN. Wild grasses in other parts of Kenya that may host MCMV and SCMV also need to be evaluated. Maize farmers need to be sensitized on the potential role of alternative hosts in spread of MLN. More work is needed to test for other potyviruses apart from SCMV that have been reported to cause MLN in other parts of the world. Finally, there is need to develop integrated disease management strategies for the mitigation of the rapid spread and effects of MLN. The role of alternative hosts of NCNV and SCMV in the epidemiology of MLN should be considered during the development of these strategies.

REFERENCES

Adams, I. P.; Harju, V. A.; Hodges, T.; Hany, U.; Skelton, A.; Rai, S.; Deka, M. K.; Smith, J.; Fox, A.; Uzayisenga, B.; Ngaboyisonga, C.; Uwumukiza, B.; Rutikanga, A.; Rutherford, M.; Ricthis, B.; Phiri, N.; Boonham, N. (2014) First report of maize lethal necrosis disease in Rwanda. New Disease Reports 29 pp. 22

Adams, I. P., Miano, D., Kinyua, W. Z. M., Wangai, A., Kimani, E., Phiri, N., Reeder, R., Harju, V., Glover, R., Hany, U., Souza-Richardsa, R., Deb Natha, P., Nixon, T., Fox, A., Barnes, A., Smith, J., Skelton, A., Thwaites, R., Mumford, R. and Boonham, N. (2012). Use of next-generation sequencing for the identification and characterization of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* causing maize lethal necrosis in Kenya *Plant Pathology* Doi: 10.1111/j.1365-3059.2012.02690.x

Alegria OM, Royer M, Bousalem M, Chatenet M, Peterschmitt M, Girard JC, Rott P. (2003). Genetic diversity in the coat protein coding region of eighty-six sugarcane mosaic virus isolates from eight countries, particularly from Cameroon and Congo. Arch Virol 148:357–372.

Bockelman, D.L.., Claflin, L. E. And Uyemoto, J.K. (1982). Host range and seed-transmission studies of *Maize chlorotic mottle virus* in grasses and corn. *Plant Dis*. 66: 216-218.

Bond, W.P. and Pirone, T.P. (1969). Evidence for soil transmission of *Sugarcane mosaic virus*. *Kentucky agricultural experiment station journal* 61: 11-101.

Brunt, A.A., Crabtree, K., Dallwitz, M.J., Gibbs, A.J., Watson, L. and Zurcher, E.J. (eds.) (1996). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups/MES/vide/

Cabanas, D., Watanabe, S., Higashi, C.H.V., and Bressan, A. (2013). Dissecting the Mode of Maize Chlorotic Mottle Virus Transmission (Tombusviridae: Machlomovirus) by *Frankliniella williamsi* (Thysanoptera: Thripidae) *Economic Entomology* 106: 16-24.

Calvert, L.A. and Ghabrial, S.A. (1983). Enhancement by soybean mosaic virus of bean pod mottle virus titer in doubly infected soybean. *Phytopath* 73: 992–997

Carrera-Martínez, H., Losoya-Saldaña, H., Mendoza-Zamora, C., Alvizo-Villasana, H. (1989). Inmunoabsorción enzimática (ELISA) en la identificación y distribución del virus moteado clorótico del maíz (VMCM) en el estado de México. *Rev. Mex. Fitopatol*., 7:20-25 (in Mexican Spanish).

Castillo, J. (1977). Maize virus and virus-like diseases in 4- Peru. Pages 40-44 *in* L. E. Williams, D. T. Gordon, and L. R. Nault, eds. Proc. Int. Maize Virus Dis. Colloq. Workshop, 16-19 Aug 1976, Ohio Agric. Res. And Develop. Center, Wooster, OH. 145 p.

Castillo, J. and Hebert, T.T. (1974). Nueva enfermedad virosa afectando al maiz en el Peru. *Fitopatologia* 9: 79-84.

