REACTION OF DIFFERENT MAIZE GENOTYPES TO INFECTION BY MAIZE LETHAL NECROSIS DISEASE AND TRANSMISSION OF VIRUSES CAUSING THE DISEASE FROM SOIL AND PLANT DEBRIS

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my children, Andrew Momanyi, Christine Kwamboka and Emmanuel Akuma who endured my absence and supported me throughout the course of this study.

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I return all glory to the Almight, for according me the strength and good health throughout this study. I thank Him in all ways for His provision.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

AEZ	Agro Ecological Zones
ANOVA	Analysis of Variance
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
AUDPC	Area under disease progress curve
BMV	*Brome mosaic virus*
BSMV	*Barley stripe mosaic virus*
BYDV	*Barley yellow dwarf virus*
CAN	Calcium Ammonium Nitrate
CCSV	*Cynodon chlorotic streak virus*
CIMMYT	International Maize and Wheat Improvement Center
CP	Coat protein
CRD	Completely randomized design
CSV	Cocksfoot streak virus
DAC-ELISA	Direct antigen coating Enzyme Linked Immuno-sorbent assay
DAP	Di-ammonium Phosphate
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immuno-sorbent Assay
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany)
FAO	Food and Agricultural Organization
FAOSTAT	Food Agricultural Organization and statistics
GEB	General Extraction Buffer

GGMV	*Guinea grass mosaic virus*
ICIPE	International Centre of Insect Physiology and Ecology
IPPC	Intergovernmental Panel on Climate Change
JGMV	*Johnson grass mosaic virus*
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least significant difference
MCMV	*Maize chlorotic mottle virus*
MCMV-K	*Maize chlorotic mottle virus*-Kansas strain
MCMV-NE	*Maize chlorotic mottle virus*-Nebraska strain
MCMV-P	*Maize chlorotic mottle virus*-Peru strain
MCMV-YN	*Maize chlorotic mottle virus*-Yunnan (China) strain
MMCSV	*Maize mottle/chlorotic stunt virus,*
MDMV	*Maize dwarf mosaic virus*
MDRAT	Multi-Disciplinary Rapid Assessment Team
MESV	*Maize eyespot virus*
MLND	Maize lethal necrosis disease
MSV	*Maize streak virus*
MYSV	*Maize yellow stripe virus*
NGS	Next generation sequencing
NPPOs	The National plant protection organizations
ORFs	Open reading frames
PBS-T	Phosphate buffered saline with tween
PCR	Polymerase chain reaction

PFBV	*Pelargonium flower break virus*
$_{P}$NPP	Para-nitrophenyl-phosphate
PMV	*Panicum mosaic panicovirus*
PVY	*Potato virus Y*
PVX	*Potato virus X*
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RSD	Ratoon stunting disease
RT-PCR	Reverse transcription polymerase chain reaction
SCMV	*Sugarcane mosaic virus*
SCMV-A	*Sugarcane mosaic virus* serotype A.
SCMV-BC	*Sugarcane mosaic virus* serotype BC
SCMV-MDB	*Sugarcane mosaic virus* serotype MDB
SCMV-MB	*Sugarcane mosaic virus* serotype MB.
SCMV-SP	*Sugarcane mosaic virus* -Spain strain
SrMV	*Sorghum mosaic virus*
SSA	Sub-Saharan Africa
+ssRNA	Positive sense single stranded RNA
TCV	*Turnip crinkle carmovirus*
TAS-ELISA	Triple antibody sandwich Enzyme Linked Immuno-sorbent assay
USA	United States of America
WSMV	*Wheat streak mosaic virus*
ZeMV	*Zea mosaic virus*

ABSTRACT

Maize (*Zea mays*) is a key cereal crop in sub-Saharan Africa (SSA). It is the staple food for more than 1.2 billion people in the developing countries. Maize production faces many pests and disease challenges. In 2011, there was an outbreak of a disease in Kenya, Maize lethal necrosis (MLN), caused by a synergistic interaction of two viruses, the *Maize chlorotic mottle virus* (MCMV) and the *Sugarcane mosaic virus* (SCMV). The disease causes up to 100% crop loss and has threatened food security in Kenya. This study aimed at determining how commonly grown genotypes react to infection by the viruses causing the disease and to determine the role of soil and crop debris in disease epidemiology.

Five maize genotypes H614, H513, Duma 43, Kikamba and Kinyanya were used. The genotypes were selected based on the Agro Ecological Zones (AEZ) they are grown and whether it is a hybrid or a landrace genotype. The genotypes were planted in a screenhouse at the University of Nairobi, Upper Kabete campus Field Station and inoculated with MCMV, SCMV, the combination of the two (MCMV+SCMV) and with inoculum from plants with combined viruses (MLN). The plants were assessed for disease incidence and severity and data analysis done using Genstat package version 12. The area under disease progress curve (AUDPC) was used to compare the severity of the different treatments. Serological analysis on the collected samples was done using double antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA). On completion of the experiment, the plant debris were cut into small pieces and mixed with the soil. Half of the bags were left without the debris to assess if there was any significant difference if infested soil alone or with debris enhanced the acquisition of the viruses from the soil. The same soil was used again for a third planting to check if the viruses could still be viable to cause infection.

All the genotypes evaluated developed disease symptoms after viral inoculations, although the landraces developed symptoms earlier than the hybrids. The landraces also had higher disease incidences in earlier weeks and showed more severity. All genotypes had >90% disease incidence with the combination of the two viruses (MCMV+SCMV) recording the highest disease severity of 4.94 on the landrace Kinyanya. Significant differences in both incidence and severity were noted in MCMV+SCMV treatment while the other treatments had no significant differences. The combination also recorded the largest area under disease, with the Elisa results indicating the doubling of the MCMV virus titer in the presence of SCMV while it remained unaffected in single infections.

There was no significant difference when soil had infected debris or only infested soil in the efficiency of transmiting the virus to the the newly planted maize. However, the landraces showed symptoms of the acquired viruses immediately on emergence. Higher disease incidence was reported on the landraces than on hybrids with severity being high also on the landraces. However, no significant difference was noted among the tested maize genotypes. In the third planting to check if any plants acquire the viruses; disease incidence was low with the highest being 36.1% for SCMV on Kikamba and lowest at 0% for Duma 43 in MLN treatment. All the genotypes tested were infected by MLN disease and its causal viruses with the landraces being the worst affected. The genotypes also acquired the viruses from the soil with the landraces showing symptoms immediately on emergence. From the above findings, use of certified seeds, crop rotation, and field hygiene including removal and burning of infected plant should be practiced to avoid repeated infections of maize plants and buildup of viral inoculum.

CHAPTER ONE

1.0 Introduction

1.1 Background information to the Maize lethal necrosis disease

Maize (*Zea mays* L., corn) belongs to the family Poaceae and is ranked the second largest crop in the world after wheat. The annual yield of maize was recorded at 1.038 billion tons in 2014 (FAOSTAT, 2017). Of this amount, Africa recorded a yield of 78 Million tons with East Africa contributing 30.7 Million tons and Kenya recording a total of 3.5 million tons (FAOSTAT, 2017). In Kenya, the area under maize production in 2014 was 2,116,141 ha.

Maize is the main food crop in Kenya where and forms a major component of meals in form of either porridge, *ugali or githeri* or and indirectly as animal feed (Khan *et al*., 2001; De Groote, 2002; Owino, 2009). It is also used in industry for starch, ethanol production, oil extraction and its products are used in the food manufacturing industry (Tsaftaris, 1995). The daily calories for the Kenyan population are accounted for by this crop at an estimate of about 40% (Kibaara, 2005). The largest production area is in Trans Nzoia County although the crop is grown in other areas in the country (Mwangi *et al.,* 2002; Owino, 2009). The other areas with high production include Wareng, Eldoret East and West sub counties of Uasin Gishu and Nakuru Counties. These areas are often referred to as the 'Granary of Kenya' because of the maize surplus they produce. Due to its wide use, maize dominates all national food security considerations and contributes highly to agricultural employment (Nyoro, 2002; Jayne *et al.,* 2005).

Maize production levels in Kenya have been declining since 2006 (Ong'amo *et al.,* 2006). The main reasons for this include, drought, high input costs, low soil fertility and attack from pests,

weeds and diseases (Ong'amo *et al.,* 2006). The above constrains account for 15-40% and 20-100% yield losses in the East and South African regions, respectively. For the stored grains, the larger grain borer (*Prostephanus truncatus*), maize weevils, Angoumois grain moth (*Sitotroga cerealella)* and rodents are the main problem. Principally, 30% yield losses occur due to stem borers (Kfir*et al.,* 2002), weeds especially striga (Khan *et al.,* 2002) and *Maize streak virus* (Martin *et al.,* 2001).

In the African continent, other viruses infecting maize include; *Guinea grass mosaic virus (potyvirus)* (GGMV), *Maize mottle/chlorotic stunt virus* (MMCSV), *Maize eyespot virus (*MESV), *Brome mosaic virus (bromoviridae)* (BMV), *Cynodon chlorotic streak virus (rhabdoviridae)* (CCSV), *Barley stripe mosaic virus (hordeivirus)* (BSMV), *Maize yellow stripe virus (tenuivirus)* (MYSV) *and Barley yellow dwarf virus* (*luteovirus)* (BYDV) (Thottappilly *et al*., 1993).

In 2011, a strange disease that caused 100% crop loss in early infected fields was reported in the 'granary of Kenya' regions (Wangai *et al*., 2012). The disease was confirmed to be caused by two viruses, the *Maize chlorotic mottle virus* and the *Sugarcane mosaic virus* acting in a synergistic manner (Wangai *et al*., 2012; Adams *et al*., 2013). Since the first report of the disease, it has become a major setback to maize production in Kenya and the East African region (Wangai *et al*., 2012; Adams *et al*., 2014; Mahuku *et al*., 2015; Lukanda *et al*., 2017).

1.2 Problem statement

Since the outbreak of the Maize lethal necrosis (MLN) disease in 2011, the disease has continued to spread all over Kenya and in the East African region (Wangai *et al*., 2012, Adams *et al*., 2014; Mahuku *et al*., 2015; Lukanda *et al*., 2017). The disease had affected over 26,000 ha of maize by early 2012, amounting to US$52m in losses in Kenya alone (MDRAT, 2012). Eight of the major growing areas had been affected and quarantined in Tanzania while in Uganda, eight districts had been affected (IPPC, 2014).

The outbreak of the Maize lethal necrosis disease especially in Kenya's 'grain basket' areas is a serious threat to food security in the country. Any shortages in maize production will have major implications on the economy, since the government will use huge sums of the country's foreign exchange to import maize to bridge the production gap. To effectively manage this disease, the effects of different viruses and viral interactions causing this disease on commonly planted genotypes needs to be determined. Additionally, the survival mechanisms of viruses causing MLN disease both in the soil and the plant debris need to be understood to come up with viable management strategies.

1.3 Justification

In East Africa, the reaction of the different genotypes (commercial and landraces) to single and co-infections of the viruses causing the MLN disease have not been fully evaluated. The evaluation will go a long way in identifying the tolerant genotypes which will in turn help in the identification of the responsible genes for resistance to the viruses in these lines. Several studies done in Europe and Asia identified two major quantitative trait loci (QTLs) responsible for SCMV resistance in the maize (Xia *et al*., 1999). Similar studies need to be done for the East

African maize genotypes and this will be guided by the disease reaction experiments of different maize genotypes grown in the region. Das *et al*. (2015) noted that maize variety H614D was more tolerant to MLN disease compared to the landraces and other commonly used genotypes. This study, therefore, is aimed at evaluating the reaction of different maize genotypes to MLN disease and to the viruses causing the disease.

Elsewhere, repeated outbreaks of MLN disease have been attributed to the MCMV virus surviving in the soil and hence causing disease outbreaks season after season (Uyemoto and Claflin, 1981; Phillips *et al*., 1982; Uyemoto, 1983). The spread was however enhanced by the presence of the corn rootworms and the spread by the corn thrips (Uyemoto, 1983; Jiang *et al*., 1990; Jiang *et al*., 1992; Cabanas *et al*., 2013). The viruses can lie dormant and survive in maize residues when maize is off season (Uyemoto, 1983; Montenegro and Castillo, 1996). In similar studies, crop rotation was proved to be an efficient way of managing the MLN disease and its causal viruses (Phillips *et al*., 1982; Uyemoto, 1983). Non-inoculated sorghum plants became infected with the SCMV when grown in containers with SCMV infected plants indicating the possibility of soil transmission for the virus (Bond and Pirone, 1970). In Kenya, there have been repeated outbreaks of the disease in maize since the first outbreak, similar to what was observed in the USA.

The role of the soil and plant debris in the spread and survival of the viruses causing MLN in the tropics has not been fully studied. Understanding their role is critical in making management decisions to avert recurrences. This study is thus motivated by these gaps in screening maize genotypes for resistance/tolerance against the two viruses and evaluating the role of soil and plant debris in MLN epidemiology. The results will greatly contribute to devising proper management practices for the disease.

1.4 Objectives

1.4.1 Broad objective

The main aim of the study is to contribute towards management of maize lethal necrosis (MLN) disease through understanding the interaction of the viruses causing the disease in different maize genotypes and their transmission from soil and plant debris.

1.4.2 Specific objectives

1. To determine the reaction of different maize genotypes to maize lethal necrosis disease and its causal viruses.
2. To determine the possibility of transmission of viruses causing maize lethal necrosis disease from crop debris and soil.

1.4.3 Null hypothesis

1. The reaction of the different maize genotypes to the two viruses causing maize lethal necrosis disease is the same.
2. There is no transmission of the viruses causing the maize lethal necrosis disease from plant debris and soil.

CHAPTER TWO

2.0 Literature review

2.1 Origin, classification and morphology of maize

Maize is a crop in the grass family of plants. Its in the family Poaceae, subfamily Panicoideae and the tribe Andropogoneae, genus *Zea* and species *Z. mays* (OECD, 2003; USDA, 2005). It's one of the oldest cultivated grains (Paliwal *et al*., 2000). Maize originated from the Mesoamerican region especially in the Mexican highlands (Watson and Dallwitz, 1992) from where it spread rapidly all over the world. Its domestication is estimated to date 6000 years back (Piperno and Flanner, 2001; Matsouka *et al*., 2002).

The plant is a monocot grass which is tall usually 1-4 m high and has a single erect stem. The plant has nodes and internodes bearing broad single leaves on each node alternating at opposite sides. The plant has seasonal adventitious roots and the brace roots. Maize is a monoecious plant and has the shanks develop from the leaf axis. These terminate into female inflorescence called the ear while the tassel (male inflorescence) develop at the top of the plant (Paliwal *et al*., 2000). Maize fruit is caryopsis. They are called either grain, seed or kernel. The seed is made up of the embryo, endosperm and the fruit wall (Paliwal *et al*., 2000;). The crop's main vegetative growth stages can be classified as; stage 1 forms the germination and emergency; stage 2 is the early vegetative stage while stage 3 is the late vegetative stage. Maize has a C4 photosynthetic pathway (Hanway, 1963; Purseglove, 1972; Colless, 1992; Paliwal *et al*., 2000).

2.2 Types and uses of maize

Maize is a highly adaptable crop which can do well in many climatic zones of different latitudes (58^oN -40^oS) and altitudes ranging from sea level up to 3000m high and rainfall as low as 250mm and above 5000mm (Shaw, 1988; Dowswell *et al.,* 1996). The different maize types include: flint and flourly maize which are mainly used for food, dent maize which is used for silage and grain, waxy maize which is mainly an industrial crop with a high amylopectin starch content, pop maize which is mainly used as a snack and sweet maize which is usually consumed as food while green (Purseglove, 1972; Paliwal *et al*., 2000; Darrah *et al*., 2003). Uses of maize vary from region to region. In the developed countries, it's used mainly for animal feed and as an industrial raw material while in the developing countries, it's the main staple food (Galinat, 1988; Shaw, 1988; Morris, 1998).

2.3 Constraints to maize production

Maize production is affected by many factors including both abiotic and biotic factors. Drought forms the major abiotic factor affecting production especially in the tropics. Most production systems are rain-fed and water is always a limitation leading to plant death (Meisey and Edmeades, 1998). The second factor is the poor soil fertility. This is especially affected by the repeated artificial fertilizer use which leads to acidity hence limiting maize production (Morris *et al*., 2007). The last major constraint in this category is the low adoption of improved maize genotypes hence leading to poor production (Shiferaw *et al*., 2011).

Different pests and diseases reduce maize production drastically (Shiferaw *et al*., 2011). The plant parasitic weed, striga (Martin *et al.,* 2001; Khan *et al.,* 2002) is a major problem of the African region. Insect pests cause direct damage to the plants by feeding on the different parts of the plant. Root pests include seed corn maggots, wireworms, rootworms and white grubs

(Ortega, 1987). The above-ground pests include spider mites, aphids, thrips, grasshoppers, stem borers, termites, ear worms, adult rootworms and army worms (Ortega, 1987). The stem borers can cause up to 30% yield loss (Kfir *et al.,* 2002). On mature grain either in the field or storage, the grain weevils and grain borers are the major problems (Tefera, 2012). Of all the pests listed, the stem borer (*Chilo partellus* and *Busseola fusca*) causes the most devastating yield losses of the crop (De Groote, 2002). The post-harvest pests including the angoumois grain moth (*Sitotroga cereallela*), the larger grain borer (*Prostephanus truncatus*), the grain weevils (*Sitophilus zeamais*) and lesser grain weevil (*Sitophilus oryzae*) together, they can lead to yield losses of between 20-30% of the stored grain (Markham *et al*., 1994; Abebe *et al*., 2009; Yuya *et al*., 2009).

Fungal diseases of economic importance globally include Southern corn leaf blight (*Bipolaris maydis*), Northern corn leaf blight (*Exserohilum turcicum*), Common rust (*Puccinia sorghi*), Southern rust (*Puccinia polysora*), Gray leaf spot (*Cercospora* species), Kernel, Stalk and ear rots (*Diplodia* spp., *Fusarium* spp. *and Aspergillus* spp.) (Macdonald and Chapman, 1997; Kedera *et al.,* 1999; Shiferaw*et al*., 2011). The *Fusarium* spp. and *Aspergillus* spp. causing kernel and ear rots lead to food contamination through mycotoxin production hence reducing the quality and safety of the seed (Njuguna *et al*., 1990; Macdonald and Chapman, 1997).

Diseases caused by viruses include Maize streak disease (*Maize streak virus* (MSV)) (Fajemisin and Shoyinka, 1976) and Maize lethal necrosis (MLN) disease, occurring due to a synergistic interaction of two viruses, the *Maize chlorotic mottle virus* (MCMV) and the *Sugarcane mosaic virus* (SCMV) (Niblett and Claflin, 1978; Wangai *et al*., 2012; Adams *et al*., 2014). Other viral diseases include, Maize dwarf mosaic disease whose causal agent is the *Maize dwarf mosaic*

virus (MDMV) and maize stripe disease caused by the *Maize stripe virus* (MStpV) (Kulkarni, 1973), Guinea grass mosaic disease caused by *Guinea grass mosaic virus* (GGMV) and Maize eyespot virus disease caused by *Maize eyespot virus (*MESV) (Fauquet and Thouvenel, 1987), Maize mottle/ chlorotic stunt virus disease caused by *Maize mottle/ chlorotic stunt virus* (MMCSV) (Rossel and Thottappilly, 1983; Rossel, 1984) and Maize mosaic virus disease caused by *Maize mosaic virus* (MMV) (Kulkarni, 1973).

2.4 Maize lethal necrosis disease

Maize lethal necrosis (MLN) disease was originally called the Corn lethal necrosis (CLN) disease (Niblett and Claflin, 1978; Uyemoto *et al*., 1981; Goldberg and Brakke, 1987; Scheets, 1998) and was first reported in Kansas in 1976 (Niblett and Claflin, 1978). The disease comes about as a co-infection between the *Maize chlorotic mottle virus* (MCMV), a Machlomovirus and any of the gramineae infecting viruses in the genus *potyvirus* or the *Wheat streak mosaic virus* (WSMV) genus rymovirus (Niblett and Claflin, 1978; Uyemoto *et al*., 1981). The potyviruses involved include *Maize dwarf mosaic virus* (MDMV) (Niblett and Claflin, 1978) and the *Sugarcane mosaic virus* (SCMV) (Uyemoto *et al*., 1980; Goldberg and Brakke, 1987). The co-infections are usually very severe as compared to the single viral infections (Scheets, 1998).

2.4.1 History and distribution of the Maize lethal necrosis disease in the world

Corn lethal necrosis disease was first reported in Kansas USA in 1976 by Niblet and Claflin (1978). The outbreaks were mainly in North Central Kansas (Uyemoto *et al*., 1980) and South Central of Nebraska (Doupnik and Wysong, 1979). The disease is caused by the co-infection of the MCMV and the WSMV **(**Niblett and Claflin, 1978; Uyemoto and Claflin, 1981). The disease has since been reported in Peru (Castillo, 1977; Uyemoto, 1983) and Hawaii US (Kauai) (Jiang *et al*., 1992; Nelson *et al*., 2011). Other areas where the disease has been reported include Brazil

and Texas (Uyemoto, 1983), Argentina (Gordon *et al*., 1984), China (Xie *et al*., 2011) and East Africa (Wangai *et al*., 2012; Adams *et al*., 2014; Lukanda *et al*., 2017). In China and East Africa, the disease is caused by the co-infection of the MCMV and the SCMV (Wangai *et al*., 2012; Xie *et al*., 2011; Adams *et al*., 2013).

In Kenya, the disease was first reported in 2011 in the Rift valley region (Wangai *et al*., 2012; Adams *et al*., 2013). The disease led to severe leaf chlorosis, mottling and necrosis of the affected plants (Wangai *et al*., 2012). The disease has since been reported in the Northern and Lake Zones of Tanzania (Makumbi and Wangai, 2013), Uganda in the Busia region (ASARECA, 2013), Rwanda in the Northern and later in the Western provinces (ASARECA, 2013; Adams *et al*., 2014), the Democratic Republic of Congo (Lukanda *et al*., 2017), South Sudan (ASARECA, 2013) and Ethiopia (Mahuku *et al*., 2015).

2.4.2 Maize lethal necrosis disease development and symptoms

The symptoms displayed by MLN disease depend on the stage of the crop affected. The infected plants develop intense chlorosis from the base of young whorl leaves upward to the leaf tips (Plate 2.1) (Niblett and Claflin, 1978; Goldberg and Brakke, 1987; Scheets, 1998, Wangai *et al*., 2012) As the disease progresses, the leaves become extremely chlorotic and necrosis sets in starting from the leaf tips and edges progressing inwards (Goldberg and Brakke, 1987; Scheets 1998). This leads to a "dead heart" symptom (Wangai *et al*., 2012). Early infections lead to complete plant death (Plate 2.2A) (Goldberg and Brakke, 1987) while late infections lead to plants aging prematurely (Gordon *et al*., 1984), male sterility and malformed or no ears or production of deformed seed (Uyemoto *et al*., 1981; Gordon *et al*., 1984), no grain fill and cob rotting (Plate 2.1) (Wangai *et al*., 2012) and general stuntedness (Plate 2.2B) (Goldberg and Brakke,1987).

Environmental factors also play a key role in the development of the disease. High temperatures favor the development of the MCMV and hence the development of the MLN disease (Scheets, 1998). At high temperatures, disease symptoms develop early and disease severity is aggravated (Scheets, 1998). Serological analysis of the infected plants reveal that the concentration of MCMV is higher in plants infected with both MCMV and SCMV than in singly infected plants. MCMV titer concentration ranges from 3-11 times higher in coinfections as compared to single MCMV infection (Goldberg and Brakke, 1987; Scheets, 1998).

Plate 2.1 Maize plants expressing different symptoms due to infection by the MLN disease, A: Severe chlorosis and setting in of necrosis from leaf margins, B: Disease progress leading to 'dead heart' symptom, C: Poor seed set in late infections and D: Premature dryng of the husks and poor grain fill.

Plate 2.2 The severe symptoms on maize crop affected by the MLN disease and the symptoms on the single and co- infected plants, A: Complete death of crop stand B: Maize infected with SCMV (left), MCMV (middle) and synergistic effect of mixed infection of MCMV and SCMV (Right)(Adopted from DSMZ Plant Virus Department).

2.4.3 Losses associated with the Maize lethal necrosis disease

Losses due to MLN disease depend on the stage of infection, plant variety, the strain of the viruses involved and the prevailing environmental conditions (Uyemoto *et al*., 1980). In Eastern Uganda, 31-70% yield losses have been reported (Kagoda *et al*., 2016). Huge losses of between 50-90% have been reported in North central Kansas and South Central Nebraska in maize crop in both natural and artificial infections (Niblett and Claflin, 1978; Uyemoto *et al*., 1980). Farmers in Kenya lost their entire crop due to the disease (Wangai *et al*., 2012). All maize stages are vulnerable to infection but the seedling stage is most susceptible and infection at this stage can lead to complete crop loss (Goldberg and Brakke, 1987; Wangai *et al*., 2012). For early control of the disease, planting seed has to be treated with both a fungicide and an insecticide (Redinbaugh and Zambrano-Mendoza, 2014; Kagoda *et al.,* 2016). As a consequence, the cost of production of certified seed maize goes up. Subsequently, this cost is passed on to the farmers (Kiruwa *et al.,* 2016) thus reducing access of the certified seed to the farmer and hence low adoption.

2.5 Viruses causing Maize lethal necrosis disease

2.5.1 *Maize chlorotic mottle virus*

The virus is the only established Machlomovirus, in the family *Tombusviridae* infecting the *Gramineae* group of plants (Falquet *et al*., 2005; King *et al.*, 2011). It shares some similarity with the *Carnation mottle carmovirus* (CarMV) and *Turnip crinkle carmovirus* (TCV) members of the genus *Carmovirus* (Nutter *et al.*, 1989).

2.5.1.1 Genome organization and composition of *Maize chlorotic mottle virus*

The virus has an icosahedral/isometric positive sense single stranded RNA particle (+ssRNA) (Plate 2.3). It has a genome of 4437 nucleotides (nt) and is about 28-34nm in diameter (Nutter *et al*., 1989; Lommel *et al*., 1991; Stenger and French 2008; Xie *et al*., 2011). It has a smooth coat protein of 25kDa which is usually articulated from a subgenomic RNA (sgRNA) and is spherical or hexagonal in shape (Plate 2.3) (Lommel *et al*., 1991; Scheets, 2010). The genome is composed of six open reading frames (ORF) (Plate 2.4), five of these are needed for replication and virus spread within the plant (Scheets, 2004). The reading frames are usually overlapping (Plate 2.4) (Scheets, 2004). The virus particles are found mainly in the cytoplasm and vacuoles of the infected plant cells (Xie *et al*., 2011).

