Breeding Investigations for Developing Durable Resistance to Maize Lethal Necrosis Disease (MLND) and its Causal Viruses in Kenya

By

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ABSTRACT

Maize is an important staple food in Kenya, grown in almost all agro-ecological zones, and accounts for about 40% of daily calories. The country produces an average of 3.51 million tons of maize grain (39 million x 90 kg-bags) which does not match the demand. The production is limited by emerging pests and diseases, such as fall army worm (FAW) and maize lethal necrosis (MLN) disease. The MLN disease is caused by synergisim between maize chlorotic mottle virus (MCMV) and sugar cane mosaic virus (SCMV). The FAW and MLN cause serious challenges for maize production due to the lack of suitable cultural control methods and tolerant maize germplasm. As a result, farmers and government agencies have challenged maize breeders to develop varieties that are resilient to climage change. This study focused on **breeding investigations for developing durable resistance to maize lethal necrosis disease and its causal viruses in Kenya.** The specific objectives were to 1) identify maize germplasm lines that are tolerant to MLN and its causative viruses (SCMV and MCMV) for use in making hybrid combinations; 2) to understand the genetic divergence and background of these genotypes to aid in developing high yielding and stable MLN resistant maize hybrids; and 3) to assess the impact of MLN, SCMV, and MCMV on maize production in Kenya. Experiments were conducted in Kenya during the 2015, 2016, 2017 and 2018 seasons in both screen house and open fields. Diallel crosses were used to characterize maize inbred lines, from CIMMYT, KALRO, and Ohio, for reactions to SCMV, MCMV and MLN disease. A set of selected and pre-commercial maize hybrids were used to assess MLN impact on maize production in Kenya. The study revealed that MLN is important for maize production, with high incidence and severity levels observed in all commercial maize varieties in Kenya. Disease severity, yield and its attributes significantly varied ($P < 0.05$) at V_3, V_7 and V_T inoculation stages. The greatest effect was observed at V_3 stage with increased number of rotten cobs observed at V_T. Percentage yield loss was proportional to the percentage of disease incidence, severity and effect on yield attributes which varied from variety and season. This calls for the development and deployment of improved maize hybrids with MLN tolerance/resistance in the MLN-prone areas to enhance maize production. The study further identified eight inbred lines with low disease severity < 3.0 at 56dpi indicating their tolerance/resistane to SCMV, MCMV and MLN. The maize inbred lines MLN001 and MLN006, displayed high levels of resistance to MCMV, while MLN042 and MLN041 had the highest resistance to SCMV. Strong sources of MLN resistance, such as MLN013, MLN019, N211, and KS23-6, should be used in developing MLN-resistant

hybrids. Inbred lines CML312, CML442, MLN013, N211 and MLN 019 are identified as good combiners for yield and earliness. The nature of gene effects was established as one including additive, and that genotype x location effects were significant for conditioning MLN resistance in maize hybrids. The presence and impact of SCMV and MCMV viruses in the field need to be investigated to aid in developing more robust MLN resistant varieties. MNL001, MLN013, MLN018, and MLN019 and their derived crosses need to be evaluated across maize agro-ecologies and seasons to understand MLN disease, the role of environment and the interaction of MCMV and Potyviruses.

DECLARATION

I, James Kamau Karanja, declare that:

The research reported in this thesis, except where otherwise indicated, and is my original research.

1. This thesis has not been submitted for any degree or examination at any other university.
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Signed:

……Date……

KARANJA James Karanja

As the candidate's supervisors we agree to the submission of this thesis

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Finally, I express sincere thanks to my family for their immense concern, love, patience, encouragement and prayers during this study.

THANK YOU ALL.

DEDICATION

This work is dedicated to my grandmother Hannah Wangui MUGO,

To my mother Mary Njeri MUGO,

To my wife Elizabeth

And

To our children, Fredrick, Kelvin, Sylvia, Joseph, Emmanuel and Rose.

Their love, support, guidance and prayers made all this possible

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INTRODUCTION TO THESIS

1. Background

Maize, *Zea mays* L., is an important staple crop grown widely by smallholder farmers in East Africa. Studies show that about 90% of the Kenyan population depends on maize for food, income, and employment. For instance, in 2017, the area under maize production was 2,266,196 ha which amounted to 4 mt (Ministry of Agriculture, 2017). The national average maize yields are estimated at 1.8 t ha^{-1} (20 bags of 90-kilogram bags). These yields are about 20% of those attained internationally in countries such as Argentina. In the early 80s, the maize yields started to increase following the adoption of hybrid maize varieties and the accompanying high fertilizer use to the extent that by 1986, the average national yields were over 2 t ha^{-1} (Nyoro, 2004). However, this growth was not sustained. Yields started to fall gradually and stagnated at 1.9 t ha^{-1} by the end of 2018 (Kang'ethe, 2011; Kirimi *et al*., 2017; FAOSTAT, 2018). Some of the farmers in the high potential maize growing zones in the Rift Valley have achieved between 4 and 6 t ha^{-1} representing only 50% (or less) of the genetic potentials of the hybrids (One Acre Fund, 2015; Schroeder *et al*., 2013). Highland maize varieties are grown on some 40-50% of the total maize area, representing 600,000 - 800,000 ha. Since almost all the arable land is under cultivation in Kenya, future increase in maize production will heavily depend on technical efficiency and yield improvement to support intensive production rather than expansion in the area under production (Kariuki *et al*., 2020). The government policy on the maize subsector is to increase production so that self-sufficiency and food security can be achieved. One way of realizing this goal is to increase farm output by improving technical efficiency and access to crop varieties.

In Kenya, hybrid maize accounts for 70-80% of total maize seed demand and is completely within the formal sector which is produced by both public and private entities. The average age of maize hybrids utilized in Kenya is >15 years, with heavy reliance upon varieties released from the 1980s (Munyua *et al*., 2010; Marenya *et al.*, 2021). Open-pollinated maize varieties (OPVs) account for 20-30% of total maize demand, with ~10% produced formally and 20% informally. Private seed company involvement in OPV production remains low, with the Kenya Agricultural and Livestock Research Organization (KALRO) Seed Unit (KSU) holding a significant market share. Demand for maize hybrid seed is high in the high, medium, and mid-altitude regions, with >90% adoption, while OPVs are left for the lower, dry, and coastal regions (Karanja *et al*., 2020).

Despite the economic importance of maize, the crop faces several constraints in its production. Yield losses of 60%, for example, have been attributed to biotic factors, such as stem borers (Kfir *et al*., 2002), fall army worm, weeds such as Striga (Khan *et al.,* 2004), and diseases like maize streak, gray leaf spot, *turcicum* leaf blight (Martin *et al.,* 2001), and maize lethal necrosis (Karanja *et al.,* 2018). Abiotic stresses include drought, heat, soil acidity, and low soil nitrogen (Ministry of Agriculture, 2017).

Maize lethal necrosis (MLN) was reported in Bomet County in 2011 (Wangai *et al*., 2012) and later spread to Eastern, Western and Central parts of Kenya (Joint Assessment Report, 2012). MLN was later reported in Tanzania, Uganda, Southern Sudan (FAOSTAT, 2018), Rwanda (Adams *et al*., 2014), and in Congo (Lukanda *et al*., 2014). Following this rapid spread across eastern and central Africa, maize lethal necrosis (MLN) disease gained importance and was considered a priority disease threatening maize production in Africa. Since its first report in Kenya's Rift valley in Bomet County, successive studies and surveys were carried out, and results showed that the disease is distributed across all the major maize growing areas in Kenya. The level of damage varied from season to season, locations, and agro-ecologies, with the most serious damage being recorded in Bomet, Baringo, Subukia, Naivasha, Kisii, and Molo regions (Wanaga *et al*., 2012).

The spread of MLN in central and eastern Africa has been facilitated by seed, continuous maize cropping, especially with a bimodal rainfall pattern. This leads to a constant build-up of virus inoculum. The spread is also caused by seed contamination by MLN-causing viruses, especially Maize chlorotic mottle virus (MCMV), insect vectors, and growing susceptible varieties.

In curbing further spread and management of MLN, farmers, breeders, regulators, seed growers, and merchants were advised by the MLN task force to adhere to the following:

i) Improving the cropping system diversification, especially by introducing grain legumes. MLN does not occur on leguminous crops, so farmers are advised to avoid growing maize after maize but rather diversify their farm enterprise by planting different crops each season. Crop rotation for at least two seasons with alternative non-cereal crops such as potatoes (*Solanum tuberosum*), sweet potatoes (*Ipomoea batatas*), cassava (*Manihot esculenta*), beans (*Phaseolus vulgaris*), bulb onions (*Allium cepa*), spring onions (*Allium fistulosum*), and garlic (*Allium*

sativum) will diversify farm enterprises. Manure and basal/top dressing fertilizers can be applied to boost plant vigour.

ii) Strengthening MLN surveillance and diagnostic capacity at farm level. Farmers are advised not to plant a new maize crop near an infected field. Wind-blown insect vectors can transmit the disease from the infected field to the new crop. Keeping adequate isolation from MLN-infected fields can prevent the spread of the disease. Maize planting at the onset of the main rainy season, rather than during the short rain season, creates a break between maize crops and interrupts the disease cycle. Immediately remove diseased plants from your fields. Infected plants should be removed from the field and fed to livestock. However, infected ears or grains should not be consumed by humans or animals but rather burned, for they may contain secondary fungal infections and harmful mycotoxins.

iii) Managing the insect vectors and virus co-hosts. Weeding the fields regularly eliminate alternate hosts for insect vectors, using appropriate insecticide (at weekly intervals) to control vectors, keeping unnecessary machines/people out of the field. Chemicals should target soilborne and early season vectors and combine long residual and fast-acting control agents to achieve faster knockdown and longer protection.

iv) Individual plants with MCMV or sugar cane mosaic virus (SCMV) alone show milder symptoms; therefore, seed production fields must be carefully inspected and plants that appear infected removed immediately. Practicing MLN-free seed production through compulsory phytosanitary seed testing, rigorous disease management practices in seed production plots, and safe exchange of certified seeds.

v) Developing and deploying MLN tolerant/resistant maize varieties, ultimately would be the most effective means of managing MLN.

Efforts towards the development of tolerant/resistant maize germplasm have seen over 200,000 diverse maize germplasm screened against MLN under artificial inoculation at the KALRO-CIMMYT screening facility established in 2014 at KALRO-Naivasha. Of these, 63% are from CIMMYT, 16% from the National Agriculture Research System (NARS), and 21% from the private sector (Prasanna *et al*., 2020).

2. Rationale for research focus

MLN stands out as one of the significant threats to African food security. In addition to the loss of farmer harvests, MLN affects investment in seed production, loss of animal feed, and increased risk of aflatoxin due to increased maize rots. The number of strains or isolates for MCMV that are in Kenya that could be combining with SCMV to cause the MLN disease symptoms observed in maize fields are not known. The disease has been reported in almost all the major maize growing agro-ecological zones in Kenya. However, the relationship between the disease and other environmental factors is not known. Currently, there is no single method of control for MLN. All commercial varieties are susceptible to the disease and, therefore, need replacement. The use of tolerant or resistant varieties would be the ultimate and the most effective means of managing the disease. Breeding for resistance to MCMV in tropical maize seed stocks would provide the best control for this disease. This would be feasible. According to Nelson *et al*. (2004), trials performed in Hawaii found many tropical inbred lines and varieties highly resistant to MCMV. Thirty (30) out of 40 (75%) of University of Hawaii-bred field maize inbred lines tested positive for resistance. However, no complete immunity to MLN was observed. In Kenya, almost all commercial hybrids are currently highly susceptible to MLN (Karanja *et al*., 2020). Reaction and response of maize inbred lines and hybrids to SCMV and MCMV virus is not well understood. This means that yield losses may be going unnoticed since the symptoms of infection with SCMV and MCMV alone are usually not very dramatic compared to thosed caused by the two viruses. In the long run, the deployment of varieties that are resistant to both MCMV and SCMV will be the best means of managing MLN. Through conventional breeding, resistance to SCMV and MCMV can be incorporated into the susceptible maize varieties within a 3-years period. However, little has been done to determine the type of resistance expressed by the promising hybrids and inbred lines in Kenya. Resistant hybrids often become infected upon inoculation in the greenhouse and fields, but the symptoms appearance is usually delayed (Jones and Tolin, 1972; Scott *et al*., 2015). There is a need to compare the relative virus concentration in the susceptible and resistant accession by dilution end point assay. This will help in understanding pathogen colonization, their interaction and the degradation of defence systems in host plants in relation to disease progression.

This study was designed to validate and understand the inconsistency in tolerance/resistance for the maize accessions evaluated in Olerai and Narok. In addition, the results will guide further studies to understand the defensive mechanisms in MLN host plants. Preliminary

inheritance studies on the inheritance of traits suggest polygenic control of the disease, with resistance being recessive. It is believed that an approach that integrates all these options could provide information to be used in designing an effective way to control MLN. This will include developing suitable maize varieties with MLN tolerance or resistance and other effective preventive measures.

3. Research objectives

Overall objective

The breeding investigations were aimed at identifying the sources of resistance to MLN and its causative viruses, quantify yield losses, assess the performance of MLN tolerant crosses, and establish the mode of resistance and its inheritance in maize hybrids. The information would be useful to develop improved MLN resistant maize varieties.

Specific objectives

The following specific objectives were pursued to:

1. Determine the level of resistance of selected maize germplasm to mechanical inoculation with MCMV and SCMV individually and combined (MLN).
2. Determine the yield loss caused by MCMV, SCMV, and MLN in selected maize germplasm
3. Assess grain yield and stability, and MLN tolerance in F_1 maize hybrids developed from a 12 x 12 maize half-diallel cross.
4. Determine the mode of inheritance for MLN resistance gene to MCMV

4. Research Hypotheses

To achieve high genetic gains with the available maize germplasm and to improve resistance to field protection levels, the following assumptions were made:

1. Selected maize inbred lines have genes for resistance to SCMV, MCMV and MLN which can be transmitted effectively to progeny, and that disease progress is uniform among genotypes
2. SCMV, MCMV and MLN are of economic importance and need individual attention

3. The selected inbred lines have stable performance across the target regions, which can be transmitted to stable and highly adapted F_1 hybrids and hybrids with high MLN resistance are high yielding and have stable yield performance and wide adaptation
4. Inheritance of MCMV resistance is controlled by simple mechanisms that can be introgressed into susceptible backgrounds and/or stacked into an elite maize line for hybrid development

5. Outline of thesis

The specific objectives mentioned in the foregoing were achieved and addressed in the various chapters that constitute this thesis. Each chapter is an independent, potential manuscript or already published in refereed journal, and as such, overlapping of content and reference may be inevitable. The referencing system used in the chapters is based on the Journals of the Open Agriculture Journal, Journal of Food, Agriculture and Environment, and Journal of the Science of Food and Agriculture. The work in Chapter 2 and Chapter 3 have been published as follows:

1. Karanja, J, J. Derera, A. Gubba, S. Mugo, A. Wangai, 2018. Response of selected maize inbred germplasm to maize lethal necrosis disease and its causative viruses (sugarcane mosaic virus and maize chlorotic mottle virus) in Kenya. The Open Agriculture Journal DOI: 10.2174/1874331501812010215, 2018, *12*, 215-226 www.benthamopen.com/TOASJ/
2. Karanja J, J. Derera, A. Gubba, S. Mugo and A. Wangai, 2020. Effect of maize lethal necrosis and its causative viruses (maize chlorotic mottle virus and sugar cane mosaic virus) on the growth and yield of maize as influenced by varietal tolerance/susceptible levels and plant stage at time of inoculation. Journal of Food, Agriculture & Environment Vol.18 (1) : 2 3 - 2 9.

The publication for the work in Chapters 4 and 5 is underway.

The chapters are divided as follows:

1. Introduction to thesis.
2. Chapter 1: Literature review.
3. Chapter 2: Response of Selected Maize Inbred Germplasm to Maize Lethal Necrosis Disease and its Causative Viruses (sugarcane mosaic virus and maize chlorotic mottle virus) in Kenya.

4. Chapter 3: Effect of maize lethal necrosis and its causative viruses (maize chlorotic mottle virus and sugar cane mosaic virus) on the growth and yield of maize as influenced by varietal tolerance/susceptible levels and plant stage at time of inoculation.
5. Chapter 4: Genotype x environment interaction and stability analysis of half-diallel crosses in maize
6. Chapter 5: Heritability and gene effects controlling MLN resistance in selected maize germplasm
7. Chapter 6: General Overview

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CHAPTER 1

Literature review

1.1 Introduction

This review covers aspects relevant to maize research with emphasis on disease tolerance/resistance breeding in tropical maize germplasm to create a frame of reference for the research study. The importance of maize diseases, primarily maize lethal necrosis (MLN) disease, is discussed, including control and breeding strategies to curb the disease. Combining ability analyses, stability analyses, mode of gene action, and economic importance of the disease and its causative viruses are also reviewed.

1.2 Importance of maize

Maize (*Zea mays* L.) belongs to the grass family Poaceae and tribe Maydeae. The maize plant has wide adaptability in the different growing conditions. Maize together with wheat (*Triticum* aestivum L.) and rice, *Oryza sativa* L.), are the three most important cereal crops in the world. Prior to the British colonization, the Portuguese had already introduced maize to the Kenyan coast, where it became common among Arab traders. The domestic demand for maize grew as Kenyans went to work on settler farms, in mines, and industrial plants (Mosley, 1983; Jansen, 1977). Maize gradually became a staple food in the Kenyan diet which was previously dominated by finger millets, (*Eleusine coracana* L.) , sorghum (*Sorghum bicolor* L.), tubers, and legumes commonly found in Kenyan traditional farming systems. For urban populations, maize "received a boost" over native millet and sorghum in the 1920s with the introduction of the hammer mill. The prevalence of large-scale, industrial processors contributed to preferences for the dent type (Jansen, 1977).

In 1955, the first "scientific" maize research program began in Kitale, "the center of maize production in the White Highlands." The chief maize breeder, M. N. Harrison, was funded by the Rockefeller Foundation to visit Mexico and Colombia in 1958 and brought maize seed back with him. This resulted in the first "modern" improved maize variety released in 1961 from a combination of Kenyan and Ecudorian germplasm. Since then, maize has remained a major crop in the high potential areas (highland tropics and moist transitional zone) where hybrids are adopted by over 90% of the farmers and account for a large proportion of the maize area planted (Njoroge *et al*., 1997). A large proportion of small- holder farmers in

marginal areas still use local varieties and prefer improved open-pollinated varieties (OPVs) over hybrids. It has, however, been shown that well-adapted maize hybrids could perform profitably in terms of yield and yield stability even in marginal production environments under low input conditions (Hassan *et al*., 1998; Mugo *et al*., 2002).

In Kenya, agriculture is and will continue to be the cornerstone of the economy for a considerable time in the future. The sector accounts for 31.5% of the country's GDP, 75% of the labour force and over 50% of total revenue from exports and provides 18% and 60% of formal and total employment, respectively (Kariuki *et al*., 2020). However, over the last five years, agricultural sector growth has been on a downward trend from 5.4% in 2013 to 1.6% in 2017, leading to a decrease in food in the country. Notably, the production of maize has decreased from 40.7 million bags in 2013 to 35.8 million bags in 2017, significantly lower than the national consumption of 45 million bags per annum (Ministry of Agriculture, 2017). This is because Kenya's food production has been the burden of small-scale farmers who produce 75% of the total maize and the remaining 25 % by large scale commercial producers. Small scale producers mainly grow the crop for subsistence, retaining up to about 58% of their total output for household consumption (Mbithi and Huylenbroeck, 2000; Export Processing Zone Authority, 2005).

Maize is the main staple food crop of over 85% of the population in Kenya, comprising a significant part of the diet of millions of people. The per capita consumption ranges between 98 to 100 kilograms which translates to at least 2700 thousand metric tonnes, per year (Nyoro *et al.*, 2004; Keya and Rubaihayo, 2013). Over 38% of the food crop producers in Kenya grow the maize (Republic of Kenya, 2003a). The trend in total maize output in Kenya for the period 1981 to 2018 (Fig. 1.1), reveals that output has increased slowly from about 1.5 Million tonnes in 1980 to 4.2 million tonnes in 2018, but with significant fluctuation over the years.

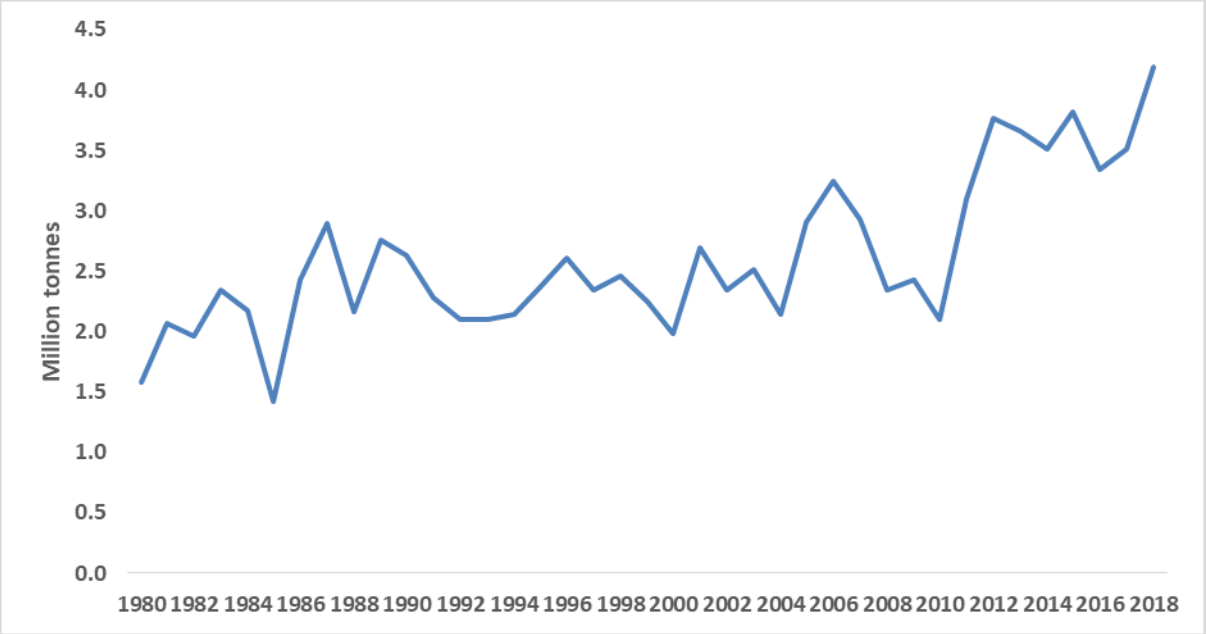

Figure 1.1: Maize production (MT) in Kenya (1981 – 2018); Data source: Republic of Kenya. Economic Surveys (various issues). Nairobi: Government Printer

The total land area under maize production is about 1.5 million hectares (ha), with an annual average production estimated at 3.0 million metric tonnes, giving a national mean yield of 2 tha^{-1}. Although breeders and agronomists have developed high yielding maize, its production has not kept pace with the population increase. Crop protection through entomology, pathology, and weed science has identified disease and pest problems. Research has over the years generated a good number of maize hybrids with the potential for increasing food production and rural incomes. Typically, grain yields range from 4-6 tha^{-1} in the high potential highlands of Kenya, representing only about 50% of the genetic potentials of 10-12 tha^{-1}. Highland maize varieties, however, are grown on some 40-50% of the total maize area, representing 600,000 - 800,000 ha. In the other regions, production stands at 1.5- 2tn ha^{-1} (De Groote and Mugo 2005; FAOSTAT, 2017).

The decline in maize production is occasioned by a number of factors, such as drought, limited agricultural land expansion, low and declining soil fertility, inadequate use and delayed supply of quality seeds, high fertilizer cost and pests such as the fall army worms. Other constraints include prolonged rainfall during harvest, contributing to high post-harvest losses and lack of ready markets (Ministry of Agriculture, 2017). The incidence and severity of most of these pests and diseases can be reduced by chemical control methods ranging from

seed dressing to foliar spraying, but host plant resistance provides the most economical management option.

The present study focuses on maize lethal necrosis disease in ensuring maize productivity growth of 10% per year by intensifying production in endemic areas.

1.3 Maize lethal necrosis disease

1.3.1 Biology and distribution of MLN

Maize lethal necrosis (MLN) disease was first reported in Kansas, USA, in 1976 (Niblett and Claflin, 1978). The disease is caused by double infection of maize plants by Maize chlorotic mottle virus (MCMV) (Machlomovirus: Tombusviridae) in combination with any of the several viruses in the Potyviridae group, sugarcane mosaic virus (SMV) (Potyvirus: Potyviridae), maize dwarf mosaic virus (MDMV) or wheat streak mosaic virus (WSMV). Maize is usually susceptible to MLN at all stages of its growth, with the component viruses being transferred from plant-to-plant and field-to-field by insect vectors (Nault *et al*., 1978; Redinbaugh and Stewart, 2018). MLN disease-causing viruses are transmitted by vectors such as Thrips (*Frankliniellawilliamsi* Hood) and beetles for MCMV (CIMMYT, 2012; Nault *et al*., 1978), and aphids for SCMV (Brandes 1920; Pemberton and Charpentier 1969). Transmission of MCMV via seed from infected plants is normally very low, about 0.04-0.17% (CIMMYT, 2012; Jensen *et al*. 1991, Karanja *et al*., 2020). Before MCMV had spread to other islands in Hawaii, it had been controlled for several years in the island of Kaua'i through crop rotation (Nelson *et al*., 2011).

MLN disease outbreak in east and central Africa was first reported in September 2011, at lower elevations (1900m ASL) in Longisa Division of Bomet County, Southern Rift Valley of Kenya (Wangai *et al*., 2012). Later the disease was noted in Bomet Central Division, spreading into the neighbouring Chepalungu, Narok South, North and Naivasha sub-counties. Farmers in Bomet County called it, '*Koroito*' a vernacular name for a plague which indicates a sudden phenomenon that could not be explained, cause unknown, and which has a devastating effect to the community and with no cure. By April 2012, the disease was reported in altitudes of 1279 to 2300 masl and in various parts of the country (Wangai *et al*., 2012, Karanja *et al.*, 2018). Since then, the disease has spread to other parts of the country as well as neighbouring countries including; Tanzania, Uganda, Rwanda, South Sudan, DR

Congo and Ethiopia (Mahuku *et al*., 2015, b; Adams *et al*., 2013 Lukanda *et al*., 2014). The disease has further spread to Taiwan, Ecuador and Spain (Deng *et al*., 2014; Quito-Avila *et al*., 2016; Achon *et al*., 2017). The outbreak of MLN has been a serious challenge to maize production and still poses a big threat to food security, primarily due to the introduction of MCMV (Prasanna *et al*., 2020).

Other host plants for MCMV and SCMV include velvet crabgrass (*Digitaria velutina* L.), couch grass (*Digitaria abyssinica* L.), star grass (*Cynodon dactylon* L.), kikuyu grass (*Pennisetum clandestinum* L.), Sugarcane (*Saccharum officinarum* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.) and signal grass (*Brachiaria brizantha* L.). MCMV has also been detected in Nut grass (*Cyperus rotundus* L.) and Napier grass (*Pennisetum purpureum* L.) (Kusia *et al*., 2014).

1.3.2 Economic importance of maize lethal necrosis disease

In Kansas, crop losses due to corn lethal necrosis (CLN) was estimated to be 50-90% (Niblett and Claflin, 1978; Uyemoto *et al*., 1980) depending on the variety of maize and the year. In Peru, losses in floury and sweet corn maize varieties due to maize chlorotic mottle virus have been reported to an average of 10 to 15% (Hebertt and Castillo, 1974). MLN emerged as a serious threat to maize production and the livelihoods of smallholders in eastern Africa since 2011, primarily due to the introduction of MCMV. MLN nearly affected 26,000 ha by mid-2013, an estimated loss of 57,000 tonnes of maize worth $23.5 million. The disease has a serious implication in the counties of Trans-Nzoia, which produces 60% of the country's maize, Bomet, Uasin Gishu, Nakuru, Narok, Kisii, Nyamira, Meru, Embu, Busia, Murang'a, Kirinyaga, and Kericho. Field observations indicated that the disease was affecting all maize varieties grown in these regions (Ministry of Agriculture surveillance report, 2012). MLN and its causative viruses have a serious impact on maize production and grain yields in eastern Africa (De Groote *et al*., 2016; Marenya *et a*l., 2018; Karanja *et al*., 2020). A survey conducted in 2015/2016, indicated that MLN incidence was at 35 to 90%, prevalence, 44 to 72%, and symptoms severity 3.0 -7.0 on a 1-9 severity scale (Karanja *et al*., 2018; Prasanna *et al*., 2020). The disease is still a major threat to the maize crops in eastern Africa, and its threat still looms in Sub-Saharan Africa regions (Isabirye and Rwomushana, 2016).

