SCREENING MAIZE GERMPLASM FOR RESISTANCE TO SUGARCANE MOSAIC VIRUS

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FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

DECLARATION

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

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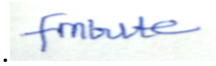
Dr. Felister Mbute Nzuve …  ……Date…17th June, 2022…

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Prof. Douglas W. Miano  Date: 17th June, 2022

DEDICATION

This work is to my parents, thanking them for their prayers. If not for you, I would have given up. Thank you. To my niece, Kayla and nephews Ethan and Nathan, you can be whatever you want to be.

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LIST OF ABBREVIATIONS AND ACRONYMS

F_1	First filial generation
F_2	Second filial generation
GCA	General combining ability
MCMV	*Maize chlorotic mottle virus*
MLN	*Maize lethal necrosis*
QTL	Quantitative trait loci
SSR	Single sequence repeats
siRNA	Small interfering ribonucleic acid
SCMV	*Sugarcane mosaic virus*

ABSTRACT

The first report of Maize lethal necrosis (MLN) in Kenya was in 2011 in Bomet County. The disease quickly spread to nearby counties causing devastating damage to maize crop yield. The causative agents of MLN are two viruses MCMV and SCMV. The study's objectives were i) to identify germplasm with resistance to SCMV and ii) to identify the mode of gene action associated with tolerance to the virus. To achieve objective one, 42 parental maize genotypes were planted in a screen house of the Faculty of Agriculture, University of Nairobi, in a completely randomized design to identify the ones with resistance to SCMV using the CIMMYT SCMV disease severity scale. Analysis of variance (ANOVA) tests were conducted on disease severity, disease incidence and Area under disease curve progression (AUDPC) scores using GENSTAT statistical software and showed significant differences among the genotypes for all the parameters. The genotype means were separated using least significant differences (LSD) at 0.05 significance level. Four genotypes had no symptoms of SCMV and 27 genotypes had a score of between 2 to 2.8 and were therefore classified as resistant or tolerant to SCMV, respectively. The rest had a score of 3 and above, and classified as highly susceptible. The resistant/tolerant genotypes are valuable sources of resistance to SCMV and could be employed in development of MLN resistant maize varieties. To achieve objective two, 448 maize genotypes consisting of 60 parents and 388 F_1s were planted in the short rains season of 2016 and were self-pollinated and each cob harvested singly to give F_2 population. Two populations namely 384 (parents UON-2015-50 × UON-2015-115) and 385 (parents UON-2015-50 × UON-2015-117) with the common parent 50 previously identified as resistant in MLN screening were selected for further genetic analysis studies. For genetic studies,

150 seeds of each F_2 derived families were planted in the screen house in plastic pots and artificially inoculated with SCMV and evaluated for disease symptoms for 6 weeks using the CIMMYT SCMV disease severity scale and then categorized as either resistant or susceptible for based on the F_2 generation. Resistant plants had a disease score of 2 and below and susceptible plants had a score of 3 and above. Chi-square goodness-of-fit test was then conducted to find conformity to various genetic ratios. The results of this study showed conformity to the 15:1 ratio which means the resistance to SCMV in these crosses could be controlled by major genes with complementary epistatic effects. These parents could be exploited in developing maize hybrids with resistance to SCMV, and therefore contribute towards management of MLN disease.

CHAPTER 1

INTRODUCTION

1.1 Maize production and its challenges

Maize is an important cereal crop globally (Ekpa *et al*., 2018). About 85% of the populace in Eastern and Southern Africa relies on maize for food (Boddupalli *et al.,* 2020). It is cultivated by mostly smallholder farmers for human consumption, animal feed and processed to produce vegetable oils (Nyaligwa *et al*., 2017).

The sub Saharan Africa (SSA) population is estimated to increase threefold by 2050 (Ekpa *et al*., 2018), thus increasing demand for maize. However, the maize yield in SSA is below the global average at 1.8 t/ha (Semagn *et al*., 2014). The major reasons for the low production are use of landraces and obsolete hybrids (Ekpa *et al*., 2018), low use of fertilizer, poor agronomic practices and abiotic factors such as poor soils and erratic rainfall. Biotic factors such as pests like the fall army worm, stalk borers and *Striga* weed (Keno *et al*., 2018) and diseases such as Grey Leaf Spot, *Maize streak virus* (MSV) and the Northern Leaf Blight (Sibiya *et al*., 2013) are prevalent with Maize lethal necrosis being the latest scourge in the eastern Africa region (Beyene *et al*., 2017).

Maize lethal necrosis occurs when maize plants are infected by *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) simultaneously (Hilker *et al.*, 2017). The disease can occur by double infestation of MCMV and other potyviruses *(*Gowda *et al.*, 2018). The earliest report of MLN was in Peru in 1976, then later in USA and China (Wu *et al*., 2013). SCMV was reported in Kenya in 1973 (Kulkarni, 1973; Louie, 1980) and MCMV in 2011 (Wangai *et al*., 2012; Mahuku *et al*., 2015). MLN was initially

recorded in Kenya in September 2011 (Mahuku *et al*., 2015; Wangai *et al*., 2012) in Bomet County and quickly spread to nearby counties and by 2012, other counties in the Rift valley, Nyanza, Western and Eastern regions had reported the disease (Wangai *et al*., 2012). MLN also spread to nearby countries namely Uganda in 2013 (Kagoda *et al*., 2016) and Tanzania, Rwanda (Adams *et al*., 2014), Congo, Ethiopia and South Sudan (Mahuku *et al*., 2015). Several management practices have been attempted such as rouging and use of pesticides to target vectors such as aphids and thrips but there's a danger of causing ecological damage and is not affordable for majority of smallholder farmers. Use of germplasm that is tolerant to MLN, MCMV and SCMV is the most durable, cost-effective way to manage the disease and has least environmental impact.

1.2 Statement of the problem

About 77,000 hectares under maize production in Kenya was affected by MLN in 2012, translating to 52 million US dollars in losses (Mahuku *et al*., 2015). In 2014/2015 season, 10% yield losses were reported which amounted to US$ 50 million (Beyene *et al*., 2017). The disease was reported to affect most of the commercial varieties with losses in yield ranging from 30% to 100% subject to variety and phase of infection (Mahuku *et al*., 2015). When a field is diseased early in the season, 100% yield loss can occur (Beyene *et al*., 2017). CIMMYT screened about 95,000 maize germplasms including elite commercial hybrids like H614D from Eastern and Southern Africa and reported high susceptibility to MLN (Beyene *et al*., 2017).

The prevalence to MLN has been aggravated by a number of factors such as favourable weather which promotes survival and spread of the viruses' vectors; maize monoculture which leads to build-up of the viruses and occurrence of new and more virulent strains of MCMV and SCMV (Manje, 2015) and recycling of infected seed (Beyene *et al*., 2017). The presence of a potyviruses increases the concentration of MCMV particles up to five times in a co-infected plant. The increase in MCMV concentration is due to synergism which results in increased severity of symptoms than in a single virus infection. The potyvirus has the ability to suppress the host plant's mechanisms that limit MCMV multiplication in cells consequently permitting easier spread of MCMV and subsequently heightened symptoms.

Since maize is a staple food to 98% of the Kenya's population with a consumption rate of 125 kg per capita (Kariuki, 2015), loss of yield due to MLN threatens food and economic security of the many households' dependent on maize cultivation. In addition, 90% of commercial varieties of maize grown in eastern Africa region are susceptible to MLN (Manje, 2015). Managing MLN is multifaceted.

The use of closed seasons such as use of gap years between planting seasons in Kenya may not be a viable solution for smallholder farmers (Kariuki, 2015). Use of chemical pesticides to manage the virus vectors may not be affordable to the resource-constrained small-holder farmers and may have undesirable effects on the environment. Use of chemicals to contain spread of SCMV is also difficult due to the non-persistent manner of virus spread by the aphids. MCMV has also been shown to be seed transmitted a very low rates (Sanchez *e*t

al., 1994). A long term and sustainable approach could involve the use of germplasm with resistance to viruses causing MLN disease.

