DETERMINING THE ROLE OF SEED AND SOIL IN THE TRANSMISSION OF VIRUSES CAUSING MAIZE LETHAL NECROSIS DISEASE

By

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DECLARATION

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

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DEDICATION

To the almighty God for imparting me with his grace along this journey.

To my wife Angelique for her support and prayers, and all kinds of encouragement.

My sons, Alain, Kevin, Yves, Cedric and my beloved daughter Kevine Bihogo, whom I missed for long periods when I was away from them.

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ACRONYMS

A.I Active Ingredient

CIMMYT International Maize and Wheat Improvement Center

CLND Corn lethal necrosis disease

CRD Completely randomized design

C.S Clean seeds

DAP Days After Planting

DPI Days Post Inoculation

DAC-ELISA Direct antibody coating- Enzyme Linked immunosorbent assay

DAS-ELISA Double antibody sandwich -Enzyme Linked Immunosorbent Assay (ELISA)

DRC Democratic Republic of Congo

DSMZ Deutsche Sammlung von Mikroorganimen und Zellkulturen

FAO STAT Food and Agriculture Organization Statistics

GDP Gross Domestic Product

GEB General Extraction Buffer

GIEWS Global Information and Early Warning System

GM Gram

GPS Global Positioning System

HA Hectare

IgGAg Immuno globulins type G Antigen

I.S Infected seeds

JGMV *Johnson grass mosaic virus*

KALRO Kenya Agricultural and Livestock Research Organization

KDA Kilo Dalton

KFSSG Kenya Food Security Steering Group

KNBS	Kenya National Bureau of Statistics
L	Litre
LSD	Least significant difference
MALF	Ministry of Agriculture, Livestock and Fisheries
MCMV	*Maize chlorotic mottle virus*
MDMV	*Maize dwarf mosaic virus*
MG	Milligram
MINAGRI	Ministry of Agriculture and Animal Resources
ML	Milliliter
MLN	Maize lethal necrosis
MRDV	*Maize rough dwarf fijivirus*
MT	Metric ton
NM	Nanometer
OD	Optical density
ORF	Open reading frame
PNP	Para-nitrophenyl-Phosphate
QTL	Quantitative trait locus
SCMV	*Sugarcane mosaic virus*
SrMV	*Sorghum mosaic virus*
T	Ton
USA	United States of America
µl	Microliter
WSMV	*Wheat streak mosaic virus*
SSA	Sub-Saharan Africa
SSRNA	Single Stranded Ribonucleic Acid

ABSTRACT

Maize is an important food crop in sub-Saharan Africa (SSA) and is consumed by over 90% of Kenyan people. The outbreak of Maize lethal necrosis (MLN) disease in Kenya in 2011 resulted in drastic reduction in maize production. The disease, a result of synergistic interaction between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV), is also reported to be present in Uganda, Tanzania, Rwanda, DRC-Congo and Ethiopia. The two viruses causing MLN are reported to be transmitted by vectors, through mechanical means, and by seeds though at very low rates. However, the rate of spread of the disease in the eastern Africa region has been very high, indicating a potential role of seed transmission. There is also little information on the role of contaminated soil in the transmission of the two viruses. The aim of this study was therefore to determine the rate of MCMV and SCMV transmission through seed and soil and to determine the combined effect of mechanical and seed transmission in MLN disease development.

In order to determine the rate at which the two viruses are transmitted through seed and soil, forty-eight inbred lines were evaluated, where seeds from infected plants were planted in clean soil, and seeds from non-infected plants (clean seeds) planted in soil where MCMV or SCMV-infected maize plants were harvested. Seeds harvested from infected plants were first tested for both viruses using DAS-ELISA. Soil was collected in pots from fields where infected susceptible plants were grown and all debris removed before planting clean seeds. To determine the combined effect of seed and mechanical transmission in MLN disease development, maize were grown from clean seeds which were not inoculated with any virus; other plants were grown from clean seed but inoculated with both viruses, and other plants were grown from seeds obtained from infected plants and inoculated with one virus first and later with the other virus. The experiments were laid out in a completely

randomized design with three replications in the greenhouse. Plants were evaluated for incidence and severity, and tested with DAS-ELISA to determine the presence of MCMV and SCMV.

All seeds harvested from MCMV-infected plants tested positive for MCMV while all seeds tested negative for SCMV, even in those obtained from SCMV-infected plants. There were also no observable symptoms in plants grown from infected seeds or in contaminated soil. However, DAS-ELISA results confirmed MCMV to be present in leaves from plants grown from infected seeds at a rate of 4.17% in CMCMV111, a susceptible inbred line, and at a rate of 8.34% in CMV066 (tolerant inbred line), and CMCMV111 (susceptible inbred line) grown on contaminated soil. Transmission of SCMV by seed or through soil could not be confirmed by use of DAS-ELISA. Results also indicated that all combinations of artificial inoculations with MCMV or SCMV resulted in development of typical symptom associated with the viruses. Resistant genotypes resulted in low rates of infection, particularly where MCMV was artificially inoculated. However, no symptoms were observed in plants from infected seeds but not inoculated with either of the viruses. MCMV was detected in all genotypes inoculated with MCMV or both viruses, while there was no detection of SCMV or MCMV in non-inoculated plants, even when seeds were grown from infected plants.

Based on these findings, it can be concluded that MCMV is transmitted through seed and contaminated soils though at low rates, information that could be of importance to farmers and all stakeholders interested in maize production; while SCMV was not. The results also indicate that mechanical inoculation (and probably any form of secondary infection such as by vectors) of viruses, causing MLN plays a significant role in disease development. While mechanical transmission of viruses is naturally rare in maize, the findings are helpful to farmers in that prevention of secondary infection in the field may help in reducing MLN incidence and severity. Further studies need to be conducted to determine the localization of the virus in seed.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 World maize production

Maize is an indispensable food and source of nutrition (Adebayo and Omodele, 2015) and 60-70% of world production is used to feed animals, only 30-40% account for human consumption (Mdangi *et al*., 2017). It is widely used for different reasons in human and animal feeding (Ranum and Casal, 2014; KFSSG, 2015). Production of maize in 2015 was estimated at 1037.79 MT in the world and the consumption was reported to reach 965 MT (European Union, 2017). Worldwide yield was also reported to decline due to drought, flooding, cold and winds (Priya, 2016) or weeds infestation (Mdangi *et al*., 2017). Weather conditions, stress and lack of inputs (Ngonkeu *et al*., 2017), rainfall regime and soil quality have contributed to yield reduction (Danda *et al*., 2015).

1.2 Maize production in Kenya

Maize is ranked among the chief cereal crops in sub-Saharan Africa (SSA), and is produced on over 33 million hectares. Above 90% of the population in Kenya subsist on maize (Charles, 2014). The production of maize was estimated at 42.2 million 90 kg bags in 2011, which decreased to 39.9 and 39.0 million in 2013 and 2014, respectively (Kingori *et al*., 2016). The decline was partly due to Maize lethal necrosis (MLN) disease, first reported in Bomet region (Wangai *et al.*, 2012) in 2011 and with 100% losses in affected farms (Kenya Ministry of Agriculture, 2012; Snipes and Gitonga, 2014). Other factors like poor rainfall distribution due to global climate change have aggravated and decreased the production acreage from 2.12 to 2.1 million hectares (Kamau, 2013; MALF, 2015; Vimla *et al.*, 2016). The Food insecurity drought-dependent assessment report estimated the humanitarian need at 2.2 million people in dry pastoral areas in January 2017 (GIEWS, 2017). The

recent outbreak of fall armyworm in the main maize production areas also contributed to the decline in maize production (GIEWS, 2017).

1.3 Problem statement

Maize grains support a large population in the world, through consumption and income generation (Ranum *et al*., 2014). Maize production is limited by a number of constraints including pests such as stem borers, fall army worm, aphids, thrips, beetles, leaf hoppers; diseases such as gray leaf spot, corn lethal necrosis, rust and smut; weeds, and poor agronomic practices (Nault *et al.*, 1978; Joseph *et al.*, 2013; Charles, 2014). The emergence of MLN disease in the East African region (Wangai *et al.,* 2012), due to the interaction of *Maize chlorotic mottle virus* with *Sugarcane mosaic virus* (Makone *et al.*, 2014) aggravated the maize production challenges.

In Kenya, MLN has been reported to cause up to 100% loss of maize yields in affected farms (Kenya Ministry of Agriculture, 2012; Wangai *et al*., 2012). The disease was reported to be managed by using resistant genotypes and crop rotation (Ramadjita and Harold, 2015). However, no resistant maize varieties are available for use by farmers in Kenya. Meanwhile, of 62,518 maize genotypes screened between 2012-2015 by the International Maize and Wheat Improvement Center (CIMMYT) ï Kenya, only 10% were reported as tolerant, while 90% were susceptible to MLN (Prasanna 2016, unpublished data).

Sugarcane mosaic virus is endemic in Kenya, and has not been reported to cause major losses in maize production (Louie, 1980). *Maize chlorotic mottle virus,* on the other hand, has been reported to cause significant yield losses in maize production even when there is no interaction with any other pathogen (Jensen, 1992). Understanding the role played by seed and soil in transmission of MCMV and SCMV and the effect of this interaction when mechanically inoculated is necessary in screening genotypes for breeding purposes.

1.4 Justification

Maize is consumed by 96% of Kenyans, with 125 Kg per capita consumption (Kariuki, 2015). Diseases and weather conditions have compromised maize yield, and MLN outbreak has significantly contributed to the yield reduction (Omoyo *et al.*, 2015). *Maize chlorotic mottle virus* was reported to be soil transmitted at a very high rate (Mahuku *et al.,* 2015) and very low seed transmission (Nelson *et al.,* 2011). In Eastern Africa, maize growers and stakeholders have little information on the role of seed and soil in transmission of MCMV and SCMV. There are conflicting reports on the specific vectors involved, and the rate of seed transmission has not been fully confirmed. This study was conducted to determine the rates of transmission of MLN-causing viruses through seed and soil.

Huge losses of crop and yield has been attributed to virusôs synergism (Mbega *et al*., 2016). Available knowledge suggests that understanding the mechanism behind synergy in mechanical transmission can contribute to more effective management of the disease and reduce losses (Miano, 2014). Responses of inbred lines and hybrids to viral infection or co-infections have not been fully explored. Effective management of MLN causing viruses will require working on this gap and understanding the reaction of different genotypes in disease epidemiology. The study investigated the combined effect of mechanical and seed transmission in MLN development. These results will be of importance to maize producers and will contribute to available information on MLN management and in crop breeding purposes as well.

1.5 Objectives

1.5.1 Overall objective

The overall objective of the study was to contribute to reduction of losses attributed to Maize lethal necrosis disease through understanding the role of soil and seed in the transmission of viruses causing the disease.

1.5.2 Specific objectives

The specific objectives of the study were:

1. To determine seed transmission rates and the role of soil in transmission of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in different maize inbred lines.
2. To determine the combined effect of mechanical and seed transmission on Maize lethal necrosis disease development.

1.6 Null hypothesis

1. There is no difference in seed transmission rates of viruses causing MLN disease in different maize genotypes.
2. Infected soil has no role in transmission of viruses causing MLN disease.
3. There is no interactive effect of mechanical and seed transmission in MLN disease development.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of maize production

Maize (*Zea mays* L.*)* is cultivated for different purposes including human consumption as staple food, animal feed and industrial purposes (Baffour and Fakorede, 2017). The crop originated from Parviglumis, in the highlands of Meso-american region, before its dissemination to other countries (Ranum *et al.*, 2014). The crop is suggested to have been domesticated 6,000 years ago (Willy, 2010). The discovery of new lands was the major pathway of maize spreading around the world by Europeans explorers and traders in the 15th century (Baffour and Fakorede, 2017). Nutritional composition rates are protein 10%, starch 72% and vitamins B, B_{12} and C as well (Ranum *et al.*, 2014).

2.2 Maize production in the world

Maize is one of the chief cereal crops grown worldwide, with the estimated production of 1038.33 million metric tons in 2014, and declined to 1010.61 MMT in 2015, while production in 2016 increased to 1060.11 MMT (FAOSTAT, 2018; Table 2.1).

Table 2.1 Worldwide maize production in Million Metric tons from 2008 to 2016

Region, Country/Year	2008[a]	2009[a]	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]	2016[a]
World	829.24	820,07	851.34	886.01	874.24	1015.40	1038.33	1010.61	1060.11
Africa	58.37	59.99	66.23	65.90	71.09	70.28	78.24	72.32	70.56
Americas	437,84	440.57	445.27	437.23	418.18	519.65	526.71	521.92	547.42
Asia	238.30	234.28	253.79	271.20	288.42	305.28	303.73	311.70	324.09
Europe	94.09	84.59	85.53	111.10	95.88	119.46	129.00	103.92	117.41
Oceania	0.61	0.63	0.53	0.58	0.68	0.73	0.65	0.74	0.63
Eastern Africa	20.29	20.79	26.19	27.43	29.15	28.05	31.40	28.70	28.42

Source: FAOSTAT (2018), [a]: Aggregate, include official, semi-official, estimated or calculated data

In Africa, 70 million tons of maize were produced in 2012 (Harold and Ramadjita, 2015),which increased to 70.14 million tons the following year and to 78.01 million tons in 2014 (FAOSTAT, 2017). Thirteen African countries produced about half of the total yield from the continent in 2014 and Ethiopia ranked first with 7.23 million metric tons, followed by Uganda with 6.74 million metric tons (Table 2.2).

