STATUS OF MAIZE LETHAL NECROSIS IN SEED PRODUCTION SYSTEMS AND INTERACTION OF VIRUSES CAUSING THE DISEASE IN KENYA

Joyce Waithira Eunice
B.Sc. (Philosophy in Applied Biology)-Technical University of Kenya

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Crop Protection

Department of Plant Sciences and Crop Protection,

Faculty of Agriculture.

University of Nairobi

2023

DECLARATION

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

Joyce Waithira Eunice

Sign…….....…….....Date…….....17/08/2023

This thesis has been submitted with our approval as university supervisors:

Prof. Douglas W. Miano
Department of Plant Science and Crop Protection
University of Nairobi

Sign……..……. Date……..……17/08/2023

Prof. William Maina Muiru
Department of Plant Science and Crop Protection
University of Nairobi

Sign……..…….. Date…….19/08/2023

Prof. Eunice W. Mutitu
Department of Plant Science and Crop Protection
University of Nairobi

Sign…… Date……21/08/2023

DECLARATION OF ORIGINALITY

Name of Student:	JOYCE WAITHIRA EUNICE
Registration Number:	A56/76509/2014
Faculty/School/Institute:	Agriculture
Department:	Plant Science and Crop Protection
Course Name:	Master of Science in Crop Protection
Title of the work:	Status of Maize Lethal Necrosis in Seed Production Systems and Interaction of Viruses Causing the Disease in Kenya

DECLARATION

1. I understand what Plagiarism is and I am aware of the University's policy in this regard

2. I declare that this **Master's thesis** is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work, or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

3. I have not sought or used the services of any professional agencies to produce this work

4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own work

5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy.

Signature ……. Date 17/08/2023

DEDICATION

This work is dedicated to my loving Mum Eunice Nduta Kagwe, my husband Moses Njore and my beloved children Judah, Joy and Caleb for their patience and moral support in every step of the way.

ACKNOWLEDGEMENTS

My heartfelt honor and appreciation goes first to the Sovereign God for his grace, ability and knowledge during my entire study period. Secondly, my appreciation and thanks go to my supervisors Prof. Douglas W. Miano, Prof. William Maina Muiru and Prof. Eunice W. Mutitu for their guidance, patience and invaluable advice from the time of selection of the project, proposal writing, and execution of the research work and writing of this thesis. Much appreciation to Dr. Isaac Macharia for his special support and guidance throughout the study period. A lot of thanks go to Dr. James Karanja of Kenya Agricultural and Livestock Research Organization (KALRO) for his assistance and guidance in experiments set up, data analysis and interpretations. Many thanks to Ms. Edith Avedi and Dr. Kimaru for their assistance in data interpretations and publications. Through the process, I have been transformed into a thoughtful and critical researcher and scientist. Thirdly, I thank my employer Kenya Plant Health Inspectorate Services (KEPHIS) for financial support that made this study possible. Finally my special thanks go out to my family for their prayers, moral support and encouragement during my studies. It is because of you that I had the fire that kept me focused all the way.

TABLE OF CONTENTS

DECLARATION ii
DECLARATION OF ORIGINALITY iii
DEDICATION ii
ACKNOWLEDGENTS v
TABLE OF CONTENTS vi
LIST OF TABLES viii
LIST OF FIGURES ix
ABBREVIATIONS AND ACRONYMS x
ABSTRACT xi
CHAPTER ONE 1
INTRODUCTION 1
1.1 Background information 1
1.2 Statement of the problem 2
1.3 Justification 3
1.4 General objectives 4
CHAPTER TWO 5
LITERATURE REVIEW 5
2.1 Origin of maize 5
2.2 Maize production and consumption 5
2.3 Constraints in maize production 6
2.4 Maize lethal necrosis disease 7
2.5 History of Maize lethal necrosis disease, its spread and impact 7
2.6 Maize lethal necrosis disease symptoms 8
2.7 Viruses causing Maize lethal necrosis disease 9
2.8 Transmission of viruses causing Maize lethal necrosis disease 11
2.9 Cycle of infection of viruses causing Maize lethal necrosis disease 14
2.10 Synergistic interactions of MCMV and potyviruses 15
2.11 Diagnosis and detection of viruses causing Maize lethal necrosis disease 17
2.12 Management of Maize lethal necrosis disease 19
CHAPTER THREE 23
STATUS OF MAIZE LETHAL NECROSIS DISEASE IN SEED PRODUCTION SYSTEM IN KENYA 23
Abstract 23
3.1 Introduction 23
3.2 Materials and Methods 25
3.3 Results 30
3.4. Discussion 38
CHAPTER FOUR 43
EFFECT OF INTERACTIONS OF MAIZE CHLOROTIC MOTTLE VIRUS AND SUGARCANE MOSAIC VIRUS ON MAIZE LETHAL NECROSIS DISEASE DEVELOPMENT IN INFECTED MAIZE 43
Abstract 43
4.1 Introduction 44
4.2 Materials and Methods 47
4.3 Results 49
4.4 Discussion 56
CHAPTER FIVE 61
GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS 61

5.1 Discussion 61
5.2 Conclusion 63
5.3 Recommendations 64
6.0 REFERENCES 65

LIST OF TABLES

Table 2.1 Approximated losses due to maize lethal necrosis (MLN) disease (2013 to 2016) in sub-Saharan Africa 9
Table 3.1 Maize lethal necrosis (MLN) disease incidence and severity across agro-ecological regions.....31
Table 3.2 Maize lethal necrosis (MLN) incidence and severity in Kenyan agro-ecological regions over time 30
Table 3.3 Mean maize lethal necrosis (MLN) disease incidence and severity of sampled maize growing counties in Kenya.....32
Table 3.4 Maize lethal necrosis (MLN) disease incidence and severity for selected Kenyan counties over years 32
Table 3.5 Mean maize lethal necrosis (MLN) disease incidence and severity on commonly grown maize varieties in Kenya 33
Table 3.6 Maize lethal necrosis (MLN) disease incidence and severity on commonly grown maize varieties in Kenya.....34
Table 4. 1 Mean disease severity score of maize genotypes infected with maize chlorotic mottle virus (MCMV), sugarcane mosaic virus (SCMV), and SCMV+MCMV (MLN) assessed over time. 51
Table 4. 2: Plant heights of maize genotypes infected with maize chlorotic mottle virus (MCMV), sugarcane mosaic virus (SCMV), and and SCMV+MCMV (MLN) assessed over time.....54
Table 4. 3: Virus CT values of maize chlorotic mottle virus (MCMV) in maize genotypes infected with MCMV alone or in mixed infections (MLN) with sugarcane mosaic virus (SCMV). 55

LIST OF FIGURES

Figure 2.1 (a) MCMV T=3 octahedral protein shell (b) genomic organisation of MCMV,(c) SCMV composition, (d) SCMV molecular arrangement. 12

Figure 3.1 Maize lethal necrosis (MLN) disease incidence and severity for various maize growth stages 35

Figure 3.2 Detection of maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) using Real time PCR assays……..…36

Figure 3.3 Real time PCR image showing positive and negative results of maize chlorotic mottle virus (MCMV) detection……..36

Figure 3.4 Distribution of maize lethal necrosis (MLN) disease causing viruses in major seed production counties surveillanceed in 2015, 2017, 2018, and 2019…….. 37

Figure 3. 5 Detection of maize chlorotic mottle virus (MCMV) using Immunostrip assays ..38

Figure 4. 1 Percent disease incidence over time in maize varieties DK8031 plants inoculated with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV) and maize lethal necrosis (MLN) disease 52

Figure 4. 2 Percent disease incidence over time in maize varieties Duma 43 plants inoculated with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV) and maize lethal necrosis (MLN) disease. 52

Figure 4.3: Mean area under disease progress curve (AUDPC) as calculated from disease severities for DK8031 and Duma 43 infected with sugarcane mosaic virus (SCMV), maize chlrotic mottle virus (MCMV) and MLN (MCMV+SCMV) inoculation.....55

ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
BPMV	Bean pod mottle virus
CIMMYT	International Maize and Wheat Improvement Centre
CP	Coat protein
CT	Cycle Threshold
ELISA	Antibody Sandwich Enzyme-linked Immunosorbent Assay
DAS-ELISA	Double Antibody Sandwich Enzyme-linked Immunosorbent Assay
TAS-ELISA	Triple Antibody Sandwich Enzyme-linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FAOSTAT	Food Agricultural Organization and statistics
KEPHIS	Kenya Plant Health Inspectorate Service
MCMV	Maize chlorotic mottle virus
MDMV	Maize dwarf mosaic virus
MDRAT	Multi-Disciplinary Rapid Assessment Team
MLND	Maize lethal necrosis disease
MP	Movement protein
NGS	Next generation sequencing
NPPOs	The National plant protection organizations
ORFs	Open reading frames
PVX	Potato Virus X
PVY	Potato Virus Y
PCR	Polymerase chain reaction
q-PCR	Quantitative Real Time Polymerase Chain Reaction
QTLs	Quantitative trait locus
RNA	Ribonucleic acid
SCMV	Sugarcane mosaic virus
SSA	Sub-Saharan Africa
+ssRNA	Positive sense single stranded RNA
USA	United States of America
WSMV	Wheat streak mosaic virus

ABSTRACT

Maize is cultivated in different climatic and ecological conditions in Africa. In Kenya, it's a principal crop with an average of 90% of residents in maize production areas relying on it for food and employment. Production of maize has been on decline due to infection by Maize lethal necrosis (MLN) disease that is triggered by infestation of MCMV and a potyvirus, mainly Sugarcane mosaic virus (SCMV). Transmission of MCMV and SCMV through seed and soil may occur though at a very low rate. This research was done to determine the status of MLN disease in maize growing farms in Kenya and the effects of interactions of MCMV and SCMV in maize plant.

A survey was conducted in seed production fields in 5 agro-ecological regions in 13 counties that produce maize seed in 2015, 2017, 2018, and 2019. Collection of samples was done for non-symptomatic and symptomatic maize by applying a standard protocol with testing for the MCMV virus using immunostrips and both viruses by real-time quantitative reverse transcription polymerase chain reaction (qRT- PCR). A total of 2550 ha was surveyed where 21% of maize was found to having varied degrees of severity of MLN infection. High MLN disease incidences and severities were recorded in Sub-humid agro ecological regions constituting of Embu, Elgeyo Marakwet, Uasin Gishu and Nakuru counties. Out of the total samples analyzed using qRT PCR, 38% had MCMV alone, 14% having SCMV alone and 18% with dual MCMV and SCMV. Out of the 185 samples analyzed with immunostrip from 2017-2019, 29 (16%) were positive for MCMV.

To determine the effects of interactions, maize plants were infected with MCMV and SCMV single infections and in combination of the two viruses (M+S). Two varieties Duma 43 and DK 8031 were used in the experiment which was carried out in two seasons. Disease assessment for severity and heights were recorded 7 days post inoculation (7 dpi) and after

every 7 days for a period of 56 days. Sampling of leaves was also done in each treatment in the same interval and leaves preserved at -80°C. Stored leaves were tested for MCMV using q-PCR and CT values recorded for each sampling point for each treatment. Treatment M+S recorded the lowest mean heights while SCMV recorded higher mean heights compared with MCMV except at 7 dpi. The M+S treatments recorded the highest disease severity score throughout the data collection period. There was significant difference ($P < 0.05$) in Ct values between the treatments for all the days of data collection. Treatments M+S recorded the lowest Ct values which is inversely proportional to the virus titer in the infected maize. The concentration of the MCMV increased in mixed infections compared to single inoculations. Similarly growth was retarded in mixed infections and disease severity was increased compared to single infections. Disease incidence was high in mixed infection in the initial stages of plant growth.

The above findings show MLN disease is still a threat and measures need to be put in place to help minimize the introduction and spread of the disease. Monitoring of disease causing viruses through inspection and testing is effective in reducing the disease impact which is a result of the synergistic interactions of the viruses. Potyviruses play a major role in increased MLN outbreaks and hence require attention as part of management of the disease and development of resistant or tolerant varieties.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize (*Zea Mays* L.) belongs to family *Poaceae* which is widely cultivated and is third in importance after wheat and rice (FAO, 2018). Maize is cultivated in different climatic and ecological conditions in Africa. In Sub-Saharan Africa (SSA) maize covers close to 30 million hectares with smallholders producing close to 70 million metric tons (FAO, 2018). Africa recorded an annual production of 78 million tons in 2014 with Kenya contributing 3.5 million tons of the total yield (FAO, 2018). In Kenya, it's a key basic food with over 90% of livelihood relying on it for food and employment. In SSA as well as Latin America, maize forms a reliable energy source for over 1.2 billion people. Utilization of maize is majorly as human staple food, animal feed and for industrial production. In developing countries, consumption is usually as solid or as porridge while in countries which are developed, it is majorly used as animal feed as well as raw material for industries.

Production of maize has been on decline due to various reasons which includes drought, soil infertility, lack of inputs, pests and diseases (Prasanna *et al.*, 2020). Pests and diseases, whether viral, bacterial, fungal or insects affect significantly the production, resulting in huge loses. A serious challenge is the emergence of Maize lethal necrosis (MLN) disease, originally described by Wangai *et al.* (2012) in Kenya in 2011. The disease outbreak spread to other eastern Africa countries resulting in huge yield losses and decrease in maize production (Mahuku *et al*., 2015b; Marenya *et al*., 2018). The MLN disease is a result of synergistic reaction of maize chlorotic mottle virus (MCMV) with potyviruses which include sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), and wheat streak mosaic virus (WSMV) (Niblet and Claflin,

1978). Sugarcane mosaic virus has been endemic in Kenya for many years with minimal effects on maize (Louie, 1980), however, MCMV is a new disease in the region, and when in combination with SCMV results in MLN disease epidemics. The two viruses have been identified as major among the viruses that infect maize all over the world (Lubberstedt *et al*., 2006).

1.2 Statement of the problem

In African continent maize is a main source of food, accounting for over 50% of small income generating population. Utilization of maize in the world is approximately 116 million tons with Africa taking 30% out of which 21% is utilized in SSA. In Kenya maize is a major staple food with over 90% depending on it for food and employment. Maize lethal necrosis disease is of great concern to the agricultural sector in Kenya and poses a risk to the national food security and economy. Huge yield loses have been reported in Kenya, impacting negatively to the livelihoods of many people. Losses from MLN have impacted not only small-scale producers, but also other important stakeholders within maize production value chain, such as middle and large scale maize producers, seed agribusinesses and processing units. Losses due MLN disease epidemic has resulted to maize shortage in Kenya which has led to an upsurge in importation of maize from Malawi and Zambia and further increase in food prices (MDRAT, 2012). Total destruction of maize crops have been reported in Kenyan fields, with an estimated value of USD 52 million (Prasanna *et al.*, 2020).

Spread of viruses that cause MLN disease is through insect vectors, where MCMV is transmitted by thrips, especially *Frankiniella williams*i (Zhao *et al*., 2014) and a variety of beetle species from the Chysomelidae family (Nault *et al*., 1978) whereas SCMV is transmitted by aphids

mainly *Myzus persicae* (Cabanas *et al*., 2013). Seed have also been associated with MCMV propagation (Mahuku *et al*., 2015). Despite being reported at very low rates of transmission, it is of great significance since it can contribute to introduction and spread of the disease. In order to control the disease, knowledge on spread, dissemination, survival and role of seed need to be determined. Interactions associated with MLN causing viruses require attention as part of understanding the disease outbreaks. There are various options available for management and control of the disease including resistant varieties and phytosanitary procedures. Exchange and production of virus free maize germplasm is vital in curbing the spread of the disease. Production of seed is critical in the avoidance of MLN since it is a pathway of outbreaks. The status of MLN disease and its causative viruses in seed production has not been determined. Monitoring of the disease during production is key towards ensuring availability of clean seed.

1.3 Justification

Different management options have been deployed to control disease spread. Seed certification is one of the ways to curb the spread and introduction of disease in new areas, ensuring production, distribution and exchange of virus free germplasm. Monitoring of disease presence, severities and incidences in maize seed production is important in ensuring sanitation is maintained throughout the production period.

Interaction of the viruses in maize plants is also important in contributing to the control of the maize causing pathogens and reduction of disease surge. During the interactions, one virus may weaken the resistance of the plant, making it highly susceptible to another virus. A study on the role of SCMV and effect on MCMV titer in inoculated maize plant is significant in order to know whether symptoms expression relates to the disease in both dual and single infections. This

is of great importance in seed certification especially in determining the tolerant and resistant varieties. It may also help in determining which virus to target in the development of virus resistant plants

1.4 Broad objective

To contribute to increased production of maize by determining the status of viruses that cause Maize lethal necrosis (MLN) disease in maize seed production and the effect of interactions of the viruses in infected maize.

1.4.1 Specific objectives

i. To determine the status of viruses that cause Maize lethal necrosis disease in major maize seed production areas in Kenya
ii. To determine the effects of interactions of Sugarcane mosaic virus and Maize chlorotic mottle virus on plant growth and MLN disease development in infected maize.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of maize

Maize (*Zea Mays L.*) is one of the oldest cultivated grains that belongs to the family *Poaceae* (Paliwal *et al.*, 2000). The crop originated from the American highlands and spread all over the world. It is an annual crop with a single thick erect stem and seasonal adventitious roots. It is a monoecious plant, meaning that distinct flowers on the same plant bear both the male and female inflorescence. Eggs and pollen usually are produced in pistillate and staminate inflorescence respectively. Pollination can be both self and cross through wind and pollinated pollens can remain viable up to 30 minutes in optimal conditions (Coe *et al*., 1988). Maize fruit is called kernel, grain or seed and consists of embryo, endosperm and fruit wall. Maize crop is adaptable to different climatic environmental zones of various altitudes and latitudes. Utilization of maize varies in different continents where developed countries mainly use it for animal feed and industrial use whereas in developing countries it is the main staple food. The crop is thrives to a vast range of climatic conditions and can be produced as dry or irrigated crop.

2.2 Maize production and consumption in the world

Maize is an important plant grown in sub Saharan Africa occupying more than 30 million ha with production at over 70 MMT (FAO, 2018). Maize is a food security crop and has a value of over 8% compared to other crops in Southern Sahara Africa. In Africa 85% of maize is utilised as food (FAOSTAT, 2014). In Africa, over 70 million livelihoods depend on maize either directly or indirectly. Consumption of maize is over 950 MMT in the world with Kenya consuming approximately 98 kilograms per capita. Production of maize in Kenya is one of the

key economic activities, acting as a source of income and employment, with Rift valley region being the hub of maize production. Maize kernels provide approximately 86% of calorie requirements as well as a reliable source of fibre, Vitamin B, Vitamin B5. The starch from the crop can be converted into plastic fabrics and adhesives. Maize can be consumed as snack inform of popcorns, fresh /green boiled or roasted maize. Maize is also used as a domestic feed for animals by feeding on stalk leaves. Stalk and cobs are also used as domestic fuel and organic manure.

2.3 Maize production constraints

Maize production is constraint by both biotic and abiotic factors. Abiotic factors include poor soil fertility due to acidity, inadequate availability of seed and fertilizer and low adoption of improved varieties, drought (Shiferaw *et al.*, 2011). Biotic factors include pests and diseases which result to decline in maize yields. These include weeds such as striga, insects like corn maggots, wire worms, root worms, and white grabs which directly damage maize plant by feeding on it (Ortega, 1987). Above ground pests include spider mites, aphids, thrips, grasshoppers, stem borers, termites, ear worms, and armyworms. Post-harvest pests include weevils, larger grain borer, and anguinous grain moth which are cited to resulting to over 40% yield decline (Zorya *et al.*, 2011; Aulakh *et al*., 2013). Fungal infections are also a challenge to production and include gray leaf spot, common rust (Shiferaw *et al.*, 2011). Maize safety and quality is also affected by infection with *Fusarium spp* and *Aspergillus spp* which result in mycotoxin production (Njuguna *et al*., 1990; Macdonald and Chapman, 1997). Viral infections also constrain maize production resulting in great yield losses. Emergence of a new viral disease

called Maize lethal necrosis (MLN) has significantly affected production of the crop in the region (Wangai *et al.,* 2012).