1.3 Justification

The best approach to manage MLN and viruses causing the disease is use of resistant varieties. Development of new varieties is crucial (Makone *et al*., 2014). However, this requires that sources of resistance are continuously identified and then deployed into adapted maize varieties. In 2013, KALRO together with CIMMYT set up a MLN screening site in Naivasha. In efforts to identify resistant maize germplasm, about 95,000 maize genotypes sourced from different organizations assessed for their response to MLN reported high susceptibility (Mahuku *et al.,* 2015). To manage MLN, it's important to identify sources of resistance to the singular viruses namely SCMV and MCMV and also MLN since SCMV presence exacerbates symptom severity of MCMV resulting in higher yield losses. This should be followed by knowing the nature and manner of inheritance of the resistance to facilitate deployment of such resistance in maize breeding programs and in development of superior varieties. Commercial seed companies can use these materials to develop tolerant or resistant maize varieties and avail them to farmers thus ensure food security and income to households.

1.4. Main objective

The major goal of this study was to contribute towards control of MLN disease by finding of sources of resistance to *Sugarcane mosaic virus* that will be useful in breeding programs.

1.4.1 Specific objectives

1. To identify maize germplasm with resistance to *Sugarcane mosaic virus*.
2. To determine the nature and number of genes conferring resistance to *Sugarcane mosaic virus* among F_2 segregating populations.

1. 4.2 Hypothesis

1. Maize varieties with resistance to SCMV exist among the available germplasm.
2. Resistance to Sugarcane mosaic virus in maize is provided by single genes with major effect.

CHAPTER 2

LITERATURE REVIEW

2.1 Maize production, importance and constraints

Maize (*Zea mays* l.) is a major cereal crop in Sub-Saharan Africa (SSA) where it covers 25 million hectares of land in production where it is mainly cultivated by smallholder farmers primarily for food (Smale *et al.*, 2011). It is cultivated in different climatic and ecological zones in the region and is staple food and major cash crop for over 300 million people (Nyaligwa *et al.*, 2017; Beyene *et al.*, 2016). Globally, maize provides 33% of daily calories to over 4.5 billion people and its demand is projected to be twice the current by 2050 (Nyaligwa *et al.* 2017).

Despite its apparent importance, maize yields are still very low. For instance, from 2011-2013 maize grain yields in SSA was approximately 1.8 t/ha while production in Mexico was at 3.1 t/ha and Thailand at 4.4 t/ha (Beyene *et al.*, 2016) against the international average yield of 4.5 t/ha (Nyaligwa *et al.*, 2017). Abiotic stresses have contributed to the yield discrepancies namely inadequate use of fertilizers, erratic rainfall in the growing season and declining soil fertility. Other yield limiting factors include low uptake of improved varieties and use of low yielding landraces. Of major concern are biotic factors namely weed infestation, pests and diseases. Pests such as the fall armyworm (*Spodoptera frigiperda*) have had devastating effects on maize yields with losses estimated to amount to $3 billion (Center for Agriculture and Biosciences International, 2017).

2.2 Maize lethal necrosis disease

Maize lethal necrosis (MLN) occurs through double infection of maize plants with MCMV and viruses in the *Potyviridae* genus such as SCMV and MDMV (Mekureyaw, 2017). In the case of Kenya and Eastern Africa, MLN has been brought about by the coinfection of SCMV and MCMV (Wangai *et al*., 2012; Adamas *et al*., 2013). The two viruses have a synergistic interaction that results in adverse symptoms that lead to reduced yields and even death of the plant (Redinbaugh *et al.*, 2004).

2.2.1 History of Maize lethal necrosis and the causative viruses

Globally, MLN was first recorded in Peru in 1973 and in Kansas in U.S.A in 1977 (Niblett and Claflin, 1978) and thereafter in Nebraska, Hawaii (Jiang *et al.*, 1992) and China in 2010 (Wu *et al.*, 2013; Xie *et al.,* 2011). MCMV was first recorded in Peru in 1973, then in Kansas in the U.S.A in 1976, Latin America and later in China (Wu *et al.*, 2013). In 1990, the virus was recorded in Hawaii (Jensen *et al.,* 1991), China in 2010 (Xie *et al.,* 2011). It's quite new virus in Africa, Kenya reporting the first incidence which later spread to Tanzania, Uganda and Rwanda. The rapid spread across the borders is due to poor phytosanitary systems and porous borders (Isabirye and Rwomushana, 2016).

Sugarcane mosaic virus was first recorded in USA, then Indonesia in 1922 (Wakman *et al.*, 2001). In Africa, the first report was in 1960s (Chaves-Bedoya *et al.*, 2011). In Kenya, Uganda and Tanzania it was reported in 1973 in sugarcane and maize (Louie, 1980). Originally, the SCMV viral isolates from sugarcane were labeled as SCMV strains and those in maize were identified Maize dwarf mosaic virus (MDMV) strains but later

reclassified after realization that both SCMV and MDMV can infect maize (Espejel *et al.*, 2005).

2.2.1.1 Distribution of MLN Disease in East Africa

Maize lethal necrosis poses a great threat to food security given that it may cause yield loses of up to 100% especially where infection occurs at early crop growth stages (Wangai *et al.,* 2012; Ritte *et al.,* 2017). After the initial reports in Bomet County in 2011 it later spread to Narok north, south and Naivasha districts, and Nyamira, Trans Nzoia, Embu, Uasin Gishu, Kisii, Busia, Meru, Nyeri and Murang'a (Wangai *et al.,* 2012; CIMMYT, 2012). In 2012, MLN was reported in Tanzania in Mwanza, Lake Victoria region and later spread to the central region in Dodoma and Singida and the northern region in Kilimanjaro, Arusha and Manyara (Wangai *et al.,* 2012; Ritte *et al.,* 2017). For Uganda, initial reports were reported across the districts bordering Kenya namely Busia, Tororo, Iganga and Mbale (Kagoda *et al.,* 2016). Most of the countries in Africa like Rwanda, Democratic Republic of Congo and Ethiopia have reported high incidences of MLN (Adams *et al.,* 2014; Lukanda *et al.,* 2014; Mahuku *et al.,* 2015).

The fast progression of MLN within the region can be attributed to repeated cultivation of maize crops throughout the year, use of MLN susceptible varieties presently in cultivation and presence of major hot spots for MLN which are favorable environment for the vector *Frankliniella wiliamsi*. Seed recycling has been reported to aid in transmission of MCMV (Jensen *et al.*, 1991; Gatunzi, 2018). Nationally, poor phytosanitary measures and porous borders have aided in the spread of MLN across the region (Isabirye and Rwomushana, 2016).

2.3 *Maize chlorotic mottle virus*

Maize chlorotic mottle virus, is an RNA virus in the genus Machlomovirus and Tombusviridae family. It's a single-stranded genome that's positive sense and a linear RNA containing 4437 nucleotides. It has a non-enveloped icosahedral virion that's 30nm in diameter (Wu *et al.*, 2013; Lommel *et al.*, 1991). MCMV symptoms include: chlorotic stripes on leaves which run parallel to leaf veins, leaf mottling and necrosis, stunted growth, short male inflorescences with reduced spikes and short and malformed ears. In severe cases, when the plant is infected in early stages, death might occur (Adams *et al.*, 2014; Mahuku *et al.*, 2015).

Machlomovirus MCMV is transmitted by 6 species of chrysomelid beetles, family *Chrysomelidae* (Nault *et al.*, 1978) which includes the corn flea beetle (*Chaetocnema pulicaria)*, cereal leaf beetle (*Oulema melanopa*), the flea beetle (*Sytema frontalis*) and members of the genus Diabrotica including *D. longicornis*, *D. virgifera* and *D. undecimpunctata* (Mahuku *et al.*, 2015; Nault *et al.*, 1978). Several species of thrips including maize thrips (*Frankliniella williamsi*) have been reported to transmit MCMV as was the case in Hawaii (Nelson *et al.*, 2011). Thrips were found in huge numbers in maize fields in Kenya and could have been possibly around even before the outbreak of MLN (Mahuku *et al.*, 2015). Both the thrips and chrysomelid beetles can transmit MCMV after 3 hours of contact with no latent period. In both, their larvae and adults do the transmission (Mahuku *et al.*, 2015; Cabanas *et al.*, 2013).

Transmission of MCMV through seeds was recorded to be low at a rate of 0.04% in Hawaii (Jensen *et al.*, 1991). However earlier research from Peru and Kansas have reported that

MCMV is not seed-borne (Gordon *et al.*, 1984). Soil transmission has also been recorded in Zimbabwe where maize hybrid SC513 had a 70% infection when it was grown in a field where the maize crop had been infected by MCMV. Only 4% of the same hybrid was infected when grown in sterile soil (Mahuku *et al.*, 2015). Infected plant debris can also cause spread of the disease since MCMV can survive in the plant residue (Kagoda *et al.*, 2016). It can be controlled by use of integrated pest and disease management such as use of insecticides to control thrips in Hawaii (Nelson *et al.*, 2011). Other methods to control spread of MCMV include removal of grassy weeds which act as alternative hosts to reduce the population of vectors, crop rotation and rouging of infected plants. However, developing and availing tolerant or resistant varieties to farmers is the most effective way of managing the disease (Gowda *et al*., 2018).