Table 2.2 Maize production of thirteen countries in Africa (Million Metric tons) from 2008 to 2014

Country/Year	2008	2009	2010	2011	2012	2013	2014
Burundi	0.12	0.12	0.13	0.13	0.14	0.16	0.13
Cameroon	1.39	1.63	1.67	1.57	1.75	1.65	*1.6
DRC	1.16	1.16	1.16	1.16	1.17	1.18	1.17
Ethiopia	3.78	3.90	4.99	6.07	6.16	6.49	7.23
Kenya	2.37	2.44	3.46	3.38	3.75	3.59	3.51
Malawi	2.63	3.58	3.42	3.70	3.62	3.64	2.78
Rwanda	0.17	0.29	0.43	0.53	0.57	0.67	0.58
Senegal	0.40	0.33	0.19	0.12	0.24	0.23	0.18
South Sudan	-	-	-	-	*0.13	*0.17	*0.27
Tanzania	2.31	2.35	2.37	2.55	2.73	2.75	2.76
Uganda	5.44	3.33	4.73	4.34	5.10	5.36	6.74
Zambia	1.21	1.89	2.80	3.02	2.85	2.53	3.35
Zimbabwe	0.50	*0.7	1.19	1.01	1.00	0.86	*1.456

Source: FAOSTAT (2017),*: Unofficial data, : FAO data based on imputation methodology, DRC: Democratic Republic of Congo,-: Data not available.

2.3 Worldwide constraints to maize production

Maize production all over the world is constrained by both abiotic and biotic factors, among them being climate change, especially drought and frosts which have decreased maize yield tremendously (Cairns *et al.*, 2013; Gobin, 2017) with losses reaching 100% in Belgium (Gobin, 2017), and 40-80% in India (Priya *et al.*, 2016). Flooding and water logging also constrain maize production particularly in Asia and cause losses of 25-30% in Southeast Asia, while high temperature, wind and cold have decreased arable areas. Increased population pressure is increasing maize prices while the area for

production is decreasing (Peter *et al.*, 2017). Latin America, Asia, and Sub-Saharan Africa face challenges related to poor purchasing power of farmers to acquire agricultural inputs (Prasanna, 2015). Poor soil fertility has led many farmers to switch to other crops that do not require a lot of soil amendments. In European maize farms, yield and quality was reduced by 40% by fungi, insects, viruses, bacterial diseases and parasitic plants (Ricroch *et al.*, 2015).

Fungal diseases are considered as economically important due to the losses they cause. *Fusarium* spp and *Aspergilus* spp have compromised maize grains safety, through contamination of kernel and ear with mycotoxin. Grey leaf spot (*Cercospora species*), Northern corn leaf blight (*Exserohilum turcicum*) and southern corn leaf blight (*Bipolaris maydis*) are also destructive (Rose, 2017). Viruses have also contributed to losses in maize yields. Transmitted by leafhopper (*Cicadulina* spp), *Maize streak virus* (MSV) causes maize streak disease and can cause 100% yield loss (Rybicki and Pietersen, 1999), maize rayado fino disease (MRFD), caused by *Maize rayado fino virus*, an ssRNA virus was reported to cause 100% losses in America, vectored by *Dalbulus maidis,* a corn leafhopper. In China, *Maize dwarf mosaic virus* (MDMV) was responsible for 30% of losses, while *Maize rough dwarf fijivirus* (MRDV) caused between 70 and 80% of losses (Rybicki and Pietersen, 1999). Stem borer is reported to be a challenge with losses reaching 30% (Rose, 2017). Among other challenges, lack of resistant maize varieties and access to fertilizers and pesticides contributed to reduction of production due to stresses and diseases (Prasanna, 2015).

2.4 Maize lethal necrosis disease and its economic importance

Maize lethal necrosis (MLN) disease is caused by synergistic interaction of *Maize chlorotic mottle virus* (MCMV) and any maize potyvirus, including *Sugarcane mosaic virus* (SCMV), *Wheat streak mosaic virus* (WSMV) and *Maize dwarf mosaic virus* (MDMV) (Uyemoto *et al.*, 1981; Yan *et al.*, 2016), leading to serious yield losses (Adenya and Frenken, 2014). In Kansas ï USA, MLN (which is

referred to as corn lethal necrosis disease (CLND) caused 91% losses in 1977 (Nault *et al*., 1978). Mechanical infection resulted in yield loss of 70% compared to the natural infection with 50% (Uyemoto, 1980). In 1978, it was reported in Nebraska (Uyemoto *et al*., 1981) and in China in 2009 (Xie *et al*., 2011). From here, it is believed to have spread to other parts of Eastern Africa. It was reported in Tanzania in 2012 (Eduin and Frenken, 2014), Uganda in 2013 with yield loss of 50.5% (Kagoda *et al.,* 2016*)* and in Rwanda in 2013 (Adams *et al.*, 2013) with up to 100% crop loss (MINAGRI, 2016). The disease was later reported in Democratic Republic of Congo (DRC) in 2014 (Lukanda *et al*., 2014) and in Ethiopia in 2014 (Mahuku *et al*., 2015, unpublished data).

Maize lethal necrosis is now confirmed to be present in seven Eastern Africa countries (Table 2.3) of Kenya, Tanzania, Rwanda, Uganda, DR-Congo, Ethiopia and South Sudan (FAO, 2013; Mahuku *et al*., 2015). The disease has not yet been reported in Burundi.

Table 2.3 World distribution of Maize lethal necrosis (MLN) disease

Country	**Disease**	**Year reported**	**Reference**
Peru	MLN	1973	Castillo and Hebertt (1974)
USA	CLN	1976	Niblett and Cafflin (1976)
Argentina	CLN	1982	Teyssandier *et al*. (1982)
Mexico	MLN	1987	Delgadillo and Gaytan (1987)
Thailand	MLN	1983	Uyemoto (1983)
Brazil	MLN	1983	Cited in Uyemoto (1983)
China	MLN	2011	Xie *et al*. (2011)
Kenya	MLN	2012	Wangai *et al*. (2012)
Tanzania	MLN	2012	Eduin and Frenken (2014)
Uganda	MLN	2012	Kagoda *et al*. (2013)
Rwanda	MLN	2013	Adams *et al*. (2013)
DRC	MLN	2014	Lukanda *et al*. (2014)
Ethiopia	MLN	2015	Mahuku and Wangai, (2015)
South Sudan	MLN	2015	Mahuku and Wangai, unpublished results (2015)

Source: Regional platform report on plant pest and diseases, 2014 improved with my inputs, 2017

2.5 Production of maize in Kenya

Maize was introduced in Coastal Kenya in the 15th century by Portuguese (Kingori *et al*., 2016). The crop is now grown in all provinces of the country with major regions like Rift Valley producing 50%, Nyanza 14% and Central region with 6% (Chemiat and Makone, 2015). Agriculture sector is ranked the most prominent and contributed up to 30% of Kenyaôs GDP, with maize accounting for 9% after coffee and tea (KNBS, 2016). Climate change affected the sector with 3.5% of decline from 5.2% recorded in 2013 (Wiggins and Keats, 2015). Maize production in Kenya has fluctuated since 2011 (Fig.1). The production was 3.46 million tons in 2010, which decreased to 3.38 million tons in 2011 during the MLN outbreak. The following years, production increased to a peak of 3.82 million tons in 2015, before dropping to a low of 3.33 million tons in 2016 (FAOSTAT, 2018). In 2014, Kenya ranked among the top ten highest maize consuming countries at position seven with 171 g/person/day after Lesotho (328), Malawi (293), Zambia (243), Zimbabwe (241) and South Africa (222) (Ranum *et al.*, 2014)

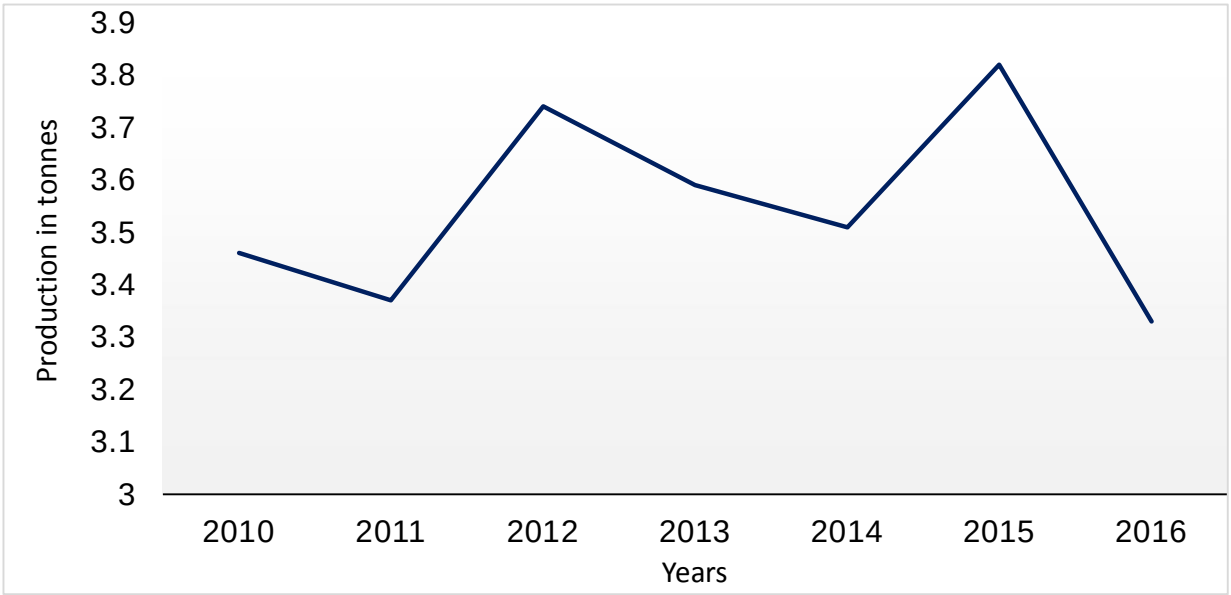

Figure 2.1 Maize production in Kenya (in Million tons) from 2010 to 2016 (Adapted from FAOSTATA DATA, 2018).

2.6 Constraints to maize production in Kenya

Poor quality seeds used by farmers, weevils, rodents and high costs of inputs constrains maize production in Kenya (Chemiat and Makone, 2015). Fungal diseases have contributed to yield losses of maize not only in Kenya but also worldwide. Among the leading disease is northern leaf blight which causes yield losses estimated at 70%. In the Rift valley, losses due to gray leaf spot caused by *Cercospora zeae-maydis* reached 30-50%, while damage due to head smuts caused by *Sphacelotheca reiliana* was estimated at 10-15%. *Maize streak virus* which is a viral disease can cause 100% of yield losses (Charles, 2014). *Striga hermonthica* L., a plant parasitic weed endemic in Kenya since 1936 near Lake Victoria basin (Ndwiga *et al.*, 2013), affects maize with yield losses reaching 100% (Charles, 2014). Maize crop requires enough water during critical growth stages, especially at flowering and early grain filling, as it is important for evapotranspiration of plants and determines the yield trend such as number of ears per plant and number of kernels per ear (Omoyo *et al.,* 2015). Kenya is not self-sufficient in maize due to persistent droughts, pests, diseases and weather conditions, which reduce the yield to 1.1t ha^{-1} (Omoyo *et al.*, 2015).

2.7 Maize lethal necrosis disease in Kenya

Maize lethal necrosis disease was first reported in Kenya in September 2011 in Bomet County (Wangai *et al.,* 2012). The disease spread to other six counties of the country within five months (Kenya Ministry of Agriculture, 2012). By 2012, the disease was reported to be present in 22 counties (FAO, 2013). Naivasha and Bomet experienced losses of up to 100% (Wangai *et al.*, 2012; Adams *et al*., 2013), Sotik, Chepalungu and Borabu were also heavily affected (Joint Assessment Report, 2012). Losses of 90% were reported in 2012 and about 77,000 ha were affected (Mahuku *et al*., 2015) with 10% of losses in 2014-2015 (Snipes and Gitonga, 2014; Table 2.4).

Table 2.4 Maize production in different ecological zones and MLN losses in Kenya, 2015

Ecological zones	Production in tons	Estimated losses in tons	Average losses in %
Moist mid-altitude	304,994	96,707	32
Moist transitional West	1,040,794	298,277	29
Highland tropical	583,681	87,750	15
Moist transitional East	490,03	2,649	5
Dry mid-altitude	157,159	5,021	3
Dry transitional	27,409	762	3
Lowland tropical	8,228	1,227	15
Total	**2,122,268**	**492,393**	**23**

Source: Distribution and impact of maize lethal necrosis in Kenya, DE Groote, working paper, May 2015.

2.7.1 Symptoms of Maize lethal necrosis disease

Major symptoms of MLN were described by Uyemoto (1980) and Mahuku *et al.* (2015), and consist of chlorotic mottle on leaves, beginning from the lower base of the leaf to the meristem of young leaves (Plate 2.1A). Symptoms development start with mild to severe mottling. Leaf margins display necrosis which continues to the mid-rib and result in drying out of the whole leaf or stunting, in some cases plants fail to fill the grain, while others die prematurely (Plate 2.1B).

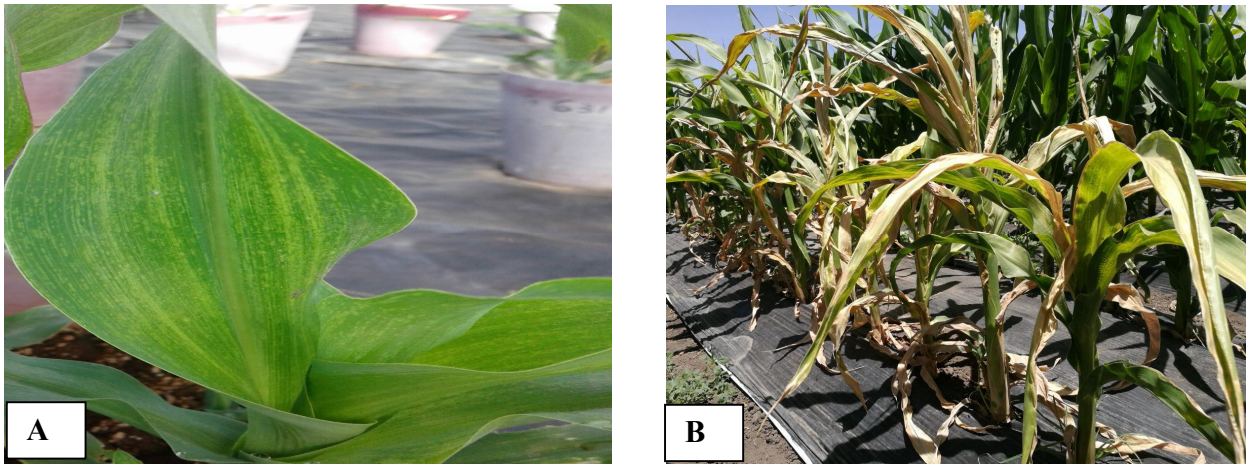

Plate 2.1 Maize lethal necrosis symptoms with moderate chlorotic mottling in leaf margin (**A**), and excessive necrosis of plants and completely necrotic (**B**), Pictures taken in Naivasha, April 2017.