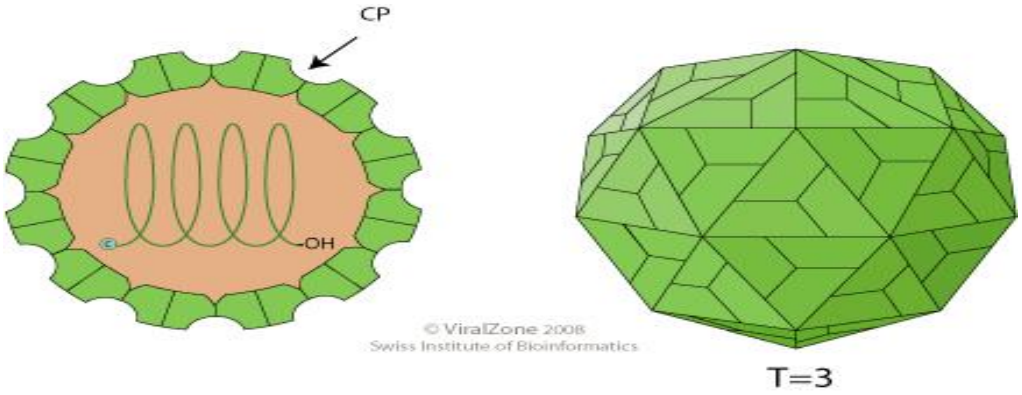

Plate 2.3: The spherical icosahedral *Maize chlorotic mottle virus* particle with capsid with a T=3 icosahedral symmetry.
Source: **http://viralzone.expasy.org**

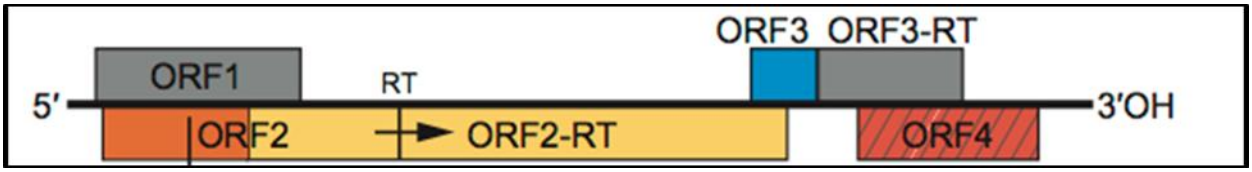

Plate 2.4 *Maize chlorotic mottle virus* genome showing the 6 Open reading frames (ORFs)
Source**:** **https://talk.ictvonline.org**

2.5.1.2 World distribution of *Maize chlorotic mottle virus*

The virus is endemic in Peru where it was first reported (Castillo and Hebert 1974; Nault *et al*., 1979), North central Kansas, South central Nebraska especially in fields in Small river valleys and irrigation districts and has also been identified in the high plains of Texas (Kessler,1979). It is widely spread in the North and South America, Brazil, Mexico (Gordon *et al*., 1984), and the United States (Niblett and Claflin, 1978; Uyemoto *et al*., 1980; Doupnik, 1979; Jiang *et al*., 1992). The virus is present in Asia , Thailand (Scheets, 2008) and China (Xie *et al*., 2011). It is currently widespread in the East African region especially Kenya, Uganda, Tanzania, Rwanda and DRC (Adams *et al*., 2014; Mahuku *et al*., 2015; Lukanda *etal*., 2017) since its first report in 2012 in Kenya (Wangai *et al*., 2012).

2.5.1.3 Strains of *Maize chlorotic mottle virus*

There are several strains of the MCMV and these are quite distinct. MCMV-P and MCMV-K are isolates from Peru and Kansas, respectively (Uyemoto, 1983) while MCMV-NE is the isolate from Nebraska (Stenger and French, 2008) with MCMV-YN being the Chinese isolate from Yunnan (Xie *et al.,* 2011). The US isolates (K and NE) share 99.5% Nucleotide (nt) sequence identity, a clear indication the two isolates are related (Nutter *et al*., 1989; Stenger and French, 2008) while the MCMV-Hi (Kauai isolate) sequence identity has 98% nucleotide similarity to the Nebraska and Kansas strains, indicating both have a very recent common ancestor (Cabanas *et al*., 2013). The isolates from Peru and US are quite distinct as they are separated both genetically and geographically from each other (Nyvall, 1999). The Yunnan isolate (MCMV-YN) shares nucleotide sequence identity with MCMV-NE and MCMV-K of 97.3 % and 97.1%, respectively (Xie *et al*., 2011) while the Thailand and China isolates had 98% - 99.6% identity indicating they evolved from a common ancestor (Stenger and French, 2008).

Results from East Africa indicate that the Kenyan isolate is more than 96% similar to the Yunnan isolate from china (Xie *et al*., 2011; Adams *et al*., 2013) but it was different from the US strains (Scheets, 2000; Stenger and French, 2008; Xie *et al*., 2011; Wangai *et al*., 2012; Adams *et al*., 2013). On the other hand, the Rwandan isolate had 99% homology to the Kenyan and Chinese isolates with 96-97% homology to the US (NE and K) isolates (Adams *et al*., 2014). Both the Kenyan and Rwandan isolates share a common origin (Adams *et al*., 2014). Partial coat protein sequences of isolates from Tanzania and Democratic Republic of Congo showed 99% identity to the Kenyan and Rwandan isolates (Mahuku *et al*., 2015). This is a clear indication that the EA and some Chinese isolates share a common origin (Mahuku *et al*., 2015).

Recently, the virus was isolated from Johnson grass and the 519 bp segment sequence had a high identity with the American isolates while the 950 bp amplicon had a high identity with the African isolates (Achon *et al*., 2016).

2.5.1.4 Losses due to the *Maize chlorotic mottle virus* infection

Yield loss due to MCMV is dependent on the maize variety, production season, infection age of the plants, prevailing environmental conditions and if it's single or co-infections (Wangai *et al*., 2012). In single infections, losses of up to 10-15% have been reported in the field while in experiments, higher losses have been recorded (Castillo and Hebert, 1974; Castillo, 1977; Uyemoto *et al*., 1981). However, in combination with any of the potyviruses infecting maize, there is severe disease development leading up to 91% crop losses in natural infections (Niblet and Claflin, 1978).

2.5.1.5 Symptoms caused by *Maize chlorotic mottle virus*

In maize, various symptoms have been reported. These are mainly influenced by the crop genotype, age of plant at infection and environmental conditions (Wangai *et al*., 2012). These can be from mild chlorotic mottle to severe mottling and stunting of the affected plants; chlorotic stripes which end up as chlorotic blotches as they coalesce also occur in the affected plant leaves (Castillo and Hebert, 1974; Castillo, 1977; Niblett and Claflin, 1978; Uyemoto *et al.,* 1981). In severe infections, especially in elevated temperatures and susceptible genotypes; leaf necrosis occur leading to premature plant death. The virus also leads to malformed and partially filled ears with shortened male inflorescences and few spikes (Castillo and Hebert, 1974; Castillo, 1977; Niblett and Claflin, 1978; Uyemoto *et al.,* 1981).

2.5.1.6 Host plants for *Maize chlorotic mottle virus*

MCMV was initially thought to be restricted to the poaceae family (Gordon *et al*., 1984) but has since been reported in *Cyperus rotundus* which belongs to the *Cyperaceae* family (Kusia, 2014). Maize (*Zea mays)* is a natural host of the virus (Scheets, 2004). The virus has been reported in finger millet (*Eleusine coracana*) and sugarcane (*Saccharum officinarum)* (Kusia *et al*., 2015; Wang *et al*., 2016). Other hosts are mainly in the gramineae family including fox tail millet (*Setaria italical*), barley (*Hordeum vulgare* L), wheat (*Triticum aestivum* L) and proso millet (*Panicum milliearceum* L) which are systemic hosts (Castillo and Hebert, 1974; Niblett and Claflin, 1978; Bockelman *et al*., 1982; Brunt *et al*., 1996). These are among the nineteen grass species reported as systemic hosts to the MCMV-K while 15 species others were systemic hosts to the MCMV-P. The virus has recently been isolated from the *Sorghum halepense* (Johnson grass) (Achon *et al*., 2016).

2.6.1.7 Transmission of *Maize chlorotic mottle virus*

The virus is transmitted by different means which include seed transmission, vectors and through mechanical means. Low rates of MCMV transmission have been reported in seed. Transmissions rates of 0.03-0.33% have been reported when seed from infected plants are used for planting (Jensen *et al*., 1991). The virus concentration in the seeds is quite low (Jensen *et al*., 1991; Johansen *et al*., 1994). Studies carried out in East Africa indicated that seeds harvested from MCMV infected fields had 72% of them being positive to MCMV using RT-PCR. This is a clear indication of seed contamination, which might lead to some level of seed transmission (Mahuku *et al.,* 2015). Samples of commercial seed lots showed 46% infection (Mahuku *et al.,* 2015). These infection rates can lead to epidemics since the disease can easily be spread by the vectors from the few infected plants acting as focal points (Nault *et al.,* 1978; Jensen *et al.,* 1991; Delgadillo-Sanchez *et al.,* 1994).

The virus is reported to be vectored by six species of Chryosmelid beetles in a semi-persistent manner. The species involved are; the northern corn rootworm *Diabrotica barberi*, the southern corn rootworm *Diabrotica undecimpunctata* howardi, the western corn rootworm *Diabrotica virgifera*, the flea beetle *Systena frontalis*, the corn flea beetle *Chaetocnema pulicaria*, the cereal leaf beetle *Oulema melanopus* and *Diabrotica viridula* (F.) (Nault *et al*., 1978; Castillo, 1983; Gordon *et al*., 1984; Jensen, 1985; Jensen *et al*., 1991).

Thrips have also been reported to transmit MCMV. *Frankliniella williamsi* Hood (Corn thrips) has been associated with MCMV transmission in a semi- persistent manner (Jiang *et al*., 1990; Jiang *et al.,* 1992; Cabanas *et al*., 2013). Both the adults and the larvae of thrips have same transmission efficiency (Nault *et al*., 1978; Cabanas *et al*., 2013).

MCMV is capable of surviving in the soil and in the plant debris (Uyemoto and Claflin, 1981; Phillips *et al*., 1982; Uyemoto, 1983). MCMV disease prevalence has been shown in plots that had corn year after year while plots that practiced crop rotation had very low or no disease (Phillips *et al*., 1982; Uyemoto, 1983). Mechanical transmission of the virus from plant debris has also been demonstrated (Uyemoto *et al*., 1980; Jensen, 1985) and plant residues collected from the field had infective virus (Uyemoto, 1983).

Studies conducted in East Africa indicated that when seeds produced in an MCMV non endemic country were planted in contaminated soil in an endemic country, 70% of the emerging seedlings were positive of the virus (Mahuku *et al*., 2015). This is a clear indication that the disease survives in plant debris and soil.

The MCMV is found in all maize parts (sheath, anther, leaf, stem, roots, cob, husks, silk and kernel) including the seed with 13-30% moisture content. This was confirmed through infectivity and Elisa tests (Jiang *et al*., 1992; Scheets, 2004).

2.6.2 The *Sugarcane mosaic virus*

Sugarcane mosaic virus is a member of viruses in the family *Potyviridae* and genus *Potyvirus* (Mattheus, 1982; Pirone, 1972; Teakle *et al*., 1989; Wu *et al*., 2013; Zhu *et al*., 2014). The virus (SCMV) was formerly known as the *Maize dwarf mosaic virus* strain B (Uyemoto *et al.*, 1980; Goldenberg and Brakke, 1987). It causes mosaic disease in maize, sugarcane, sorghum and other Poaceous plants (Pirone, 1972; Persley *et al*., 1985; Teakle *et al*., 1989).

The virus is part of the *Sugarcane mosaic virus* complex comprising the following viruses; the *Johnson grass mosaic virus* (JGMV), the *Maize dwarf mosaic virus* (MDMV), the *Sorghum*

mosaic virus (SrMV) and the *Sugarcane mosaic virus* (SCMV) (Shukla *et al*., 1989; McKern *et al*., 1991; Shukla *et al*., 1994), *Zea mosaic virus* (ZeMV) (Seifers *et al*., 2000), *Sugarcane streak mosaic virus* (SCSMV), *Pennisetum mosaic virus* (PeMV) (Fan *et al*., 2003) and *Cocksfoot streak virus* (CSV) (Gotz and Maiss, 2002).

SCMV is predominant over MDMV on maize crops in Central European countries (Oertel *et al*., 1997; Pokorny and Porubova 2000), while the latter is the predominant potyvirus in the Mediterranean region (Signoret and Alliot, 1995; Achon *et al*., 1995; Ivanovic *et al.*, 1995).

2.6.2.1 History and geographical distribution of *Sugarcane mosaic virus*

The virus is widespread in the world and is more where sugarcane is grown (Gillaspie and Mock, 1987). It was first observed in Puerto Rico in 1916 (Brandes, 1919) and is one of the key viruses of maize in European countries (Huth and Lesemann, 1991; Krstic and Tosic, 1995; Pokorny and Porubova, 2001). It has been reported in Poland (Trzmiel and Jeżewska, 2008; Trzmiel, 2009), Brazil (Goncalves *et al*., 2012) in maize, China (Xu *et al*., 2008; Wu *et al*., 2012) and Argentina (Perera *et al*., 2009) in sugarcane.

In East Africa, the virus was first reported in sugarcane and maize in 1973 (Kulkarni, 1973). Currently, the disease is endemic both in Kenya and South Africa (Louie, 1980; Handley *et al*, 1998). Overall, the disease has affected over 25 countries (Koike and Gillaspie, 1989; Wu *et al.*, 2012). The virus has been the main cause of the Maize lethal necrosis disease in the East African countries in co-infection with the MCMV (Wangai *et al*., 2012; Adams *et al*., 2013; Adams *et al*., 2014; Lukanda *et al*., 2017).

2.6.2.2 Structure and genome organization of the *Sugarcane mosaic virus*

The SCMV particle is composed of a positive sense, single stranded RNA (+ssRNA) genome (Plate 2.5). It is approximately 10kb long (Wu *et al.,* 2012). The particle has a large ORF, and two untranslated regions (UTRs); the 5'terminus (5`UTR) which is covalently linked to a virus genome-linked protein while at its 3' terminus (3`UTR) it is linked to polyadenylated tail (poly A) (Plate 2.5) (Wu *et al*., 2012; Gell *et al*., 2015). The ORF has 10 functional proteins which include; protein 1 (P1), protein 3 (P3), 6K1, 6K2, CI (cylindrical inclusion protein), CP (coat protein), HC-Pro (helper component proteinase), NIa-Pro (major protease of the small nuclear inclusion protein, NIa), NIb (large nuclear inclusion protein) and VPg (viral protein genome linked) (Plate 2.5) (Shukla *et al*., 1988; Padhi and Ramu, 2011). Additionally, there are PIPO protein (ORF2) (Chung *et al*., 2008; Wei *et al*., 2010). The members of the SCMV group have a conserved DAG motif at the N- terminal region of the CP. This is responsible for aphid transmission in potyviruses (Chen *et al*., 2002).

The SCMV particles are filamentous and flexuous measuring 700-750nm by 11nm length and width respectively (Plate 2.6) (Harrison *et al*., 1971; Adams *et al*., 2005). The suspending media affects the length of the particles (Govier and Woods, 1971). They are longer and straight in Ca^{2+} or Mg^{2+} presence while they are shorter and more flexious with ethylene diamine tetra acetic acid. In infected cells, the SCMV induces cylindrical pinwheel inclusions (Edwardson and Christie, 1978; Lesemann *et al*., 1992).

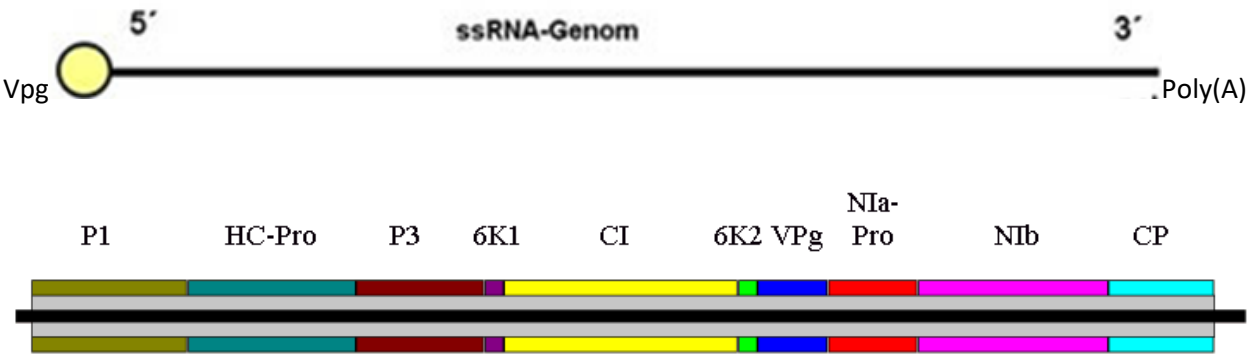

Plate 2.5 The linear, (+) sense ssRNA potyvirus genome showing the 10 functional proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, Nib and CP). The 5' terminus has a genome linked protein (Vpg) and 3'terminus has a poly (A) tail.

Source: http://www.dpvweb.net/potycleavage/

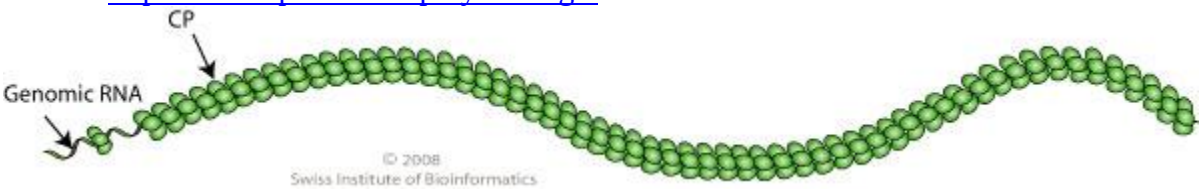

Plate 2.6 Filamentous flexuous particles characteristic of the Potyviridae group of viruses (*Sugarcane mosaic virus)* with genomic RNA and coat protein (CP)

Source: **http://viralzone.expasy.org**

2.6.2.3 The *Sugarcane mosaic virus* strains

Most of the time, strains of individual viruses reveal sequence homologies of greater than 90% (Shukla and Ward, 1988). It's important to note that relationships between different strains of the SCMV is more related to the host plants of isolation than the geographical origin of the virus (Achon *et al*., 2007). Some strains of SCMV include; SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-SC, SCMV-BC, SCMV-Sabi, SCMV-Brisbane, SCMV-Bundaberg, SCMV-Isis and MDMV-B (Shukla *et al.*, 1989; Gough and Shukla, 1981; Oertel *et al*., 1997; Shukla *et al*., 1987). The Kenyan isolate is closely related to the Chinese isolate at 98-99% sequence

homology (Xie *et al*., 2011; Adams *et al*., 2013) while the Rwandan isolate is closer to the US isolate at 95% sequence identity (Adams *et al*., 2014).

2.6.2.4 Host range of *Sugarcane mosaic virus*

The host plants for SCMV are mainly the poaceous species (Brandes, 1920; Pirone, 1972; Teakle *et al.*, 1989). Sugarcane, sorghum and maize are natural hosts for the virus (Teakle and Grylls, 1973; Scheets, 2004; Koike and Gillaspie, 1989; Toler and Bockholt, 1969). *Sorghum verticilliflorum* (wild sorghum) and *Brachiaria piligera* (hairy arm grass) have been reported to be natural hosts (Srisink *et al.*, 1993). Generally, any weed in the grass family growing near sugarcane infected with the virus will be infected eventually through the vectors (Koike and Gillaspie, 1989). Experimentally, the virus affects most *gramineae* species (Abbott and Tippett, 1964; Ford and Tosic, 1972; Penrose, 1974).

2.6.2.5 Symptoms of *Sugarcane mosaic virus*

The disease symptoms are diverse and depend on crop variety, growing conditions especially the prevailing temperatures, strains of virus involved and the plant stage at infection (Perera *et al.*, 2012). Usually young or rapidly growing plants are more prone to the virus than older or slow growing plants (Brandes, 1920; Comstock and Lentini, 2005). In sorghum, genotype and temperature influence the red leaf reaction (Penrose, 1974). The sorghum genotype determines the mosaic and red stripe reactions while temperatures control the red leaf reaction (Seifers and Hackerott, 1987). In maize, symptoms of infection appear first in younger leaves. They include mosaics or mottles with uneven shades of light or dark green which lead to narrow, yellowish or light green streaks (different with the maize streak) along the veins (Wu *et al*., 2012). The mosaic usually fades as the plant matures and in high temperatures. With early infections, there is poor seed set and bushiness of the plants with general stunting (Wu *et al*., 2012).

On sugarcane, pale green or yellow chlorotic stripes are visible on the leaves usually with a dark green background while on the stems, white stripes are seen (Perera *et al*., 2012; Balarabe *et al.*, 2014). The symptoms are more conspicuous on the leaf base and sheath (Comstock and Lentini, 2005). Symptoms disappear as the plant ages (Abbott, 1961; Bailey and Fox, 1980). Latent infections in sugarcane lead to slowed plant growth with production of small size cane (Gillaspie, 1967; Kulkarni, 1973; Waterworth and Hadidi, 1998). This kind of infection poses a threat to the sugarcane industry (Rybicki and Pietersen, 1999).

In sorghum, the virus leads to the development of a yellow to dark green mosaic on the leaves (Seifers and Hackerott, 1987). In advanced stages this turns into a necrotic red leaf reaction, on some genotypes when the temperatures fall below 21^{o}C (Toler, 1968; Henzel *et al*., 1979). Infected plants show reduction in plant height with the red leaf reactor genotypes being more stunted (Seifers and Hackerott, 1987).

2.6.2.6 Losses and impact of *Sugarcane mosaic virus* infection

Crop losses due to the SCMV vary from negligible to severe and are mainly dependent on various factors among them being cultivar susceptability, SCMV strains involved, prevailing environmental conditions, stage of infection for the plants, disease incidence and the type of crop affected (Jones, 1987; Goodman, 1999; Balamuralikrishnan, 2003). The disease threatened the sugarcane industry in the mid-1920s in Argentina, Brazil and Louisiana (Abbott, 1961; Koike and Gillaspie, 1989; Yang and Mirkov, 1997).

The disease causes significant losses in both the production quantity and quality (Shukla *et al*., 1989; Gan *et al*., 2010). Based on the influence of the above mentioned factors, yield losses of 0.5% to 100% have been reported from different countries worldwide from sugarcane (Abbott

and Stokes, 1966; Costa and Muller, 1982; Jones, 1987; Bailey and Fox, 1987; Koike and Gillaspie, 1989; Singh *et al*., 1997; Rao *et al.*, 2002; Viswanathan and Balamuralikrishnan, 2005).

The disease also affects the growth parameters in sugarcane including internode numbers, cane diameter and cane weight (Viswanathan and Balamuralikrishnan, 2005) which are usually reduced in the infected plants. On the sugarcane quality, sucrose content, brix, commercial cane and purity are greatly reduced (Koike and Gillaspie, 1998; Balamuralikrishnan, 2001; Viswanathan and Balamuralikrishnan, 2005). The disease also affects the growth characteristics of the sugarcane where it leads to reduced sett germination, tiller production, plant vigor and photosynthetic rates in infected plants (Tu and Ford, 1968; Tu *et al*., 1968; Moline and Ford, 1974; Viswanathan and Balamuralikrishnan, 2005).

In maize crop, yield losses of between 6-46% have been reported from various countries (Kulkarni, 1973; Louie and Darrah, 1980; Kovacs *et al*., 1994). In sorghum, reduced grain weights were noted in the red leaf reactors while the mosaic prone genotypes recorded no weight loss (Seifers and Hackerott, 1987). Additionally, the viral infection led to higher viral titers in the red leaf reactor genotypes, a clear indication of high virus replication (Seifers and Hackerott, 1987).

2.6.2.7 Transmission of *Sugarcane mosaic virus*

SCMV is naturally transmitted in a non-persistent mode by several aphid species (Brandes, 1920; Pemberton and Charpentier, 1969; Brunt *et al.,* 1996; Xia *et al.*, 1999; Zhang *et al.,* 2008). The aphid species involved include *Aphis craccivora*, *Aphis maidis*, *Dactynotus ambrosiae*, *Hysteroneura setariae, Rhopalosiphum maidis, Rhopalosiphum padi, Myzus persicae, Schizaphis*

graminum, *Toxoptera graminum*, and a number of other aphid species (Brandes, 1920; Kennedy *et al.*, 1962; Pemberton and Charpentier, 1969; Teakle and Grylls, 1973; Knoke *et al*., 1983).

Several factors influence the spread of the disease by the aphid vectors; the aphid acquisition time (this affects the retention time), the age of the host plants, viral concentrations in the plant (leaves of different ages on the same plant have different viral concentrations), state of the aphid, aphid population, aphid behavior and the prevailing environmental conditions especially the temperatures (Abbott, 1961; Bailey and Fox, 1980; Sahi et *al*., 2003).

The virus is infectious and can thus be spread mechanically through sap (Brandes, 1920). Its easily transmitted from or to sorghum and maize than its is to or from sugarcane (Brandes, 1920; McMartin and King, 1948; Pirone, 1972; Noone *et al.*, 1994).

Seed transmission for the virus has been reported at very low rates of 0.4% to 3.9% in maize seedlings (Li *et al*., 2011). Low rates have also been recorded for the MDMV a related virus to the SCMV at 0.005% to 0.4% (Williams *et al*., 1968; Hill *et al*., 1974; Sherpherd and Holdeman, 1965; Mikel *et al*., 1984) in maize. In infected seeds, the virus is usually found in the kernel, silks, glumule, whole anthers but none in pollen (Mikel *et al*., 1984). In the immature seeds; the virus was found in the embryo and endosperm in low levels while none was found in the embryo in mature seeds although low levels were detected in the endosperm and the pericarp (Mikel *et al*., 1984). Latent infections also occur and these plants can act as focal points for spread to healthy plants (Hill *et al*., 1974). The virus seems to be inactivated as the seed matures (Ford, 1966).