Cheng, Y., Chen, J. and Chen, J. (2002).The complete sequence of a sugarcane mosaic virus isolate causing maize dwarf mosaic disease in China. *Science in China* 45: 3.

CIMMYT, (2012). Maize lethal necrosis (MLN) disease in Kenya and Tanzania: Facts and actions. Retrieved from CIMMYT website http://intranet.cimmyt.org.

Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34:475–483.

FAO, (2013). Maize lethal necrosis disease (MLND). Food security and nutrition working group.

FAO, (1997.)Country Pasture/Forage Resource Profiles.

Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E., and Vetten, H. F. (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology*. 47: 95-02.

Grisham, M. P. (2000). Mosaic. In: A guide to sugarcane diseases (eds Rott P, Comstock JC, Croft BJ and Saumtally AS. CIRAD/ISSCT, Montpellier.

Goldberg, K.B. and Brakke, M. K. (1987). Concentration of *Maize chlorotic mottle virus* increased in mixed infections with *Maize dwarf mosaic virus*, strain B. *Phytopathology* 77:162-16.

Gordon, D. T., O. E. Bradfute, R. E. Gingery, L. R. Nault, and I. K. Uyemoto. 1984. Maize chlorotic mottle virus. Descriptions of Plant Viruses, No. 284 Commonwealth Mycological Institute and Association of Applied Biologists, Kew, Surrey, England.

Jensen, S., Wysong, D., Ball, E. and Higley, P. (1991). Seed transmission of *maize chlorotic mottle virus*. *Plant Disease* 75(5):497-498.

Jiang, X. Q., L. J. Meinke, R. J. Wright, D. R. Wilkinson, and J. E. Cambell. 1992. Maize chlorotic mottle virus in Hawaiian-grown maize: vector relations, host range and associated viruses. Crop Prot. 11: 248-254.

Joint Assessment Report, (2012). Status of maize lethal necrosis disease and general maize performance; stakeholders maize tour. Retrieved from http://www.disasterriskreduction.net 2012

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, and Drummond A.(2012). Geneious Basic: an integrated and extendable desktop software for the organization and analysis of sequence data. *Bioinformatics*: 28 (12):1647-9.

Kfir, R., Overholt, W.A., Khan, Z.R. and Polaszek, A. *(*2002*)* Biology and management of economically important lepidopteran cereal stemborers in Africa*. Annual Review of Entomology* 47*:* 701–731.

Khan, Z. R., Hassanali, A., Pickett, J.A., Wadhams, L. J. and Muyekho, F. (2003). Strategies for control of cereal stemborers and striga weed in maize-based farming systems in eastern Africa involving 'push-pull' and allelopathic tactics, respectively. *African Crop Science Conference Proceedings*,6: 602-608.

Li, L, Wang, X.F. and. Zhou, G.H (2011). Effects of Seed Quality on the Proportion of Seed Transmission for *Sugarcane mosaic virus* in maize. *Cereal Research Communications* 39(2):257–266

Lommel, S. A., Kendall, T.L., Siu, N.F. and Nutter, R.C. (1991) Characterization of *Maize chlorotic mottle virus*. *Phytopathology* 81:819–823

Lommel, S.A., McCain, A.H., and Morris, T.J. (1982). Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. *Phytopathology* 72: 1018–1022.

Louie, R. (1980) *Sugarcane mosaic virus* in Kenya. *Plant Disease* 64: 944–947.

Lukanda., M. Owati., A. Ogunsanya., P. Valimunzigha., K. Katsongo K. Ndemere H. and Kumar, L. P. (2014). First Report of Maize chlorotic mottle virus Infecting Maize in the Democratic Republic of the Congo. *Plant Disease* 98: 1,448.2 - 1,448.2.

Mantel, S. and van Engelen, V.W.P. (1994). The impact of land degradation on food productivity. Case studies of Uruguay, Argentina and Kenya, Volume 1, Volume 1: main report.