2.7.2 Management of maize lethal necrosis

Enforcement of local and regional quarantine measures is the best way to delay infection and infestation of maize plants by MLN (Mahuku *et al*., 2015). Tolerant and or resistant varieties with promising yields were developed by CIMMYT under the framework of screening and development of MLN resistant genotypes (Prasanna, 2016). The use of resistant genotypes can mitigate the development of MLN disease (Gitonga, 2014). Maize seeds dressed with chemicals such as Imidacloprid 350g/L (1.0 mg a.i/kernel) before sowing can also reduce contamination on seeds (Kibaki and Francis, 2013). Other effective disinfectant products can also be used to remove viral surface contaminations. Application of pesticides and insecticides like ɓ- cyfluthrin 45g/L (0.3L/ha) through sprays have also been shown to protect maize plants and also reduce the disease vectors (Kibaki and Francis, 2013). Usually, when plants are sprayed, they grow and develop vigorously which might delay the development of the disease as vectors are also managed. Crop rotation had shown to be important in reducing inoculum build-up in farms (Sitta, 2017). Crop rotation minimizes continuous cropping year round (Hugo De Groote, 2015). Synchronizing crops within the growing season and between farms positively contribute to MLN management (Mahuku *et al.,* 2015). Regular monitoring of farms, roguing and destroying infected plants have reduced the occurrence and spread of MLN to non-infected plants and farms (Joint Assessment Report, 2012). Proper harmonization and implementation of mechanisms regulating maize seed trade and phytosanitary measures within the region were also proposed as important in MLN management (Trocaire, 2014; Katrin and Zhou, 2015).

2.8 Viruses causing maize lethal necrosis disease

2.8.1 Maize chlorotic mottle virus and its distribution

Maize chlorotic mottle virus (MCMV) has a spherical single stranded ribonucleic acid (ssRNA) of 28-34 nm in diameter with 4437nt and 24.6 KDA protein capsid (Goldberg and Brakke, 1987). The virus was first identified in Peru in 1974 (Bockelman *et al.,* 1982; Jensen *et al*., 1991) and has since then been reported in other areas like the States of Kansas and Nebraska in USA (Nault *et al.,* 1978; Philips *et al*.,1982), Argentina (Jiang *et al.,* 1992a), Mexico and Hawaii (Jensen *et al.,* 1991; Brooks *et al.,* 2012), China in 2011 (Xie *et al.,* 2011), Kenya in 2012 (Wangai *et al.,*2012*)*, Tanzania in 2012 (Eduin and Frenken, 2014), Uganda in 2012 (Kagoda *et al.,* 2016), Rwanda in 2013 (Adams *et al.*, 2014), DR-Congo in 2014 (Lukanda *et al.,* 2014), Ethiopia in 2015 (Mahuku *et al.,* 2015) and South Sudan in 2015 (Mahuku *et al.,* 2015). Several serotypes have been identified, MCMV-K, MCMV-P (Uyemoto,1980) and a number of species of beetles have been reported as MCMV vectors (Jensen *et al.,* 1991). When MCMV interacts with any potyvirus infecting maize, this results to MLN disease (Wu *et al.,* 2013). The nucleotide sequence similarity of MCMV isolates from East African countries are 99% (Mahuku *et al.,* 2015), indicating that the whole region have similar MCMV viruses interacting mainly with SCMV. Kenyan isolates had 95- 98% sequence similarity (Wangai *et al.*, 2012). Ethiopia isolate was similar to East Africa isolates with 99% similarity (Mahuku *et al.,* 2015). Rwanda, Kenya, China isolates were identical with 99%, and 96-97% with USA isolates (Adams *et al.,* 2014). MCMV isolates from Thailand were closely related to China strains with 98-99.6% sequence similarity (Wu *et al.,* 2013). Kansas and Nebraska isolates similarity was 100% and Taiwan isolates were related to China at 99.1- 99.7%, though they differed from American strains at 96.9- 97.3% (Wang *et al.,* 2017), which implied a common phylogenic ancestry.

2.8.1.1 Symptomatology and host range of *Maize chlorotic mottle virus*

The host range for the virus is limited to members of the gramineae family and symptoms are mild (Plate 2.2C) to severe (Plate 2.2D). Mosaic and necrosis on leaves precede stunting and premature plant death. Short male inflorescence with few spikes and partial ear filling characterize its symptoms (Nelson *et al.*, 2011; Wu *et al*., 2013). MCMV was reported to cause losses of 10 to 15% in natural fields and 59% in trial plots (Jiang *et al.*, 1992). Other hosts of MCMV are grasses belonging to the poaceae family like *Tricicum aestivum, Sorghum bicolor* (Gordon *et al.,*1984), *Bromus japonicas, Eragrostis trichodes, Hordeum pusillum, H. vulgare* L.*., Andropogon scoparius, B. secalinus, Digitaria sanguinalis* L., *Panicum miliaceum, S. viridis, Setaria faberi,* and *Setaria italica* (Nelson *et al.,* 2011; Mahuku *et al.,* 2015). Initial inoculum accumulation of MCMV can result from alternative crop hosts, debris and increased number of vectors (Uyemoto, 1983). Moisture in seed (13-30%) influences the development of the virus (Jiang *et al*., 1992).

Plate 2.2 MCMV mild mosaic turning the leaf to dark green (**C**, Picture taken in Naivasha, April 2017) and severe mosaic stripes on both sides of the leaf with light green leaves (**D**, Nelson *et al.* (2011).

2.8.1.2 Seed transmission of *Maize chlorotic mottle virus*

Seed transmission of MCMV was reported not to occur in maize inbred lines, hybrids and three *Setaria* species tested in Peru and Kansas State (Bockelman *et al.*, 1982; Gordon *et al.,* 1984).

Contrary to this result, seed was shown to transmit MCMV in Hawaii at 0.04% and China (Jensen *et al.,* 1991; Nelson *et al.,* 2011; Wu *et al.*, 2013). The virus was also found in the entire organs of maize plant like leaf, husk, kernel, seed, anther, stem, root, cob and sheath (Jiang et al., 1992) in Kauai region. Though the rate of transmission was low, this can have significant impact in farmersô field (Johansen *et al.,* 1994). The virus is suspected to be aggravated by presence of vectors, which spread the initial virus inoculum to other places of the same field or the neighboring fields (Johansen *et al.,* 1994). Although transmission of MCMV through seed has been reported, it largely remains unresolved, especially the mechanisms surrounding the infection process and location of the virus.

2.8.1.3 *Maize chlorotic mottle virus* transmission through soil

Soil is believed to transmit MCMV in the presence of corn root worm, cutworm, beetle larvae, fungi, and plant parasitic nematodes, which may result to virus persistence in the same fieldôs residues (Jensen, 1985; Nelson *et al.,* 2011). In Zimbabwe a maize hybrid (SC513) was infected at 70% when grown in soil where MCMV infected maize were grown, while only 4% of the plants grown on sterile soil were infected with MCMV (Mahuku *et al.,* 2015). However, factors that contributed to virus transmission in the soil are not clear.

2.8.1.4 Vectors associated with *Maize chlorotic mottle virus* transmission

Maize chlorotic mottle virus is reported to be transmitted by chrysomelid beetles and corn rootworms (Nault *et al.,* 1978; Jensen, 1985) and can be spread by migratory beetles (Uyemoto, 1983). Thrips, aphid, leaf hoppers, whiteflies and scarab beetles have also been associated with MCMV transmission (Jiang *et al.,* 1992; Nelson *et al.,* 2011; Mahuku *et al.,* 2015). Corn thrips survive on crops like beans, sorghum, cassava, onions and grasses and can transmit the virus to maize plants when grown nearby *(*Nelson *et al.,* 2011).

2.8.1.5 *Maize chlorotic mottle virus* management

Maize chlorotic mottle virus can be managed using integrated pest and disease management practices including application of insecticides on a weekly basis to control vectors (Nelson *et al.,* 2011). The use of resistant varieties can be a sustainable solution, roguing the plants showing symptoms or any other alternative hosts neighboring the field and avoidance of mechanical injuries to plants with tools or un-necessary movements in the field (Nelson *et al.,* 2011). Crop rotation, and reduction of cool and wet conditions have been reported to be efficient in controlling arthropods survival rate and reproduction mechanisms thus hindering the spread of MCMV (Jiang, *et al.,* 1992b).

2.8.2 *Sugarcane mosaic virus* and its distribution

Sugarcane mosaic virus is a member of potyviridae family, positive sense single stranded RNA potyvirus of filamentous particle of 700- 750nm by 11 nm in length and width, respectively. It has one large open reading frame (ORF) and two un-translated regions (UTRS). Original isolates from maize were classified as MDMV strains and those of sugarcane as SCMV strains (Chen *et al.,* 2002). *Sugarcane mosaic virus* is widely distributed in the world and is essentially transmitted by aphids (Slykhuis, 1976). The virus was first reported in USA (Brands, 1919), then in Indonesia in 1922 (Wakman *et al.,* 2001; Muis, 2002), California in 1962 (Johnson *et al.,* 1972), in twenty districts of Kenya in 1973 (Louie, 1980), Tanzania (Louie, 1980; Wangai *et al.,* 2012) and new isolate ñ*group IV''* in China (Yan *et al.*, 2016). In India SCMV was confirmed to differ from MDMV serologically and both viruses were found present in sorghum and maize (Rao *et al.,*1998). In Thailand, mechanical inoculation of SCMV resulted in incidence of 17 to 90% on maize, 15 to 92% on sorghum and 0 to 80% on sugarcane within two weeks after artificial inoculation (Gemechu *et al.*, 2004).

The yield loss due to SCMV was 48% in Brazil (Rybicki and Pietersen, 1999). Losses caused by SCMV in sugarcane plant were estimated at 40% (Australian Government, 2004; Dangora *et al.*, 2014) and 50% in Pakistan. The potyvirus is also reported to be distributed all over the world (Shukla *et al.,* 1989). With serological and molecular characterizations, four groups were classified: MDMV (MDMV-A, MDMV-D, MDMV-E, MDMV-F), SCMV (MDMVB, SCMV-A, SCMV-B, SCMV-E, SCMVSC, SCMV-BC, SCMV-Sabi), *Johnson grass mosaic virus*, JGMV (SCMV-JG, MDMV-0) and *Sorghum mosaic virus*, SrMV (SCMV-H,SCMV-I,SCMV-M) (Lidia *et al.,* 2012).

Sugarcane mosaic virus isolate from Ethiopia was reported to be similar to Rwanda isolates with 96% nucleotide similarity (Mahuku *et al.,* 2015) and distant to Kenya isolates at 87% (Adams *et al.,* 2014). Kenyan isolates were identical at 88%-96% similarity (Wangai *et al.*, 2012). Vietnam SCMV strains shared similarities with Thailand strains at 99% (Wang *et al.,* 2017), while China SCMV group IV strains were similar at 95.1% and 99.5% between isolates (Yan *et al.,* 2016). Thailand, Vietnam, China, and East Africa strains are homologous and might have a common background.

2.8.2.1 Epidemiology and host range of *Sugarcane mosaic virus*

The virus spread is dependent on a number of vectors available during the cropping season and the alternative hosts for the vectors. Sap of injured plants may also contaminate healthy plants (William, 2015). When plant is infected, early infection becomes more damaging than late infection (Johnson *et al.,* 1972). Host range of SCMV is known to include maize, sorghum, pearl millet, millet, barley, rice, and rye (Slykhuis, 1976). The incidence of SCMV in nine maize genotypes infected naturally in Indonesia reached 7.3% (Wakman *et al.,* 2001). Other hosts include *Digitaria abyssinica*, *Tripsacum fasciculatum*, and *Brachiaria sp*. (Louie, 1980).

2.8.2.2 Symptoms of *Sugarcane mosaic virus*

Sugarcane mosaic virus symptoms are not totally different from those caused by MCMV and are most clear on younger leaves. The notable ones include green shade, green patches, yellow streak or chlorosis (Plate 2.3F), stunting and necrosis, small chlorotic spots between veins (Plate 2.3E) and mosaic (CABI, 2016). In some cases, plants are asymptomatic and may still result in yield losses (Cronje and Bailey, 1994).

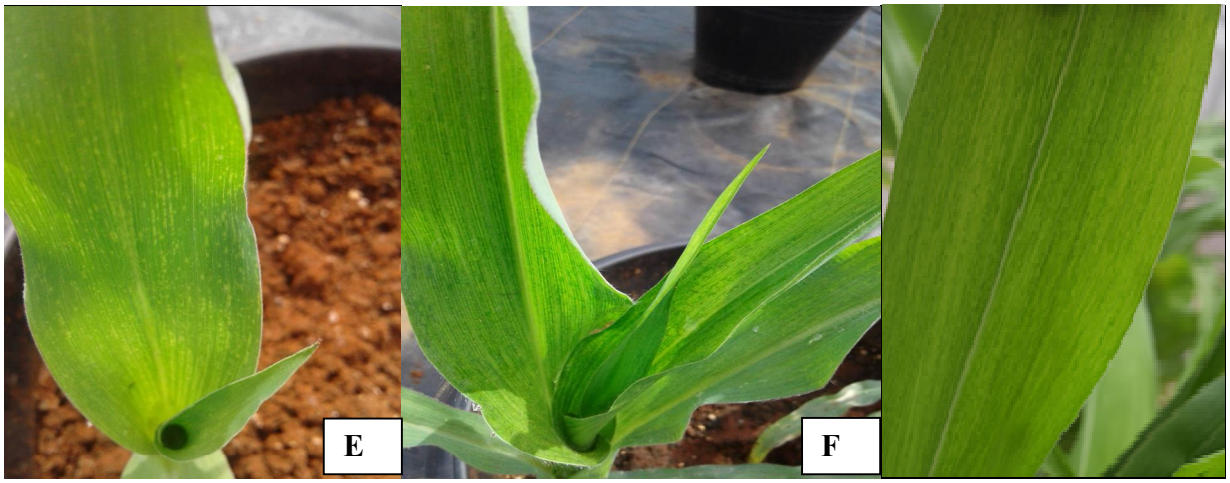

Plate 2.3 *Sugarcane mosaic virus* symptoms in infected maize plants. Small chlorotic spots between veins (**E**), and mosaic in the leaf and light green patches with some chlorosis (**F**).