2.4 *Sugarcane mosaic virus*

Sugarcane mosaic virus (SCMV) is estimated to be responsible for up to 45-48% yield losses in maize production (Chaves-bedoya *et al.*, 2011). It's in the genus *Potyviridae* which are the most damaging group of viruses in crop production with a global distribution (Lubberstedt *et al*., 2006). It's a positive sense, single strand RNA virus of filamentous particle, has a width of 11 nm and a length of 700-750 nm with a length of 9596 nucleotides and is surrounded by a non-enveloped capsid.

Sugarcane mosaic virus is passed on by aphids in both the nymph and adult stages. Diagnostic *Sugarcane mosaic virus* symptoms include stunted growth, leaf chlorosis, reduced plant biomass and consequently reduced grain yield (Ingvardsen *et al.*, 2010). SCMV is spread non-persistently which makes it difficult to control (Lubberstedt *et al*.,

2006). SCMV control using chemicals since they are harmful to the environment and not economical to small-scale farmers. Development of germplasm that's resistant is the effective way to tackle disease (Lubberstedt *et al.*, 2006; Louie, 1980).

2.5 Plant virus interactions

Mixed viral infections in plants are commonplace and the various virus interactions have resulted in a number of diseases. Viruses co-infecting the same host plants have interactions that can be categorized as synergistic or antagonistic (González-Jara *et al.*, 2009). Synergistic interactions have an enabling effect on one or both viruses and results in increase in virus multiplication in plant host. It can also be manifested by one virus facilitating the vector transmission of the other one and is termed as helper dependence (González-Jara *et al.*, 2009). On the other hand, antagonistic interactions occur when the presence and activity of one virus hinders the fitness of the other and as such only one virus benefits. Other viral interactions are cross-protection, replacement or mutual suppression (Syller, 2012). There are two ways of multiple virus infection. First is co-infection where several viruses attack a host plant at once or in quick succession (Saldana *et al.*, 2003). The second one is super-infection where diverse strains of a virus infect the host at different times (Syller, 2012).

Synergistic interactions occur when a plant infected by multiple viruses result in amplified growth of one or both viruses and when the interaction induces more severe symptoms than what would be observed if the interaction was additive. The enhanced viral pathogenicity increases crop damage in susceptible varieties resulting in higher yield loss. Such interactions have been best described in viral interactions involving *potyviruses* (Syller,

2012). This type of interaction has been reported between *Potato virus Y (*PVY) and *Potato virus X* (PVX) in *Nicotiana tabacum* which resulted in increased symptoms and a 10-fold rise in the titre of PVX in comparison to single virus infection (Rochow and Ross, 1995). Synergistic interaction leading severe disease symptoms is seen in Cassava mosaic disease, sweet potato virus disease and Maize lethal necrosis (Syller, 2012; Scheets, 1998).

2.5.1 The synergistic interaction between MCMV and SCMV

It is critical to also understand the role of each causative virus in the interaction leading to MLN which is important in the breeding programs geared toward its resistance. Xia *et al.* (2016) demonstrated that MCMV and MCMV-derived silencing RNAs (siRNAs) in maize plants was amplified when there was co-infection of MCMV with SCMV than when it was a single infection. Thus, the presence of SCMV favored not only its own multiplication but also aided multiplication of MCMV (Xia *et al.*, 2016). In synergistic interactions, the potyvirus posseses the ability to amplify virulence of the co-infecting virus, in this instance, MCMV (Mbega *et al.*, 2016).

The region in the potyviral genome facilitating synergism encodes a polyprotein that has two products, P1 and helper component-protease (HC-pro). HC-pro aids the transference of the virus in the vascular tissues and suppression of the host plant's defence mechanism against viruses, posttranscriptional gene silencing (PTGS) (Xia *et al.*, 2016). HC-pro is however not involved in the induction of MLN when the Wheatstreak mosaic rymovirus (WSMV) is involved implying that another gene is involved in suppression of PTGS (Mbega *et al.*, 2016). Scheets (1998) reports that WSMV infection is heightened when in combination with MCMV. He observed that in co-infected plants, WSMV concentrations

were averagely 2.1-3-1 times more than singly infected plants. MCMV concentrations in doubly infected plants were on average 3.3-11.2 times higher than singly infected plants (Scheets, 1998). This shows that both viruses affected the replication and spread of each other (Scheets, 1998). This suggests that several factors are involved in potyvirus synergism.

Sugarcane mosaic virus has two proteins that exacerbate MCMV's replication and increase severity of symptoms; HC-pro and nuclear inclusion protein-a and viral genome-linked protein (Nia/VPg) (Kreuze, 2014)). The SCMV VPg interacts with host maize plant's elongin C protein (ZmElc) resulting in its minimised production mainly in the leaves and pistils (Zhu *et al.,* 2014). The lowered expression of ZmElc gene which codes for the ZmElc protein results in increased replication of MCMV. The SCMV VPg also enhances movement of its own particles and MCMV from one cell to another within the plant.

The HC-pro gene in SCMV functions to suppress the expression of PTGS but it is also interracts with ferredoxin-5 (FdV) resulting in disturbance of posttranslational import into bundle sheath cells chlroplasts. Disruption of chlroplast activity due to infection by the two viruses results in low synthesis of ATP required for the Calvin cycle in photosystem I which causes poor yield and scant production of chlrophyll and enhance symptom manifestation. It is therefore important that in identifying germplasm for MLN resistance, SCMV resistance has to be achieved because of the synergistic effect SCMV has on MCMV symptoms.

2.6 Alternative hosts of Maize lethal necrosis causative agents

Hosts of MCMV and SCMV are limited to the gramineae family with *Z. mays* and *S.officinarum* being the primary hosts of each respectively (Scheets, 2004). In Hawaii, MCMV was found in soft brome (*Bromus mollis),*and broomcorn millet (*P.miliaceum)* (Brunt *et al.*, 1996). Other sources are barley, proso millet and foxtail millet. It has been identified in sorghum and wheat (Kusia, 2014).

China reported both viruses in sugarcane (Uyemoto, 1983). SCMV has been found to cause disease in sugarcane, sorghum, millet, pearl millet, barley, rice and rye (Louie, 1980). In Tanzania, SCMV was found in sugarcane, bristly foxtail, sorghum and finger millet while MCMV was found in the primary host, maize only (Mariki, 2017). In Kenya, MCMV was found maize, sorghum and napier grass (Wamaitha *et al.,* 2018).

2.7 Management of Maize lethal necrosis

Several methods have been employed in combination to manage the disease. In Hawaii, use of pesticides for insect vector populations and host tolerance was the most effective combination (Mahuku *et al.*, 2015). Seed dressing with chemicals before planting has been used to reduce surface contamination. Pesticides are widely used but it's not affordable to the majority small-scale farmers (Beyene *et al*., 2017).

In Uganda, rouging infected plants to reduce disease pressure is quite common but it's not effective when used in isolation (Kagoda *et al.*, 2016) and it requires regular monitoring. Removal of alternative hosts such as grasses near maize fields is effective in reducing the disease inoculum (Nelson *et al.*, 2011). Timely weeding and application of fertilizer during planting and top dressing increases plant vigor. Rotating maize with non-cereal crops and use of certified seeds reduces MLN occurrence (Kagoda *et al.*, 2016). Crop rotation was

used successfully in Central USA to manage MLN (Uyemoto, 1983). Crop rotation may not be feasible to farmers with small pieces of land who bank on maize production for income and farmers may not be able to afford certified seed for every planting season. Phytosanitary measures are required especially in cross-border seed trade to control spread of plant diseases from a country to another (Kagoda *et al.*, 2016).