In Kenya, maize deficit has led to increased importation of maize from countries such as Zambia and Malawi, where the disease has not been reported, which has led to further

increases in food prices. Other effects associated with the disease include food and economic security threatened, losses for seed producers (for both growers and seed companies), millers (both in quality and quantity), transporters (reduced volume of business), storage facilities (dormant capacity), middlemen all resulting to loss of business volumes. The disease has greatly affected food security, reduction on farmers' income, increased seed cost, uncertainty on period it will take for farmers to receive effective, affordable, and sustainable control measures, and difficulties for communities to shift from their dietary preference of maize in Kenya.

1.3.3 Disease symptoms

MLN symptoms expression depend on the viruses infecting the crop, concentration of the viruses, host plant infected, time and stage of infection in the crop growth, prevailing environmental conditions, and agronomic factors. Symptoms of maize chlorotic mottle virus (MCMV) in maize include; relatively mild chlorotic mottle to severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and/or shortened, malformed, partially filled ears (Castillo and Herbert, 1974; Castillo Loayza, 1977; Niblett and Caflin, 1978; Uyemoto *et al*., 1981). When MCMV co-infects maize with a potyvirus, the infected plants in the field show a diverse range of symptoms. These include chlorotic mottling of the leaves, usually starting from the base of the young leaves in the whorl and extending upwards toward the leaf tips. The leaves can experience necrosis at the leaf margins that progress to the mid-rib resulting in the drying of the whole leaf. If there is necrosis of young leaves in the whorl before expansion, then 'dead heart' symptoms will be visible. Other symptoms include premature aging of the plants, mild to severe leaf mottling, severe yellowing and leaf drying from the edges, stunting and, sterility in tassle, lack of or only a few grains in the cob, malformed or rotten grains and cobs (Fig. 2.2). The entire crop can frequently be killed before flowering (premature plant death) (Niblett and Claflin, 1978; Uyemoto *et a*l., 1980, 1981; Karanja *et al*., 2020; https://mln.cimmyt.org/).

1.3.4 MLN causative viruses

1.3.4.1 Maize chlorotic mottle virus (MCMV)

Maize chlorotic mottle virus (MCMV) is a positive-sense single-stranded RNA virus, and the sole and type member of the *Machlomovirus* genus in the *Tombusviridae* family which encompasses 16 genera of viruses that infect a broad range of plant hosts (23). The virus was first described in Peru (CABI, 2014), then reported shortly afterward in Nebraska, Brazil,

Argentina and Thailand (Uyemoto, 1983, Teyssandier *et al*., 1982; Klinkong and Sutabutra, 1982). Within the past decade, MCMV has spread globally, with first reports in China, Taiwan, Mexico, Ecuador, Spain, Kenya, Uganda, Tanzania, Rwanda, D.R Congo, Ethiopia, Spain, and Ecuador (Jiang *et al*., 1992; Scheets 2004; Xie *et al*., 2011; Wangai *et al*., 2012; Adam *et al*., 2013; Deng *et al*., 2014; Lukanda *et al*., 2014; Mahuku *et al*., 2015; Quito-Avila *et al*., 2016).

Maize is the major natural host of MCMV. MCMV has a compact positive-sense RNA (+RNA) genome of 4.4 kb that is neither capped nor polyadenylated, which generates 1.4 kb and 0.37 kb sub-genomic RNAs during infection (Scheets, 2000; Nutter *et al*., 1989). Full-length MCMV genome sequences are available for North American, Chinese, and East African isolates, but until now, there have been no data for South American and Hawaiian isolates. The virus particle is very stable and can retain infectivity at 20°C for over 30 days, with thermal inactivation at 80-5^{o}C (Uyemoto, 1981). Different isolates of MCMV have been reported, for example, MCMV-P (Peru), MCMV-KS (Kansas), and MCMV-YN (Yunnan), and different unconfirmed strains have been suspected in some parts of Africa, including Nigeria, Rwanda, Sao Tome and Principe, Tanzania, Togo, Zambia and Zimbabwe (Uyemoto *et al*., 1980; Scheets, 1998; Nelson *et al*., 2011; Misra and Sharma, 2011; Xie *et al*., 2011,).

MCMV can spread through soil, water, seed, mechanical transmission, and is semi-persistently vectored by chrysomelid beetles and thrips (*Frankliniella*) (Nault *et al*., 1978; Jensen 1985 & 1991; Cabanas *et al*., 2013; Zhao *et al*., 2014; Mahuku *et al.*, 2015). MCMV interacts synergistically with members of the *Potyviridae* family, the potyviruses sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV), and the tritimovirus, wheat streak mosaic virus (WSMV). The interaction causes a more aggressive condition known as maize lethal necrosis (MLN).

1.3.4.2 Potyviruses

1.3.4.2.1 Sugarcane Mosaic Virus *(SCMV)*

Potyviruses have an approximately 10-kb+RNAgenome encapsulated in a flexuous rod-shaped virion approximately 700–900 nm in length and 11–13 nm in diameter (Wangai *et al*., 2012; Redinbaugh and Stewart 2018). SCMV is one of the major viruses in the genus *Potyvirus*, family Potyviridae. The SCMV species are divided into four subgroups based on sequence variability (Gao *et al*., 2011).

Various organisms of the order *Hemiptera* have been reported to mechanically transmit SCMV in a non-persistent manner through sap and cuttings is also effective (Misra and Sharma and, 2011; Mahuku *et a*l., 2015; Kiruwa *et al.*, 2016). Among the vectors, aphids including *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* and *Schizaphis graminum* are the most prevalent. However, the transmission efficiency varies greatly depending upon environmental conditions, virus strains, and host plants (Misra and Sharma and, 2011). They can feed on SCMV infected maize plant for an acquisition access period of about 20-30 seconds and transmit the virus into a healthy plant in a non-persistent (stylet-borne transmission) manner within 1-2 minutes inoculation access period (Misra and Sharma and, 2011). The spread of the virus is enhanced when the aphid over-winter on infected weed hosts and transmits the virus to maize plants. The virus can also spread to a long distance when the aphid vector is carried from one location to another by the wind. SCMV can also be transmitted by seed at low rates. It can also infect grass and other cereal hosts, such as sorghum, Johnson grass, and sugarcane. The symptoms are most easily seen in young rapidly growing leaves and the symptoms tend to fade as the leaves age (Grisham, 2000). Infected plants develop distinct mosaic irregularities in the distribution of normal green color on the youngest leaf bases. Sometimes the mosaic appearance is enhanced by narrow chlorotic streaks extending parallel to the veins. Later on, the youngest leaves show a general chlorosis, and streaks are larger and more abundant. As plants approach maturity, the foliage can turn purple or purple-red. Depending on the time of infection, there may be severe stunting of the plant. Plants infected early may produce nubbins or be totally barren (Deng *et al*., 2014; https://mln.cimmyt.org/).

SCMV has been reported as a co-infecting virus resulting to MLN in China, East Africa, and Spain and Ecuador (Mahuku *et al*., 2015; Quito-Avila *et al*., 2016; Wang *et al*., 2017). The spread of MLN reflects the spread of MCMV since SCMV has been present in East and central Africa, China and South America for decades (Louie 1980; Chen *et al*., 2002; Perera *et al*., 2009).

1.3.4.2.2 Wheat Streak Mosaic Virus (WSMV)

WSMV is one of viruses in genus *Tritimovirus*, family Potyvirideae (Liu *et al*., 2002). It is single stranded positive sense RNA (ssRNA) approximately 9.4 to 9.6 kb sizes with a 3‟-poly A terminus. It has a filamentous particle of 15 nm diameter and 690 to 700 nm long (Schellenberger *et al*., 2010). WSMV is found in the continental United States, Canada,

South America, Eastern Europe, Australia, and the Middle East (Hadi *et al*., 2011) and contributes to wheat disease in these locations and to MLN in the continental United States and not East Africa or Asia. WSMV is not considered a major pathogen of maize, because genetic resistance in maize is common. It is commonly transmitted by the eriophyid wheat curl mite *Acer tulipae* Keifer (Slykhuis 1955). In single infections they cause mosaic, chlorosis and stunting in maize, very similar to the symptoms produced by MCMV infection. Symptoms can be bright or more muted, depending on the host-virus-environment interaction.

1.3.4.2.3 Maize Dwarf Mosaic Virus (MDMV)

MDMV belongs to genus *Potyvirus*, family Potyviridae (Schellenberger *et al*., 2010). The virus is a single stranded positive-sense RNA (ssRNA) with a flexuous filamentous viral particle of 750 nm long and 13 nm wide. MDMV can infect maize, Johnson grass, sugarcane, millet, sorghum species, besides several grass species and cause severe agronomical losses worldwide. In maize, the economic damage consists of the high proportion of ears with aborted ends, a reduced ear size, and a decrease in the thousand-kernel weight. MDMV can be spread through seed (0.007–0.4 %;) mechanically, or by leaf aphids in a non-persistent manner (Moreno *et al*., 1997; Hudson *et al*., 1992 & 2000).

1.3.5 Disease transmission

Continuing disease in crop hosts requires a source or reservoir and favorable conditions of virus. The prevalence and survival of MLN viruses in the tropics and subtropics are enhanced by the ideal tropical temperature conditions and relative humidity that encourage the perpetuation of both the viruses and their insect vectors (Misra and Sharma, 2011). Vectors play important roles in the pathogenicity and spread of viruses in plants because they create entry points for the viruses to get into the host cells during feeding (Fereres and Raccah, 2015). Vectors are vehicles for viruses to move from one plant to another and between fields. Virus spread is also enhanced by an increase in vector population and favorable weather conditions (Gowda *et al*., 2015; Yang *et al*., 2017).

Maize thrips (*Frankliniella williamsi* Hood) and aphid (*Rhopalosiphum maidis* Fitch) are the major vectors MCMV and SCMV, respectively in eastern, central Africa and South American (Wang *et al*., 2013; Mahuku *et al*., 2015, Kiruwa *et al*., 2016). An adult thrips has one needle-like mouth part (stylet) that is used to break the cell wall and penetrate into plant tissue while feeding. The acquisition access period for thrips feeding on MCMV-infected maize plants is 3hr, after which it is able to transmit the virus for inoculation feeding period

of up to 6 days in a non-persistent (stylet-borne) manner (Misra and Sharma, 2011). Thrips that acquired the virus at the larva stage cannot be effective at the adult stage unless it feeds afresh on an infected maize plant. MCMV sap also remains stable in both larvae and adult thrips for an inoculation feeding period of up to 6 days, with decreasing rate of transmission with time (Cabanas *et al*., 2013, ASARECA, 2014).

MCMV can also be vectored by maize flea beetle (*Chaetocnema pulicaria* Melsheimer), southern maize rootworm (*Diabrotica undecimpunctata* howardi Barber), western maize rootworm (*Diabrotica virgifera* LeConte), *Systena frontalis*, *Diabortica longcornis* and *Oulema melanopa* (Nault *et al*., 1978; Uyemoto *et al*., 1980; Misra and Sharma, 2011). The viruses can survive on different host plants such as cassava, beans, maize, sorghum, onions, rice, peppers coriander (*Coriandrum sativum* L.), peas (*Pisum sativum var.*), various grasses, black-jack (*Bidens pilosa* L.*)* and *Tithon*ia diversifolia (Nelson *et al*., 2011; Liu *et al*., 2017). Use of infected seed and plant residues also encourages spread of MLN. The spread of MCMV in east and central Africa likely to have been increased by seed. A high incidence (45–72%) of MCMV was detected on whole seeds from infected plants and on the seed being sold to farmers in local markets in Kenya (Mahuku *et al*., 2015). In earlier experiments using 800–25,000 seeds from infested fields, the rate of MCMV transmission to emerging plants was 0–0.33%, 0.00012%, 0%, and 0% transmission of virus for Hawaiian, Mexican, Peruvian, and Kansas, respectively (Zhang *et al*., 2011; Wang *et al*., 2017). Although these rates of MCMV transmission through seed are fairly low, they are likely sufficient to allow for disease outbreaks in the presence of significant vector populations (Albrechsen, 2016).
Soil transmission has been suggested after a reduced incidence of MCMV in maize after rotation to sorghum (Phillips *et al*., 1982). Preliminary experiments indicated a high incidence of MCMV in seedlings planted in soil taken from around MLN-infected maize (Mahuku *et al.,* 2015). Both chrysomelid beetles and thrips have soil-borne stages and are suspected to be effective virus vectors. Although there has been a report of SCMV transmission through the soil, it is not considered a major route for virus transmission given the normally high and ubiquitous distribution of aphid vectors (Shukla and Ward 1994).

1.3.6 MLN detection

Serological techniques including enzyme-linked immuno-sorbent assay (ELISA), polymerase chain reaction (PCR), genome-wide association (GWAS) mapping, and next-generation sequencing have been effectively used for detection and characterization of the MLN

causative pathogens. Commercial ELISA kits for detecting MCMV are available from several manufacturers and research labs (Wangai *et al*., 2012; Mahuku *et al*., 2015; Wang *et al*., 2013). Immunostrips for MCMV detection are also commercially available (https://mln.cimmyt.org/mln-diagnostic-techniques/#immunostrips). However, serological assays may not necessarily produce consistent results for SCMV due to its high diversity (Braidwood *et al*., 2019).

Monoclonal antibodies have also been used to develop different sensors for rapid MCMV detection (Huang *et al*., 2014). RT-PCR assays based on several different sets of primers are also widely used for virus detection. Similarly, several quantitative RT-PCR assays provide sensitive or quantitative detection of MCMV (Adam *et al*., 2013). As outlined above, NGS has been used to identify MCMV in samples and to assess virus population variation.

The high degree of sequence variation within potyvirus species, particularly SCMV, makes the development of diagnostics a challenge. Mahuku *et al*., 2015 observed clear differences between two polyclonal antisera in their responses to SCMV isolates, with two antisera detecting different subsets of virus isolates (Mahuku *et al*., 2015). Serological diagnostics targeted at conserved epitopes detected potyviruses in 21 of 26 samples from Kenya, three of which were not positive for SCMV, MDMV, or JGMV (Stewart *et al*., 2017). However, the potyvirus immunostrips do not detect all samples positive for JGMV. RT-PCR and quantitative RT-PCR assays have been developed for SCMV, but assays for simultaneous detection of all known isolates have not been tested.

Next-generation sequencing (NGS) analysis, while too expensive for routine analyses, is an excellent tool for characterizing potyvirus populations, although the diversity of SCMV and other potyvirus sequences is a major confounding factor. Because of their wide availability, stability, and relative insensitivity to laboratory contamination, serological diagnostics are broadly used for MCMV diagnosis. Although RT-PCR assays provide robust detection of MCMV, problems with laboratory contamination, reagent availability, and stability have been a major problem (Karanja *et al*., 2020).

1.3.7 Control measures for MLN

Currently, there is no effective control for MLN disease in Kenya. A number of approaches, including cultural, management of insect vectors, and breeding for resistance, have been recommended in order to contain the disease. However, no single method among these is effective in the control of MLN. Therefore, the best approach for the management of MLN is to employ integrated pest management practices encompassing cultural control such as closed

season (3 months), crop rotation and crop diversification, vector control using seed treatment followed by foliar sprays, and host-plant resistance (Karanja *et al*., 2018 & 2020).

Continuous maize production in the same field is the major factor that can increase the incidence of MLN. Crop rotation with non *Poaceae* crops (non-cereal) such as sweet potatoes, cassava, beans and peas for three seasons in an attempt that can reduce the spread of the virus and vectors provide an alternative food source (Uyemoto, 1983). In addition, planting different crops each season will diversify farm enterprises (ASERECA 2014). Manure and top-dressing fertilizers can be applied to boost plant vigour, which enables the plant to fight the disease (Prasanna *et al*, 2020). Use of good field management methods, such as weed control measures to eliminate alternative hosts for potential vectors, scouting and removal of infected plants from the field to reduce pathogen and vector populations. This material can be fed to livestock, but grain and cobs that are rotten should not be fed to humans or animals but be destroyed by burning (Karanja *et al*., 2018). Farmers should avoid recycling seed/grains but rather purchase certified seed only. Creating a break in maize planting seasons, e.g., planting maize during the main rainy season and not during the short rain season, also can reduce the virus load and population of vectors.

In addition, the vectors can be controlled by the use of recommended insecticides. In Hawaii producers of maize seed spray regularly after planting to control insects that spread the virus (Nelson *et al*., 2011).

There is a need to have regulation by governments to impose compulsory testing of the seeds for MCMV and SCMV contamination, quarantine on the movement of maize materials from affected areas within a country. This quarantine is important bearing in mind that MCMV can be mechanically, and seed transmitted (Jensen *et al*., 1991; Mahuku *et al*., 2015; Prasanna *et al*., 2020). Enforcing such regulations can be challenging and expensive, but alongside increased awareness by the farming community, they can help reduce the spread of the disease.

The use of resistant maize varieties is the most reliable option due to the non-persistent manner of MLN virus transmission, it's cost-effectiveness, and ease of planting resistant varieties compared to management of pesticides by local farmers (Kiruwa *et al*., 2016; Liu *et al*., 2017).

1.3.8 Mechanism of resistance to MLN

The most economically viable and environmentally sustainable method in controlling pest and diseases in crops is by availing resistant varieties. Plants prevent pest and disease infection by either restricting pathogen growth (resistance) or by reducing or moderating pathogen effects (tolerance) (Boots 2008; Roy and Kirchner 2000). Rodier *et al*. (1995) suggested that more than one mechanism is involved in a field resistance where plants are able to outgrow symptoms. A plant is referred to be resistant or tolerant if it showed severe symptoms at early stages and reduction in symptoms in subsequently emerged leaves (Mawere *et al*., 2006, Salaudeen *et al*., 2010). This indicates that these resistance mechanisms are manifested in varying degrees in different maize resistant germplasm. For maize, genes or QTL conferring moderate to total resistance to most of the major disease-causing viruses have been identified and the genetics of resistance have been defined (Redibaugh and Pratt 2008). In the few cases where mechanisms of resistance have been examined, the virus can replicate in inoculated tissues of resistant maize lines but fails to move systemically, preventing disease development. Other authors have found the mechanism of resistance to be antiviral in nature (Martin *et al*., 1999) with an antixenosis component (van Rensburg, 2001). Antiviral resistance is where host plants pose small regulating RNAs (miRNAs) for viral gene silencing and these can be engineered into plants to down regulate the expression of viral defence mechanisms (Ramesh *et al.,* 2014).

On the other hand, antixenosis (non-preference) describes resistance in which insects is either repelled from host plants. These two resistance mechanisms can operate in the same resistance source and sometimes cannot be distinguished. Combining resistance to the insect vector and resistance to the virus could further contribute to the durability of the resistance.

The susceptibility of the maize crop to MCMV is not surprising, as major genes and/or quantitative trait locus/loci (QTL) conferring maize resistance to specific viruses are not widely distributed in maize germplasm (Gowda *et al*., 2015). Once resistant or tolerant lines are identified, breeders can take advantage of substantial genomic information for brerding either through transformation, doubled haploids, and clustered equally interspaced short palindromic repeats (CRISPR)-Cas for genome editing) (Xu *et al*., 2017).

1.3.9 Source of germplasm and origin of resistance

Development and deployment of improved maize varieties with tolerance/resistance to MLN and/or its causing viruses offers an effective and important practical solution to food security.

Native genetic "resistance" to plant viruses, including MLN-causing viruses, cannot be "complete" i.e., the plants are neither immune nor do completely inhibit the replication of the disease-causing viruses. Screenings in Kenya and Ohio identified two maize lines: KS23-5 and KS23-6 as strong sources of MLN resistance. KS23 is a genetic broad-based synthetic developed by inter-mating 26 inbred lines which combined well with a strain of Suwan1 in Kasetsart University, Thailand (Jampatong *et al*., 2010). KS23 contained approximately 35% temperate germplasm and was designed to be a counterpart of Suwan1 in the hybrid breeding program. Although the exact origin of the MLN resistance in KS23 is not known, KS23-6 serve as excellent trait donor (Karanja *et al*., 2018; Prasanna *et al*., 2020). Other maize lines with contrasting response to MLN developed by CIMMYT through pedigree and doubled haploid (DH) breeding schemes include CKDHL120918, CML494, CKLTI0227, CML505 and CKDHL120312 (Beyene *et al.,* 2017; Karanja *et al*., 2018). These maize lines are also known for tolerance to various biotic and abiotic stresses as well as good agronomic performance.

1.3.10 Breeding for MLN resistance

The development of MLN-tolerant/resistant maize varieties is the most economically viable and environmentally sustainable approach for reducing yield losses in farmer's fields. This requires intensive screening of a large number of inbred lines to identify resistant donor lines, followed by the introgression of the MLN resistance together with other relevant traits into suitable genetic backgrounds. Available maize lines and varieties in the region are mostly susceptible, and yield losses due to MLN reached 100% under severe infections (Wang *et al*., 2013; Gowda *et al*., 2015; Kagoda *et al*., 2016; Olsen *et al*., 2016). The initial screening of commercial maize varieties for MLN resistance was carried out in 2013, with the opening of the joint KALRO/CIMMYT MLN Screening facility at KALRO- Naivasha. Nearly all the 25,000-maize germplasm (inbred lines, hybrids, and open pollinated) screened were highly susceptible to the disease (Prasanna 2015; Marenya *et al.,* 2018; Gowda *et al*., 2015).

SCMV resistance was first identified in lines, D21, D32, and FAP1360A in Europe, indicating that genetic resistance is indeed an economical way to control SCMV (Kuntze *et al*., 1997; Wang and Yuan, 2003). In the U.S., Pa405, B68, Oh7B, Mp339, GA209, and A239 were shown to be resistant to SCMV (Scott 1981; Roane *et al*., 1989) while Huangzaosi, Siyi, X178, and Hai9-21 displayed complete resistance to SCMV in China (Chen *et al*., 2004; Wu and Du, 2002). SCMV resistance genes were first located in inbred line GA209 on both arms

of chromosome 6 by use of translocation lines (Scott 1971). The first resistance genes Scmv1 and Scmv2 was located near the centromere of chromosome 6 of Pa405 by flanking RFLP markers Umc85 and Bnl6.29 (McMullen and Louie 1989). High-resolution mapping using progeny from the cross between FAP1360A (resistant) and F7 (susceptible) confirmed that Scmv1 and Scmv2 are two major SCMV resistance loci. Although both are required for complete resistance against SCMV, Scmv1 has a stronger effect than Scmv2 since it suppresses symptoms at all developmental stages with Scmv2 functions at later stages of infection (Xia *et al*., 1999; Dussele *et al*., 2000; Xing *et al*., 2006). The presence of resistance alleles at both loci, Scmv1 and Scmv2, is crucial for complete SCMV resistance (Dussle *et al*., 2002; Xu *et al*., 1999).

Maize lines, KS23-6, N211Oh1VI, KS23-5 and DR were identified to be MCMV-tolerant in Hawaiian, Thai, and U.S. maize breeding programs (Brewbaker and Martin 2015; Kaeppler *et al*., 1998; Jampatog *et al*., 2010; Mahuku *et al.,* 2015; Jone *et al*., 2018). MCMV resistance have been hypothesized to be associated with decreased virus accumulation in systemic leaves. Using mapping approaches, QTL associated with MCMV resistance were identified on chromosomes 3 (Oh1VI), 5 (N211), 6 (KS23-5 and KS23-6), and 10 (DR and Oh1VI). QTLs on chromosomes 5 (N211), 6 (KS23 lines), and 10 (DR) were considered to have a large-effect and the multiple smaller QTL observed in 3 (Oh1IV) (Jone *et al*., 2018; Murithi *et al*., 2019). It will be important to determine whether pyramiding of the loci identified will produce a highly tolerant or even a resistant line to MCMV.

Linkage mapping study by CIMMYT revealed three major QTL on chromosomes 3, 6 and 9 that are consistently detected for MLN using KS23-6 as a donor line. These genomic regions coincided with SCMV resistance loci (Sitonic *et al*., 2019; Gowda *et al*., 2015), suggesting that the genomic regions may either represent resistance to only SCMV or for both SCMV and MCMV. Further studies have revealed the presence of a major effect QTLs on chromosomes 3, 6, and 9 which are potential candidates for marker-assisted breeding to improve MLN resistance (Gowda *et al*., 2018).

1.3.11 Gene action

1.3.11.1 Combining ability

Mating design is a procedure of arranging different controlled crosses to produce progenies (Klein *et al*., 1973). The mating techniques and arrangements to be used depends on; i) type

of pollination (self or cross); ii) type of crossing used (controlled or natural); iii) type of pollen dissemination (wind or insect); iv) biological limitations such as cytoplasmic or genetic sterility; v) purpose of project (breeding or genetic); and vi) size of population required (Stuber, 2004). Mating designs are used to generate genetic pedigrees, genetic information, estimating information on general combining ability (GCA) and specific combining ability (SCA) and materials that can be used in a breeding program (Jenkins, 1934). In all mating designs, the individuals are taken randomly and crossed to produce progenies which are related to each other as half-sibs or full-sibs. There are two types of combining ability. General combining ability (GCA) is the average performance of a genotype in its hybrid combination, while specific combining ability (SCA) refers to the superiority of a certain hybrid compared to other hybrids derived from crossing different genotypes (Sprague and Tatum, 1942). The variance for GCA is associated with an additive genetic variance, while SCA variance relates to non-additive genetic effects, primarily dominance and epistatic deviations (Hallauer and Miranda, 1988). The effect of both additive and non-additive effects has been reported for different agronomic traits in maize including grain yield and hence the gene action conditioning most complex traits cannot be generalized.

Information on combining the ability of suitable parents is necessary for maize varieties development for diverse production in different agro-ecological zones. Combining ability studies have also been used to study the yield characters and heterotic groups for inbred lines with the aim of developing new hybrids with good quality, high yields and multiple disease tolerance (Xingming *et al*., 2001).

Other studies have identified significant GCA and SCA effects for disease resistance either conditioned by additive, non-additive gene actions or both (Menkir and Ayodele, 2005; Vivek *et al*., 2010; Lagat *et al*., 2008; Zhang *et al*., 2016). The significant GCA implies that breeders can possibly exploit the available genetic variability, while identifying elite parents with desirable traits whereas significant SCA effects suggest that promising single cross combinations could be identified. GCA deviations can be positive or negative (Kearsey and Pooni, 1996). A positive deviation can be favourable or unfavourable, depending on the trait under consideration. The identification of single crosses with high and positive GCA effects for grain yield suggested that potential parents could be exploited in the development of various hybrids, including three-way, double-cross and top cross hybrids. On the contrary, high positive values on foliar disease ratings would not be desirable. Negative GCA values on the days to anthesis are more desirable for selection of early maturing combinations. In

addition, the stability of GCA and SCA effects is key in identifying parents and hybrids with improved homeostasis to cater for environmental variations (Dehghanpour and Ehdaie, 2013). However, for an effective breeding program, the genotypes tested should exhibit enough genetic diversity (Betran *et al*., 2003; Karim *et al*., 2018). Thus, many genotypes should be evaluated to select suitable parents with desirable genotypes (El-Hosary, 2014).