Soil transmission of SCMV has been reported in non-inoculated sorghum, getting 0.7-5.4% infection through planting in containers with infected plants (soil); non-inoculated plants getting

6.2% infection when drain water from infected plants was used to irrigate them and 5% infection when the non-inoculated plants shared irrigation water (Bond and Pirone, 1970).

2.6.3 Diagnostic methods for the *Maize chlorotic mottle* and *Sugarcane mosaic viruses*

For proper management decisions to be made, rapid and reliable diagnostic methods need to be available. Earlier on, the main method used was based on symptom identification. This diagnostic method is important especially in carrying out some management practices like rouging of infected plants to avert further spread. However, use of symptoms alone isn't accurate as some symptoms overlap with nutritional deficiency symptoms like stunting and chlorosis or with other mosaic diseases while other plants don't express symptoms (Nelson *et al*., 2011; Lima *et al*., 2012).

The second diagnostic method involves the morphological assessment of virus particles in crude plant extracts through the transmission electronic microscope**.** This has been extensively used for the characterization and identification of SCMV and other potyviruses (Edwardson and Christie, 1978; Lesemann *et al*., 1992). This method needs expensive equipment hence limiting its adoption.

The third method in use is the serological diagnosis (Martin *et al*., 2000). The method is based on the antigen-antibody reactions principle (Lima *et al*., 2012). The main test in this category is the enzyme-linked immunosorbent assay (ELISA) test method. It has various forms including triple antibody sandwich elisa (TAS-ELISA), double antibody andwich elisa (DAS-ELISA) and direct antigen coating elisa (DAC-ELISA) (Kumar *et al*., 2004). In this test, the specific antibodies (IgG) recognize the virus antigens for which they have been developed (Lima *et al*., 2012). Generally, DAS ELISA has been used to detect the SCMV and MCMV using antisera raised

against the same viruses (Clark and Adams, 1977). The test method is highly sensitive, strain specific, cheap, simple, and precise and can be used on very large samples (Voller *et al.,* 1976; Clark and Adams, 1977; Lima *et al*., 2012).

Due to the need for rapid pathogen testing, a quick test method has been developed based on the same principle as Elisa of the antigen-antibody reaction above called the Lateral flow assays. The device offers a very fast and convenient on-site testing without the need for specialized and costly equipment (Ward *et al*., 2004).

The nucleic acid of the pathogen in question is also used for identification. In this category, we have the Polymerase chain reaction (PCR) which amplify small quantities of nucleic acid sequences to produce quantities that can be analyzed (Mullis and Faloona, 1987; Coleman and Tsongalis, 2006). The other is next generation sequencing (NGS), which entails generation of DNA sequences in a general manner, upon which a search for a match on similarity for identification is done against the GenBank (Adams *et al*., 2013). The PCR variants include basic or conventional PCR, Real-time PCR and RT-PCR which is common for RNA based viruses (Lopez *et al*., 2003; Kumar *et al*., 2004; Punja *et al*., 2007; Hardingham *et al*., 2012). The two have been used in the detection of many viral pathogens including MCMV and SCMV (Zhang *et al*., 2011; Wangai *et al*., 2012; Adams *et al*., 2013; Mahuku *et al*., 2015; Lukanda *et al*., 2017) where next generation sequencing with 454 sequencing was used to detect both the SCMV and MCMV isolates (Adams *et al*., 2013) causing the MLN disease in Kenya.

2.6.4 Prevention and control of maize lethal necrosis disease

2.6.4.1 Reducing the rate of infection

The rate of maize infection by MLN-causing viruses can be reduced through avoidance, plant protection or use of resistant and or tolerant genotypes. Avoidance aims at limiting contact of the pathogen and the host plant. This can be achieved through use of certified seeds, planting in fields with no disease history and having adequate plant spacing (Trigiano *et al*., 2008; Wangai *et al*., 2012). MCMV incidents were observed more in the silt to silty clay loamy soils with moisture of 0.56% while rare infections were noted on the sandy loamy soils of 0.36% moisture content (Uyemoto, 1983). The high moisture content soils leads to survival of the virus in the residues as they keep it hydrated. Thus choosing soils which will facilitate fast drying of plant debris and those with no history of the disease will guarantee a disease free crop. Crop management practices should be carefully planned to avoid having a young crop when vector populations and activity are at their peak (Bailey and Fox, 1980). Planting seeds should be treated to avoid early pest invasion (Garud *et al*., 1990; Epperlein *et al*., 1995). This should be done in conjunction with control of the alternative hosts for both the virus and its vectors (Rybicki and Pietersen, 1999).

Plant protection aims at protecting the host plants from the invading viruses. It involves the use of chemicals, improved plant nutrition to strengthen the plant to pest and disease attack (Wangai *et al*., 2012) and modification of the environment. Chemicals are used to kill the vectors. Those commonly used include Abamectin, Deltamethrin, Dimethoate, Endosalphan, Imidacloprid, Permethrin, Thiamethoxam among others (TPRI, 2011). The chemicals need to be applied frequently, once every one to two weeks, and need proper rotations to limit resistance development of the targeted vector to the chemical (Mezzalama *et al*., 2015). Use of herbicides will help in killing the alternative hosts of the viruses hence limiting spread (Victoria *et al*.,

1995). Regular spraying in Hawaii by the maize seed producers after planting, controlled the insect vectors (Nelson *et al*., 2011).

Use of resistant and or tolerant genotypes is the most reliable, effective, economical and environmentally friendly control measure for plant diseases (Xia *et al*., 1999; Dussle *et al*., 2002; Kumar *et al*., 2004). Resistance genes for both viruses were confirmed in maize (Xia *et al*., 1999; Jones *et al*., 2007; Jones *et al*., 2011) and resistant maize genotypes have been released while more efforts have been put to ensure more are released for all environmental conditions. In maize, two dominant genes are responsible for resistance to SCMV, *Scmv1* located on chromosome 6 at the short arm is responsible for symptom suppression throughout the plants' development stages and *Scmv2* found near the centromere region of chromosome 3 is responsible for symptom suppression at later stages of the plant growth (Melchinger *et al*., 1998; Xia *et al*., 1999; Xu *et al*., 1999). In Krish sorghum, resistance is dependent on a single gene K whose resistance is dominant over susceptibility (Toler, 1985). In maize, genetic resistance is polygenically controlled with partial dominance (Nelson *et al*., 2011). MLN resistance in maize is determined by multiple genomic regions which often have small to medium effects (Gowda *et al*., 2015). The genome wide association study revealed 24 Single nucleotide polymorphisms (SNPs) associated with the MLN resistance with the putative gene R being responsible for the plant response to the disease (Gowda *et al*., 2015). Eight of these SNPs were detected on chromosome 3 similar to the SCMV QTL. Overall, there are three major QTLs on chromosome 3 and 6 responsible for the MLN resistance (Olsen *et al*., 2016).

In East Africa, screening for resistant genotypes is being done in Naivasha, Kenya through the collaboration of the Kenya Agricultural Livestock Research Organization (KALRO) and

International Maize and Wheat Improvement Center (CIMMYT) to identify resistant genotypes to the MLN disease and speed up breeding. For example, H614D has some level of tolerance to MLN when compared to other genotypes in use although it is not resistant (Das *et al.,* 2015). Generally, resistance is affected by the environment, interactions between the participating genes, virus isolate and virus species more so if its conferred by resistant genes (Jones *et al*., 2011).

To manage the MLN disease, deployment of resistant genotypes to both the MCMV and SCMV will be the ultimate goal (CABI, 2012). Resistance to either of the viruses will also be a win against the disease (Makumbi and Wangai, 2013).

2.6.4.2 Reducing initial inoculum

Reducing initial inocula of MLN-causing viruses can be achieved through exclusion or eradication. Exclusion involves prevention of establishment of disease in areas where it never occurred before. This can be achieved through strict quarantine measures. All seeds and breeding material imported into a country need to be screened for the MCMV (Adams *et al*., 2014). This is the best method to use in order to avoid the introduction of the virus to new regions (Wangai *et al*., 2012). National plant protection organizations (NPPOs) should restrict movement of maize materials and products from affected areas to clean areas within a country and extend this outside the country (Kagoda *et al*., 2016).

Eradication aims at reducing pathogens from affected areas before establishment. Good field sanitation practices will help reduce the disease. They include destruction of infected maize plant debris, rouging of diseased maize plants and disposing them off by burning coupled with the cleaning of tools, equipment and clothing used in infected fields (Makumbi and Wangai, 2013;

Mawishe and Chacha, 2013) and eliminating weeds or other alternative hosts for insect vectors which can serve as virus reservoirs (Webster *et al*., 2004; Trigiano *et al*., 2008; Makumbi and Wangai, 2013).

Crop rotation with plants in the non-grass group (Wangai *et al*., 2012) can also help in reducing the viruses and hence manage the MLN disease (Uyemoto, 1983). In the US, the disease recurred in fields with corn previously while those which had a different crop were free of the disease (Philips *et al*., 1982; Uyemoto, 1983). Crop rotation not only breaks the disease cycle but it also helps diversify farm enterprises and enhances yields (Uyemoto *et al.*, 1980; Krupinsky *et al*., 2002; Makumbi and Wangai, 2013).

The most efficient method for viral elimination in sugrcene for SCMV is through chemotherapy (Balamuralikrishnan *et al*., 2002), meristem tip culture in association with thermotherapy or on its own (Balamuralikrishnan *et al*., 2002; Goncalves *et al*., 2012). The meristem size used is critical in achieving virus free cuttings (Ramgareeb *et al*., 2010) with optimal range of 0.2-1.5mm length being the best for viral elimination (Chatenet *et al*., 2001; Parmessur *et al*., 2002; Zhang *et al*., 2006).

Effective control of MLN encompasses the use of various cultural methods with chemicals and use of host resistance (Nelson *et al*., 2011). No one method will completely eliminate the disease hence combination of all the methods is paramount for the disease management and control.

CHAPTER THREE

3.0 Reaction of different maize genotypes to maize lethal necrosis disease and its causal viruses

Abstract

A greenhouse study was conducted to determine the reaction of five different genotypes of maize to infection by *Sugarcane mosaic virus* (SCMV), *Maize chlorotic mosaic virus* (MCMV) and the synergistic interaction of the two viruses which results in maize lethal necrosis (MLN) disease. The genotypes included H614, H513, Duma 43, Kikamba and Kinyanya. Their selection was based on the Agro-ecological Zones (AEZ) where they are grown in addition to whether they are hybrids or landraces. The treatments included SCMV and MCMV each inoculated separately and in combination (SCMV+MCMV) and with inoculum obtained from MLN infected plants. The plants were mechanically inoculated at four leaf stage. The plants were assessed for disease severity using a scale of 1-5 and area under disease progress curve (AUDPC) and percent disease incidence determined starting from week 1 post inoculation to week 6. Leaf samples were collected and analyzed for the presence of the viruses using Double Antibody Sandwich Enzyme Linked Immuno-sorbent Assay (DAS-ELISA). All the genotypes had >90% incidence recorded with significant differences among genotypes only for the co-infection (SCMV+MCMV) at P=0.05 while on disease severity, the SCMV+MCMV had the highest AUDPC. The landraces recorded higher disease severity with Kinyanya having an average of 4.9 and Kikamba 4.5 on the SCMV+MCMV treatment. In general, landraces showed more severity to the different viral infections. When the plants were co-infected with both SCMV and MCMV, the disease severity was quite high with more than 80% crop loss whereas the plants inoculated with MLN extracted

inoculum recorded severity of between 2.5 to 3.44. On the other hand, single viral infections were mild with severity averages ranging from as low as 2.0 to high of 2.7 and most of the plants survived. Synergistic infections had double MCMV titers compared to plants with single MCMV infections whose titers did not change or were not affected. The increased titer for the MCMV could be responsible for the severity noted in the plants infected with the two viruses. This finding is critical in the management of the MLN disease as the presence MCMV in conjunction with the SCMV leads to increased symptom expression in addition to the pathogenicity of the MCMV in coinfected plants. Hence the MCMV virus is the key player in this synergistic reaction. Its management, prevention and control will be key in managing MLN disease.

3.1 Introduction

Maize lethal necrosis (MLN) disease is caused by double infection of MCMV and any of the maize infecting potyviruses, such as *Wheat streak mosaic* rymovirus (WSMV), *Sugarcane mosaic virus* (SCMV) and the *Maize dwarf mosaic* Potyvirus (MDMV) (Goldberg and Brakke, 1987; Scheets, 1998). When the single viruses infect the plants, the severity is very low unlike in the synergistic reactions which are very severe (Goldberg and Brakke, 1987; Scheets, 1998). First reported in 2011 in Kenya, the disease has since spread to other countries in the East African region (Wangai *et al*., 2012; Adams *et al*., 2014; Lukanda *et al*., 2017). For the East African outbreak, the viruses involved are SCMV and MCMV (Wangai *et al*., 2012; Adams *et al*., 2013).

Maize lethal necrosis disease leads to leaf necrosis starting from the tips and the edges progressing inwards and is usually preceded by severe chlorosis of the plants in the early infection stages and can lead to complete plant loss (Goldberg and Brakke, 1987; Scheets 1998).

Maize chlorotic mottle virus is one of the viruses in the family *Tombusviridae*, genus Machlomovirus, infecting the *gramineae* group of plants (Fauquet *et al*., 2005; King *et al.*, 2011). The virus had never been reported in Africa until 2012 when it was reported in Kenya (Wangai *et al*., 2012) and has since spread to most of the East African countries (CIMMYT, 2012; FAO, 2013; ASARECA, 2013).

Several factors determine the range of symptoms exhibited by maize plants infected with the MCMV. These include, environmental conditions, maize genotype, age at infection and the virus strain. Generally, there is stunting associated with chlorosis and mottling. On the leaves, chlorotic stripes occur and these lead to chlorotic blotches in later stages. In severe infections, leaf necrosis occurs, leading to complete crop loss (Castillo and Hebert, 1974; Castillo, 1977; Niblett and Claflin, 1978; Uyemoto *et al.,* 1981).

Sugarcane mosaic virus is one of the main viruses in the Potyviridae group (Pirone, 1972; Mattheus, 1982; Teakle *et al*., 1989). The virus is endemic in Kenya and in Africa (Louie and Darrah, 1980). The classical symptoms of SCMV in maize leaves are presence of pale green to yellow chlorotic portions on a background of distinct shades of green. Sometimes yellow stripes and/or necrosis occur (Wu *et al.,* 2012). The particular symptoms observed can vary depending on the host, virus strain, and the prevailing environmental conditions (Perera *et al.,* 2012). External and internal stalk necrosis may occur in some cultivars. Latent infections may also occur (Frison and Putter, 1993).

Understanding how the different maize genotypes react to the different viruses will be key in selection during the breeding process. Germplasm diversity is key in any crop improvement program and more so when the improvement is to achieve resistance to specific disease causing

organisms. It is important to search for resistance and / or tolerance in the commonly used maize genotypes, which will further form the basis for selection during breeding. The goal of this work was therefore to evaluate the reaction of different maize genotypes to MLN disease and to the viruses causing the disease.

3.2 Materials and methods

3.2.1 Maize genotypes used for the study, source and planting

Five maize genotypes were used for this study. The genotypes were selected based on the Agro Ecological Zones (AEZ) they are grown and based on whether it is a hybrid or a landrace genotype. The selected genotypes were H614 which is a hybrid adopted for high altitude areas, H513 which is a hybrid grown in medium altitudes, Duma 43 a hybrid for medium and low altitude, and Kikamba and Kinyanya which are landraces grown in the medium and low altitude areas. The seeds were acquired from the University of Nairobi, Kabete Campus' field station seed store. These had been in stock before the maize lethal necrosis disease outbreak and were closely monitored for any disease symptoms before inoculations to ascertain the they were free of the MLN causing viruses.

The maize seeds were sown in soil mixed with sand and organic manure at a 2:1:1 ratio, respectively in a 5-litre bag at the rate of two seeds per bag. During planting, 2 grams of Di-ammonium Phosphate (DAP) fertilizer was applied per bag. The plants were top dressed using Calcium Ammonium Nitrate (CAN) once at two months at a rate of 2 grams per bag. The plants were maintained in a screen-house treated prior to planting with an insecticide (Abamectin - Dynamec) due its knock down effect on the insect vectors. Weekly insecticide spraying was

done to control insects especially thrips and aphids which are vectors of the two viruses. Abamectin was used for three consecutive weeks followed by pyrethrins (Karate) which has lower persistence to prevent the pests from developing resistance.

3.2.2 Source of virus inoculum, treatments and experimental design

The initial viruses used for the experiments were supplied from the Maize lethal necrosis laboratory at Kenya Agricultural and Livestock Research Organization (KALRO). The viruses were maintained in the maize plants for subsequent inoculations. When it was not possible to do so due to space limitation, the maize leaves were frozen at -40^{o}C. Inoculating young plants prior to use for further experimental inoculations activated the viruses.

Two viruses, *Sugarcane mosaic virus* (SCMV) and *Maize chlorotic mottle virus* (MCMV) were used in this experiment either as single or mixed infections. In total, there were five treatments; plants inoculated with SCMV alone, plants inoculated with MCMV alone, plants inoculated with a combination of SCMV and MCMV (abbreviated as SCMV+MCMV), and plants inoculated with both viruses from an MLN-infected plant (abbreviated as MLN) and a control (no virus). Each treatment had two bags of a variety replicated four times. These were arranged in a completely randomized design (CRD).

3.2.3 Mechanical inoculation of maize with the viruses

Ten grams of maize leaves from plants infected with a specific virus were crushed with a pestle and mortar. Addition of carborandum at the rate of 0.2g and 2mls of 0.01M phosphate buffer enhanced crushing. The finely crushed extract was transferred to a clean well-labeled jar after sieving to remove the plant debris. For the mixed infections (SCMV+MCMV), a ratio of 1:1 was used whereby one part of MCMV was mixed with one part of the SCMV. Before inoculation,

more carborandum was added at the rate of 1-5% w/v to the extract and inoculation done by stripping the leaves thrice between cheese-cloth-wrapped forefingers, which were wet with the freshly prepared inoculum. The cheese clothes were changed after each treatment and 70% alcohol hand disinfection. The coinfections were inoculated last. Inoculations were done at 3-4 leaf stage and only the three upper leaves were inoculated. The inoculated plants were washed 2 hours later using plain water to remove the carborandum.

3.2.4 Disease incidence, severity assessment and data analysis

Disease severity was assessed using a scale of 1 to 5 adopted from KALRO/CIMMYT where 1= no symptoms observed, 2= fine chlorotic streaks on upper leaves, 3= chlorotic mottling throughout the plant, 4= excessive chlorotic mottling and dead heart and 5= complete plant necrosis. The average severity per treatment combination was determined. To further assess the severity of the infections, the Area under disease (AUDPC) was determined using the formula from Shaner and Finney (1977).

$AUDPC=\sum [(0.5) (Y_{i+1} + Yi) (T_{i+1} + Ti)]$

Where;

Y=Disease severity score and

T=Time (Weeks) of the severity assessment

Scoring for symptoms for the plants was done weekly starting in the first week up to the 6^{th} week after inoculation. Samples for virus analysis were collected in the last day of data collection from both symptomatic and asymptomatic plants.

Percent disease incidence was assessed as follows;

n/N*100

Where;

N=Total No. of plants per treatment

n=Total no. of plants with disease symptoms

All data collected was subjected to Analysis of Variance using Genstat statistical package (Version 12) to determine the effects of the different treatment and differences among the means were separated using the Fischer's Protected LSD test at 5% probability level.

3.2.5 Serological assays of plant leaf samples

The samples were collected after six weeks post inoculation and assayed for viruses using the standard Enzyme Linked Immuno-sorbent Assay (DAS-ELISA) with reagents and antibodies from Agdia (Http://www.agdia.com) following the manufacturer's protocol. The test procedure was similar for both SCMV and MCMV using SCMV- and MCMV-specific antibodies and conjugates. Each well of the Nunc microtiter plate was filled with 100µl of the capture antibody. The capture antibody was prepared by adding 100µl of the concentrated antibody in 10ml of carbonate coating buffer. This was enough for one microtiter plate of 96 wells. The plate was incubated overnight at 4^{o}C. After incubation, the plate was washed four times after disposing of its contents, using 1X phosphate buffered saline with tween 20 (PBS-T) washing buffer.

One part of sample was extracted in ten parts of General Extraction Buffer (GEB). As an example, 0.3g of test samples was placed into the Agdia sample extraction bags and 3ml of General Extraction Buffer (GEB) added. The buffer was composed of the Sodium sulfite, Polyvinylpyrrolidone, sodium azide, powdered albumin and tween 20 dissolved in 1X PBS-T. The samples were then homogenized to extract the sap for pipetting. The samples were then pipetted at the rate of 100µl per well in accordance with the sample layout. Each sample was filled in duplicate. Positive and negative controls were then added to their reserve wells while the

blank wells were filled with the sample extraction buffer. The plate was placed in a humid box and incubated at room temperature for two hours. The plate was thoroughly washed as above using the PBS-T washing buffer.

The enzyme- antibody conjugate was added at the rate of 100µl per well. This was prepared by adding 100µl of concentrated enzyme conjugate to 10ml of the ECI buffer. The ECI buffer is composed of Bovine serum Albumin, Polyvinylpyrrolidone and sodium azide dissolved in 1X PBS-T. The plate was placed in a humid box and incubated for 2 hrs. at room temperature. After incubation, the plate was washed thoroughly as above using the PBS-T buffer.

The p-nitrophenyl phosphate (pNPP) solution was added to each well at the rate of 100µl per well. This was prepared by adding the PNP tablet into the substrate solution at the ratio of 1mg /ml. The plate was incubated in the dark for 60 minutes after which the plate was read at 405nm wavelength on the Elisa reader (BIO-RAD Model 550). The results were considered valid when the positive controls gave positive results and the buffer and negative control wells were colorless. Samples with values above the mean of the negative controls multiplied by 2.5 ([(A+B)/2*2.5]) were considered positive while those with values below were grouped negative.

3.3 Results

3.3.1 Incidence and symptom severity in plants infected with *Sugarcane mosaic virus*

The plants developed symptoms four days post inoculation. No significant difference (P=0.05) in disease incidence between the evaluated maize genotypes was observed (Figure 3.1). Plants inoculated with SCMV developed symptoms earlier and the incidence was higher than the rest of the treatments. The disease incidence of the SCMV was similar across the weeks except for week 1, where Kikamba had the highest incidence while H513 had the least. The symptoms observed included light green mosaic and mild mottle on the younger leaves. These appeared as specks and streaks on the leaves, respectively (Plate 3.1). As the disease progressed, symptoms developed into clear streaks but the visibility of the conspicous symptoms earlier observed eventually lessened. On the other hand, some plants showed severe chlorosis, indicating that the disease can be severe in some circumstances, depending on the variety affected. The landraces became more chlorotic than the hybrids. On disease severity, the genotypes H614 and Duma 43 recorded higher scores than the landraces in the initial weeks but this trend was reversed in the subsequent weeks. Generally, no difference within weeks (P=0.05) was noted for all the weeks of data collection (Figure 3.2).

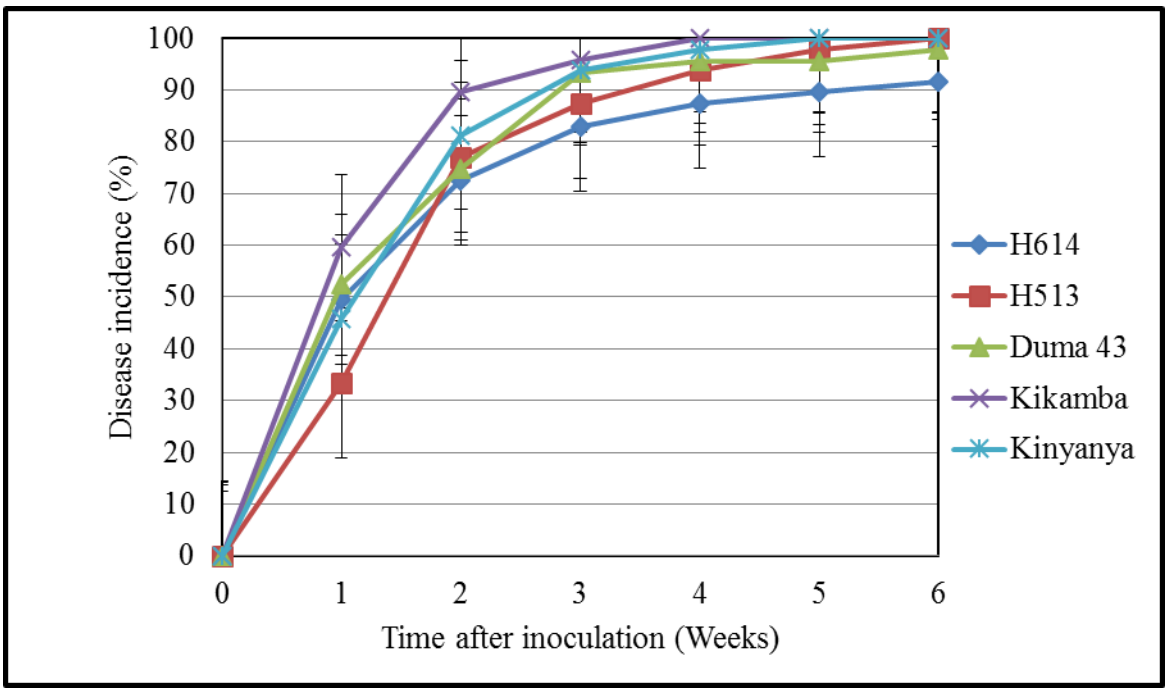

Figure 3.1 Disease incidence in different maize genotypes infected with *Sugarcane mosaic virus* (SCMV).