2.7.1 Host resistance breeding

Use of maize varieties with tolerance to MCMV and SCMV is environmental friendly and cost-effective way to manage spread of the disease. Host resistance breeding involves planting of maize varieties to be evaluated, artificial inoculation with the disease under observation in the field and greenhouse conditions, and then scoring for resistance to the disease (Mahuku *et al.*, 2015). After identifying tolerant materials, the genes are then transferred to maize varieties with desirable agronomical traits such as high yields and early maturity. Disease resistance tolerance breeding can therefore be described as the process of introgression of disease resistance or tolerance genes into plants that are susceptible to a disease (Shrestha *et al.*, 2019).

There have been efforts to find sources of resistance and or tolerance to MLN, SCMV and MCMV both locally and internationally. Mahuku *et al*. (2015) screened 63 maize inbred lines which had tolerance to other viral diseases or had shown tolerance to MCMV by exposing them to an isolate of MCMV from Kansas to a Kenyan isolate of MCMV and SCMV in field conditions. Inoculation was done artificially in both experiments and Oh28 was used as the susceptible control. In the Ohio experiment, 13 lines were advanced after the initial experiment for 2 further trials. The results showed seven lines that had

significantly delayed symptoms expression relative to Oh28 including Oh1VI and 6 recombinant inbred lines developed from a cross of OhVI x Oh28 (Mahuku *et al.,* 2015). The highest ranked plants for tolerance in controlled conditions showed significant tolerance in the field conditions that had both MCMV and SCMV inoculation thus showing probability for being sources of resistance (Mahuku *et al.,* 2015).

CIMMYT together with KALRO assessed 25,000 accessions of maize for MLN resistance in Naivasha and Bomet and reported high susceptibility to MLN among the genetic materials (Mahuku *et al.*, 2012). In 2013, CIMMYT under the 'Global Maize Program' screened 124 varieties of maize of which 122 were found to be highly susceptible to MLN (CIMMYT, 2019). A further screening of 62,000 lines showed that 90% of the lines were susceptible (CIMMYT, 2019). By 2017, only 6 lines showed tolerance to MLN. Some of them included WE5139, UUH5354, H12ML and MeruHB607 (CIMMYT, 2019). Major resistance QTLs occurring on chromosomes 3 and 6 and minor QTLs occurring on all chromosomes save for chromosome 8 were reported in previous studies (Semagn *et al.*, 2014; Gowda *et al.,* 2018). Among 6 parental lines evaluated, 3 lines namely CML543, CML539 and CML144 showed moderate tolerance to MLN with a mean disease severity score of 2.1, 2.2 and 2.1 respectively early scoring (21 days post inoculation) and 2.3, 2.5 and 2.4 for late scoring (42 days post inoculation) (Gowda *et al.,* 2018).

Maize is prone to MLN infection throughout its growth from germination to almost maturity (CGIAR, 2019). However, knowledge on the varietal resistance of maize to MLN-causing vectors and viruses is limited (Mahuku *et al.*, 2015). More lines with resistance to MLN followed by the elucidation of the mechanism underlying the host resistance have to

be identified. This will enhance the deployment of the potential parents in breeding programs for development of superior maize varieties.

2.7.1.1 Genetics of resistance to MLN and its causal viruses

Resistance to plant diseases can be natural or induced and can either be qualitative or quantitative. Qualitative resistance is provided by one dominant or recessive gene which is strain-specific and offers high degree of resistance (Maule *et al.*, 2007; Shrestha *et al.*, 2019). Quantitative resistance is aided by multiple genes giving minor contributions to the resistance. The resistance is centered on polygenic or oligogenic inheritance, controlled by additive or partially dominant genes. The resistance is durable and non-specific to race of the pathogen (Maule *et al.*, 2007).

Quantitative traits are measurable, have a continuous variation and loci that control genetics of traits are known as quantitative trait loci (QTL) (Nduwumuremyi *et al*., 2013). The polygenic inheritance of the genes with minor additive effects which are affected by the environment results in continuous variation (Poland *et al.*, 2009). These traits cannot be dissected using Mendelian methods of genetic analysis and hence require different methods for analysis such as QTL mapping or linkage mapping, association mapping and Nested association mapping (NAP). Disease resistance is a polygenic, effected by several genes and the environment (Shrestha *et al.*, 2019). As such quantitative resistance is long-lasting and when a pathogen overcomes a single allele with minor effect, it does not get an advantage over the host. The loss of one allele does not render the host plant susceptible to the pathogen (Poland *et al.*, 2009; Shrestha *et al.*, 2019).

Genetics of resistance to potyviruses including SCMV have been identified. SCMV research in *Zea mays* has been improved greatly through use of molecular markers such as single sequence repeat (SSR) markers, restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (Leng *et al.*, 2015). The first SCMV resistant genes were found in inbred line GA209 (Leng *et al.*, 2015). Inbred lines identified by Kuntze *et al.* (1997) as completely or partially resistant were crossed with susceptible lines in a separate study with the aim of identifying the genes conferring resistance. The F_2 segregations were categorized into 3 gene models based on environment and genotype of the susceptible parent. Analysis of the markers mapped 2 dominant genes *Scmv1* on chromosome 6S and *Scmv2* on chromosome 3 (Soldanova *et al.,* 2012). BC_5 progeny obtained from crossing FAP1360A and an F_7 susceptible line was analyzed and deduced that the 2 dominant genes *Scmv1* and *Scmv2* are necessary for SCMV resistance. In total, 5 quantitative trait loci (QTLs) have been identified on chromosomes 1, 3, 5, 6 and 10 for SCMV resistance (Soldanova *et al*., 2012). The U.S. line Pa405, which is resistant, the loci for Potyviridae resistance segregate as a dominant gene (Redinbaugh *et al*., 2004).

Total resistance to SCMV needs *Scmv1* and *Scmv2*. However, *Scmv1* offers a stronger effect than *Scmv2. Scmv1* is positioned on the short arm of chromosome 6 and *Scmv2* is found on chromosome 3 close to the centromere (Leng *et al.*, 2015). *Scmv1* subdues symptoms from early to late stages of infection development while *Scmv2* is effective at late phases. As for gene action, *Scmv1* is completely dominant and Scmv2 is additive (Soldanova *et al.*, 2012).

To determine the mode of gene action to any disease, chi square (χ^2) tests are used. Chi test is a test of goodness of fit between the observed and expected number of plants with disease symptoms and those that are symptomless. It is used to test an earlier stated genetic hypothesis (Weir, 1990). Since χ^2 is calculated using actual numbers of progenies and not percentages nor proportions, it is important to have sufficient sample size in the different classes to be tested. The letter 'O' represents the observed samples in a class and 'E' represents the expected samples in the same sample class as stated in the hypothesis.

2.7.2 Popular mating designs used in generation of progenies

A mating design is the procedure followed in producing progenies (Nduwumuremyi, 2013). It's also defined as a pattern or scheme used by a breeder to produce progeny and gather genetic information on the germplasm. In any plant breeding project choice of good parental materials and suitable mating designs are vital for the achievement of the breeding goals. The aim of study, space availability, time and the physiology of the plant are important to consider when choosing a mating design. Other factors to consider include the aim of the project, size of the breeding population needed, the type of pollination (self or cross), type of crossing which is either natural or artificial, pollination dissemination technique which could either be by insects or wind and presence or absence of a male sterility system. A good mating design will enable a breeder to know the genetic control of a trait of interest; it will generate a sufficient breeding population; give estimates of genetic gain and provide information to be used in evaluating the parents. There are 6 mating designs.

Topcross mating design also known as inbred variety cross was developed by Jenkins and and Brunsen in 1932 to test inbred lines of maize in cross-bred combinations. Nduwumuremyi (2013) describes it as a mating between a line or selection, and a common male parent which could be an inbred line, a variety or single cross. In an open pollination, the selected plants are then crossed with a common tester (Nduwumuremyi, 2013). The common tester usually has a well-known genetic background. While making a top cross, single cross F_1s are used since they're uniform. They're selected based on their agronomic superior traits or desirable parents. Top cross design is used in situations where the desired gene(s) is in an exotic material which means the material is from imported from a different geoclimatical condition and therefore not adapted to the local conditions or difficult, which means the material is a poor combiner or is dominant susceptible. The design acts to increase chances of obtaining the desired gene(s) (Nduwumuremyi *et al*., 2013). '

This design is applied in initial estimation of combining ability on novel inbred lines and yields only general combining ability (GCA). It's a simple design that has a low crossing load and a simple statistical analysis. It's necessary to have 5 heads per cross since the crosses will segregate in the subsequent F1 generation and not less than 80 plants to allow selection of desirable plants in F_1 (Nduwumuremyi, 2013). The disadvantages of this design are that a single tester variety may not offer sufficient genetic background to test inbred germplasm and the crosses may be too much if the inbreeding coefficient is tested (Nduwumuremyi, 2013). North Carolina design was developed by Comstock and Robinson in 1952 with the aim of getting more information on combining ability with reduced labor. There are 3 designs NC I, II and III.