2.8.2.3 Seed transmission of *Sugarcane mosaic virus*

Sugarcane mosaic virus can be transmitted to the seeds when infected males and females fertilize. This has been reported to reach 4.81% in maize plants in China and the virus was found in all parts of the seed (4.21%) of infected embryo after their pollination (Li Li *et al.,* 2007). Results from a study in Poland reported SCMV to be seed transmitted in two maize cultivars at 1.7% in arobase cultivar and 1.8% in blask cultivar (JeŨewska and Trzmiel, 2009). Seed transmission of SCMV in sorghum was not observed (Singh *et al*., 2005).

2.8.2.4 Soil transmission of *Sugarcane mosaic virus*

Sugarcane mosaic virus was reported to replicate and translocate in maize leaves at a rate of 0.46% through mechanical inoculation of roots grown in papers, though it may also result from a wounded stem (Moline and Ford, 1974). Transmission of SCMV through soil was reported in sorghum grown alternatively in the same cage and ranged at 0.7 and 5.4% for non-inoculated and inoculated plants, respectively (Bond and Pirone, 1970).

2.8.2.5 Vectors associated with *Sugarcane mosaic virus*

The most important known vectors of SCMV are aphids (Slykhuis,1976).Two types of aphids have been associated with high transmission rates of SCMV; *Rhopalosiphum maidis and Rhopalosiphum Padi* transmitted the virus at 92% on maize while *Schizaphis graminum* transmitted the virus at rate of 72% in wheat in Pakistan (Mansoor *et al.,* 2003; William, 2015). *Rhopalosiphum maidis* was shown to transmit SCMV in different areas like Argentina, Ecuador, Brazil, and Venezuela (Perera *et al.,* 2012).

2.8.2.6 Management of *Sugarcane mosaic virus* disease in maize

Due to the economic importance of SCMV and its wide distribution (Moline and Ford, 1974), the use of resistant varieties incorporated with Scmv1 and Scmv2 genes for the purpose of retaining the yield was recommended (Lübberstedt *et al.,* 2006). Quarantine of materials during the exchange when importing or moving seeds from one region to another was reported to avoid introducing the virus in clean areas (Francisca *et al.,* 2012). The use of clean varieties, removal of suspected plants have reduced the pathogenic inoculum load effectively (Perera *et al.*, 2012).

CHAPTER THREE

DETERMINATION OF RATES OF TRANSMISSION OF *MAIZE CHLOROTIC MOTTLE VIRUS* AND *SUGARCANE MOSAIC VIRUS* IN MAIZE THROUGH SEED AND SOIL

3.1 Abstract

Reduction in production and yields of cereal-based foodstuffs, particularly maize has resulted in increase in importation of maize in most Sub-Saharan countries. The outbreak of Maize lethal necrosis (MLN) disease, a result of *Maize chlorotic mottle virus* (MCMV) in association with *Sugarcane mosaic virus* (SCMV) was among reasons behind this increase. Previous studies on seed and soil transmission of the two viruses have not been conclusive. The objective of this study was therefore to determine the rate of seed and soil transmission of MCMV and SCMV in different maize genotypes. Forty eight inbred lines were evaluated in the study, where infected seeds were planted in clean soil, and clean seeds were planted in soils where MCMV or SCMV-infected maize plants had been harvested. The trial was laid out in a completely randomized design with three replications in the greenhouse. Seeds harvested from infected plants were first tested for both viruses using ELISA. All seeds harvested from MCMV-infected plants tested positive for MCMV while all seeds tested negative for SCMV, even those obtained from SCMV-infected plants. Virus-contaminated soil was collected in pots where infected susceptible plants were grown and all debris removed before growing the clean seeds. Plants were evaluated for incidence and severity, and tested with ELISA to determine the rate of MCMV and SCMV transmission. No symptom was recorded in all plants grown from infected seeds or in contaminated soil. However, ELISA results confirmed MCMV to be present in maize leaves from plants grown from infected seed at a rate of 4.17% in CMCMV111, a susceptible inbred line, and at a rate of 8.34% in CMV066 (tolerant inbred line), and CMCMV111 (susceptible

inbred line) grown on contaminated soil. Transmission of SCMV by seed or through soil could not be confirmed by the use of ELISA technique. Based on these findings, it can be concluded that MCMV can be transmitted through infected seed and contaminated soils though at low rates, while SCMV was not. This information is of importance to farmers and all stakeholders interested in maize production. Further studies should be conducted to determine the localization of MCMV in seed.

3.2 Introduction

Sub-Saharan Africa (SSA) countries are under pressure from different food challenges including shortage of grains for consumption. About 80% of people in these developing countries have difficulty in accessing low cost cereal food crops (Sasson, 2012). The average yield of maize crop in SSA has declined to 1.8t/ha in the recent years partly due to drought, diseases, insect-pests, and continuous cultivation throughout the year (Beyene *et al*., 2017). In Kenya, agricultureôs contribution to GDP dropped to 30% in 2014 from 32% in 2000, while the food imports has increased from 447 to 1216 million USD in the same period (FAO, 2015).

Maize chlorotic mottle virus (MCMV), in synergistic interaction with *Sugarcane mosaic virus* (SCMV), has been shown to result in maize lethal necrosis (MLN) disease (Isabirye and Rwomushana, 2016), and has become destructive and a real threat to farmers who have lost their produce since 2011 in Kenya (Wangai *et al*., 2012; Perera *et al*., 2012; Bulegeya, 2016). The viruses are reported to be transmitted by different means including vectors, mechanical means, and through seed and soil (Jensen *et al*., 1991; Li Li and Zhou, 2007; Mahuku *et al*., 2015).

Maize seed has been reported to transmit MCMV and *Maize dwarf mosaic virus* (MDMV) at very low rates of 0.008-0.04% (Zitter, 1984; Jensen *et al.*, 1991; Cabanas *et al*., 2013; Zhao *et al*., 2014). Maize male pollen infected with SCMV when crossed with healthy female resulted in 0.04 to 0.1% of infected offspring, while seed transmission of SCMV was reported to be 4.81% where grow-out seeds

in the net house originated from infected plants in the field (Li Li and Zhou, 2007). Early studies on soil transmission had focused on SCMV strain -H on sorghum through mechanical inoculation (Bond and Pirone, 1970). Moline and Ford (1974) reported the transmission of *Sugarcane mosaic virus* strains (MDMV-B of sweet corn, SCMV-H of sorghum and MDMV-A of Johnson grass) on sweet corn through mechanical inoculation of roots at a very low rate of 0.46%. Mahuku *et al*. (2015) reported that MCMV is soil transmitted to healthy seeds grown on infected soil at a high rate of 70%, but there was no evidence of SCMV transmission through soil. Mahuku *et al.* (2015) also reported the presence of MCMV in 72% of seeds harvested from infected plants, but argued that the result may not necessarily support the fact that the virus was transmitted from parent plants to the offsprings. The same observations were made in Rwanda where MCMV was indicated to be transmitted by seeds harvested from infected parent plants up to the third generation (Asiimwe *et al*., unpublished data, 2014).

Although previous studies have indicated the possible role of seed and soil in transmission of viruses causing MLN disease, it is not clear if the transmission rates are genotype dependent. These concerns impact farmers and seed companies all over the world. The aim of this study was therefore to determine the rate of transmission of MCMV and SCMV in different maize inbred lines through seed and the role played by soil in transmitting both viruses to inbred lines grown on infested soil.

3.3 Materials and Methods

3.3.1 Description of experimental site

The study was conducted in Naivasha district at the Kenya Agricultural and Livestock Research Organization - International Maize and Wheat Improvement Center (KALRO-CIMMYT) MLN screening facility. Coordinates (GPS) of the site are: Latitude: 0°43'00"S; Longitude: 36°26'09"E and altitude: 1884 meters above sea level. The rainfall ranges between 120 and 131 mm in two

seasons (April to May and October to January). The soil is basically alkaline while the mean annual temperature reaches 22.6°C. The Center serves Kenya as well as sub-Saharan Africa in screening maize germplasm, developing resistant varieties and developing detection methods for viruses causing MLN. The facility covers 20 hectares, where 17 hectares are allocated to research and 3 hectares for facilities including screenhouses, laboratory, and offices (Suresh *et al*., 2016).

3.3.2 Experimental planting materials

Forty eight (48) inbred lines, which were grouped as resistant, tolerant or susceptible to MCMV or SCMV (16 resistant, 16 tolerant and 16 susceptible to either of the viruses), were evaluated for seed and soil transmission of MCMV and SCMV (Table 3.1 and 3.2). The inbred lines were coded ñCimmyt *Maize chlorotic mottle virus*ò (CMCMV), and ñCimmyt *Sugarcane mosaic virus*ò (CSCMV) followed by digits to differentiate lines with specific reactions to the viruses. Seeds used for determination of seed transmission rates were harvested from maize plants artificially inoculated with MCMV or SCMV and grown in the greenhouses at the KALRO-CIMMYT Naivasha MLN screening facility. The harvested seeds were tested for the presence of the two viruses prior to planting using Double Antibody Sandwich Enzyme Linked Immunosorbent Assay (DAS- ELISA) technique. Seeds used to determine soil transmission rates were obtained from virus-free plants grown in CIMMYT- Kiboko research center.

Table 3.1 List of inbred lines used to evaluate seed transmission rates of MCMV and SCMV.

MCMV inbred lines	Phenotype	SCMV inbred lines	Phenotype
CMCMV 011	Resistant	CSCMV01	Resistant
CMCMV 022	Resistant	CSCMV02	Resistant
CMCMV 033	Resistant	CSCMV03	Resistant
CMCMV 044	Resistant	CSCMV04	Resistant
CMCMV 055	Tolerant	CSCMV05	Tolerant
CMCMV 066	Tolerant	CSCMV06	Tolerant
CMCMV 077	Tolerant	CSCMV07	Tolerant
CMCMV 088	Tolerant	CSCMV08	Tolerant
CMCMV 099	Susceptible	CSCMV09	Susceptible
CMCMV 110	Susceptible	CSCMV010	Susceptible
CMCMV 111	Susceptible	CSCMV011	Susceptible
CMCMV 112	Susceptible	CSCMV012	Susceptible

Table 3.2 List of inbred lines used to evaluate soil transmission rates of MCMV and SCMV in maize plants.

MCMV inbred lines	Phenotype	SCMV inbred lines	Phenotype
CMCMV 044	Resistant	CMCMV 044	Resistant
CMCMV 022	Resistant	CSCMV04	Resistant
CMCMV 011	Resistant	CSCMV01	Resistant
CMCMV 01220	Resistant	CSCMV 400	Resistant
CMCMV 055	Tolerant	CSCMV07	Tolerant
CMCMV 066	Tolerant	CSCMV05	Tolerant
CMCMV 01224	Tolerant	CSCMV 405	Tolerant
CMCMV 01226	Tolerant	CSCMV 407	Tolerant
CMCMV 01229	Susceptible	CSCMV011	Susceptible
CMCMV 111	Susceptible	CSCMV 408	Susceptible
CSCMV01	Susceptible	CSCMV 409	Susceptible
CMCMV 01231	Susceptible	CSCMV 444	Susceptible

3.3.2.1 Detection of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in maize seeds

Detection of viruses from seeds was done in two ways; soaking the seeds in general extraction buffer (GEB) overnight and then testing for the viruses in the buffer, or seeds were crushed in the extraction buffer and the solution used for virus detection. In the first case, a bulk of 1000 seeds per line were randomly sampled, rinsed with wash buffer and soaked overnight in general extraction buffer (GEB) 20g/15ml. In the second case, 1000 seeds of each inbred line were crushed and five grams (5gms) of powder dissolved in 15ml of GEB, and 100µl of the sample solution used for virus detection.

Virus detection was done using DAS- ELISA. Plates were coated with specific antibodies and incubated for two hours, then washed with PBS-Tween to remove the unbound antisera and dried on tissue paper to remove all bubbles according to *DSMZ* protocol version 2.0, of the ELISA-Kit purchased in Germany- Brunswick (September, 2016). A 100µl aliquots extraction buffer from soaked or crushed seeds were loaded in duplicate wells and incubated overnight at 4ºC. Plates were then washed three times and conjugate enzyme (IgG AP, 1µl/ml) added into wells and incubated at 37ºC for two hours. Three washes followed by drying on tissue papers were done. Para-nitrophenyl-phosphate (PNP) tablets were dissolved in PNP buffer 1X at pH 9.8 (1mg/ml), loaded in duplicates into wells and incubated for 60 minutes at 37ºC. The color development and results were read using a spectrophotometer at OD_{405}nm, which determines the samples that tested positive or negative.

To determine whether the sample was considered to be negative or positive, the OD value of negative control was multiplied by two (NC*2), and all sample OD values above two times the negative control (NC) were considered to be positive, which implied that the sample was infected by the virus being tested for. Color change to yellow, indicated that tested samples were infected

(positive) while absence of color development indicated that collected samples were not infected (negative).

3.3.3 Determination of seed transmission rates

To determine the rates of seed transmission, seeds harvested from the twenty four inbred lines infected with MCMV or SCMV were planted in plastic containers measuring 24 cm at bottom and 34 cm above in diameter filled with sterile top soil mixed with manure and peat moss at a ratio of 3:1:1. The experiment was carried out in a completely randomized design, replicated three times. Four pots from each inbred line were used and twenty seeds were planted per pot. Plants were observed for symptoms development of a specific virus for a period of five weeks.