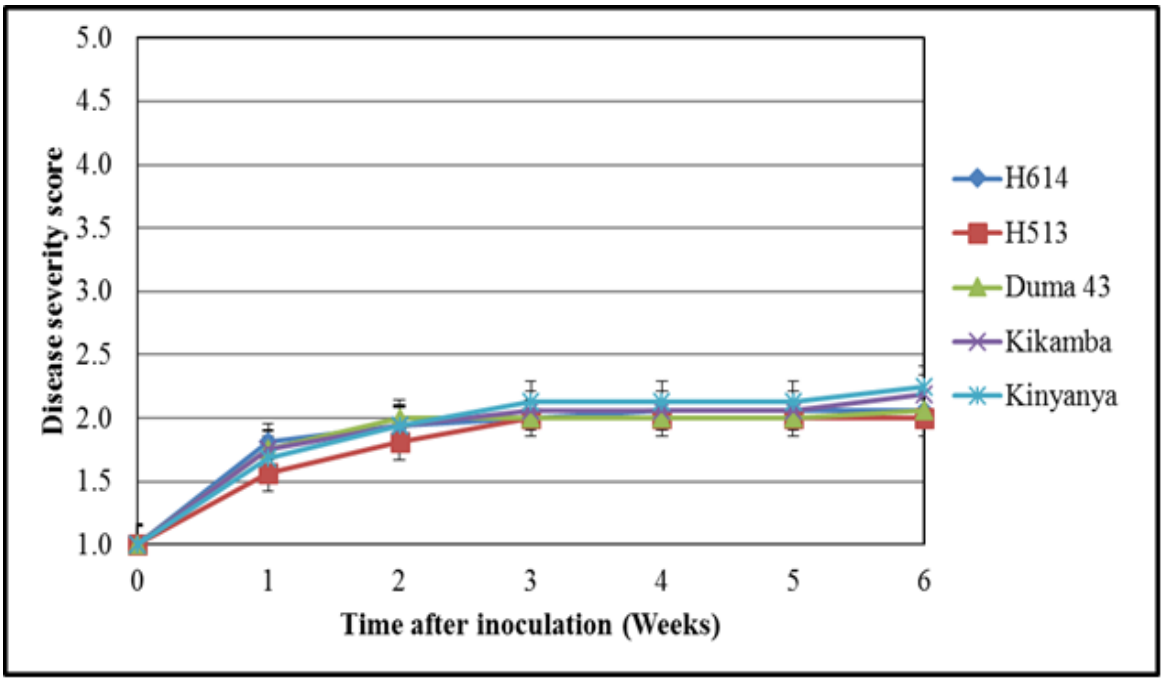

Figure 3.2 Severity of different maize genotypes inoculated with *Sugarcane mosaic virus*

Plate 3.1 *Sugarcane mosaic virus* (SCMV) symptoms in maize leaves after 4 weeks post mechanical inoculation. Initial symptoms appeared as mosaic and mild mottle depicted as specks and streaks on the upper leaves (A, B). These specks and streaks merged to form clear streaks running parallel to the veins (C).

3.3.2 Incidence and symptom severity in plants infected with *Maize chlorotic mottle virus*

All maize genotypes developed symptoms one week post inoculation (Figure 3.3). The differences in incidence were insignificant (P=0.05) between genotypes due to infection by MCMV. In the first week post inoculation, incidence was very low for all genotypes. This steadily rose at the same rate for the subsequent weeks. Kinyanya recorded higher incidence but by week 5, all the genotypes had recorded 100% disease incidence. The maize genotypes reacted almost similarly to the virus (Figure 3.4).

The symptoms observed included chlorotic mottling which appeared as streaks. The chlorosis became more severe leading to necrosis. There was general stunting in plants inoculated with MCMV due to the shortened internodes (Plates 3.2 and 3.3).

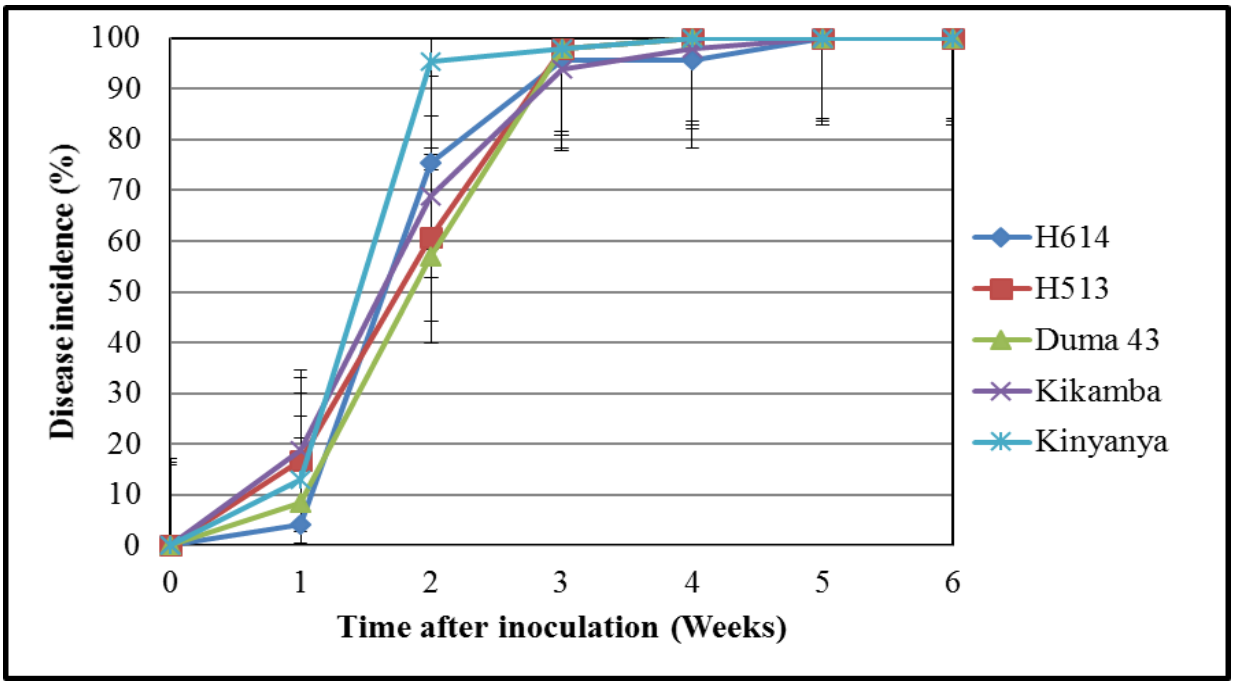

Figure 3.3 Disease incidence of different maize genotypes due to the infection by *Maize chlorotic mottle virus* (MCMV).

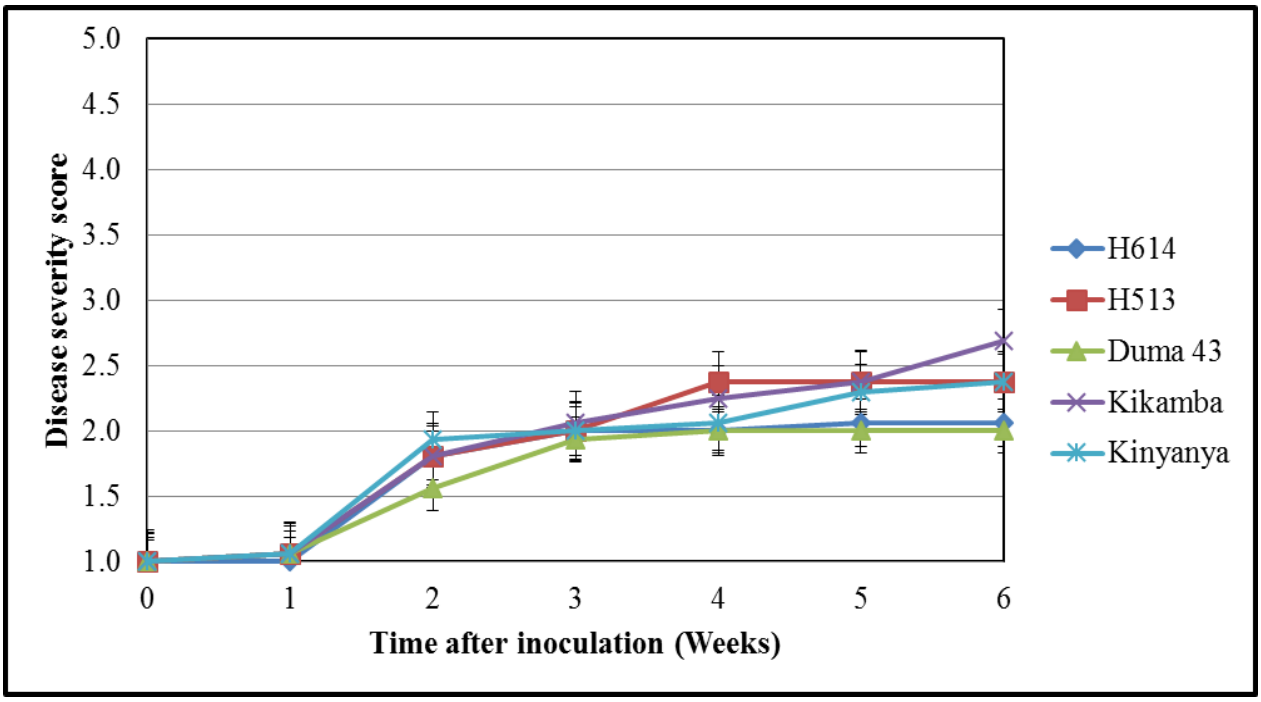

Figure 3.4 Disease severity on the genotypes infected by the *Maize chlorotic mottle virus* (MCMV). No significant difference at P=0.05.

Plate 3.2 *Maize chlorotic mottle virus* disease progression- Initial stage of symptom development-one week post inoculation. The plants had chlorotic streaks and mottles (A). As the disease progress there was merging of the chlorotic streaks and mottles leading to yellowing of the leaves (B) while others developed severe chlorosis (C) which eventually led to necrosis starting from the edges of the younger leaves (D).

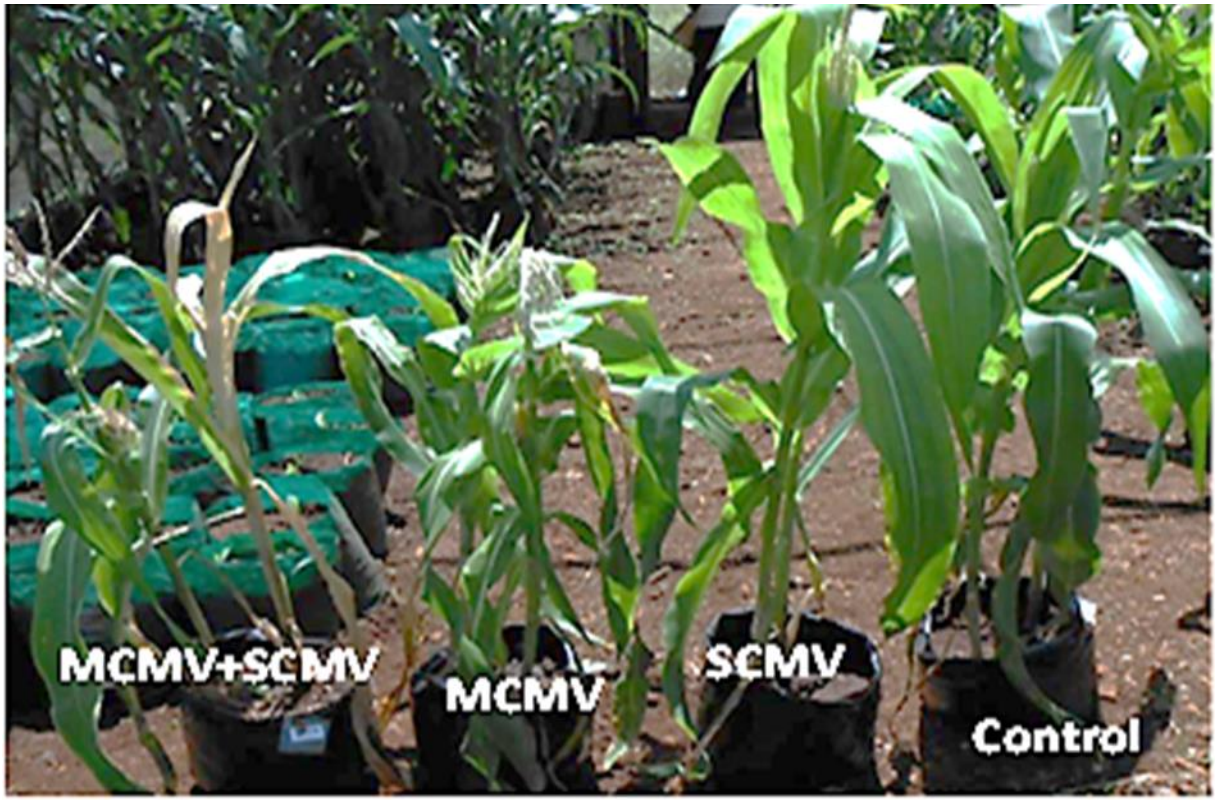

Plate 3.3 The effect of the *Maize chlorotic mottle virus* on the plants. Both the single and co-infected plants affected. There is general stunting associated with shortened internodes.

3.3.3 Incidence and symptom severity of the co-infections

Co-infection with the two viruses was evaluated in two different ways; inoculating the plants with the viruses individually extracted and mixed at the ratio of 1:1 and by getting inoculum from a plant already infected by the two viruses and inoculating healthy plants.

In the first scenario, disease symptoms were observed one week post inoculation. The effect of the synergy between the viruses was severe unlike the single infections. When the two viruses (SCMV and MCMV) were combined, there was significant difference (P=0.05) on how the maize genotypes reacted both in incidence and severity. The genotypes recorded higher disease incidences with the landraces having the highest. All plants in this treatment were affected hence by week four all the treated maize genotypes had 100% incidence (Figure 3.5). The symptoms observed included leaf chlorosis and mottling usually from the base extending upward to the leaf tips. As the disease progressed, there was severe chlorosis and mottling leading to the plants becoming bright yellow followed by necrosis from the leaf margins which led to a dead heart for the younger leaves. On the older leaves, however, the necrosis progressed inwards to the midrib leading to death of the whole leaf (Plate 3.4).

The landraces showed higher severity to the co-infection with Kinyanya recording the highest followed by Kikamba (Figure 3.6). The hybrids also reacted to the inoculation but their severity was lower. H614 had higher severity followed by H513 with Duma 43 having the least severity. There was significant difference from week two to week six. Kinyanya was significantly different from most genotypes except in week 4 where the landraces were significantly different from the hybrids. Most plants developed the maize lethal necrotic disease.

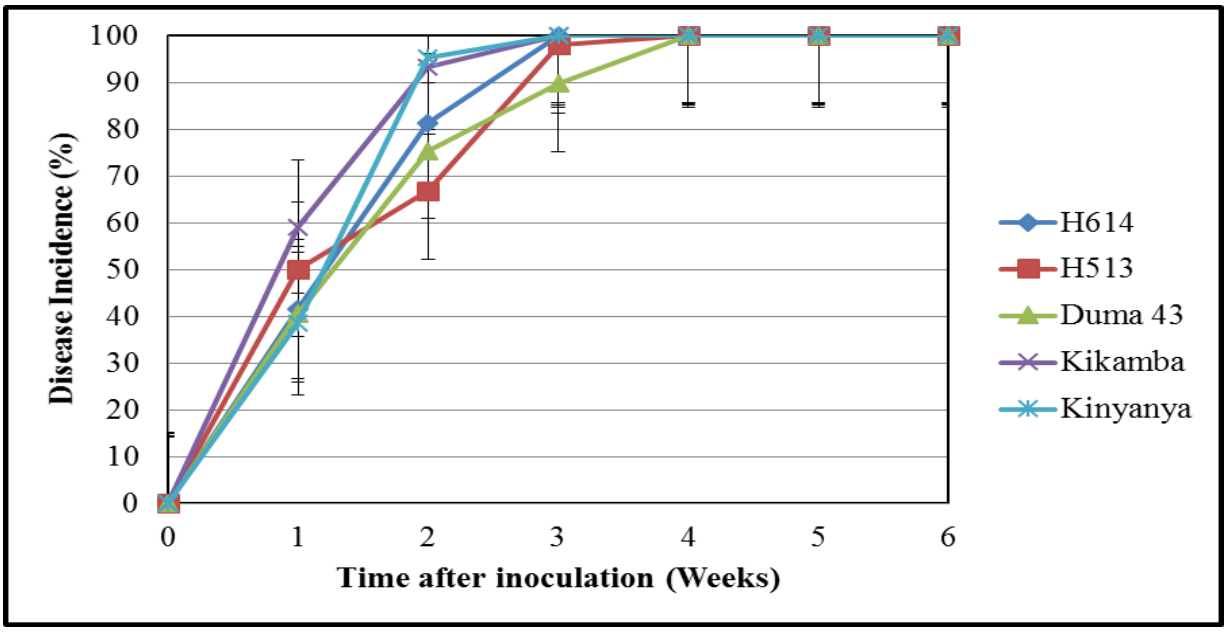

Figure 3.5 Disease incidence of different maize genotypes inoculated with a combination of MCMV+SCMV. All the genotypes had a 100% disease incidence by week four post inoculation.

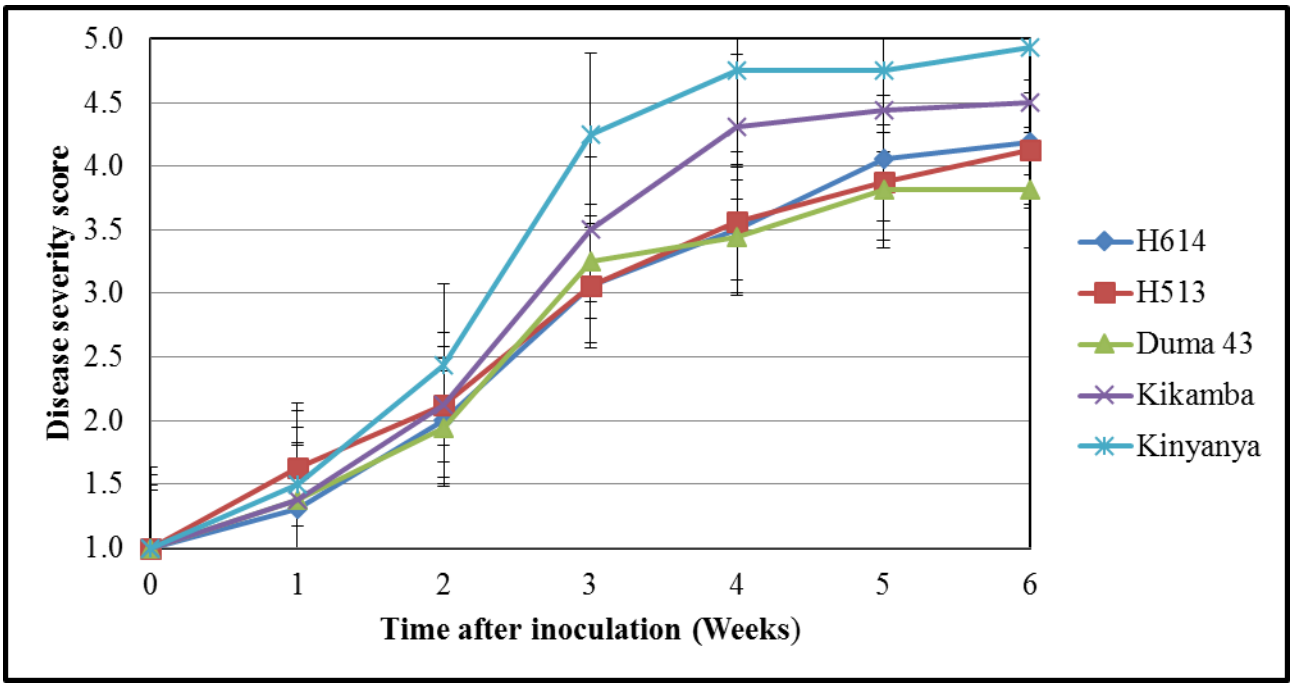

Figure 3.6 Disease severity in different maize genotypes inoculated with a combination of MCMV+SCMV.

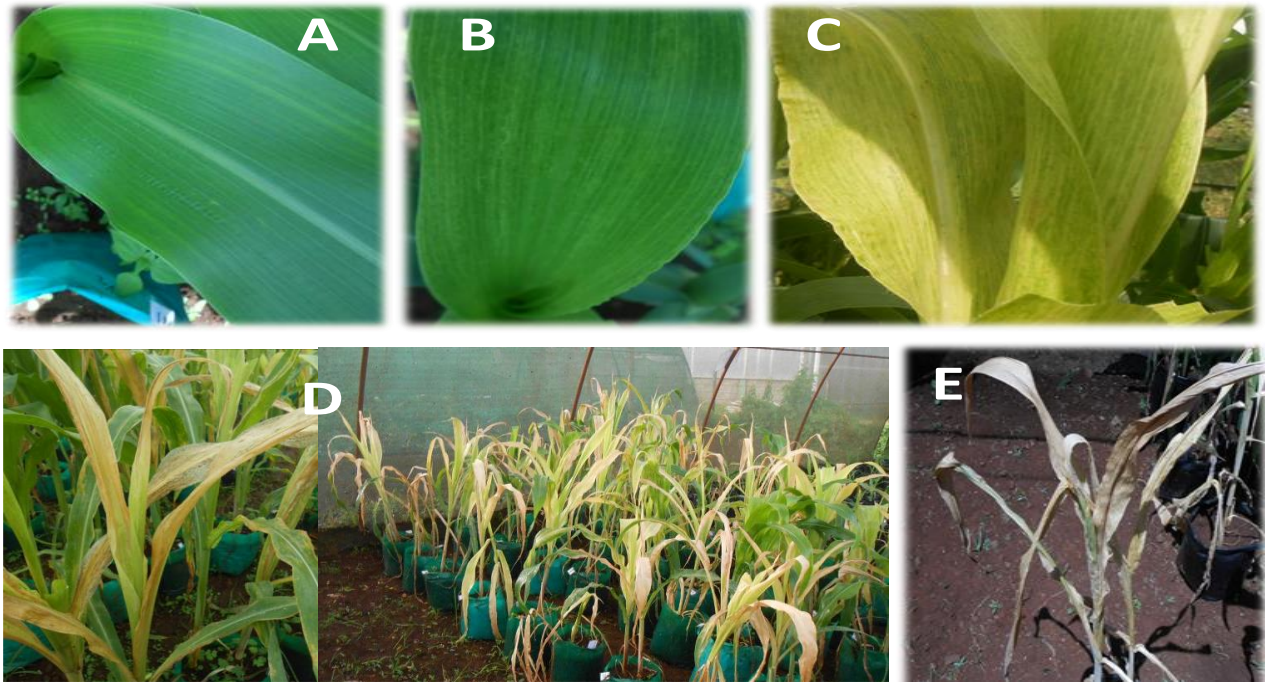

Plate 3.4 The different stages of symptom development for the Maize lethal necrosis disease. Healthy leaf (A), Chlorotic leaf (B), severe chlorosis leading to yellowing of leaves (C), setting in of necrosis from the leaf margins (D) and complete plant death (E).

In the second scenario, as the disease developed from the combination of the SCMV +MCMV in scenario one; the symptomatic leaves were harvested and used for inoculation of healthy plants. This is reffered to as the MLN treatment. The plants developed similar symptoms as the combination but the severity of the maize lethal necrosis disease was lower. Of the five genotypes, Kinyanya started off with the highest incidence followed by H513. Overall, all the genotypes attained 100% disease incidence by week five (Figure 3.7). The leaf symptoms included mild mottling and chlorosis originating from the base and extending upward to the leaf tips followed by severe chlorosis and mottling afterwards the plants became bright yellow followed by necrosis from the leaf margins. In the young leaves; the necrosis progressed

inwards leading to a death heart. The severe symptoms were observed on a few plants (Plate 3.5).

On disease severity, there was no significant difference in reaction to MLN between the different maize genotypes, except for week three where there was significant (P=0.05) difference between the landraces Kikamba and Kinyanya (Figure 3.8). Kinyanya had the highest severity followed by H513 with the landrace Kikamba showing the least. Few plants developed the maize lethal necrotic disease.

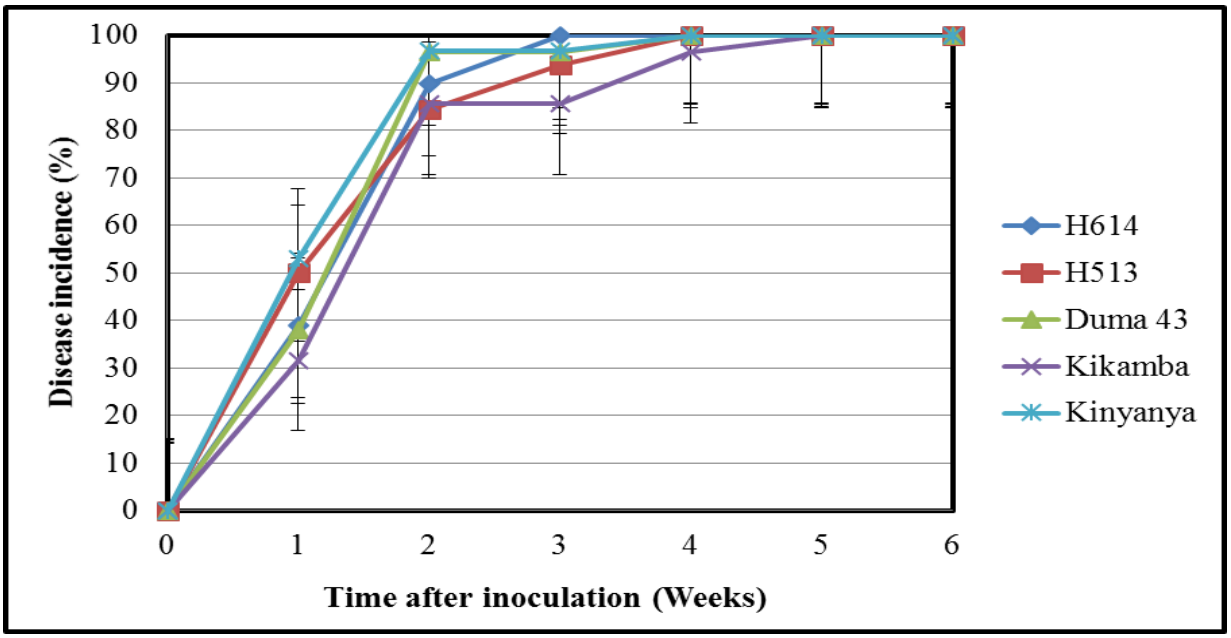

Figure 3.7 Disease incidence of different maize genotypes infected with MCMV and SCMV obtained from MLN infected plants. All the genotypes had a 100% disease incidence by week 5 post inoculation.