North Carolina I is effective for estimating additive and dominance variances. It requires replicated trials and therefore one has to have sufficient seed hence not effective for plants that cannot produce large seed quantities (Acquaah, 2012). Each member of the male parental group is crossed with a different parental group. The progenies are both full-sib and half-sib. Each set of families with a similar male parent are half-sib families and those with similar male and female parent are full-sib families. The advantage of North Carolina I is that it allows for a test of significance for additive genetic variance. It's been used in maize breeding to estimate genetic variances (Acquaah, 2012).

North Carolina II is a factorial mating design where all members of the male parent group is crossed with each member of the female parents group. It's used for evaluating combining ability among inbred lines. Blocking is necessary in this design to ensure that every single set of male and female parents to be mated remain intact (Acquaah, 2012). NC II allows for the measuring of general combining ability (GCA) and the specific combining ability (SCA) but cannot be used to assess epistasis or genotype x environment (G x E) interaction (Nduwumuremyi, 2013).

North Carolina III is assumed to be the best design among the 3. A sample of randomly selected F_2 plants is backcrossed to the 2 inbred lines it descended from. The predecessor parent testers are unique because the F_2 is segregating at the loci that the testers differ. Kearsey and Jinks improved the design by adding a third tester to the 2 predecessor parents

called the triple testcross. The triple testcross can be used to test epistasis as well as estimate additive and dominance variance (Nduwumuremyi, 2013).

2.8 Significance of SCMV resistance to MLN resistance

Maize lethal necrosis is caused by two viruses interacting in a synergistic fashion. Therefore finding resistance to MLN would be approached by targeting resistance to the individual viruses especially the potyvirus, this instance, SCMV. Mwatuni *et al* (2020) while conducting a countrywide survey in Kenya to assess the distribution of viruses causing MLN, genetic diversity and recombination, observed that whenever maize plants are diseased with MCMV and SCMV, they had severe systemic necrosis on the stems and leaves just as earlier reported in United States by Scheets (1998) where Corn lethal necrosis (CLN) was caused MDMV and WSMV. In the Kenyan study, maize infected with MCMV and SCMV had more severe symptoms of MLN.

Bulegeya (2016) observed, under artificial inoculation, reduced MLN symptoms with presence of potyvirus resistance genes. Potyvirus resistance has been established to be found on loci found on chromosome 3, 6 and 10. The loci on chromosome 3 and 6 have major effect on potyvirus resistance but the one at chromosome 3 seems to be of greater importance than the others whether in combination or singly (Bulegeya, 2016). A combination of loci on chromosomes 3 and 6 and a combination of loci on chromosomes 3 and 10 had similar effects against MLN but a combination of loci found on chromosomes 6 and 10 had more symptoms of MLN (Bulegeya, 2016). QTL on locus on chromosome 10 confer resistance to Wheat streak mosaic virus (WSMV) but not to SCMV. It was found to be inadequate to fight MLN caused by MCMV and SCMV or Maize dwarf mosaic virus (MDMV). Therefore, loci found on chromosome 10 unless combined with loci on

chromosome 3 has no effect on MLN resistance. Loci on chromosomes 3 and 6 confers resistance to MLN, even when acting singly. Loci on these two chromosomes are the ones that offer SCMV resistance and therefore show the importance of identifying germplasm with SCMV resistance which is significant for achieving MLN resistance (De Souza *et al.*, 2008).

2.9 Current status of Maize lethal necrosis in Kenya

After initial reports of MLN in 2011 and the rest of the region between 2012 to 2014, international multi-agency efforts have been put in place to combat it. Up to 2019, 18 CIMMYT-derived MLN tolerant hybrids have been released in East Africa. Of these, 14 hybrids were released in Kenya in collaboration with KALRO, Kenya Seed Company, Western Seed Company and Seed Co. Limited. Twelve hybrids have a severity score of 4 on the MLN scale of 1-9 and two have a score of 3. Other strategies that have been put in place include rigorous awareness creation on MLN has been done for all key stakeholders in the sector, capacity building of concerned institutions in the public and private sector on MLN diagnosis and monitoring. Standard operating procedures and checklists for MLN-free seed development and exchange has been put place for commercial seed production. Stricter phytosanitary procedures have been put in place to monitor trans-boundary movement of maize seed across sub-Sahara Africa. The measures put in place have proved to be working. Since 2014, no new country has reported a new case of MLN in the region.

CHAPTER 3

MATERIALS AND METHODS

3.1 IDENTIFICATION OF MAIZE GERMPLASM WITH RESISTANCE TO *SUGARCANE MOSAIC VIRUS*

3.1.1 Location and climatic description of study area

The experiment was done at the University of Nairobi Field Station (Upper Kabete), located at 1°15'' South and 36°44'' East. Its altitude is 1940 m a.s.l. and receives rains in March to June for long rains and short rains come in October to December. Mean annual precipitation is 1000 mm. Diurnal temperature range is 13°C to 23°C. The area has dark red-brown clay soils with a PH range of slightly acidic to slightly alkaline soils. They are well drained and the top soil pH ranges from 5.2 - 7.2 while the sub soil pH ranges from 5.2 - 7.7.

3.1.2 Maize germplasm used in the study

The genetic material evaluated for response to the sugarcane mosaic virus disease comprised of 42 maize genotypes which were assembled from different sources namely; 32 were obtained from KALRO, three from CIMMYT and 7 were collected from the farmers' fields.

Table 3.1 Maize germplasm used in the evaluation for response to SCMV disease

Genotypes	Designation	Source of germplasm
1	UON-2015-5	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
2	UON-2015- 25	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
3	UON-2015- 19	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
4	UON-2015- 21	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
5	UON-2015- 24	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
6	UON-2015- 9	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
7	UON-2015- 26	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
8	UON-2015- 34	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
9	UON-2015-37	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
10	UON-2015- 39	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
11	UON-2015-41	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
12	UON-2015- 47	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
13	UON-2015- 48	Kenya Agricultural and Livestock Research Organization (KALRO), Muguga
14	UON-2015-52	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
15	UON-2015- 53	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
16	UON-2015- 54	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
17	UON-2015- 55	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
18	UON-2015- 56	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
19	UON-2015- 57	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
20	UON-2015- 58	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
21	UON-2015- 59	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
22	UON-2015- 60	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
23	UON-2015-63	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
24	UON-2015- 65	Kenya Agricultural and Livestock Research Organization (KALRO), Katumani
25	UON-2015- 83	International Maize and Wheat Improvement Center (CIMMYT)
26	UON-2015-85	International Maize and Wheat Improvement Center (CIMMYT)
27	UON-2015- 86	International Maize & Wheat Improvement Centre (CIMMYT)
28	UON-2015- 87	International Maize & Wheat Improvement Centre (CIMMYT)

Table 3.1 Maize germplasm used in the evaluation for response to SCMV disease

Genotypes	Designation	Source of germplasm
29	UON-2015- 89	International Maize & Wheat Improvement Centre (CIMMYT)
30	UON-2015- 91	International Maize & Wheat Improvement Centre (CIMMYT)
31	UON-2015- 92	International Maize & Wheat Improvement Centre (CIMMYT)
32	UON-2015- 94	International Maize & Wheat Improvement Centre (CIMMYT)
33	UON-2015- 99	International Maize & Wheat Improvement Centre (CIMMYT)
34	UON-2015- 101	International Maize & Wheat Improvement Centre (CIMMYT)
35	UON-2015- 103	International Maize & Wheat Improvement Centre (CIMMYT)
36	UON-2015-105	International Maize & Wheat Improvement Centre (CIMMYT)
37	UON-2015-108	Farmer varieties/landraces
38	UON-2015-110	Farmer varieties/landraces
39	UON-2015-112	Farmer varieties/landraces
40	UON-2015-118	Farmer varieties/landraces
41	UON-2015-120	Farmer varieties/landraces
42	UON-2015-122	Farmer varieties/landraces

3.1.3 Experimental design

The 42 genotypes were planted in a completely randomized design (CRD) in two replications in a screen house at the Field Station. Seeds were planted in plastic pots measuring 28cm x 20cm x 30cm, filled with soil. The soil was obtained from farm at the Field station. It consisted of top soil and subsoil of well drained red-brown clay soil. Each pot represented a plot with four plants of each genotype. At planting, 10g of Di-ammonium phosphate (DAP) fertilizer was applied in each pot and top dressing done using 10g of Calcium ammonium nitrate (CAN) per plant and watered regularly. Two weeks post emergence; the plants were mechanically inoculated with SCMV by hand rubbing method. A second inoculation was done a week later.