2.4 History of Maize lethal necrosis disease

Maize lethal necrosis (MLN) disease was first reported in the Rift Valley region of Kenya in 2011 (Wangai *et al*., 2012). Since the outbreak, MLN has spread to other counties in Kenya and neighbouring countries in eastern African (Redinbaugh and Stewart, 2018). The disease is result of maize chlorotic mottle virus (MCMV) in combination with potyviruses infecting cereals including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), and wheat streak mosaic virus (WSMV) (Wang *et al*., 2017). Maize lethal necrosis disease in Africa is mainly associated with MCMV and SCMV (Wangai *et al*., 2012). The two viruses interact within the maize plant and are able to survive and can infect plant at same time or one before the other (Awata *et al.,* 2019). Reports from the recent work have also identified Johnson grass mosaic virus (JGMV) in the African region which is suspected to be contributing to MLN disease development (Stewart *et al*., 2017).

2.5 History of Maize lethal necrosis disease, its spread and impact

Maize lethal necrosis (MLN) was first reported in Kansas USA in 1976 where it was referred to as Corn lethal necrosis (CLN) disease (Niblet and Claffin, 1978). The disease then spread to Nebraska (Doupnik, 1979) and was later reported in Hawaii USA in 1992 (Jiang *et al*., 1992). Previous Other findings have shown MLN to have been described in other countries like Argentina (Teyssandier *et al*., 1982), Thailand (Klinkong and Sutabutra, 1982; Uyemoto, 1983), Mexico (Delgadillo and Gaytán, 1987), China (Xia *et al*., 2011), Kenya (Wangai *et al*., 2012),

Rwanda (Adams *et al*., 2014), Democratic Republic of Congo (Lukanda *et al*., 2014), Uganda (Mahuku *et al*., 2015a, b) and Ethiopia (Mahuku *et al.,* 2015b).

Maize chlorotic mottle virus was initially discovered in Peru in 1974 (Castillo and Herbertt, 1974). Since then, the virus has been identified in Africa as new and responsible for MLN outbreaks in the continent (Wangai *et al*., 2012). Viruses of the Potyviridae family were first described in Ohio and the United States in the 1960s (Redinbaugh and Zambrano, 2014). Sugarcane mosaic virus had been identified in Kenya in 1980's (Louie, 1980) existing in diverse strains with differing biological properties, pathogenicity as well as host range (Awata *et al*., 2019). The virus has been linked to MLN outbreaks in the majority of African countries.

Maize yield losses have been experienced in Kenya due to infection of MLN with national loss estimated at 0.5 MMT, equivalent to 80 million US$ (Prasanna *et al.,* 2020). Similar losses are also experienced in other east African countries (Table 2.1). In Guyas province in Ecuador, yield losses of up to 25 – 40% were experienced between 2015 and 2016 estimated at 64 - 100 million USD (Vega *et al*., 2016). Over 2 billion USD in losses have been estimated in China, which equates to more than 10 MT (Rao *et al.*, 2010). Maize lethal necrosis disease threatens production in SSA (Isabirye and Rwomushana, 2016). Losses up to 100% in some counties in Kenya were experienced equated to 126,000 MMT (Mahuku *et al.*, 2015 a, b). In 2013, farmers in the Western, Rift Valley, and Central Kenya experienced high losses, with farmers in the Western region losing more than 50% of their maize yield, accounting for 22% of Kenya's total maize yield (De Groote *et al*., 2016).

2.6 Maize lethal necrosis disease symptoms

Symptoms of MLN may vary according to the timing of the infection which may occur at early or late stages of plant growth. When infection occurs early, high intense chlorosis is observed

Table 2.1 Approximated losses caused by maize lethal necrosis (MLN) disease (2013 to 2016) in sub-Saharan Africa

Country	Production, 2013−2016 (millions of metric tons)	Estimated losses to smallholder farmers (millions of USD)	Approximate present annual cost to smallholder farmers (millions of USD)	Expected annual losses in the next 5 years (millions of USD)
Ethiopia	5.5	9.0	131–152	154–176
Kenya	3.3	3.4	124–145	141–161
Rwanda	0.5	1.3	2–3	5–6
Tanzania	4.6	3.2	20–23	40–46
Uganda	2.6	3.6	14–16	25–29
Total	16.5	20.5	291–339	365–418

Source: Pratt *et al.* (2017), Redinbaugh *et al.* (2018)

from the foundation of young whorl leaf up to the tips of the leaves (Niblett and Claflin, 1978; Wangai *et al*., 2012; Awata *et al*., 2019). The disease progresses to form long and yellow streaks which are wide and dry out from the edges to the mid rib resulting in "dead heart" symptom leading to plant death (ASARECA, 2014). Late infections result to premature ageing of the plants, male sterility, malformed ears and production of deformed seed and general stunting of the maize plants (Goldberg and Brakke, 1987; Wangai *et al*., 2012).

2.7 Viruses causing Maize lethal necrosis disease

2.7.1 Genome organization of Maize chlorotic mottle virus

Maize chlorotic mottle virus (MCMV) is an RNA virus that is single stranded with positive sense (+) ssRNA non-enveloped monopartite globular structure encased in octahedral shell, each virus having 180 sub units (Awata *et al*., 2019). The virus genome is 4-4.5kb in length and 30 mm diameter with both terminals from 5' to 3 shielded from non-coding regions) (Xia *et al*., 2016). Maize chlorotic mottle virus consists of 4436 nucleotides with 6 open reading frames (ORFs). The ORF1 (P32) participates in virion accumulation encrypts a protein of 32k Daltons. ORF2

virus assembly encodes a 50 kDa protein and also produces a read through (P111) with a 111 kDa protein with UAG stop codon at its N-terminus. Functions of ORF3 are not clearly understood. ORF4 (P7) generates a protein of 7k Daltons and in charge of cell to cell mobility. When the stop codon UAG of ORF4 is suppressed, ORF5 encodes the P31 protein. A 24 kDa coat protein from 3' terminal is involved in cell to cell movement of the virus. P31, P32, and P50 play important roles in host defense mechanisms by displaying viral silencers of RNA (Scheets, 2016).

There are four distinct strains of MCMV that are genetically and geographically distinct. These are MCMV-P reported in Peru, MCMV-KS from Kansas (Nyvall, 1999; Uyemoto, 1983), MCMV-NE from Nebraska (Stenger and French, 2008), and MCMV-YN from Yemen and China. The Kenyan isolate was found to be 96% identical to the Yunnan strain from China (Adams *et al*., 2012) while MCMV - KS isolates from Kansas compared to MCMV NE from Nebraska were 99.5% identical (Nutter *et al*., 1989; Stenger and French, 2008). Symptoms of MCMV on infected plant are mild to severe chlorotic mottling, chlorotic stripes which coalesce to form chlorotic blotches and stunting. In susceptible varieties necrosis of leaves may occur resulting to pre mature plant death. Infections with the virus can also result to partially filled or malformed ears with shortened male inflorescences and few spikes (Awata *et al.,* 2019).

2.7.2 Genome organization of sugarcane mosaic virus

Sugarcane mosaic virus (SCMV) is a single-stranded RNA virus with a positive sense (+) ssRNA which is filamentous and flexus measuring 708 nm by 11 nm length and width (Harrison *et al*., 1971; Adams *et al*., 2005). In infected cells, a characteristic pinwheel or scroll shaped inclusion structures are present (Akbar *et al*., 2017). The virus genome is approximately 10kb

long and has two untranslated regions UTRs and a large opening reading frame (ORF). The 5'terminal (UTR) is covalently linked to a virus genome linked protein whereas 3'terminal (3UTR) is linked to polyadenated tail (POLY A) (Padhi and Ramu, 2011). The ORF has 10 functional proteins which include; protein 1 (P1), protein 3 (P3), 6K1, 6K2, cylindrical inclusion protein (CI), coat protein (CP), helper component proteinase (HC-Pro), NIa-Pro (major protease of the small nuclear inclusion protein, NIa), large nuclear inclusion protein (NIb) and viral protein genome linked (VPg) (Padhi and Ramu, 2011; Shukla *et al*., 1988).

Diversity of SCMV phylogenetically is based on host and geographical origin (Li *et al.*, 2013). Symptoms of the virus include mosaic and dwarfing in maize plants and is manly transmitted by aphids (Brault *et al.*, 2010). Seeds are another mode of transmission of the virus (Li *et al.*, 2011).

Symptoms of infection with SCMV include mosaics or mottles with uneven shades of light to dark green which result to narrow streaks which are light green or yellow along the veins. This mosaic symptoms in the plant usually disappear or fade away as the plant mature. Stunting and poor seed set may occur in early infections (Wu *et al*., 2012).

2.8 Transmission of viruses causing Maize lethal necrosis disease

2.8.1 Transmission of maize chlorotic mottle virus

Virus MCMV is transmitted by beetle species both at larval and adult stages (Sharma and Misra, 2011). This is the only virus in Tombusviridae family that is transmitted by beetles and known to have no latent period. Thrips (*Frankliniella williamsi Hood*) are reported as the most common

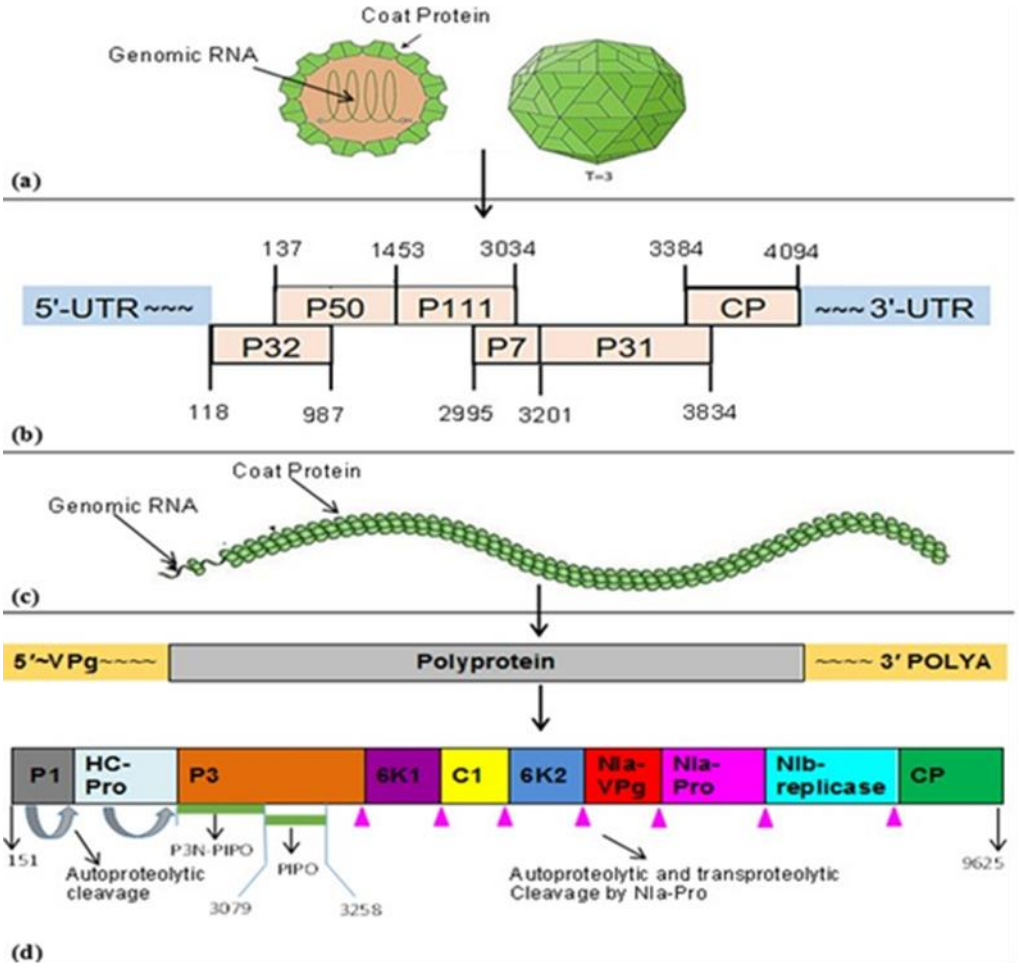


Figure 2. 1 (a) MCMV T=3 octahedral protein shell (b) genomic organisation of MCMV,(c) SCMV composition, (d) SCMV molecular arrangement.
Sources: (Awata *et al*., 2019).

vector transmitting MCMV in many maize producing parts of the continent (Kiruwa *et al*., 2016). A fully grown thrip feeds by breaking the cell wall of the plant tissue. The effectiveness of transmission of the virus is enhanced by longer duration of acquisition and inoculation (ASARECA, 2014). An adult thrip cannot be effective to transmit the virus by having fed at larval stage unless it accesses infected maize plant afresh. Maize chlorotic mottle virus persists in

thrips adult for feeding duration of 6 days, coupled with a decrease in transmission rate as time passes (ASARECA, 2014). Previous study showed virus load in the thrips body decline when they feed on a non-infected maize and as they develop from larval stage to adults (Cabanas *et al*., 2013). Most often thrips have on-going cycle and can be observed all year. This insect takes about three weeks to complete its life cycle as influenced by relative humidity and temperature. According to Sharma and Misra (2011), thrips can produce 12-15 generations in a year under greenhouse conditions. Seed transmission of the virus has been reported though at low rates of 0.03-0.33% (Jensen *et al*., 1991). A different study carried out in East Africa showed 72% of seed from infected fields to contain MCMV, which indicates possibility of transmission through contaminated seed (Mahuku *et al*., 2015a). 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 (Delgadillo *et al*., 1994).

2.8.2 Transmission of sugarcane mosaic virus

Sugarcane mosaic virus is spread in a non-persistent manner by insects of the order Hemiptera mostly aphids (Kiruwa *et al*., 2016). The vectors transmit the virus in non-persistent manner after feeding on infected maize with an acquisition period of 20-30 seconds and inoculation access period of 1-2 minutes to a healthy plant (Sharma and Misra, 2011). Several factors determine transmission efficiency which includes the host, strain of the virus as well as environmental conditions (Sharma and Misra, 2011). Presence of weeds acting as host of the aphid accelerates dissemination of the virus in the next period. The virus can also spread along significant distances by being carried by wind turbulence from one place to another. 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% (Sherpherd and Holdeman, 1965; Williams *et al*., 1968; Mikel *et al*., 1984; Hill *et al*., 1974) 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). 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).

2.9 Cycle of infection of viruses causing Maize lethal necrosis disease

The MLN viruses multiply in the host cytoplasm and function as both genome and messenger since they are (+) ssRNA (Mbega *et al*., 2016). In the host cell the viruses use the metabolic machinery to produce its own genetic material for multiplication and translation process. Virus particle penetrates the cell of the host through injuries made by vectors, mechanical or through contaminated maize pollen (Xia *et al*., 2011). Viral uncoating takes place in the cytoplasm resulting in production of virus replication proteins (Rp) and (+) ssRNA genomic material. The genomic material (+) ssRNA plays a role in which it acts as replication template, packaging material for virions during assembly of virus and mRNA for viral proteins synthesis. On the other hand RNA-dependent - RNA-polymerases are found in RP (RpRd) and replication-associated proteins, which form replication complexes by interacting with host factors. The RdRp replication produces new (+) ssRNA progeny via complementary (-) ssRNA (Kawamura-nagaya *et al*., 2014). In other cases, (+) ssRNA genetic material have been induced to generate mobility polypeptidess (MP) and coat polypeptides by host golgi apparatus. Remainder of (+) ssRNA progeny come together with CP and MP forming fresh virus components prepared to evade the next cell beginning a new infection cycle (Carrington *et al*., 1996; Scholthof, 2005). The mature MLN virions move to the next cell via plasmodesmata,

either by quasi directed motion (just genomic RNA) or non-quasi directed motion (just intact virions). Virus transport is aided by protein mobility and P3N-PIPO (Scholthof, 2005).

2.10 Synergistic interactions of MCMV and potyviruses

An interaction of two or more viruses within a plant is referred to as synergism which mostly results in more severe effects than in single infections. Two types of synergistic interactions are known to exist: Potyvirus-associated synergisms occur when one of the viruses is a potyvirus; while non-potyvirus synergisms occur when neither virus belongs to the same group. Studies have shown viral load to be high and increased symptoms in mixed infections of viruses (Mbega *et al*., 2016). Synergistic effects have been observed in bean pod mottle virus (BPMV) a potyvirus that interacts with another potyvirus soybean mosaic virus (SMV) (Lee and Ross, 1972; Calvert and Ghabriel, 1983; Anjos *et al*., 1992) and the well characterized synergistic interaction between potato virus X (PVX) and a potyvirus potato virus Y (PVY) (Lee and Ross, 1972; Calvert and Ghabriel 1983; Goodman and Ross, 1974; Vance, 1991). Previous research has found that MDMV-A and SCMV virus attacks accelerated intensity of fungal diseases including northern corn leaf spot, gray leaf spot, southern corn leaf blight, diplodia leaf streak, and eyespot while the accumulations of the both potyviruses remained constant (Meyer and Pataky, 2010). Mixed interactions of MCMV and SCMV or MDMV have been shown to result in lethal symptomatology and an upsurge in MCMV concentration while MDMV and SCMV concentrations remain unchanged in sick plants (Xia *et al*., 2016). Maize chlorotic mottle virus is considered as the primary cause of MLN outbreaks because of its high transmission prevalence and occurrence in comparison with SCMV (Awata *et al*., 2019).

Genomic concept of synergistic effects is poorly understood, nevertheless infected maize plants with high concentrations of RdRp results in stimulations of synthesis of SCMV virus-derived siRNAs (vsiRNA) in plenty. Increased vsiRNA density causes SCMV mRNA degeneration (suppressing) resulting in low SCMV densities in plants (Wang *et al*., 2017). Previous research by Xia *et al*. (2016) found that SCMV- induced vsiRNAs contribute for over fifty percent of cumulative small vsiRNAs in mixed infection, whereas MCMV-induced vsiRNA seemed to be 14.7- 19.49 percent, indicating that SCMV RNA is much selective for RNA suppression and vsiRNA buildup. In SCMV, the viral helper component protein (HC-Pro) performs an important role in synergistic relations alongside replication and movement. In mixed infections of MCMV and SCMV, replication and movement SCMV is reduced incase HC-Pro is prioritized as VSRs. In this case, SCMV concentration in mixed infections is constant, which is sustained by virus particles like P1 and VPg, which aren't powerful replication and mobility proteins boosters. This explains why the amount of SCMV in combined infestation with MCMV doesn't at all rise in constant comparison to MCMV (Ivanov *et al*., 2014). Furthermore, due to internal codon and long 3' UTRs, nonsense-mediated decay (NMD) could remove MCMV RNAs and reduce the concentration of M-vsiRNAs in MCMV in mono and combined (of SCMV) maize diseased crops (Xia *et al*., 2016). The above scenario enables a few MCMV RNAs to bypass degeneration and then be translated into valid mRNAs, causing a rise in MCMV accumulation (Wang *et al*., 2017). The RNase III enzyme plays a role in potyviral synergism interactions leading to potyviruses levels to be high in infected plant (Cuellar *et al*., 2009). According to Liu *et al*. (2017b), ZmTrxh maize gene, encoding a *h*-type thioredoxin meant for resistance at Scmv1 locus, plays a role in suppression of SCMV RNA accumulation. In general, potyvirus HC- pro is

important in the virus infection process, and also MCMV pathogenicity and replication in combined pathogens (Wang *et al*., 2017).