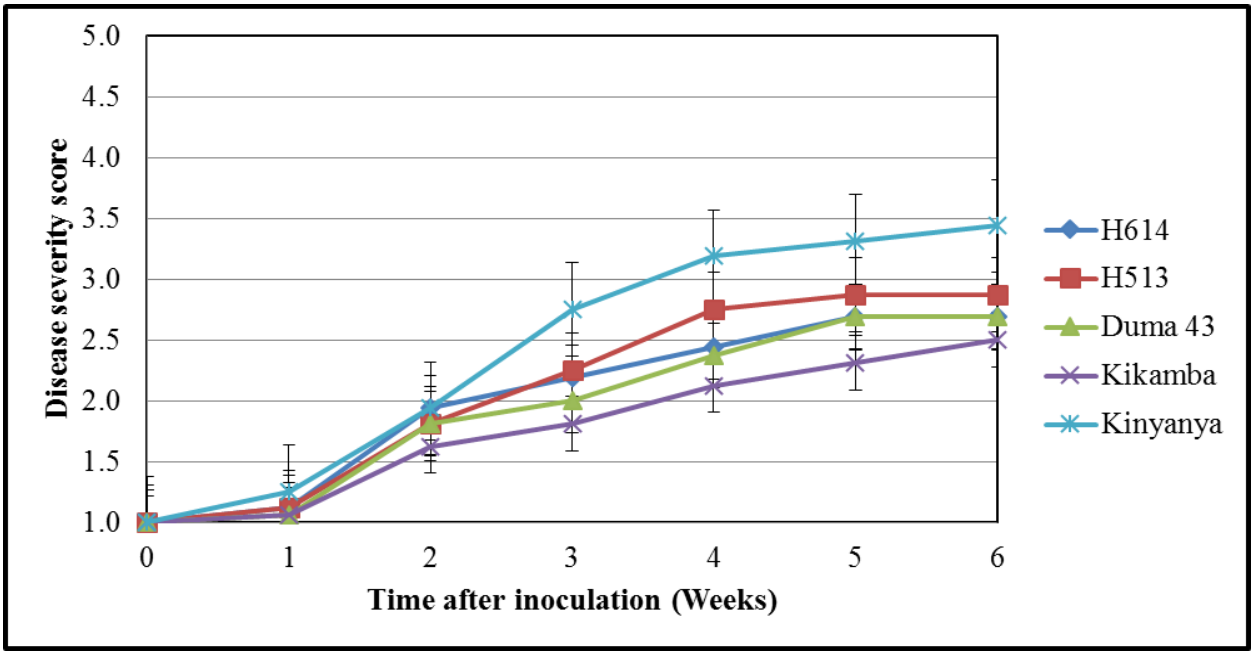

Figure 3.8 Disease severity in different maize genotypes inoculated with inoculum from MLN infected plants.

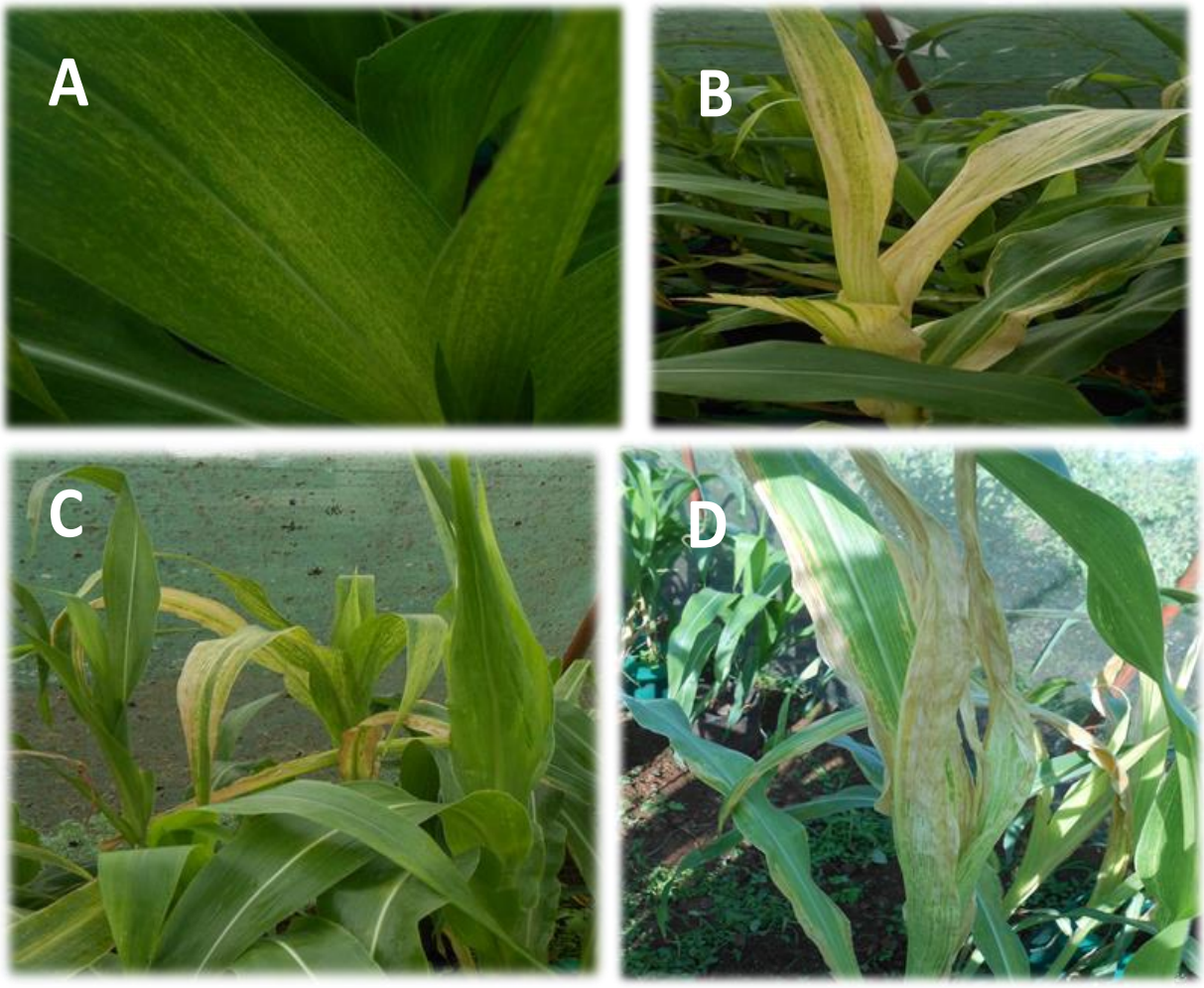

Plate 3:5 Symptoms observed on MLN infected plants (A) Chlorotic leaf (B) Severe chlorosis leading to yellowing of leaves (C, D) setting in of necrosis from the leaf margins.

3.3.5 Area under disease progress curve

The coinfections recorded the highest AUDPC with plants infected with SCMV+MCMV having the highest area under disease followed by the MLN infected plants. The single viruses recorded the least AUDPC with SCMV being the least (Table 3.1). The variety means for the single viral infections and for the MLN were not significantly different (P=0.05). However, there was some difference recorded for the doubly infected plants where the landraces had more disease compared to the hybrids.

Among the genotypes, the landraces had higher AUDPC with Kinyanya being higher followed by Kikamba. The hybrids had lower AUDPC with H513 recording the least (Table 3.2). Some differences were recorded on treatment means for H513, Kikamba and Kinyanya. For maize variety H513, the coinfection (MLN) was significantly different from all the other treatments while the coinfection (SCMV +MCMV) was different from the MCMV. For Kikamba, the coinfected plants reacted differently from the rest of the viral treatments with the same displayed by Kinyanya. Over all, the coinfections reacted differently from single infections.

Table 3.1 Area under disease for the single *Sugarcane mosaic virus*, *Maize chlorotic mottle virus* and coinfections per different maize genotypes.

Variety	H614	H513	Duma 43	Kikamba	Kinyanya
SCMV	15.00ab	14.59bc	14.78ab	14.84b	15.09b
MCMV	13.72b	13.35c	14.22b	13.94b	14.72b
MCMV+SCMV	17.10a	15.59b	17.56a	21.90a	22.70a
MLN	17.05a	17.47a	17.06ab	15.28b	20.47a
Control	0.00c	0.00d	0.00c	0.00c	0.00c
$LSD^{p=0.05}$	2.562	1.632	2.908	2.427	3.073
Pvalue	<.001	<.001	<.001	<.001	<.001

Key: The letters indicate the significance between the treatments on different varieties. Figures followed by the same letters are not significantly different at P=0.05.

Table 3.2: Area under disease for the different maize genotypes inoculated with single *Sugarcane mosaic virus*, *Maize chlorotic mottle virus* and coinfections

Variety	SCMV	MCMV	MCMV+SCMV	MLN	Control
H614	15.00a	13.72a	17.1b	17.05b	0.00a
H513	14.59a	13.35a	15.59b	17.47b	0.00a
Duma 43	14.78a	14.22a	17.56b	17.06b	0.00a
Kikamba	14.84a	13.94a	21.9a	15.28b	0.00a
Kinyanya	15.09a	14.72a	22.7a	20.47a	0.00a
$LSD^{p=0.05}$	1.332	0.941	3.612	4.193	
Pvalue	0.932	0.071	0.003	0.174	

Key: The letters indicate the significance within the treatments. Figures followed by the same letters are not significantly different at P=0.05.

3.3.6 Confirmation of the presence of the *Maize chlorotic mottle* and *Sugarcane mosaic viruses* through serological test

3.3.6.1 ELISA test results

A total of 20 samples were collected for serological analysis per treatment, four samples per variety. Maize variety Duma 43 which showed least disease severity due to infection by SCMV (Figure 3.2) recorded high OD values indicating the plants were infected but remained asymptomatic (Table 3.2). All samples for all genotypes tested positive for SCMV except for variety H614 which had half the samples positive (Table 3.2).

Similarly for the MCMV, Duma 43 which showed least disease severity recorded the highest OD values indicating the plants were infected but remained asymptomatic. Sixteen samples were positive for the virus while only two were negative (two for H614 and two for Kikamba) (Figure 3.4).

The coinfected plants (SCMV+MCMV) displayed high viral titers for the MCMV, with the genotypes showing the least disease severity recording high viral titers. For example, Kinyanya which was leading in severity had the least OD values (Figure 3.6). Overall, the hybrids; H614, H513 and Duma 43 had higher titers while the landraces, Kikamba and Kinyanya had lower viral titers. On the other hand, the viral titer for the SCMV was relatively low in the same samples (Table 3.3). The landraces recorded higher titers compared to the hybrids. The samples were subjected to both the MCMV and the SCMV virus tests.

Samples collected for the MLN treatment were subjected to both MCMV and SCMV analysis. Out of the 20 samples, 9 samples tested negative for the MCMV virus in the MLN treatment.

Variety H614 had two of its samples testing positive while the other two were negative. All samples for H513 were negative for the virus while Duma 43 had one positive sample while the other three samples were negative. The landrace Kikamba, had three positive samples while one was negative. Kinyanya had two positive samples with the other two samples giving negative results (Table 3.3).

In the coinfection (SCMV + MCMV), Kikamba had the highest SCMV titers followed by Duma 43 while H513 had the least titers. On average, the viral titers were relatively low with (Kikamba) recording the highest followed closely by H614. H513 had the least titers (Table 3.2).

Table 3.3 The number of positive samples from the serological tests for the *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* for the different viral treatments

Variety	SCMV	MCMV	SCMV+MCMV (SCMV)	SCMV+MCMV (MCMV)	MLN (SCMV)	MLN (MCMV)
H614	2	2	3	4	3	3
H513	4	4	3	2	3	1
Duma 43	4	4	3	3	3	2
Kikamba	4	2	4	4	4	3
Kinyanya	4	4	4	2	4	2
Negative controls	0	0	0	0	0	0

Footnote: In total 20 samples were collected per treatment four samples for each of the five genotypes with the samples for the coinfection treatments (SCMV+MCMV) and MLN being subjected to both the MCMV and SCMV viral analyses.

3.3.5.2 Titer comparisons between single infections and co-infections

After serological test for the samples, the ratio of SCMV to MCMV in single infections remained at 1:1. However, this changed when the plants were coinfected with both the SCMV and the MCMV. Most samples recorded two fold increase in titer concentration for the MCMV.

Some samples recorded as high as three fold increase in the titer level for the MCMV in coinfected plants. This was not affected in the singly infected plants (Table 3.4).

Sugarcane mosaic virus (SCMV) viral titers remained at the ratio of 1:1 at both the single and coinfection a clear indication of no significant change if its in single infection or in synergistic infection with the *Maize chlorotic mottle virus* (Table 3.4).

Table 3.4 Mean viral titers for the maize genotypes inoculated with SCMV, MCMV and the coinfections.

Varieties	SCMV	MCMV	SCMV + MCMV (SCMV)	SCMV + MCMV (MCMV)	MLN (SCMV)	MLN (MCMV)
H614	1.140	1.180	1.380	2.350	1.226	1.101
H513	1.370	1.260	1.193	1.987	1.143	0.760
Duma 43	1.139	1.720	1.237	1.930	1.210	1.420
Kikamba	1.120	1.206	1.444	2.500	1.260	1.860
Kinyanya	1.049	1.410	1.293	1.398	1.157	1.320
Control (Healthy Plants)	0.313	0.426	0.313	0.426	0.313	0.426
Negative Control (kit)	0.300	0.466	0.357	0.516	0.357	0.516

Footnote: The MCMV co-infection had two fold the titer levels for the single virus.The SCMV remained at the ratio of 1: 1 in both infections.

3.3.7 Discussion

Different factors determine the reaction and symptom expression of the maize plants to viral infection. These include, the maize genotype, the viruses and their combinations and the prevailing environmental conditions (Wangai *et al*., 2012; Perera *et al.,* 2012).

The reaction of the selected maize genotypes to the viral inoculations varied between the landraces and the hybrids. The hybrids exhibited more tolerance as they developed symptoms later than the landraces. A similar observation was made in a previous study where H614D hybrid was reported to be tolerant to the MLN disease as compared to landraces and other commonly used genotypes (Das *et al.,* 2015). Hybrids are known to have superior performance both in yields and tolerance to the pests and diseases (Russell, 1991). Some hybrid plants even remained symptomless to the infection but showed high viral titers during the Elisa viral diagnosis.

All genotypes developed symptoms due to the viral infections albeit at different levels of severity and time. Varietal reaction to SCMV was rapid after the inoculations with symptoms developing within four days post inoculation. The rapid expression of symptoms by SCMV can be attributed to the HC-pro protein present in the potyvirus that inhibits the antiviral defence mechanism in the plants while assisting in the vascular transport of the virus (Savenkov and Valkonen, 2001). The plants developed light green mosaic and mild mottles on the younger leaves. The observed symptoms were similar to those earlier described by Wu *et al.* (2012) on maize plants. The plants infected with the MCMV were however stunted and there was general chlorosis while some of them developed necrosis on the upper leaves. Similar symptoms had been described by Uyemoto *et al.* (1981). Kikamba landrace had a higher severity while Duma 43 hybrid recorded least severity. Viruses rely on the metabolism of the plant cells for their replication (Cronin *et al*.,

1995). In the process of the infection, there is disruption of the chloroplast function which leads to inadequate chlorophyl production hence mottling and mosaic symptom development (Mbega *et al*., 2016).

In the coinfection of SCMV and MCMV where the viruses were mixed in equal ratios after extraction, the reaction was more severe leading to the development of the Maize lethal necrosis (MLN) disease. The plants developed severe chlorosis accompanied by leaf necrosis on the young leaves starting from the leaf margins leading to a dead heart. This is attributed to reduced level of chlorophyll and a reduced ratio of chloroplasts to cytoplasmic rRNA in the plant cells (Goldberg and Brakke, 1987). The coinfected cells also have small starch grains in the chloroplasts and disrupted mitochondria leading to reduced respiration and photosynthesis (Wang *et al*., 2017). This combination of reduced respiration rate and photosynthesis leads into leaf necrosis and eventual plant death (Wang *et al*., 2017). This is exactly what happened in the earlier reported incidences of the disease in the USA (Niblet and Claflin, 1978; Uyemoto *et al*., 1981). When the two MLN-causing viruses were isolated from the same plant and used for inoculations, a relatively lower disease severity was observed. This is a clear indication that one of the two viruses involved in the coinfection was relatively reduced hence the reduction in the severity.

The serological results showed a difference in the concentration of MCMV in the co-infected plants. Whereas SCMV titers remained almost constant in the single and the double infections; that for MCMV was in some samples up to three fold the titer of the single MCMV infections. The hybrids had higher titers for the MCMV in the coinfection SCMV and MCMV as compared to the landraces. When in synergistic infection with the potyvirus; the MCMV replication is enhanced hence the increased accumulation of MCMV in the plant tissues. This high

accumulation has epidemiological implications on the spread and persistence of the virus. The rate of transmission by vectors and mechanically will be increased. The virus is also likely to persist for long due to its high concentrations (Fondong *et al.,* 2000). On average, the concentration of MCMV in dual infections was two fold. This tallies with what was reported earlier by Goldberg and Brakke (1978) when the MCMV was in co-infection with MDMV-B, although the titer concentrations for the MCMV are relatively low as compared to what they reported. The difference could probably be due to the different ratios used in preparing the viral cocktail used for inoculations.

The hybrids were not severely affected and the viruses were able to replicate and hence have high accumulation of the viruses unlike the landraces which start dying earlier hence no much virus accumulation. Plants have developed some resistance mechanisms to help them overcome pathogen invasion (Dangl and Jones, 2001). They can use various methods including avoidance, resistance and tolerance for protection (Vale *et al*., 2001). Resistance in plants can either be qualitative where there is complete resistance and the resistance is brought about by a major or single gene or quantitative or incomplete resistance where resistance is controlled by one to multiple minor genes (Poland *et al*., 2009). In maize, resistance to the *Sugarcane mosaic virus* has been mapped to major QTLs in chromosome 3 and 6. A minor QTL has also been mapped on Chromosome 10 and this has additional modifier effect on the major QTLs which work in an additive and dominance way (Xu *et al*., 1999; Xia *et al*., 1999). Resistance to the MLN disease has been identified in three major QTLs in chromosome 3 and 6 (Olsen *et al*., 2016). The hybrids could be having more QTL for SCMV resistance unlike the landraces hence the less infection. Resistance in maize is also owed to the posttranscriptional gene silencing (PTGS) mechanism which is involved in the degradation of the viral RNA (Incarbone and Dunoyer, 2013). The

viruses have to overcome this for infection to occur. There could be differences between the hybrids and the landraces on the strength of the PTGS mechanism hence the differences in susceptibility.

In synergistic reactions there is increased severity due to the accumulation of the most virulent virus. In this scenario, the MCMV seems to be the most virulent virus and its accumulation in the host plants when in co-infection with the SCMV is enhanced. The potyvirus could be facilitating the replication and the rapid movement of the MCMV within the tissues, which when on its own is not rapid (Calvert and Ghabrial, 1983; Goldenberg and Brakke, 1987; Vance, 1991). The SCMV provides two proteins (HC-Pro and Nuclear inclusions, protein a (NIa) and viral genome linked protein (VPg) that enhance MCMV replication leading to severe symptoms (Kreuze, 2002). The VPg also interacts with the maize elongin C protein (ZmElc) reducing its production (Zhu *et al*., 2014) which enhances the replication of the MCMV (Mbega *et al*., 2016). The VPg protein also enhances the cell to cell translocation of both the SCMV and the MCMV (Barker, 1989; Cronin *et al*., 1995).

During the cropping period, farmers should be encouraged to maintain field sanitation/hygiene especially on plant handling to minimise spread of the MLN causing viruses from plant to plant since some plants/ genotypes like Duma 43 have been demonstrated to harbour the viruses in single infections and show no symptoms at all.

In conclusion, MCMV and SCMV infected plants produced more severe symptoms leading to the MLN disease development. Disease development, be it the single infections or coinfections is highly dependent on the maize genotypes involved. The landraces were highly susceptible while the hybrids showed a degree of tolerance. Farmers prefer planting the landraces because they are

readily available and cheap. This practice should, however, be discouraged and farmers encouraged to acquire the more tolerant certified hybrid seeds to increase food security and help in combating the MLN disease.

CHAPTER FOUR

4.0 Transmission of Maize lethal necrosis disease -causing viruses from crop debris and soil

Abstract

The role of plant debris and contaminated soil in the epidemiology of viruses causing maize lethal necrosis (MLN) disease is of importance to the management of the disease. A greenhouse study was carried out to determine the transmission of viruses causing MLN from crop debris and soil to healthy young plants. The treatments included plants inoculated with *Sugarcane mosaic virus* (SCMV), *Maize chlorotic mottle virus* (MCMV), co-infections (SCMV+MCMV) and inoculum obtained from MLN-infected plants. The genotypes used were H614, H513, and Duma 43 representing the hybrids and Kikamba and Kinyanya representing the landraces. The plants were first inoculated with the respective viruses and grown for two months after which the plant materials were chopped and incorporated into one set of planting bags while another was left without debris but had the soil previously holding infected plants. In the third season, all plant debris were removed from the bags and replanted with same maize genotypes to assess if the viruses were still present in the soil. Disease severity was scored on a scale of 1-5 and area under disease progress curve determined. Viral infection was confirmed using double antibody sandwich enzyme linked immuno-sorbent assay (DAS-Elisa). There was no significant difference in infection of plants by viruses either from the soil with debris or with infested soil without debris. However, the landraces recorded high disease incidences for most of the treatments and higher disease severity. Additionally, they had a bigger area under disease. On the viruses, SCMV+MCMV combination had a bigger area under disease. On the Elisa test results, most of the samples (58.3%) tested positive for MCMV while none were positive for the SCMV.

On the third planting; there were only few plants that showed disease symptoms. Of the tested samples, 28% were positive for MCMV, while none were positive for SCMV. The SCMV symptom which included light green mosaic and mild mottle on the younger leaves, were however very distinct and clear on the plants. The above experiment demonstrates that viruses causing MLN disease can be acquired easily from the soil irrespective of whether there is debris or not or the variety is hybrid a or a landrace so long as there were plants infected before in the same soil. Hence crop rotation with crops not in the grass family like beans, carrots, cabbage and onions will help in reducing the recurrence of the disease.

4.1 Introduction

Maize lethal necrosis (MLN) is a complex disease caused by synergistic interaction between the *Maize chlorotic mottle Machlomovirus* (MCMV) and any of the maize infecting potyviruses (Goldberg and Brakke, 1987; Scheets, 1998). The potyviruses involved include *Wheat streak mosaic Rymovirus* (WSMV), *Maize dwarf mosaic Potyvirus* (MDMV) and *Sugarcane mosaic virus* (SCMV). Maize lethal necrosis disease is widespread all over the world including Peru, USA, Argentina, Mexico and China (Castillo and Hebert, 1974; Niblett and Clafin, 1978; Gordon *et al.,* 1984; Carrera-Martínez *et al.,* 1989; Xie *et al.,* 2011). In Kenya, the disease was confirmed to be caused by the synergistic interaction between MCMV and SCMV (Wangai *et al.,* 2012; Adams *et al.,* 2013).

In the USA, repeated outbreaks of the MLN were attributed to MCMV virus surviving in the soil hence causing disease outbreaks season after season. The spread was enhanced by the presence of the corn rootworm (Uyemoto, 1983). The virus can overwinter and survive in ploughed in corn stubble and maize residues in the absence of maize (Uyemoto, 1983; Montenegro and

Castillo, 1996). Experiments to demonstrate the efficiency of crop rotation confirmed that disease incidences were high in plots that had maize the previous year while those that had other crops remained free of the disease. This is a clear demonstration that the virus overwinters in the soil and plant debris (Phillips *et al*., 1982; Uyemoto, 1983).

Sugarcane mosaic virus (SCMV) has been isolated from most parts of infected plants. These include leaf, stem, roots, cob, seed, sheath tissues, kernel, anther, husks and silk. The virus was also detected in immature kernel, root and terminal leaf tissues of dry eared plants (Uyemoto, 1980; Jiang *et al*., 1992). In studies done to confirm soil transmission of the virus, non inoculated sorghum plants became infected with the SCMV when grown in containers with infected plants indicating the possibility of soil transmission (Bond and Pirone, 1970). The scenario in Kenya since the outbreak of MLN disease is similar to that observed in the USA where there was an outbreak after every season of maize planting. Thus for proper management of this disease, it is important to determine the role of the soil and plant debris in continuous cropping systems to the disease outbreak.

4.2 Materials and methods

4.2.1 Source of plant materials and genotypes used

Maize plant debris from the previous trial (described in Section 3.2) were severed into pieces and the debris incorporated into the same soil used earlier for planting. The previous trial consisted of *Sugarcane mosaic virus* (SCMV), *Maize chlorotic mottle virus* (MCMV), co-infections (SCMV+MCMV and MLN inoculum) and the negative control. The trial was used to confirm if the viruses survive in the plant remains and soil or not. The debris-soil mixture was

then planted with the maize genotypes earlier planted in the same pot. These genotypes had earlier been acquired from the University of Nairobi, Kabete Campus' field station seed store. These had been in the store before the MLN disease outbreak. These were monitored for any disease symptoms after planting and before inoculations. The genotypes included H614, H513, Duma 43, Kikamba and Kinyanya. Planting was done two weeks after debris incorporation. In each treatment, half of the bags (4) were planted without the debris while half of the bags (4) had the debris. The treatments were SCMV + debris, SCMV-No debris, MCMV+debris, MCMV-No debris, MCMV+SCMV+debris, MCMV+SCMV-no debris, MLN+debris, MLN-no debris, Control+debris and control-no debris. These were replicated 4 times with one bag per treatment and arranged using the CRD experimental design. After six weeks of data collection, the crop was destroyed and the soil re-used for planting the same genotypes for the third time. There was no debris incorporation this time round. This constituted the third planting on the same soil.

4.2.2 Disease assessment and data analysis

Determination of disease severity, incidence, serological tests and data analysis were done as described in section 3.2.4 and 3.2.5 above.

4.3 Results

4.3.1 Rate of transmission of viruses from soil with debris and soil alone

4.3.1.1 Disease incidence, symptoms and severity of SCMV in maize planted in soils with infected plant remains

The plants in contaminated soil plus debris generally had more affected plants than those in contaminated soil alone. However, no difference was noted between genotypes except in weeks 4, 5 and 6. Plants in debris started off with a higher severity when compared to those in the soil alone (Figure 4.2) although there was no significance in severity among the different maize genotypes. The landraces showed disease symptoms immediately on emergence while the hybrids exihibited symptoms later (Figure 4.1). The landraces also had a higher incidence than the hybrids. Kinyanya in contaminated soil plus debris had more affected plants than those in the soil alone. The same trend was observed on the disease severity whereby the landraces recorded more severity than the hybrids.