3.1.4 Source of the SCMV inoculum

Sugarcane mosaic virus was isolated from infected maize leaves showing clear symptoms. The infected maize leaves were obtained from KALRO Biosafety screen houses (BSH) at Kabete and prepared at the University of Nairobi laboratory.

3.1.5 Inoculum preparation and inoculation

The SCMV inoculum was prepared by crushing 10 mg of SCMV-infected maize tissues in 1 ml of phosphate buffer which consisted of potassium phosphate dibasic (K_2HPO_4) and potassium phosphate monobasic (KH_2PO_4) at pH 7 in ratios of KH_2PO4 = 4.8g, K_2HPO_4= 10.6g and Na_2SO_3=0.6g. Inoculation was done by leaf rubbing. The Carborandum powder ($SiCO_3$) was used as an abrasive agent to induce microscopic injuries on the plants to increase SCMV virus penetration into plant cell.

3.1.6 Data collection

3.1.6.1 Assessing for disease incidence and severity

The SCMV symptoms severity was estimated based on a scale of 1 to 5 adopted from CIMMYT where 1=no symptoms and 5=dead heart symptoms or complete death (CIMMYT, 2012).

Severity of the disease was assessed and recorded weekly for eight weeks with the first one being at seven days after the second inoculation. Kuntze *et al.* (1995*)* recommends that for experiments on evaluation of disease resistance, disease scoring should be done for a minimum of seven weeks after inoculation so that complete information on the material tested can be obtained. Disease incidence on the other hand was assessed by obtaining the percent count of diseased plants over entire number of plants in a pot.

3.1.7 Data analysis

3.1.7.1 Analysis of Variance

All data collected on disease severity and incidence was subjected to analysis of variance (ANOVA) using GenStat (15th Edition) to obtain genotypes means which were separated using Fischer's least significant differences (LSD) test at P ≤0.05. The model for ANOVA is shown on equation 1.

Equation 1

$$Y_{ij} = \mu + \beta_i + r_j + \varepsilon_{ij}$$

μ = is overall means; r_j = is the effect of jth replication and βi = is the effect of ith treatment

3.1.7.2 Area under disease progress curve (AUDPC)

AUDPC is a quantitative value of disease intensity over time (Mariki, 2017). It can be used to make comparisons across locations, time and management practices. It is estimated

using the trapezoidal method which is done by discretizing the variable of time which could be hours, days, weeks, months or years and find the mean of the disease intensity between the pairs of head-to-head time points (Simko and Piepho, 2012). The formula for AUDPC defined by Campbell and Madden (1990) is as shown on below:

Equation 2

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where; n = Number of consecutive reading; $Y_{i+1} =$ Average coefficient of infection of i+1th observations; $(t_{i+1} - t_i)$ = Number of days between ith and i+1th observations

3.2 DETERMINING THE NUMBER AND NATURE OF GENES CONTROLLING RESISTANCE TO *SCMV* IN F_2 MAIZE POPULATION

3.2.1 Development of the F_2 maize population used in the genetic studies

In 2016 short rains season, seeds from F_1 crosses were planted in an un-replicated field nursery at the Faculty of Agriculture Field Station' under disease free conditions. At flowering stage, the F_1s were selfed and the resultant F_2 seeds from each cob harvested separately.

3.2.2 Assessment of maize genotypes for resistance to SCMV

For genetic studies, two genotypes 384 and genotype 385 were selected (Table 3.2) because of their disease resistance based on their previous MLN scores. For each F_2 genotype, 150 seeds with their respective parents and H614D (check) were planted at the University of Nairobi Faculty of Agriculture Field Station's screen house (Table 3.2) in plastic pots measuring 28cm x 20cm x 30cm. Each pot had four plants from the same genotype. At planting, 10g of DAP fertilizer was applied per plant. For top dressing, 10mg of CAN was applied per pot. Watering was done adequately.

The SCMV inoculum was sourced from KALRO and prepared as described in section 3.1.4. The seedlings were then artificially inoculated by hand rubbing with the SCMV inoculum at seven days after germination as described in section 3.1.5 and was repeated to ensure all plants were infected and that there were no disease escapes. The virus symptoms were estimated based on the CIMMYT disease severity scale ranging from 1-5 as described in Section 3.1. Disease scoring began a week after second inoculation. The observed results in the F_2 population were categorized as either resistant (R) or Susceptible (S). Those which

displayed SCMV severity score of 3 or higher were considered susceptible while those with a score of 2 and below were considered resistant.

Table 3.2: List of maize populations used for the genetic study to identify the genes and their number for resistance to Sugarcane mosaic virus

Designation	Origin	Parentage	MLN severity score (scale of 1-5)
Population 384	Cross	UON-2015-50 × UON-2015-115	2
Population 385	Cross	UON-2015-50 × UON-2015-117	2
Parent 50	Parent	UON-2015-50	2
Parent 115	Farmer variety	UON-2015-115	2.3
Parent 117	Farmer variety	UON-2015-117	1.5
H614D	Commercial variety	Check/control	3

(Disease severity scores obtained from Sitta *et al*., 2017)

3.2.2.1 Data analysis

To explain the mode of inheritance of SCMV resistance among the maize parents, segregation data was collected by scoring for the individual responses of each maize seedlings to the disease. The individual responses to SCMV were grouped into either resistant or susceptible based on the segregation model of an F_2 population following Mendelian ratios. A chi square test (Equation 3) was done to test the goodness of fit of observed (O) segregations to expected (E) genetic ratios of resistance to susceptible (Lobo, 2008) and to establish how many genes control SCMV resistance among the F_2 derived families.

Equation 3

$$x^2 = \frac{\sum(Observed - Expected)^2}{Expected}$$

CHAPTER 4

RESULTS

4.1 ASSESSMENT OF PARENTAL MAIZE GERMPLASM FOR RESPONSE TO SCMV INFECTION

The 42 maize parents (genotypes) assessed in the screen house showed varied responses to *Sugarcane mosaic virus* infection (Table 4.1) over a period of eight weeks. For weekly disease severity scores, three genotypes namely entry UON-2015- 65, UON-2015-110 and UON-2015-63 had a value of 1 implying they were immune to the SCMV disease. Resistant responses were exhibited among 26 maize genotypes whereas the remaining 13 maize genotypes had susceptible responses. (Table 4.1). The mean weekly disease severity score at the end of the evaluation period was 2.07 with no significant difference at $p<0.05$ except for weeks 4 and 5.

For the disease incidence, showed 18 entries displayed 100% infection whereas 28 entries showed over 75% of the plants were infected. The least infected genotypes comprised of UON-2015-5 and UON-2015-21 with disease incidences of 12.5%.

The disease severity scores were used to calculate the area under disease progress curve (AUDPC) values where the categorical scale of genotypes were groups as follows; AUDPC value ≥ 70 implied resistant responses whereas AUDPC value71 ≥ 120 implied moderate resistant responses (Shah *et al*., 2020).