2.11 Diagnosis and detection of viruses causing Maize lethal necrosis disease

Detection and identification is key towards intervention and management of disease causing viruses.Visual observation of disease symptoms is not adequate since it cannot identify the causative agent. Electron microscopy has been used to identify virus particles but has the challenge of more work to prove that the particles are the ones causing the disease (Sharma and Misra, 2011). There are various methods that have been effective in detection of MLN viruses which include serology, molecular techniques and sequencing techniques (Xia *et al*., 2016).

2.11.1 Symptomatology

Symptoms expression has been used to describe MLN disease resulting from infection of MCMV in combination with SCMV. The disease symptoms typically manifest on maize plant parts where the virus replication is occurring and viruses move from the inoculation site to the phloem (Xia *et al*., 2016). Due to accumulation of the viruses in young tissue and upper leaves as a result of high replication, symptoms are manifested strongly (Kiruwa *et al*., 2016). Symptoms due to infection by viruses causing MLN vary due to a variety of factors such as variety, plant age, environmental conditions, and virus strain. Infection by multiple viruses could also induce similar symptoms on a host (Wang *et al*., 2017). This makes it difficult to confirm with certainty that the plant is infected with a particular virus. Signs could be confirmed by rubbing non-infected leaves against healthy leaves and transferring a viral infection from an infected to a healthy plant. This can then be determined using electron microscopy or serological

tests (Xia *et al*., 2016).

2.11.2 Enzyme-linked immunosorbent assay

Plant viruses have been identified and analyzed using enzyme-linked immunosorbent assay (ELISA) methods (Wu *et al.,* 2013). Viruses causing MLN disease have been to detected using ELISA method which is easy to adopt (Thorat *et al*., 2015). Serological tests include the Double Antibody Sandwich Enzyme-linked Immunosorbent Assay (DAS-ELISA), Triple Antibody Sandwich Enzyme-linked Immunosorbent Assay (TAS-ELISA), and Direct antibody capture (DAC-ELISA). Many studies of plant viral disease identification have used ELISA because it is specific, simple, sensitive, and inexpensive (Lima *et al*., 2012). ELISA was used in MCMV and SCMV identification studies (Mahuku *et al*., 2015a). Coat protein of the virus has antigens which react with specific antibodies in a specific way. In positive reactions, yellow coloration results from the reaction of the immunogenic indicator (epitope) with the antibody's scripting area (paratope) (Xia *et al*., 2016).

2.11.3 Polymerase Chain Reaction Reverse-Transcription (RT-PCR)

This has been one of the more recent techniques for amplifying specific nucleic acid sequences to quantifiable levels. PCR, which is highly sensitive and specific, is used in plant virus diagnostics and PCR product sequencing. The use of qPCR has made easy the detection of plant viral diseases such as MCMV and SCMV in maize seeds (Wangai *et al.*, 2012).

It is a sensitive technique that is nucleic acid based that amplifies small quantities of nucleic acid for ease of detection. Viruses MCMV and SCMV are RNA based so reverse transcription followed by amplification is used in detection of the two viruses using specific primers (Thorat *et al*., 2015). In Kenya, Wangai *et al*. (2012) used RT PCR to detect SCMV and MCMV in

maize, as did Kusia *et al*. (2015) in finger millet. This was also seen in Congo in detection of MCMV in maize (Lukanda *et al*., 2014) as well as Adams *et al*. (2014) in real time PCR to detect MCMV and SCMV in Rwanda. Real time PCR has also been used to detect MCMV in corn thrips *Frankliniella williamsi*) (Cabanas *et al*., 2013).

2.11.4 Next generation sequence (NGS)

This method is the most accurate, fast and generates reliable genomic information where a large accurate amount of data in form of sequences is acquired. This technique uses nucleic acid sequences which are similar to already known viruses in the Genbank (Adams *et al*., 2012). The technique was used to identify MCMV isolates obtained from Kenyan samples where a full-length MCMV sequence (4452bp: accession number KP 8519970) showed 99% genome identity to sequences of 12 MCMV isolates deposited in GenBank (Adams *et al*., 2012) together with four from Rwanda (Adams *et al*., 2014). There was also 99% identity to MCMV isolates from maize and sugarcane collected in Yunnan and Sichnan, China; 98% identical with another isolates from Yunnan, China (GU138674) and 96 to 97% identity to MCMV isolate from Kansas and Nebraska in the United States. Coat protein sequences of isolates from Tanzania had 99% identity to those from Kenya, DR Congo and Rwanda (Mahuku *et al*., 2015b). The close relationship between isolates of MCMV in Kenya and China means possibility of MMCMV from Kenya could have originated from china.

2.12 Management of Maize lethal necrosis disease

Disease management entails using appropriate techniques to manage a particular disease. The primary goal of managing a viral disease is to minimize damage and infection to the plant

(Maloy, 2005). Knowledge of the disease's cause and effect aids in the disease's effective and proper management.

2.12.1 Genetic resistance and tolerant varieties

Several studies on genetic resistance to SCMV, WSMV, and MDMV have identified a number of quantitative trait loci (QTLs) for resistance in chromatids 1, 2, 3, 6, 7, and 10 (Yang *et al*., 2017). These QTLs can be modified by a number of genes with additive impacts (Beyene *et al*., 2017). Resistance of maize to SCMV has been linked to two loci (Scmv1 and Scmv2) located in chromosomes 6 and 3, respectively. In SCMV defense, Scmv1 plays a role in early infections, while Scmv2 plays a role in late infections, and two loci differentiate as dominant alleles (Liu *et al*., 2017a). A recessive gene (Scm3 on chromosome 3) was also reported by Zhang, *et al*. (2008) which was seen to provide resistance throughout maize growth period.

2.12.2 Use of clean seed

Planting of certified seed with appropriate insecticide is important and farmers should avoid recycling of seeds. Use of certified seed contributes greatly to the management of MLN disease since they have been declared free from the viruses. Seed treatment has been shown to give protection against attack by the vectors during early stages of maize crops (Alford, 2000).

2.12.3 Integrated disease management

Different approaches has been deployed for control for MLN disease in Kenya but none has been shown to be effective. Cultural measures, insect vector management, and resistance breeding have all been incorporated into efforts to contain the disease. Management of vectors by use of

insecticides targeting the insects results to disease decline. The use Imidacloripid as a seed dressing together with foliar sprays was reported to result to disease decline .The use of maize hybrids and cultivars that are high-yielding and have strong and durable resistance to MLN is the best option for disease control. There has been a lot of efforts and good progress has been made in developing hybrids which are tolerant to MLN in East Africa. Production of maize continuously in same field contributes greatly to MLN incidence. Crop rotation has been shown to contribute to control of the MLN disease by breaking the cycle of the vectors with crops belonging to different families. These include beans, peas, cassava, Irish potatoes, and sweet potatoes which also have economic value to the farmers (Uyemoto, 1983). Planting during long rains and avoiding cultivation of maize during short rains can help to reduce the virus load and vectors in the farm.

2.12.4 Quarantine and regulatory measures

Regulatory measures as a management strategy aims to avoid disease introduction into new areas through quarantine procedures. Both the entry and exit ports of trading countries inspect maize seeds. One of the most efficient ways to prevent the spread of MCMV is to implement quarantine laws, which are widely accepted (Adams *et al.,* 2014). Boosting quarantine measures and procedures will also help in limiting the spread of MLN into new areas, reducing threats to sustainability of food.

2.12.5 Eradication and avoidance

This is a strategy of eradicating a pathogen or removing it from a location before it becomes established (Maloy, 2005). Good hygiene is one method of pathogen elimination that includes

washing farm equipment, roguing diseased maize plants, clearing alternate hosts, and other field hygiene measures (Mawishe and Chacha, 2013). According to Wangai *et al.* (2012), crop rotation may be done with non-host crops including cassava, onion, bean, garlic and potatoes. This will help in breaking the cycle for the breeding of the vectors by introduction of non-host crop in between the seasons. Insect vectors may also be lowered by utilizing methods such as sticky traps and reflective mulches for aphids, which minimize their movement and thus the amount of disease inocula transmitted. This management strategy reduces contact between the host and plant viruses. One of the avoidance strategies is to plant maize in non-infested fields and also through early planting when the disease pressure is low. Early planting allows the planting to germinate and develop before the vector population has increased hence less infection. Since viruses enter plants through wounds, adequate spacing is another mechanism for reducing plant injury. Wangai *et al*. (2012) observed that a closed season, along with the use of certified seeds reduced the vector population, resulting in lower disease infection rates and severity.

CHAPTER THREE

STATUS OF MAIZE LETHAL NECROSIS DISEASE IN SEED PRODUCTION SYSTEM IN KENYA

Abstract

Production of maize in Kenya is threatened by maize lethal necrosis (MLN) disease in the field. This study was carried out to assess the status of the disease in fields of maize seed producers. A surveillance was conducted in 5 agro-ecological regions in 13 counties in 2015, 2017, 2018, 2019. Sampling for symptomatic and non-symptomatic maize in fields producing seed was done using a standardized protocol. On site MCMV testing was performed using immunostrips followed by real time Polymerase Chain Reaction (qRT- PCR) test in the laboratory. A total of 2550 ha of fields producing maize seed was visited where 21% were found to have MLN disease at varying levels of severity. The incidence and severity was not significantly different ($P>0.05$) in different agro-ecological regions, counties, maize varieties and growth stages. High MLN disease incidences and severities were observed in Sub-humid region comprising of Embu, Uasin Gishu, Nakuru and Elgeyo Marakwet counties which form the hotspots for MLN disease. The most common MLN-causing viruses detected using q-RT- PCR were MCMV and SCMV. In total, 38% of the samples tested positive for MCMV alone, 14% for SCMV alone, and 18% for both MCMV and SCMV. From the 185 samples analyzed with immunostrip from 2017-2019, 29 (16%) were positive for MCMV. Phytosanitary programs should be included in seed legislation for legal adoption and at the same time controlling the spread of MLN disease should focus on high-risk agro-ecological regions and counties.

3.1 Introduction

Maize is a significant crop in Africa's sub-Saharan regions and a basic food to approximately 70 million of the population, with its production occupying over 25 million ha (Melinda *et al*., 2013). In Kenya, maize constitutes a significant source of food and is cultivated by both large and small-scale farmers. More than 90% of Kenyans rely on it for their livelihood, human consumption and raw material for industrial uses (Manje *et al.,* 2015). Amongst the challenges, maize lethal necrosis (MLN) disease has been rated as a major constraint to production of maize (Yang *et al.,* 2017). Maize lethal disease is triggered by a combined infection of (MCMV) and a maize infecting potyvirus like maize dwarf mosaic virus (MDMV) or sugarcane mosaic virus (SCMV) (Wangai *et al.,* 2012).

Maize lethal necrosis disease was first described in the USA and symptoms such as chlorosis, mosaic and necrosis were seen in infected plants, resulting to either stunted growth or death of the plant (Niblett and Claflin, 1978; Uyemoto *et al.,* 1980). In Africa, MLN has been reported in Rwanda, the Democratic Republic of the Congo, and the border districts of Uganda (Adams e*t al*., 2014). The disease has spread to all the eastern Africa countries and most of the SSA countries, with significant effect on maize production (Manje *et al.,* 2015). The infection was first discovered in Kenya in 2011 in the Bomet area (Wangai *et al*., 2012), resulting in a US$ 67 million maize loss in 2012 (Prasanna, 2014). The disease has since expanded to other regions of the country, including the Central, Nyanza, Western, and Rift Valley regions (Wangai *et al.,* 2012; Miano, 2014).

In Kenya, MLN disease is brought about by a combination of MCMV and SCMV (Adams *et al*., 2014). The two viruses are transmitted by different vectors (Jiang *et al*., 1992; Cabanas, 2013). Thrips and beetles are reported to transmit MCMV (Zhao *et al.,* 2014). On the other hand SCMV

is spread by Aphids (*Myzus persicae* and *Aphis gossypii*) (Cabanas *et al*., 2013). Previous study has also shown that infected seed has a very low transmission rate of MCMV and SCMV (Jensen *et al*., 1991; Zhang *et al*., 2011b; Mahuku *et al*., 2015a). Jensen *et al*. (1991) reported seed dissemination of MCMV in America while Mahuku *et al*. (2015a) reported the same in Africa. Li *et al.* (2011) reported that SCMV could also be transmitted through seed. Infection of maize with MCMV and SCMV can be in single or combined (Guadie *et al.,* 2018).

In order to put strategies that are effective for the control of MLN disease, there is need to have information on the development and factors contributing to its spread. This will provide accurate information to allow for implementation of integrated disease management. There is currently no information on the incidence and severity of the disease in Kenya's major seed production areas. This has made it difficult to employ the right and effective measures to the disease management. Phytosanitary procedures need to be strengthened to enhance the delivery of virus-free seeds due to correlation between vectors' presence and MLN epidemics (Prasanna *et al*., 2020). The use of field validated protocols and techniques like immunostrips can also enhance on the disease's surveillance. The goal of this research was to document the presence of MLN in seed maize crop fields in Kenya.

3.2 Materials and Methods

3.2.1 Study sites

Surveillance was were conducted in 2015, 2017, 2018 and 2019 to evaluate the MLN disease status in key maize seed production regions in Kenya from small and large scale seed companies and producers. The study covered 13 Kenyan counties in 5 agro-ecological regions categorized according to vegetation, altitude and climatic conditions. The humid zone covered the following

counties; Kakamega, Transnzoia and Meru with elevation between 1980-2700 m and minimum rainfall of 1000 mm. The sub-humid zone covered Embu, Uasin Gishu, Nakuru and Elgeyo Marakwet Counties with elevation between 900 – 1800 m and annual rainfall of between 950 – 1500mm. The semi humid zone covered West Pokot and Machakos counties with elevation of 900 – 1800 m and annual rainfall of 500 – 1000 mm. The semi-arid zone covered Makueni and Kajiado with an annual rainfall of 300 – 600 mm. The arid zones covered Baringo and Taita Taveta counties with an annual rainfall of 200 -400 mm.

3.2.2 Surveillance design

In order to document the condition of the fields as well as the temporal and geographic distribution of MLN disease in the counties, maize seed fields were assessed directly and producers were interviewed. Fields in seed-producing regions were purposefully chosen every 10-20 km. In determining the incidence and severity of MLN, maize variety, agro-ecological and growth stages were considered during the survey periods. These growth stages were; V1 to V9 – vegetative stages, VT-Tassling, R1- flowering/silking, R2- Blistering, R3- Milky, R4-Dough, R5- physiologically mature, R6– Harvesting stage. In each seed field, evaluation for incidences and severities scoring was done along a quadrat counting 20 plants and crossing the field in two diagonals forming an X pattern. The counts were defined by the size of the field and used to calculate the number of plants within a transect. 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

Severity was assessed and recorded based on a scale of 1-5 (Gowda *et al.,* 2015) where 1= no symptoms, 2 = <10% of leaf surface showing symptoms, 3 = 1–30% plant surface showing symptoms, 4 = 31-50% of plant surface showing symptoms, 5 = >51% of plant surface showing symptoms. Disease severity scores were converted into a percentage severity index (PSI) for analysis (Wheeler, 1969).

3.2.3 Detection of MCMV using Immunostrip in the field

Surveillances were carried out using CIMMYT and partners standardized protocols for MLN in which an average of 6 leaves were obtained from each field and screened on site using immunostrips (Bioreba). A bulk homogenized sample was made by pooling the six samples which was employed to detect a presence of MCMV. The bulk samples were ground in buffer to get the sap and an immnostrip dipped into the solution to detect the virus. The result is an easy to read pattern of bands (single band for negative, double-band for positive). Using GPS, all sampled fields were geo-referenced and mapping information for sampling points and associated incidence and severity was created.

3.2.4 Detection of MLN viruses using real time Polymerase Chain Reaction

3.2.4.1 Isolation of RNA

By using modified Cetyl trimethylammonium bromide (CTAB) method, total RNA was extracted from samples of maize leaves (Adams *et al.,* 2009). Leaves approximately100 mg were crushed in 1 ml of 0.1M Tris base (pH 8) CTAB buffer, 2% CTAB w/v, 0.02 M EDTA and 1.4M NaCl, 2% polyvinylpyrrolidone (PVP) and 1% Na_2SO3 added before use. The extract of

leaves were loaded into a 1.5 ml sterile microfuge tube and incubated for 10 min at 65°C. The extracts were diluted with an equivalent amount of chloroform: isoamyl alcohol (24:1) after incubation and centrifuged at 12 000 rpm for 10 min.

The intermediate aqueous phase was transferred in sterile Eppendorf tube mixed with an equivalent amount of 4 M LiCl and incubated at –20°C for one hour. Samples were vortexed and centrifuged for 10 minutes at 12,000 rpm once again. In a different sterile eppendoff tube, 450 µl supernatant was obtained and 300 µl of cold iso-propanol was transferred to the tube and incubated for one hour at -20°C. The samples were then centrifuged at 12,000 g for 25 minutes. The resultant pellets were washed in 70% ethanol, air-dried and re-suspended in 50 µL of water and stored at -20^{o}C for further analysis. Quantity and quality checks were implemented using a NanoDrop (Thermo fisher scientific, Madison, USA).

3.2.4.2 Virus detection by Polymerase Chain Reaction

The RNA from the positively identified samples by immunostrips were analysed to confirm the presence of MCMV using real time Polymerase Chain Reaction (qRT-PCR) as described by Adams *et al*. (2012). Real time qPCR was done in 1 µL of RNA of 25 µL reaction volume containing 2.5 µL of 10x PCR buffer, 5.5 µL of 25 mM $MgCl_2$, 2.0 µL of 6.25 Mm of dNTPs, 1.1 µL of 7.5 uM of forward primer and reverse primer, 0.5 µL of Taqman probe, 0.05 µL of MMLV, and 0.125 µL of Taq polymerase enzyme. Thermal requirements for PCR amplification were set as follows: 48°C for 30 minutes for cDNA synthesis, 95°C for 10 minutes to deactivate MMLV and activate taq polymerase proceeded by denaturation at 95°C for 15 seconds and annealing/extension at 60°C for one minute for 40 cycles. Nucleic acids from a known infected

plant material, from a healthy plant known to be virus free, and a non-template control were also included.

The assay was performed using primers and probes targeting coat protein region which are MCMV F: 5’ – CCGGTCTACCCGAGGTAGAAA – 3’ MCMV R: 5’ – TGGCTCGAATAGCTCTGGATT T – 3’. The Probes involved were MCMV Pe 5’ - [FAM] – CAGCGCGGACGTAGCGTGGA - [BHQ1] - 3’. SCMV F: 5’ CCA GGC CAA CTT GTA ACA AAG C - 3’, SCMV R: 5’ - CAT CAT GTG TGG ATA AAT ACA GTT GAA - 3’and SCMV pe (FAM)-TGT CGT TAA AGG CCC ATG TCC GCA-BHQ1. Data from the tests were obtained in the form of values of Ct (cycle thresholds), which is typically inversely proportional to the amount of virus in the samples. Ct values less than 29 indicated strong positive reactions showing high amounts of the target MCMV virus concentrations whereas Ct of 30 to 35 indicated low virus concentration. All samples with Ct values less than 35 were deemed positive and those with value above 35 were classified as being negative for the virus (Zhang *et al.*, 2011b).

3.2.5 Data analysis

A general analysis of variance (ANOVA) was used to determine the significance of the effects of agro-ecological condition, maize variety, and growth stage on MLN disease severities and incidence using GenStat 15th edition statistical software (VSN International, UK). The test statistic considered the sample sizes, means, and standard deviations in each of the comparison groups.