1.3.11.2 Diallel mating designs

A diallel cross is a set of parents and their crosses in all possible combinations. Diallel mating models developed by Griffing (1956) and Gardner and Eberhart (1966), are the major models used in combining ability analyses. Diallel crosses have been used extensively for studies of quantitative characters with the methods developed mainly by Hayman (1954), Jinks (1954), Griffing (1956), Kempthorne (1957) and Gardner and Eberhart (1966). It helps in explaining the genetic control of important plant traits, while enhancing breeding and selection of promising parents. The diallel crosses enable breeders to predict progeny performance from parental performance (Iken and Olakojo, 2002; Ojulong *et al*., 1996). Pairs of parental lines that yield heterotic crosses identified have been pivotal in the development of appropriate hybrids (Vega and Chapman, 2006).

The theory of homozygous diallel crosses is based on the following assumptions: (i) homozygous parents, (ii) diploid segregation, (iii) no difference between reciprocal crosses, (iv) independent gene action of non-allelic genes, (v) no multiple allelism, (v) no linkage and (v) random selection of parents.

Griffing (1956) presented diallel crossing schemes depending on the inclusion of reciprocal crosses and/or parental lines. Method 1 includes the parents, one set of F_1's, and the reciprocal F_1's (all $p2$ combinations); method 2 has parents and one set of F_1's without reciprocals ($12(p+1)$ combinations); method 3 has one set of F_1's and reciprocals but not the parents (($p-1$) combinations); while method 4 has one set of F1's but neither the parents nor reciprocal F1's are included ($12p$ ($p-1$) combinations. Since methods 3 and 4 do not involve selfing; only these methods can be used to estimate variance components (Wricke and Weber, 1986). Further, the fixed model of method 3 or 4 is the most appropriate for obtaining unbiased estimates of combining abilities and gene action (Shattuck *et al*., 1993). Further, reciprocal or maternal effects can be determined with Griffing method 1 and 3 (Wricke and Weber, 1986; Brahmbhatt *et al*., 2018).

The diallel thus provides an assessment of the relative merits of parents and crosses and information on the relative contribution of additive and non-additive gene action to guide selection and testing methodologies. Traits controlled by additive effects respond efficiently to selection while those controlled by non-additive effects, mainly dominance and epistasis, manifest in hybrid combinations (Fasahat *et al*., 2016).

1.3.11.3 Generation mean analysis

Generation mean analysis (GMA) is used in estimating gene action. Generation mean analysis (GMA) was developed by Hayman (1958). It utilizes six populations P_1, P_2, F_1, F_2, BCP_1 and BCP_2 to estimate genetic effects (Hayman, 1958; Carson, 1995). Generation mean analysis has been employed in various crops and traits to estimate genetic effects in contrasting characteristics. The method is efficient in partitioning epistasis and non-allelic gene effects (Hettiarachchi *et al*., 2009). Thus, it is used to study populations that have distinct wide contrasting traits like disease resistance because it analyses one trait at a time (Frank and Hallauer, 1997; Derera *et al*., 2007).

The knowledge about the magnitude and behaviour of genetic components for a quantitative character being investigated is essential for a plant breeder since gene action and effects are key factors for understanding the inheritance of quantitative traits (Lamkey and Lee, 1993; Arora *et al*., 2010; Moharramnejad *et al*., 2018).

Quantitative traits are usually controlled by multiple genes and are considerably influenced by the interaction of the environment (Hallauer and Miranda, 1981; Kearsey and Pooni, 1996). In maize, GMA has been used for twin cobs study (Frank and Hallauer, 1997) and inheritance of disease resistance and other agronomic traits in cereals (Boling and Grogan, 1965; Bernardo *et al.*, 1992; Chungu *et al*., 1996; Frank and Hallauer, 1997; Hakizimana *et al*., 2004; Bucheyeki, 2012; Butrón *et al.*, 2015; Madhav *et al*., 2019).

Several studies have shown that maize disease inheritance is mainly controlled by additive gene action while dominance and epistasis contributions are normally non-significant (Carson, 1995; Jenkins and Robert, 1952). Other studies observed the significant contribution of additive, dominance and non-allelic gene interaction in controlling the disease resistance in maize (Rahangdale *et al*., 2019).

1.3.12 Genotype by environment interactions

The association of the environment (non-genetic factors, such as locations, seasons, years, rainfall, temperature) and the phenotypic (visible characteristics resulting from genetic

makeup and environment) expression of a genotype constitute the genotype x environment (G x E) interaction (Fan *et al*., 2007). The presence of G x E interaction complicates the selection of superior genotypes for a target population of environments (TPE). However, the absence of G x E would imply that superior genotypes would be suitable in all environments in the TPE.

There are two possible strategies for developing varieties with low G x E interaction. The first is the stratification of the environment into homogenous regions with varieties developed for specific sub-regions. This approach is costly and ineffective since some environmental variations cannot be reliably predicted (Allard and Bradshaw, 1964). The second strategy for reducing G x E interaction involves selecting genotypes with good stability and adaptability across a wide range of environments to better predict behavior (Eberhart and Russell, 1966).

Genotype x environment interactions can either be qualitative (i.e., crossover type) or only quantitative (i.e., non-crossover type) (Singh *et al*., 1999). Changes in the genotype ranks across environments suggest the existence of crossover genotype by environment interactions. Breeders are concerned with crossover interactions as it results in changes of genotypes ranks across environments and makes it difficult to recommend a single best genotype for all environments (Fehr, 1987).

Genotype and genotype × environment interaction (GGE) Biplot analysis results can discriminate between expected and realized responses of genotypes and has been widely used in recent years to determine the stability of disease resistance through multi-location trials. It allows visual examination of the relationships among the test environments, genotypes, and the G x E interactions. It is an effective tool for: (i) mega-environment analysis, whereby specific genotypes can be recommended for specific mega- environments (Yan and Kang, 2003; Yan and Tinker, 2006), (ii) genotype evaluation (the mean performance and stability), and (iii) environmental evaluation (the power to discriminate among genotypes in target environments; Ding *et al*., 2007).

Kenya is endowed with six main agro-ecologies. This wide agro-ecological variability is the major challenge for maize research which has resulted in high genotypes by environment interaction effect. This results in the need to develop different varieties for these six maizes growing ecologies. Previous studies have confirmed a strong significant genotype (G), locations (L), genotypes by locations interaction (GLI), and GEI effects for maize genotypes in Kenya (Rao *et al*., 2002; Fekadu *et al*., 2009).

1.3.13 Determination of G x E interactions

There are a variety of statistical procedures available for the determination of G x E in multi-environment trials. These are based on analysis of variance, linear regression or non-linear analysis, multivariate analysis, biplots and/or non-parametric statistics. Parametric methods have limitations including the need to satisfy the assumptions of normality, the homogeneity of variance, and the additivity (linearity) of the effects of genotypes and environment (Yue *et al*., 1997). Non-parametric models which are based on the relative classification of genotypes across different environments do not require these assumptions and are often viewed as good alternatives for parametric measurements (Huehn, 1990). Combinations of these groups have been used to identify the most suitable genotypes (Balestre *et al*., 2009; Scapim *et al*., 2010). Multivariate statistical methods have been used to identify and group genotypes with a similar environmental response. The effect of the genotype (G) and sites (E) and GE are intpreted by additive main effect and multiplicative interaction (AMMI) and principal component analysis (PCA) (Zobel *et al*., 1988). A more recent modification of the AMMI analysis is the GGE analysis proposed by Yan et al. (2000) that instead pools together the genotype effect and GE (multiplicative effect) and submits these effects to PCA. Bi plots have been primarily used for analyzing multi-environment trials and studying genotype x environment interactions (Crossa and Cornelius 1997; Yan *et al*., 2000). They can also be used for studying response patterns of entries when crossed with testers, that is, line x tester interactions (Narro *et al*., 2003) and diallel crosses (Yan and Hunt 2002; Bhatnagar *et al*., 2004). The GGE biplot method is useful for identifying genotypes with dynamic stability, since its principal component axis I (PC_1) shows genotypes that are highly adapted while PC_2 axis denotes genotype stability. In comparison, to other methods of estimating G x E, the GGE biplot has been considered superior to AMMI1 in explaining sums of squares of GE and G + GE and in predictive accuracy (Balestre *et al*., 2009). The method is also highly correlated and provides similar results with other "traditional" stability methods such as Shukla's Stability variance and Eberhart-Russell regression model (Blanche *et al*., 2002). Thus, the GGE biplot is more versatile and allows easily comprehensible presentation of the data, since it includes both mean performance and stability.

1.3.14 Stability and adaptability analyses

Stability has been defined as static, where a genotype remains unchanged regardless of the environmental conditions, or dynamic where a genotype changes in a predictable manner across a wide range of environmental conditions (Becker and Leon, 1988). The ultimate goal

of stability analysis is developing of consistently responding superior genotypes for broad adaptability (Kang, 1998). Therefore, stability is the measure of the ability of a genotype to maintain relative performance across a wide range of environments. In the assessment of stability, association of different models helps breeders choose the best adjusted and most informative stability parameter(s) to fit the static and dynamic concepts of stability (Scapim *et al*., 2010). A stable genotype is described as the one having a predictable response across diverse environments. An appropriate stable genotype can utilize resources that are available in high yield environments, while maintaining above average yield in all other environments (Finlay and Wilkinson, 1963). There are indications that static stability is most useful for qualitative traits such as diseases or stress resistance while for quantitative traits such as yield, the dynamic concept of stability is most practical (Norden *et al.*, 1986). It is reported that grain yield stability is a heritable trait controlled by additive gene action and can be improved through selection (Lee *et al.*, 2003). A major goal in many breeding programs is selection of high yielding genotypes with wider adaptation. However, achieving this goal is generally difficult due to the probability of significant GEI effect (Gauch and Zobel, 1996).

1.3.15 Summary

From the review of literature, it is noted that maize yields are still very low in Kenya, mostly less than 2 tha^{-1} in the dry lowlands and 4 tha^{-1} in the highlands. This is far below the potential yields of 4 tha^{-1} and 8 tha^{-1} in the dry lowlands and highlands, respectively. This has resulted in food insecurity in the country and the importation of maize to bridge the gap. Maize production can only be increased by intensifying production within the existing areas and through the mitigation of many constraints that plague high maize productivity.

MLN disease has been found to one of the major contributors of maize shortage in all maize growing zones in Kenya. The conditions that exacerbate increased MLN incidence and severity are likely to increase as maize production is intensified through irrigation, seed recycling and because of climate change.

There is evidence of the vulnerability of commercial varieties to MLN whenever epidemics occur. This suggests the need to develop and deploy MLN resistant varieties to curb the challenge.

Knowledge of the mode of gene action for MLN diseases is still scanty. However, it is possible that non-additive effects play a major role in complicating the process of selection. Further, the kinds of gene action involved depend on test germplasm and environment including the levels of disease pressure present. A thorough investigation on the kinds of

gene action controlling MLN resistance, its causative viruses and their interaction will help in selecting suitable germplasm and especially where the development of a variety with multiple traits is targeted.

Identification of suitable genotypes in a breeding program is a continuous process due to changing of environmental conditions and the development of new hybrid combinations. Germplasm exhibiting dynamic stability for quantitative traits such as grain yield and static stability for qualitative traits such as disease resistance would be most beneficial for breeding and commercialization.

The genetic control of yield stability is not widely researched but is noted to be under additive gene action. There are numerous methods of estimating G x E interaction and stability and a comparison among methods can be a useful strategy to identify superior genotypes. This will greatly influence the adoption of new varieties by the farmers. The replacement of old varieties with new ones in Kenya is worrying, as farmers are stack with varieties developed in 1960s. The introgression of desirable traits and engagement of key players in the maize value-chain in variety development ensures increased and sustained maize production in Kenya.

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CHAPTER 2

Response of selected maize inbred germplasm to maize lethal necrosis disease and its causative viruses (Sugarcane Mosaic Virus and Maize Chlorotic Mottle Virus) in Kenya

Response of selected maize inbred germplasm to maize lethal necrosis disease and its causative viruses (sugarcane mosaic virus and maize chlorotic mottle virus) in Kenya. Karanja, J, J. Derera, A. Gubba, S. Mugo, A. Wangai, 2018. The Open Agriculture Journal 12: 215-226

Abstract

Maize lethal necrosis (MLN), caused by the synergistic interaction between Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV) continue to reduce maize production threatening food security in Kenya and other Eastern and Central African countries. Depending on the time of infestation, it causes yield losses of 30–100% in farmers' fields and an annual loss of USD198 million. Most of the commercial maize varieties being grown by the farmers are susceptible whether under natural or artificial conditions. In combination with recommended integrated pest management practices, development and adopting MLN tolerant maize varieties is an important step towards safeguarding food security, income and livelihoods of resource-poor smallholder farmers. The objective of this study was to identify sources of resistance to MCMV, SCMV and MLN in a set of selected maize genotypes that can support further research for genetic analysis and development of durably resistant maize lines and hybrids with good agronomic performance under MLN. Sixty five (65) maize genotypes were evaluated in a potted experiment with the pots arranged in a randomized complete block design of three replications. Seeds were sown at a rate of five seeds per pot, giving a total of 15 plants per genotype and artificially inoculated using virus strains collected from Bomet County in Kenya at the 3-4 leaf stage. Data was recorded on disease severity and incidence and used to calculate area under disease progress curve (AUDPC), flowering, yield and yield attributes. Data was analysed using multi-environment trial analysis, GenStat and breeding management system software. From the result, there were significant differences among inbred lines for SCMV, MCMV and MLN responses. Based on area under disease progressive curve score and ELISA analysis, genotypes MLN001 and MLN006 had the lowest score of 270, whereas OH28 had a maximum at 1259 under MCMV. Genotypes MLN042 and MLN041 were identified as the most promising sources of resistance against SCMV. However, no genotype was identified to have acceptable

levels of tolerance to MLN, but MLN001 and MLN013 were identified as the best performers under MLN. This study also validated the presence of MLN tolerance in MLN013 (CKDHL120312) and MLN001 (CKDHL120918) as earlier reported by CIMMYT. The identified inbred lines would be recommended for use in varietal development, MLN management and to enhance maize productivity in the MLN endemic regions and further research in understanding the mode of gene action for MLN tolerance.

2.1 Introduction

Maize accounts for more than 20% of total agricultural production, and 25% of agricultural employment in Kenya (Muasyia and Diallo, 2001). Thus, Kenya's national food security is strongly linked to the production of adequate quantities of maize to meet increasing domestic demand (De Grroote, *et al*., 2001; FAOSTAT, 2010). The total land area under maize in Kenya is about 1.5 million ha, with 70-80% of maize being produced by small-scale farmers with an average on-farm production of 1.5-2.6 tha^{-1}.

The major biotic causes of stress in maize have in the past included *Striga*, a parasitic weed, insect pest, diseases mainly northern corn leaf blight, maize streak virus (MSV), and common leaf rust, gray leaf spot (GLS), stalk and ear rot. Maize lethal necrosis (MLN), is a new disease in Kenya with its first incidences observed in 2011 (Adams *et al*., 2014; Wangai *et al*., 2012a&b). Earlier, the disease was observed in Kansas, USA, in 1978 Niblett and Claflin (Niblett and Claflin, 1978) where it was identified as corn lethal necrosis (CLN) disease (Doupnik, 1979).

Both MLN and CLN are caused by double infection of maize plants by maize chlorotic mottle virus (MCMV) (*Machlomovirus: Tombusviridae*) in combination with any of the cereal viruses in the Potyviridae group, Sugarcane mosaic virus (SCMV) (*Potyvirus: Potyviridae*), Maize dwarf mosaic virus (MDMV) or Wheat streak mosaic virus (WSMV). MCMV is transmitted by vectors such as Thrips (*Frankliniella williamsi* Hood) and beetles (Cabanas, *et al*., 2013) while SCMV is transmitted by Aphids (Brandes, 1920). Transmission and spread of MCMV has also been reported to be through seeds from infected plants (Jensen, *et al*., 1991) at a rate of 0.0003% which can translate into high number of infected plants resulting in epidemics. MCMV is a threat on its own and may cause significant yield loss even in the absence of the other viruses.

Since its first reports in Bomet County in 2011, the disease has spread into other areas (Wangai, *et al*., 2012) (Fig. 1). Yield losses of up to 100%, which is an estimated grain loss of 126,000 metric tonnes valued at $52 million have been reported in Kenya (De Groote, *et*

al., 2001), while yield losses of up to 59% due to MCMV had been reported in Peru (Castillo-Loayza, 1977). A survey conducted in the maize growing regions of Kenya in 2013/2014 indicated that 60% of the 2,467 randomly selected samples were positive for MCMV with more than 40% of these being infected with MCMV alone. In Democratc Republic of Congo (DRC), MCMV was detected in 40 to 80% of symptomatic samples collected from the Beni, Lubero, and Rutshuru territories of North Kivu Province in 2013 (Mahuku, *et al*., 2015; Lukanda *et al*., 2014). Other than seed, MCMV has also been reported to be transmitted through soil with 70% of emerging plants found to be infected (Mahuku, *et al*., 2015).

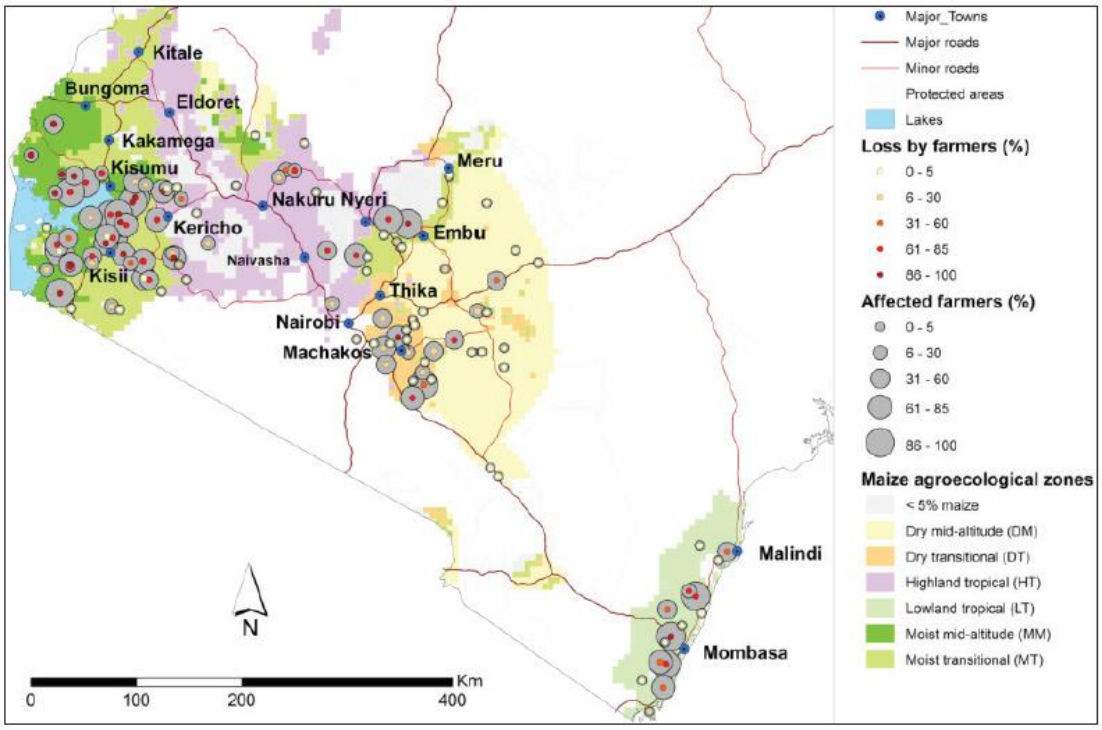

Figure 2.1: Distribution and losses affiliated by MLN in Kenya; Source: De Groote *et al*., 2016

Depending on the maize variety, a number of viruses infecting the plant, part of the plant infected, time of infection and prevailing environmental conditions, infected plants show a wide range of symptoms (Redinbaugh and Pratt, 2009). Common symptoms include; chlorotic mottle on the leaves usually starting from the base of the young leaves in the whorl and extending upwards toward the leaf tips, mild to severe leaf mottling, dwarfing and premature aging of the plants, necrosis of young leaves in the whorl before expansion leading to a "dead heart" and drying up of the whole plant. Severely affected plants form small cobs with little or no grain set. The entire plant can frequently be killed before flowering (Niblett and Claflin, 1978; Wangai *et al*., 2012 a&b; Uyemoto *et al*., 1980; Uyemoto 1983) (Fig. 2.2).

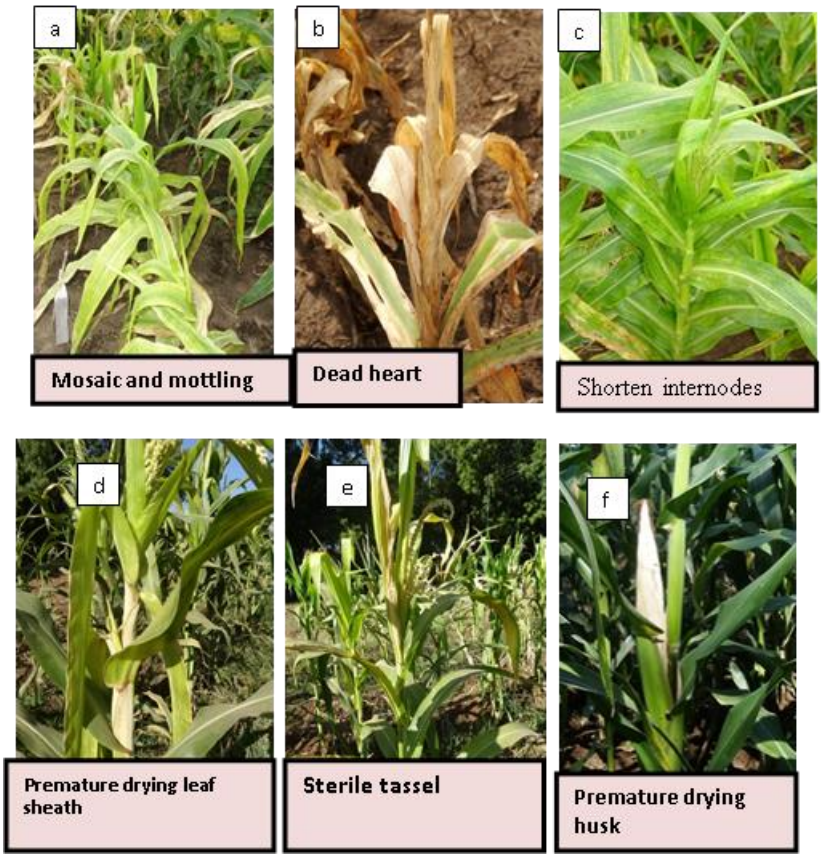

Figure 2. 2: Disease symptoms of MLN

Poor agricultural practices like leaving infected maize crop residues in the field, relay maize planting, and maize monoculture aid in inoculum build-up which increases the disease transmission season after season. In addition, poor crop rotation and lack of proper weed management practices have increased maize susceptibility and also aided in offering alternative hosts to the vectors where the weeds are susceptible to the MCMV and SCMV. The management of MLN disease could be achieved through integrating cultural methods, use of chemicals such as seed dressing and vector control with host resistance breeding. However, the use of chemicals is uneconomical and environmentally unfriendly especially among the resource constrained smallholder maize farmers (Wangai *et al*., 2012a).

Other measures include effective monitoring, rigorous implementation of a maize-free period of at least three months, timely planting, use of certified MCMV-free seed and crop rotation with non-cereal crops (Phillips *et al*., 1982). However, these approaches are difficult to implement in Bomet, Kisii and Narok counties where relay planting of maize is a common practice due to frequent rainfalls. Therefore, the most efficient control method for MLN, SCMV and MCMV, is the use of resistant maize genotypes. The development of virus-

resistant maize germplasm and hybrids is the most cost-effective and environmentally friendly approach to control disease (Gururani *et al*., 2012; Ali and Yan, 2012). The utilization of disease nurseries and trials in the screen houses and field infestation is the easiest approach to screen for MLN resistance in maize.

The objective of this study was to identify sources of resistance to MCMV, SCMV and MLN in a set of selected maize genotypes that can support further research for genetic analysis and development of durably resistant maize lines and hybrids with good agronomic performance under MLN.

2.2 Materials and methods

2.2.1 Host plants

Seeds of 65 selected maize genotypes were obtained from KALRO, CIMMYT (Manje *et al*., 2015) and from USDA, ARS Corn, Soybean and Wheat Quality Research Unit (CSWQRU) in Wooster, Ohio. The germplasm were selected based on previous studies and data on resistance to other diseases, such as maize streak virus, grey leaf spot, turcicum leaf blight, common leaf rust among others and MLN. Four yellow maize lines: P405, OH28, N211 and KS23-6 were used as checks. The line PA405 is resistant to SCMV but susceptible to MCMV and MLN, OH28 (CI.112-1 X Oh920) X (I11.A x I11.B), which was released in 1943 (Zambrano *et al*., 2013) and it is susceptible to maize dwarf mosaic virus (MDMV), SCMV, wheat streak mosaic virus (WSMV), maize chlorotic dwarf virus (MCDV), maize mosaic virus (MMV), and maize fine streak virus (MFSV) (Jones *et al*., 2007; Louie, 1995; McMullen and Louie, 1989; Redinbaugh and Pratt PC, 2009; Zambrano *et al*., 2013) was used as a susceptible check. The lines N211 and KS23-6 were used as tolerant checks for both MCMV and MLN (Mahuku, *et al*., 2015).

In the second and third screening, germplasm that had severity scores higher than OH28 in MCMV and MLN, were removed, leaving only 30 genotypes. Plastic pots measuring 30 cm in diameter filled with autoclaved (heat-sterilised) silt- loam soil and manure mix (6:1, v/v) were arranged in a randomized complete block design of three replications. Seeds were sown at a rate of five seeds per pot, giving a total of 15 plants per genotype. Di Amonium Phosphate (DAP) and Calcium Ammonium Nitrate (CAN) were applied to give 60kg N and 60kg P_2O_5 ha^{-1} at planting and at eight weeks after planting (WAP) respectively.

2.2.2 Virus isolates/ Inoculum preparation and inoculation

MCMV and SCMV isolates used in this study were originally collected from Bomet County in South Rift valley in Kenya and have been maintained at KALRO-Kabete, National Agricultural Research Laboratories (NARL) by serial passage on to susceptible maize hybrid H614 in separate green houses. Virus strain identity was verified at each passage time inoculum was prepared for a test by also inoculating susceptible maize germplasm OH28 and H614, *Sorghum bicolor* (L.) Moench *cv*. Atlas and Sart. Atlas and Sart sorghums are resistant to MCMV while Sart is susceptible to MDMV and SCMV but resistant to WSMV (Jones *et al*., 2007). As in other crops, it is very difficult to diagnose virus diseases in maize based solely on symptoms, as these vary significantly based on plant genotype, time of infection, environmental conditions and the potential for multiple infections. Therefore, the serological assay, ELISA, was used to check the virus purity, inoculation and disease assessment for MCMV and SCMV.

At the 3 to 4 leaf stage (10 days after planting) all plants were mechanically inoculated twice within a 1-week interval by rubbing the two youngest leaves (Louie, 1986). Virus inoculum was prepared from freshly harvested infected symptomatic young plants (infected 10-15 days prior to the main inoculation). Before inoculation, leaves were homogenized in 0.01 M phosphate buffer (pH 7.0) in 1:8 dilutions and 0.6% of 22µm carborundum was added prior to inoculation. The inoculum was kept on ice during inoculation time. The plants were watered daily until all the plants flowered.

2.2.3 Symptoms identification/rating

Disease incidences, severity and days to 50% anthesis (pollen shade) were recorded. Plants were diagnosed for virus symptoms from 7 days post inoculation (dpi) every two days interval up to 56 days, using a 1 to 5 rating scale, where; 1= no symptoms, 3= mild symptoms and 5= Severe chlorosis (die back of the plant) (Fig. 2.3). A score of 1 and 2 represents a resistant variety with mild visible symptoms of the disease, while a score of 5 signifies extreme susceptability.

Included in the diagnoses were observations of whether symptoms were local lesions on inoculated leaves or systemic infections that were limited or general and consisted of mosaics, mottles, or flecks and streaks.