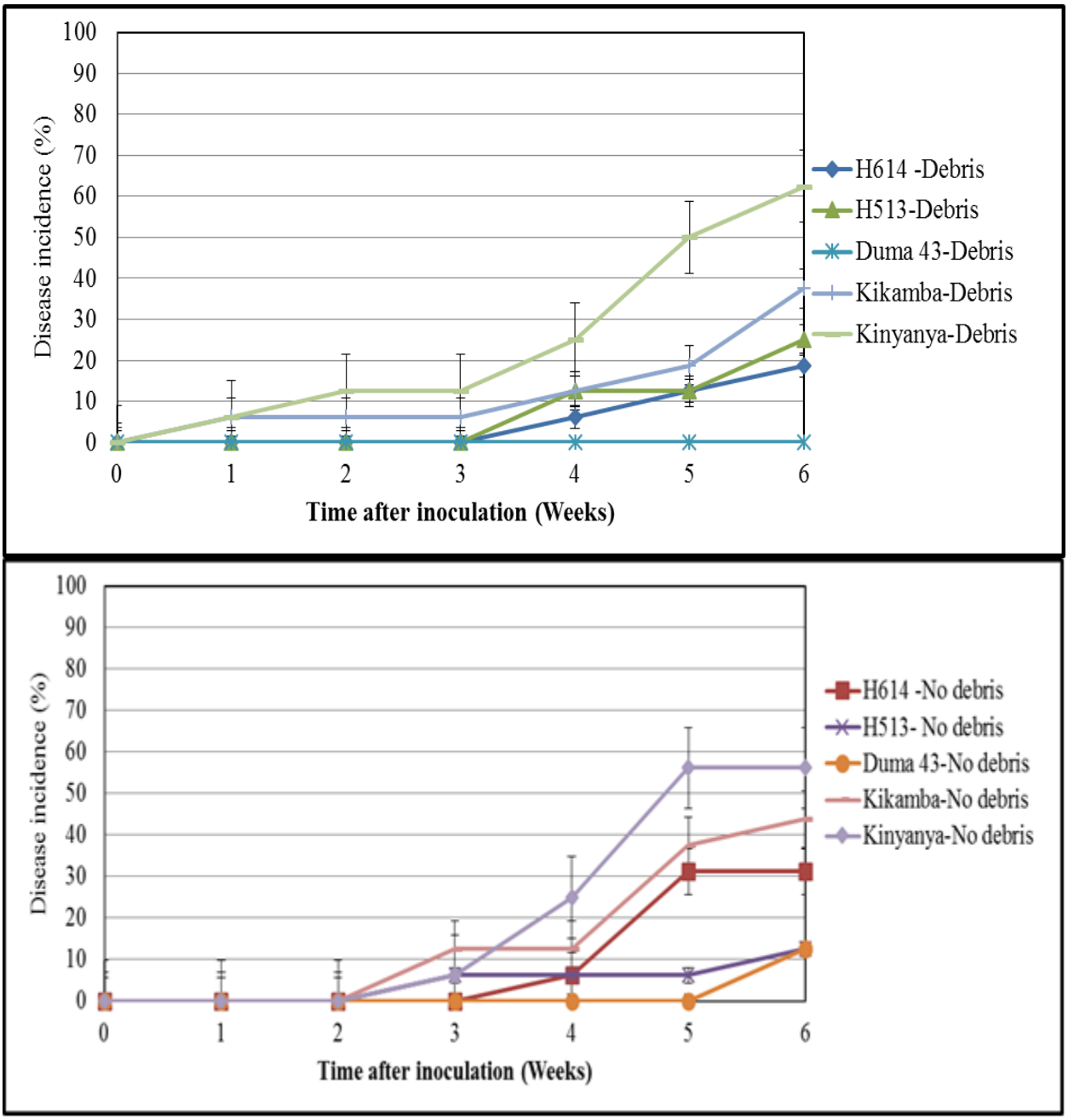

Figure 4.1 Disease incidence in maize genotypes planted in *Sugarcane mosaic virus* contaminated debris (Top) and soil (Bottom).

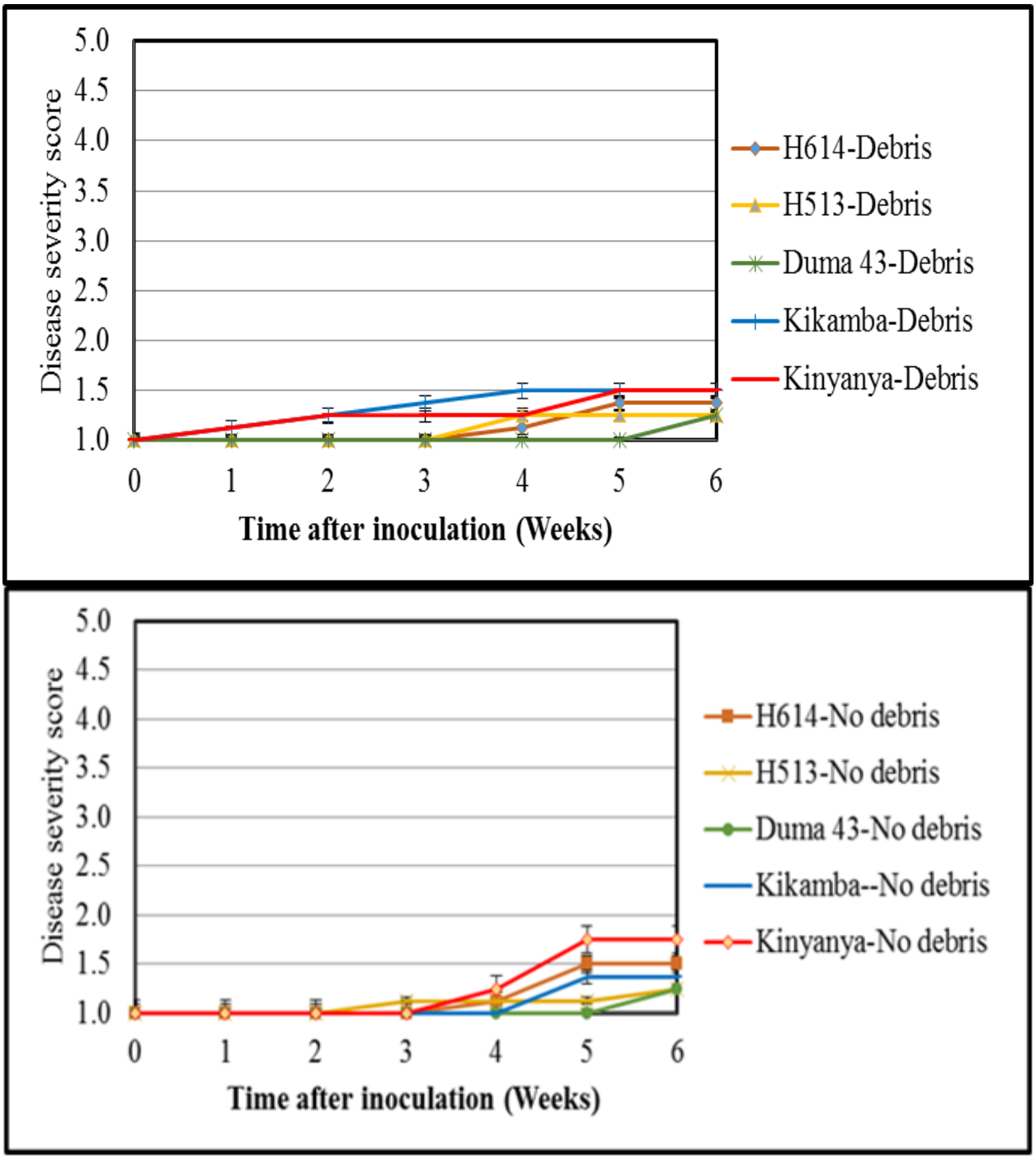

Figure 4.2 Disease severity recorded for *Sugarcane mosaic virus* for maize genotypes planted in soil with contaminated debris (Top) and without debris (Bottom).

4.3.1. 2 Disease incidence, symptoms and severity for MCMV contaminated soil and debris

Genotypes planted in soil with debris showed symptoms earlier than those in soil alone (Figure 4.3). The symptoms seen included chlorosis and mottling of the leaves. The symptoms were observed in H614, H513 and Kikamba by week 1. No disease incidence was reported for Duma 43 in soil with debris. Kikamba had high incidences in the soil incorporated with debris although those without debris followed closely. Kikamba without debris displayed some difference from H614, Kinyanya and Duma at week 5. This was similar in the disease severity whereby Kikamba in soil with debris had higher severity as compared to that without debris (Figure 4.4). Generally, the maize genotypes acquired the viruses almost equally from the soil and debris.

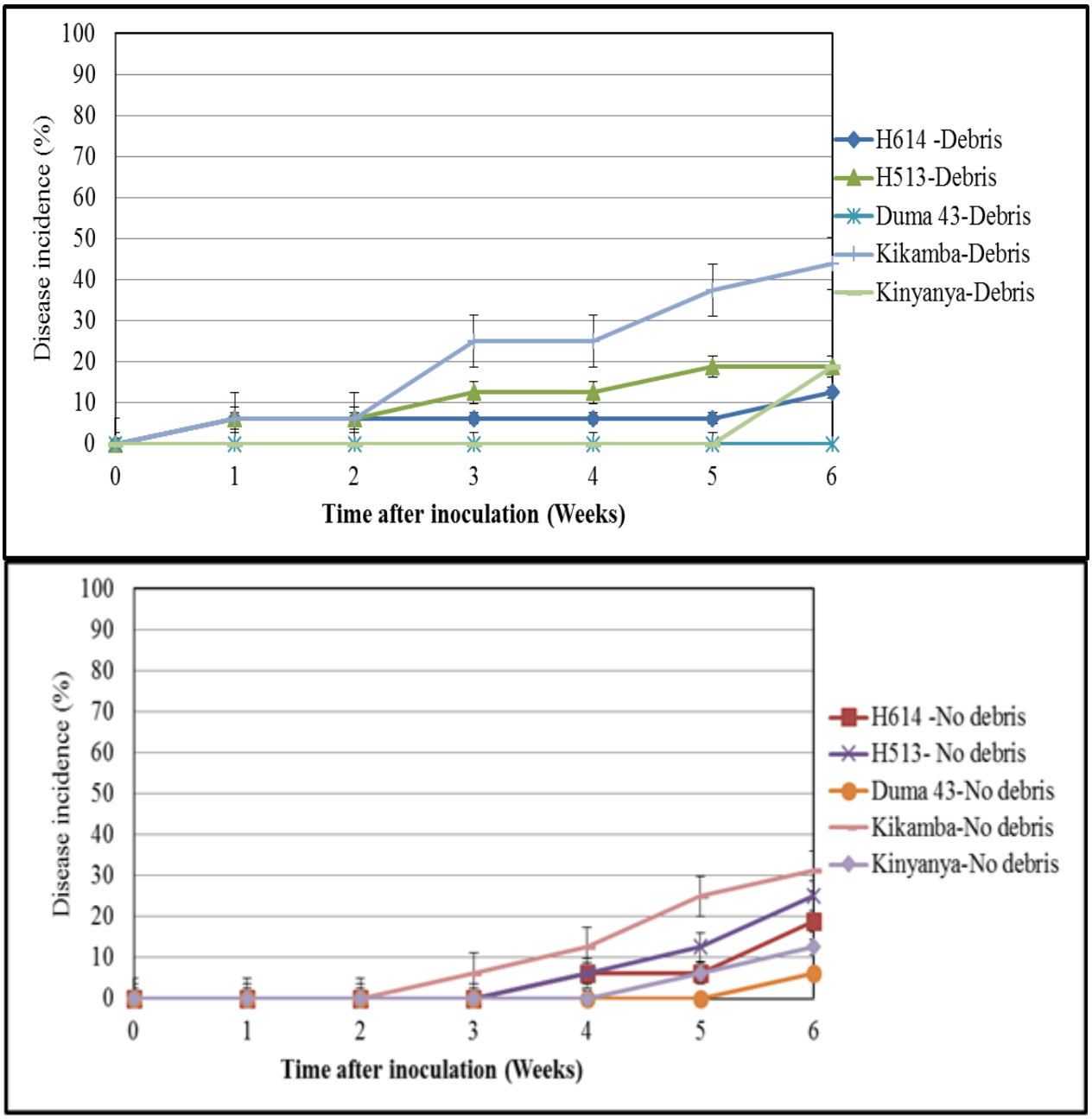

Figure 4.3 Disease incidence in maize genotypes planted in *Maize chlorotic mottle virus* debris (Top) and infested soil (Bottom.

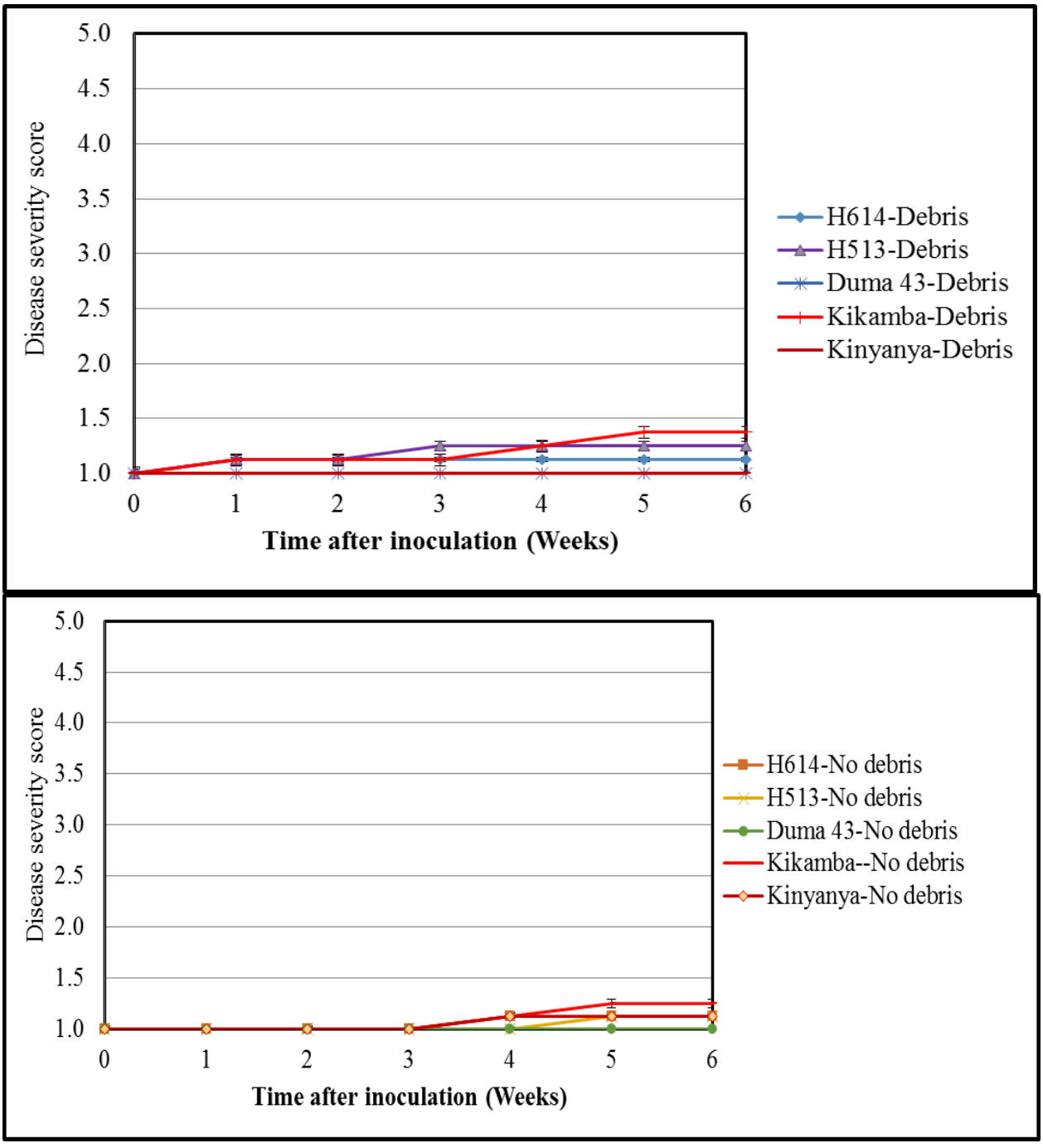

Figure 4.4 Disease severity recorded for maize genotypes planted in *Maize chlorotic mottle virus* contaminated debris (Top) and without debris (Bottom).

4.3.1.3 Disease incidence, symptoms and severity for MCMV+SCMV contaminated soil and debris

Plants for the landraces started showing symptoms immediately after emergence for both in the soil with debris for Kikamba as well as for those in soil alone for Kinyanya (Figure 4.5). There was general chlorosis which led to severe necrosis of some of the infected plants. Those with no debris were more as compared to those in the soil with debris albeit not significantly (P=0.05) different. Overall, Kinyanya had more plants affected than the other genotypes.

On disease severity, the same trend was observed where the plants in soil without debris were more affected and showed higher severity. Kikamba was more affected than the other genotypes and had some of its plants dying from the infection (Figure 4.6). There was however no significant difference among the genotypes.

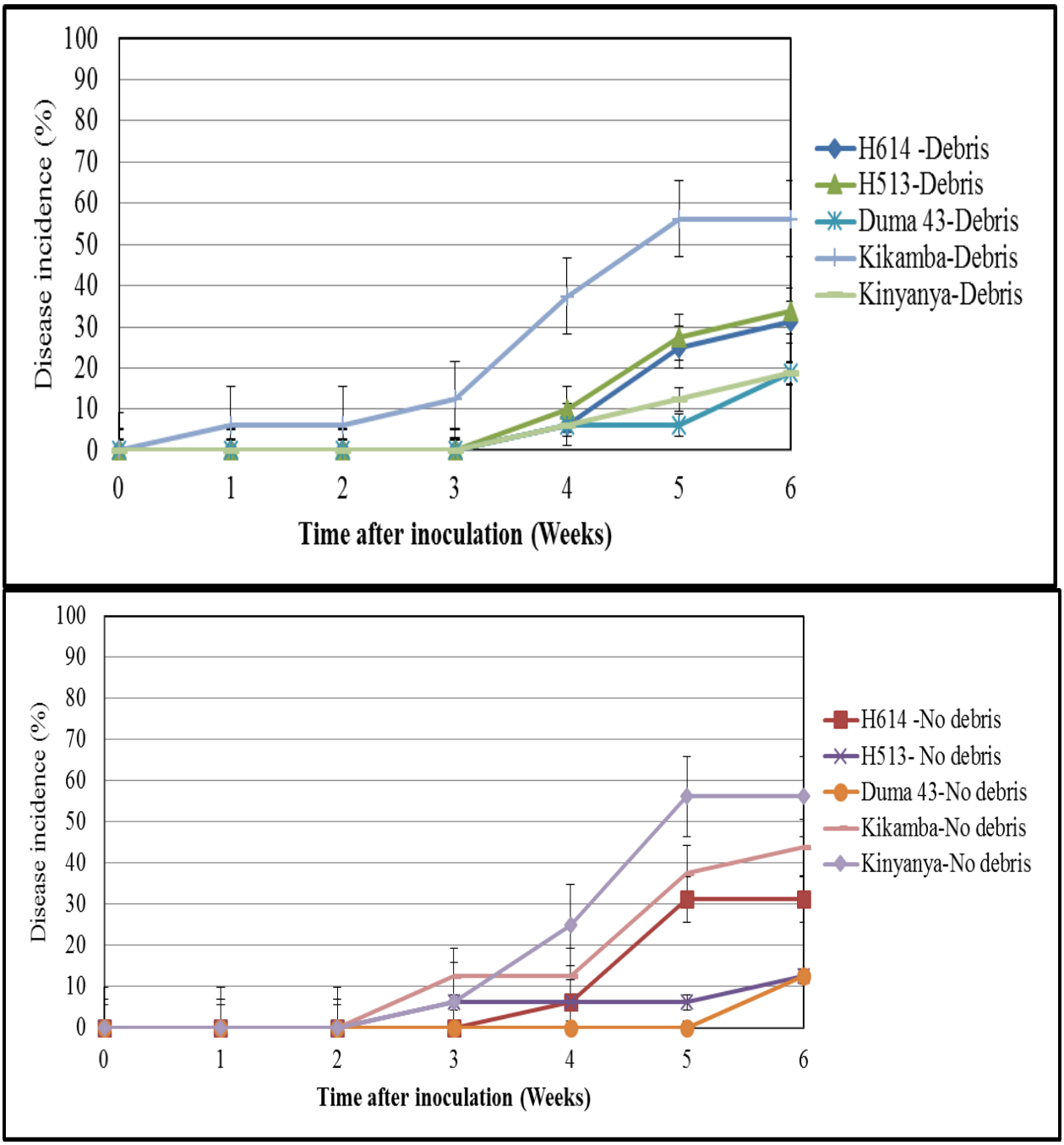

Figure 4.5 Disease incidence for maize genotypes planted in *Maize chlorotic mottle virus* + *Sugarcane mosaic virus* contaminated debris (Top) and soil (Bottom).

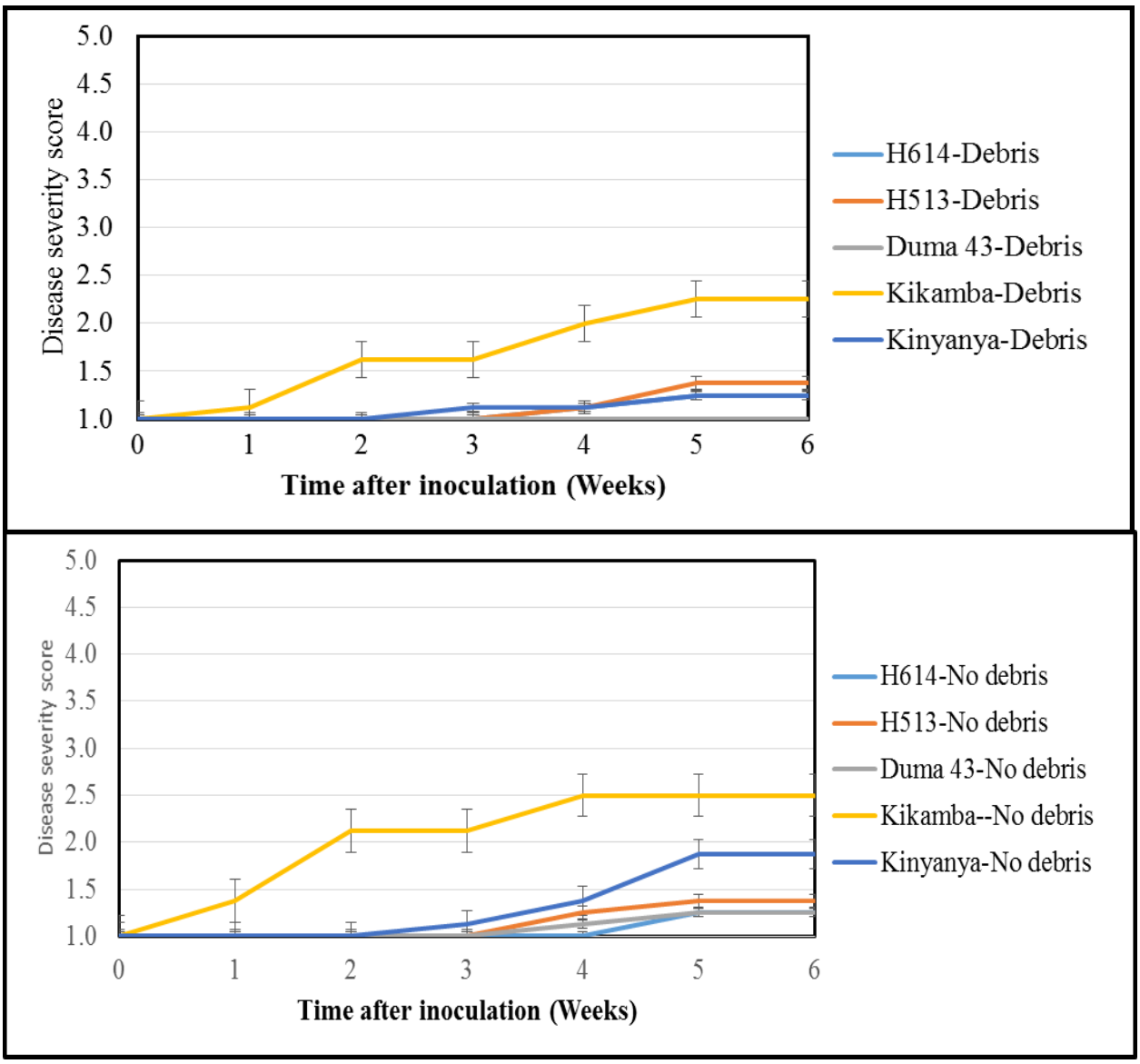

Figure 4.6 Disease severity recorded for maize genotypes planted in contaminated debris (Top) and soil (Bottom) coinfected with *Maize chlorotic mottle virus* and *Sugarcane mosaic virus.*

4.3.1.4 Disease incidence, symptoms and severity for MLN contaminated soil and debris

For disease incidence, Kikamba had more affected plants than the other genotypes and it was significantly different from all the others through weeks 4-6 for the plants in soil without debris (Fgure 4.7). For disease severity, there was no difference noted among the genotypes, however those in the soil without debris recorded more severity when compared to those in the soil with debris (Figure 4.8).