3.3 Results

3.3.1 Maize lethal necrosis disease incidence and severity in Kenyan agro-ecological regions

In the year 2019, there was no MLN disease occurrence noted in all agro-ecological regions (Table 3.1). The highest MLN incidence of 6.8 was recorded in the year 2015 in the semi humid agro-ecological region. The highest MLN severity index of 2.3 was documented in the year 2015 in semi humid agro-ecological region while the lowest severity index of 1 was recorded in all agro-ecological region of Kenya. The study reported no MLN disease incidence and severity in semi-arid regions in 2015 and arid regions in 2018.

Table 3.1 Maize lethal necrosis (MLN) incidence and severity in Kenyan agro-ecological regions over time

Agro-ecological regions	2015		2017		2018		2019	
	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)
Arid	0.8	2.1	0.1	1.8	0	1	0	1
Humid	0.8	1.6	0.6	1.6	0.3	1.2	0	1
Semi-arid	0	1	0.04	1.4	2.3	1.3	0	1
Semi humid	6.8	2.3	0.4	1	0.7	1.8	0	1
Sub humid	6.3	2.2	0.7	1.7	3	1.3	0	1

The mean incidence of MLN disease in various agro-ecological regions in Kenya was highest in sub-humid region that recorded 2.5, followed with semi humid region with 1.98, semi-arid agro-ecological region had 0.59, humid with 0.43 and least MLN incidence was documented in arid region with incidence of 0.23 (Table 3.2). Sub humid agro-ecological region recorded the highest mean severity index of 1.55, then semi humid with 1.53, arid with 1.48, humid with 1.35 and lowest mean severity index of 1.18 was noted in semi-arid agro-ecological region (Table 3.2).

There was no significant difference (P>0.05) on MLN incidence [F (4, 15) = 1.05, P=0.416] and severity [F (4, 15) = 0.42, P=0.789] across the five agro-ecological areas.

Table 3.2 Maize lethal necrosis (MLN) disease incidence and severity across agro-ecological regions

Agro ecological regions	Annual rainfall (mm)	Counties	Mean incidence +SEM	Mean severity +SEM
Semi-arid	300 – 600 mm	Makueni and Kajiado	0.59 ±0.57	1.175 ±0.10
Humid	1000 1100 mm.	Kakamega, Transnzoia and Meru	0.43 ±0.18	1.35 ±0.15
Arid	200 -400 mm	Baringo and Taita Taveta	0.23 ±0.19	1.475 ±0.29
Semi humid	500 – 1000 mm	West Pokot and Machakos	1.98 ±1.61	1.525 ±0.32
Sub humid	950 – 1000mm	Uasin Gishu, Nakuru , Elgeyo Marakwet	2.50 ±1.42	1.55 ±0.26
LSD			3.02	0.716

3.3.2 Incidence and severity of MLN in major maize production counties in Kenya

For the four years, a total of 13 counties consistently produced seed during this duration. Embu County had the highest MLN mean incidence of 5.32 while Kajiado County had zero MLN mean incidence (Table 3.3). Elgeyo Marakwet had the highest mean MLN severity index of 1.8 followed by Embu County with 1.7. The lowest mean MLN severity index of 1.0 was documented in Kajiado County. There was no significant difference (P>0.05) on incidence among the counties in Kenya for the 13 counties [F (12, 39) = 0.71, P=0.733]. There was no significant difference on MLN severity (P>0.05) among the counties in Kenya for the 13 counties [F (12, 39) = 0.6, P=0.828].

The highest MLN incidence of 21 was documented in the year 2015 in Embu County while the lowest MLN incidence of zero was recorded in all counties of Kenya in the year 2019 (Table 3.4). Embu and Pokot counties recorded the highest MLN severity index of 2.6 in the year 2015 followed by Elgeyo Marakwet with 2.5. The least MLN severity of 1 was noted in all the

counties in the year 2019. Distribution of viruses within the counties in the consecutive years showed decline in MLN disease.

Table 3.1 Mean maize lethal necrosis (MLN) disease incidence and severity of sampled maize growing counties in different agro ecological regions in Kenya

County	Agro ecological Regions	Mean Incidence +SEM	Mean Severity +SEM
Embu	Sub humid	5.32 ±5.32	1.7 ±0.39
Nakuru	Sub humid	3.04 ±2.79	1.4 ±0.21
Uasin Gishu	Sub humid	0.16 ±0.15	1.3 ±0.24
E.Marakwet	Sub humid	1.45 ±0.70	1.8 ±0.35
Machakos	Semi humid	2.66 ±2.45	1.4 ±0.24
Pokot	Semi humid	1.24 ±0.77	1.7 ±0.39
Makueni	Semi-arid	1.17 ±1.14	1.4 ±0.21
Kajiado	Semi-arid	0.00 ±0.00	1.0 ±0.00
Transzoia	Humid	0.54 ±0.54	1.3 ±0.30
Meru	Humid	0.38 ±0.18	1.6 ±0.25
Kakamega	Humid	0.33 ±0.24	1.4 ±0.17
Baringo	Arid	0.42 ±0.36	1.6 ±0.38
Taita Taveta	Arid	0.04 ±0.04	1.3 ±0.28
LSD		5.27	0.805

Table 3.2 Maize lethal necrosis (MLN) disease incidence and severity for selected Kenyan counties over years

County	2015		2017		2018		2019	
	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)
Baringo	1.5	2	0.16	2.5	0	1	0	1
E.Marakwet	3	2.5	2.2	2.3	0.6	1.5	0	1
Embu	21	2.6	0.29	2	0	1	0	1
Kajiado	0	1	0	1	0	1	0	1
Kakamega	0	1.3	1	1.8	0.3	1.3	0	1
Machakos	10	2	0	1	0.63	1.5	0	1
Makueni	0	1	0.07	1.8	4.6	1.6	0	1
Meru	0.15	2	0.7	2	0.67	1.3	0	1
Nakuru	0.61	1.6	0.15	1	11.4	1.8	0	1
Pokot	3.5	2.6	0.69	1	0.77	2	0	1
T.Taveta	0.17	2.1	0	1	0	1	0	1
Transzoia	2.16	2.2	0	1	0	1	0	1
Uasin Gishu	0.6	2	0.16	1.3	0	1	0	1

3.3.3 Disease incidence and severity in maize varieties under maize seed production

A total of 8 maize varieties that were consistently produced were sampled during the surveillance period. The highest mean MLN incidence of 10.9 was recorded in DK 8031 maize variety while Duma 43 and DH04 recorded the least mean incidence of zero (Table 3.5). The MLN severity of 1.7 was the highest in variety DK 8031 while the lowest mean MLN severity index of 1 was recorded in DH04. There was no significant difference (P>0.05) on MLN incidence among the 8 varieties sampled [F (7, 24) = 0.89, P=0.057]. There was no significant difference (P>0.05) on MLN severity among the 8 maize varieties sampled [F (7, 24) = 0.62, P=0.732]

Table 3.3 Mean maize lethal necrosis (MLN) disease incidence and severity on commonly grown maize varieties in Kenya

Variety	Mean Incidence +SEM	Mean Severity +SEM
DK 8031	10.9 ±10.4	1.7 ±0.45
WE 1101	5.1 ±5.0	1.5 ±0.24
H 624	1.3 ±1.2	1.4 ±0.36
H 6213	1.0 ±0.3	1.5 ±0.23
H 614	0.2 ±0.1	1.4 ±0.26
H 513	0.1 ±0.1	1.2 ±0.20
DH04	0.0 ±0.0	1.0 ±0
DUMA 43	0.0 ±0.0	1.0 ±0.
LSD	11.95	0.81

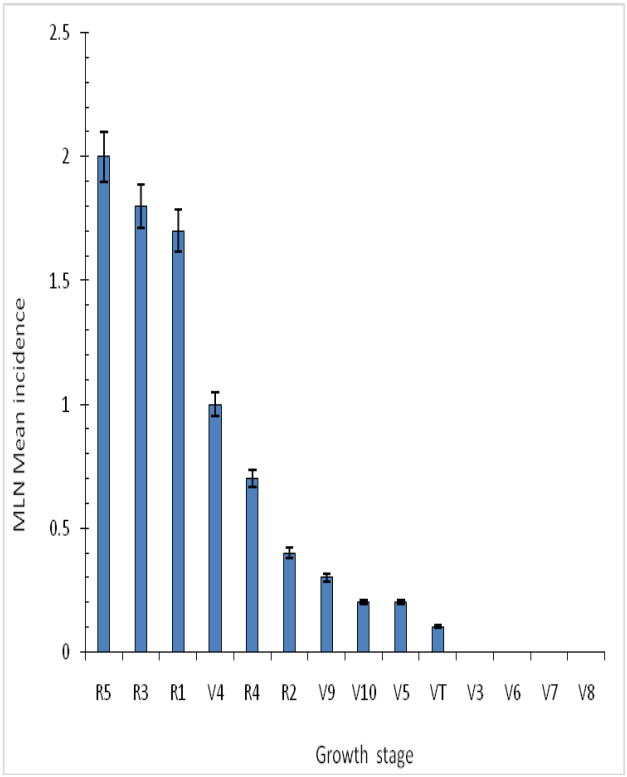
In the year 2018, variety DK 8031 recorded the highest incidence of 42 followed by WE1101 that recorded 20.1 MLN incidence in 2015 (Table 3.6). The MLN severity index of 3 was highest for variety DK 8031 in the year 2018 followed by severity index of 2.5 for H624 in the year 2015.

Table 3.4 Maize lethal necrosis (MLN) disease incidence and severity on commonly grown maize varieties in Kenya

Variety	2015		2017		2018		2019	
	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)	MLN Incidence (%)	MLN Severity (1-5)
DH04	0	1	0	1	0	1	0	1
DK 8031	0.6	1.7	0.1	1.2	42	3	1	1
DUMA 43	0	2	0	1	0	1	0	1
H 513	0	1	0.3	1.8	0	1	0	1
H 614	0.4	2.1	0.3	1.5	0	1	0	1
H 6213	1.8	2.1	1.3	1.5	0.4	1	0.5	1.5
H 624	5	2.5	0.15	1.2	0	1	0	1
WE 1101	20.1	2	0.2	1.6	0	1	0	1

3.3.4 Disease incidence and severity in different maize growth stages

In the assessment of MLN incidence and severity, different growth stages were considered during the surveillance periods. These stages were; V1 to V9 vegetative stages, VT-Tasseling, R1- flowering/silking, R2- Blistering, R3- Milky, R4-Dough, R5- physiologically mature, R6– Harvesting stage. Majority of the crops surveyed were at R4 (dough stage) at 23% followed by R3 (milk stage) at 17%. Flowering and physiologically mature stages were also recorded during the surveillances covering 14% of the total growth stages. Growth stage R5 (physiologically mature) recorded the highest incidence of 2 followed by R3 with MLN mean incidence of 1.8. The least MLN mean incidence was seen in V3, V6, V7 and V8 maize growth stage that recorded zero MLN mean incidence (Fig 3.1). Growth stage R4 recorded the highest severity 1.8 with the lowest at (V3) vegetative stage (Fig. 3.1). There was no significant difference on MLN incidence ($P>0.05$) among maize growth stages for all the growth stages sampled [$F(13, 42) = 0.71$, $P=0.723$]. There was no significant difference ($P>0.05$) on MLN disease severity among maize growth stages for the 14 growth stages sampled [$F(13, 42) = 0.61$, $P=0.807$].



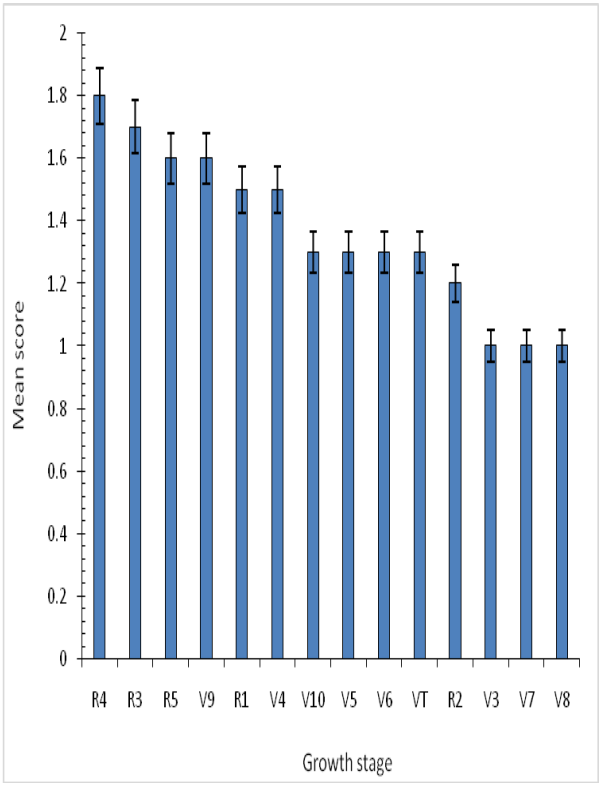


Figure 3.1 Maize lethal necrosis (MLN) disease incidence and severity for various maize growth stages

3.3.5 Detection of MLN causing viruses using Real time qRT-PCR

In 2015, samples were tested for the presence of MCMV and SCMV using qRT-PCR. A total of 118 samples were collected out of which 38% were found to have MCMV, 14% with SCMV, 18% with both MCMV and SCMV and 30% did not have any of the viruses (Figure 3.3). Figure 3.4 shows ct values for some samples that were positive while others negative using specific primers and labelled primer probes.

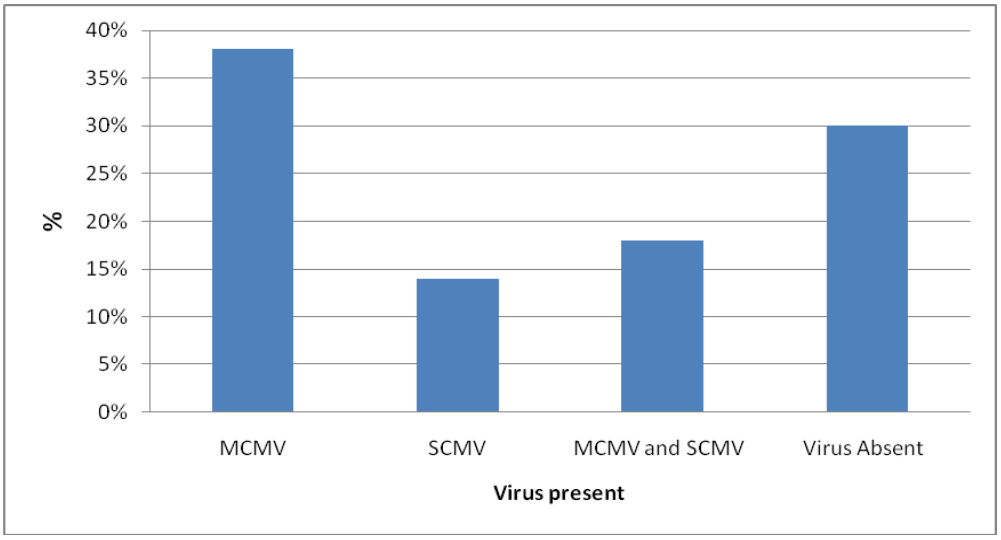


Figure 3.2 Detection of maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) using Real time PCR assays

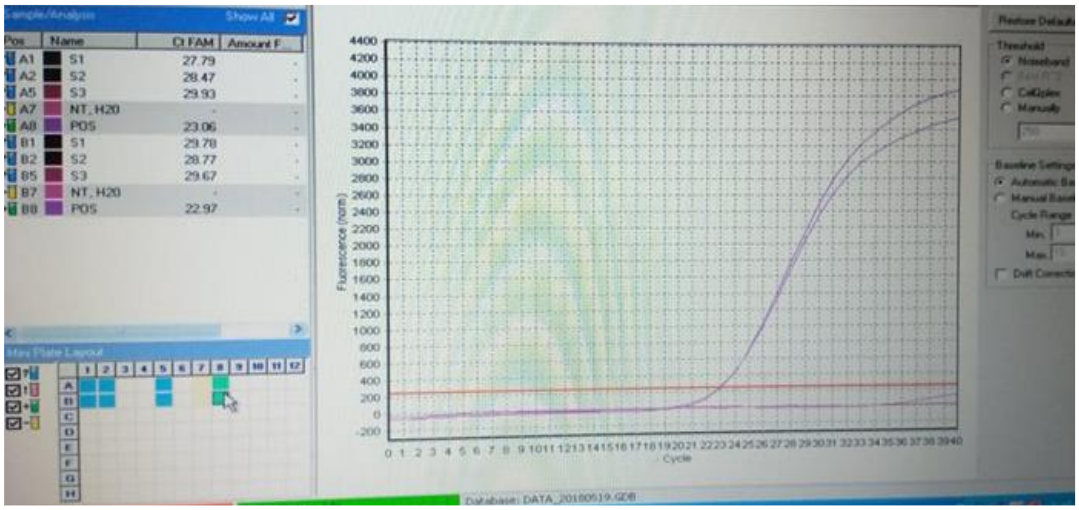
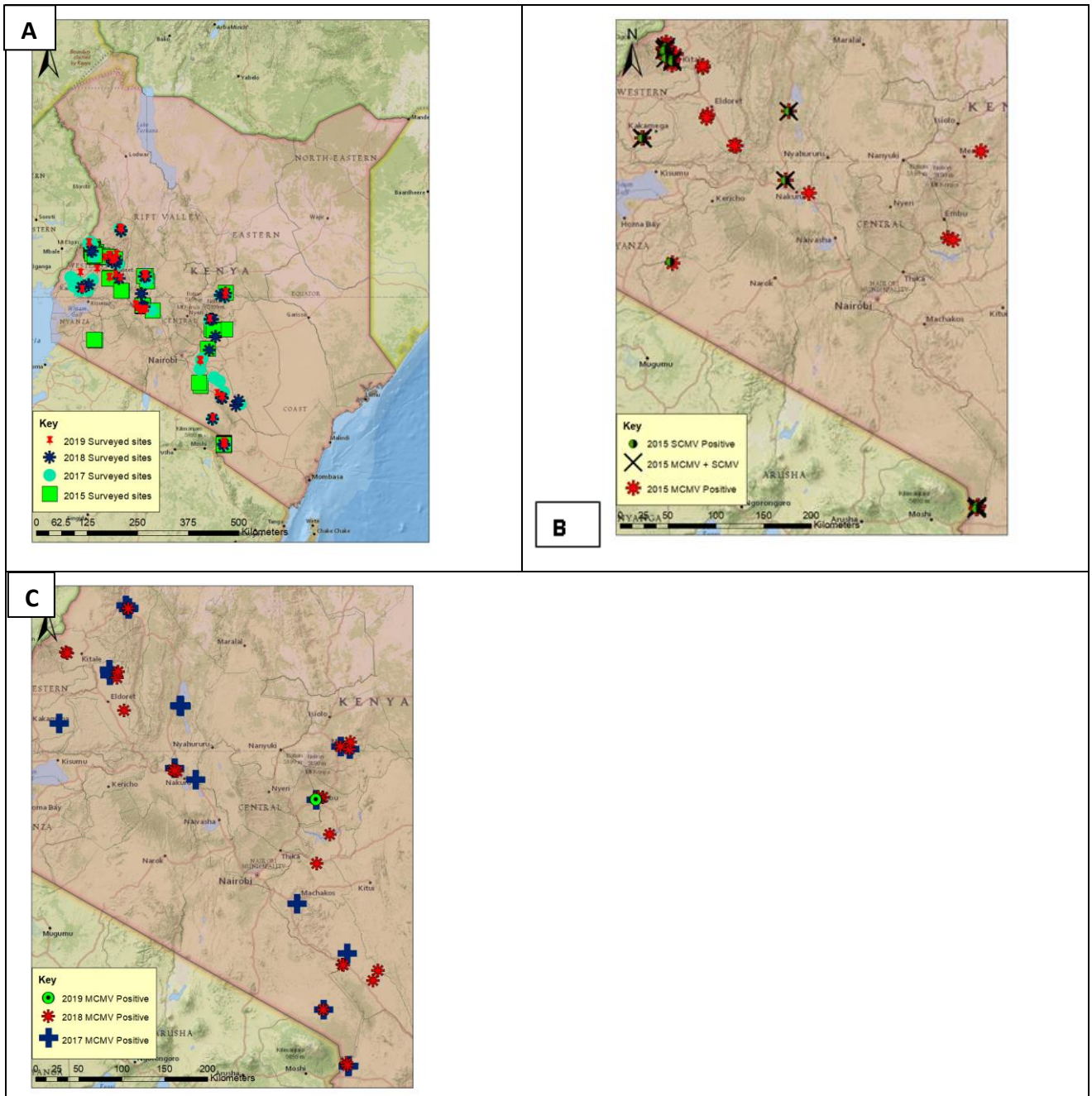


Figure 3.3 Real time PCR image showing positive and negative results of maize chlorotic mottle virus (MCMV) detection



NOTE: A= counties visited during the surveillance, B= Distribution of MCMV, SCMV AND M+S, C= MCMV IN 2017, 2018, 2019

Figure 3.4 Distribution of maize lethal necrosis (MLN) disease causing viruses in major seed production counties visited during surveillance in 2015, 2017, 2018, and 2019

3.3.5 Detection of MCMV virus using by Immunostrips assay

Surveillance carried out in 2017 to 2019 was incorporated with onsite testing of samples using immunostrips for the presence of MCMV. This was real time results in the field for the virus detection. The results indicate 45% MCMV detection in Kakamega County followed by 44% in West Pokot County (Fig 3.5). In Transzoia, Uasin Gishu and Machakos counties no MCMV virus was detected. In total 16% of the total samples analyzed on site turned positive for MCMV virus.