Figure 2. 3: Disease severity rating scale for SCMV, MCMV and MLN in maize

In the scale, 1= no disease symptoms/clean; 2, chlorotic mottling on the lower leaves; 3= chlorotic mottling and mosaic throughout the whole plant; 4=excessive chlorotic mottling, mosaic, plant necrosis, and /or dead heart; and 5= dead plant and complete plant necrosis

Double-antibody sandwich ELISA (DAS-ELISA) analysis was carried out to confirm virus identity prior to inoculation and non-symptomatic plants. 500 mg of tissue were taken from the tip of 6^{th} and 7^{th} leaf and homogenized in 2.5 ml of 1x extraction buffer; PBST, pH 7.2 (137 mM NaCl, 3 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4), containing 2% BSA and 0.05% Tween 20. The ground samples were placed at –20°C for long-term storage. The samples were thawed to room temperature before loading plates. Plates were coated with a 1:1000 dilution of virus -specific IgG (1mg/ml) in carbonate coating buffer, pH 9.6, and the secondary antibody; alkaline phosphatase conjugated IgG (1 mg/ml, Sigma) at a 1:5000 dilution, both of which proved to be optimal in a previous evaluation. After incubation of the plates containing primary antibody for 1 hour at 37°C, the wells were washed with 200 µl of PBST, 200 µl of the diluted samples were added and plates were incubated at 37°C for 1 hour. Washing was done as above and secondary antibody was added, plates were incubated for 1 hour at 37°C. Finally, 200 µl of a 0.6 mg/ml solution of p-nitrophenyl phosphate (Sigma) was added to each well and color development was allowed to continue for 1 hour at room temperature. ELISA Nunc (Inter Med., Denmark) microtiter plates were used and absorbance was recorded at 405 nm in a MR700 Dynatech spectrophotometer (Dynatech, UK). Blank, buffer, positive control from the primary inoculum and health sample were used on all plates as controls. The readings were carried out at 60 minutes after addition of the substrate. In cases of doubt, the reading overnight was compared to the reading at 1 hour. A sample was judged as positive when the reading was greater than three times the absorbance value of a healthy (negative) plant extract reaction.

2.3 Statistical analysis

Analysis of variance for each trial and combined analysis across years was performed using multi-environment trial analysis with R, version 5.0, GenStat 15th edition (Genstat, 2017) and Breeding management system software, version 3.0.9. Area under the disease progress curve (AUDPC) which is a better indicator of disease expression over time was calculated on a single plant basis by trapezoidal integration over the whole observation period as follows:

$AUDPC = \sum_i [(DS_i + DS_{i-1}) \times (t_i - t_{i-1})]/2$

Where "*i" ={6, 8, 10, 12, 14, 16, 18, 20, 22, 31, 38, 45, 56}* are the days of observation, "*DS*" is the disease score using the above severity score of 1 to 5 and "t" represents the number of days post-inoculation (Campbell and Madden,1990; Jegger and Viljanen-Rollinson, 2001). Genotypic correlations (R_g) between treatments were estimated according to Coopre *et al*., 1996 as:

$R_g = R_{p(12)}/(H_1 \times H_2)^{1/2}$

In which $R_{p(12)}$ is the phenotypic correlation between the traits measured in Treatments 1 and 2, H_1 and H_2 are the broad-sense heritability for the trait measured in screen houses 1 and 2, respectively.

2.4 Results

Infection was observed in all the inoculated plants, but the maize lines differed significantly ($P < 0.001$) for the resistance to SCMV, MCMV and MLN. Disease severity differed significantly with plant growth with first disease symptoms in susceptible genotypes observed at 5 to 6 days post-inoculation (dpi) for SCMV, 10 to12 days for MLN and 14 to 15 days for MCMV. Disease severity was rated up to 56 dpi a time at which most of the lines had attained 50% flowering. There were significant differences ($p \leq 0.001$) among the genotypes at 14, 35 and 56 dpi, for disease severity, mean disease score and AUDPC (Figures 2.4, 2.5, 2.6 and Table 2.1). Only 17% of the total germplasm screened under SCMV had a mean score < 2.5. Two maize genotypes, MLN041 and MLN 042, had the lowest AUDPC value of 286 units compared to mean AUDPC value of 1338.4 units for the susceptible germplasm (Table 2.1).

The symptoms were first observed at 14 dpi from the lower leaves and spread slowly to the newly emerging leaves in the MCMV experiment. At 20 dpi, 45% of the germplasm screened had a disease severity score < 3, while at 56 dpi, only 15% of the germplasm were <3. Only

9% of the total germplasm in the two years period of screening showed acceptable levels of tolerance to MCMV. Among the top performing were MLN001, MLN006 MLN012, MLN016, MLN008, MLN007, and MLN009, which had a disease severity score <2.0 at 56dpi. N211, unlike the others, developed symptoms slowly with the first symptoms observed at 20 dpi and with moderate symptoms at 56dpi (Figure 2.5).

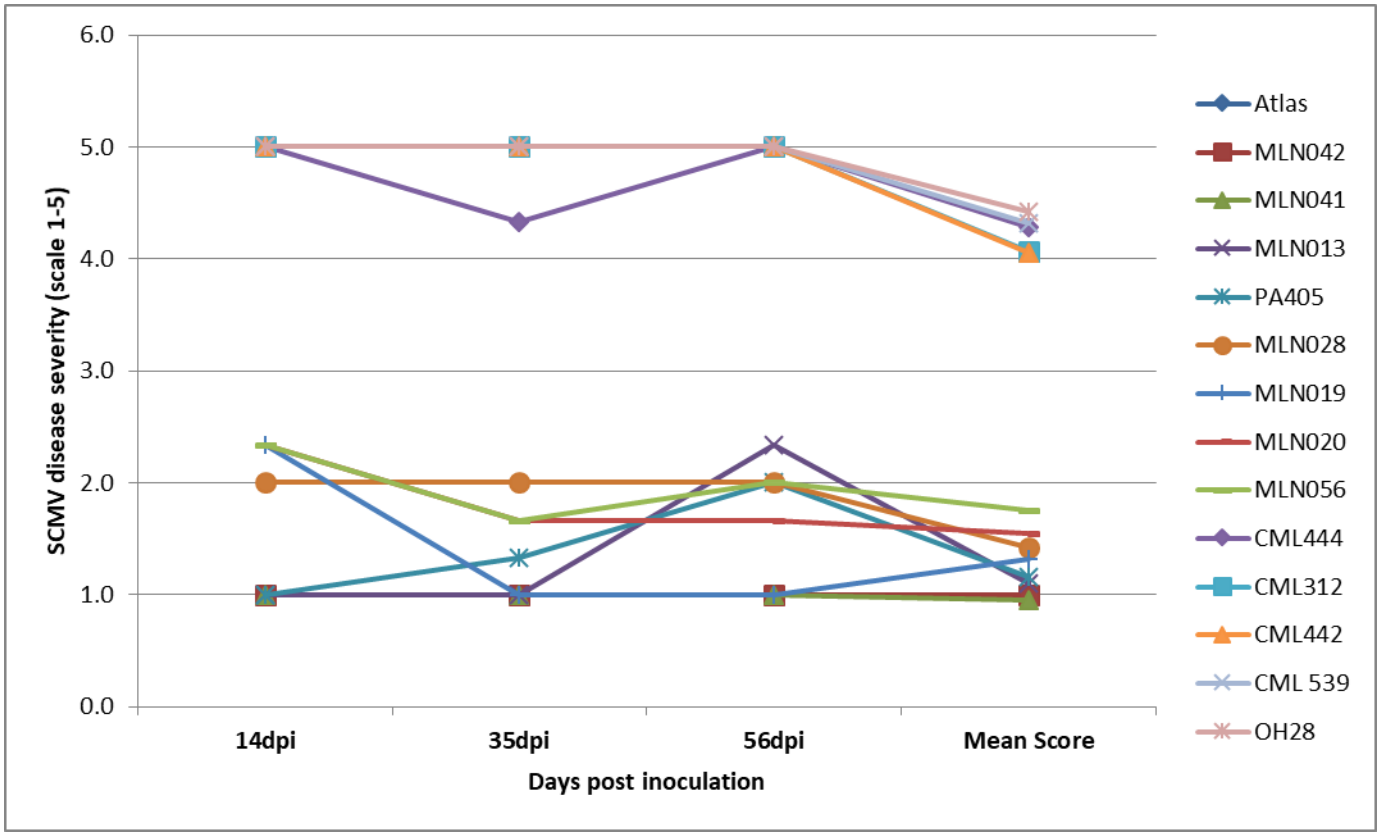

Figure 2.4: SCMV disease severity progress for the top performing germplasm (bottom) compared to the susceptible (top) obtained by plotting disease severity scores against the number of days post inoculation at 14 (early), 35 (middle) and 56 days (late)

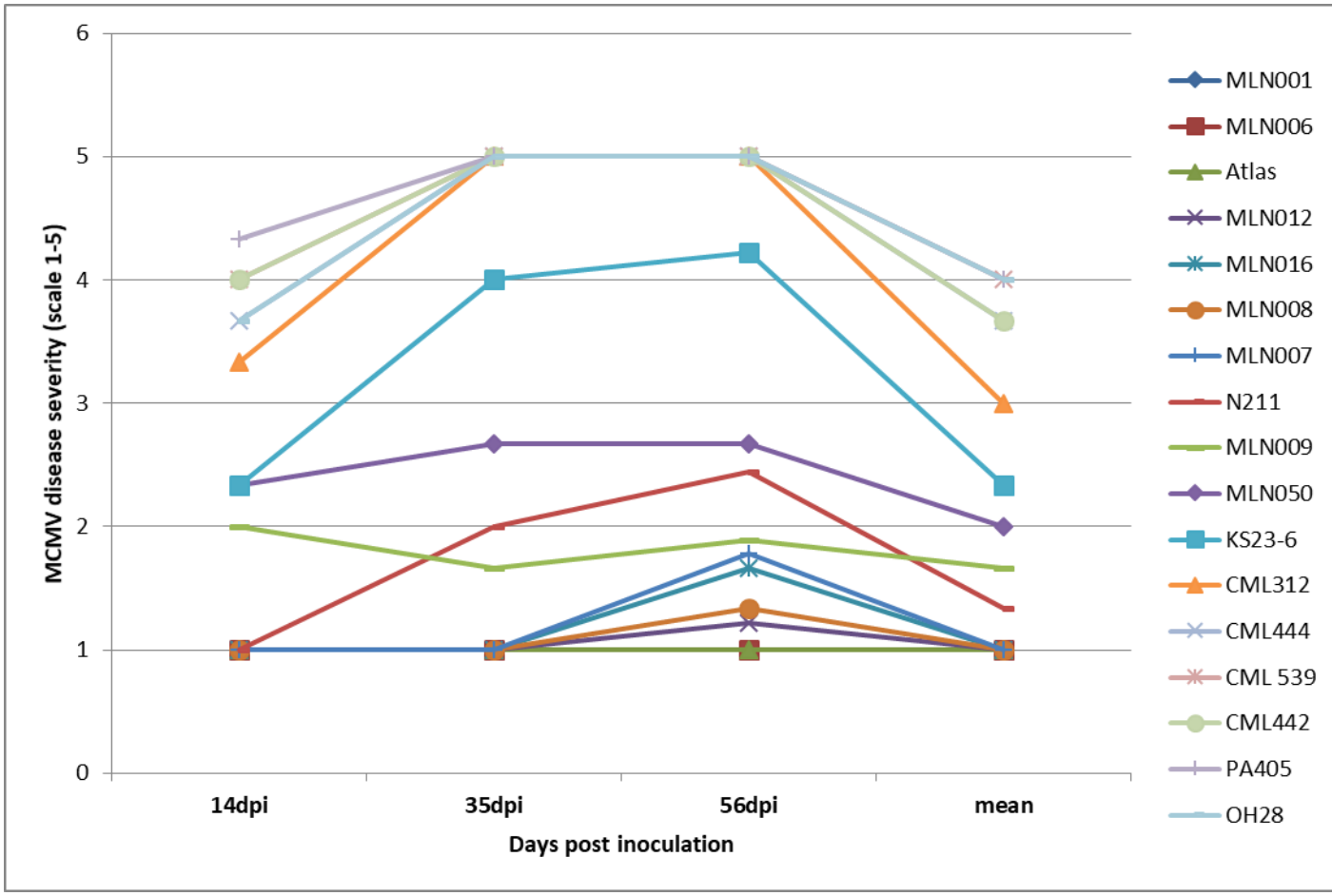

Figure 2. 5: MCMV disease severity progress for the resistant germplasm (bottom), tolerant (middle) and susceptible (top) obtained by plotting disease severity scores against the number of days post inoculation at 14 (early), 35 (middle) and 56 days (late)

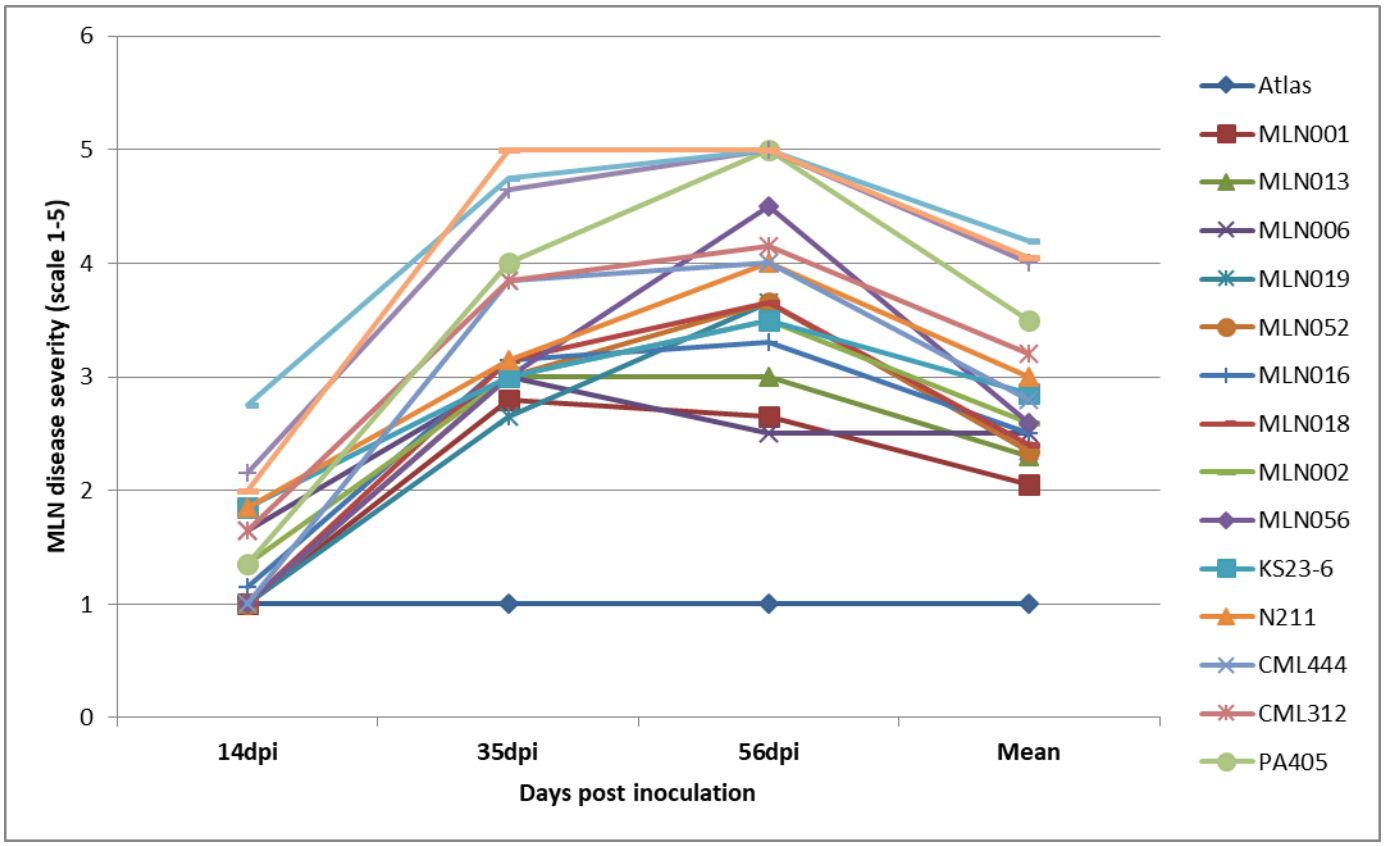

Figure 2. 6: MLN disease severity progress for the tolerant germplasm (middle) and susceptible (top) obtained by plotting disease severity scores against the number of days post inoculation at 14 (early), 35 (middle) and 56 days (late)

Nearly all plants inoculated with MLN (SCMV and MCMV combined) developed symptoms within 10 dpi. At 35dpi, 95% germplasm screened had a mean disease severity score of < 2.5. However, symptoms for genotypes MLN001 and MLN006 developed moderately with a final score of < 3.0 at 56 dpi (Figure 2.6). The disease severity on susceptible germplasm was high and reached a maximum score of 5 and highest AUDPC value of >650 (Table 2.1). More than 60% of germplasm under MLN showed stunted growth and dead heart (Figure 2.2b) or died before reaching knee height. Since the plants could grow to maturity, >50% of the germplasm did not produce tassels. Those that managed to produce tassels did not shed pollen (sterile) (Figure 2.2e). Most of the germplasm did not produce silks. The tolerant germplasm N211, KS23-6, MLN001, MLN006, MLN013 and MLN019 managed to tassel and produced silk. However, the ears were either deformed, partially filled or had husk cover senesced prematurely. If a maize field is infected with MLN late in the growing cycle, an increased dry husk (Figure 2f) and high number of rotten ears is observed (CIMMYT 2012, Wangai *et al*., 2012a).

Other than Sart and Atlas sorghums, no other germplasm was found to be completely immune to MCMV based on ELISA tests (Table 2.2). However, N211 and MLN016 can be moderately tolerant to MCVM given the low ELISA readings (Table 2.2). Moreover, inbred lines MLN013, MLN041 and MLN019 were found to be resistant to SCMV as no virus was detected with ELISA analysis. In germplasm MLN042, MLN020, PA405 and CML 444, SCMV virus was detected when analysed with ELISA suggesting low virus titre hence tolerance to SCMV.

However, there is a need to use real time quantitative PCR (RTqPCR) to confirm these results given the low sensitivity of ELISA. A negative genetic and phenotypic correlation was observed under MLN between 56 days post inoculation (dpi), disease severity scores pooled mean (dspm), area under disease progress curve (AUDPC) and days to 50% anthesis (Anth) and silking (SLK) (Table 2.3). Most of the plants in MLN experiment were stunted or died resulting in missing data for anthesis and silking, thus high cv (%) of 51.2 (Table 2.1). However, majority of the plants under SCMV and MCMV managed to flower despite the severe symptoms even for the checks (Table 2.1).

Table 2. 1: Mean performance of 10 lowest in disease severity from among 65 maize germplasm and 5 susceptible checks evaluated under SCMV, MCMV and MLN in 2015 P≤ 0.001

MCMV				**SCMV**				**MLN**	
Pedigree	**AUDPC**	**Days to 50 % anthesis**	**Days to 50% silking**	**Pedigree**	**AUDPC**	**Days to 50 % anthesis**	**Days to 50% silking**	**Pedigree**	**AUDPC**
MLN001	270	71.33	73.67	Atlas	286	68	73.67	Atlas	196
MLN006	270	59.67	62	MLN042	286	66	69	MLN001	471.3
Atlas	270	70	74.33	MLN041	288	69	70.67	MLN013	518
MLN012	286	75	77	MLN013	321	77.33	80.67	MLN006	535.5
MLN016	287	81	83	MLN019	378	71.33	70.33	MLN019	551.2
MLN008	292	77.33	78.67	PA405	385	61.67	62.67	MLN052	555.9
MLN007	298	76	82.67	MLN028	453	70.67	73	MLN016	565.8
N211	454	64.67	66.33	MLN020	474	63	60	MLN018	573.4
MLN009	459	72.33	75	MLN056	566	66	70.33	MLN002	582.8
MLN050	636	58.33	59.33	CML444	1311	73.67	74.33	MLN056	601.1
KS23-6	844	77.33	77.67	CML312	1323	70	70.33	KS23-6	606.7
CML312	1132	70.67	73.33	CML442	1326	70.67	73.33	N211	663.8
CML444	1166	84.33	86	CML 539	1363	66	68.67	CML444	666.8
CML 539	1165	69.33	71.33	OH28	1369	69.67	72.33	CML312	722.2

MCMV				**SCMV**				**MLN**	
Pedigree	**AUDPC**	**Days to 50 % anthesis**	**Days to 50% silking**	**Pedigree**	**AUDPC**	**Days to 50 % anthesis**	**Days to 50% silking**	**Pedigree**	**AUDPC**
CML442	1178	76.33	75.67	MLN006	960	58.67	63.76	CML 539	890.2
PA405	1182	67.33	70.67					OH28	913.5
OH28	1259	73.33	75					CML442	922.8
Mean	1019.94	73.099	76.12	Mean	1116.7	69.932	72.609	Mean	740.38
s.e.	69.15	5.993	6.159	s.e.	196.7	3.142	3.352	s.e.	79.94
cv%	6.8	8.2	8.1	cv%	17.6	4.5	4.6	cv%	10.8

Table 2.2: Enzymes-linked immunosorbent assay (ELISA) validation results for selected maize genotypes

MCMV			**SCMV**		
Genotype	**Elisa reading**	**Reaction type [a]**	**Genotype**	**Elisa reading**	**Reaction type [a]**
Buffer	0.18		MLN013	0.09	R
Atlas	0.25	R	MLN041	0.09	R
Health control (*3)	0.41		MLN019	0.09	R
MLN006	0.42	T	Buffer	0.09	
N211	0.47	T	MLN042	0.1	R
MLN016	0.66	S	Atlas	0.1	R
CML442	0.74	S	MLN020	0.11	R
MLN009	0.74	S	PA405	0.13	R
OH28	0.87	S	CML444	0.15	R
MLN007	0.94	S	OH28	0.2	T
MLN001	0.95	S	CML442	0.23	T
CML 539	1.03	S	MLN028	0.24	T
MLN012	1.03	S	CML 539	0.25	T
CML312	1.09	S	CML312	0.25	T
CML444	1.11	S	Health Control (*3)	0.26	
Positive control	1.15		Sart	0.28	Highly Susceptible
CML395	1.2	Highly Susceptible	MLN006	0.29	Highly susceptible
MLN050	1.25	Highly Susceptible	Positive control	1.07	
Heritability	0.85			0.96	
Mean	0.86			0.2	
LSD0.05	0.38			0.08	
CV (%)	25.17			22.66	

**Three times the reading for health control;* [a] R=Resistant; T=Tolerant; S=Susceptible

Table 2.3: Genotypic (upper triangle) and phenotypic (lower triangle) correlation estimates for 56 days post inoculation (dpi), disease severity score pooled mean (dspm), Area Under Disease Progress Curve (AUDPC) and days to 50% anthesis (Anth) and silking (SLK) measured in the screen house under; a) MCMV, b) MLN and c) SCMV

MCMMV					
Traits	**52dpi**	**dspm**	**AUDPC**	**Anth.**	**Slk**
52dpi	1	0.92***	0.99***	0.11*	0.12*
Dspm	0.95***	1	0.96***	0.16*	0.16*
AUDPC	0.99***	0.99***	1	0.13*	0.14*
Anth.	0.14*	0.20*	0.17*	1	0.93**
SLk[e]	0.15*	0.20*	0.17*	1.00	1
MLN					
52dpi	1	0.89***	0.93***	-0.51**	-0.60**
Dspm	0.92***	1	0.99***	-0.48*	-0.52**
AUDPC	0.96***	0.99***	1	-0.51**	-0.56**
Anth	-0.57**	-0.52**	-0.56**	1	0.80***
Slk	-0.73**	-0.62**	-0.67**	0.93***	1
SCMV					
52dpi	1	0.97***	0.99***	0.04ns	0.08ns
Mean	0.97***	1	0.99***	0.03ns	0.06ns
AUDPC	0.99***	0.99***	1	0.04ns	0.07ns
Anth	0.04ns	0.03ns	0.04ns	1	0.96**
Slk	0.09ns	0.07ns	0.08ns	0.98***	1

**significant at P<0.05; **significant at P<0.01; ***significant at P<0.001; ns, not significant*

2.5 Discussion

The analysis of disease severity revealed that genotypic effects of SCMV, MCMV and MLN were highly significant on all assessment/scoring dates as well as on the pooled mean and AUDPC values. The susceptible genotypes, CML444, CML 312, CML442, CML 539 and OH 28, showed the highest disease scores at all assessment dates, pooled mean and AUDPC. Atlas sorghum was immune to MLN, but no maize genotype was observed to be immune to MLN. Evaluation of the germplasm suggests that MLN041 and MLN042 has the desirable genes for SCMV resistance, while MLN001, MLN006, MLN016 and N211 have the genes for MCMV tolerance.

This study has also confirmed that PA405 developed in Pennsylvania is tolerant to SCMV strain used in this study. Inbred PA405 was reported to show complete resistance to MDMV and SCMV inoculation under both field and greenhouse conditions (Louie *et al*., 1990) with six major genes conferring resistance to SCMV and MDMV being identified (Dussle *et al*., 2000; Dussle, *et al*., 2002; Gemechu, *et al*., 2006; Rosenkranz and Scott, 1986; Uyemoto *et al*., 1981). Further studies need to be conducted on MLN042 and MLN041 to confirm and identify the number of genes conforming to resistance.

Inbred line N211, which showed delayed symptoms expression with the first symptoms appearing at 20 dpi, could be considered as partial tolerant to MCMV, since a low viral load was detected at 25 dpi using ELISA. The delayed symptoms development on N211 was also observed by Mahuku *et al*. (2015). Similar observations were also made by Kovacs *et al.* (1994) after screening different maize inbred lines and hybrids with MCMV. Nelson and Brewbaker (2011) also observed that the level of resistance to MCMV varied widely among the maize lines screened suggesting that MCMV resistance is controlled by many minor genes. The extended incubation period observed in N211 is an indication of the presence of resistance genes which are providing a certain degree of MCMV resistance. Similar results of delayed symptoms were observed under MLN for the inbred N211, KS23-6, MLN016 and MLN009. This could be associated to reduce synergistic interaction between SCMV and MCMV given their high level of tolerance to MCMV. Zihao *et al*. (2016) reported that the co-infection of MCMV and SCMV increases the accumulation of MCMV. The accumulation of MCMV genomic RNAs and the expression of MCMV CP have been observed to be higher in MLN-infected leaves than single-infected leaves, with no obvious difference observed for

SCMV RNA and SCMV CP expression levels between MLN and single-infected leaves (Zihao *et al*., 2016). This suggests that the symptoms expression in MLN for the inbred MLN042 and MLN041 were for MCMV given their high level of resistance to SCMV.

This study also validated the presence of MLN tolerance to MLN013 (CKDHL120312) and MLN019 (CKDHL140918) as earlier reported by CIMMYT (https://mln.cimmyt.org). However, more study needs to be conducted on these germplasm lines together with N211, KS23-6, MLN001, MLN006, MLN009 and MLN016 to understand the mechanism underlying the synergistic interaction between MCMV and SCMV (Beyene *et al*., 2017). The inbred lines should also be used to conduct further research on mechanism responsible for the healthy pollen-diseased silk relationship, SCMV and MCMV synergism as earlier suggested by Mikel *et al*. (1982).

The failure of >50 % of screened germplasm to reach flowering and/or delayed flowering, male sterility, poorly filled cobs and high number of rotten ears under MLN trials is an indication of the high impact of the disease on yield. Previous studies have reported intense susceptibility to MLN in East Africa (EA) among the pre-commercial and commercial maize varieties (Gowda *et al.*, 2015; Semagn *et al*., 2015).