3.3.4 Determination of soil transmission rates

In order to determine the rate of soil transmission of the two viruses, seeds of 24 healthy inbred lines (referred to as ócleanseedsô) were planted in soil obtained from MCMV or SCMV greenhouses where susceptible artificially inoculated inbred lines were previously grown (referred to as óinfected soilô). The soil (with no debris) was collected and filled in plastic pots as described in section 3.3.3. Controls consisted of clean seeds and sterile soil. The experiment was laid out in a completely randomized design with three replications. Four pots per line were used and twenty seeds were sown in each pot. The experiment had two sub-components established according to the viral isolates (MCMV or SCMV) and insect-proof net, insect traps (yellow for thrips and blue for aphids) were installed between sets to prevent cross contamination.

3.3.5 Assessment of disease incidence and severity

Plants were assessed for incidence and severity of MCMV and SCMV for five weeks and data collected from week three to week five after planting. Incidence was determined by computing

the number of plants with typical and visible symptoms, out of the total plants observed in each set of the experiments.

$$\text{Disease incidence (DI)} = \frac{\text{Number of infected plants}}{\text{Number of plants assessed}} x100$$

Severity on the other hand was assessed by scoring plants using a scale of 1-9 (Suresh, unpublished data, 2017) where 1= No visible symptom, 2= Fine chlorotic spots on base of leaves, 3= Fine chlorotic streaks on lower leaves, 4= Fine chlorotic streaks on lower and upper leaves, 5= Chlorotic mottling through the whole plant, 6= Excessive chlorotic mottling through but no necrosis, 7= Excessive chlorotic mottling through plant and dead heart or necrosis symptoms, 8= Excessive chlorotic mottling through plant, plus excessive necrosis, 9= Complete plant necrosis.

3.3.6 Leaf sampling for virus assays

Asymptomatic and symptomatic second top and tender leaves were collected twice, on the third and fifth weeks after planting at 6 and 10 leaf stages, respectively. Twenty pieces ($2mm^2$) from each of the leaves were collected per pot and considered as one composite sample, and bulked in a ziplock sampling bag. Samples were immediately taken to the laboratory and kept in a -20ºC freezer before being tested for the presence or absence of the viruses using DAS-ELISA technique as described in Section 3.3.2.1.

3.4 Results

3.4.1 Seed testing for presence of viruses

Maize seeds in bulk of 1000 of each inbred line were harvested from SCMV and MCMV infected plants of twenty four inbred lines, which totaled 24,000 seeds comprising of 8000 resistant, 8000 tolerant and 8000 susceptible seeds of both viruses. *Maize chlorotic mottle virus* was detected in all seed samples of MCMV infected plants soaked or crushed (Table 3.3). However, no

Sugarcane mosaic virus was detected from any seed samples of SCMV infected plants soaked or crushed (Table 3.4).

Table 3.3 Detection of *Maize chlorotic mottle virus* in seeds of resistant, tolerant and susceptible lines to MCMV harvested from infected maize plants and soaked or crushed in general extraction buffer

		MCMV infected seeds in GEB buffer	
Inbred line	Phenotype	Soaked seeds	Crushed seeds
CMCMV 011	Resistant to MCMV	+	+
CMCMV 022	Resistant to MCMV	+	+
CMCMV 033	Resistant to MCMV	+	+
CMCMV 044	Resistant to MCMV	+	+
CMCMV 055	Tolerant to MCMV	+	+
CMCMV 066	Tolerant to MCMV	+	+
CMCMV 077	Tolerant to MCMV	+	+
CMCMV 088	Tolerant to MCMV	+	+
CMCMV 099	Susceptible to MCMV	+	+
CMCMV 110	Susceptible to MCMV	+	+
CMCMV 111	Susceptible to MCMV	+	+
CMCMV 112	Susceptible to MCMV	+	+

GEB: General extraction buffer, MCMV: *Maize chlorotic mottle virus*, +: Positive means sample was infected with the virus tested for, - : Negative means sample was not infected with the virus tested for, **CMCMV**: Cimmyt *Maize chlorotic mottle virus*, **CSCMV**: Cimmyt *Sugarcane mosaic virus*.

Table 3.4 Detection of *Sugarcane mosaic virus* in seeds of resistant, tolerant and susceptible lines to SCMV harvested from infected maize plants soaked or crushed in general extraction buffer

		SCMV infected seeds in GEB buffer	
Inbred line	Phenotype	Soaked seeds	Crushed seeds
CSCMV01	Resistant to SCMV	-	-
CSCMV02	Resistant to SCMV	-	-
CSCMV03	Resistant to SCMV	-	-
CSCMV04	Resistant to SCMV	-	-
CSCMV05	Tolerant to SCMV	-	-
CSCMV06	Tolerant to SCMV	-	-
CSCMV07	Tolerant to SCMV	-	-
CSCMV08	Tolerant to SCMV	-	-
CSCMV09	Susceptible to SCMV	-	-
CSCMV010	Susceptible to SCMV	-	-
CSCMV011	Susceptible to SCMV	-	-
CSCMV012	Susceptible to SCMV	-	-

GEB: General extraction buffer, SCMV: *Sugarcane mosaic virus,* +: Positive means sample was infected with the virus tested for, - : Negative means sample was not infected with the virus tested for. **CMCMV:** Cimmyt *Maize chlorotic mottle virus*, **CSCMV**: Cimmyt *Sugarcane mosaic virus.*

3.4.2 Incidence and severity of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* on maize plants that originated from infected seed

Observations of symptoms of MCMV and SCMV viruses transmitted through seed were done for a period of five weeks on 24 inbred lines evaluated. There were no typical symptom expressed which related to incidence or severity of any of the two viruses studied in all inbred lines evaluated (Tables 3.5 and 3.6).

Table 3.5 Incidence and severity of *Maize chlorotic mottle virus* in maize plants grown from seeds harvested from MCMV-infected plants and grown in virus-free soil

Inbred line	Phenotype	MCMV Incidence	MCMV Severity
Infected plants			
CMCMV 011	Resistant to MCMV	0	1
CMCMV 022	Resistant to MCMV	0	1
CMCMV 033	Resistant to MCMV	0	1
CMCMV 044	Resistant to MCMV	0	1
CMCMV 055	Tolerant to MCMV	0	1
CMCMV 066	Tolerant to MCMV	0	1
CMCMV 077	Tolerant to MCMV	0	1
CMCMV 088	Tolerant to MCMV	0	1
CMCMV 099	Susceptible to MCMV	0	1
CMCMV 110	Susceptible to MCMV	0	1
CMCMV 111	Susceptible to MCMV	0	1
CMCMV 112	Susceptible to MCMV	0	1
Clean plants			
CMCMV 113	Resistant to MCMV	0	1
CMCMV 114	Tolerant to MCMV	0	1
CMCMV 115	Susceptible to MCMV	0	1

CMCMV: Cimmyt *Maize chlorotic mottle virus*

Table 3.6 Incidence and severity of *Sugarcane mosaic virus* in maize plants grown from seeds harvested from SCMV-infected plants and planted in virus-free soil

Inbred line	Phenotype	SCMV Incidence	SCMV Severity
Infected plants			
CSCMV01	Resistant to SCMV	0	1
CSCMV02	Resistant to SCMV	0	1
CSCMV03	Resistant to SCMV	0	1
CSCMV04	Resistant to SCMV	0	1
CSCMV05	Tolerant to SCMV	0	1
CSCMV06	Tolerant to SCMV	0	1
CSCMV07	Tolerant to SCMV	0	1
CSCMV08	Tolerant to SCMV	0	1
CSCMV09	Susceptible to SCMV	0	1
CSCMV010	Susceptible to SCMV	0	1
CSCMV011	Susceptible to SCMV	0	1
CSCMV012	Susceptible to SCMV	0	1
Clean plants			
CSCMV013	Resistant to SCMV	0	1
CSCMV014	Tolerant to SCMV	0	1
CSCMV015	Susceptible to SCMV	0	1

CSCMV: Cimmyt *Sugarcane mosaic virus*

3.4.3 Incidence and severity of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* on maize plants that originated from plants grown on infected soil

Symptoms of MCMV and SCMV were observed on plants of the 24 inbred lines for a period of five weeks. No symptom of the two viruses was recorded in all inbred lines studied (Tables 3.7 and 3.8).

Table 3.7 Incidence and severity of *Maize chlorotic mottle virus* in plants grown from seeds harvested from virus-free plants and grown in soil obtained from MCMV-infected maize greenhouse

Inbred line	Phenotype	MCMV Incidence	MCMV Severity
Infected soil x Healthy lines			
CMCMV 044	Resistant to MCMV	0	1
CMCMV 022	Resistant to MCMV	0	1
CMCMV 011	Resistant to MCMV	0	1
CMCMV 01220	Resistant to MCMV	0	1
CMCMV 055	Tolerant to MCMV	0	1
CMCMV 066	Tolerant to MCMV	0	1
CMCMV 01224	Tolerant to MCMV	0	1
CMCMV 01226	Tolerant to MCMV	0	1
CMCMV 01229	Susceptible to MCMV	0	1
CMCMV 111	Susceptible to MCMV	0	1
CSCMV01	Susceptible to MCMV	0	1
CMCMV 01231	Susceptible to MCMV	0	1
Clean soil x Healthy Lines			
CMCMV 01232	Resistant to MCMV	0	1
CMCMV 01243	Tolerant to MCMV	0	1
CMCMV 01254	Susceptible to MCMV	0	1

CMCMV: Cimmyt *Maize chlorotic mottle virus*

Table 3.8 Incidence and severity of *Sugarcane mosaic virus* in plants grown from seeds harvested from virus-free plants and grown in soils obtained from SCMV-infected maize greenhouse

Inbred line	Phenotype	SCMV incidence	SCMV severity
Infected soil x Healthy lines			
CMCMV 044	Resistant to SCMV	0	1
CSCMV04	Resistant to SCMV	0	1
CSCMV01	Resistant to SCMV	0	1
CSCMV 400	Resistant to SCMV	0	1
CSCMV07	Tolerant to SCMV	0	1
CSCMV05	Tolerant to SCMV	0	1
CSCMV 405	Tolerant to SCMV	0	1
CSCMV 407	Tolerant to SCMV	0	1
CSCMV011	Susceptible to SCMV	0	1
CSCMV 408	Susceptible to SCMV	0	1
CSCMV 409	Susceptible to SCMV	0	1
CSCMV 444	Susceptible to SCMV	0	1
Clean soil x Healthy Lines			
CSCMV445	Resistant to SCMV	0	1
CSCMV446	Tolerant to SCMV	0	1
CSCMV447	Susceptible to SCMV	0	1

CSCMV: Cimmyt *Sugarcane mosaic virus*

3.4.4 Detection of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in maize leaf samples of plants grown from seeds harvested on infected plants

To confirm if the viruses were transmitted to the seedlings through the seeds, maize leaf samples were harvested from plants in the greenhouse and all the seven hundred and twenty (720) samples tested for the presence of the viruses. Each virus had 360 samples, each inbred line had 24 composite samples and each sample was made of 20 leaf pieces. DAS-ELISA tests were done using 24 composite samples which originated from 480 maize leaf samples.

Although no symptoms were observed on plants grown from infected seeds, one of twenty four **(**1/24) samples of the susceptible inbred line (CMCMV 111), had *Maize chlorotic mottle virus* transmitted at the rate of 4.166% (Table 3.9). However, ELISA results did not result in any sample testing positive for *Sugarcane mosaic virus* (Table 3.10).

Table 3.9 Detection of *Maize chlorotic mottle virus* in maize plants grown from seeds harvested from MCMV-infected maize

Inbred line	Phenotype	Number of samples tested	Samples positive to MCMV	% rate of infection
CMCMV 011	Resistant to MCMV	24	0/24	0
CMCMV 022	Resistant to MCMV	24	0/24	0
CMCMV 033	Resistant to MCMV	24	0/24	0
CMCMV 044	Resistant to MCMV	24	0/24	0
CMCMV 055	Tolerant to MCMV	24	0/24	0
CMCMV 066	Tolerant to MCMV	24	0/24	0
CMCMV 077	Tolerant to MCMV	24	0/24	0
CMCMV 088	Tolerant to MCMV	24	0/24	0
CMCMV 099	Susceptible to MCMV	24	0/24	0
CMCMV 110	Susceptible to MCMV	24	0/24	0
CMCMV 111	Susceptible to MCMV	24	1/24	4.17
CMCMV 112	Susceptible to MCMV	24	0/24	0
Healthy lines x Clean soil				
CMCMV 113	Resistant to MCMV	24	0/24	0
CMCMV 114	Tolerant to MCMV	24	0/24	0
CMCMV 115	Susceptible to MCMV	24	0/24	0

CMCMV: Cimmyt *Maize chlorotic mottle virus*, MCMV: *Maize chlorotic mottle virus*.

Table 3.10 Detection of *Sugarcane mosaic virus* in maize plants from seeds harvested from SCMV-infected plants

Inbred line	Phenotype	Number of samples tested	Number positive to SCMV	% rate of transmission
Infected +clean soil		360		
CSCMV01	Resistant to SCMV	24	0/24	0
CSCMV02	Resistant to SCMV	24	0/24	0
CSCMV03	Resistant to SCMV	24	0/24	0
CSCMV04	Resistant to SCMV	24	0/24	0
CSCMV05	Tolerant to SCMV	24	0/24	0
CSCMV06	Tolerant to SCMV	24	0/24	0
CSCMV07	Tolerant to SCMV	24	0/24	0
CSCMV08	Tolerant to SCMV	24	0/24	0
CSCMV09	Susceptible to SCMV	24	0/24	0
CSCMV010	Susceptible to SCMV	24	0/24	0
CSCMV011	Susceptible to SCMV	24	0/24	0
CSCMV012	Susceptible to SCMV	24	0/24	0
Healthy lines x clean soil				
CSCMV013	Resistant to SCMV	24	0/24	0
CSCMV014	Tolerant to SCMV	24	0/24	0
CSCMV015	Susceptible to SCMV	24	0/24	0

CSCMV: Cimmyt *Sugarcane mosaic virus*, SCMV: *Sugarcane mosaic virus.*

3.4.5 Detection of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in maize leaf samples of plants grown in soil collected from MCMV and SCMV-infected maize plants

Maize leaf samples were harvested in the greenhouse from seedlings grown on soil collected from pots where inoculated plants were grown. Seven hundred and twenty (720) leaf samples were collected, as described in section 3.3.6 and were brought to the laboratory to test for the presence of MCMV and SCMV. ELISA results indicated that one tolerant (CMCMV066) and one susceptible (CMCMV111) inbred lines had one sample testing positive to *Maize chlorotic mottle virus* each (Table 3.11). However, all the samples tested negative for SCMV (Table 3.12).