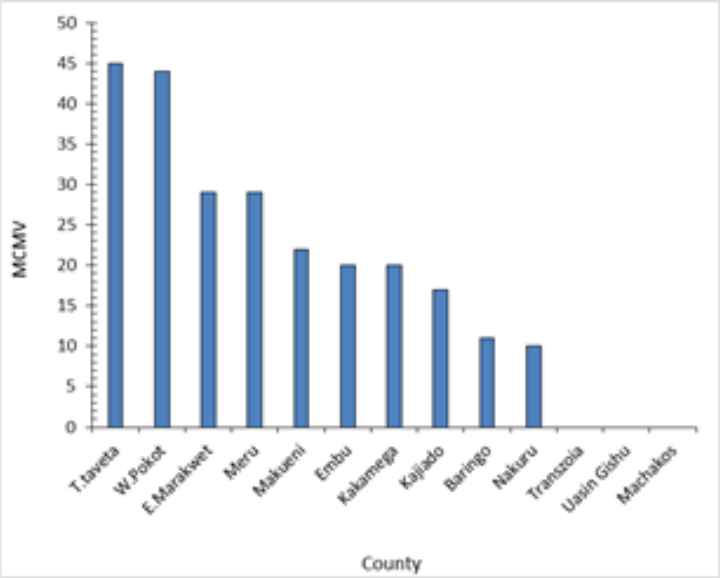


Figure 3. 5 Detection of maize chlorotic mottle virus (MCMV) using Immunostrip assays

3.4. Discussion

The purpose of this study was to document the severity, incidence and distribution of Maize lethal necrosis (MLN) disease in seed farms in Kenya's key maize growing agro-ecological zones. No significant differences in MLN disease incidences and severity among agro-ecological regions were observed. Disease incidences in seed farms is a major problem and threat since this can be a pathway for introduction and spread of diseases even in low levels, hence requiring intervention. The impact of presence of MLN viruses in maize seed is great due to the movement of seeds within and across local and regional borders. Given the

reasonably high incidence and severity of infections in maize, in the sub-humid and semi humid agro-ecological areas they require priority for management and research and control. Plant virus occurrence and persistence in the tropical and subtropical areas are aided by optimal temperature and relative humidity, conditions that promote the plant growth and therefore virus infections and survival of vectors (Macauley, 2015). In this case disease management may include different strategies such as vector control, crop rotation, weeding and planting on time, application fertilizer/manure and rouging of infected plants including adoption of voluntary MCMV monitoring through harmonized protocols.

The highest incidence of MLN recorded in Embu County could be attributed to extensive and continual maize production in the County. The elevated MLN incidence rate reported in Embu could also be due to the two seasons for maize cultivation (long and short rains). Increase in MLN causing viruses can be due to farmers planting maize infected seeds between the seasons (Shiferaw *et al.,* 2011). Embu has favorable climate for vector propagation. The incidence of MLN disease is strongly correlated with rainfall, warm temperatures and high relative humidity which favor disease development (Mudde *et al.,* 2018; Kusia, 2014). The elevated disease pressure of MLN in Nakuru, Elgeyo Marakwet, Pokot, Transzoia, and Baringo Counties could be attributed to their close vicinity to the disease's first reported location (Mahuku *et al*., 2015a; IPPC, 2014; Asea, 2013; Kagoda *et al*., 2016).

Low incidences in Makueni and Kajiado semi-arid agro ecological regions may be attributed primarily to climate patterns and the cropping system. This region receives intermittent rainfall and the production of maize is seasonal with fields left for a dry period of 3-5 months. Cropping systems, on the other hand, have been shown to increase the population of insect vectors and the development of maize MLN pandemics A large number of evaluation studies

have found that disease declines in intercropped systems outperform monocropped systems (Boudreau, 2013; Gopal and Jagadeeswar, 2010; Ramkat *et al*., 2008).

In the three consecutive surveillances, the report suggests increased incidence of MCMV in West Pokot and Taita Taveta, which could be caused by continuous maize production through irrigation, thus increasing insect vector burden and inoculum build-up in these fields. Lack of rain, soil infertility, and wrong farming practices may all contribute to the increased incidence in West Pokot and Taita Taveta. This is consistent with the findings of Isabirye and Rwomushana (2016), who reported that abiotic factors like lack of rain, poor soil fertility, and limited farming practices increase the severity of MLN. This supports the findings of Guadie (2018), who reported an increased disease incidence in key maize-growing regions at low and mid-altitude ranges. The study documented MCMV, SCMV and combined infection of MCMV and SCMV in maize seeds. Viruses that cause MLN disease, particularly MCMV, have been shown to be seed-borne, albeit at a very low level of 0.04% (Jensen *et al*., 1991; Mikel *et al*., 1984). Guadie *et al*. (2018), Fentahun *et al*. (2017), and Demissie *et al*. (2016) discovered a high prevalence of both MCMV and SCMV infections, either alone or in combination, in Ethiopia's main maize-growing regions. Kusia (2014) explicitly stated that MCMV and SCMV were found to be hosted either individually or in combination in maize, wild grasses, domesticated grasses, and crop cereals. This also concurs with findings of Wangai *et al.* (2012) who noted that mixed infections had been previously reported in Kenya.

Notably, there were viruses which were detected in some areas which had shown no disease incidence by visual observation. Symptoms due to viruses causing MLN can also varies according to the developmental stage of the maize plant, the variety involved, and the surrounding conditions, MCMV and SCMV virus strain, and different viruses can cause similar symptoms in the same plant (Wang *et al.,* 2017). This study found that disease symptoms were identified in some cases despite the absence of MCMV or SCMV. This result

supports the likelihood of other Potyviruses and Poleroviruses being involved in outbreak of MLN disease in Kenya or incorrect symptoms (Wamaitha *et al.*, 2018).

A total of 8 varieties were found to be majorly under production during the surveillance period. It was observed that out of the 8 varieties DH04 and Duma 43 were found to have no MLN symptoms and viruses. The absence of incidence and severity of diseases in DUMA 43 and DH 04 could be due to an effective production management approach, as most Kenyan varieties are susceptible to MLN disease (Karanja *et al.*, 2019). The involvement of passive and active defense mechanisms hinders the replication and dissemination of viruses that affect either the germplasm's vulnerability or its resistance (Zambrano *et al.*, 2014). Most of the varieties grown in Kenya have previously shown susceptibility to the disease which is reported in this study. According to Manje *et al*. (2015), nearly 90% of maize landraces in East Africa is highly susceptible to MLN disease. Reports on analysis on previous studies has confirmed the presence of MCMV which is the key virus in spread of the disease in Kenya. Kagoda *et al*. (2016) previously discovered the existence of MCMV in Eastern Uganda. This scenario shows that MCMV has the capability to cause manifestation of MLN disease symptoms. This supports the findings of Mahuku *et al*. (2015b) that MCMV alone can cause MLN disease. Real time PCR assays on samples from the farmer's field were found to have MLN viruses with 38% being positive for MCMV. On the other hand samples that had showed positive reaction for SCMV showed no amplification with qRT-PCR. This could be due to the appearance of new strains with different capsid protein sequences than the primers that were designed. This was also seen where isolates from Rwanda failed to be detected with primers designed from Kenyan SCMV isolate (Adams *et al.*, 2014).

High disease incidences and severities in 2015 across most of the counties could be attributed to the lack of effective measures for regulation of MLN disease since the disease was still new and intervention measures were still in discussion. During this period rejection of maize

crops was based on 10% threshold on visual inspection and laboratory testing was not mandatory for locally produced seeds. This could have resulted in infected seed lots due to the high threshold resulting in increased spread of the disease through seed. Seed production companies were still struggling with management options and not much had been validated to be adopted for the control of the disease. Decrease in MLN incidences in the subsequent surveillances could be attributed to the efforts put in place by National Plant Protection Organisation (NPPO) to curb the spread of MLN which included decreasing the threshold of rejection to 1% during field inspection and zero tolerance of MLN viruses through laboratory testing. There was also mandatory testing of all locally produced seed before processing for the existence viruses causing MLN disease.

CHAPTER FOUR

EFFECT OF INTERACTIONS OF MAIZE CHLOROTIC MOTTLE VIRUS AND SUGARCANE MOSAIC VIRUS ON MAIZE LETHAL NECROSIS DISEASE DEVELOPMENT IN INFECTED MAIZE

Abstract

Synergistic interactions occur when one virus affects a co-infecting virus resulting in increase in multiplication in the host due to either replication or transportation within the plant tissues. Maize lethal necrosis (MLN) disease results from infection of maize with maize chlorotic mottle virus (MCMV) together with any potyviruses affecting maize. Sugarcane mosaic virus (SCMV) is the most common potyvirus that cause of MLN in Kenya. A greenhouse experiment was set up to ascertain the effects of interactions of MCMV and SCMV on plant growth, virus multiplication and MLN disease development in infected plants. Plants of maize were treated with MCMV and SCMV both as a single infection and as a mixture of the two viruses (M+S). Two varieties Duma 43 and DK 8031 were used in the experiment which was carried out in two seasons. Disease assessment for severity and heights were recorded from 7 days post inoculation (dpi) and after every 7 days up to 56 days dpi. Sampling of leaves was also done in each treatment in the same interval and leaves preserved at -80˚C. Stored leaves were analysed for the presence of MCMV using real time quantitative polymerase chain Reaction (qRT-PCR) and Cycle threshold (Ct) values recorded for each sampling point for each treatment. For all days of data collection, there was a significant difference ($P<0.05$) in heights between treatments, except at 7 dpi, where there was no difference between MCMV and SCMV. The treatment with mixed infection (M+S) recorded low mean heights while SCMV recorded higher mean heights compared with MCMV except at 7 dpi. There was a significant difference ($P<0.05$) in disease severity between the treatments for all the days of data collection except at 7 dpi where there was no difference

between M+S and SCMV as well as between MCMV and control. The M+S treatments recorded the highest disease severity score throughout the data collection period. For all data collection days, there was a significant difference (P 0.05) in Ct values between treatments. Treatments M+S recorded the lowest Ct values which is inversely proportional to the virus titer in the infected maize. The concentration of the MCMV was seen to increase in mixed infections compared to single inoculations. Similarly growth was retarded in mixed infections and disease severity was increased compared to single infections. This study showed effects of interactions of MCMV and SCMV on plants growth, symptoms expressions and concentration of the MCMV virus. This is mostly due to the synergistic interaction of the potyviruses which plays a role in increasing the MCMV accumulation in plants. This effects of the interactions are the one leading to the lethal and necrotic effects of the MLN disease in maize plants. The study shows that targeting development of plants that are resistant to MCMV may reduce the severity of MLN in dually infected plants.

4.1 Introduction

Maize is an important grain crop around the world, ranking third place in significance after rice and wheat (Mbega, 2016). In Eastern and Central Africa over 300 million people depend on the crop as a mainstay diet. Production of the crop is faced by challenges associated with both abiotic and biotic factors. Maize lethal necrosis (MLN) infection has been reported as a major disease constraining maize cultivation causing up to 100% loss in yields (Wangai *et al.,* 2012). This has great impact on food security especially for small holder farmers.

Maize lethal necrosis disease is caused by a dual infection of maize by maize chlorotic mottle virus (MCMV) and one of the maize-infecting potyviruses such as maize dwarf mosaic virus (MDMV), wheat streak mosaic virus (WSMV), or sugarcane mosaic virus (SCMV). In

Kenya, the potyvirus associated with MLN is mainly SCMV, which was first reported in the country in 1970s (Louie, 1980).

Co-infections of viruses in natural environment involves simultaneous infection by distinct viruses or by one virus with different strains in the same host in which interactions may or may not occur (Mbega *et al*., 2016). In neutralism or interactions scenario, viruses replicate, accumulate or are transported within the plant due to their influence on each other. Co-infections mostly occur in antagonistic or synergistic manner. In synergistic interactions, one virus affects a co- infecting virus resulting to increase in its accumulation in the host due to either replication or transportation within the plant tissues (Mbega *et al*., 2016). Synergistic interactions of viruses in plants are common, with two types known to occur: potyvirus-associated synergisms, where one virus is potyvirus, and non-potyvirus synergisms, where neither of the viruses is a potyvirus (Mbega *et al*., 2016). Majorly, a potyvirus group of plant viruses has been seen to be involved in most of the synergistic interactions. An example where a potyvirus is involved is in sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV; Kreuze, 2002); bean pod mottle virus (BPMV) and a potyvirus soybean mosaic virus (SMV) (Anjos *et al*., 1992); and the classical interaction of potato virus X (PVX) and a potyvirus potato virus Y (PVY) (Vance, 1991).

Among the viruses associated with MLN disease, indications have shown MCMV to be the primary disease causing virus. The virus can establish itself in warm, semi-arid, and sub-humid climates (Isabirye and Rwomushana, 2016). Sugarcane mosaic virus is the most widely distributed potyvirus and is well adapted to interactions with the host plant, having developed resistance to the virus's attack, resulting in less impact (Redinburg and Zambrano, 2014). However, MCMV virus is the latest virus in the crop's system in the region, and the host is unprepared for the attack, and the impact is exacerbated when a potyvirus is present.

The maize chlorotic mottle virus was first discovered in Peru in 1971 and later in Kansas in co-infections with potyviruses (Castillo, 1974). The virus is a member of family *Tombusviridae,* genus *Machlomovirus.* Maize chlorotic mottle virus particle is stable and is readily mechanically transmissible. Symptoms include mild mosaic, mottling leaf chlorosis and stunting of plants, male blossoms with few spikes and prematurely mortality of plants. Complete genome of MCMV isolates globally have shown limited diversity and no differences in pathogenicity have been observed among the variants. Sugarcane mosaic virus was described as early as 1924 in maize and sugarcane and later reported in East Africa as maize pathogen and subsequently reported in Kenya and Tanzania in 1980. Sugarcane mosaic virus belongs to family *Potyrividae* genus potyvirus. In single infections they cause mottling pattern on leaves produced by contrasting light green to yellow and dark green patches. Patches are irregular in shape and have diffused margins; plants appear paler and more yellow. In dual infections of synergy between MCMV and SCMV symptoms include intense chlorosis from base of young whorl leaves developing upward to the leaf tips. As infection progresses severe chlorosis and necrosis sets in starting from leave edges and tips towards the midrib resulting to 'dead heart' symptoms and plant death.

The classic process of virus infection in a plant includes virus entry into the cell, virus capsid disassembly, genomic replication and transcription, and viral RNA translation (Mbega *et al*., 2016). Maize crop resistance to virus infection is due to the role played by posttranscriptional gene silencing (PTGs) and to cause disease, viruses must use a gene silencing strategy. Synergistic interactions results in more severe symptoms in the infected plants than in single infections resulting in severe disease outbreaks (Mathews, 1991; Untiveros *et al*., 2007). In synergistic interactions of MCMV with a potyvirus, the outbreaks and effects are expected to be higher than in single infections. MCMV and MCMV-derived siRNA buildup has been shown in studies to be higher during synergistic infections (Xia *et al.*, 2016). This study was

undertaken to determine the effects of interaction of MCMV and SCMV in virus multiplication, disease development and plant growth in single and mixed infections.

4.2 Materials and Methods

4.2.1 Source of virus isolates and seeds

The MCMV and SCMV isolates used in the study were obtained from KALRO-NARL where they are maintained in separately to avoid contamination. The experiment was set up using Duma 43 seeds from seedco and DK 8031 from Bayer Seed Company.

4.2.2 Experimental design and set up

Two experiments were carried out in the greenhouse in May to July and October to November 2019. Two maize varieties were used in the experiment, namely Duma 43 and DK 8031 which are known to be susceptible to the two virus causing MLN disease in the region. Plastic pots of 4 kg were filled with sterilized soil at three quarter level and mixed with Ammonium Phosphate (DAP) fertilizer at a rate of 5 g per plant. Seven seeds were planted in each pot at a depth of 2.5 cm below the soil surface. Germinated plants were later thinned to four in each pot to avoid overcrowding. Completely randomized design (CRD) with four replicates per treatment and four plants in each replicate was used.

4.2.3 Mechanical inoculation of the maize plants with viruses

Two viruses were used in the experiment, MCMV and SCMV, in both single and combined infections. In total, there were four treatments plants inoculated with SCMV, plants inoculated with MCMV, plants inoculated with a combination of SCMV and MCMV (MLN) and a control with no virus inoculation. Virus inoculum for each virus was prepared by grinding infected maize leaves in 0.1M phosphate buffer pH 7.0 at a ratio of 1:10 (w/v). The

MCMV and SCMV inoculations were mixed at a 1:9 ratio. Inoculum was sieved with a muslin cloth to remove plant debris and added carborandum powder. Plants were mechanically inoculated twice at 3 and 4 leaf stage by rubbing with the inoculum. Plants were maintained and watered regularly for ideal growth and symptoms expressions**.**

4.2.4 Disease assessment and sampling

Disease assessment for severity and plant height were recorded from 7 days post inoculation (dpi) and after a time span of 7 days up to 56 dpi. Sampling of leaves was also done in each treatment in the same interval and leaves preserved at -80°C. Severity was assessed and recorded based on a scale of 1-5 (Gowda *et al.,* 2015) as described in Section 3.2.2. Disease incidence was calculated according to with Section 3.2.2.

4.2.5 Virus assays

Leaf samples collected from 7 dpi to 56 dpi were analyzed for MCMV using q-RT PCR assays to determine the CT values of the viruses at each sampling points. Concentrations of SCMV could not be done due to challenge in real time detection assay where it was not working due to the variability of the virus. As described in 3.2.4.1, total RNA was extracted from leaves of maize samples. The RNA from the infected samples was analysed to confirm the presence of MCMV and SCMV using real time q- PCR as described in 3.2.4.2**.** Data in CT values of the viruses was recorded to determine the virus concentration at each sampling points where CT is inversely proportional to the virus concentration.