2.6 Conclusion

The results presented here revealed enough variability for response to SCMV, MCMV and MLN. Atlas sorghum was immune to MLN, but no maize genotype was observed to be immune to MLN. MLN041 and MLN042 indicated presence of desirable genes for SCMV resistance, while MLN001, MLN006, MLN016 and N211 have the genes for MCMV tolerance. SCMV seems to play an important role in increasing MLN symptoms in MCMV susceptible maize genotypes and can be considered as a catalyst to its multiplication. This indicates that in developing MLN tolerant/resistant maize varieties more focus should be in identifying and using SCMV tolerant maize germplasm. The tolerant genotypes would be recommended for use as donors in the introgression of the tolerance into the Kenyan adapted maize backgrounds and development of improved MLN tolerant varieties combined with multiple abiotic and biotic stress tolerance traits.

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CHAPTER 3

Effect of maize lethal necrosis and its causative viruses (maize chlorotic mottle virus and sugarcane mosaic virus) on the growth and yield of maize as influenced by varietal tolerance/susceptible levels and plant stage at time of inoculation

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Abstract

Despite the development of maize lines and hybrids with improved resistance to MLN, little is known about the impact that these viruses have on grain yield losses. A field study was conducted in 2015-2017 using 25 improved maize varieties to estimate the yield loss caused by MLN and its causative viruses by comparing uninfected and infected plants in the field. The maize varieties were planted in an alpha lattice design with three replications. The plants were mechanically inoculated with MCMV, SCMV and dual (MCMV+SCMV) at three stages of growth; V_3, V_7, and V_T. Disease severity scores, grain yield attributes and grain yield were collected and mean performance and comparison for grain yield calculated. In the susceptible varieties, all measured variables were significantly reduced in V_3, V_7 and V_T inoculations, with the greatest reductions occurring in the V_3 inoculation stage. The greatest reductions occurred on plant height, ear height, days to 50% anthesis, number of ears at harvest, rotten ears and grain yield. Hybrid KH500-33A was significantly affected in the MLN and SCMV treatments recording a yield reduction of 96.6 and 90.3%, respectively, with WE1101 recording 85.4% in MCMV. Hybrid CKLMLN146038 was the best performer across the three treatments recording the lowest yield reduction of 17.0%, 29.7% and 31.5% in MCMV, MLN and SCMV, respectively. The results from this study indicate that under field conditions, (i) MCMV tolerant maize hybrids are less affected by MLN, and (ii) dual inoculation of most of the hybrids with MCMV and SCMV exacerbates symptom expression and yield loss in MCMV susceptible hybrids. The percentage of yield loss is likely to be proportional to the percentage of disease incidence and severity since plant densities in the

experiment and farmers' fields are probably too low to allow uninfected plants around the infected ones to compensate for the yield losses of infected plants.

3.1 Introduction

Maize is the staple food crop for more than 80% of the Kenya population, accounting for about 65% of the total food consumption. About 95% of the maize produced in the country serves the subsistence needs, with per capita consumption of 103kg per person. Maize accounts for 3% of Kenya's GDP, and it's the single most extensively grown in Kenya. It is grown from the coastal lowland at 0m asl to more than 2,300m asl in the highlands. Maize production has, however, been declining though it oscillates between bumper harvests and poor yields (Karanja *et al*., 2018).

The decreased yields are occasioned by a spectrum of challenges ranging from climatic hazards of droughts and flooding, low quality inputs, pests, and diseases. Fall Armyworm (FAW) insect pest and maize lethal necrosis (MLN) disease are the most recently emerged constraints. Farmers are also confronted by a double bind of soil acidification due to the continued use of Di-ammonium Phosphate (DAP) fertilizer which is necessitated by declining soil fertility due to monoculture. Other challenges include high production costs making the crop less competitive compared to prices from neighboring countries. For example, according to Tegemeo Institute, the cost of producing a 90kg bag in Kenya is about Ksh 1,800 compared to Uganda's at Ksh1, 000.

Good estimates of the economic impacts of MLN and its causative viruses is likely to point to the development of effective control measures by farmers, technology developers, and the governments.

It seems whichever way this pendulum swings, the farmer is always left holding the short end of the straw (FAOSTAT, 2018; Farmers Trend, 2018).

The new, improved maize varieties therefore come as a relief for farmers with statistics from the Ministry of Agriculture indicating that 75% of the 52 million bags (90kg per bag) harvested in 2018 came from smallholder farmers located in the Rift Valley and Western regions. This is just months after the county witnessed a massive shortage of maize due to a prolonged drought that affected farming in key producing areas. This was a 30% increase from the projected 40 million bags.

The nature of entry and replication of the MCMV virus into the plants also aggravates the MLN menace (Ingvardsen *et al*., 2010; Gowda *et al*., 2015). The virus has also been detected

in velvet crabgrass velvet crabgrass (*Digitaria velutina* L.), couch grass (*Digitaria abyssinica* L.), star grass (*Cynodon dactylon* L.), kikuyu grass (*Pennisetum clandestinum* L.), Sugarcane (*Saccharum officinarum* L.), finger millet (*Eleusine coracana* L.), sorghum (*Sorghum bicolor* L.) and signal grass (*Brachiaria brizantha* L.). MCMV has also been detected in Nut grass (*Cyperus rotundus* L.) and Napier grass (*Pennisetum purpureum* L.) (Kusia *et al*., 2014).. Other viruses that have been found to interact with MCMV in Kenya include; the polerovirus *Maize yellow dwarf virus* (MYDV-RMV) which was found to be widely distributed and the polerovirus Barley virus which was detected in 11 out of 68 samples analyzed (Wamaitha *et al*., 2018). MYDV-RMV was always found as part of a complex that included MCMV and SCMV, or MCMV, SCMV and MSV. The wide distribution of poleroviruses infecting maize in Rwanda (Adams *et al*, 2017) and in Kenya complicates the management of the disease.

Yield reduction in maize caused by maize chlorotic mottle virus (MCMV), sugar cane mosaic virus (SCMV) and maize lethal necrosis (MLN) is as a result of reduced kernel number per ear, reduced kernel weight, and increased number of rotten ears. Early infection of maize by the disease has been observed to cause reduced plant and ear height (stunted growth), delayed maturity (days to 50% anthesis and silking), smaller ears, reduced diameter and ear length, reduced ear weight and sterility expressed as missing kernels (James Karanja unpublished data). Under MCMV, SCMV and/or MLN infection, missing kernels could result from reduced pollen vigour, an alteration of silk receptivity, stigma and style receptivity for pollen germ tube growth, or an alteration in ovule, further complicating its management (Nelson *et al*., 2011).

MLN is known to kill plants before they can grow. MCMV and SCMV viruses are usually transmitted by insect vectors, contaminated soil debris, crop residue and seed (Mahuku *et al*., 2015). The Food and Agriculture Organization (FAO) of the United Nations reports that the first signs of the disease were reported in Longisa, Bomet County in September 2011 further spreading to other parts such as Nyamira, Kisii, Naivasha, Narok, Trans-Nzoia, Uasin Gishu, Busia and parts of Eastern region (Embu and Meru) (Karanja *et al*., 2018).

Notable symptoms of the viruses in maize include mild severe mosaic and mottling on the leaves, usually starting from the base of young leaves in the whorl and extending upwards towards the leaf tips, shortened internodes, chlorotic mottle, ‘dead heart’, necrosis starting from the leaf margin, premature drying of the husk and poor or no grain filling. New maize varieties released in 2016 and 2017 have proved their potential of overcoming MLN under natural infection following on-farm demos conducted in Bomet, Narok and Kisii counties (Karanja *et al.,* 2018). Initially, farmers in the region planted various maize seed varieties

such as H6213 and H614 but lost nearly 100% of their crops due to MLN infestation, according to the agricultural extension officer in the region.

The main objective of the study was to determine percentage yield loss caused by MCMV, SCMV and MLN at different plant growth stages at the time of inoculation.

3.2 Materials and methods

3.2.1 Germplasm

Twenty five (25) maize hybrids (10 commercial and 15 pre-commercials) were assembled and used to assess the effect/impact of SCMV, MCMV and MLN on growth and yield as influenced by varietal resistance / tolerance/ susceptibility levels and stages of growth at inoculation time. The maize varieties were selected based on their performance in the MLN screening conducted in 2013 at the KALRO/CIMMYT MLN screening facility at Naivasha (**Table 3.1).**

Table 3.1: List of maize hybrids used in the study

Entry	Pedigree	Entry	Pedigree
1	PAN 67	14	CKLMLN146038
2	WE1101	15	CKLMLN146092
3	DK8053	16	CKLMLN146281
4	KH500-33A	17	CKLMLN146025
5	DK9089	18	CKLMLN146027
6	SC Tembo 73	19	CKLMLN146032
7	WH403	20	CKLMLN146063
8	DH09	21	CKLMLN146065
9	H614	22	CKLMLN146082
10	PH 30G19	23	CKLMLN146096
11	CKLMLN146016	24	CKLMLN146405
12	CKLMLN146030	25	CKLMLN146407
13	CKLMLN146036		

3.2.2 Experimental sites, design and management

The KALRO/CIMMYT MLN Screening Facility site is located at 0º 41' 22" S; 36º 23' 43" E, 1884 m ASL, 85km from Nairobi at Naivasha along the Nairobi-Nakuru Highway. The station receives between 120 and 143 mm of rainfall in two seasons. It is a hot, dry region with a mean annual temperature of 22.6º C, mean annual maximum of 28.6º C and mean annual minimum of 16.5º C. The soils are well drained, very deep, dark reddish-brown to dark red, friable sandy clay to clay (Acri-Rhodic Ferrassols) developed from undifferentiated basement system rocks. The site is preferred for MLN, MCMV and SCMV screening because of isolation since no maize is grown in the surrounding fields. The site was established in 2013 after the outbreak of MLN in 2011 through funds from Bill & Melinda Gates Foundation (B&MGF) and Syngenta Foundation for Sustainable Agriculture (SFSA), with the first screening conducted in 2014 (CIMMYT, 2016).

KALRO-Kiboko research farm is located at 2º15' S; $37^0$45' E, 993 m a.s.l, in the dry mid altitude agro-ecological zone of eastern Kenya and experiences mean annual temperature ranges of 28 to 37^0C, with February and October being the hottest months. Kiboko receives a mean annual rainfall of approximately 530 mm. The soils are well-drained, Fluvisols, Ferralsols, and Luvisols, with soil pH of about 7.9 (Kivuva *et al*., 2014).

All trials were planted in an incomplete block design (alpha lattice design) with two replications. Each genotype was planted in a two-row plot of 5 meters long and spacing of 0.75 meters between rows and 0.25 meters between hills. Two seeds were sown per hill and later thinned to one plant per hill to maintain plant population density of 53000 plants ha^{-1}. During planting, phosphate fertilizers (P_2O_5) at a rate of 60 Kg per hectare was applied, and nitrogen fertilizer (N) at the knee height stage of plants was used for topdressing at a rate of 60 Kg per hectare. Weeds were removed throughout the growth cycle of plants and supplemental irrigation done when required to ensure plants grew healthily.

The trials at KALRO Kiboko (optimum) was sprayed with duduthrin 250EC (carbosulfan 250g/l), Thunder OD 145 (imidacloprid 100g/l + Beta-cyfluthrin 45g/l), and Escort® 19EC (Metsulfuran methyl) at weekly intervals the vectors aphids, thrips and FAW.

The trials at KALRO/CIMMYT MLN screening site were inoculated at V_3 stage (3rd leaf stage), V_7 (7 leaf stage) and V_T (tasselling). Two trials were left non-inoculated (controls) to assess role of vectors in MLN transmission (natural transmission). Two optimal trials were conducted at Kiboko and Naivasha (MLN-free) to assess and record the potential yields of

the test hybrids. Disease severity were observed weekly and recorded at 26, 33, 40, 54, 61- and 72-days post inoculation (dpi). Severity was evaluated on the two uppermost leaves within a row using 5-point scale; where 1= absence of symptoms or chlorotic spots covering <25% of the leaf surface with no stripes and 5= a previously symptomatic plant dead heart. Other data collected included plant stand at harvest, days to 50% flowering (anthesis and silking), plant height, ear height, number of infected ears within a row, number of ears harvested, number of rotten ears, grain weight and grain moisture content.

3.3 Data analysis

The SAS and Genstat version 18.2 computer programs were used to perform analysis of variance for severity scores, AUDPC, grain yield and yield related characters (SAS, 2013; Baird *et al.*, 2017). The programs computed mean performance and comparison for grain yield and yield-related traits. The respective standard errors were also calculated (Dabholkar, 1992). Analysis of variance for each trial and combined analysis across years was performed using Multi-environment Trial analysis with R; version 5.0 to determine the level of significant difference between genotypes for severity scores, AUDPC, grain yield and yield related characters. Area Under the Disease Progress Curve (AUDPC) which is a better indicator of disease expression over time was calculated on a single plant basis by trapezoidal integration over the whole observation period as follows:

$AUDPC = \sum_i [(DS_i + DS_{i-1}) \times (t_i - t_{i-1})]/2$

Where "*i" ={26, 33, 40 and 61}* are the days of observation, "*DS*" is the disease score using the above severity score of 1 to 5 and "t" represents the number of days post-inoculation (Campbell and Madden, 1990; Jegger and Viljane-Rollinson, 2001). Genotypic correlations (R_g) between treatments were estimated according to Cooper and Delacy, 1996 as:

$R_g = R_{p(12)} / (H_1 \times H_2)^{1/2}$

In which $R_{p(12)}$ is the phenotypic correlation between the traits measured in Treatments 1 and 2, H_1 and H_2 are the broad-sense heritability for the trait measured in season 1 and 2, respectively.

Maize grain loss percentages were calculated according to the following formula modified for the formula described by Zahid *et al*., (2008):

$\%\text{yield loss} = [(Y_{op} - Y_{t2\ldots5} / Y_{op}) \times 100]$

Where: Y*op* = optimal yield, which is corresponding to T1 where the maize plants were sprayed with Chlorantraniliprole. Y*t2*... *4* = yield for each SCMV, MCMV and MLN treatment.

3.4 Results

The results obtained in the experiment clearly showed the effect of SCMV, MCMV and MLN on plant growth which significantly varied with the time of inoculation. The effect of MLN and its causative viruses on yield, plant height, 50% days to flowering and ear rot was significantly different at *P =0.05*. The highest plant height was recorded for WH403 (211.3cm) and the lowest was recorded for CKLMLN146281 (146.6cm) in optimum treatment. Hybrid WH403 was the most affected with a reduction in plant height of 42.2% (MLN), 38.3% (MCMV) and 37.9% (SCMV), with the tolerant varieties WE5135 and CKLMLN146281 least effected (Table 3.2). Most of the maize hybrids were able to perform well under optimum, MCMV, SCMV, and MLN recording an average yield of 6.5, 2.4, 1.6 and 1.4 tha^{-1}, respectively (Table 3.3). The results showed that hybrids CKLMLN146036, DK9089, CKLMLN146096 and WE1101 resulted in a higher mean yield of > 9.0 tha^{-1}, while CKLMLN146038 (3.2 tha^{-1}) was the least responsive hybrid under optimum conditions. Hybrids CKLMLN146030 and CKLMLN146038 recorded the highest yield of > 2 tha^{-1} under MLN with WE1101, PH30G19, DH04, H614 and KH500-33A significantly recording lowest yield of <1 tha^{-1} (Table 3.3). Similarly, hybrid CKLMLN146030 was the top performer in MCMV and SCMV with a yield of 3.6 and 2.5 tha^{-1}, with H614 performing poorly in SCMV (1 tha^{-1}) and MCMV 2tha^{-1}).

The yield decrease was highly significant in all treatments recording a mean of 71.2%, 58.9% and 75.3%, in SCMV, MCMV and MLN, respectively, below the health control trials (Table 3.3). CKLMLN146038 was the least affected in the three treatments with a mean reduction of 31.5%, 17% and 29.7% in SCMV, MCMV and MLN, respectively. This hybrid also recorded the lowest yield of 3.2 tha^{-1} under Kiboko and Naivasha MLN free trials. Hybrids WE1101, H614 and KH500-33A were the most affected, recording an average of > 80% yield reduction across SCMV, MCMV and MLN trials (Table 3.3). However, the yield loss decreased with the time of inoculation with the least effect observed at $V_{T.}$ Inoculation at flowering (V_T) had more effect on the cobs resulting in pre-mature drying of the husk cover and increased ear rots (Figure 3.2). However, more effect was observed at V_7 in SCMV trials compared to the other treatments (Figure 3.1). Number of ear rots increased with time of inoculation in all the treatments with V_T recording the highest of 52.3%, 39.4% and 37.3% in MLN, SCMV and MCMV respectively (Table 3.2).

Table 3.2: Effect of SCMV, MCMV and MLN yield and yield attributes and disease severity at V_3, V_7 and V_T inoculation time

Treatment	Yield ($tonha^{-1}$)	Days to 50% anthesis	Anthesis-silking interval	Plant height	Ear height	Husk cover	Ear rot	dpi26	dpi33	dpi40	dpi61	Mean	AUDPC
V_3													
SCMV	1.7***	108.0^{ns}	3.2^{ns}	134.5^{ns}	81.9^{ns}	22.8^{ns}	N/A	2.4^{ns}	2.8^{ns}	3.1^{ns}	3.4^{ns}	3.0^{ns}	374.5^{ns}
MCMV	1.8***	101.6***	3.1^{ns}	120.4***	68.8^{ns}	N/A	32.2***	1.8^{ns}	3.1^{ns}	3.4^{ns}	3.8^{ns}	3.1^{ns}	430.8^{ns}
MLN	1.1***	102.6^{ns}	4.2***	103.4***	55.4***	8.8^{ns}	13.4^{ns}	2.4^{ns}	3.3^{ns}	3.6^{ns}	3.9^{ns}	3.4^{ns}	555.5^{ns}
V_7													
SCMV	1.5***	106.8^{ns}	2.8^{ns}	148.5***	89.2^{ns}	21.2***	36.8***	2.4^{ns}	2.7^{ns}	3.1^{ns}	3.5^{ns}	3.0^{ns}	363.2^{ns}
MCMV	2.2^{ns}	103.5^{ns}	2.9^{ns}	137.3^{ns}	84.8***	N/A	37.9^{ns}	2.6^{ns}	3.0^{ns}	3.1^{ns}	3.4^{ns}	3.0^{ns}	362.9^{ns}
MLN	1.6***	80.5^{ns}	1.0^{ns}	129.2***	80.2***	18.3^{ns}	29.2***	2.4^{ns}	2.9***	3.4***	3.7***	3.3***	385.2***
V_T													
SCMV	1.8***	106.8***	2.1^{ns}	148.8^{ns}	90.6***	60.1^{ns}	39.4^{ns}	2.5^{ns}	2.5^{ns}	2.3^{ns}	2.8^{ns}	2.6^{ns}	202.5^{ns}
MCMV	3.1^{ns}	211.4^{ns}	2.2^{ns}	138.5***	86.6^{ns}	N/A	37.3^{ns}	2.5***	3.1^{ns}	2.5^{ns}	2.8^{ns}	2.6^{ns}	205.2^{ns}
MLN	1.5^{ns}	104.0^{ns}	2.1***	145.7^{ns}	91.9	49.9***	52.3***	2.3^{ns}	2.5	3.1^{ns}	3.3	2.7^{ns}	213.9^{ns}
Health (Kiboko)	4.84***	61.7***	2**	202.9***	98.1^{ns}	5.1^{ns}	5.1^{ns}	N/A	N/A	N/A	N/A	N/A	N/A
Health (Naivasha)	6.5^{ns}	88^{ns}	1.9^{ns}	175.3***	98.1^{ns}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Disease symptoms were observed in all the inoculated plants, but the maize genotypes differed significantly ($P < 0.05$) for the resistance to SCMV, MCMV and MLN and time of inoculation. Disease symptoms also differed significantly for plant growth, with commercial hybrids being more susceptible than the pre-commercial hybrids (Table 3.4). Disease severity was lowest in V_T in all the trials at 26, 33, 40- and 61-days post inoculation with a mean score of 2.6 for SCMV and MCMV and 2.7 for MLN. It was also at V_T where the lowest AUDPC was recorded (Table 3.4). The overall performance for the test hybrids was low in the MLN trials with hybrids CKLMLN146405, CKLMLN146016, CKLMLN6281 and CKLMLN6068 performing better than WE5135 with a score of <3.0 (Table 3.4). They also showed some vigour and were devoid of moderate symptoms of the disease. CKLMLN6405 was the most tolerant in both SCMV (2.6) and MLN (2.7) trials, with CKLMLN146016 and CKLMLN6281 emerging as top performer under MCMV and MLN. DH04 was the most affected hybrid in SCMV, MCMV and MLN with a score >3.5 (Table 3.4).

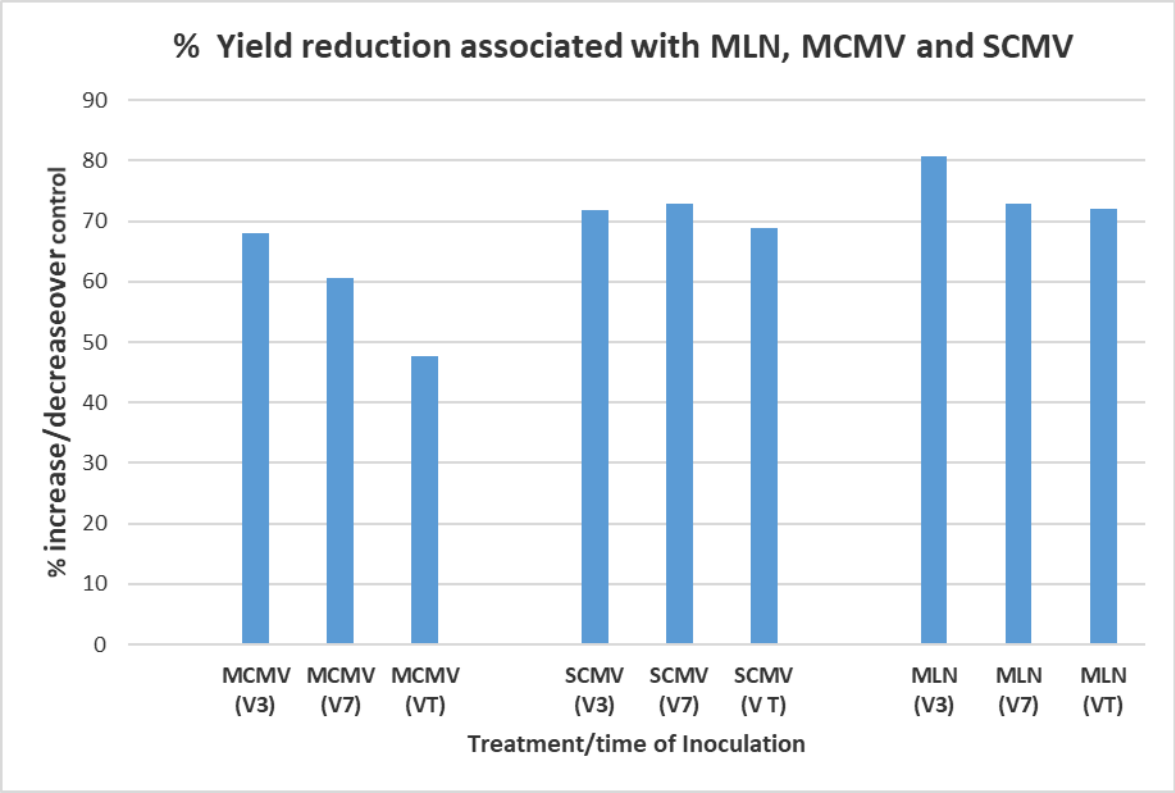

Figure 3. 1: Percentage increase /decrease in yield as effected by time of inoculation

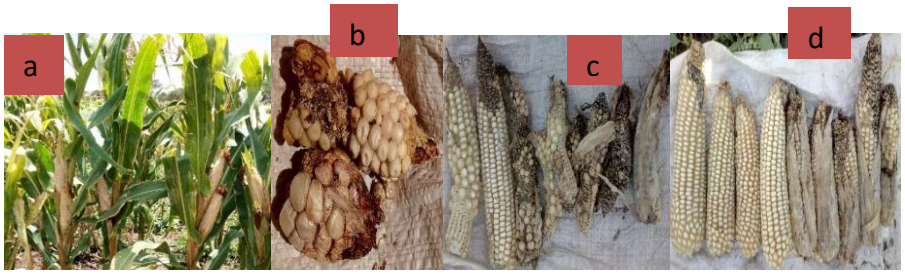

Figure 3. 2: (a) Pre-mature husk cover drying at V_T; (b) Ear rots; (c) V_3; (d) V_7 for CKLMLN146281

Table 3.3: Effect of SCMV, MCMV and MLN on plant growth and yield

	Health				SCMV					MCMV					MLN				
Hybrids	Yield (ton/ha)	50% days to anthesis	Plant Height	Ear Height	Yield (ton/ha)	50% days to anthesis	Plant Height	Ear Height	% yld loss	Yield (ton/ha)	50% days to anthesis	Plant Height	Ear Height	% Yield loss	Yield (ton/ha)	50% days to anthesis	Plant Height	Ear Height	% yld loss
CKLMLN146016	7.8	90.8	150.4	97.1	2.4	104.6	149.9	92.0	69.5	2.8	135.7	135.8	81.6	63.9	1.9	88.6	133.8	79.4	75.5
CKLMLN146025	4.3	87.3	175.3	88.9	1.8	107.1	138.4	82.2	59.0	2.1	136.8	128.3	77.3	52.1	1.5	94.6	131.9	78.7	64.6
CKLMLN146027	4.2	86.3	167.2	102.9	1.8	103.4	139.4	87.9	56.8	2.0	131.9	134.0	84.3	52.1	1.6	97.3	126.7	74.7	63.0
CKLMLN146030	7.6	90.8	189.1	100.7	2.5	107.8	152.2	92.6	66.8	3.6	110.6	145.4	94.3	53.1	2.4	110.6	148.1	88.1	68.9
CKLMLN146032	6.0	91.2	165.4	94.5	1.5	101.7	146.9	91.5	75.7	2.0	140.9	131.3	83.4	66.4	1.5	94.6	129.6	78.6	74.7
CKLMLN146036	9.6	86.3	204.7	96.7	2.1	104.6	153.8	94.8	77.9	6.7	140.6	134.9	83.8	30.4	2.0	82.3	134.8	88.0	79.2
CKLMLN146038	3.2	87.9	167.9	86.4	2.2	101.7	144.8	81.6	31.5	2.6	140.5	128.2	75.3	17.0	2.2	92.7	126.3	73.4	29.7
CKLMLN146063	7.2	86.3	183.5	114.5	1.8	111.8	144.9	87.5	75.3	2.3	139.0	135.7	82.1	68.3	1.4	94.7	128.8	75.4	80.4
CKLMLN146065	4.3	87.2	158.4	106.6	2.0	107.3	148.7	118.9	53.2	2.4	137.0	139.0	84.5	43.8	1.9	95.4	134.6	80.9	54.8
CKLMLN146082	7.0	88.4	178.4	102.1	2.1	104.8	153.9	96.6	70.4	2.3	137.7	133.4	84.1	66.7	1.6	95.2	130.6	77.1	76.7
CKLMLN146092	6.6	89.1	166.9	98.8	2.8	110.2	138.1	78.5	57.4	3.0	139.6	137.8	81.3	54.8	1.9	125.0	130.2	76.3	71.1
CKLMLN146096	9.3	86.1	195.9	98.5	2.2	101.9	149.3	92.0	75.9	2.2	138.1	132.3	79.6	76.5	1.4	95.9	129.2	78.2	84.9
CKLMLN146281	4.6	87.8	146.6	80.9	2.5	117.6	156.8	92.2	45.4	3.4	106.9	154.2	94.6	26.4	1.7	112.6	136.4	82.4	62.2
CKLMLN146405	5.4	85.8	163.3	91.0	1.6	102.0	145.5	88.6	71.0	2.4	138.4	132.9	80.2	55.7	1.6	95.1	127.3	78.0	71.0
DH04	5.2	89.3	176.4	107.2	0.7	104.3	131.3	76.3	87.1	2.0	138.4	113.5	55.7	60.8	0.6	98.0	115.8	68.3	87.8
DK8053	5.9	93.5	188.7	106.6	1.1	105.4	129.2	76.6	81.2	1.7	139.4	130.4	75.6	71.0	1.6	94.2	111.3	69.4	72.2
DK9089	9.5	86.7	161.3	95.3	1.4	104.2	139.9	85.6	85.0	2.1	137.6	121.2	73.4	77.6	1.0	93.3	115.7	70.2	89.2
H614	5.7	86.1	197.0	99.7	0.9	104.7	153.4	99.5	84.2	1.5	142.0	131.7	83.6	73.2	0.5	96.1	135.1	91.4	90.7
KH500-33A	8.9	89.0	159.7	83.4	0.9	110.1	132.0	84.9	90.3	3.0	139.8	133.9	92.1	66.4	0.3	98.4	118.3	72.0	96.6
PAN 67	7.9	84.9	166.2	90.2	1.8	109.8	135.6	76.1	76.9	1.8	149.7	126.9	68.6	76.9	1.2	92.0	120.0	70.0	84.8
PH 30G19	5.6	88.4	167.1	96.0	0.8	117.0	153.7	82.3	86.2	2.2	120.3	133.2	76.7	60.1	0.7	97.1	129.1	73.9	87.5
SC Tembo 73	4.5	93.8	204.4	123.0	1.8	115.3	134.0	87.7	59.0	2.3	131.5	135.0	82.8	47.8	1.3	102.2	126.9	74.7	71.5
WE1101	9.1	85.5	175.5	92.0	1.2	116.7	123.4	71.4	87.2	1.3	141.9	117.7	68.9	85.4	0.9	96.2	106.9	58.4	90.5
WE5135	6.0	85.7	162.5	85.7	1.8	128.7	200.7	90.6	69.3	2.3	91.5	149.4	93.4	61.9	1.8	95.4	149.1	87.8	69.8
WH403	7.3	86.7	211.3	113.2	0.8	109.1	131.2	81.8	89.1	2.7	145.0	130.4	65.9	63.5	1.1	102.6	122.1	72.4	85.4
Grand Mean	**6.5**	**88.0**	**175.3**	**98.1**	1.6	107.2	143.9	87.2	71.2	2.4	138.8	131.6	79.5	58.9	1.4	95.7	126.1	75.9	75.3
LSD		**5.6**	**26.1**	**30.4**	0.8	15.1	35.3	14.0		2.5	66.1	16.3	10.1		1.0	22.5	19.6	16.1	
CV	**30.4**	**3.2**	**11.4**	**18.0**	27.8	8.6	15.0	9.8		64.1	29.1	7.2	7.4		41.1	14.4	9.5	13.0	
p	ns	+	***	ns	***	***	ns	***		*	ns	***	***		***	***	***	*	