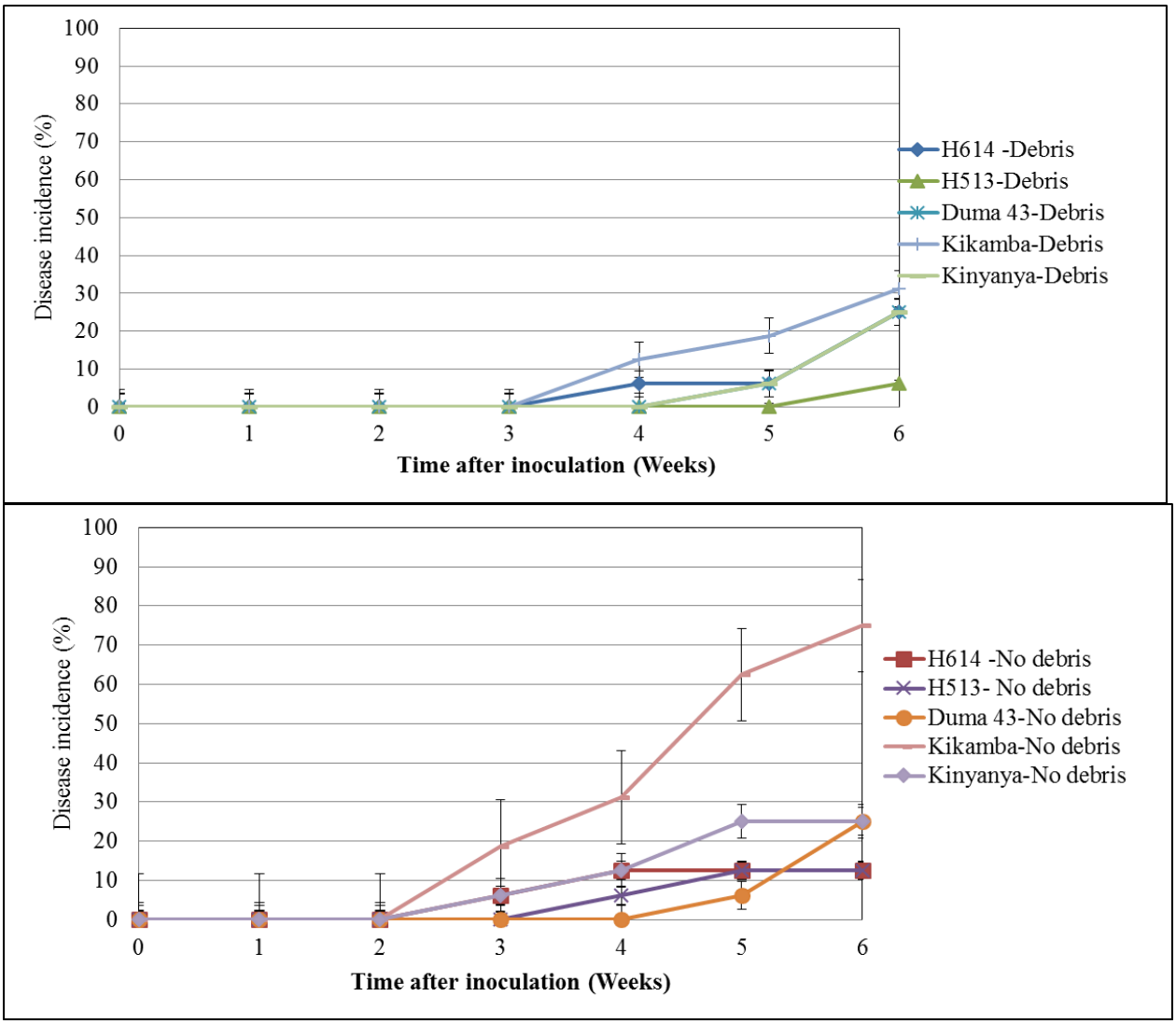

Figure 4.7 Disease incidence in maize genotypes planted in coinfection of maize lethal necrosis contaminated debris (Top) and soil (Bottom).

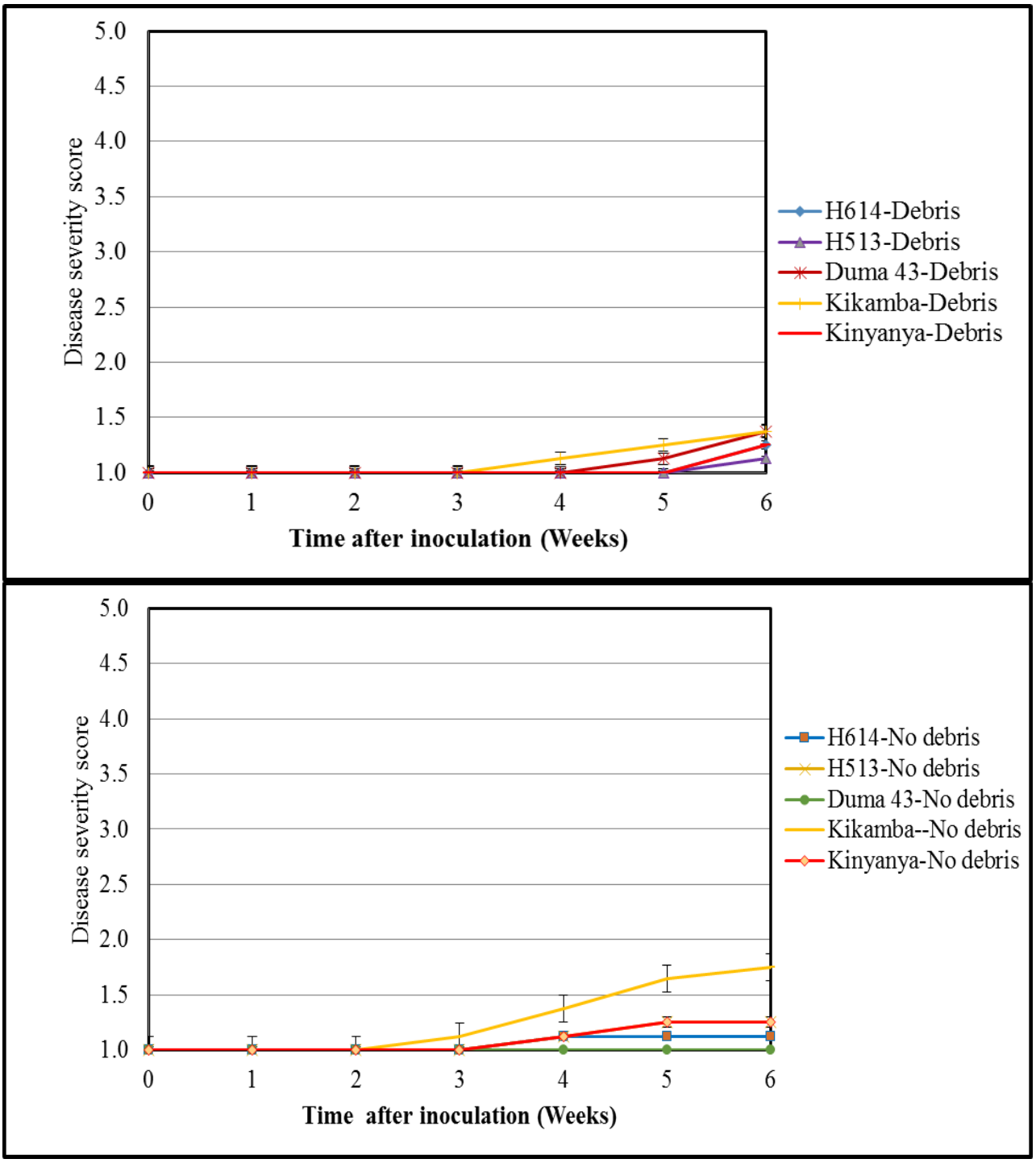

Figure 4.8 Disease severity recorded for MLN for maize genotypes planted in debris following coinfection of maize lethal necrosis (Top) and without debris (Bottom).

4.3.1.5 Area under disease progress curve

The AUDPC was high in plants in the bags where the SCMV and the MCMV were combined whereas those in soil plus debris and without debris had no significant difference. Plants in the SCMV infested soil and debris were second (although they were negative on serological test) and closely followed by those in the MCMV infested soil and debris. Plants in soil and debris infected with MLN coinfection had the least area (Table 4.1). The coinfections (SCMV+MCMV and MLN, were significant different (P=0.05).

Assessing the AUDPC for the five genotypes used in the experiment, Kikamba without debris had a large area under disease followed closely by Kikamba with debris. Duma 43 had the least AUDPC (Table 4.2). Some difference was recorded among the different variety treatments in Duma 43 with and without debris, H614 without debris and Kikamba without debris. In the first three scenarios, MLN was significantly different from the rest while in the last case, the combination was significantly different from the rest of the treatments.

Table 4.1 Area under disease for the single *Sugarcane mosaic virus*, *Maize chlorotic mottle* virus and coinfections for different maize genotypes

Variety	H614-No debris	H614-With debris	H513- No debris	H513- With debris	Duma 43 No debris	Duma 43 With debris	Kikamba No debris	Kikamba With debris	Kinyanya- No debris	Kinyanya With debris
SCMV	7.94a	7.88ab	7.50a	8.00ab	7.50a	7.50a	7.69b	9.62a	8.25a	9.06a
MCMV	7.75a	8.31a	7.56a	8.75a	7.50a	7.50a	7.81b	8.62a	7.56b	7.50b
MLN	6.50b	6.69b	7.62a	6.88b	6.50b	6.69b	7.62b	6.88b	8.06a	7.88b
MCMV+SCMV	7.63a	7.63ab	8.06a	7.80ab	7.81a	7.50a	14.44a	11.69a	8.50a	7.50b
Control (-Ve)	0.00c	0.00c	0.00b	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c	0.00c
$LSD^{p=0.05}$	0.43	1.29	0.85	1.74	0.43	0.16	4.34	4.90	0.65	2.04
Pvalue	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Key: The letters indicate the significance between the viral treatments on different varieties. Figures followed by the same letters are not significantly different at P=0.05.

Table 4.2: Area under disease for different maize genotypes inoculated with single *Sugarcane mosaic virus*, *Maize chlorotic mottle* virus and coinfections

Variety	SCMV	MCMV	MLN	MCMV+SCMV	Control (-Ve)
H614-No debris	7.94a	7.75a	6.50d	7.63c	0.00a
H614-With debris	7.88a	8.31a	6.69cd	7.63c	0.00a
H513-No debris	7.50a	7.56a	7.62abc	8.06bc	0.00a
H513-With debris	8.00a	8.75a	6.88bcd	7.80c	0.00a
Duma 43-No debris	7.50a	7.50a	6.50d	7.81c	0.00a
Duma 43-With debris	7.50a	7.50a	6.69cd	7.50ca	0.00a
Kikamba-No debris	7.69a	7.81a	7.62abc	14.44a	0.00a
Kikamba-With debris	9.62a	8.62a	6.88bcd	11.69ab	0.00a
Kinyanya -No debris	8.25a	7.56a	8.06a	8.50bc	0.00a
Kinyanya-With debris	9.06a	7.50a	7.88ab	7.50c	0.00a
$LSD^{p=0.05}$	**2.45**	**1.53**	**1.03**	**3.63**	
Pvalue	**0.69**	**0.57**	**0.02**	**0.01**	

Key:The letters indicate the significance within the treatments. Figures followed by the same letters are not significantly different at P=0.05.

4.3.1.6 Confirmation of the *Maize chlorotic mottle* and *Sugarcane mosaic viruses* through serological tests

For each treatment, two samples were collected for serological analysis making a total of four samples per variety and twenty samples per each virus treatment. None of the samples for SCMV virus tested positive upon serological analysis while all the samples for H614 were positive for MCMV (Table 4.3). Samples from H513 in debris all tested positive while one of the samples from no debris was negative. All Duma 43 samples from no debris were positive while one was positive for the samples from no debris. Kikamba had one sample from no debris positive while those for debris were positive. None of the samples for Kinyanya was positive for MCMV. Out of the twenty samples tested for MCMV, thirteen of them were positive while the rest were negative. Seven of the positive samples were from the soil with the infected debris while the other six were from the soil without debris (Table 4.3).

In the coinfection of MCMV + SCMV treatment, all the samples for H614 without debris were positive for MCMV while only one was positive for the soil plus debris. This was similar for the H513. Samples for Duma 43 without debris were all negative for the MCMV while only one was positive for those with debris. For the landraces, Kikamba had one positive for plants with debris and one without debris while Kinyanya had all those samples without debris positive while those with debris were all negative. None of the samples was positive for the SCMV (Table 4.3).

Lastly, on the coinfection of MLN treatment serological analysis for MCMV, samples for H614 from debris contaminated soil were positive while one sample was positive from the contaminated soil alone. H513 had all its samples from contaminated soil alone positive while one from the infested soil plus debris was positive. Duma 43 had only one positive sample from those in the soil with debris while the rest were negative. Kikamba had two of its samples from

soil without debris positive while one was positive for the samples from soil with debris. Kinyanya had only one sample positive. This was from the sample from the soil with debris. In summary, eleven samples were positive for the MCMV while nine were negative. Out of the positive samples, six were from the soil with debris while five were from the soil without debris. No sample was positive for the SCMV (Table 4.3).

Table 4.3 The number of positive samples after serological analysis for the MCMV for single and coinfections for each variety treatment

Variety	MCMV	SCMV+MCMV (MCMV)	MLN (MCMV)
H614-No debris	2	2	1
H614-With debris	2	1	2
H513-No debris	1	2	2
H513-With debris	2	1	1
Duma 43-No debris	2	0	0
Duma 43-With debris	1	1	1
Kikamba-No debris	1	1	2
Kikamba-With debris	2	1	1
Kinyanya-No debris	0	2	0
Kinyanya-With debris	0	0	1
Negative controls-No debris	0	0	0
Negative controls-With debris	0	0	0

Footnote: In total 20 samples were collected per viral treatment four samples for each of the five genotypes (Two for debris and two for without debris) with the samples for the coinfection treatments (SCMV+MCMV) and MLN being subjected to both the MCMV and SCMV viral analyses. None of the samples tested positive for SCMV.

4.4 Third planting with no debris incorporation

4.4.1 Disease incidence, symptoms and severity for *Sugarcane mosaic virus* contaminated soil

Duma 43 and Kikamba showed symptomatic plants one week after planting (Figure 4.9). However, disease incidence was low. The symptoms included fine chlorotic streaks on all the leaves. By week 3, at least all the genotypes had some plants exhibiting the SCMV symptoms with Kikamba leading while Duma 43 had the least affected plants. On incidence, Kikamba was significantly different from the rest of the genotypes at P=0.05 as it had more affected plants. Generally, the severity on the affected plants was low with most of the plants showing only the fine chlorotic streaks. However some few plants for Duma 43, Kikamba and Kinyanya proceeded to record severe chlorosis and dead heart (Figure 4.10). No difference was recorded on severity between the different genotypes and none of the collected samples tested positive for SCMV (Table 4.3).

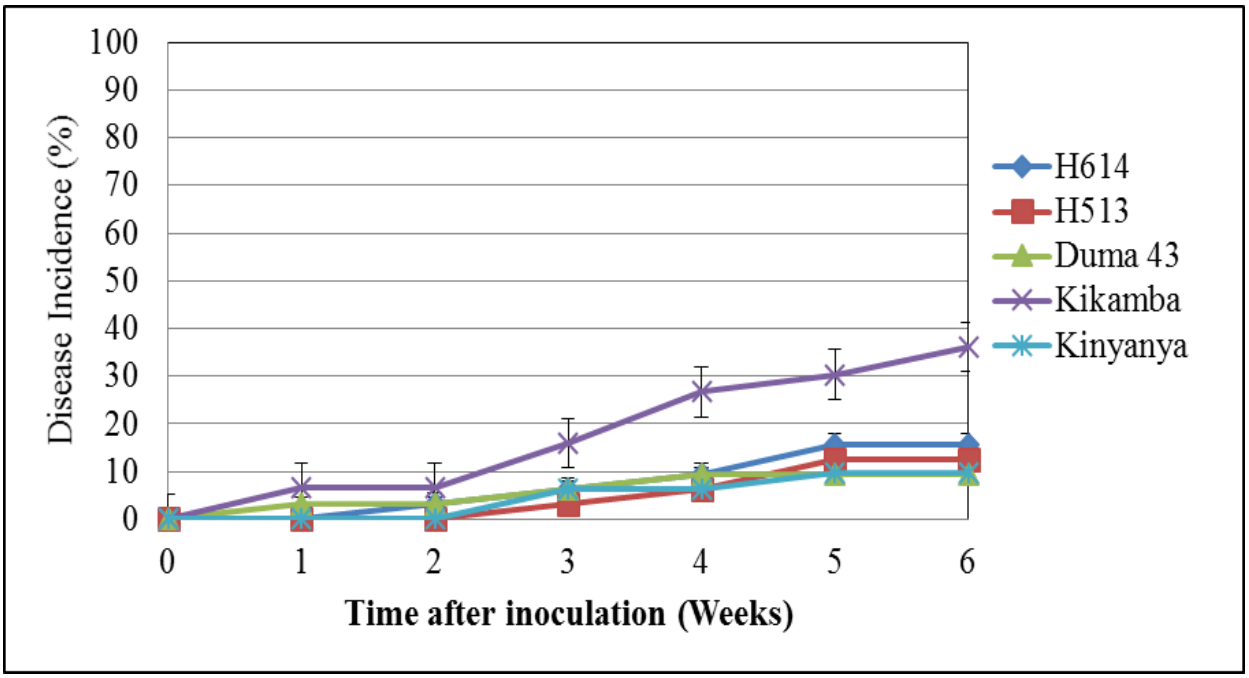

Figure 4.9 SCMV incidence recorded for different maize genotypes planted on *Sugarcane mosaic virus* infested soil without plant rotation for the 3rd continuous season

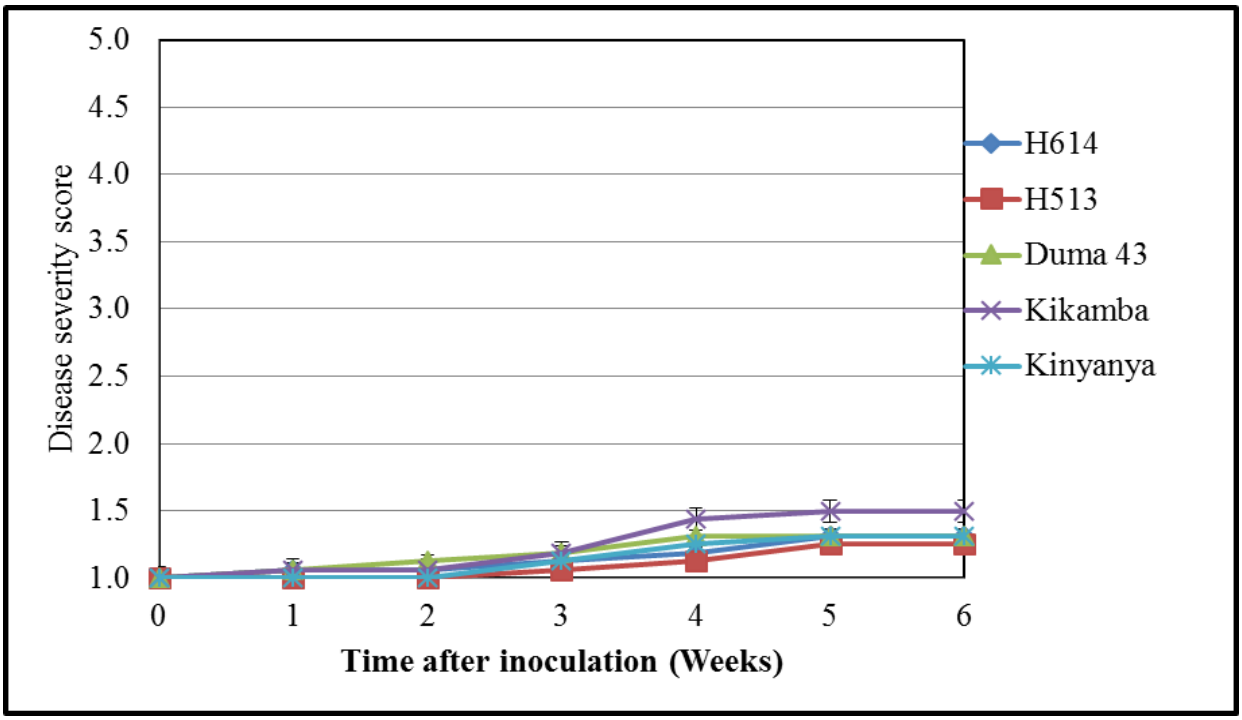

Figure 4:10 Disease severity recorded for different maize genotypes planted on *Sugarcane mosaic virus* infested soil without plant rotation for the 3rd continuous season.

4.4.2 Disease incidence, symptoms and severity for *Maize chlorotic mottle virus* contaminated soil

The genotypes started exhibiting symptoms from week 1 after planting (Figure 4.11). By week 3 all the genotypes had plants showing chlorotic mottling and stunting which is characteristic for the MCMV. The landraces recorded higher disease incidence with Kinyanya leading but no significant difference was recorded among the genotypes. Mild severity was recorded on the affected genotypes with Kinyanya leading while Kikamba recorded least severity (Figure 4.12). There was however no significant difference (P=0.05) between the different maize genotypes tested.

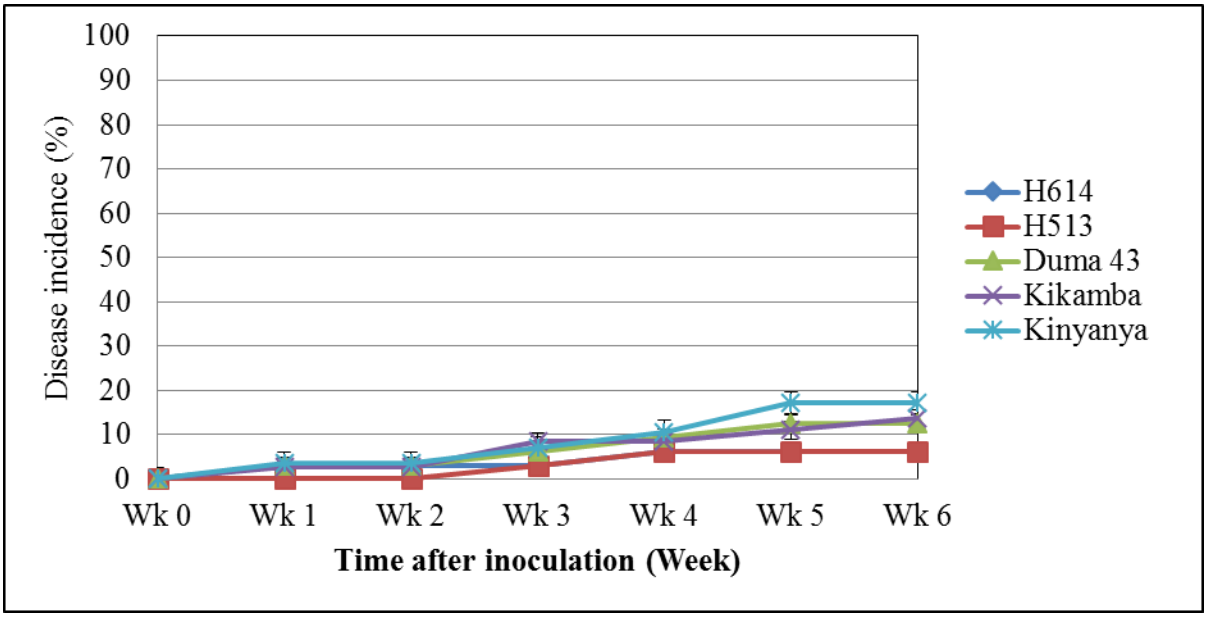

Figure 4.11 Disease incidence for maize plants in *Maize chlorotic mottle virus* infested soil

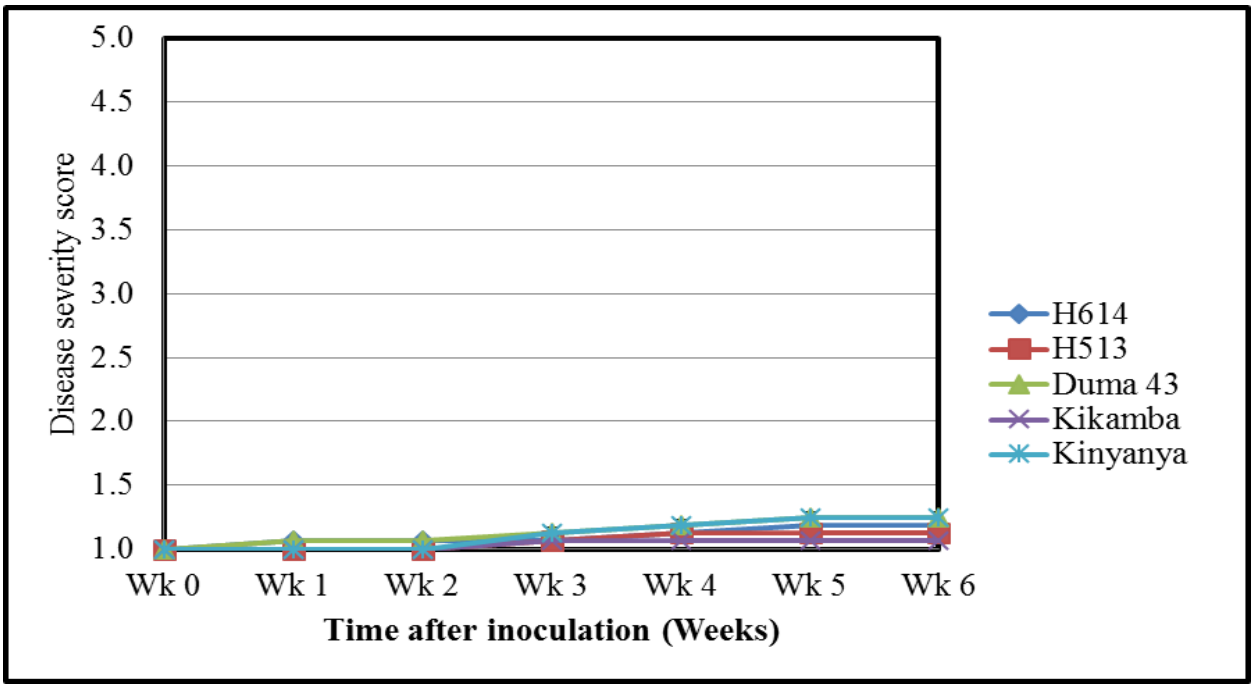

Figure 4.12 Disease severity recorded for different maize genotypes planted on soil with *Maize chlorotic mottle virus* without plant rotation for the 3rd continuous planting.