Table 3.11 ELISA detection of *Maize chlorotic mottle virus* in seedlings transmitted through contaminated soil.

Inbred line	Phenotype	Number of samples tested	Number of positive to MCMV	% rate of transmission
Infected soil x Healthy lines				
CMCMV 044	Resistant to MCMV	24	0/24	0
CMCMV 022	Resistant to MCMV	24	0/24	0
CMCMV 011	Resistant to MCMV	24	0/24	0
CMCMV 01220	Resistant to MCMV	24	0/24	0
CMCMV 055	Tolerant to MCMV	24	0/24	0
CMCMV 066	Tolerant to MCMV	24	1/24	4.17
CMCMV 01224	Tolerant to MCMV	24	0/24	0
CMCMV 01226	Tolerant to MCMV	24	0/24	0
CMCMV 01229	Susceptible to MCMV	24	0/24	0
CMCMV 111	Susceptible to MCMV	24	1/24	4.17
CSCMV01	Susceptible to MCMV	24	0/24	0
CMCMV 01231	Susceptible to MCMV	24	0/24	0
Clean soil x Healthy Lines				
CMCMV 01232	Resistant to MCMV	24	0/24	0
CMCMV 01243	Tolerant to MCMV	24	0/24	0
CMCMV 01254	Susceptible to MCMV	24	0/24	0

CMCMV: Cimmyt *Maize chlorotic mottle virus*, **I**: Infected, **H**: Healthy

Table 3.12 Detection of *Sugarcane mosaic virus* in seedlings grown in contaminated soil using DAS-ELISA

Inbred line	Phenotype	Number of samples tested	Number positive to SCMV	% rate of transmission
Infected soil x Healthy lines				
CMCMV 044	Resistant to SCMV	24	0/24	0
CSCMV04	Resistant to SCMV	24	0/24	0
CSCMV01	Resistant to SCMV	24	0/24	0
CSCMV 400	Resistant to SCMV	24	0/24	0
CSCMV07	Tolerant to SCMV	24	0/24	0
CSCMV05	Tolerant to SCMV	24	0/24	0
CSCMV 405	Tolerant to SCMV	24	0/24	0
CSCMV 407	Tolerant to SCMV	24	0/24	0
CSCMV011	Susceptible to SCMV	24	0/24	0
CSCMV 408	Susceptible to SCMV	24	0/24	0
CSCMV 409	Susceptible to SCMV	24	0/24	0
CSCMV 444	Susceptible to SCMV	24	0/24	0
Clean soil x Healthy Lines				
CSCMV445	Resistant to SCMV	24	0/24	0
CSCMV446	Tolerant to SCMV	24	0/24	0
CSCMV447	Susceptible to SCMV	24	0/24	0

CSCMV: Cimmyt *sugarcane mosaic virus*

3.5 Discussion

To determine the transmission rates of MCMV and SCMV through seed and soil, pre-test results of infected seeds used in this study detected *Maize chlorotic mottle virus* in seed samples, which result confirmed that the seeds were infected, but *Sugarcane mosaic virus* was not detected. Lack of symptom expression on plants grown from infected seeds and those grown on contaminated soil might be due to low titer of viral particles, which were not translocated to other parts of the plants. It is not clear how plants that originated from infected seeds were not able to express symptoms. DAS-ELISA test, which was used in this study to detect the presence of MCMV and SCMV, had revealed that MCMV was seed and soil transmitted at low rates in susceptible and tolerant inbred lines, while *Sugarcane mosaic virus* could not be detected in all plant leaf samples

tested. These results support prior reports which indicated that MCMV was transmitted through maize seed (Jensen *et al.,* 1991; Li Li *et al.*, 2007; Sastry, 2013; Cabanas *et al.,* 2013). The results agreed with Mahuku *et al.* (2015), though the rate of transmission of MCMV in soil that he reported was very high.

Lack of SCMV in samples implied that the virus could probably not be seed and soil transmitted. These results complement work by Mink (1993), who suggested removing SCMV from the list of seed transmitted viruses. However, these findings contradict reports by Zitter (2001) who reported SCMV or MDMV to be seed transmitted in dent corn and more of seed transmission was reported on MDMV strains than SCMV strains (Chaves *et al.,* 2012). *Maize dwarf mosaic virus* races were used interchangeably as SCMV in most early reports because of similarity in biochemical properties (Chaves and Giovanni, 2012). Some examples include Spanish isolates which shared 85% of nucleotides identity (Achon *et al.,* 2007) and China isolates that shared 98.6 and 97.6% similarity (Zhong *et al.,* 2005). Available information on *Sugarcane mosaic virus* transmission through soil in sweet corn, sorghum and Johnson grass were reported to be through mechanical inoculation of roots (Ford and Moline, 1974), which differed from the method used in this study where no mechanical inoculation was used. No differences were observed between inbred lines in the field, although ELISA results indicated that susceptible and tolerant inbred lines may transmit MCMV at low rates.

Though this study indicates MCMV to be seed and soil transmitted at low rates, these rates are sufficient to serve as initial inoculum for the virus to spread and become a problem to farmers in the presence of vectors and any other transmission methods. Famers should therefore be aware about the threat in seed usage. Further studies should be conducted to determine the localization of MCMV virus in seed to rule out the possibility of seed transmission or if, it is just a contaminant on the seed coat.

CHAPTER FOUR

COMBINED EFFECT OF MECHANICAL AND SEED TRANSMISSION OF VIRUSES CAUSING MAIZE LETHAL NECROSIS ON DISEASE DEVELOPMENT

4.1 Abstract

Synergistic interaction of plant viruses have been reported since the beginning of the 19th century. Interaction of viruses causing Maize lethal necrosis (MLN) disease in Eastern Africa have been reported to occur between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). The two viruses are reported to be transmitted in different ways including vectors, through seed, and by mechanical means. This study investigated the combined effect of seed and mechanical transmission of the two viruses on nine maize genotypes. Treatments consisted of plants grown from seeds obtained from healthy plants (referred to as ócleanô seeds) which were not inoculated with any virus, plants from clean seeds inoculated with both viruses, and plants grown from seeds obtained from infected plants and inoculated with one virus first and later with the other virus. Incidence, severity and ELISA detection of MCMV and SCMV were recorded. Results indicated that all combinations of artificial inoculations with MCMV or SCMV resulted in development of typical symptoms associated with either of the viruses, respectively. Resistant genotypes resulted in low rates of infections, particularly where MCMV was artificially inoculated. No symptoms were observed on plants grown form clean seeds and plants from infected seeds but not inoculated with either of the viruses. MCMV was detected in all genotypes inoculated with MCMV or both viruses. There was no detection of SCMV or MCMV in non-inoculated plants, even when they were grown from seeds obtained from infected plants. These results indicate that mechanical transmission (and probably any form of secondary infection such as by vectors) of viruses causing MLN plays a significant role in disease development. While

mechanical transmission of viruses may be rare in natural conditions, the findings are helpful to farmers in that prevention of secondary infection in the field may help in reducing MLN incidence and severity. Further studies need to be conducted to understand the reason behind the lack of virus infection and symptom development in plants from infected seeds.

4.2 Introduction

Wheat, maize and rice account for 94% of cereals consumed world over, although the southern and eastern parts of Africa prefer maize as staple food (Ranum *et al.*, 2014). The emergence of maize lethal necrosis (MLN) disease in maize has affected livelihoods of farmers who depend on maize products in the region (Wangai *et al*., 2012; Mahuku *et al*., 2015). Maize loss due to MLN disease was estimated at 0.5 million metric tons in 2015 in Kenya (Hugo, 2015). Maize lethal necrosis disease is a result of synergetic interaction of two viruses, *Maize chlorotic mottle virus* (MCMV), a machlomovirus (principal virus) and *Sugarcane mosaic virus* (SCMV), a potyvirus (Gowda *et al*., 2015). Each of the two viruses can also cause significant quality and yield losses separately (Zhu *et al*., 2014; Yu *et al*., 2014).

Maize chlorotic mottle virus is reported to be transmitted by chrysomelid beetles and corn rootworms (Nault *et al*., 1978; Uyemoto, 1983; Jensen, 1985). Thrips, aphids, leaf hoppers, whiteflies and scarab beetles have also been associated with the transmission of MCMV in maize (Jiang *et al*., 1992; Nelson *et al*., 2011; Mahuku *et al*., 2015). *Maize chlorotic mottle virus* was also reported to be transmitted by seed and through soil (Jensen *et al*., 1991; Mahuku *et al*., 2015). On the other hand, two types of aphids have been associated with the transmission of SCMV, *Rhopalosiphum maidis* and *Schizaphis graminum rond* (Mansoor *et al*., 2003; William, 2015). Seed transmission of SCMV has also been reported (Li Li *et al*., 2007).

Seed transmission of MCMV and SCMV is reported to occur at a very low rate (Nelson *et al*., 2011), resulting in minor symptoms being exhibited in infected plants. On the other hand, artificial (mechanical) and vector inoculations result in higher symptom expression and the induced symptoms leads to loss of yield and quality (Redinbaugh *et al*., 2001; Sastry, 2013; Bulegeya, 2016). Mechanical transmission in nature is rare but may happen by contact of infected materials with healthy ones, by rubbing, or using any tool that can injure a plant and create the entry point for the virus, especially when weeding (Sastry, 2013). Majority of maize farmers in the region use simple tools such as jembes, pangas or animal drawn ploughs for weeding, which may result in injury of roots. Such injuries may result in transmission of viruses if present in the soil. Vectors for the viruses are also present throughout the year. This study aimed at evaluating the effect of both seed and mechanical transmission of viruses causing MLN on disease development.

4.3 Materials and Methods

4.3.1 Planting materials and experimental setup

Seeds from maize plants infected with MCMV, SCMV and with a combination of both viruses were harvested from artificially inoculated maize plants grown in greenhouses at the KALRO-CIMMYT Naivasha MLN screening facility (described in Section 3.3.1) and were tested for the presence of the two viruses prior to planting using DAS-ELISA as described in Section 3.3.2.1, while clean seeds were obtained from CIMMYT- Kiboko research center.

Nine genotypes which were grouped as susceptible, tolerant or resistant to MCMV, SCMV or MLN (Table 4.1) were used in this study. The experiment consisted of nine treatments as described in Table 4.2. All genotypes were grown on soil collected from the forest and were artificially inoculated at six and ten leaf stages and evaluated for the effect of both seed and mechanical transmission. Completely randomized design (CRD) was used with four replications.

Each replication consisted of one pot with four plants. Genotypes infected with one virus through the seed were inoculated twice with the other virus 14 and 21 days after planting (DAP). Treatments consisting of plants from clean seeds were inoculated twice with each virus, the first inoculation with one virus being done 14 and 21 DAP, and with the second virus 28 and 35 DAP. For MLN infected seeds, inoculations were done at the same time for both viruses 14 and 21 DAP.

Table 4.1 List of hybrids and inbred lines used in the mechanical and seed transmission experiment. Seeds were obtained from healthy plants in CIMMYT-Kiboko research center and Karlo- CIMMYT Naivasha.

Hybrids/Inbred lines	**Phenotypes**
CMLN500	Resistant hybrids to MLN
CMLN601	Tolerant hybrids to MLN
CMLN702	Susceptible hybrids to MLN
CMCMV011	Resistant inbred line to MCMV
CMCMV088	Tolerant inbred line to MCMV
CMCMV099	Susceptible inbred line to MCMV
CMCMV044	Resistant inbred line to SCMV
CSCMV07	Tolerant inbred line to SCMV
CMCMV099	Susceptible inbred line to SCMV

Table 4.2 Treatments used to study the effect of seed and mechanical transmission of viruses causing Maize lethal necrosis disease in maize and their abbreviations

NO	Treatments	Abbreviations
1	Clean seeds non-inoculated	C.S non-inoculated
2	Clean seeds inoculated with *Maize chlorotic mottle virus* first and *Sugarcane mosaic virus* later	C.S+MCMV+SCMV
3	*Maize chlorotic mottle virus* infected seeds non-inoculated	MCMV I.S ONLY
4	*Maize chlorotic mottle virus* infected seeds artificially inoculated with *Sugarcane mosaic virus*	MCMV I.S+SCMV
5	Clean seeds inoculated with *Sugarcane mosaic virus* first and *Maize chlorotic mottle virus* later	C.S+ SCMV+MCMV
6	*Maize chlorotic mottle virus* infected seeds non-inoculated	SCMV I.S ONLY
7	*Sugarcane mosaic virus* infected seeds artificially inoculated with *Maize chlorotic mottle virus*	SCMV I.S+ MCMV
8	Maize lethal necrosis infected seeds non-inoculated	MLN I.S ONLY
9	Maize lethal necrosis infected seeds artificially inoculated with *Sugarcane mosaic virus* combined with *Maize chlorotic mottle virus*	MLN I.S+ MLN

4.3.2 Inoculum preparation and plant inoculation

Infected leaves were collected from pure virus isolate-infected plants in respective greenhouses, chopped into small pieces or discs, and measured to a ratio of 1:10 (leaf tissue to buffer) of either MCMV or SCMV, respectively. The ratio of MCMV and SCMV leaf materials used to prepare MLN inoculum was 1:4 for MCMV: SCMV. Bainô s inoculation method (Singh *et al*., 2005) was used where upper leaves were rubbed with forefingers using celite (0.02g/ml) to enhance abrasion on leaf surface and allow the virus to enter the leaf tissues easily. All inoculated plants were washed under running water for 10 minutes to avoid any mechanical injury to the plant.

4.3.3 Evaluation of disease incidence and severity

Disease incidence was assessed four times in eight (8) weeks at two week intervals. Emerged seedlings were evaluated for incidence and severity of MCMV, SCMV and MLN as described in section 3.3.5. Levels of disease damage was assessed by computing scores into area under disease progress curve (AUDPC) (Simko and Piepho, 2012; Sitta *et al*., 2017).