Table 3.4: SCMV, MCMV and MLN severity on selected maize varieties under artificial inoculation

	SMCV			MLN			MCMV		
Genotype	dpi61	Mean	AUDPC	dpi61	Mean	AUDPC	dpi61	Mean	AUDPC
CKLMLN146016	2.93	2.67	294.73	3.33	2.83	343.93	2.8	2.47	282.97
CKLMLN146025	3.1	2.8	306.3	3.47	2.97	359.2	3.13	2.73	320.33
CKLMLN146027	3.33	2.9	319.5	3.9	3.27	401.27	3.33	3.03	344.03
CKLMLN146030	3.7	3.27	396.17	3.9	3.1	416.5	3	2.6	328.53
CKLMLN146032	3.1	2.77	308.03	3.6	2.87	364.83	2.87	2.47	279.67
CKLMLN146036	2.9	2.77	298.03	3.4	2.93	364.5	3.13	2.77	312.93
CKLMLN146038	2.9	2.7	298.23	3.43	2.97	365.23	2.83	2.53	282.33
CKLMLN146063	3.23	2.83	312.07	3.47	2.83	354.7	3.1	2.63	304.6
CKLMLN146065	3.03	2.77	301.67	3.37	2.9	369.2	3.17	2.73	315.97
CKLMLN146082	3.13	2.8	309.3	3.4	3.07	376.47	3.13	2.8	325.6
CKLMLN146092	3.03	2.67	294.3	3.4	2.93	361.1	2.87	2.6	296.1
CKLMLN146096	3.03	2.8	309.13	3.5	3.17	391.17	3.27	2.87	326.5
CKLMLN146281	3.17	2.8	340.57	3.47	2.83	394.73	2.47	2.27	311.47
CKLMLN146405	2.93	2.6	286.1	3.2	2.7	335.4	3	2.73	320.8
DH04	3.8	3.27	365.27	4.63	3.83	470.67	4.17	3.63	428.57
DK8053	3.63	3.17	360.57	4.57	3.8	459.5	3.93	3.4	396.47
DK9089	3.57	3.13	353.83	4.13	3.43	422.13	3.67	3.17	367.83
H614	3.67	3.2	341.7	3.97	3.37	417.3	5.3	3.93	438.1
KH500-33A	4	3.37	384.2	4.47	3.6	439.9	3.87	3.33	399.33
PAN 67	3	2.83	307.77	3.77	3.3	402.27	3	2.73	307.67
PH 30G19	3.67	3.13	357.1	4.3	3.57	431.87	3.63	3.03	378.47
SC Tembo 73	3.2	2.8	307.97	3.1	2.97	395.87	3	2.87	326.03
WE1101	3.63	3.13	345.23	4.47	3.77	463.23	3.83	3.47	404.3
WE5135	3.13	2.6	279.37	3.27	2.87	303.47	3.17	2.73	304.07
WH403	3.63	3.17	347.8	4.3	3.6	446.4	4.03	3.53	404.27
Grand Mean	3.21	2.85	313.38	3.65	3.12	384.88	3.3	2.9	332.96
LSD	0.48	0.37	58.31	0.68	0.47	52.31	0.9	0.44	70.17
CV	9.12	7.89	11.38	11.34	9.24	8.31	16.62	9.37	12.88
p	***	***	***	***	***	***	***	***	***

3.5 Discussion

The results obtained in the experiment showed that extreme chlorosis and necrosis are the root cause for yield loss in maize productivity either in SCMV, MCMV or MLN infected fields. A significant interaction between variety and time of inoculation indicated that varieties were differently affected by SCMV, MCMV, and MLN in relation to the growth stage. Plant age at the time of inoculation had a significant effect on yield, and growth characters, with earlier infection resulting in greater disease severity and yield reduction in

MCMV and MLN. SCMV, MCMV and MLN disease reduced yield and growth in all years, but the varieties differed significantly in the amount of loss, disease severity and incidence. For disease rating, the pre-commercial maize varieties were moderately tolerant to SCMV and MCMV with a disease severity <2.8. CKLMLN146281, CKLMLN146016, CKML146032, and CKLMLN146038 were the best in terms of MCMV tolerance. There was no variety that was found to be best in terms of MLN tolerance as the hybrids were scored > 2.5. All commercial hybrids were susceptible to SCMV, MCMV and MLN. , The yield of susceptible varieties was significantly more, by 60 - 90%. A screening done for all commercial hybrids in 2013/2014 revealed that they were highly susceptible to MLN (unpublished data). This confirms the quick spread of the disease to 43 counties within a year from the first report (Wangai *et. al*., 2012). The susceptible hybrids need to be replaced by the new hybrids, such as KATEH16-01, KATEH16-02 and KATEH16-03 from KALRO, WE5135, WE5140, WE7117 and WE7118, WE6109 with a score < 2.5 for better adaptation to MLN endemic belt to enhance higher yield. The use of these hybrids offers many advantages, especially if grown with integrated pest management practices (Yuanful *et al*., 2007). However, the yield potential of these hybrids is not guaranteed under single virus-infected fields as proved by WE5135, with yield that was reduced by 69.3% and 61.9% due to damage by SCMV and MCMV, respectively. Multi-seasonal testing is required before settling on a suitable hybrid for release and registration as it recorded an average of 69.8% across the four seasons of testing.

3.6 Conclusion

Results of this study have indicated that infestation by SCV, MCMV and MLN could result in yield losses in both early and late growth period maize, although, the extent of yield loss in time of infestation may vary from variety and season to season. The study also revealed that the MLN tolerant hybrids were susceptible to SCMV, and the time of infection does not matter. The improved maize varieties tested are generally suitable for production in areas that have SCMV and MCMV infestation. Within a given infestation time, the incidence of disease severity and consequent yield loss tended to decrease. Based on the observed time of infestation in all the treatments, it is concluded that infestation at V_T was responsible for the increased number of rotten cobs. In the farmers' condition infestation at V_T may commence earlier depending on the inoculum load in the soil, residual crop and vectors. The viability season break and rotational cropping should be investigated, since reports have shown that the practices are less prone MLN and yield loss. To increase gains in maize yields under

MLN, increased emphasis needs to be placed on SCMV screening which can be used to discard susceptible hybrids at an early stage before conducting the expensive multi-location testing and nomination for registration. CKLMLN146038 was identified as fairly resistant to MLN and its causative viruses despite its low yield under optimum conditions. However, the reality is that for most farmers in Kenya, the time of planting is largely dependent on the onset of rainfall. As such, during a growing season when the rains are late, and if high vectors activity occur, significant levels of yield loss may be experienced. Therefore, better estimates occurrence and effect of SCMV and MCMV viruses in the fields will facilitate the development of more robust strategies for MLN control.

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CHAPTER 4

Half-Diallel Cross Analysis of MLN Tolerant Maize Inbred Lines

Abstract

Breeding for disease resistance can offer a solution but takes a lot of time before varieties are released. Hybrid breeding is an effective approach to enhance crop productivity and yield stability. The development of hybrids involves the selection of inbred parents with desired attributes and either good general or specific combining ability. This study aimed to evaluate crosses among MLN tolerant elite maize lines for their general and specific combining abilities to identify those with high combining ability for MLN tolerant/resistant hybrid formation. Fifty-five (55) crosses were obtained from a partial diallel that involved 12 inbred lines. The 55 F_1 crosses were evaluated for grain yield, plant height, ear heights, days to anthesis, days to silking and MLN severity at four locations in Kenya in an alpha lattice design with three replications in each location in 2016, 2017 and 2018. Genotypes, general and specific combining ability mean square were highly significant at all traits. The inbred lines MLN001, MLN019, OH001 and OH002 had good GCA for both MLN resistance and high grain yield qualifying them as suitable candidates for use in breeding for the disease. The contributions from general combining ability (GCA) to the sum of squares of the F_1 crosses showed additive effects for yield, days to 50% anthesis and ear position. The GCA contribution was less than 50%, indicating that the non-additive effects were more important for plant and ear height. The GCA to SCA ratio was close to a unity for grain yield, further indicating the predominance of additive over non-additive gene action for grain yield in these inbred lines. The MLN disease severity ratings on the maize hybrids and inbreds ranged from 2.0 to 8.0. The top performing cross under MLN was MLN001/MLN019. Lines MLN001 and MLN 019 were earlier identified to be highly resistant to MLN. The highest yielding F_1 hybrid was CML312 x CML442 with 6.5 tha^{-1} and recording a 33.6% yield advantage above the mean of commercial checks. Other crosses that showed superior yield performance were CML444 x MLN019, CML442 x CML444, MLN018 x MLN019, MLN018 x OH002, CML444 x MLN018 and MLN008 x MLN013 all recording a grain yield advantage of > 20% above the mean of commercial checks in descending order. CMLN442 and CML444 had been earlier identified to be SCMV resistant, OH002 and MLN008 MCMV resistant while MLN019 and MLN013 as highly resistant to SCMV and MLN. These results indicate that SCMV resistance is important in breeding for MLN resistance. Environment x GCA

interactions was highly significant (P<0.001) for grain yield suggesting changes in rank of GCA and SCA across environments. Genotype and genotype ×environment (GGE) biplots indicated an absence of crossover interaction and revealed positive associations among testing environments, signifying the suitability of all the environments for earliness and yield. Crosses CML442 x CML444, CML312 x CML444, CML444 x MLN019 and MLN018 x MLN019 had high yield means and stability. Inbred lines CML312, CML442, MLN013, OH001 and MLN 019 are identified as good combiners for yield and earliness. MLN013 and MLN019 should have been identified as a donor for MLN tolerance and should be exploited in developing MLN tolerant varieties. However, other technologies such as gene editing should be used in converting CML312 and CML442 to MLN resistance.

4.1 Introduction

Maize lethal necrosis (MLN) caused by the synergistic interaction between *potyvirus* and maize chlorotic mottle virus (MCMV) was added to this list of endemic diseases of maize in the tropical Kenya (Wangai *et al.*, 2012b). MLN is now widely distributed in the eastern and central Africa (ECA) region and causes yield losses of more than 70% in susceptible genotypes (Karanja *et al.*, 2020). Therefore, this disease has become important in the region, and the incidence and severity of epidemics have been increasing (Prasanna *et al*., 2020).

A wide range of chemical control methods ranging from seed dressing to vector spraying, early planting, and practicing maize free window can reduce incidence and severity of MLN disease, but host plant resistance offers the most economical and easy-to-use management option. Farmers in Hawaii and other endemic areas of the United States have been using MCMV-tolerant maize hybrids (Nelson and Brewbaker 2011; Jardine, 2018). In Kenya, nearly 200,000 genotypes have been screened at the MLN screening facility under artificial inoculation using isolates that were collected from the hot spot areas (CIMMYT 2012; https://mln.cimmyt.org/). MLN tolerant germplsm identified from the screenings have been used to develop 18 hybrids; 4 first-generation with a mean MLN severity score of 5.5 and 14 second-generation with MLN severity scores of 4.0 on a scale of 1–9 that have been released in East Africa in collaboration with CIMMYT, public and private partners. With 70-80% yield penalty on commercial MLN-susceptible hybrids, the first-generation and second-generation hybrids recorded 3.4 tha^{-1} and 4.6 tha^{-1} respectively (Prasanna *et al*., 2020; Karanja *et al*., 2020). Breeding for MLN resistance is now an integral component of public and private institutions, including international maize breeding institutions.

Evaluation of maize germplasm collected from Kenya in search of MLN resistance suggests that they are susceptible to MLN. However, the germplasm were not tested against individual viruses (Mahuku *et al*., 2015). To date, new resistance sources for SCMV, MCMV, and MLN have been identified from diverse germplasm. Identification of best parental lines with either resistance to SCMV, MCMV or combined is crucial for the successful development of MLN resistant hybrids. Maize hybrids with resistance to *Potyviruses* and MCMV are have been developed in some parts of the world (Nelson *et al*., 2011). More MLN resistant sources and their causative viruses need to be identified and the mode of inheritance of the resistance be investigated. The new lines with high level to moderate resistance to SCMV, MCMV, and MLN identified can be used by partners both through coordinated projects and individually to develop more superior MLN resistant varieties.

Hybrid performance prediction involves the estimation of the breeding value of the crosses. The hybrids are developed by utilising elite lines. As a result, improved hybrids developed through crossbreeding of elite inbred lines followed by a subsequent evaluation in multiple environments to identify superior varieties adapted to different agro-ecologies. However, developing inbred lines with high performance is time-consuming. For this reason, and considering the high demand for maize in Kenya, KALRO, CIMMYT, and private companies have focused on developing superior hybrids with high performance and stable adaptation throughout different regions, climates, and planting times (Wangai et al., 2012a). With the availability of molecular tools and doubled haploid (DH) technology in maize, breeders are quickly and accurately developing a large number of inbred lines with a high level of homozygosity (Chaikam *et al*., 2019). It was important to estimate the value of MLN tolerant lines in maize hybrid development. For complex traits such as MLN resistance, line per se is a poor predictor of hybrid performance. The mean values of parents and F1 combinations are important for estimating combining ability, evaluating the performance of hybrids, and selecting superior parents.

The diallel design has been widely used for estimating GCA and specific combining ability (SCA) effects as well as other genetic parameters for grain yield (GY) and other traits (Reif *et al*., 2013; Beyene *et al*., 2017). A commonly used diallel analysis to estimate GCA and SCA effects of parents and crosses is the Griffing's method (Griffing, 1956) and its modified versions. Biplots have been primarily used for analyzing multi-environment crop trials and

studying genotype x environment interactions (Crossa and Cornelius, 1997; Yan *et al*., 2000). They can also be used for studying response patterns of entries when crossed with testers, that is, line X tester interactions (Narro *et al*., 2003) and diallel crosses (Yan and Hunt 2002; Bhatnagar *et al*., 2004).

It is essential to know the extent and pattern of genetic diversity among germplasm lines for identifying and selecting parents to develop heterotic F_1 hybrids and to identify sources of useful alleles for introgressive hybridization (Badu-Apraku et al., 2016). Information on the type of gene action controlling yield and related traits under stress is key for identifying useful parents and hybrids, as well as for designing suitable strategies to breed multiple stress-tolerant hybrids. This information is available through combining ability studies conducted by several researchers (Elmyhun *et al*., 2020, Oyekunle *et al*., 2013; Badu-Apraku *et al*., 2011; Derera *et al*., 2008) who have reported conflicting observations.

The first report on heterosis in maize was reported by Shull (1908), while the concept of the superiority of a progeny over the performance of its parents was described by Meena *et al*., (2017). A heterotic pattern is a specific pair of heterotic groups expressing high heterosis and hybrid performance upon crossing. Fan *et al*. (2018) described a "heterotic group" as a group of genotypes, related or otherwise, that displayed similar combining ability and heterotic response upon crossing with genotypes from another distinct heterotic group. According to Lee (1995), a heterotic group is a collection of germplasm exhibiting higher heterosis in combination with germplasm from an external group than when crossed with a member of its own group. According to Hallauer *et al*. (1988), the temperate germplasm is more clearly classified into heterotic groups than tropical germplasm. Therefore, Wen *et al*. (2012) proposed introgression and the use of distinct heterotic patterns to increase heterosis in tropical germplasm. To satisfy the increasing demand for MLN resistant maize varieties in Kenya, there is a need to identify inbred combinations for the exploitation of heterosis for increased grain yield under MLN stress to enhance food availability. A heterotic group classification method is either quantitative or molecular-based (Wu *et al*., 2007; Badu *et al*., 2016). Quantitative methods of classification, such as GCA and SCA (Fan *et al*., 2009), combining ability estimates of pedigree lines and hybrid field data to classify inbreeds. However, Menkir *et al*. (2004) and Barata and Carena (2006) recommended a molecular-based approach as an important preliminary tool in field evaluations for combining ability studies to create distinct heterotic groups having higher within-group than between-group genetic similarities. Lines that expressed negative SCA effects with the two testers could be

discarded (Vasal *et al*., 1992; Parentoni *et al.*, 2001; Gauch, 2006). Positive SCA effects indicate that inbred lines are in different heterotic group, whereas negative SCA indicates the genetic similarity of the parents (Kanyamaoro *et al*., 2012). Maximum genetic variability and hybrid vigor (heterosis) can be exploited by crossing lines from different heterotic groups. Lines in the same heterotic group accompanied by desirable GCA effects can be used for the development of improved hybrids. The combining ability of several inbred lines that were bred for MLN resistance has not been established. This makes it difficult to design new hybrids for cultivation and to develop the population to derive new inbred lines. Therefore the objectives of this study were to (i) investigate the general combining ability (GCA) and specific combining ability (SCA), (ii) evaluate the efficiencies of GCA based prediction and hybrid performance using the cross-validation procedure; (iii) to classify the MLN lines into heterotic groups (iv) estimate trait correlations in hybrids; (v) identify MLN tolerant single cross combinations that could be used as female parents for three-way cross hybrid production and (vi) identify new breeding starts (crosses) for MLN tolerant inbred lines development from diallel cosesses involving several MLN resistant and susceptible inbred lines.

4.2 Methodology

4.2.1 Germplasm

Eleven (11) maize inbred lines with varying levels of tolerance to MLN, MCMV, SCMV and commercial hybrid checks that are prefereed by the farmers but MLN susceptible, were used as checks in the study (Table 4.1).

Table 4.1: Features of selected maize inbred lines for diallel crossing

Ent.	**Pedigree**	**Pedigree**	**Source**	**SCMV resistant sore (1-5)**	**MCMV resistant score (1-5)**	**MLN resistance score (1-5)**	**Grain Endosperm Colour**
1	MLN 001	CKLMLN140283	CIMMYT	2.9	1.0	2.1	White
2	MLN 008	CKLMLN140289	CIMMYT	4.2	1.1	3.3	White
3	OH001	N211	OARDC-OH Wooster	4.3	1.4	3.0	Yellow
4	OH002	KS23-6	OARDC-OH, Wooster	4.4	2.5	2.8	Yellow
5	MLN 013	CKDHL120312	CIMMYT	1.1	2.9	2.2	White
6	MLN 003	HIFIL-57	KALRO	3.7	3.0	3.6	White
7	MLN019	CKDHL120918	CIMMYT	1.3	3.1	2.3	White
8	MLN 018	CKSBL10060	CIMMYT	2	3.5	2.4	White
9	CML 312		CIMMYT	4.1	3.3	3.3	White
10	CML 442		CIMMYT	4	3.3	4.0	White
11	CML 444		CIMMYT	4.3	3.5	2.8	White

Key; 1-2-Resistant; 2.5-3-Tolerant; >3.5-Susceptible, (Source: Karanja *et al*., 2018)

4.2.2 Experimental sites

KALRO Katumani is in Machakos County at 1º 35'S and 37º 32'E with an elevation of 1580 meters above sea level (masl). It is located in the dry mid-altitude agro-ecological zones of eastern Kenya and experiences mean annual temperature ranges of 17-27^{0}C and receives annual rainfall ranging between 690 and 1262 mm that comes in two short seasons. The main soil types are Cambisols and Ferralsols (Tefera *et al*., 2013).

KALRO Kiboko is located at 2º15' S 37^{0}45' E, 993 metre above sea level, in the dry mid-altitude agro-ecological zone of eastern Kenya and experiences mean annual temperature ranges of 28 - 37^{0}C, with February and October being the hottest months. Kiboko receives a mean annual rainfall of approximately 530 mm that comes in two short seasons. The soils are well-drained, Fluvisols, Ferralsols, and Luvisols, with soil pH of about 7.9 (Kivuva *et al*., 2014).

KALRO Kitui is located at 1º22' S, 38^0 1'E, 1115 metre above sea level, in the dry mid-altitude agro-ecological zone of eastern Kenya and experiences a mean annual temperature of 24^{0}C, with February and October being the hottest months. Kitui receives a bimodal mean annual rainfall of approximately 775 mm that comes in two short seasons. The soils are well-drained, clay loam and sandy clay.

The MLN Quarantine and screening facility is located at the KALRO Dairy Research Centre at Naivasha (latitude 0°43′S, longitude 36°26′E, 1896 metre above sea level and receives 120 - 131 mm of rainfall per year. It is a hot, dry region with a mean annual temperature of 22.6ºC, with a mean annual maximum of 28.6ºC and a minimum of 16.5ºC. The soils are well-drained, very deep, dark reddish-brown to dark red, friable sandy clay to clay (Acri-Rhodic Ferrasols) developed from undifferentiated basement system rocks.

4.2.3 Experimental design and management

Eleven (11) maize inbred lines were crossed in a diallel design, excluding the reciprocals at KALRO-Kiboko research farm in 2015. Among the crossing lines were testers CML312, CML442 (T1), and CML444 (T2). Testers utilized were initially developed by CIMMYT and have been widely used to study combining ability and heterotic grouping of newly developed inbred lines by national maize breeding programs in sub-Saharan Africa. The crossing was done by two staggered sowings of lines at an interval of five days to capture different flowering dates. The ears of each line were covered with a transparent polythene bag (shoot bag) before the emergence of silk to control unnecessary cross-pollination. Tassel bagging

was done with Khaki bag (Pollination bags) made of heavy craft paper with waterproof glue to collect pure pollen from the desired male lines. Pollination was performed by dusting pollen collected in the pollen bag on the silk of the specific ear and covered. The resulting 55 F_1's crosses together with five commercial varieties were planted and the experiment laid as an alpha (0, 1) lattice design with three replications in each environment. The trials were evaluated for three years under optimal conditions at KALRO-Kiboko, random drought stress at KALRO-Katumani and KALRO Kitui, and MLN artificial inoculation at KALRO Naivasha sites in 2016, 2017 and 2018. Each entry was planted in two rows plot of 5 m long, with rows spaced at 0.75 m between rows. Two seeds were planted in each hole at 0.25 m intervals and thinned down to one plant per hill at four weeks after planting to obtain a final plant population density of 53,333 plants per hectare. During planting, phosphate fertilizer (P_2O_5) was applied at a rate of 60 kg ha^{-1}. Nitrogen fertilizer (N) was applied at the knee height stage of plants as a top-dress at a rate of 60 kg ha^{-1}. Weeds were removed manually throughout the growth cycle of plants, and supplemental irrigation was applied at Kiboko and Naivasha when required.

4.3 Data collection

Data were collected according to CIMMYT protocols as described by Magorokosho *et al*., (2010). The following data were collected: days to 50% anthesis: the date when 50% of plants in a plot shed pollen, days to 50% silking: the date when 50% of plants in a plot show silks, plant height: average height of 10 randomly selected plants from the ground to first tassel branch at physiological maturity, ear height: average height of 10 randomly selected plants from the ground to ear placement at physiological maturity, plant number: total plants counted before harvest after removing the first on each side of the row, ear aspect: quality of ears done on a scale of 1-5, where; 1= nice and uniform cobs with good grain texture and 5= ugly cobs with undesirable texture, field weight: weight of ears per plot recorded immediately after harvest and grain yield: weight of shelled grain in each plot.

4.4 Statistical analysis

All the data collected was organized and analysed using SAS statistical package (SAS, 2013). Analysis of variance across location was conducted with PROC GLM procedure by considering location, replication, and blocks as random and genotypes as fixed factors. The significance of mean squares for crosses and location in the combined analysis were tested

against the mean squares for their corresponding interaction with location as an error term, while their interaction with location were tested against their corresponding pooled error.

The statistical model for the combined diallel analysis across environments is as follows: $Yijk = \mu + gi + gj + sij + lk + glk + sijlk + \varepsilon ijk$

Where $Yijk$ is the observed measurement of the $ijth$ cross grown in the kth environment, μ is the grand mean; gi and gj are the GCA effects; sij is the SCA effects; glk is the interaction effect between GCA and the environment; $sijlk$ is the interaction effect between SCA and the environment, and εijk is the error term associated with the $ijth$ cross evaluated in the kth replication and $l\ th$ environment (Hallauer and Miranda, 1988).

Based on a general analysis of variance, traits that showed significant differences among the crosses were further analysed according to the diallel analysis using the analysis of Genetic Designs with R (AGD-R) version 3.0 procedures for individual and combined data (Francisco *et al*., 2015).

Genotypic means of individual locations were used for the determination of GCA and SCA effects. The "Heritability" is a statistic that estimates the degree of variation in a phenotypic trait in a population that is due to genetic variation among individuals in that population. Heritability is expressed as $H^2 = V_g/V_p$, where H is the heritability estimate, V_g the variation in genotype, and V_p the variation in phenotype. Heritability estimates range in value from 0 to 1. If $H = 1$, then all variation in a population is due to differences or variation between genotypes (i.e., there is no environmentally caused variation). If $H = 0$, there is no genetic variation; in this case, all variation in the population comes from differences in the environments experienced by individuals. The SAS computer program performed analysis of variance for every location for grain yield and yield-related characters. Bartlett's test evaluated the homogeneity of error variances before the combined analysis of variance across locations.

The standard heterosis (SH) for mean values of each genotype relative to the checks and the respective standard error was performed (Fehr, 1987).

$$Standard\ Heterosis\ (SH) = \left[\frac{Genotype - mean\ of\ check}{mean\ of\ check}\right] \times 100$$

$$SE\ of\ Heterosis = \sqrt{\frac{\sigma^2 e}{2}}$$

Where by; $\sigma^2 e$ = environmental variance

The stability and adaptability of hybrids were determined using parametric and nonparametric stability as well as multivariate analysis. Multivariate analysis was done using GGE biplot as well. The regression stability parameters, regression coefficient (βi), and deviation from regression (S^2di) regressed according to the method proposed by Eberhart and Russell (1966). The t-test tested significant differences among the βi values and unity, while the F-test tested the significance of the S^2di values.