4.2.6 Data analysis

For all continuous variables, descriptive data (frequency, percentages, and mean values) were used to generate summaries and tables, as well as analysis of variance using SAS version 9.1

at a significance level of p 0.05 (SAS Institute, 2004).Differences between means was determined using Fischer's Protected LSD at P = 5%. Disease severity was further determined through Area under disease progression curve (AUDPC) as follows:

$AUDPC = \Sigma i[(DSi + DSi\text{-}1) \times (ti\text{-}ti\text{-}1)]/2$

where i ={7,14,21} are the days of observation, "DS" is the disease score using the above severity score of 1 to 5 and "t" represents the number of days post-inoculation.

4.3 Results

4.3.1 Effects of interaction of MCMV and SCMV in single and mixed infection on disease severity

In variety DK 8031, there was significant differences ($P < 0.05$) in disease severity between the treatments for all the days of data collection except at 7 dpi where there was no significant difference between MCMV and control (Table 4.1). Severity and progress of the disease varied across the treatments and at different days of post inoculation. At 7dpi SCMV had developed symptoms of the disease together with mixed infection of SCMV and MCMV (MLN) with a disease severity score of 1.86 and 2, respectively. In plants infected with SCMV symptoms observed included light green mosaic and mild mottle on the young leaves which appeared as specs and streaks on leaves which developed into clear specs. However, as the disease progressed the visibility of the conspicuous symptoms reduced. Plants inoculated with MCMV developed symptoms later at 14 dpi compared with SCMV and MLN treatments. Plants inoculated with MCMV delayed in symptoms expression but later showed chlorotic mottling which appeared as streaks progressing subsequently with intense and excessive chlorotic mottling and necrosis of leaf margins. However at 28 dpi the MCMV treatment recorded a higher disease severity compared to SCMV with 3.70 and 3.27, respectively. Plants with SCMV showed a slow disease progress compared with MCMV

despite the early onset of the disease. Plants inoculated with MCMV showed a rapid increase in disease severity up to 56 dpi with score of 4.59. Plants infected with MLN viruses had the highest severity score in all days of post inoculation and at 49 dpi they had a score of 5. In MLN infections symptoms were observed as early as seven days post inoculation showing leaf chlorosis, mottling from the base extending upwards to leaf tip. Severe chlorosis and mottling with plants becoming bright yellow with necrosis from the leaf margins leading to dead heart symptoms. Severe stunting and eventual plant death was later observed.

In Duma 43 there was significant difference between the treatments within the days post inoculation (Table 4.1). Plants inoculated with SCMV exhibited symptoms as early as 7dpi same case with MLN treated plants with a severity score of 1.91 and 1.97, respectively. Plants inoculated with MCMV treatment started to exhibit symptoms at 14 dpi with a score of 1.70. The SCMV treatment recorded higher disease severity score at earlier days compared to MCMV but this was reversed at 35 dpi with the latter recording higher than SCMV for the rest of the period. In MLN treatment the severity of the disease remained high compared to the rest of the treatments throughout the observation period. Disease progression was rapid and the symptoms were lethal leading to stunted growth and death of the plants. At 56 dpi in all treatments except control the severity became retarded and there was no progress.

Table 4. 1 Mean disease severity score of maize genotypes infected with maize chlorotic mottle virus (MCMV), sugarcane mosaic virus (SCMV), and SCMV+MCMV (MLN) assessed over time

Variety	Treatment	7dpi	14dpi	21dpi	28dpi	35dpi	42dpi	49dpi	56dpi
DK 8031	Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	SCMV	1.86	2.50	3.06	3.27	3.52	3.97	3.81	3.81
	MCMV	1.02	1.92	2.84	3.70	4.09	4.26	4.44	4.59
	M+S	2.00	2.67	3.28	3.87	4.26	4.58	5.00	5.00
Duma 43	Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	SCMV	1.91	2.45	3.00	3.33	3.61	3.72	3.98	4.00
	MCMV	1.05	1.70	2.53	3.23	3.75	4.16	4.50	4.56
	M+S	1.97	2.59	3.34	3.72	4.19	4.62	4.94	5.00
	LSD	0.07	0.17	0.27	0.38	0.30	0.21	0.16	0.16
	CV	10.07	16.00	14.72	12.16	11.32	10.31	5.96	5.50

4.3.2 Disease incidence of SCMV, MCMV and MLN on genotypes DK 8031 and Duma 43 maize varieties

Throughout the growth period, there were no significant differences in disease incidence between DK 8031 and Duma 43 (Figure 4.1). Disease symptoms in plants inoculated with SCMV were observed as early as 7 days post inoculation, recording 93.8% and 96.9% for DK 8031 and Duma 43, respectively and attained 100% at 14 days post inoculation (Figure 4.2). Plants inoculated with MCMV had low disease incidence of 9.4% for Duma 43 and none for DK 8031 at seven days post inoculation. However, the incidence increased steadily attaining 100% at 21 dpi. Plants infected with a combination of MCMV and SCMV (MLN) recorded high disease incidence of 100% at 7dpi with severe symptoms of necrosis spreading fast to all leaves of the plants compared to SCMV and MCMV treatments. There was no disease incidence in the controls in both genotypes for the entire disease observation period (Figure 4. 2).

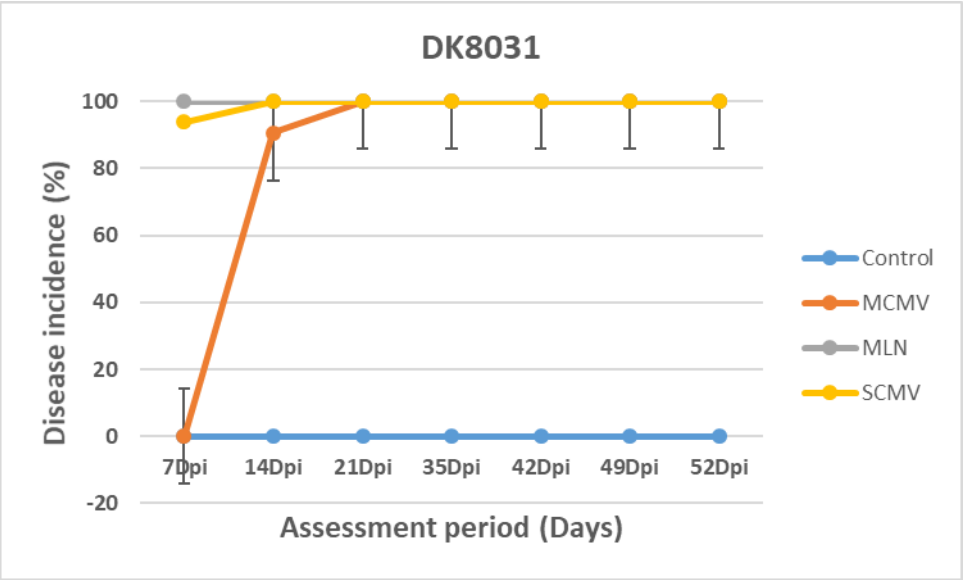


Figure 4. 1 Percent disease incidence over time in maize varieties DK8031 plants inoculated with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV) and maize lethal necrosis (MLN) disease

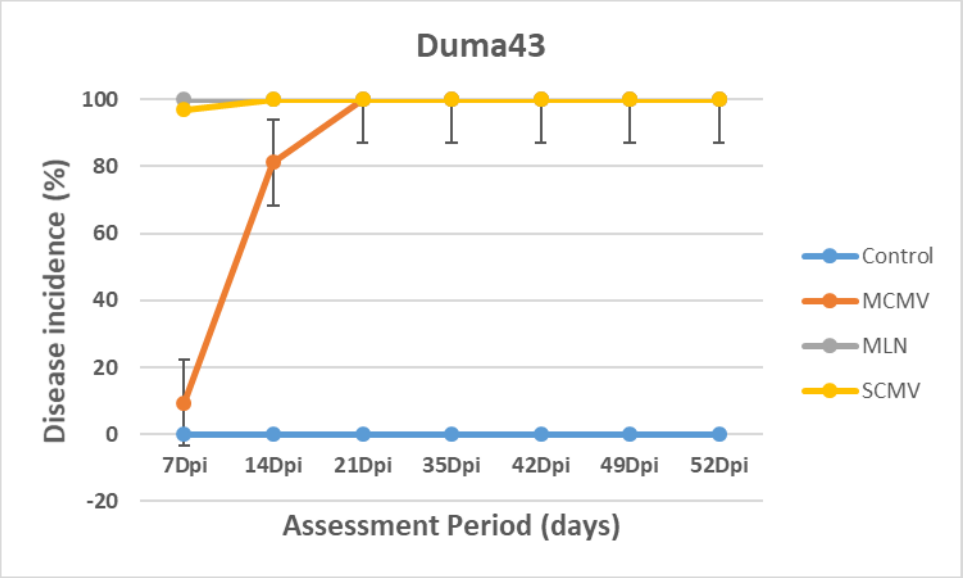


Figure 4. 2 Percent disease incidence over time in maize varieties Duma 43 plants inoculated with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV) and maize lethal necrosis (MLN) disease.

4.3.3 Effects of interaction of MCMV and SCMV in single and mixed infection on plant heights

Plant heights differed significantly (P 0.05) between treatments in variety DK 8031 for all data collection days (Table 4.2). The control treatment had the highest plant heights while MLN had the lowest height throughout the experimental period. At 7dpi MCMV recorded higher plant height compared with SCMV 48.8 and 48.6. However this was reversed at 14dpi where SCMV treatment recorded higher plant height of 59.8 compared with MCMV 56.7. This plant growth continued with the same trend with SCMV recording higher plants heights than MCMV up to dpi 56 (Table 4.2). In MLN treatment, growth was affected and were shorter up to dpi 56 with 62.5 while the control had the highest with 132.5 which is significantly different.

In variety Duma 43 there were significant differences ($P < 0.05$) in plant heights between the treatments for all the days of data collection (Table 4.2). At 7dpi there was significant difference in plant heights between the control and MLN but the differences were not significant between SCMV and MCMV. At 28 dpi however, SCMV recorded slightly higher plant heights compared to SCMV which continued up to 56dpi. The MLN treatment recorded lowest plant heights throughout the data collection period with most plants being retarded and nearly dead.

4.3.4 Virus titer of MCMV in single and mixed infections of MCMV and SCMV plants

In variety DK 8031, there was significance difference ($P < 0.05$) in Ct values between the treatments for all the days of data collection (Table 4.3). The Ct value is inversely proportional to the virus titer which means the smaller the Ct value the higher the MCMV titer. The control treatments recorded highest Ct values of MCMV virus throughout the study period. Treatments MLN recorded the lowest Ct values throughout the period except at 7 dpi.

Table 4. 2: Plant heights of maize genotypes infected with maize chlorotic mottle virus (MCMV), sugarcane mosaic virus (SCMV) and SCMV+MCMV (MLN) assessed over time

Variety	Treatment	7dpi	14dpi	21dpi	28dpi	35dpi	42dpi	49dpi	56dpi
DK 8031	Control	50.60	61.46	70.80	81.33	92.79	109.56	120.75	132.48
	SCMV	48.58	59.83	66.07	74.98	83.17	95.69	112.60	123.29
	MCMV	48.81	56.68	65.01	72.49	79.54	88.51	99.24	112.90
	MLN	46.37	52.64	56.80	61.13	62.44	62.55	62.64	62.53
	LSD	0.90	1.24	1.92	1.94	1.87	2.12	2.38	2.39
	CV	4.77	5.42	7.52	6.70	5.90	5.95	6.03	5.54
Duma 43	Control	50.44	62.31	70.97	81.85	93.02	103.49	115.07	126.68
	SCMV	49.26	58.13	65.30	73.88	83.26	97.90	109.01	119.56
	MCMV	49.16	58.28	65.64	72.38	82.64	91.19	101.45	111.86
	MLN	47.47	54.54	57.46	59.99	62.58	62.97	63.19	63.28
	LSD	0.57	1.27	1.78	2.05	2.38	2.31	2.27	2.30
	CV	2.94	5.52	6.95	7.14	7.43	6.52	5.85	5.47

At 7 dpi MLN treatment recorded higher Ct value compared with MCMV but at 14 dpi this was reversed and MCMV recorded a higher Ct value. The Ct values in treatments MCMV and MLN at 42, 49 and 56 dpi did not increase rapidly as compared to the initial dpi.

In variety Duma 43 there was significance difference ($P<0.05$) in Ct values between the treatments for all the days of data collection. At 7 dpi, MLN recorded a higher Ct value compared to MCMV but it was relatively lower in the rest of the dpis'. Treatments of MCMV recorded the highest Ct values except at 7 dpi. Both MLN and MCMV maintained standard Ct values at 42, 49 and 56 dpi.

4.3.5 Area under disease progress in maize genotypes DK 8031 and DUMA 43 inoculated with SCMV, MCMV and MLN disease

Plants inoculated with mixed viruses (MCMV and SCMV) recorded the highest mean AUDPC (>800) in both varieties. The lowest mean AUDPC scores of 697 and 703 was recorded in plants inoculated with SCMV in DK 8031 and Duma 43, respectively (Figure 4.

3). The mean AUDPC for MCMV was moderate with DK 8031 recording 772 compared to that of Duma 43 of 740 (Figure 4.3).

Table 4. 3: Virus CT values of maize chlorotic mottle virus (MCMV) in maize genotypes infected with MCMV alone or in mixed infections (MLN) with sugarcane mosaic virus (SCMV)

Variety	Treatment	7dpi	14dpi	21dpi	28dpi	35dpi	42dpi	49dpi	56dpi
DK 8031	Control	38.27	37.91	39.10	39.28	39.48	39.48	39.41	39.49
	MCMV	18.92	17.84	15.06	14.38	13.53	12.54	12.39	12.47
	MLN	23.37	15.41	13.43	12.23	10.26	10.98	10.24	10.40
Duma 43	Control	38.71	39.43	39.43	39.53	39.55	39.57	39.63	39.54
	MCMV	19.04	17.99	14.78	14.35	13.58	12.18	12.09	12.37
	MLN	22.90	15.76	13.11	12.07	10.17	10.88	10.49	10.48
	LSD	1.09	1.51	1.20	0.98	0.86	0.62	0.78	0.61
	CV	5.25	8.53	9.74	7.55	6.58	3.50	4.03	3.81

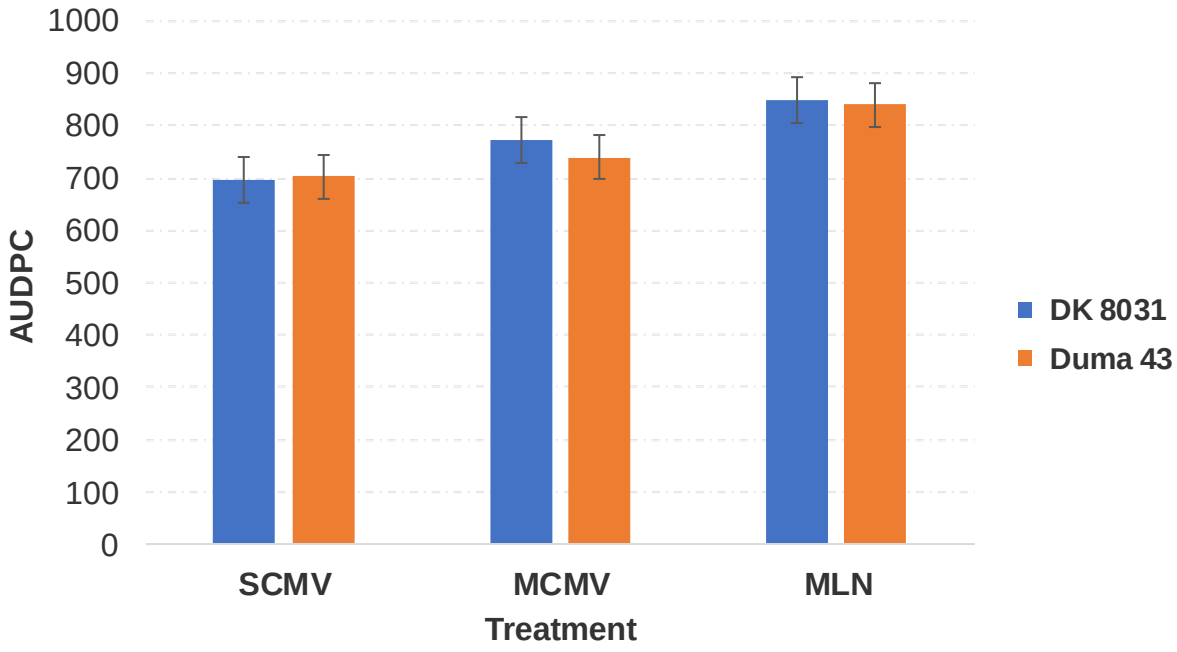


Figure 4.3: Mean area under disease progress curve (AUDPC) as calculated from disease severities for DK8031 and Duma 43 infected with sugarcane mosaic virus (SCMV), maize chlorotic mottle virus (MCMV) and MLN (MCMV+SCMV) inoculation

4.4 Discussion

This study aimed at assessing the response of interactions of MLN causing viruses in single and mixed infections in inoculated plants in two maize varieties and the effect of the interactions on MCMV concentrations in infected plants**.** The impact was determined by assessment of disease severity, incidence, plant heights and virus titer.

In single infections in is study, disease severities were observed with SCMV showing symptoms earlier than MCMV which shows the ability of SCMV virus to colonize and establish immediately after an infection as compared to MCMV. Results from this study showed a change in the severity trend where MCMV severity increased compared with SCMV as time progressed and eventually SCMV inoculated plants were seen to start recovering from the infection by the end of data collection. This could be contributed to the fact that MCMV is more virulent and once it colonizes the plant it has strong ability to cause disease while on the other hand SCMV is less virulent and the plants are able to resist the latter than the former virus (Awata *et al*., 2019).

Disease severity was observed in maize plants in both single and mixed infections of the two viruses i.e. MCMV and SCMV. The symptoms were more severe in mixed infections as compared to the single infections. Progression of the disease was observed with time and the severity scores were high in mixed infections compared to single infections. Symptoms of the virus infections are observed as a result of colonization and establishment of the pathogen within the host plant. Mixed viral infections are to a great extent determined by the relationship and interactions between the host, virus and vectors besides their individual characteristics (Moreno and Moreno, 2019). Interactions which are synergistic are known to exacerbate pathogenicity and symptom expressions compared to single infections of the same viruses. In MLN synergistic infections, the virus is transcribed to mimic itself to the maize DNA where it is replicated to produce many copies which are then transported within the

cells to the phloem and finally colonizes the whole plant, hence disease symptoms. Effective and successful colonization of the plants by viruses requires the presence of viral suppressors of RNAs (VSRs). These VSRs are expressed at multiple stages resulting in suppression of the gene silencing strategy, hence virus infection and diseases in plants (Mbega *et al*., 2016). Cell-to-cell movement and transportation of the virus through the vascular tissues is required for viral infection to be successful. This movement function in this synergistic interaction is facilitated by SCMV VPg which enhances long distance movement of its own virus and that of MCMV. Therefore, the remarkable increase in disease severities in mixed infections compared to single infection in this study is attributed to the synergistic reaction of SCMV and MCMV. This is similar to interactions between a potyvirus sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (Kreuze, 2002). Disease incidences were also high in mixed infections compared to single infections. Co-infections with MCMV and SCMV resulted in random stipple mottle, mosaic, narrow streaks, and shortened internodes, which persisted with apoptosis and dying of the leaves inward from the margin, resulting in crop mortality. This could be due to the hypoplasia condition, in which the damaged leaf lamina thins out and has few chloroplasts and less distinguishable mesophyll (Awata *et al*., 2019). Infections by viruses affects the ultrastructural structure of maize leaf bundle sheath cells by directly affecting the chloroplasts and thylakoid membranes (Zhao *et al*., 2016). Severe MLN observations in the co-infections in this study could be due to reduced chlorophyll production due to reduced chloroplasts. Similar observations were observed by Wang *et al*. (2017) where co-infected plants showed reduced starch grains in the chloroplast compared to single MCMV inoculated plants with MLN symptoms expression by the plants. Lower levels of pyruvate orthophosphate dikinase (PPDK), a genotype for Co2 sequestration in C4 photosynthesis processes, result in mosaic manifestations due to anthocyanin loss and internal content leakage due to mitochondrial disruption in co-infected

plants (Wang *et al*., 2017). Systemic necrosis in co-infected plants is also due to disruption of photosynthesis and mitochondria respiration systems (Awata *et al*., 2019).