4.4.3 Disease incidence, symptoms and severity for coinfected contaminated soil

One week after planting, some plants for Duma 43 and Kikamba showed disease symptoms. These included mild mottling and streaks on all the leaves. Kinyanya did not record any symptomatic plant while H513 showed a symptomatic plant after week 4 (Figure 4.13). The plants that showed symptoms late had only their upper young leaves with symptoms while those that showed one week after planting had all the leaves symptomatic. On the disease severity, Kikamba recorded a higher score while Kinyanya did not record any (Figure 4.14). Overall, no difference was noted among the genotypes.

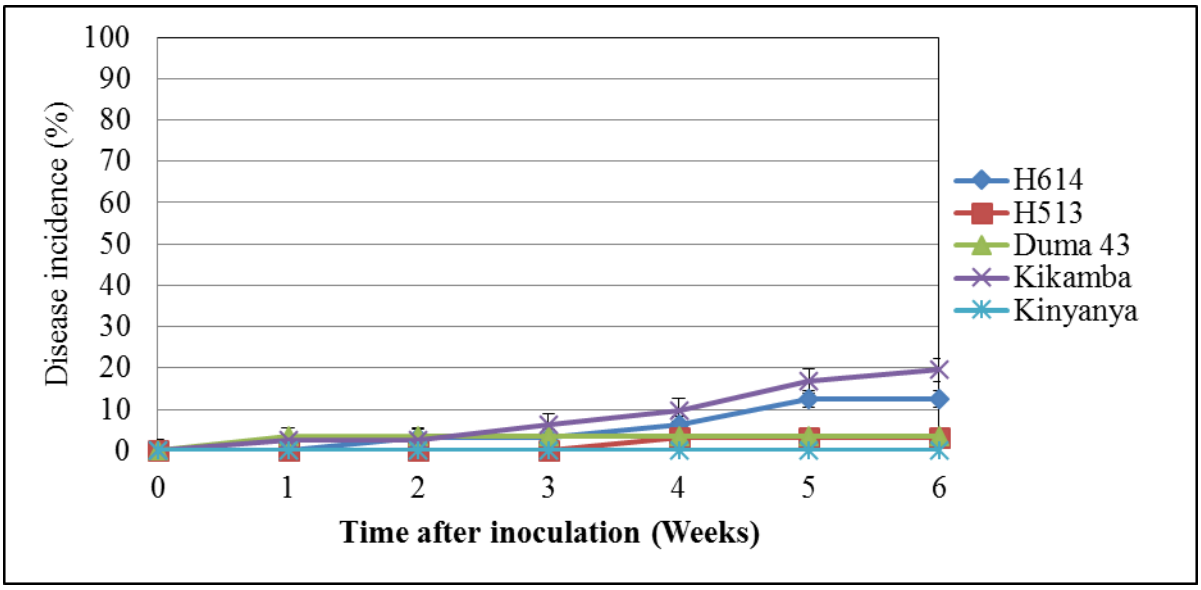

Figure 4.13 Disease incidence for different maize genotypes planted in contaminated soil coinfected with *Maize chlorotic mottle virus* + *Sugarcane mosaic virus*.

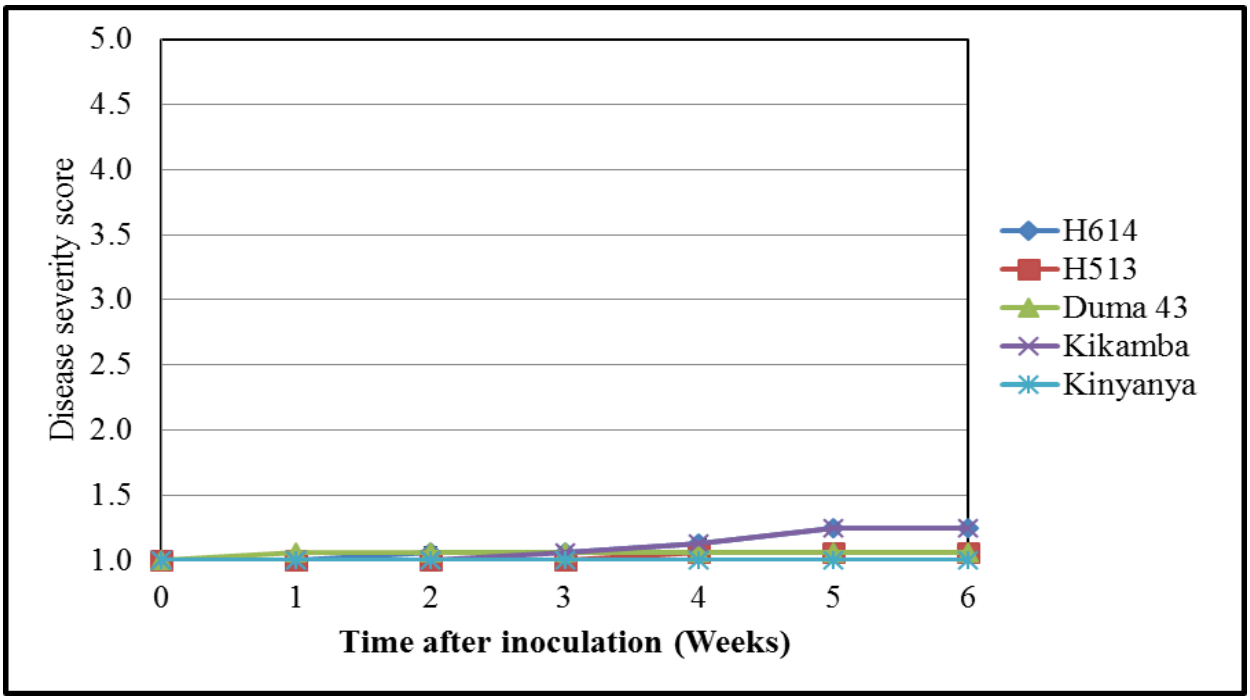

Figure 4.14 Disease severity recorded for plants in contaminated soil coinfected with *Maize chlorotic mottle virus* + *Sugarcane mosaic virus*.

4.4.4 Disease incidence, symptoms and severity for maize lethal necrosis disease contaminated soil

Only the landraces had plants showing disease symptoms after emergence. None of the hybrids had a symptomatic plant up to the 5th week of data collection when H614 had a plant with disease symptoms. H513 showed a symptomatic plant only in the last week of data collection (Figure 4.15). Some difference was noted in weeks 4 and 5 when the incidences for the landraces were significantly different from those for the hybrids. The disease severity recorded was so low for this treatment with Kikamba recording a higher severity score but no significant difference between the genotypes (Figure 4.16).

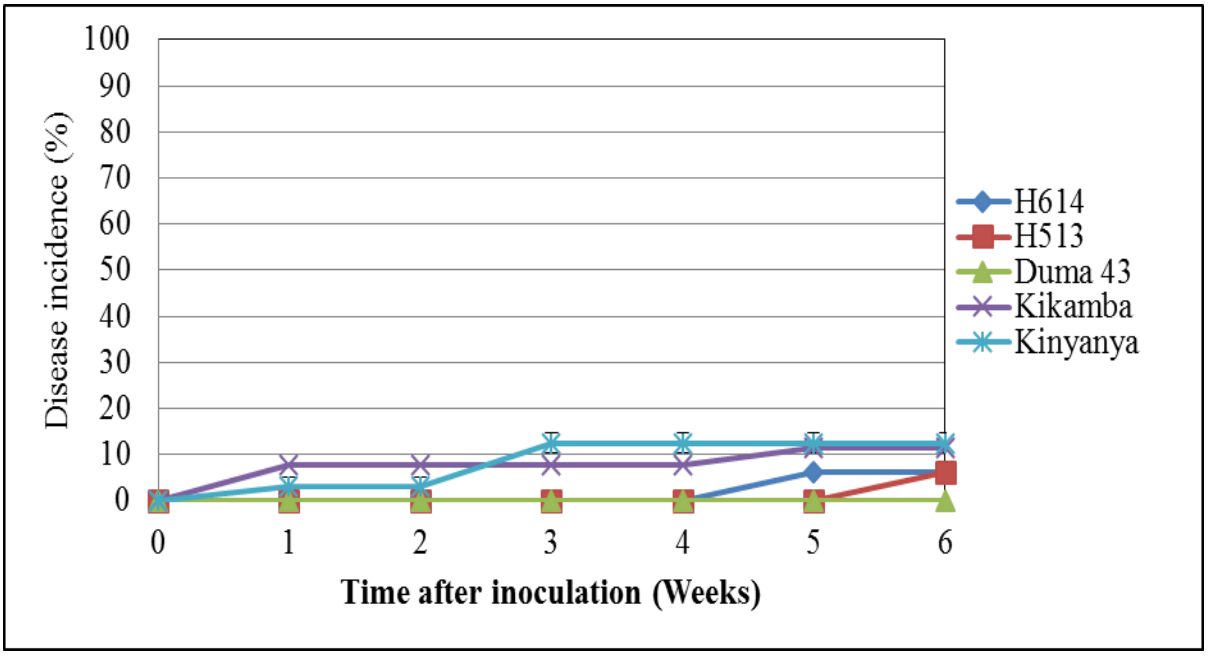

Figure 4.15 Disease incidence for maize genotypes in coinfection of maize lethal necrosis disease contaminated soil

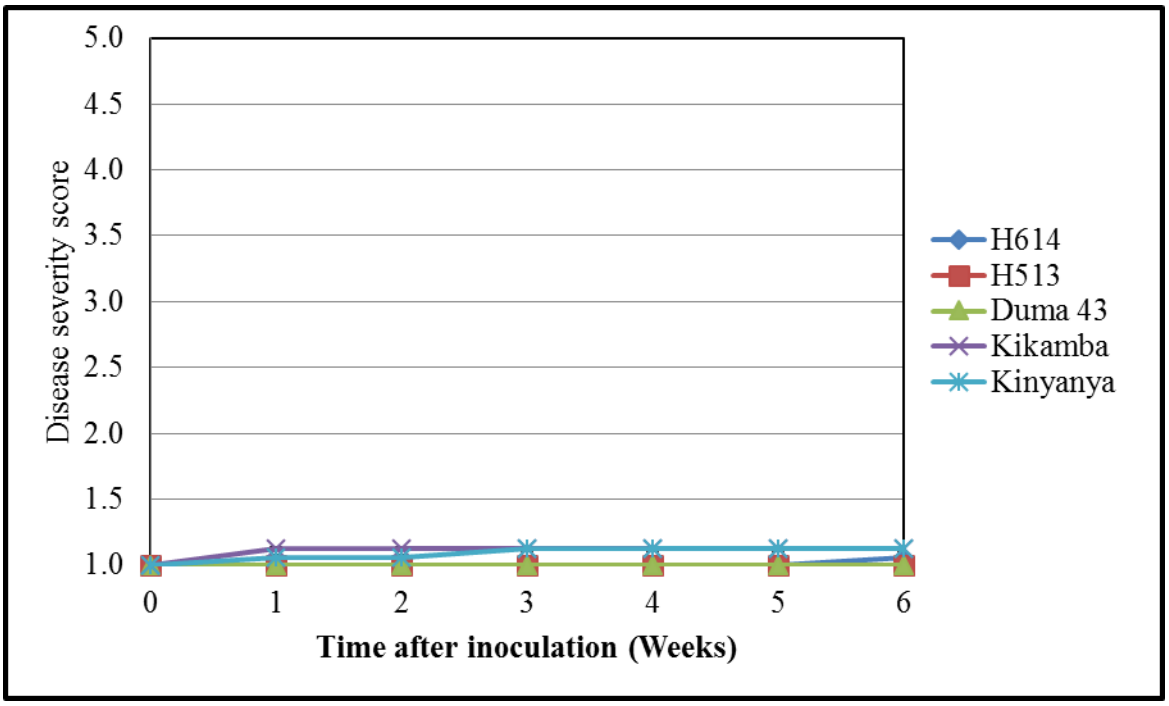

Figure 4.16 Disease severity recorded for different maize genotypes planted on soil for the maize lethal necrosis treatment without plant rotation for the 3rd continuous planting.

4.4.5 Area under disease progress curve

The *Sugarcane mosaic virus* had a large AUDPC as compared to the rest of the treatments, followed closely by the MLN and the combination respectively, while MCMV had the least area (Table 4.4). There was however no variation between the treatments. On genotypes, Kikamba had a large area under disease followed closely by Duma 43. Kinyanya was third with H513 recording the least (Table 4.5).

Table 4.4 Area under disease for the single *Sugarcane mosaic virus*, *Maize chlorotic mottle virus* and coinfections per different maize genotypes.

Variety	H614	H513	Duma 43	Kikamba	Kinyanya
SCMV	8.22a	7.91a	8.69a	8.88a	8.22a
MCMV	8.06a	7.84a	8.28a	7.72a	8.09a
SCMV+MCMV	8.50a	8.12a	8.41a	8.41a	8.00a
MLN	8.00a	8.00a	8.00a	8.88a	8.62a
Control	0.00b	0.00b	0.00b	0.00b	0.00b
$LSD^{P=0.05}$	0.97	0.72	1.34	1.39	1.19
Pvalue	<.001	<.001	<.001	<.001	<.001

Key: The letters indicate the significance between the viral treatments on different varieties. Figures followed by the same letters are not significantly different at P=0.05.

Table 4.5: Area under disease for different maize genotypes planted in soil infected previously with single *Sugarcane mosaic virus*, *Maize chlorotic mottle virus* and coinfections

Variety	SCMV	MCMV	SCMV+MCMV	MLN	Control
H614	8.22a	8.06a	8.50a	8.00a	0.00a
H513	7.91a	7.84a	8.12a	8.00a	0.00a
Duma 43	8.69a	8.28a	8.41a	8.00a	0.00a
Kikamba	8.88a	7.72a	8.41a	8.88a	0.00a
Kinyanya	8.22a	8.09a	8.00a	8.62a	0.00a
$LSD^{P=0.05}$	**1.612**	**0.974**	**0.802**	**0.977**	
Pvalue	**0.694**	**0.744**	**0.623**	**0.202**	

Key:The letters indicate the significance within the treatments. Figures followed by the same letters are not significantly different at P=0.05.

4.4.6 Confirmation of the presence of the *Maize chlorotic mottle* and *Sugarcane mosaic viruses* through serological tests

In the single virus treatments, the hybrid maize genotypes, H614 and H513 had three positive samples each for *Maize chlorotic mottle virus*, while Duma 43 had two. On the other hand, the landrace Kikamba had no positive result while Kinyanya had only one sample testing positive for the MCMV. Only 9 samples were positive while the rest (eleven) were negative (Table 4.6). All samples for the SCMV tested negative on Elisa.

Viral coinfection for SCMV + MCMV had only two samples positive for MCMV one for H614 and another for H513 respectively. None of the samples was positive for SCMV. While for MLN coinfection, none of the H614 were positive for MCMV while one of the H513 tested positive and two samples for Duma 43. Of the landraces, Kikamba had one positive sample while Kinyanya had two. Of the twenty samples, only six were positive for the MCMV. None of them tested positive for the SCMV (Table 4.6).

Table 4.6 Number of positive samples after serological analysis for the MCMV in the single and coinfection viral treatments

Variety	MCMV	SCMV+MCMV (MCMV)	MLN (MCMV)
H614	3	1	0
H513	3	1	1
Duma 43	2	0	2
Kikamba	0	0	1
Kinyanya	1	0	2
Negative controls	0	0	0

Footnote: In total 20 samples were collected per treatment, four samples for each of the five genotypes with the samples for the coinfection treatments (SCMV+MCMV) and MLN being subjected to both the *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* analyses.

4.4.7 Discussion

Recurrence of maize lethal necrosis (MLN) disease season after season is a worrying trend threatening food security in Kenya and the other developing countries, as more than 1.2 billion depends on maize for food. There is a clear indication that viruses causing the disease survive either in the soil, plant debris or both and most of the genotypes planted are susceptible. The rate at which maize plants are able to acquire the viruses from the soil with or without infected debris formed the core of this work.

When infected plant debris was incorporated into the soil, the plants were able to acquire the viruses one week after emergence in the coinfection of MCMV and SCMV. All genotypes showed plants exhibiting symptoms related to the two viruses, MCMV and SCMV. The landraces seemed to acquire the viruses more easily as they showed symptoms earlier than the hybrids. There was however no major difference among the genotypes and if the soil had debris or not. Plants that were planted in bags previously containing plants coinfected with the two viruses (MCMV+SCMV) had the largest area under disease, a clear indication of the effect of continous planting in MLN infected fields. On the variety level, the landrace Kikamba without debris had a large area under disease followed closely with that for the same variety but with debris. Duma 43 had the least area under disease. Albeit not significant, the addition of debris seem to enhance disease acquisition as has been demonstrated by MCMV (Philips *et al.*, 1982; Uyemoto, 1983). Plants that had acquired the viruses immediately on emergence developed severe chlorosis resulting in necrosis and eventually a dead heart. These plants were generally stunted. This is a clear demonstration that the disease can cause severe damage if it attacks early.

Irrespective of whether the soil is incorporated with the plant debris or not, the viruses can still be acquired from the soil that has not been given a rest to allow for the viruses to degrade. The small roots left behind after harvest and uprooting of the maize plants are significant in the survival of the viruses. As earlier demonstrated (Uyemoto, 1980; Jiang *et al.*, 1992), both viruses can be found in any part of the maize plant so long as the plant was infected. This is important in planning the disease management strategy. The plant residues have also been demonstrated to play a very crucial role in the survival of the MCMV when the maize plants are off season (Uyemoto, 1983; Montenegro and Castillo, 1996). In earlier experiments the role of crop rotation was emphasised in managing the outbreaks (Philips *et al*., 1982; Uyemoto, 1983). In the third planting in the infested soil without any debris incorporation, few plants were able to acquire the viruses too. The genotypes acquired the viruses equally from the soil.

On the serological analysis of the collected samples however, all the samples for the SCMV tested negative despite the fact that they had very clear symptoms on the plants during data collection. The propable reason to this could be, the antisera used for this test could have been of a diffferent strain from the one used for this experiment as we have varied strains of SCMV in East Africa where the Kenyan strain has 87% identity to the Rwandan strain which is 95% related to the SCMV-DMB strain (Adams *et al.*, 2014). Most samples were positive for the MCMV which indicated the antiserum used was raised against an isolate closely related to the Kenyan strain or Kenyan isolate itself. More investigations need to be done to explain the scenario for the SCMV.

The acquired viruses on the few plants can act as focal points for the different viruses as they act as sources of inoculum which can then be spread to other plants through other means especially

mechanically and through the insect vectors. The maize plants in the SCMV infested soil clearly aquired the virus as there were clear symptoms on the plants. Earlier study on sorghum had shown that the virus can be easily aquired from infested soil or contaminated containers (Bond and Pirone, 1970).

From the above results, its evident that infested soil and debris are crucial in the survival and spread of the viruses causing MLN disease. Their management is critical in addressing the spread and control of this disease. It's therefore important to put measures in place to ensure maize debris is properly managed and the farmers encouraged to carry out crop rotation to reduce the chances of picking the viruses fom the infested soil. The role of few infected plants in the field should also be addressed to avert spread to other non infected plants through other means especially the vectors and mechanically through the crop management processes. Asymptomatic plants also play a role in recurrence of the outbreak hence proper field sanitation should be encouraged during the crop production period to avoid unnecessary spread of the disease.

CHAPTER FIVE

5.0 General discussion, conclusions and recommendations

5.1 General discussion

Understanding the reaction of the various genotypes to viruses causing MLN disease is key in breeding for resistance. Five maize genotypes were subjected to the various viral treatments and then evaluated for disease incidence and severity under screenhouse conditions.

The hybrids showed slowed development of disease symptoms unlike the landraces. This correlates with an earlier observation by Das *et al.* (2015) where a hybrid variety H614D was reported to be more tolerant to MLN disease compared to landraces and commonly used genotypes. In addition, hybrids have been shown to have superior performance over landraces in both yields and resistance to pests and diseases (Russell, 1991).

Plants infected with *Sugarcane mosaic virus* showed symptoms earlier than the other treatments with plants with coinfection with both viruses having the highest severity. The same reaction had earlier been reported on SCMV and MCMV synergistic reaction (Niblet and Claflin, 1978; Uyemoto *et al* 1980; Uyemoto *et al.,* 1981; Wangai *et al.,* 2012). Viral titer for MCMV was also doubled in the coinfections. This agrees with earlier reports on the effect on non-potyvirus in synergistic reactions whereby the potyvirus remains unaffected while the titres of the non-potyvirus are enhanced (Goldberg and Brakke, 1978).

When viruses from co-infected plants were used for inoculation of healthy plants, there was disease development although not as severe as when the two viruses are mixed directly for inoculation. This could be due to the different ratios of the SCMV: MCMV in the two treatments. The MLN diseased plants pose a great risk to healthy young plants as they will act as

the source of inoculum for these plants through mechanical and vector transmission. In this treatment; the level of MCMV was not enhanced as in synergistic infections. Chances are that the level of SCMV was too low to have any effect on the synergy as the completely chlorotic leaves were used for inoculations. The ratio remained at 1:1 for the single to co-infections respectively. More studies need to be done to explain why this was so.

To understand the role of plant debris and contaminated soil in MLN disease development, screenhouse experiments were conducted using the infected plant materials obtained from previous experiment. What came out clearly is the fact that the plants were able to acquire viruses from the soil and debris. Hence repeated cropping of maize in the same field poses a great risk of MLN disease persistence. This finding is in agreement with earlier findings by Uyemoto (1983) and Philips *et al.* (1982).

When maize genotypes were planted for the third time in the same soil, the plants were able to acquire the viruses but at a lower rate of 28% than they did in the second planting (55%) when there was also debris incorporation. This is really worrying where crop rotation is not practiced as the disease will recur year in year out. When there is incomplete debris decomposition, viral acquisition is high while there could be a reduction in completely decomposed debris. Acquisition of the viruses is also enhanced by the presence of roots in the soil. Earlier studies have demonstrated the fact that the two viruses can be found in any part of the maize plants (Uyemoto, 1980; Jiang *et al.,* 1992). Disease management options should focus on this aspect and more studies should be done to confirm how long the persistence can take both in the plant debris and in the soil. Furthermore; understanding the mechanisms of viral acquisition is critical and hence more studies should be done to ascertain this and also find out the enhancing conditions and factors that influence this.

The landraces developed disease symptoms earlier than the hybrids. Disease symptoms were also visible earlier in the soil incorporated with debris than those in soil alone although the differences were not significant. The plant debris enhances disease acquisition through increased virus concentration and this is critical as the earlier acquisitions could be potential focal points of spread to healthy neighbouring plants and or plots.

Serological test results showed some plants which were negative on symptom scoring, testing positive for MCMV. This is quite risky as some genotypes might have plants not exhibiting any symptoms but contain the virus hence acting as focal points for spread to susceptible healthy plants or genotypes. These genotypes could however be key in the development of tolerant genotypes since they exhibit some form of tolerance. Plants which had high disease severity had low viral titers which can be attributed to the low multiplication and deterioration of the viruses in plants which are dying unlike the rapid and continued multiplication in the less severely affected plants. This can also be attributed to the sensitivity of the genotypes.

All the samples collected from plants testing for virus transmission from the soil and debris, tested negative on the ELISA for SCMV although they scored highly during symptom assessment. The samples had been in storage before diagnostics and this could have contributed greatly. MCMV was however not affected by the storage. MCMV seems to be very stable and hence poses very high risk to production if it's not well managed. Studies have shown that the SCMV easily mutates due to its weak proof reading activity of the RNA dependent RNA polymerase. Further diagnostic options need to be evaluated specifically for this particular virus due to its dynamics. Additionally, more studies need to be done to understand what exactly happens when the SCMV is in storage in general. How long MCMV remains virulent while in storage also need to be confirmed as this could be key to MLN disease management.

5.2 Conclusions

The different maize genotypes reacted differently to the viruses causing the MLN disease whereby landraces exhibited more susceptility to infection while hybrids displayed some degree of tolerance. Due to breeding programs for hybrids, their performance has been enhanced hence they display more resistance to the various diseases and pest invasion (Russell, 1991). The evaluated landraces were more vulnerable to the effect of the disease while the hybrids seemed slow to disease development especially in synergistic infections. From this study; its evident that different maize genotypes play a key role in the development of MLN disease. Proper variety selection will guarantee less disease damage and hence more productivity of crops affected by the MLN disease. It's critical therefore to do an evaluation of all landraces available in East Africa and screen them for resistance against MLN disease. Similarly, farmers need to understand the role of these genotypes in the MLN disease development in addition to the importance for them to use the certified hybrid seeds to lessen the effect of MLN disease. Repeated planting in the same soil and incorporation of plant debris enhanced the occurrence of MLN disease and the acquisition of the viruses. The study has shown clearly that MLN disease-causing viruses survive in the soil and in the plant debris.

5.3 Recommendations

From this study it's evident that the maize genotypes used in production play a very great role in the development or severity of the viruses causing MLN disease. It's therefore recommended that

- Farmers should prioritize the use of certified hybrid seeds for planting other than landraces. However, landraces can be used in fields which have never had incidences of the MLN disease and with proper field sanitation. Additionally, more studies need to be done to evaluate reaction of commonly grown landraces in East Africa and especially in Kenya to MLN disease and its causal viruses.

- The role of debris and soil should not be underestimated as they play a critical part in the spread and survival of viruses causing MLN disease. These act as disease reservoirs hence their presence is highly risky. It's therefore important for farmers to practice crop rotation to avoid repeated epidemics of disease and to avoid buildup of inoculum. Proper management of crop debris after harvesting or upon crop destruction is therefore critical in managing this important disease. Debris need to be disposed off well especially by burning so that it doesn't get its way back to maize or crop fields.

- Crop rotation with non-host plants need to be emphasized to help in breaking disease cycle hence lessen the severity of MLN disease. The crops for rotation include cabbage and other vegetables in this family, onions and related crops, beans and other crops not in the grass family.

- To get a better understanding on when viral titers are optimal and especially for comparison of titers in co-infected plants versus single infections; different sample

collection times and diagnosis should be done. This will help in determining the best sampling stage for the viruses.

- Different genotypes were used in these experiments. These are grown in diverse agro ecological zones (AEZ) when in production. Hence for further assessment; these genotypes should be evaluated at different ecological zones to determine the influence of environmental conditions on disease severity.

- The virus titers obtained in synergistic interaction of SCMV + MCMV for the MCMV were a bit lower than what was earlier reported from other studies (Goldberg and Brakke, 1987). This can be attributed to the mix ratios used of the two viruses. More studies therefore need to be done to determine the optimal ratios of SCMV: MCMV to obtain optimal viral titer concentrations.

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