Martin, D.P. Willment, J.A. Billharz, R., Velders, R., Odhiambo, B., Njuguna, J., James, D. and Rybicki, E.P. (2001). Sequence Diversity and Virulence in *Zea mays* of Maize Streak Virus Isolates. *Virology* 288: 247-255.

Ministry of agriculture (2013) Food security assessment report.

Muchena F.N., Mbuvi, J.P. and Wokabi, S.M. (1988). Report on soils and land use in arid and semi-arid lands of Kenya. Ministry of Natural Resources, National Environment Secretariat, Nairobi, Kenya.

Nault, L., Styer, W., Coffey, M., Gordon, D., Negi., L. and Niblett, C. (1978). Transmission of *Maize chlorotic mottle virus* by chrysomelid beetles. Phytopathology, 68:1071-1074.

Nelson, S. Brewbaker, J. and Hu, J. (2011) Maize Chlorotic Mottle. Retrieved from www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-79

Niblett, C.L. and Claflin, L. E. (1978) Corn lethal necrosis – a new virus disease of corn in Kansas. *Plant Disease Reporter* 62: 15–19.

Nutter, R.C., Scheets, K., Pangnaniban, L.C. and Lommel, S.A (1989). The complete nucleotide sequence of the *Maize chlorotic mottle virus* genome. *Nucleic Acids Res* 17:3163–3177

Nyvall, R. 1999. Field crop diseases (Third Edition). Iowa State University Press, Ames, Iowa.

Pruss, G., Ge, X., Shi, X. M., Carrington, J. C., and Vance, V. B. 1997. Plant viral synergism: The potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* 9:859-868.

Queensland Herbarium, (2013) Collection and preserving plant specimens, a manual Department of Science, Information Technology, Innovation and the Arts.

Rochow, W.F., and Ross, A.F. (1955). Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology* 1:10-27.

Scheets, K. (2004). Maize chlorotic mottle. In: H. Lapierre and P.-A. Signoret (eds.) *Viruses and virus diseases of Poaceae (Gramineae)*. Institut National de la Recherche Agronomique, Paris.

Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology 7:539 doi:10.1038/msb.2011.75

Stenger, D. C., and R. French. (2008). Complete nucleotide sequence of a Maize chlorotic mottle virus isolate from Nebraska. *Arch. Virology.* 153: 995-997.

Phillips, S. J., Anderson, R. P. and Schapire. R. E. (2006). Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190:231-259.

Ritchie, S.W., Hanway J.J., and Benson G.O. (1992). How a corn plant develops. Spec. Rep. 48 (rev.). Iowa State Univ. Coop. Ext.

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution:30 2725-2729.

Uyemoto, J. K., (1983) Biology and control of *Maize chlorotic mottle virus*. Plant *Disease* 67:7-10.

Uyemoto, J. K.; Bockelman, D. L. and Claflin, L. E. (1980). Severe outbreak of corn lethal necrosis disease in Kansas. *Plant Disease* 64:99-100

Vance, V.B. (1991). Replication of potato virus X RNA is altered in co-infections with potato virus Y. *Virology* 182:486-494.

Wang, Q., Zhou X. P., and Wu J. X (2014)First Report of *Maize chlorotic mottle virus* Infecting Sugarcane (*Saccharum officinarum*). 98: 572.2 - 572.2.

Wangai, A W., Redinbaugh, M. G., Kinyua, Z. M., Miano, D. W., Leley, P. K., Kasina M., Mahuku G., Scheets K. and Jeffers D. (2012). First Report of *Maize chlorotic mottle virus* and Maize lethal necrosis in Kenya. *Plant Disease* 96: 1582.

Xie, L., Zhang, .J, Wang, Q., Meng, C., Hong, J. and Zhou, X. (2011). Characterization of Maize chlorotic mottle virus associated with maize lethal necrosis disease in China. Journal of Phytopathology 159, 191–3.

Xu, D. L., Park, J.W., Mirkov, G.H., and Zhou, G. H. (2008). Viruses causing disease mosaic in sugarcane and their genetic diversity in southern China. *Arch Virol* 153: 1031-1039