Results from this study shows a remarkable effect on plant growth in mixed infections of MCMV and SCMV compared to single infections. Plant growth in the mixed infections was retarded with stunting and shortened internodes and to an extent plant death. This scenario resembles previous reports with most of MLN outbreaks in the fields as result of synergistic infection between MCMV and a potyvirus (Redinbaug and Stewart, 2018). The results from this study concurs also with the findings by Karanja *et al*. (2019) where MLN inoculated plants recorded the lowest plant heights compared with single virus inoculations. Interactions in mixed infections have been shown to affect plant vigor and productivity, resulting in economic losses. Synergistic interactions of MCMV and SCMV results in reduced growth due to interference with Ferrodoxin -5 (FDV) which plays an important role in the electron distribution transmitted from photosynthesis photosystem 1 to a variety of electron acceptors. The potyvirus SCMV's HC Pro interacts with FDV, resulting in less ATP production required to drive the Calvin cycle via electron flow around photosystem 1, resulting in low yields, poor growth, and insufficient chlorophyll (Mbega *et al*., 2016).

This study's findings revealed MCMV Ct values to be lower in mixed infections of MCMV and SCMV compared with single inoculations of MCMV. This indicates high virus titer of MCMV in mixed infections compared to single infections. Plants inoculated with both viruses showed remarkable MCMV titer throughout the observation period. This results agree with previous findings by Xia *et al*. (2016) who found MCMV RNA and MCMV- derived siRNAs to be higher in synergistic interactions of MCMV and SCMV compared to single inoculations. Combined relations between MCMV and SCMV cause serious MLN symptoms as well as a rise in MCMV concentration, whereas SCMV concentration remains constant in single infected cereals crops (Xia *et al*., 2016). Sugarcane mosaic virus VPg protein reacts

with maize elongin C protein (ZmElc), resulting in decreased production in leaves and pistils, as well as other maize organs. Reduced expression of the ZmElc gene, which generates ZmElc protein, causes an increase in MCMV multiplication (Mbega *et al*., 2016). In another scenario, the potyvirus helper component and nuclear inclusion protein genes are known to decrease maize plants' capacity to prevent MCMV replication (Rajamaki and Volkonen, 2009). These two scenarios explains the remarkable increase in MCMV concentration in maize plants.

The concentration of SCMV was not determined in this study. However, previous reports indicate that in maize plants co-infected with MCMV, there is high concentration of potyvirus RdRp which induces production of SCMV virus-derived small interfering RNAs (vsiRNA) in high numbers. A high concentration of vsiRNAs causes SCMV mRNA degeneration (suppression), resulting in a reduced density of SCMV in crops (Wang *et al*., 2017).

There are pathways for RNA silencing and post-transcriptional gene silencing are a major component of responses of plants towards virus infection (Moreno and Moreno, 2019). All eukaryotes have RNA silencing machinery that uses various types of small RNAs to regulate chromatin modification, DNA methylation, and transposon activity. One of the most important antiviral defense mechanisms induced in plants in response to RNA and DNA virus infection is RNA silencing. In synergistic interactions Hc Pro of the potyvirus plays a major role in suppression of antiviral defense mechanism and vascular movement leading to accumulation of MCMV virus and enhancement of pathogenicity (Awata *et al*., 2019). These mechanisms and scenario explains the increased MCMV titer in mixed infections with SCMV observed in this study. The results are in agreement with previous research finding where the concentration of MCMV virus is high in mixed infections involving SCMV compared with single infections.

The varieties used in this study showed no significant differences in growth, severity and MCMV concentration. This shows that the two varieties were susceptible to MLN causing viruses and the disease. This is consistent with previous findings in Kenya, where all commercial maize cultivars have been found to be susceptible to MLN in the both natural and artificial inoculation (Boddupalli *et al*., 2020). Similar results research by Karanja *et al*. (2019) found that all SCMV, MCMV, and MLN were found in commercial hybrids. The study indicates that targeting development of plants that are resistant to MCMV may reduce the severity of MLN in dually infected plants.

CHAPTER FIVE

GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

In Eastern Africa, maize lethal necrosis (MLN) disease has had a significant impact on yields of maize as well as production (De Groote *et al*., 2016; Marenya *et al*., 2018), with reported losses ranging from 23 to 100 percent in Kenya (Boddupalli *et al*., 2020). The disease continues to pose a significant threat to the region's maize crop (Isabirye and Rwomushana, 2016), with the risk of spreading to other SSA regions looming.

Sugarcane mosaic virus (SCMV) and maize chlorotic mottle virus (MCMV) have the potential to spread through contaminated seed, resulting in widespread infection (Zhang *et al*., 2011b). In this regard since infected seed is a pathway for introduction of the virus, it requires attention to ensure the spread is curbed. Monitoring of the disease through surveillance combined with diagnostics is one of the phytosanitary approach that contributes greatly towards tracking the disease and minimizing its spread. Surveillance was carried out in seed production fields in Kenya to check the status of the MLN disease. Field diagnostics using immunostrips was incorporated within the surveillance and real time data submission was done. This surveillance, diagnostics and data management was done using standardized and validated protocols by CIMMYT and NPPO.

Disease incidence and severity was high in 2015 and 2017 but the disease declined to zero incidence in 2019. This was contributed greatly to the monitoring scheme adopted by the seed companies through voluntary use of checklists standard operating procedures (SOPs) for MLN pathogen-free seed production. Also the ability to create more awareness and management to seed production companies and stakeholders contributed to the disease decline in seed fields. The majority of the varieties were discovered to be vulnerable to the viruses that cause MLN symptoms. This is compatible with previous findings in Kenya, in

which all commercial maize cultivars had been observed to be vulnerable to MLN both in naturally occurring and artificial inoculation (Boddupalli *et al*., 2020). Karanja *et al*. (2019) reported comparable findings in which all a commercial hybrids were found to be susceptible to SCMV, MCMV, and MLN. The various growth stages encountered during the surveillance showed different disease incidence and severity. According to previous findings, maize crops are vulnerable to MLN infection at any and all phases, with severity increasing with plant maturity level at the period of infestation, in addition to genetic makeup and other non - living factors (Awata *et al*., 2019). Mixed viral infections are significant since they do not entirely rely on the properties of the actors involved but rather their relationship and interactions (Moreno *et al*., 2020). Synergistic interactions of viruses have been in discussion since 1920s (Mbega *et al*., 2016). In Kenya, maize lethal necrosis disease has been linked to a synergistic relationship between MCMV and a potyvirus (Redinbaug and Stewart, 2018).In this study, plants inoculated with mixed viruses (SCMV and MCMV) showed reduced/retarded growth and in some cases death of the plants. This could be due to interference with Ferrodoxin -5 (FDV), which is important in the distribution of electrons from photosystem 1 of photosynthesis to a variety of electron acceptors. The potyvirus SCMV's HC Pro interacts with FDV, resulting in less ATP production required to drive the Calvin cycle via electron flow around photosystem 1, resulting in low yields and poor growth (Mbega *et al*., 2016). The effects of the synergistic interactions become lethal and necrotic resulting to plant retardation and death.

Notably there was high disease severity in mixed infections compared to single infections which could be attributed greatly by the ultrastructural changes in bundle sheath of the maize cells containing the chloroplasts. Physiological changes within the plant cells results to malfunction of chloroplast resulting to decrease in production of chlorophyll hence expression of MLN symptoms. Maize chlorotic mottle virus concentrations was found to be

higher in mixed infections compared to single infections. This is in agreement with the earlier report by Xia *et al*. (2016) who found increased accumulations of MCMV in synergistic interactions of MCMV and SCMV. Synergistic interactions involving a potyvirus have been linked to an upsurge in one of the viruses involved while its concentrations remain the same.

5.2 Conclusion

Based on the findings, MLN disease is a major impediment to maize production. The presence of severities and incidences in the seed maize production fields indicates a pathway of spread of disease through seed. Efforts should be made to and incorporate combined strategies in the control and management of MLN disease. Strategies management of vectors, field hygiene, and phytosanitary measures .Monitoring of the disease can be enhanced by use of immunostrips which can effectively be adopted in field inspections to assist in detection especially latent infections.

This study showed disease decline in seed production fields due to surveillance and monitoring of the disease over a period of time and at the same time adoption of voluntary MLN checks during production. It also showed the effectiveness of the phytosanitary approach towards management of the disease by combination of symptoms observations with field diagnostic tools. This is to ensure there is no virus escape due to latent virus infections and rejections or acceptance of seed crop to be based on reliable observations and results. In this regard monitoring of the viruses and disease should be enhanced to facilitate the exchange of only clean maize germplasm within the boundaries. Phytosanitary approach should be strengthened to ensure safe exchange of seed within regions and also during production.

This study showed effects of interactions of MCMV and SCMV viruses on plants which affects the growth, symptoms expressions and concentration of the MCMV virus. This

indicates the presence of the viruses needs to be given attention and should be closely monitored to avoid dual occurrence. It was evident that both viruses play a significant role in causing the MLN disease in maize hence both require attention during management. Management of the two viruses in order to avoid the attack and spread would contribute greatly in ensuring healthy maize crops. In the breeding for tolerant and resistant varieties approach could consider the use of the susceptibility genes of the maize and engineer towards increasing the plants immunity.

5.3 Recommendations

Based on the findings of this study, it is suggested that

i. Monitoring of the viruses causing the disease should be considered especially during seed production and in processing of the seed for distribution
ii. Combined efforts in control of the disease to be enhanced including integrated pest management as well a phytosanitary approach.
iii. There is need to understand the susceptibility of the maize plants and exploration of the response towards disease attack can be utilized in development of resistance varieties
iv. Climatic factors contributing to the disease surge should be studied further to determine the epidemiology of the disease
v. More work should be done to understand the interactions of the other potyviruses as a way of managing the viruses.

6.0 REFERENCES

Adams, I. P., Glover H. H., Monger W. A., Mumford, R., Jackeviciene, E, Navalinskiene, M., Samuitiene, M. and Boonham, N. (2009). Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. Molecular Plant Pathology, 10(4): 537–545.

Adams, I.P., Harju, V.A., Hodges, T., Hany, U., Skelton, A., Rai, S., Deka, M.K., Smith, J., Fox, A., Uzayisenga, B., Ngaboyisonga, C., Uwumukiza, B., Rutikanga, A., Rutherford, M. Ricthis, B., Phiri, N. and Boonham, N. (2014). First report of maize lethal necrosis disease in Rwanda. New Disease Reports, 29: 22. https://doi.org/ 10.5197/j.2044-0588.2014.029.022.

Adams, I.P., Miano, D.W., Kinyua, Z.M., Wangai, A., Kimani, E., Phiri, N., Reeder, R., Harju, V., Glover, R., Hany, U., Souza-richards, R., Nath, P., Nixon, T., Fox, A., Barnes, A., Smith, J., Skelton, A., Thwaites, R. and Mumford, R. (2012). Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. Plant Pathology, 62:741-749.

Adams, M.J., Antoniw, J.F. and Beaudoin, F. (2005). Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. Molecular Plant Pathology, 6(4): 471-487.

Akbar, S., Tahir, M., Wang, M. and Liu, Q. (2017). Expression Analysis of Hairpin RNA Carrying Sugarcane mosaic virus (SCMV) Derived Sequences and Transgenic Resistance Development in a Model Rice Plant. BioMed Research International, 2017:1-10.

Alford, D.V. (2000). Pest and disease management handbook. Blackwell Science Ltd., London, UK 624 p.

Anjos, J.R., Jarlfors, U. and Ghabrial, S.A. (1992). Soybean mosaic virus (potyvirus) enhances the titer of two comoviruses in dually infected soybean plants. Phytopathology, 82: 1022-1027.

ASARECA, (2014). Taking on the maize monster. Retrieved March 2015 from http://www.asareca.org/~asareca/news/taking-maize-monster.

Asea, G. (2013). MLN in Uganda: A disease on the move. Workshop on Maize Lethal Necrosis and its management, February 12-14th, 2013, Nairobi Safari Club.

Aulakh, J. and Regmi, A. (2013). Post-harvest food losses estimation-Development of consistent methodology and workshops/GSSAC2013/Improving methods for estimating postharvest losses/Final PHLs Estimation 6-13-13.pdf. http://www.fao.org/fileadmin/templates/ess/documents/meetings (Accessed on January 14, 2017).

Awata, L.A.O., Ifiee, B.E.,Tongooona, P., Danquah, E., Jumbo, M.B., Gowda, M., Marchello-D'ragga,P.W., Chelangat, S. and Suresh, L.M. (2019). Maize lethal necrosis and the molecular basis of variability in concentrations of the causal viruses

in co-infected maize plant. General and Molecular Virology, 9:1-19. DOI: 10.5897/JGMV2019.0073.

Beyene, Y., Gowda, M., Suresh, L. M., Mugo, S., Olsen, M., Oikeh, S. O. and Prasanna, B. M. (2017). Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. Euphytica, (9.)213–224). doi: 10.1007/s10681-017-2012-3

Boddupalli, P., Suresh, L., Mwatuni, F., Beyene, Y., Makumbi, D., Gowda, M., Olsen, M., Hodson, D.Worku, M. and Mezzalama, M. (2020). Maize lethal necrosis (MLN): Efforts toward containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa. Virus Research, 197943.

Boudreau, M. A. (2013). Diseases in intercropping systems. Annual Review of Phytopathology, 51: 499–519. Doi: 10.1146/annurev-phyto-082712-102246

Brault, V., Uzest, M., Monsion, B., Jacquot, E., and Blanc, S. (2010). Aphids as transport devices for plant viruses. Comptes Rendus Biologies, 333: 524-538.

Cabanas, D., Watanabe, S., Higashi, C.H.V. and Bressan, A. (2013). "Dissecting the mode of Maize chlorotic virus (Tombusvidae: Machlomovirus) transmission by *Franliniella williamsi* (Thysanoptera: Thripidae)", Journal of Economical Entomology, 106: 16-24.

Calvert, L.A. and Ghabrial, S.A. (1983). Enhancement by *Soybean mosaic virus* of *Bean pod mottle virus* titre in doubly infected soybeans. Phytopathology, 73(7): 992-997.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K. and Schaad, M.C. (1996). Cell-to-cell and long-distance transport of viruses in plants. The Plant Cell, 8:1669-1681.

Castillo, J. and Hebert, T.T. (1974). A new virus disease of maize [Zea mays] in Peru [Maize chlorotic mottle virus, Fitopatologia 9 (2):79-84.

Coe, E.H. Jr, Nueffer, M.G. and Hoisington, D.A. (1988). The genetics of maize. American Society of Agronomy: Madison, Wisconsin.No. 18; pp. 81-236

Cuellar, W., Kreuze, J.M., Rajamaki, M., Cruzado, K., Untiveros, M. and Valkonen, J. (2009). Elimination of antiviral defense by viral RNase III. PNAS, 106:10354-10358.D.F: CIMMYT.

De Groote, H., Oloo, F., Tongruksawattana, S. and Das, B. (2016). Community-surveillance based assessment of the geographic distribution and impact of maize lethal necrosis (MLN) disease in Kenya. Crop Protection, 82: 30–35.

Delgadillo, S.F., Hernández, J.L. and Torreón, A.D. (1994). Seed transmission of *Maize chlorotic mottle virus*. Revista Mexicana de Fitopatología, 12(1): 7-10.

Delgadillo, S.F. and Gaytan, B.R. (1987). Identificacion de la enfermedad de la "necrosis letal"del maiz enel estado de Guanajuato. Revista Mexicana de Fitopatología, 5, 21–26.

Demissie, G., Deressa, T., Haile, M., Bogale, G., Dida, M., Chibsa, T. and Azmach, G. (2016). Prevalence, distribution and impact of maize lethal necrosis disease (MLND) in Ethiopia. Pest Management Journal of Ethiopia, 18, 37–49.

Doupnik, B. 1979. Status of corn lethal necrosis update. In Proceedings, 34th Annual Corn and Sorghum Research Conference. American Seed Trade Association. Corn and Sorghum Division. Corn and Sorghum Research Conference, Chicago, IL. pp. 16-24.

FAO, (2018). Faostat. Food and Agriculture Organization of the United Nations, Rome,Italy. http://www.fao.org/faostat/en/#data.

FAOSTAT, (2014). Food and Agricultural Commodities Production .Food and Agriculture Organization of the United Nations.

Fentahun, M., Feyissa, T., Abraham, A. and Kwak, H.R. (2017). Detection and characterization of maize chlorotic mottle virus and sugarcane mosaic virus associated with maize lethal necrosis in Ethiopia: An emerging threat to maize production in the region. European Journal of Pathology, 149: 1011–1017. doi 10.1007/s100658-017-1229–2 Fitopatologia, 9: 79–84.

Ford, R.E. (1966). Recovery of pea streak virus from pea seed parts and its transmission by immature seed. Phytopathology, 56(7): 858.

Goldberg, K.B. and Brakke, M.K. (1987). Concentration of Maize chlorotic mottle virus increased in mixed infections with Maize dwarf mosaic virus, strain-B. Phytopathology, 77: 162–167.

Goodman, R.M. and Ross, A.F. (1974b). Enhancement of Potato virus X synthesis in doubly infected tobacco occurs in doubly infected tobacco depends on the timing of invasion by the viruses. Virology, 58: 263-271.

Gopal, K., Muniyappa, V. and Jagadeeswar, R. (2010). Management of peanut bud necrosis disease in groundnut through intercropping with cereal and pulse crops. Archives of Phytopathology and Plant Protection, 43: 883–891. doi:10.1080/03235400802075666.

Gowda, M., Das, B., Makumbi, D., Babu, R., Semagn, K., Mahuku, G., Olsen, M. S., Bright, J. M., Beyene, Y. and Prasanna, B. M. (2015). Genome-wide association and genomic prediction of resistance to maize lethal necrosis disease in tropical maize germplasm. Theoretical and Applied Genetics, 128: 1957-1968.

Guadie, D., Knierim, D., Winter, S., Tesfaye, K. and Abraham, A. (2018). Surveillance for the identification and geographical distribution of viruses and virus diseases of maize (Zea mays L.) in Ethiopia. European Journal of Plant Pathology. doi:10.1007/s10658-018.

Harrison, B.D., Finch, J.T., Gibbs, A.J., Hollings, M., Shepherd, R.J., Valenta, V. and Wetter, C. (1971). Sixteen groups of plant viruses. Virology, 45(2): 356-363.

Hill, J.H., Martinson, C.A. and Russell, W.A. (1974). Seed transmission of *Maize dwarf mosaic* and *wheat streak mosaic viruses* in maize and response of inbred lines. Crop Science, 14(2): 232-235.

IPPC. (2014). *New Pest of Maize: Maize Lethal Necrosis in Uganda*. IPPC Official Pest Report, No. UGA-01/2.Rome, Italy: FAO. Retrieved from https://www.ippc.int

Isabirye, B.E., and Rwomushana, I. (2016). Current and future potential distribution of maize chlorotic mottle virus and risk of maize lethal necrosis disease in Africa. Journal Crop Protection 5: 215–228.