Table 4.1 Weekly disease severity progression, AUDPC scores and disease incidence of the different maize genotypes in the study

Entry	Entry Name	Weekly Disease Severity Progression (scale of 1-5)								Disease Incidence	AUDPC
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8 FDS		
1	UON-2015-5	1.0	1.0	1.0	1.0	1.0	1.0	2.0	2.0	12.5	49
2	UON-2015- 25	1.0	1.1	1.6	1.6	1.6	1.6	2.5	2.5	75	56
3	UON-2015- 19	1.4	1.9	2.5	2.5	2.5	2.5	3.0	3.0	100	88.8
4	UON-2015- 21	2.0	2.0	3.0	3.0	3.0	3.0	3.0	3.0	12.5	57.8
5	UON-2015- 24	1.8	2.0	2.5	2.4	2.4	2.5	2.6	2.6	50	98.4
6	UON-2015- 9	1.5	1.4	1.5	2.6	2.8	2.8	2.9	2.9	100	91.9
7	UON-2015- 26	1.4	1.0	1.0	1.1	1.5	1.5	2.0	2.0	62.5	53.4
8	UON-2015- 34	1.0	1.1	1.3	2.0	2.0	2.0	2.5	2.5	100	62.1
9	UON-2015-37	1.0	1.3	1.3	1.6	1.8	2.3	3.0	3.0	100	68.7
10	UON-2015- 39	1.6	1.9	2.4	2.5	2.5	2.5	2.8	2.8	100	80.1
11	UON-2015-41	1.5	1.5	1.5	1.5	2.0	2.0	2.0	2.0	100	53.8
12	UON-2015- 47	1.3	1.5	2.5	2.5	2.5	2.5	2.5	2.5	100	77.9
13	UON-2015- 48	1.8	1.8	1.8	2.0	2.0	2.5	2.5	2.5	100	74.8
14	UON-2015-52	1.6	1.6	1.6	1.6	1.6	1.8	1.8	1.8	75	67.8
15	UON-2015- 53	1.5	1.5	1.5	1.6	1.6	2.0	2.0	2.0	100	70
16	UON-2015- 54	1.0	1.1	1.5	1.5	1.5	1.5	1.5	1.5	75	54.7
17	UON-2015- 55	1.3	1.8	1.9	1.9	1.9	1.9	2.0	2.0	100	53.4
18	UON-2015- 56	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5	50	43.8
19	UON-2015- 57	1.0	1.1	1.3	1.5	1.5	1.5	1.5	1.5	100	56
20	UON-2015- 58	1.5	1.5	1.8	2.0	1.5	1.5	2.0	2.0	100	61.7
21	UON-2015- 59	1.5	1.6	1.8	1.9	2.0	2.0	2.0	2.0	100	43.8
22	UON-2015- 60	1.4	1.4	1.4	1.8	1.8	1.8	1.8	1.8	87.5	58.6
23	UON-2015-63	1.6	1.3	1.3	1.3	1.3	1.3	1.3	1.3	25	53.8
24	UON-2015- 65	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0	55.1
25	UON-2015- 83	1.2	1.3	1.3	1.4	1.4	1.5	1.9	1.9	37.5	56.4

Table 4.1 Weekly disease severity progression, AUDPC scores and disease incidence of the different maize genotypes in the study

Entry	Entry Name	Weekly Disease Severity Progression (scale of 1-5)								Disease Incidence	AUDPC
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8 FDS		
26	UON-2015-85	2.0	2.0	2.0	2.0	2.5	2.5	2.5	2.5	100	92.8
27	UON-2015- 86	1.0	1.0	1.6	1.6	2.1	2.1	2.1	2.1	62.5	70.4
28	UON-2015- 87	1.0	1.4	1.5	1.5	1.8	1.8	1.8	1.8	50	64.8
29	UON-2015- 89	1.0	1.5	1.5	1.5	2.0	2.0	2.0	2.0	50	70
30	UON-2015- 91	1.0	1.0	1.1	1.3	1.6	2.0	2.0	2.0	50	58.6
31	UON-2015- 92	1.1	1.4	1.4	1.4	1.4	1.4	2.0	2.0	62.5	87.1
32	UON-2015- 94	1.6	1.9	2.0	2.0	2.0	2.0	2.4	2.4	100	83.1
33	UON-2015- 99	1.3	1.4	1.4	1.8	2.3	2.0	2.3	2.3	100	74.4
34	UON-2015- 101	1.0	1.0	1.0	1.0	1.0	1.0	1.8	1.8	75	44.6
35	UON-2015- 103	1.4	1.4	1.4	1.4	1.4	1.4	1.9	1.9	87.5	59.5
36	UON-2015-105	1.1	1.3	1.3	1.3	1.3	1.3	1.8	1.8	75	54.7
37	UON-2015-108	1.3	1.3	1.4	1.4	1.5	1.5	1.8	1.8	75	59.5
38	UON-2015-110	1.0	1.0	1.1	1.1	1.1	1.1	1.1	1.1	25	45.9
39	UON-2015-112	1.1	1.1	1.6	1.6	1.6	1.6	1.8	1.8	87.5	63.9
40	UON-2015-118	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	50	62.1
41	UON-2015-120	2.0	2.0	2.5	2.5	2.5	2.5	2.5	2.5	100	99.8
42	UON-2015-122	1.9	1.9	1.9	2.0	2.0	2.0	2.5	2.5	100	82.7
	Mean	1.34	1.43	1.62	1.73	1.80	1.84	2.07	2.07	74.1	66
	L.S.D	0.76	0.79	1.01	1.05	1.29	1.37	1.45	1.45	69.85	29.71
	%C.V	28.1%	27.4%	31.0%	29.9%	35.6%	36.0%	34.6%	34.6%	46.70%	37.40%
	P-value	0.131	0.141	0.032	0.056	0.287	0.372	0.525	0.525	0.182	<.001

L.S. D= least significant differences; %CV= percentage Coefficient of Variation; FDS = final disease score; AUDPC= area under disease progress curve, P-value <5%

4.2 Performance of the F_2 Populations

Population 384 had 128 plants that were screened for eight weeks, of which 119 plants of genotypes 128 were resistant (R) and nine plants were considered susceptible (S). Population 385 had 147 plants that were evaluated of which 139 plants were considered resistant while 8 plants were considered susceptible. Segregation data revealed that the resistance to SCMV in parent UON-2015-50 was controlled by 2 genes following the genetic ratio 15:1 implying epistatic gene effect with chi square values of 0.13 and 0.13 respectively (Table 4.2 and Table 4.3).

Table 4.2 Segregation data among the F^2 derived families in population 384 (UON-2015-50 × UON-2015-115)

Responses among families	**Observed responses (counts)**	**Genetic ratios**			
		03:01	**09:07**	**13:03**	**15:01**
Resistant Families	119	5.5	30.7	2.2	0.01
Susceptible Families	9	16.5	39.4	9.4	0.13
Total Families	128				
Calculated Chi Square (X^2) value		**22.0**	**70.1**	**11.5**	**0.13**

Chi square(X^2) table value at 1d.f, $p<0.05$, $X^2 = 3.84$;

Resistance is predicted to be controlled by 2 genes with epistatic effect following 15:1 genetic ratio $X^2 = 0.13 < 3.84$

Table 4.3 Segregation data among F^2 derived families in population 385 (UON-2015-50 × UON-2015-117)

Responses among families	**Observed responses (counts)**	**Genetic ratios**			
		03:01	**09:07**	**13:03**	**15:01**
Resistant Families	139	7.6	37.8	3.4	0.01
Susceptible Families	8	22.7	49.0	14.3	0.11
Total Families	147				
Calculated Chi Square (X^2) value		**30.4**	**86.8**	**17.6**	**0.12**

Chi square(X^2) table value at 1d.f, $p<0.05$, $X^2 = 3.84$; Resistance is predicted to be controlled by 2 genes with epistatic effect following 15:1 genetic ratio $X^2 = 0.12 < 3.84$

CHAPTER 5

DISCUSSION

5.1 Response of the maize genotypes to sugarcane mosaic virus disease

When maize genotypes were assessed for their reaction to the SCMV disease, there were varied responses ranging from immune to susceptible ones. The susceptible genotypes showed irregular light green to yellowish mosaic symptoms on the leaves to chlorotic symptoms on the veins. General the entries showed a varied response in disease severity, disease incidence and AUDPC implying genetic variation among the genotypes and is useful in crop improvement as it provides a varied genetic pool that can be exploited to develop resistant lines (Karanja *et al*., 2018). Karanja *et al.* (2018) screened corn inbred lines for MLN, SCMV and MCMV resistance and reported significant variability in their response to the viruses.

Figure 5.0: Image showing maize plant with *Sugarcane mosaic virus* symptoms.

The low AUDPC values were associated with low severity scores. AUDPC is a measure of the disease progress over time and the genotypes with low disease severity and incidence scores as well as low AUDPC scores show a high level of tolerance to SCMV. Sugarcane mosaic virus is spread systemically through the plant and the mosaic symptoms were presented in the younger leaves first. During infection, the virus replicates and is translocated to the younger leaves. The mosaic on the infected leaves represented by yellowing and chlorotic symptoms show the altered structure and pigmentation of the chloroplasts which leads to reduced photosynthetic activity (Addy *et al*., 2017). When the

chloroplasts are affected, other processes such as photosynthetic efficiency and photo-assimilate accumulation are affected (Addy *et al*., 2017) resulting in reduced yields.