GGE analysis was performed using the software GEA-R version 4.1 (Angela *et al.*, 2015) with the model equation:

$Y_{hij} = \mu + E_h + G_i + GE_{hi} + B_{j(h)} + e_{hij}$,

Where μ = population mean, E_h = environmental effect,

G_i = genotypic effect, GE_{hi} = genotype-by-environment interaction effect, $B_{j(h)}$ = block effect, and e_{hij} = random error.

Heterotic grouping was determined according to the CIMMYT heterotic classification system as A, B and AB. Depending on the direction of the SCA estimates, lines displaying positive SCA with tester A were grouped towards the opposite heterotic group, and vice versa, whereas lines exhibiting positive SCA to both testers were grouped under AB heterotic group (Vasal, 1992).

4.5 Results

4.5.1 Analysis of variance

4.5.1.1 Hybrids

The F_1 crosses (Genotype (G)), location (L), and year (Y) main effects showed significant differences ($p \leq 0.001$) for grain yield and other traits. This implied substantial variations among crosses across sites and years in which the trials were conducted (Table 4.2). The crosses responded differently to locations and years as depicted by the significant ($p \leq 0.001$) G x L, G x Y, and G x L x Y interactions. Most of the variation was due to differences in locations and year of evaluation. The highest percentage of the total variation for grain yield was explained by the environment (33.07%). The interaction G x L x Y (23.23%) contributed

more variation than G x L (13.7%), G x Y (12.4%) and L x Y (4.29%) interactions (Table 4.3). The genotypic variance was higher than G x E for plant height, ear height, and husk cover. The presence of genotype x environment interaction effects calls for the need to select for high-yielding and stable hybrids.

The highest yielding hybrid across sites was CML312 x CML442 with 6.5 t ha^{-1} and recording a 33.6% yield advantage above the mean of commercial checks (Table 4.4). This hybrid had early flowering and a longer grain filling period. Other hybrids that showed superior yield performance were CML444 x MLN019, CML442 x CML444, MLN018 x MLN019, MLN018 x OH002, CML444 x MLN018 and MLN008 x MLN013 all recording a grain yield advantage of > 20% above the mean of commercial checks (Table 4.4). In this study, MLN018 was a common parent in the top 20 crosses. These high-performing crosses were also among the early maturing entries since they took < 70 days to attain 50% anthesis.

Table 4.2 Analysis of variance for grain yield and other agronomic traits of 55 F1 hybrids and four checks tested in four locations in 2015, 2016, and 2017 and the contribution of different main and interaction effects to the total sums of squares

Source	**d.f.**	**GY**	**AD**	**PH**	**EH**	**EPP**	**HC**	**RL**	**SL**
Genotyp	58	10.2**	1001.7	1860.5*	1264.1*	0.2547	786.6*	128.4*	56.2^{ns}
Location	3	695.7*	4401.1	9475.3*	1724.6*	3.98**	1567.3	1575.5	2982.3
Year (Y)	2	124.3*	24818.	28042.2	13100.9	0.76**	4989.5	1295.3	679.7*
GxL	174	5.0*	476.3*	588.9**	350.5**	0.14**	309.8*	93.0**	91.3**
GxY	116	6.8***	574.5*	1074.7*	494.6**	0.15**	484.6*	86.3**	76.3^{ns}
LxY	6	45.2**	1686**	3114.2*	1941.5*	0.6***	140.1^{ns}	389.0*	416.7*
GxLxY	342	4.3***	461.6*	781.6**	413.3**	0.1***	388.7*	76.3*	70.6^{ns}
Residual	736	1.5	188.1	308.2	183.1	0.1	199.80	61.9	60.4
Total	143	5.2	401.3	667.0	364.9	0.1	370.00	85.1	84.9
Trial Statistics									
Heritabil		0.38	0.37	0.45	0.59	0.35	0.57	0.15	0.0
Grand		5.2	74.0	211.4	107.7	1.1	8.9	6.6	
%cv		34.0	19.0	8.8	13.2	22.2	114.4	152.7	197.9
Contribution to total SS (%)									
G		9.36	13.25	15.91	20.28	15.25	28.15	12.90	7.03
L		33.07	3.01	4.19	1.43	12.35	2.90	8.19	19.63
Y		3.94	11.32	8.27	7.25	1.56	6.16	4.49	2.98
GxL		13.70	18.90	15.11	16.87	24.56	26.00	27.54	31.63
GxY		12.41	15.20	18.38	15.87	17.72	26.91	16.91	15.90
LxY		4.29	2.31	2.76	3.22	3.40	0.52	4.04	5.49
GxExY		23.23	36.01	35.38	35.09	25.15	9.36	25.93	17.35

*, **, *** significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (GY = grain yield (tha^{-1}), AD = days to 50% anthesis, PH= plant height (cm),

EH= ear height (cm), EPP = number of ears per plant, HC= Bad husk cover (%), RL= Root lodging and SL= Stem lodging

Table 4.3: Means for grain yield, rank, and selected agronomic traits of the 20 top, the lowest ten single cross hybrids and commercial checks evaluated across four sites in 2015, 2016, and 2017.

Genotype	**Pattern**	**GY (**t ha-1)	**Rank**	**%AMC**	**AD (Days)**	**PH (cm)**	**EH (cm)**	**EPP (number)**	**HC (%)**	**TEX (score 1-5)**
Diallel crosses										
CML312 x CML442	S x S	6.5	1	33.6	64.1	215.1	106.6	1.1	NA	3.5
CML444 x MLN019	S x R	6.4	2	30.5	75.8	216.4	118.1	1.0	12.6	3.5
CML442 x CML444	S x S	6.2	3	25.6	79.0	208.2	115.0	0.9	NA	3.5
MLN018 x MLN019	R x R	6.2	4	25.6	78.7	225.9	118.7	1.2	4.3	2.9
MLN018 x OH002	R x S	6.0	5	23.2	71.2	209.2	104.5	1.2	NA	2.8
CML444 x MLN018	S x R	5.9	6	20.6	72.7	228.4	127.2	1.1	-2.5	3.9
MLN008 x MLN013	R x R	5.9	7	20.0	75.3	220.8	117.0	1.1	3.4	3.4
CML442 x MLN008	S x S	5.9	8	19.8	70.9	229.3	110.0	1.2	-0.6	4.5
CML312 xMLN001	S x R	5.9	9	19.4	71.5	208.2	106.3	1.0	3.0	3.3
OH001 x OH002	R x R	5.8	10	19.2	88.0	225.6	124.5	1.1	19.9	3.5
MLN019 x OH002	R x R	5.8	11	18.2	73.9	213.3	106.2	1.2	NA	3.2
MLN018 x OH001	R x R	5.7	12	17.0	64.2	198.6	98.6	1.1	10.6	4.3
CML312 x OH001	S x R	5.7	13	17.0	66.4	207.6	99.1	1.2	NA	4.1
CML444 x MLN001	S x R	5.7	14	16.1	79.6	206.2	111.0	1.0	NA	NA
MLN008 x MLN018	R x R	5.7	15	15.8	76.7	223.9	115.9	1.3	5.7	3.6
MLN001 x MLN013	R x R	5.6	16	15.0	75.1	222.8	109.3	1.2	10.4	3.3

Genotype	**Pattern**	**GY (**t ha-1)	**Rank**	**%AMC**	**AD (Days)**	**PH (cm)**	**EH (cm)**	**EPP (number)**	**HC (%)**	**TEX (score 1-5)**
MLN013 x MLN018	R x R	5.6	17	13.4	68.8	208.1	107.4	1.0	4.9	3.5
MLN003 x MLN018	S x R	5.5	18	12.6	77.8	220.1	116.6	1.2	4.2	3.7
MLN003 x MLN 008	S x R	5.4	19	10.7	78.3	226.8	116.4	1.2	9.4	4.3
CML442 x OH001	S x R	5.4	20	10.5	65.4	215.6	98.3	1.1	7.4	3.9
CML444 x MLN013	S x R	4.7	46	-4.6	69.9	202.2	104.2	1.1	NA	3.5
MLN013 x OH002	R x R	4.7	47	-5.0	66.4	210.3	104.0	1.2	10.6	3.0
MLN001 x MLN008	R x R	4.6	48	-5.6	77.8	213.4	112.4	1.2	NA	4.2
CML312 x OH002	S x R	4.5	49	-7.2	68.6	198.0	96.8	1.1	NA	NA
CML442 x MLN013	S x R	4.4	50	-9.3	82.8	200.5	99.6	0.9	NA	3.9
CML442 x MLN019	S x R	4.4	51	-10.7	66.7	217.6	102.9	1.1	NA	3.2
CML444 x MLN008	S x R	4.2	52	-13.7	76.0	202.7	109.5	1.0	5.3	NA
CML312 x MLN003	S x R	4.2	53	-14.1	69.9	199.4	96.9	0.9	12.5	3.1
CML312 x MLN018	S x R	4.0	54	-19.3	69.2	193.6	99.5	0.9	NA	2.6
CML442 x MLN003	S x R	3.6	55	-25.8	67.3	203.8	100.8	0.9	NA	3.8
Check hybrids										
WE1101	Susceptible	6.0			83.0	205.5	106.5	1.0	13.0	NA
DH04	Susceptible	4.7			77.2	205.1	108.1	1.0	NA	3.8
DK8031	Susceptible	3.6			75.7	206.2	104.8	0.8	NA	3.5
DUMA 43	Sucspetible	5.3			74.3	216.6	104.6	1.0	NA	3.1
Statistics										

Genotype	**Pattern**	**GY (**t ha-1)	**Rank**	**%AMC**	**AD (Days)**	**PH (cm)**	**EH (cm)**	**EPP (number)**	**HC (%)**	**TEX (score 1-5)**
Heritability		0.4			0.4	0.5	0.6	0.3	0.6	0.5
Grand Mean		5.2			74.0	211.4	107.7	1.1	8.9	3.5
LSD		1.0			10.7	16.5	10.6	0.2	17.1	0.8
CV (%)		34.0			25.4	10.9	15.9	26.4	195.0	23.6
Genotype significance		***			***	***	***	***	***	***

*, **, *** indicates the term is significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, ns indicates non-significant ($p \leq 0.05$); GY = grain yield (t ha-1), %AMC= % advantage of the GY above the mean of checks, AD = days to 50% anthesis, ASI = anthesis silking interval, PH= plant height (cm), EH= ear height (cm), EPP = number of ears per plant, ER= total number of rotten ears (%), HC= Bad husk cover (%), RL= Root lodging and TEX= Grain texture

Table 4.4: Mean yield, PC_1 and PC_2 scores and ranks for top 20 and bottom 10 F_1 maize crosses evaluated in four environments for three seasons

Entry No.	Cross	Mean	Rank	PC1	Rank	PC2	Rank
1	CML312x CML442	6.53	1	1.39	2	0.04	1
25	CML444 x MLN019	6.38	2	1.19	1	0.69	9
11	CML442 x CML444	6.15	4	1.78	5	5.43	56
50	MLN018 x MLN019	6.04	5	1.47	3	2.23	23
24	CML444 x MLN018	5.97	6	2.42	12	4.51	52
54	MLN019 x OH002	5.96	8	1.92	7	2.66	32
52	MLN018 x OH002	5.92	9	1.63	4	2.53	31
41	MLN008 x MLN013	5.9	3	2.74	17	6.51	58
55	OH001x OH002	5.89	10	2.01	8	3.41	38
14	CML442 x MLN008	5.86	11	2.41	11	4	46
3	CML312 x MLN001	5.84	12	2.75	18	1.16	11
9	CML312x OH001	5.79	13	2.1	9	1.34	12
51	MLN018 x OH001	5.7	14	2.56	16	2.5	30
30	MLN001 x MLN013	5.64	15	2.78	20	4.15	49
20	CML444 x MLN001	5.62	16	2.48	13	3.17	36
46	MLN013 x MLN018	5.55	17	2.54	15	2.12	19
37	MLN003 x MLN018	5.54	18	2.52	14	2.45	27
42	MLN008 x MLN018	5.45	19	2.28	10	1.41	13
8	CML312 x MLN019	5.43	20	3.17	25	0.72	10
49	MLN013 x OH002	4.61	49	5.36	50	1.8	16
29	MLN001 x MLN008	4.58	50	5.17	49	1.48	15
2	CML312 x CML444	4.53	51	6.24	53	4.08	47
10	CML312 x OH002	4.5	52	5.75	51	0.09	3
15	CML442 x MLN013	4.44	53	5.98	52	3.42	39
17	CML442 x MLN019	4.36	54	7.94	57	6.65	59
22	CML444 x MLN008	4.21	55	6.77	55	2.41	26
4	CML312 x MLN003	4.21	56	6.46	54	1.93	17
7	CML312 x MLN018	3.93	57	7.81	56	3.46	41
13	CML442 x MLN003	3.61	58	9.12	59	3.13	35

Entry No.	Cross	Mean	Rank	PC1	Rank	PC2	Rank

Mean Grain yield (GY) =5.2; pearson correlation GY vs PC1 = 0.759 (P < 0.001); GY, PC2 = 0.651

4.5.2 Stability and adaptability

Stability and adaptability were assessed based on the values of PC1 and PC2 scores of the biplot (Table 4.5) as there were high correlation coefficients of genotypic effects and PC1 and PC2 scores (r=0.76 and 0.65 respectively at, $p \leq 0.01$). The grain yield across the years, seasons, and locations ranged from 6.5 t ha^{-1} to 3.6 t ha^{-1} for CML312 x CML442 and CML422 x MLN003, respectively (Table 4.5). High PC1 scores denote high genotypic means and indicate genotype adaptability, especially when highly correlated with yield rankings, while PC2 scores denote stable genotypes (Yan *et al*., 2000). Due to a high correlation between mean yield, PC1, and PC2, the top two crosses using the three parameters were CML312 x CML442 and CML444 x MLN009 (Table 4.5), indicating high yield potential and high stability. Cross CML312 x OH002 had low PC1 scores (5.75) and high PC2 scores (0.09), indicating low yielding potential but stable across environments. There was also some high yielding (PC1 scores) and unstable (high PC2 scores) hybrids, such as MLN008 x MLN013 and CML442 x CML444, as they are ranked 3 and 4 for grain yield and 58 and 56 on stability, respectively.

Disregarding yield performance for the inbred lines, CML312, CML442, and OH001 are the common parents of the most stable hybrids based on the PC2 scores (Table 4.5); while the least stable lines were MLN003, ML001, MLN019, and MLN008. Following this, the specifically adapted cross CML312 x CML442 was constituted by stable x stable lines, while CML444 x MLN003 was formed from unstable x unstable lines.

4.5.3 GGE biplot analysis of F_1 crosses

Since the genotype x location interaction was significant ($P \leq 0.001\%$) (Table 4.3), the data on grain yield was subjected to a biplot analysis. The GGE biplots for the first two PCs explained 66.48% (PC1=43.16 and PC2=23.32%), also referred to as primary and secondary effects, respectively. Results of the polygon view for the F_1 crosses performance on (i) which-won-where and (ii) Ranking of the crosses on the bases of grain yield and stability 2015, 2026, and 2017 were drawn (Figures 4.1, 4.2, and 4.3) (Yan and Kang, 2003). The

GGE biplots were derived by subjecting environment-centered yield data, i.e., the yield variation due to GGE, to singular value decomposition (Yan, 1999; Yan *et al*., 2000).

The biplot represents a polygon view (Fig. 4.1) with the same vertex crosses, while the rest are inside the polygon. The crosses at the vertex are the most responsive since they have the longest distance from the biplot origin (Yan and Rajcan, 2002). This shows crossover G x E, and thus environments could be divided into mega environments. The rays of the biplot divided the plot into nine sectors, with the four environments appearing in four sectors and the crosses falling in all the eight sectors (Fig 4.1). However, the environments fell in four sectors thus, there were three mega-environments and an indication of the presence of crossover interactions. Thus, crosses CML312 x CML442 and CML444 x MLN019 were the highest yielding at Katumani and Kitui; while MLN008 x MLN013 and CML442 x CML444 were the best performers at Naivasha (Fig. 1). The three crosses seemed to be widely adapted across the locations. None of the locations fell in the sectors with CML444 x MLN003, CML312 x MLN018, CML442 x MLN003 and CML442 x MLN019 as the vertices genotypes, indicating that these crosses were not best in any of the locations.

The horizontal axis drawn to pass via biplot origin and average genotypes was the average tester coordinate (ATC) line used for visual displaying of both means vs. stability (Fig. 4.2 and 4.3). This ATC performance line was used for genotypes ranking according to the mean and stability (Yan and Kang, 2003). The average means for genotypes were estimated by projection onto ATC horizontal axis (Fig. 4.2). The projection is equal to the longest vectors of all genotypes. The center of concentric circles shows the virtual ideal genotypes (Fig. 4.2). The ideal genotypes could be high yielding and better for stability (Yan and Kang, 2003; Pavel *et al*., 2015). The smaller distance from the ideal genotype indicates absolute stability. Crosses CML442 x CML444 (11), CML312 x CML44 (1), CML44 x MLN019 (25) and MLN018 x MLN019 (50) have both high yield means and stability. These closely positioned crosses were highly desirable due to high means and stability (Pavel *et al*., 2015; Richmond *et al*., 2015; Fayeun *et al*., 2016). These hybrids were the highest yielding across test environments and absolutely stable in performance (Olayiwola *et al*., 2015; Ashraful *et al*., 2017). CML442 x MLN003 (13) was highly projected from the center of the concentric circle to unstable. Moreover, CML312 x MLN019 (41) and CML442 x MLN008 (14) are not different from apparently inferior MLN019/OH001 (53) (Fig. 4.2). These highly projected genotypes were found to be the poorest and unstable (Edmore *et al*., 2015; Massaine *et al*.,

2018). An inter-relationship was observed among crosses 50, 54, 52, and 24. The crosses are positively and moderately correlated with the most favorable 11 (Fig. 4.2). Similar results of strong correlation among the genotypes were reported by Ashraful *et al*. (2017). They confirmed that the crosses being positioned close to each other on the GGE biplot responding together similarly to the environments were found near these crosses.

The Kiboko and Katumani form one of the mega-environments, with Kitui and Naivasha falling as individual environments but crossly related (Fig. 4.1).

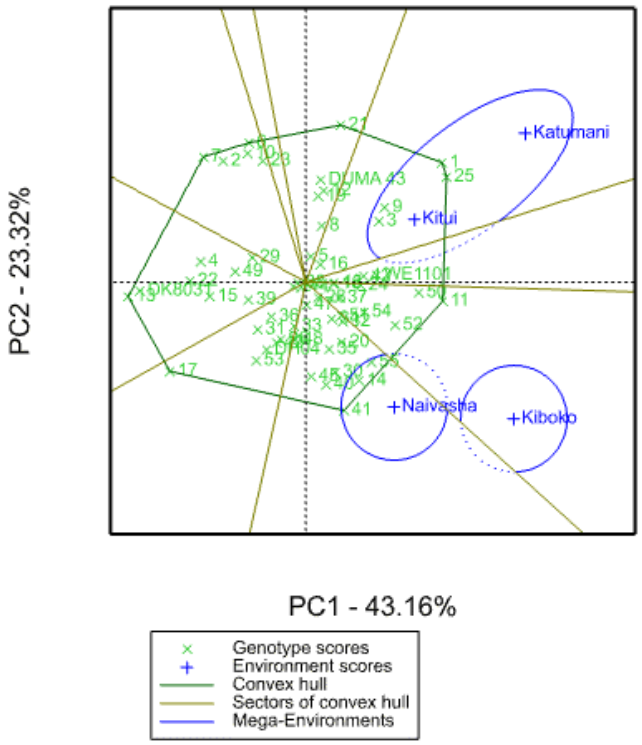

Figure 4. 1: GGE biplot based on grain yield (tha^{-1}) for Kiboko, Katumani, Kitui and Naivasha (Location x Year) for crosses in 2016, 2017 and 2018 showing "which won where and which is the best for what".

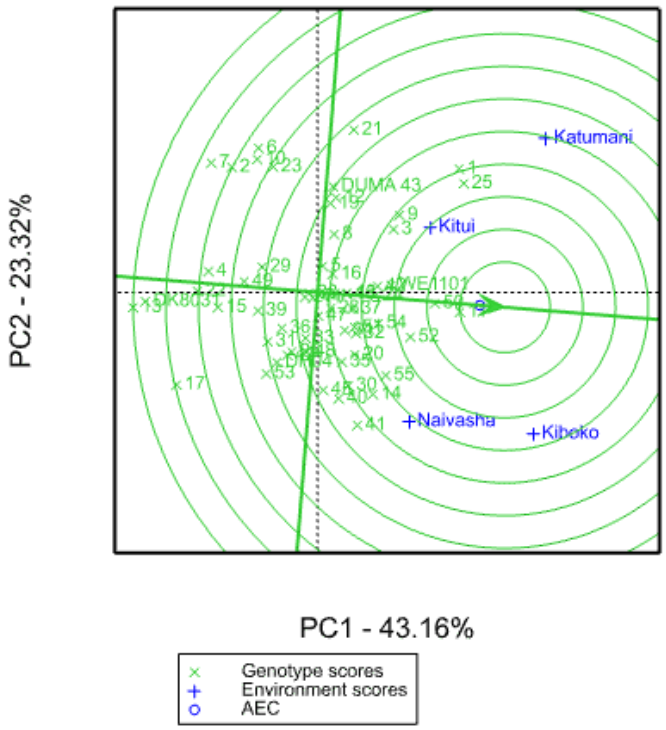

Figure 4. 2: GGE biplot showing the comparison of the F_1 crosses with the ideal cross.

4.5.4 Stability of combining abilities for grain yield

The combined analysis of variance across environments indicated highly significant ($p<0.001$) L x GCA interactions for grain yield suggesting changes in the rank of GCA and SCA across locations (Table 4.5).

Table 4.5: Mean squares (MS) from the diallel analyses for grain yield (GY), days to 50% anthesis (AD), anthesis/silking interval (ASI), Plant height (PH), ear height (EH), and Ear position (EPO)

Source of variation	GY (tha^{-1})	AD (cm)	ASI	PH (cm)	EH (cm)	EPO
Site (L)	545.9^{***}	3726.5^{ns}	719144.2^{ns}	7535.6^{ns}	1480.8^{***}	0.05^{ns}
Cross (G)	8.8^{**}	1022.9^{***}	841374.5^{ns}	1891.4^{ns}	1327.8^{ns}	0.01^{ns}
GCA	2.9^{*}	1007.8^{**}	528424.9^{ns}	1953.3^{*}	1781.9^{***}	0.02^{ns}
SCA	4.8^{***}	355.8^{*}	535485.2^{ns}	931.8^{ns}	537.7^{ns}	0.01^{*}
L x G	4.9^{ns}	486.8^{***}	893197.2^{**}	597.7^{ns}	369.7^{*}	0.01^{**}
L x GCA	3.9^{*}	302.7^{ns}	531176^{ns}	411.4^{ns}	266.3^{ns}	0.01^{ns}
L x SCA	2.2^{ns}	236.1^{ns}	534620.7^{ns}	355.8^{ns}	228.2^{ns}	0.003^{ns}
GCA %	12.12	39.16	18.32	32.27	42.96	45.04
SCA %	87.88	60.84	81.69	67.73	57.96	54.96

*, **, *** indicates the term is significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, ns indicates non-significant ($p > 0.05$)

The general combining ability (GCA) was highly significant ($p \leq 0.05$) for all traits. Nevertheless, no parent had high GCA for all the characters measured thus, the parents involved were genetically diverse. The specific combining ability (SCA) was significant for the traits grain yield, Anthesis day, and ear position. For all traits, the additive and non-additive effects are relevant, except for plant and ear height, where SCA was non-significant.. Inbred line CML312, CML442, MLN013, OH001, and OH002 contributed negative GCA effects for 50% days to anthesis, plant, and ear height. Inbred lines MLN001, MLN008, and MLN019 recorded positive GCA effects for days to 50% anthesis, plant, and ear height (Table 4.6).

GCA for grain yield showed inbred lines CML444, MLN001, MLN008, MLN018, MLN019, OH001 and OH002 had a positive effect (Table 4.6). At least 31 crosses had positive SCA effect for grain yield, while the others had negative SCA effects (Table 4.7). The positive SCA effects resulted from the crossing of lines from different heterotic groups but negative SCA effects due to the crossing of lines from the same heterotic group.

Table 4.6: General combining ability of the eleven lines for anthesis/silking interval (ASI), days to 50% anthesis (AD), ear height (EH), grain yield (GY) and Plant height (PH)

Parents	**Reaction to MLN**	**ASI**	**RANK**	**AD**	**RANK**	**EH**	**RANK**	**GY**	**RANK**	**PH**	**RANK**	
CML312	S	33.54^{ns}	3	-5.62***	11	-4.60***	10	-0.17^{ns}	9	-4.90**	10	**
CML442	S	32.68^{ns}	5	-2.71*	9	-3.60**	9	-0.004^{ns}	8	-0.52^{ns}	5	ns
CML444	S	34.13^{ns}	2	3.16**	2	4.65***	2	0.09^{ns}	4	-1.05^{ns}	6	ns
MLN001	R	29.50^{ns}	7	3.74**	1	3.58**	4	0.05^{ns}	5	4.86**	3	**
MLN003	T	28.27^{ns}	8	3.10*	3	-2.13*	7	-0.31*	11	-2.83^{ns}	8	ns
MLN008	R	29.65^{ns}	6	1.60^{ns}	5	5.31***	1	0.03^{ns}	6	7.07***	1	***
MLN013	R	32.79^{ns}	4	-0.02^{ns}	6	-1.30^{ns}	6	-0.18^{ns}	10	-1.41^{ns}	7	ns
MLN018	R	-143.79**	11	-1.57^{ns}	8	4.09***	3	0.26*	1	2.65^{ns}	4	ns
MLN019	R	34.30^{ns}	1	2.069^{ns}	4	2.59*	5	0.14^{ns}	2	4.93**	2	**
OH001	T	27.92^{ns}	9	-3.38**	10	-5.25***	11	0.01^{ns}	7	-3.42*	9	*
OH002	R	-138.99*	10	-0.36^{ns}	7	-3.33**	8	0.09^{ns}	3	-5.38***	11	***

S-susceptible, T-tolerant and R-resistant, *, **, *** indicates the term is significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, ns indicates non-significant ($p > 0.05$)

Table 4.7: Specific combining ability of fifty five single crosses for grain yield (GY), days to 50% anthesis (AD), anthesis/silking interval (ASI), Plant height (PH), ear height (EH) and Ear position (EPO)

Crosses	**Pattern**	**ASI**	**RANK**	**AD**	**RANK**	**EH**	**RANK**	**GY**	**RANK**	**PH**	**RANK**
CML312 x CML444	SxS	-36.31^{ns}	39	-1.31^{ns}	30	7.00^{ns}	4	1.47**	1	7.42^{ns}	9
CML444 x MLN008	S x R	-34.44^{ns}	34	-6.80^{ns}	52	-1.12^{ns}	30	-1.29**	54	-4.84^{ns}	42
MLN001 x MLN008	R x R	-40.09^{ns}	50	15.22**	2	5.07^{ns}	11	0.38^{ns}	13	0.17^{ns}	27
MLN008 x MNL013	R x R	-34.33^{ns}	33	-0.22^{ns}	22	5.37^{ns}	8	0.47^{ns}	11	12.43*	4
CML444 x MLN018	S x R	-31.98^{ns}	28	11.75*	3	-3.25^{ns}	39	-0.61^{ns}	48	-9.51^{ns}	50
CML442 x MLN019	S x R	141.06^{ns}	6	2.61^{ns}	12	-7.76^{ns}	53	-1.38**	55	-14.06*	54
MLN001 x MLN019	R x R	137.02^{ns}	12	-2.71^{ns}	42	10.70*	3	0.31^{ns}	18	18.62**	2
CML444 x OH001	S x T	-37.28^{ns}	40	-6.39^{ns}	51	-3.95^{ns}	40	-1.01*	52	1.07^{ns}	23
MLN001 x	R x T	-35.61^{ns}	36	-3.10^{ns}	44	2.95^{ns}	17	0.91*	2	5.17^{ns}	13

Crosses	Pattern	ASI	RANK	AD	RANK	EH	RANK	GY	RANK	PH	RANK
OH001											
MLN003 x OH001	T x T	-33.73^{ns}	31	5.61^{ns}	5	11.63**	2	-0.07^{ns}	33	17.92**	3
OH001 x OH002	T x R	-35.76^{ns}	37	-1.05^{ns}	26	-6.48^{ns}	48	-0.52^{ns}	45	-15.06*	55
CML312 x OH002	S x R	141.48^{ns}	5	18.00*	1	25.25^{ns}	1	0.50^{ns}	10	24.31**	1

S-susceptible, T-tolerant and R-resistant, *, **, *** indicates the term is significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively, ns indicates non-significant ($p > 0.05$)

4.5.5 Heterotic orientation of MLN tolerant lines

Inbred lines heterotic groups could be identified based on the value of SCA effects for grain yield. Lines displaying positive SCA effects with the known testers CML312 (A), CML442 (A) and CML444 (B) could be grouped to the opposite heterotic group. The lines that expressed negative SCA effects to the testers would be grouped in the same heterotic group with the respective tester hence (Table 4.8). Three heterotic groups were identified depending on the direction of the SCA estimates. Results in this study showed that nine hybrids combinations revealed positive significant SCA effects for grain yield with tester A and five with tester B while the other eight expressed negative SCA effects with tester A and four with tester B (Table 4.8).