Ivanov, K.I., Eskelin, K., Lohmus, A., Makinen, K. (2014). Molecular and cellular mechanisms underlying potyvirus infection. Journal of General Virology, 95:1415–29.

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

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

Kagoda, F., Gidoi, R., and Isabirye, B. E. (2016). Status of Maize Lethal Necrosis in Eastern Uganda. African Journal of Agricultural Research, 11(8), 652-660. https://doi.org/10.5897/AJAR2015.10616

Karanja, J., Derera, J., Gubba, A., Mugo, S. and Wangai, A. (2019). Response of selected maize inbred germplasm to maize lethal necrosis disease and its causative viruses (sugarcane mosaic virus and maize chlorotic mottle virus) in Kenya. Open Agriculture Journal, 12: 215–225. doi:10.2174/1874331501812010215.

Kawamura-nagaya, K., Ishibashi, K., Huang, Y., Miyashita, S. and Ishikawa, M. (2014). Replication protein of tobacco mosaic virus cotranslationally binds the 5 ′ untranslated region of genomic RNA to enable viral replication. PNAS, 111(16):1620-8

Kiruwa, F.H., Feyissa, T. and Ndakidemi. P.A. (2016). Insights of maize lethal necrotic disease. A major constraint to maize production in East Africa. African Journal of Microbiology Research, 10:271-279.

Kreuze, J., (2002). Molecular studies on the sweet potato virus disease and its two causal Agents. Doctor's dissertation, ISSN 1401-6249, ISBN 91-576-6180-4

Kusia, E. S. (2014). Characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis disease and spatial distribution of their alternative hosts in Kenya (Master's thesis). Institute for Basic Sciences Technology and Innovation, Pan African University.

Kusia, E.S., Subramanian, S., Nyasani, J.O., Khamis, F., Villinger, J., Ateka, E. and Pappu, H.R. (2015). First report of lethal necrosis disease associated with co-infection of finger millet with *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in Kenya. Plant Disease, 99(6): 899-900.

Lee, Y.S., and Ross, J.P. (1972). Top necrosis and cellular changes in soybean doubly infected by soybean mosaic and bean pod mottle virus. Phytopathology, 62: 839-845.

Li, Y.Q., Liu, R.Y., Zhou, T. and Fan Z.F. (2013). Genetic diversity and population structure of Sugarcane mosaic virus. Virus Research, 171: 242-246.

Li, L., Wang, X.F. and Zhou, G.H. (2011). Effects of seed Quality on the proportion of seed

transmission for *Sugarcane mosaic virus* in maize. Cereal Research Communications, 39: 257-266.

Lima, J.A.A, Nascimento, A.K.Q., Radaelli, P. and Purcifull D.E. (2012). Serology applied to plant virology. Serological diagnosis of certain human, animal and plant diseases. Rijeka Croácia. In Tech pp. 71-94.

Liu C, Li X, Meng D, Zhong Y, Chen C, Dong X, Xu X, Chen B, Li W, Li L, Tian X, Zhao H, Song W, Luo H, Zhang Q, Lai J, Jin W, Yan J, Chen S (2017a). A 4-bp insertion at ZmPLA1 encoding a putative phospholipase A generates haploid induction in maize. Molecular Plant 10:520-522.

Liu Q, Liu H, Gong Y, Tao Y, Jiang L, Zuo W, Xu M (2017b). An atypical thioredoxin imparts early resistance to sugarcane mosaic virus in maize. Molecular Plant pp. 483-497.

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

Lubberstedt,T,Ingvardesen,C.R.,Melchinger,A.E.,Xing,Y.,Salomon,R.and Redinbaugh,M.G. (2006). Two chromosome segments confer multiple potyvirus resistance in maize. Plant Breeding, 125 (4):352-356.

Lukanda, M., Owati, A., Ogunsanya, P., Valimunzigha, K., Katsongo, K., Ndemere, H. and Kumar, P. L. (2014). First report of Maize chlorotic mottle virus infecting maize in the Democratic Republic of the Congo. Plant Disease, 98(10), 1448-1449. https://doi.org/10.1094/PDIS-05-14-0484-PDN.

Macauley, H. and Ramadjita, T. (2015). Cereal Crops: Rice, Maize, Millet, Sorghum, Wheat. https://www.afdb.org/fileadmin/uploads/afdb/Documents/Events/DakAgri2015/Cereal_Crops-_Rice__Maize__Millet__Sorghum__Wheat.pdf pp. 1-36.

MacDonald, M.V. and Chapman, R. (1997). The incidence of *Fusarium moniliforme* on maize from Central America, Africa and Asia during 1992–1995. Plant Pathology, 46(1): 112- 125.

Mahuku, G., Lockhart, B.E., Wanjala, B., Jones, M.W., Kimunye, J.N., Stewart, L.R., Cassone, B.J., Sevgan, S., Nyasani, J.O., Kusia, E., Kumar, P.L., Niblett, C.L., Kiggundu, A., Asea, G., Pappu, H.R., Wangai, A., Prasanna, B.M. and Redinbaugh, M. (2015a). Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. Phytopathology 105(7): 956-965. https://doi.org/10.1094/PHYTO-12-14-0367-FI.

Mahuku, G., Wangai, A.W., Sadessa, K., Teklewold, A., Wegary, D., Adams, I., Smith, J., Braidwood, L., Feyissa, B., Regassa, B., Wanjala, B., Kimunye, J.N., Mugambi, C., BoTtomley, E., Bryce, S., Ayalneh, D. and Prasanna, B.M. (2015b). First report of Maize chlorotic mottle virus and Maize lethal necrosis on maize in Ethiopia. Plant Disease http://dx.doi.org/10.1094/PDIS-04-15-0373-PDN.

Maloy, O.C. (2005). Plant Disease Management. The Plant Health Instructor. doi: 10.1094/PHI-I-2005-0202-01 Retrieved February, 2015 from http://www.apsnet.org/edcenter/intropp/topics/Pages/PlantDiseaseManagement.aspx .

Manje, G., M., Das, B., Makumbi, D., Babu, R., Semagn, K., Mahuku, G. and Prasanna, B. M. (2015). Genome-wide association and genomic prediction of resistance to Maize lethal necrosis disease in tropical maize germplasm. Theoretical and Applied Genetics, 128(10), 1957-1968. https://doi.org/10.1007/s00122-015-2559-0

Marenya, P.P., Erenstein, O., Prasanna, B., Makumbi, D., Jumbo, M., and Beyene, Y. (2018). Maize lethal necrosis disease: Evaluating agronomic and genetic control strategies for Ethiopia and Kenya. Agricultural Systems, 162: 220 - 228.

Mawishe, R. and Chacha, E. (2013). Uproot maize plants with lethal necrosis disease. *Plantwise Factsheets for Farmers, CABI*.

Mbega, E.R., Ndakidemi, P.A., Mamiro, D.P., Mushongi, A.A., Kitenge, K.M. and Ndomba, O.A. (2016). Role of Potyviruses in Synergistic Interaction Leading to Maize Lethal Necrotic Disease on Maize. International Journal of Current Microbiology and Applied Sciences, 5(6): 85-96.

MDRAT, (2012). The status of Maize lethal necrosis disease and general maize performance in Kenya. Multi-Disciplinary Rapid Assessment Team, Ministry of Agriculture, Kenya.

Melinda, S., Derek, B. and Jayne, T. (2013). Maize revolutions in sub-Saharan Africa. An African green revolution (pp. 165-195). Springer Netherlands. https://doi.org/10.1007/978-94-007-5760-8_8

Meyer, M. and Pataky, J. (2010). Increased severity of foliar diseases of sweet corn infected with Maize dwarf mosaic and Sugarcane mosaic viruses. Plant Disease, 94: 1093-1099.

Miano, D. W. (2014). Maize lethal necrosis disease: A real threat to food security in the eastern and central Africa region. Nairobi, Kenya. Retrieved from http://www.nuruinternational.org.

Mikel, M.A., D'Arcy, C.J. and Ford, R.E. (1984). Seed transmission of Maize dwarf mosaic virus in sweet corn. Journal of Phytopathology, 110(3): 185-191. https://doi.org/10.1111/j.1439-0434.1984.tb00746.

Moreno, B.A., and Moreno-Moya, J.J. (2019). When viruses play team sports: mixed infections in plants. Phytopathology 110: 29-48.

Mudde, B., Olubayo, F.M., Miano, D.W., Asea, G., Kilalo, D.C., Kiggundu, A. and Adriko, J. (2018). Distribution, incidence and severity of maize lethal necrosis disease in major maize growing agro-ecological Zones of Uganda. Journal of Agricultural Science, 10(6), 72–85. doi:10.5539/jas.v10n6p72

Nault, L.R., Styer, W.E., Coffey, M.E., Gordon, D.T., Negi, L.S. and Niblett, C.L. (1978) Transmission of maize chlorotic mottle virus by chrysomelid beetles. Phytopathology, 1978; 68:1071–4.

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

Njuguna, J.G.M., Kedera, C.J., Muriithi, L., Songa, S. and Odhiambo, B. (1990). November. Overview of maize diseases in Kenya. In Proceedings of a Workshop on Review of

the National Maize Research Program. KARI/ISNAR Management Training Linkage Project: 19-23.

Nutter, R.C., Scheets, K., Panganiban, L.C. and Lommel, S.A., (1989). The complete nucleotide sequence of the Maize chlorotic mottle virus genome. Nucleic acids research, 17(8): 3163-3177.

Nyvall, R. F. (1999). Field crop diseases, 3rd ed. Iowa State University Press, Ames, IA.

Ortega, A., 1987. Insect pests of maize: a guide for field identification. CIMMYT.

Padhi, A. and Ramu, K., (2011). Genomic evidence of intraspecific recombination in Sugarcane mosaic virus. Virus Genes, 42(2): 282-285.

Paliwal, R.L., Granados, G., Lafitte, H.R. and Violic, A.D., (2000). Tropical maize: improvement and production. Food and Agriculture Organization (FAO).

Prasanna, B. M. (2014). *Maize Lethal Necrosis (MLN) in eastern Africa Tackling a Complex Challenge throughPartnerships*. AFSTA Conference, March 6, 2014. Tunis, Tunisia.

Prasanna, B.M., Suresh, M., Mwatuni, F., Beyene, Y., Makumbi, D., Gowda, M., Olsen, M., Hodson, D., Worku, M., Mezzalama, M., Molnar, T., Dhugga, K., Wangai, A., Gichuru, L., Angwenyi, S., Alemayehu, Y., Grønbech-Hansen, J. and Lassen, P. (2020). Maize lethal necrosis (MLN): Efforts toward containing the spread and impact of adevastating trans-boundary disease in sub-Saharan Africa. lsevier B.V. DOI: 10.1016/j.viruses.2020.197943

Pratt, C.F., Constantine, K.L. and Murphy. S.T. (2017). Economic impacts of invasive alien species on African smallholder livelihoods. Global Food Security14:31–37.

Rajamaki, M. and Valkonen, J.P.T. (2009). Control of nuclear and nucleolar localization of nuclear inclusion protein a of Picorna-like Potato virus Ain Nicotiana species. The Plant Cell, 21: 2485–2502.

Ramkat, R. C., Wangai, A. W., Ouma, J. P., Rapando, P. N. and Lelgut, D. K. (2008). Cropping system influences Tomato spotted wilt virus disease development, thrips population and yield of tomato (*Lycopersicon esculentum*). Annals of Applied Biology, 153: 373–380. doi:10.1111/aab.2008.153.issue-3.

Rao, Y., You, Y., Zhu, S.F., Yan, J., Huang, G. and Wei, G. (2010). The loss evaluation index system and the direct economic losses of Maize chlorotic mottle virus invading China. Plant Quarantine, 24 (2) 5-8.

Redinbaugh, M. G., and Zambrano, J. L. (2014). Control of virus diseases in maize. Pages 391-429 in: Control of Plant Virus Diseases—Seed-Propagated Crops. Advances in Virus Research, Vol. 90. G. Loebenstein and N. Katis, eds. Academic Press, Cambridge, MA.

Redinbaugh, M.G. and Stewart, L.R. (2018). Maize Lethal Necrosis: An emerging, synergistic viral disease, Annual Reviews., 5:301-322

Scheets K (2016). Analysis of gene functions in Maize chlorotic mottle virus. PMD Virus Research 222:71-79.

Scholthof, H.B. (2005). Plant virus transport: motions of functional equivalence. Trends in Plant Science, 10: 376-382.

Sharma, K., and Misra, R.S. (2011). Molecular approaches towards analyzing the viruses infecting maize (*Zea mays* L.). Journal of General and Molecular Virology, 3: 1-17.

Shepherd, R.J. and Holdeman, Q.L. (1965). Seed transmission of the Johnson grass strain of the *Sugarcane mosaic virus* in corn. Plant Disease Reporter, 49: 468-469.

Shiferaw, B., Prasanna, B.M., Hellin, J. and Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Security, 3(3): 307.

Shiferaw, B., Prasanna, B.M., Hellin, J. and Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. Food Security, 3(3): 307.

.Shukla, D.D., Strike, P.M., Tracy, S.L., Gough, K.H. and Ward, C.W. (1988). The N and C termini of the coat proteins of potyviruses are surface-located and the N terminus contains the major virus-specific epitopes. Journal of General Virology, 69(7): 1497-1508.

Stenger, D., French R.C., (2008). Complete nucleotide sequence of a Maize chlorotic mottle virus isolate from Nebraska. Plant Pathology, 153(5): 995-997.

Stewart, L.R., Willie, K., Wijeratne, S., Redinbaugh, M.G., Massawe, D., Niblett, C.L., Kiggundu, A. and Asiimwe, T. (2017). Johnsongrass mosaic virus contributes to maizelethal necrosis in East Africa. Plant Disease 101: 1455–1462.

Teyssandier, E.E., Nome, S.F., Gordon D.T., Knoke J.K., Nault L.R. and Ritter (Eds.) R.M., (1982) .Maize virus diseases in Argentina. Proceedings of the International Maize Virus Disease Colloquium and Workshop, Ohio Agriculture. Research Development Centre, Wooster, OH (1982), pp. 93-99

Thorat, A.S., Pal, R.K., Shingote, P., Kharte, S.B., Nalavade, V.M., Dhumale, D.R., Pawarn, B.H. and Babu, K.H. (2015). Detection of sugarcane mosaic virus in diseased sugarcane using ELISA and RT-PCR technique. Journal of Pure and Applied Microbiology 9: 1-9.

Untiveros, M., Fuentes, S. and Salazar, L.F. (2007). Synergistic interaction of sweet potato chlorotic stunt virus9crinivirus) with Carla-, cucumo-,ipom-, and potyviruses infecting sweet potato.Plant Diseases 91: 29-48.

Uyemoto, J.K. (1983). Biology and control of *Maize chlorotic mottle virus*. Plant disease, 67(1): 7-10.

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

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

Vega H, Beillard M.J. (2016). Ecuador declares state of emergency in corn production areas. Glob. Agric. Inf. Network. Representative USDA Foreign Agriculture. Service, Quito, Ecuador

Wamaitha, M. J., Nigam, D., Maina, S., Stomeo, F., Wangai, A., Njuguna, J. N., Holton, T. A., Wanjala, B. W., Wamalwa, M., Lucas, T., Djikeng, A. and Garcia-Ruiz, H. (2018). Metagenomic analysis of viruses associated with Maize Lethal Necrosis in Kenya. Virology Journal, 15(1): 90. doi:10.1186/s12985-018-0999-2.

Wang, B., Jiao, Y., Peluso, P., Shi, J., Liang, T. and Stitzer, M.C. (2017). Improved maize reference genome with single-molecule technologies. Nature, 546, 524– 527.

Wang, Q., Zhang, C., Wang, C., Qian, Y., Li, Z., Hong, J. and Zhou, X. (2017). Further characterization of *Maize chlorotic mottle virus* and its synergistic interaction with *Sugarcane mosaic virus* in maize. Scientific Reports, **7**, 39960.doi: 10.1038/srep39960

Wangai, A., Redinbaugh, M.G., Kinyua, Z., Miano, D., Leley, P., Mahuku, G., Scheets, K. and Jeffers, D. (2012). First report of *Maize chlorotic mottle virus* and maize (corn) lethal necrosis in Kenya. Plant Disease, 96: 1582-1583.

Wheeler, B.E.J. (1969). An introduction to plant diseases, John Wiley and Sons Limited, London, 374 pp.

Williams, L.E., Findley, W.R., Dollinger, E.J. and Ritter, R.M. (1968). Seed transmission studies of *Maize dwarf mosaic virus* in corn. Plant Disease Reporter, 52: 863-864.

Wu, J.X., Wang, Q., Liu, H., Qian, Y.J., Xia, Y. and Zhou, X.P. (2013). Monoclonal antibody- based serological methods for *Maize chlorotic mottle virus* detection in China. Journal of Zhejiang University Science B, 14(7): 555-562.

Wu, L., Han, Z., Wang, S., Wang, X., Sun, A., Zu, X. and Chen, Y. (2013). Comparative proteomic analysis of the plant–virus interaction in resistant and susceptible ecotypes of maize infected with *Sugarcane mosaic virus*. Journal of Proteomics, 89: 124-140.

Wu, L., Zu, X., Wang, S. and Chen, Y., (2012). *Sugarcane mosaic virus*–long history but still a threat to industry. Crop Protection, 42: 74-78.

Xia X, Chen W, Fu Q, Zhang P, An T, Cui A (2016). Molecular Variability and Distribution of Sugarcane Mosaic Virus in Shanxi, China. PLOS-One, 11:1-12.

Xia, L., Zhang, J.Z., Wang, Q.A., Meng, C.M., Hong, J.A. and Zhou, X.P. (2011). Characterization of maize chlorotic mottle virus associated with maize lethal necrosis disease in China. Journal of Phytopathology, 159: 191-193.

Xia, X., Chen, W., Fu, Q., Zhang, P., An, T. and Cui, A. (2016). Molecular variability and distribution of sugarcane mosaic virus in Shanxi, China. PLOS-One, 11: 1–12. DOI:10.137/journal.pone.0151549.

Yang, Q., Balint-kurti, P. and Xu, M. (2017). Quantitative disease resistance: dissection and adoption in maize. Molecular Plant, 10(3):402-413.

Zambrano,J.L.,Jones,M.W.,Brenner,E.,Francis,D.M.,Tomas,A.,Redinbaugh,M.G.,(2014).Genetic analysis of resistance to six virus diseases in a multiple virus-resistant maize in bredline. Theoretical. Application. Genetics, 127: 867–880. https://doi.org/10.1007/s00122-014-2263-5

Zhang, J., Xia, L., Wang, Q., Meng, C., Hong, J. and Zhou X. (2011a). Characterization of Maize chlorotic mottle virus associated with maize lethal necrosis disease in China Journal Phytopathology, 159: 191–937.

Zhang, M.Q., Rao, G.P., Gaur, R.K., Ruan, M.H., Singh, M., Sharma, S.R., Singh, A. and Singh, P. (2008). Characterization, diagnosis and management of plant viruses. Industrial Crops, 1:111-144.

Zhang, Y., Zhao, W., Li, M., Chen, H., Zhu, S. and Fan, Z., (2011b). Real-time TaqMan RT-PCR for detection of *Maize chlorotic mottle virus* in maize seeds. Journal of Virological Methods, 171(1): 292-294.

Zhao, M., Ho, H., Wu, Y., He, Y. and Li, M. (2014). Western flower thrips (*Frankliniella occidentalis*) transmits maize chlorotic mottle virus. Journal Phytopathology, 162: 532–536.

Zorya, S., Morgan, N. and Rios, L.D. (2011). Missing food: The case of postharvest grain losses in Sub-saharan Africa. The International Bank for Reconstruction and Development / The World Bank. Report No. 60371-AFR.