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{X_i + X_{i+1}}{2}\right](t_{t+1} - t_i)$$

Where n = Total number of plants assessed, x_i=injury intensity at the *i*th observation and t= time at the *i*th observation.

4.3.4 Leaf sample collection and detection of viruses

Asymptomatic and symptomatic leaf tissues were collected from second leaf from the top of maize plants after the fifth to eighth week from planting. Composite samples of four pieces of leaves were collected in each pot and bulked in a ziplock sampling bag. In total, 264 samples were collected and immediately taken to a laboratory for DAS-ELISA test as described in sections 3.3.2.1 and 3.3.6.

4.3.5 Statistical analysis

All data including AUDPC values were statistically analyzed using GenStat software 14th edition (2014). Means were separated using Fischerôs protected least significance difference (LSD) at 5%.

4.4 Results

4.4.1 Detection of MLN viruses in maize genotypes

Maize seeds collected from kernels of nine genotypes infected with MLN-causing viruses (three hybrids with different reactions to MLN and six inbred lines with different reactions to MCMV or

SCMV) were tested for MCMV or SCMV using DAS-ELISA. *Maize chlorotic mottle virus* was present in all the three hybrids (Table 4.3), while SCMV was not detected in GEB buffer of both soaked and crushed seeds.

Table 4.3 Detection of MCMV and SCMV in seeds harvested from MLN-infected hybrid and MCMV or SCMV-infected inbred lines, soaked or crushed in GEB buffer

		MCMV		SCMV	
Genotype	Phenotype	Seeds soaked in GEB	Seed crushed in GEB	Seeds soaked in GEB	Seeds crushed in GEB
CMLN500	MLN resistant	+	+	ī	ī
CMLN601	MLN tolerant	+	+	ī	ī
CMLN702	MLN susceptible	+	+	ī	ī
CMCMV011	MCMV resistant	+	+	NA	NA
CMCMV088	MCMV tolerant	+	+	NA	NA
CMCMV099	MCMV susceptible	+	+	NA	NA
CMCMV044	SCMV resistant	NA	NA	ī	ī
CSCMV07	SCMV tolerant	NA	NA	ī	ī
CMCMV099	SCMV susceptible	NA	NA	ī	ī

CMLN: Cimmyt Maize Lethal Necrosis, code of inbred lines and hybrids used in this trial, **CMCMV**: Cimmyt *Maize chlorotic mottle virus,* **CSCMV**: Cimmyt *Sugarcane mosaic virus,* **MCMV:** *Maize chlorotic mottle virus***, SCMV**: *Sugarcane Mosaic Virus*, **GEB**: General Extraction Buffer, **+**: indicates that samples were positive to virus being tested, **-**: indicates that sample were negative to virus being tested, NA: Not Applicable as only one virus was tested at each specific level.

4.4.2 Disease incidence and severity assessment

4.4.2.1 Response of maize genotypes to SCMV inoculation on plants grown from MCMV-infected seeds and clean plants inoculated with MCMV first and SCMV later

Plants grown from seeds obtained from healthy maize and plants grown from seeds obtained from MCMV-infected maize plants but not inoculated with SCMV did not exhibit any observable symptoms (Plate 4.1-AIP; Table 4.4). Maize plants mechanically inoculated with MCMV or both viruses were all severely affected with disease incidence of 100% and a severity score of 2 and above at 28dpi (Plate 4.1 MC-SIP). The expression of symptoms in plants grown from MCMV infected seeds artificially inoculated with SCMV started earlier than symptoms of MCMV inoculated in plants grown from SCMV infected seeds. Symptoms started as small fine chlorotic spots in some plants, clear and distinctive symptoms were observed 14dpi in all plants inoculated and had expanded to the entire leaf area of susceptible and tolerant lines. However, plants obtained from MCMV-infected seeds of resistant and tolerant inbred lines had not expressed any symptoms by 14th dpi, even when inoculated with SCMV (Table 4.4). All plants where both viruses were present, recorded AUDPC of over 100, with the highest being obtained in MCMV-susceptible inbred lines.

Plate 4.1 Uninoculated infected maize inbred line tolerant to *Maize chlorotic mottle virus* without symptoms (MC-AIP) and tolerant plants grown from MCMV infected seeds, artificially inoculated with SCMV showing excessive chlorotic mottling and necrosis (MC-SIP), **MC-AIP**: *Maize chlorotic mottle virus*-asymptomatic infected plants, MC-SIP: *Maize chlorotic mottle virus*- symptomatic-infected plants.

Table 4.4 Incidence and severity of SCMV and MCMV inoculated on MCMV-infected plants and clean plants inoculated with both viruses

		Mean incidence (%) at different dpi				Mean severity at different dpi				
Phenotype	Treatments	14	29	44	59	14	29	44	59	AUDPC
Resistant	C.S non-inoculated	0	0	0	0	1	1^{e}	1^{d}	1^{d}	44^{f}
to MCMV	C.S+MCMV+SCMV	100	100	100	100	3	4^{b}	4^{b}	5.75^{a}	182^{c}
(CMCMV011)	MCMV I.S	0	0	0	0	1	1^{e}	1^{d}	1^{d}	44^{f}
	MCMV I.S+SCMV	0	100	100	100	1	2^{d}	3c	4.5^{c}	115^{e}
Tolerant	C.S non-inoculated	0	0	0	0	1	1^{e}	1^{d}	1^{d}	44^{f}
to MCMV	C.S+MCMV+SCMV	100	100	100	100	3	4.5^{a}	4.75^{a}	5.25^{ab}	115^{e}
(CMCMV 088)	MCMV I.S	0	0	0	0	1	1^{e}	1^{d}	1^{d}	44^{f}
	MCMV I.S+SCMV	0	100	100	100	1	3.5^{c}	4^{b}	5.25^{ab}	157^{d}
Susceptible	C.S non-inoculated	0	0	0	0	1	1*e*	1^{d}	1^{d}	44^{f}
to MCMV	C.S+MCMV+SCMV	100	100	100	100	3	4^{b}	5^{a}	5^{bc}	192^{bc}
(CMCMV099)	MCMV I.S	0	0	0	0	1	1^{e}	1^{d}	1^{d}	44^{f}
	MCMV I.S+SCMV	100	100	100	100	4	4.25^{ab}	5^{a}	5.75^{a}	208^{a}
	$LSD_{(0.05)}$	*	*	*	*	*	0.40	0.40	0.50	13.48
	P-value	*	*	*	*	*	<.001	<.001	<.001	<.001

Key symbols: CMCMV: Cimmyt *Maize chlorotic mottle virus*, **MCMV:** *Maize chlorotic mottle virus*, **SCMV**: *Sugarcane mosaic virus*, **dpi**: Days post-inoculation, **IS**: Infected seed, **C.S: Clean** seed,*: Data not generated with ANOVA.

4.4.2.2 Response of maize genotypes to MCMV inoculation on plants grown from SCMV-infected seeds and clean plants inoculated with SCMV first and MCMV later

Plants grown from seeds obtained from healthy maize and plants grown from SCMV-infected seeds but not inoculated with MCMV did not exhibit any observable symptoms (Plate 4.2 SC-AIP; Table 4.5). Plants grown from seeds obtained from healthy SCMV-resistant genotypes and inoculated with SCMV first and MCMV later had significantly lower disease incidence and severity than all other plants grown from SCMV-infected seeds and inoculated with MCMV (Plate 4.2 SC-SIP). Similar observations were recorded even in AUDPC.

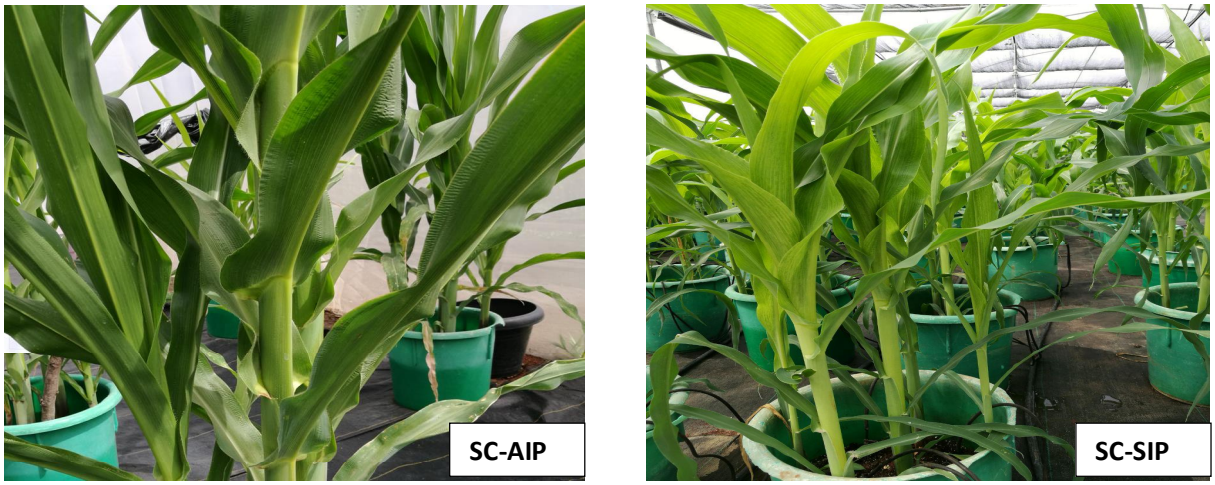

Plate 4.2 Plants grown from seeds obtained from SCMV-infected but tolerant inbred lines but not inoculated with any virus showing no symptom (SC-AIP) and plants from SCMV-infected seeds inoculated with MCMV exhibiting chlorotic mottling through the whole plant (SC-IP), **SC-AIP**: *Sugarcane mosaic virus*- asymptomatic infected plants, **SC-SIP**: *Sugarcane mosaic virus*-symptomatic infected plants.

Table 4.5 Incidence and severity of MCMV and SCMV on SCMV infected plants and clean plants inoculated with both viruses

		Mean incidence in dpi (%)				Mean severity in dpi				
Phenotype	Treatments	14	29	44	59	14	29	44	59	AUDPC
Resistant to SCMV	C.S non-inoculated	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	C.S+SCMV+MCMV	0	50^{b}	62.5^{b}	93.75^{b}	1^{b}	2.5^{c}	2.5^{e}	3.75^{c}	109^{d}
(CMCMV 044)	SCMV I.S	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	SCMV I.S+MCMV	0	87.5^{a}	93.75^{a}	100^{a}	1^{b}	3.5^{b}	3.75^{d}	4.75^{b}	177^{b}
Tolerant to SCMV	C.S non-inoculated	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	C.S+SCMV+MCMV	100	100^{a}	100^{a}	100^{a}	3^{a}	4^{ab}	4.5^{bc}	5^{b}	184^{b}
(CSCMV07)	SCMV I.S	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	SCMV I.S+MCMV	100	100^{a}	100^{a}	100^{a}	3^{a}	4^{ab}	4^{cd}	5^{b}	150^{c}
Susceptible to SCMV	C.S non-inoculated	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	C.S+SCMV+MCMV	100	100^{a}	100^{a}	100^{a}	2.75^{a}	4.5^{a}	5.25^{a}	5.75^{a}	207^{a}
(CMCMV099)	SCMV I.S	0	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{f}	1^{d}	44^{e}
	SCMV I.S+MCMV	100	100^{a}	100^{a}	100^{a}	3^{a}	4^{ab}	4.75^{ab}	5.75^{a}	179.4^{b}
	LSD(0.05)	*	15.81	18.66	5.17	0.27	0.57	0.72	0.57	20.38
	P-value	*	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Key symbols: Means followed by the same letters along columns are not significantly different (P= 0.05), **CMCMV**: Cimmyt Maize Chlorotic Mottle Virus, **CSCMV**: Cimmyt Sugarcane Mosaic Virus, **MCMV:** *Maize chlorotic mottle virus*, **SCMV**: *Sugarcane mosaic virus*, **IS**: Infected seed, **DPI**: Days post-inoculation, **C.S**: Clean seed. *: Data not available with ANOVA test.

4.4.2.3 Incidence and severity of MCMV+SCMV inoculated to plants grown from infected with both MLN causing viruses

Plants grown from seeds harvested from healthy maize and plants grown from seeds harvested from MLN-infected maize plants but not inoculated with any viruses did not express any typical symptoms (Plate 4.3 ML-AIP; Table 4.6). For the combination of both viruses inoculated at the same time, all plants were infected and some susceptible plants were heavily infected, while others died completely after 29dpi (Plate 4.3 ML-SIP). All artificially inoculated plants were infected, but resistant hybrids had lower incidence and severity scores (Table 4.6).

Plate 4.3 Maize hybrids tolerant to Maize lethal necrosis (MLN) grown from seeds obtained of MLN-infected plants but not mechanically inoculated with any virus and showing no symptoms (ML-AIP), and infected maize hybrids artificially inoculated with MCMV+ SCMV, expressing excessive chlorotic mottling (ML-SIP); **ML-AIP**: Maize lethal necrosis ï asymptomatic- infected plants, **ML-SIP**: Maize lethal necrosis- symptomatic-infected plants.