Karanja *et al.* (2018) recommended that when developing maize varieties with resistance to MLN, there was need to use maize germplasm with established resistance to SCMV. SCMV plays an important role in increased MLN symptoms 'expression with co-infection with MCMV. Mbega *et al.* (2016) discussed the role of potyviruses in synergism and stated that potyviruses cause enhanced infection of the plant by MCMV. HC-pro protein is an important protein that plays the key role in potyvirus infectivity, contains the mechanism that counters the host's defences against viral infection by suppressing the expression of PTGS. The potyvirus presence in a co-infection favours not only its own multiplication but also of MCMV infection. Zhao *et al.* (2016) observed that in double infection of SCMV and MCMV, there is amplified accumulation of MCMV genomic RNAs thus increased expression of its symptoms. There is however no difference observed for SCMV RNA accumulation levels in MLN infection and single virus infection. Thus, SCMV plays a critical role in synergism leading to MLN disease infection. Thus, there is need to screen for SCMV resistance on any germplasm intended to be used as sources for MLN resistance. The disease severity progression for genotype UON-2015-5 was slow as shown by the delayed symptoms' expression suggesting probability of resistance against SCMV. Long incubation periods have been associated with virus resistance among plants (Kuntze *et al.,* 1995; Karanja *et al.,* 2018). Delayed symptom expression and reduced disease incidence is dependent on the number of resistant genes present with a higher probability of major genes being involved (Kuntze *et al.,* 1995).

Immune responses were observed among the genotypes UON-2015- 65, UON-2015-110 and UON-2015-63 and this could be attributed to major gene or vertical resistance. Among the resistant genotypes with severity scores of 2 and 3, some showed 100% disease incidences with regard to their response to SCMV disease. The germplasm identified to have resistance to SCMV from this study would be ideal candidates for further screening to find resistance to MLN.

5.2 Segregation for the SCMV disease among the F2 derived families for population 384 and 385

The observed responses among the families for SCMV disease showed that the chi square corroborated with 15:1 genetic ratio. Thus the resistance to SCMV is conditioned by two non-allelic genes with epistatic gene effect. The 15:1 genetic ratio indicates presence of duplicate dominant epistasis with duplicate gene action which occurs when expression of the recessive alleles at the two loci are masked by a dominant allele at either of the two loci the (Miko, 2008). Epistasis is an interaction of alleles of two or more genes that affect the phenotypic expression of a trait. It occurs when several loci interact and a new phenotype is observed or when an allele at a locus masks expression of alleles at other loci or when an allele changes the effects of other alleles in other loci (Miko, 2008).

Xing *et al.* (2006) stated that two genes providing resistance to SCMV, *Scmv1* and *Scmv2* interacted epistatically. They stated that for a high degree of resistance to be achieved, a minimum of one resistant allele from each of the two loci must be present. This was shown when *Scmv1* which had indicated early dominant gene action became partially dominant.

Scmv2 on the other hand showed dominant gene action first then additive gene action at a late stage (Xing *et al.,* 2006).

Melchinger *et al.* (1998) observed that *Scmv1* and *Scmv2* are vital to total resistance against SCMV. However, *Scmv1* was observed to have a stronger effect after crossing resistant line D32 and susceptible line D145. Epistatic effects were observed between the two QTLs in this cross. *Scmv1* represses manifestation of SCMV symptoms throughout the development phases.

Dussle *et al.* (2000) tested 121 F_3 lines obtained from a cross of susceptible F_7 and resistant FAP1360A and also found the same QTLs to be responsible for SCMV resistance. Gene action was however additive in the *Scmv2* region and complete dominance in the *Scmv1* region. Duble *et al.* (2008) confirmed presence of two QTLs for SCMV resistance, the first on chromosome 6 (*Scm1*) with additive gene action and the other on chromosome 2 (*Scm2*) which had complete dominance.

Wu *et al.* (2007) conducted a study to identify the genetic basis in line Siyi which conferred complete resistance to *Sugarcane mosaic virus*. They observed that the parents, F_1 and F_2 and backcross populations had two complementary genes conditioning the resistance. The gene *Rscmv1* was located on chromosome 6 while *Rscmv2* was in chromosome 3 (Ding *et al*., 2012). De Souza *et al.* (2008) identified three QTLs conferring resistance to SCMV by crossing tropical lines L520 (resistant) and L19 (susceptible). They tested 150 F_2 families that were artificially inoculated in field conditions and genotyped using microsatellites markers (Simple Sequence Repeats). Multiple interval mapping was employed for QTL detection and found two QTLs (*Scm2a* and *Scm2b*) on chromosome 3 and QTL*Scm1* on

chromosome 6 (De Souza *et al*., 2008). Epistatic effects were also observed on the major QTLs on chromosome 3 and minor QTL on chromosome 6. Gene action of QTLs on chromosome 3 was noted as additive for *Scm2b* and overdominant for *Scm2a* and on chromosome 6 QTL *Scm1*, gene action was overdominant (De Souza *et al.*, 2008).

Redinbaugh *et al.* (2018) also recorded two major genes, *Scmv1* and *Scmv2* interacting epistatically are required simultaneously for the whole resistance to SCMV although *Scmv1* offers resistance through all growth phases while *Scmv2* is effective later. A single gene is insufficient to offer resistance to SCMV and other *Maize dwarf mosaic virus* (Redinbaugh *et al.,* 2018). Xia *et al*. (1999) observed other resistance QTLs in chromosomes 1, 5 and 10.

Awata *et al* (2019) found seven major QTL for resistance to MLN across 7-biparental populations through linkage mapping and joint association mapping. The QTLs were found to be stable across environments and genetic backgrounds. CIMMYT in Kenya in collaboration with KALRO, Kenya Seed Company, Western Seed Company and Seed Co. Limited released 18 first and second generation hybrids. Twelve hybrids have a severity score of 4 on the MLN scale of 1-9 and two have a score of 3. A score of 4.0 and below denotes resistance to MLN.

Many QTLs have been found that provide resistance to SCMV and other potyviruses in varying populations and environments but the findings differ because different populations, dissimilar genetic markers, different experimental designs and statistical methods are employed (Lu *et al*., 2008).

CHAPTER 6
GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Maize lethal necrosis is a major concern in maize producing regions in East Africa, being responsible for losses of up to 100% dependent on the phase of infection (Ritte *et al.*, 2017), /and therefore posing a food security challenge for the region as many households depend on maize for food and income generation. *Sugarcane mosaic virus* was reported in Kenya in 1980 (Louie, 1989). However, it's co-infection with MCMV (Karanja *et al.*, 2018) necessitates identification of maize germplasm that are resistant to SCMV and MCMV.

Under artificial SCMV disease infection, the study identified maize genotypes with immune responses namely UON-2015- 65, UON-2015-110 and UON-2015-63 and this could be attributed to major gene or vertical resistance. Also, 18 genotypes showed resistant responses coupled with low disease severity scores and AUDPC scores hence their potential for use as promising lines for development of MLN resistant varieties. More assessment of these lines for their response to MCMV and MLN disease could offer decisive information for their successful deployment in breeding programs.

Promising populations with the line UON-2015-50 which was found resistant to MLN in previous research revealed the role of two genes with epistatic gene effect in conditioning the resistance to SCMV. The 15:1 genetic ratio indicates presence of duplicate dominant epistasis with duplicate gene action which occurs when expression of the recessive alleles at the two loci are masked by a dominant allele at either of the two loci the (Miko, 2008). Xing *et al.* (2006) found two genes providing resistance to SCMV, *Scmv1* and *Scmv2*

interacted epistatically. They stated that for a high level of resistance to be achieved, a minimum of one resistant allele from each of the two loci must be present. This was shown when *Scmv1* which had indicated early dominant gene action became partially dominant. *Scmv2* on the other hand showed dominant gene action first then additive gene action at a later stage (Xing *et al.,* 2006). The exact nature and mode of the resistance genes established in this genotype could guide on the right breeding method to exploit in developing superior maize varieties with resistance to SCMV.

6.2 RECOMMENDATIONS

Following this study, the following is recommended:

i. Germplasm with resistance to *Sugarcane mosaic virus* identified could be used to develop MLN resistant maize varieties to reduce maize yield losses in Kenya
ii. Further research could be done to test the resistant lines for their resistance to MCMV and by extension to MLN disease
iii. The Maize UON-2015- 50 which has resistance to SCMV could be integrated in maize breeding projects to develop superior varieties

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