Table 4.6 Incidence and severity of MLN in plants inoculated both viruses (MCMV+SCMV) at the same time on MLN infected plants

		Mean incidence in dpi (%)				Mean severity in dpi				
Phenotype	Treatments	14	29	44	59	14	29	44	59	AUDPC
Resistant to MLN	C.S non-inoculated	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN I.S only	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN I.S+MLN	0^{c}	93.75^{b}	93.75^{b}	93.75^{b}	1^{b}	2.75^{c}	3.75^{b}	5.25^{b}	143^{c}
Tolerant to MLN	C.S non-inoculated	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN I.S only	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN IS+MLN	75^{b}	100^{a}	100^{a}	100^{a}	2.5^{a}	3.75^{b}	5^{a}	5.25^{b}	186.25^{b}
Susceptible to MLN	C.S non-inoculated	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN I.S only	0^{c}	0^{c}	0^{c}	0^{c}	1^{b}	1^{d}	1^{c}	1^{c}	44^{d}
	MLN IS+MLN	100^{a}	100^{a}	100^{a}	100^{a}	2.75^{a}	4.5^{a}	5.25^{a}	6.5^{a}	212^{a}
	LSD(0.05)	13.96	6.04	6.04	6.04	0.37	0.44	0.52	0.59	14.40
	P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Key symbols: Means followed by the same letters along columns are not significantly different (P= 0.05), **MLN**: Maize Lethal Necrosis, **dpi**: Days post-inoculation (14-59), **IS**: Infected seed**, C.S**: Clean seed**.**

4.4.3 Detection of MCMV and SCMV viruses using DAS - ELISA

4.4.3.1 Detection of MCMV and SCMV in plants grown from MCMV-infected seeds inoculated with SCMV and clean plants inoculated with MCMV first and SCMV later

DAS - ELISA results indicated the presence of MCMV in all samples at 100% of seeds grown from MCMV infected plants artificially inoculated with SCMV (Table 4.7), while SCMV was found in 12 samples out of 24. Plant grown from clean seeds obtained from MCMV-resistant, tolerant and susceptible inbred lines artificially inoculated with MCMV first and SCMV later indicated the presence MCMV in all samples tested, while SCMV was detected in 5 samples out of 24. No virus was detected in all samples obtained plants of seeds infected with MCMV and samples of plants grown from certified seeds non-inoculated. ELISA results of samples from MCMV infected plants inoculated with SCMV and clean plants inoculated with MCMV first and SCMV later indicated MCMV to be positive in all samples, while SCMV was detected in some samples.

4.4.3.2 Detection of MCMV and SCMV in leaf samples from SCMV-infected seeds inoculated with MCMV and samples from clean seeds inoculated with SCMV first and MCMV later

Maize chlorotic mottle virus was detected in all leaf samples (24) collected from SCMV infected seeds inoculated with MCMV, while *Sugarcane mosaic virus* was not detected (Table 4.8). Plant leaves from certified seeds artificially inoculated with SCMV first and MCMV later tested positive for MCMV with 19/24 and SCMV with 15/24. None of the two viruses were found in un-inoculated samples of SCMV infected plants and clean plants not inoculated with either of the viruses. ELISA results of SCMV inoculated first and MCMV later indicated MCMV to be present in all samples, while SCMV was detected in some samples.

Table 4.7 Detection of MCMV and SCMV viruses in plants grown from seeds of MCMVinfected plants inoculated with SCMV and clean plants inoculated with MCMV first and SCMV later

			MCMV		SCMV	
Phonotype	Genotype	Type of Inoculation	Positive	%	Positive	%
MCMV011	Resistant to MCMV	C.S Only	0	0	0	0
CMCMV088	Tolerant to MCMV	C.S Only	0	0	0	0
CMCMV099	Susceptible to MCMV	C.S Only	0	0	0	0
CMCMV011	Resistant to MCMV	C.S+MCMV+SCMV	8	100	1	12.5
CMCMV088	Tolerant to MCMV	C.S+MCMV+SCMV	8	100	2	25
CMCMV099	Susceptible to MCMV	C.S+MCMV+SCMV	8	100	2	25
CMCMV011	Resistant to MCMV	MCMV I.S	0	0	0	0
CMCMV088	Tolerant to MCMV	MCMV I.S	0	0	0	0
CMCMV099	Susceptible to MCMV	MCMV I.S	0	0	0	0
CMCMV011	Resistant to MCMV	MCMV I.S+SCMV	8	100	4	50
CMCMV088	Tolerant to MCMV	MCMV I.S+SCMV	8	100	2	25
CMCMV099	Susceptible to MCMV	MCMV I.S+ SCMV	8	100	6	75

Key symbols: CMCMV: Cimmyt *Maize chlorotic mottle virus,* **MCMV:** *Maize chlorotic mottle virus***, SCMV**: *Sugarcane mosaic virus,* **IS**: Infected seed, **C.S**: Clean seed.

Table 4.8 Detection of SCMV and MCMV in plants grown from seeds of SCMV-infected plants inoculated with MCMV and clean plants inoculated with SCMV first and MCMV later

			MCMV		SCMV	
Genotypes	Phenotype	Type of Inoculation	Positive	%	Positive	%
CMCMV044	Resistant to SCMV	C.S Only	0	0	0	0
CSCMV07	Tolerant to SCMV	C.S Only	0	0	0	0
CMCMV099	Susceptible to SCMV	C.S Only	0	0	0	0
CMCMV044	Resistant to SCMV	C.S+SCMV+MCMV	5	62.5	3	37.5
CSCMV07	Tolerant to SCMV	C.S+SCMV+MCMV	7	87.7	6	75
CMCMV099	Susceptible to SCMV	C.S+SCMV+MCMV	7	87.5	6	75
CMCMV044	Resistant to SCMV	SCMV I.S	0	0	0	0
CSCMV07	Tolerant to SCMV	SCMV I.S	0	0	0	0
CMCMV099	Susceptible to SCMV	SCMV I.S	0	0	0	0
CMCMV044	Resistant to SCMV	SCMV I.S+MCMV	8	100	0	0
CSCMV07	Tolerant to SCMV	SCMV I.S+MCMV	8	100	0	0
CMCMV099	Susceptible to SCMV	SCMV I.S+MCMV	8	100	0	0

Key symbols: CMCMV: Cimmyt *Maize chlorotic mottle virus,* **CSCMV**: Cimmyt *Sugarcane mosaic virus,* **MCMV:** *Maize chlorotic mottle virus***, SCMV**: *Sugarcane mosaic virus* **IS**: Infected seed, **C.S**: Clean seed.

4.4.4 Detection of MLN viruses from leaves of plants grown from seeds infected plants of MLN inoculated with both viruses

Maize chlorotic mottle virus was present in all samples that originated from MLN infected seeds inoculated with both viruses, but no SCMV was detected (Table 4.9). No MCMV or SCMV was detected in all samples that originated from certified seeds and MLN infected seeds non-inoculated.

Table 4.9 Detection of MCMV and SCMV viruses on leaf samples of plants grown from seeds of MLN infected plants inoculated with MCMV+SCMV the same time.

			MCMV		SCMV	
Genotypes	Phenotype	Type of Inoculation	Positive	%	Positive	%
CMLN500	Resistant to MLN	C.S Only	0	0	0	0
CMLN601	Tolerant to MLN	C.S Only	0	0	0	0
CMLN702	Susceptible to MLN	C.S Only	0	0	0	0
CMLN500	Resistant to MLN	MLN I.S	0	0	0	0
CMLN601	Tolerant to MLN	MLN I.S	0	0	0	0
CMLN702	Susceptible to MLN	MLN I.S	0	0	0	0
CMLN500	Resistant to MLN	MLN I.S+MLN	8	100	0	0
CMLN601	Tolerant to MLN	MLN I.S+MLN	8	100	0	0
CMLN702	Susceptible to MLN	MLN I.S+MLN	8	100	0	0

Key symbols: CMLN: Cimmyt Maize Lethal Necrosis, **MCMV:** *Maize chlorotic mottle virus*, **SCMV**: *Sugarcane mosaic virus*, **IS**: Infected seed, **C.S**: Clean seed.

4.5 Discussion

Maize lethal necrosis (MLN) disease is one of the major threats to maize production in the region, yet the mechanism behind the spread of causal agents has not been fully elucidated. The aim of this study was to determine the combined effect of seed and mechanical transmission of the viruses (MCMV and SCMV) causing MLN in disease development. Maize seeds harvested from infected plants tested MCMV-positive and SCMV-negative, which shows the potential of

MCMV to be seed transmitted. Evaluated infected hybrids and inbred lines which were not artificially inoculated with either of the viruses did not show any symptoms and ELISA tested negative for both viruses. Lack of symptom expression could imply the failure of the virus to multiply in the seed. This indicates that seed alone without other disseminating agents has very low probability to transmit the virus.

Plants grown from seeds obtained from MCMV-infected plants and inoculated with SCMV, and clean plants inoculated with MCMV first and SCMV later developed typical MLN symptoms, with susceptible inbred lines showing the highest incidence and severity. This agrees with Mbega *et al*. (2016) who reported on the synergistic effect of the potyvirus (SCMV), which enhances its own protein replication as well as that of the partner virus (MCMV). In the interaction with MCMV, SCMV was reported to increase MCMV titer up to 5 times and also enhanced cell-to-cell movement (Wang *et al*., 2017). This could be through the initiation of the helper gene or a gene for nuclear inclusion proteins which were reported to silence the mechanism of the host and allow MCMV to replicate and translocate in all plants tissues (Mbega *et al*., 2016). Five of six open reading frames (ORF) of MCMV are ready to help the virus to replicate and translocate in the plants through long distance movement (Scheets, 2000).

In co-infection of SCMV first and MCMV later, only SCMV symptom could be visible with light green mosaic and some yellowish color along the veins. In the interaction where SCMV was inoculated first, virus expression was low compared to the interaction where MCMV was first. Despite exhibition of SCMV symptom, serological tests with DAS-ELISA revealed that only MCMV could be detected from SCMV infected plants inoculated with MCMV, suggesting that SCMV might not be seed transmitted. *Sugarcane mosaic virus* genome may contain genes or proteins which enhance its expression. *Sugarcane mosaic virus* is also known to be active in

young tissues, resulting in changes of chlorophyll and mosaic patterns in leaves (Addy *et al.*, 2017).

Interaction of both viruses inoculated at the same time in plants grown from MLN-infected seeds started to exhibit symptoms later in resistant compared to susceptible and tolerant hybrids. Susceptible infected hybrids inoculated with both viruses were heavily infected and had the highest severity scores, implying that the viruses had overcome the resistance of the variety. *Sugarcane mosaic virus* infected plants inoculated with MCMV had all samples testing MCMV positive while SCMV tested negative with DAS- ELISA. This could support the previous findings of Mink (1993) that SCMV is not seed transmitted. The same pattern was recorded in treatments where the two viruses were inoculated at the same time, indicating all samples to be MCMV positive. The failure to detect SCMV in all MLN samples artificially inoculated at the same time remains unclear. Since SCMV was found to be present in samples of MCMV+SCMV and SCMV+MCMV treatments, this could not be attributed to the variation between the antisera and the strain of the virus used in this study.

The findings of this study are of significant importance to farmers and all stakeholders in maize production as it offers an excellent guide in developing integrated approaches for MLN management. Avoiding the use of uncertified seeds, especially in regards with MCMV which was found in all mechanically inoculated samples and confirmed serologically could minimize the build-up and spread of the virus.

Further studies should be conducted using other techniques to detect viruses and understand the reason behind the lack of detection of SCMV, even after the virus was artificially inoculated. Further investigation should also be conducted to understand the mechanism or reactions that favor the high rates of MCMV in all types of inoculation compared to SCMV.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 General discussion and conclusion

This study was undertaken to determine the rate of seed transmission and the role of soil in transmission of *Maize chlorotic mottle virus* and *Sugarcane mosaic virus* in different maize inbred lines, and the combined effect of mechanical and seed transmission on Maize lethal necrosis disease development. ELISA results indicated that seeds obtained from MCMV- and MLN-infected plants carry MCMV, a finding that was also reported by Mahuku *et al*. (2015). However, SCMV could not be detected in seeds or plants grown from seeds obtained from SCMV- or MLN-infected plants, which contradicts findings by Li Li *et al.*, (2007), who reported SCMV to be seed transmitted.

Understanding the role played by soil, seed and the combined effect of mechanical and seed transmission of viruses causing MLN disease is critical in the management of the disease. In sub-Saharan Africa, 66.67% of farmers obtain their seeds through informal sector (Erenstein *et al.*, 2011), which implies that some farmers use non-certified seeds. The similarity of MCMV strains in eastern African countries could be an indication of seed transmission through seed movements in the region (Adams *et al*., 2014; Flett and Mashingaidze, 2016). The aim of this study was therefore to determine the combined effect of mechanical and seed transmission in MLN disease development. Results indicated that plants grown from seeds obtained from MCMV- or SCMV-infected inbred lines could not express symptoms of the viruses regardless of whether the inbred lines were resistant, tolerant or susceptible to MCMV and SCMV. Similarly, plants grown in soils collected from pots where susceptible inbred lines were previously grown developed no virus symptoms. However, ELISA results indicated that MCMV was transmitted by seed and

through contaminated soil at very low rate, which could be attributed to low viral titer of inoculum in seed or soil. These findings agree with Jensen *et al*. (1991) who reported that the frequency of seed and soil transmission of MCMV is very low, but contradicts Zitter (2001) who reported that SCMV is seed transmitted, and Moline and Ford (1975) who reported SCMV to be soil transmitted.

Reaction of genotypes towards different modes of inoculation revealed that all maize genotypes artificially inoculated with the two viruses expressed typical symptoms regardless of phenotypic characteristics of inbred lines or hybrids. Both viruses were detected with ELISA, which implies that artificial inoculation of combined viruses activates the present viral particles in the seeds to multiply and increase the titer of the two viruses.

ELISA results also indicated that SCMV-infected plants inoculated with MCMV, and MLN-infected plants inoculated with both viruses at the same, MCMV was detected alone in all samples. This supports previous reports which indicated that SCMV is not transmitted through seed, though the negative result of SCMV in the case of MLN infected plants inoculated with both viruses is not explained.

5.2 General conclusion

Seed and soil were found to play an important role in the epidemiology of MLN disease through transmission of MCMV, though at very low rates. However, SCMV could not be detected in seeds. Mechanical inoculation of viruses is important in the spread of the two viruses and can be critical in MLN disease development.

5.3 General recommendations

Based on findings of this study, it is recommended that:

i. The use of resistant certified maize hybrids can delay infection, and therefore reduce losses, caused by MLN to maize production.

ii. Avoidance of any mechanical injury will drastically reduce the secondary infection caused by sharp objects or vectors, among others.

iii. Determination of the location of the virus in seeds is important to understand the reason behind the failure of viral symptom development in plants from infected seeds. The result will determine whether MLN causing viruses are really seed transmitted or surface contaminants which can be addressed with seed treatment using chemicals.

iv. Implementation of these findings can help as guide in developing strategies for MLN disease management.

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