Table 4.8: Heterotic orientation 11 inbred maize lines based on specific combining ability effects with the established tropical testers

Crosses	**Line**	**Grain yield (t ha^{-1})**	**SCA**	**Heterotic group**
CML312 x CML442	CML442	6.5	-0.63^{ns}	A
CML312 x CML444	CML444	4.7	1.47**	B
CML444 x MLN001	MLN001	5.7	0.82^{ns}	A
CML312 x MLN001	MLN001	5.9	-0.42^{ns}	A
CML442 x MLN001	MLN001	5.3	-0.424	A
CML312 x MLN003	MLN003	4.2	-0.28^{ns}	A
CML442 x MLN003	MLN003	3.6	0.73^{ns}	B
CML444 x MLN003	MLN003	5.4	0.03^{ns}	A
CML312 x MLN008	MLN008	5.3	0.32^{ns}	B
CML442 x MLN008	MLN008	5.9	-0.55^{ns}	B
CML444 x MLN008	MLN008	4.2	-1.29**	B
CML312 x MLN013	MLN013	4.7	0.01^{ns}	AB
CML442 x MLN013	MLN013	4.4	0.20^{ns}	AB
CML444 x MLN013	MLN013	4.7	0.61^{ns}	AB
CML312 x MLN018	MLN018	4.0	-0.50^{ns}	AB
CML442 x MLN018	MLN018	5.4	-0.21^{ns}	AB
CML444 x MLN018	MLN018	5.9	-0.61^{ns}	AB
CML312 x MLN019	MLN019	5.4	0.44^{ns}	B
CML442 x MLN019	MLN019	4.4	-1.38**	AB
CML444 x MLN019	MLN019	6.4	-0.10^{ns}	AB
CML312 x OH001	OH001	5.7	0.31^{ns}	B
CML442 x OH001	OH001	5.4	0.16^{ns}	B
CML444 x OH001	OH001	4.7	-1.01*	B
CML312 x OH002	OH002	4.5	0.50^{ns}	AB
CML442 x OH002	OH002	5.2	0.65^{ns}	AB
CML444 x OH002	OH002	5.3	0.17^{ns}	AB

4.5.6 Performance under MLN infestation

The maize genotypes differed significantly (P <0.05) on disease severity with the symptoms observed 10 days post inoculation on susceptible hybrids. Significant differences between lines and interactions of lines and crosses for most traits indicated wide range of variability present among them. There was a high correlation between MLN mean scores and AUDPC with the tolerant crosses recording the lowest score and AUDPC values (Figure 4.3).

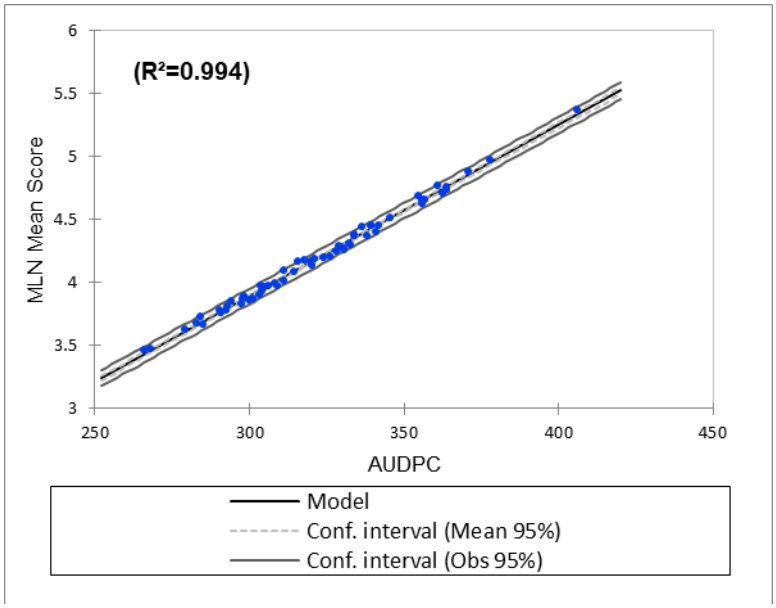

Figure 4. 3: Regression of MLN Mean Score by AUDPC for 55 F_1 crosses, pre and commercial checks

Disease severity was lowest in MLN resistant lines MLN013 x MLN0019 and CML442 x MLN013 with a mean score of <3.5 on a 1-2 (resistant), 3-tolerant and 4-5 (susceptible) rating scale and AUDPC <267, while the bottom performing crosses were combination between MLN tolerant line, OH002 and susceptible lines CML312, CML442 and CML444. It was also noted that the OH001 and OH002 plants stayed green longer in the field and in the greenhouse. There was no significant difference between the pre-commercial MLN tolerant hybrids; KATEH16-01, WE5140, KTEH16-02 and WE5135 compared to MLN013 x MLN019, CML442 x MLN013, CML442 x MLN003, MLN018 x MLN019 and CML444 x MLN013. Crosses between MLN resistance and susceptible lines also showed some vigour and was devoid of moderate symptoms of the disease with susceptible x Susceptible crosses scoring >5 at 43days post-inoculation. The susceptible commercial checks Duma43 and DK8031 were the most affected hybrid with a mean score >6 at 43dpi (Table 4.9). The Area Under the Disease Progress Curve (AUDPC) was calculated based on four dates (representing the beginning at four weeks after the first inoculation and the time the crosses

were at 50% flowering (peak of the disease severity) also separated the MLN tolerant versus susceptible crosses. The top-performing crosses under optimal conditions (no MLN) were CML312 x CML442, CML444 x MLN019, CML442 x CML444, MLN018 x MLN019 and MLN018 x OH002 recording >6t/ha and >20% yield advantage over the mean of checks. Hybrids MLN001 x OH002, CML444 x MLN003, CML444 x MLN013 and MLN003 x MLN001 recorded a mean yield >5tha^{-1} (Table 4.9) under MLN inoculation. In addition, thirteen hybrids recorded a yield advantage of >10% over the commercial checks.

In comparison to the best performing crosses under MLN and Non-MLN, hybrids, MLN018 x OH002, MLN008 x MLN013, CML312 x OH001 and CML444 x MLN003 were the best performers recording a yield of >4tha^{-1} under both conditions (Table 4.9). It's worth noting that the yield penalty between the MLN and non-MLN condition varied from resistance x resistance, resistance x susceptible, susceptible x susceptible and the commercial checks. Hybrid CML444 x MLN003 was the least affected recording a yield of 5.7 and 5.4tha^{-1} together with commercial check DH04 with 4.5 and 4.7tha^{-1} respectively. Late maturing hybrids were the most affected with WE1101 losing up to 70% of its yield under MLN inoculation.

Table 4.9: Performance of top and bottom 10 F_1 maize crosses, and commercial checks evaluated under optimum and MLN infestation condition in 2016, 2017 and 2018

Genotype	Pattern	Optimum condition			Artificial inoculated condition								
		GYG	AD	PH	GYG	AD	PH	14dpi	24dpi	34dpi	43dpi	Mean	AUDPC
CML312 x CML442	S x S	6.5	64.1	215.1	2.70	116.13	148.87	3.01	4.58	5.22	5.61	4.63	356.08
CML444 x MLN019	S x R	6.4	75.8	216.4	3.21	127.51	139.25	3.05	4.57	5.31	5.90	4.73	363.91
CML442 x CML444	S x S	6.2	79.0	208.2	3.54	117.25	149.13	2.57	3.93	4.48	4.93	3.97	306.22
MLN018 x MLN019	R x R	6.2	78.7	225.9	3.16	115.27	140.63	2.43	3.81	4.62	5.05	3.97	309.08
MLN018 x OH002	R x R	6.0	71.2	209.2	4.79	120.00	155.00	2.67	4.06	4.97	5.33	4.26	330.73
CML444 x MLN018	S x R	5.9	72.7	228.4	3.53	120.00	146.75	2.61	4.33	4.99	5.60	4.40	341.04
MLN008 x MLN013	R x R	5.9	75.3	220.8	4.15	113.00	158.00	2.54	3.84	4.55	5.05	3.99	308.38
CML442 x MLN008	S x R	5.9	70.9	229.3	1.92	113.50	132.02	2.46	3.80	4.36	5.18	3.96	304.84
CML312 x MLN001	S x R	5.9	71.5	208.2	3.37	118.43	138.75	3.05	4.46	5.35	5.86	4.71	362.49
OH001 x OH002	R x T	5.8	88.0	225.6	3.37	120.64	151.98	3.25	4.75	5.30	5.60	4.76	363.77
CML444 x MLN013	S x R	4.7	69.9	202.2	5.43	114.84	156.50	2.48	3.50	4.21	4.62	3.67	282.79
MLN013 x OH002	R x T	4.7	66.4	210.3	3.25	118.99	138.69	2.45	3.51	4.49	4.95	3.83	297.41
MLN001 x MLN008	R x R	4.6	77.8	213.4	2.69	114.00	129.25	2.75	3.72	4.45	5.01	3.97	303.60
CML312 x OH002	S x T	4.5	68.6	198.0	3.38	122.04	129.08	3.10	4.08	4.96	5.59	4.46	339.34

CML442 x MLN013	S x R	4.4	82.8	200.5	3.72	120.75	142.25	2.35	3.41	4.01	4.32	3.47	267.94
CML442 x MLN019	S x R	4.4	66.7	217.6	2.83	115.34	130.96	2.90	4.11	4.53	5.16	4.18	317.65
CML444 x MLN008	S x R	4.2	76.0	202.7	2.40	121.97	120.50	2.91	4.34	4.82	5.60	4.45	339.42
CML312 x MLN003	S x R	4.2	69.9	199.4	2.29	117.02	136.37	2.71	3.67	4.31	4.85	3.85	294.26
CML312 x MLN018	Sx R	4.0	69.2	193.6	3.35	115.50	135.50	2.39	3.89	4.41	4.90	3.86	299.85
CML442 x MLN003	S x R	3.6	67.3	203.8	2.36	85.02	124.87	2.25	3.17	4.02	5.23	3.62	279.34
	Checks												
WE1101		6.0	83.0	205.5	1.85	122.00	134.14	2.38	3.70	4.51	5.04	3.90	303.17
DUMA 43		5.3	74.3	216.6	4.43	121.50	157.25	2.76	4.21	5.04	5.40	4.37	338.20
DH04		4.7	77.2	205.1	4.45	118.75	155.25	2.59	3.92	4.90	5.60	4.27	330.66
DK8031		3.6	75.7	206.2	2.30	0.59	0.00	3.01	4.43	5.19	5.77	4.65	356.76
	Statistics												
Heritability		0.4	0.4	0.5	0.43	0.59	0.00	0.66	0.62	0.63	0.59	0.68	0.66
Grand Mean		5.2	74.0	211.4	3.36	118.64	140.80	2.71	3.97	4.67	5.22	4.14	318.60
LSD		1.0	10.7	16.5	2.78	8.00	45.25	0.47	0.71	0.69	0.79	0.61	101.22
CV		34.0	25.4	10.9	89.25	4.76	17.90	28.01	25.48	21.21	21.29	20.03	20.16
Genotype significance		***	***	***	Ns	***	ns	***	***	***	***	***	***

4.6 Discussion

The significant specific combining ability (SCA) for the traits GY, AD, and EPO allows us to infer that there were crosses combination that had a performance different from that expected only on the GCA effects. GCA analysis identified inbred lines CML312, CML442, MLN013, OH001, and OH002 as good general combiners for earliness and CML444, MLN001, MLN008, MLN018, MLN019, OH001, and OH002 as good general combiners for grain yield. Thus, efficient breeding methods should first accumulate favorable genes in a homozygous state while breaking the linkage blocks, and this will greatly help reduce the grain yield losses associated with MLN. These superior hybrids also had either MLN008, OH002, ML018, MLN019 and/or OH001 as one of the parents further confirming that these lines are good sources of MLN tolerance genes. Most of the top ten were crosses between good combiners for grain yield and MLN tolerant, MLN018, MLN001, MLN019, OH001 and OH002. Thus, the additive gene action in the inbred lines for grain yield and MLN and the non-dditive gene action in the good crosses complement each other to favor MLN tolerance and improve yields (Karanja *et al*., 2018; Prasanna *et al*., 2020).

In addition, MLN013 and MLN019 produced the best cross highly resistant to MLN and best performing under MLN and non-MLN. Therefore, in MLN019 and MLN013, the MLN resistance could be conditioned by both additive and recessive gene actions (Beyene *et al*., 2017; Gowda *et al*., 2015; Prasanna *et al*., 2020). The delayed symptoms expression by OH002 and OH001 was associated to their stay green characteristics which could also be associated with tolerance to the viruses as reported by Karanja *et al*. (2018). SCA effect analysis recognized that CML312/CML442 has good specific combining ability for grain yield. Heterotic grouping classified lines in group three lines in group A, eight in B and eleven in AB. The high general combining ability (GCA) suggests the presence of both additive and non-additive gene action in conditioning the inheritance of MLN tolerance/resistance of lines. The contributions from GCA to the sum of squares of the F_1 crosses, showed that the additive effects for all traits. The insignificant correlations of MLN mean score and grain yield indicated that each reaction was influenced by a separate genetic system. Furthermore, the G x L interpretations could be based on the GCA effects enabling breeders to select stable inbred parents across environments. Considering the interaction, the significance of the GCA vs. environment interaction for grain yield indicates that the general combining abilities of the inbred lines were altered by the environmental conditions the crosses were submitted to. On the other hand, the interaction SCA vs. environment was non-

significant for all traits, which allows us to infer that the specific crosses combinations were stable across environments. The low GCA contribution indicated that the additive and non-additive effects were important for the traits. The negative GCA effect for days to 50% anthesis, plant, and ear height indicates that CML312, CML442, MLN013, OH001 and OH002 can be utilized for developing short and early maturing hybrids to minimize yield loss due to erratic rains and prolonged drought periods as compared to those with positive GCA effect that can be used for developing tall and late maturing maize hybrids. Similar results were reported by shah *et al.* (2015) and Andayani *et al.* (2018). Positive GCA effects for maize lines indicated that they are desirable parents for maize hybrid development and involvement in the maize breeding program as they can be good allele sources in the process of varietal development (Rawi, 2016).

The predominance of GCA verified in this study for all traits can be explained by the fact that the inbred lines utilized were selected for both per se and testcross performance, which is directly associated with additive effects. Similar results for positive and negative SCA effects for grain yield have been reported by Ahmed *et al*. (2017), Melkamu *et al.* (2018), Chemada *et al*. (2015) and Natol (2017). Positive significant SCA effects indicated that the produced crosses were good specific combiners for developing high-yielding maize hybrids. Hybrid CML312x CML444 provided high mean grain yield and possessed desirable significant high SCA effects, revealing good correspondence between mean grain yield and SCA effects. But MLN001/OH001 had significant positive high SCA effects, which was not consistent with high mean grain yield performance. Similar results were reported by Abebe *et al*. (2018) and Gichuru *et al*. (2011). SCA effects also relate to dominance, epistatic and non-additive component of variations.

4.7 Conclusion

The study identified F_1 crosses with high tolerance MLN which can serve as singles crosses for MLN tolerant inbred lines development and three-way cross formation, while those with high grain yield but MLN susceptible can serve as appropriate testers for future evaluation of top-crosses to develop elite lines and hybrids for other traits. The lines and crosses may be exploited further for release in Kenya high yielding MLN tolerant maize hybrids MLN hot spots regions to avoid risks associated with MLN. Breeding efforts to increase resistance to SCMV, MCMV, and MLN will consequently require a good choice of parents. This information is imperative for breeders and farmers in the adaptation of varieties with MLN tolerance and which can be grown in different agro-ecological zones.

The overall importance of additive gene action for MLN resistance suggests that selection for the causative viruses would be an effective procedure to develop maize germplasm with resistance to MLN. This calls for further work to determine the biochemical pathways and regulations associated with MLN tolerance.

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CHAPTER 5

Determining inheritance of maize chlorotic mottle virus (MCMV) resistance in tropical maize germplasm

Abstract

MCMV is one of the causal agents of MLN in sub-Saharan Africa. The spread of MLN reflects the spread of MCMV since SCMV has been reported for decades. Due to its rapid spread, the ability of MCMV to combine and result in synergistic interaction with other Potyviruses poses a big challenge to maize production. Therefore, understanding the genetic of MCMV resistance is crucial in developing improved maize varieties with MLN resistance. This study aimed to determine the inheritance of resistance to MCMV in six breeding starts bi-parental populations. The segregating generations (F_2, BCP_1, BCP_2) were developed from three maize F_1 crosses which were derived from resistant (P_2: MLN013, MLN019, MLN018) and susceptible (P_1:CML539) inbred lines. The analysis of variance showed significant differences among generations in all crosses. Both additive and non-additive, including epistasis gene effects, played roles significant for the inheritance of MCMV resistance. This was further confirmed by the observation of moderate narrow-sense heritability (h^2) estimates. A simple additive and additive-dominance model could be fitted for the population involving CML539 x MLN013. However, this model could not be readily fitted for the populations involving CML539 x MLN019 and CML539 x MLN018 as the resistant parents, suggesting that breeding complications might arise due to the involvement of genes with epistatic effects to governance MLN resistance.

5.1 Introduction

Maize (*Zea mays L.*) is a major cereal crop, providing food and raw material for the starch industry and for livestock feed. Its cultivation extends over a wide range of geographical and environmental conditions in Kenya. Maize has a high yield potential compared to the other cereals, and it offers the best option to enhance food and feed availability in Kenya. With static and sometimes shrinking land resources due to loss to settlement, and an increase in human population, outbreak of new diseases, such maize lethal necrosis (MLN), Kenya is left with no option but to intensify maize production and increase yield (Kagethe *et al*., 2011; Karanja *et al*., 2018; Kariuki *et al*., 2020). The past considerable efforts made to increase maize productivity were hampered by MLN outbreak in 2012, fall armyworm (FAW) in

20216 and their fast spread in the maize growing areas (Wangai *et al.,* 2012; Kumule *et al*., 2018). MLN is believed to spread mainly through the contaminated seed since its transmission from infected seeds to seedlings can vary from 0.003 to 20% (Jensen *et al*., 1991; Mahuku *et al*., 2015). Once the disease is established in the field, it is spread further by insects and by contaminated farm equipment. In areas where maize is grown all year around, the viruses always have a host to survive and infect the crop in the following season.

MLN disease has been described as the most damaging disease of maize in Eastern and Central Africa, causing yield losses approaching 100% whenever an epidemic occurs (Wangai *et al.,* 2012, Mahuku *et al*., 2015). Yield loss due to MLN depends on the growth stage at which infection occurs. Below vegetative 5 stage infection leads to 100% yield loss, whilst infection at the grain filling stage causes negligible effects. However, late infection by the disease results in secondary fungal and bacterial infections, which means that the few grains that may be formed may contain toxins and, therefore, should not be eaten (Karanja *et al*., 2020, Mwasame *et al*., 2021). The recent spread of MLN reflects the spread of MCMV since SCMV has been present in Kenya for decades (Mahuku *et al*., 2015).

Maize chlorotic mottle virus is the only species in the genus *Machlomovirus* (family *Tombusviridae*). The virions of this single-stranded RNA virus are isometric, and the single-component particles have a smooth spherical or hexagonal shape (Scheets, 2010). The virus was first reported to infect *Z. mays* in Peru, Nebraska, Kansas, and Hawaii (Hebert and Castillo, 1973). The virus has also been reported in Argentina, Mexico, Kenya, Uganda, Tanzania, DR Congo, Rwanda, China, Taiwan, and Ecuador (Kitenge, 2012; Wangai *et al*., 2012; Asea, 2013; IPPC, 2014; Lukanda *et al*., 2014; and Adams *et al*., 2014; Deng *et al*., 2014; Quito-Avila *et al*., 2016 and Xie *et al*., 2011). At least two genetically and geographically distinct strains of MCMV have been reported, MCMV-P (Peru) and MCMV-K (Kansas) (Nyvall 1999). The Kenya isolates have shown low genetic variation with at least 96% similarity to the Kansas isolate (X14736.2) (Wamaitha *et al*., 2019).

Due to its rapid spread and interaction with local viruses and the absence of resistant commercial and inbred lines in East and Central Africa, MCMV represents a significant threat to maize production in sub-Saharan Africa. The most effective management of MCMV is through the integration of cultural practice with insecticides and host plant resistance. These include the use of resistant maize lines to MCMV, spraying with recommended insecticides to control the vectors, and control of alternate hosts. The most

effective management strategy for MCMV is to use MCMV-resistant maize varieties as the core of integrated pest management. However, the levels of variation and mode of inheritance for the MCMV has not been established in the MLN tolerant lines.

Over 3000 maize genotypes have been screened for MLN resistance, but little has been done on resistance to the component causal viruses, SCMV and MCMV (Karanja *et al*., 2020, Prasanna, *et al*., 2020). Many tropical maize inbred lines evaluated in Hawaii and Kenya showed some resistance to MCMV (Nelson *et al*., 2011, Karanja *et al.,* 2018). Complete immunity to MLN has not been observed. This means that there is still a lot of work needed to exploit the genetic potential of the maize crop.

The determination of the nature of gene action governing MLN resistance and that of the causal viruses allows maize breeders to optimize their breeding programs (Melchinger *et al*., 12014). The choice of selection and breeding procedures for genetic improvement of maize or any other crop depends largely on the knowledge of the type of gene action for different traits in the plant materials under investigation. Breeding for improved varieties requires a thorough understanding of the genetic mechanisms governing the trait of interest and trait components (Saleem *et al*., 2002; Unay *et al*., 2004).

Grain yield and yield components are known to be quantitative characters controlled by a large number of genes in maize, but little is known about the genetic resistance to MLN and its causative viruses. The phenotypic expression of most traits mainly depends upon the type of gene action, mainly whether it is controlled by a gene with dominance and/or additive effects. However, there could be complications that are associated with the role of non-allelic interactions in the inheritance of quantitative characters as reported in the literature (Hinz and Lamkey, 2003; Azizi *et al*., 2006; Ravikant *et al.,* 2006; Sofi *et al*., 2006). This has not been established for the breeding germplasm sources in East Africa. Therefore, the present study was conducted to determine the gene action and other aspects of inheritance for MCMV resistance in maize breeding populations.

5.2 Materials and methods

5.2.1 Germplasm

The experimental material comprised of five maize inbred lines, OH001, MLN018, CML539, MLN019, and MLN013. All the inbred lines were selected based on results from the previous

study, which was conducted by Beyene *et al*. (2017); Karanja *et al*. (2018); Jone *et al*. (2020). QTL associated with MCMV resistance was identified on chromosome 5 in N211 (OH001) (Jone *et al*., 2018). MLN019 (CKDHL120918) and MLN013 (CML584) were identified to have good resistance to maize streak virus, drought tolerant and good combining ability for grain yield and resistance to MLN, while MLN018 (CKSBL10060) has also been reported to be resistant to MLN, stemborer and fall armyworm (Mwololo *et al*., 2015). Although CML584 has been widely used in developing MLN tolerant maize varieties, it is moderately susceptible to turcicum blight and grey leaf spot and should be used in hybrid combinations with lines having good foliar disease resistance (Karanja *et al*., 2018).

5.2.2 Experimental design and management

The germplasm was sown at the KALRO-Kiboko research farm during the short rains of October-December in 2017. At the time of flowering, crosses were made to produce F_1 generations. Two rows of five-meter length of each F_1 hybrid and their parents were sown during the long rain season of March-April in 2018. Parental lines were crossed to develop fresh F_1 hybrids (only direct crosses) to increase the seed. To produce F_2 generation, the F_1 plants in each cross were self-pollinated. Simultaneously, the BCP_1 and BCP_2 generations were also developed by crossing the F_1 hybrid plants with both parents (P_1 and P_2)

The F_1, F_2, BC_1, and BC_2 generations, along with parents (P_1 and P_2) were established at the KALRO-Naivasha MLN screening site and KALRO-Kabete MLN screen house during the short rains of October to December in 2018. The planting was replicated three times. A single row for parental lines and F_1 hybrids, two for each backcross, and four for F_2 generations were planted in each replication. Each replication consisted of 40 plants from each parent and F_1s, 80 plants from each of the backcrosses, and 160 plants from each of the F_2s. The length of each row was five meters with row to row and plant to plant spacing of 0.75 m and 0.25m, respectively. Overall, 120 plants were evaluated for the non-segregating generations, P_1, P_2 and F_1 crosses. For the segregating generations 240 plants were evaluated for each of the BCP_1 and BCP_2, and 480 plants for the F_2 poluations.

To achieve and maintain the above plant poluations, three seeds were planted in each hill and thinned to one plant per hill 15 days after seeding. Plots were irrigated as needed to maintain good crop growth. The plots were kept weed-free by hand weeding. DAP fertilizer (at the rate

of 206 kg N, 85 kg P and 62 kg K per hectare) was applied in the field to meet the nutritional requirements of the crop. Regent insecticide @ 20 kg per hectare at the time of sowing was applied to control chamber grabs and cutworms. Six weeks after sowing Tremor, Escort and voliam Targo were applied alternating after every two weeks to control stem borer, aphids, and fall armyworm.

MCMV isolates used in this study were originally collected from Bomet County in South Rift valley in Kenya and have been maintained at KALRO-Kabete, National Agricultural Research Laboratories (NARL) by serial passage onto susceptible maize hybrid H614 in separate greenhouses. Virus strain identity was verified at each passage time inoculum was prepared for a test by also inoculating susceptible maize germplasm, OH28, and H614, *Sorghum bicolor* (L.) Moench *cv.* Atlas and Sart. Atlas and Sart sorghum varieties are resistant to MCMV, while Sart is susceptible to MDMV and SCMV, but resistant to WSMV and are always used to check for MCMV and SCMV contamination (Jones *et al*., 2007).

As in other crops, it is very difficult to diagnose virus diseases in maize-based solely on symptoms, as these vary significantly based on plant genotype, time of infection, environmental conditions, and the potential for multiple infections. Therefore, the serological assay, ELISA, was used to check the virus purity, inoculation and disease assessment for MCMV and SCMV.

At the 3 to 4 leaf stage (10 days after planting) all plants were mechanically inoculated twice within one-week interval by rubbing the two youngest leaves (Louie, 1986). Virus inoculum was prepared from freshly harvested infected symptomatic young plants (infected 10-15 days prior to the main inoculation). Before inoculation, leaves were homogenised in 0.01 M phosphate buffer (pH 7.0) in 1:8 dilutions, and 0.6% of 22µm carborundum was added prior to inoculation. The inoculum was kept on ice during inoculation time. The plants were watered daily until all the plants flowered.

All individual plants were visually scored at two weeks after initial inoculation for MCMV symptoms on a scale of 1 to 9; where 1 represented highly resistant with normal green leaves and 9 representing highly susceptible plants with serve necrosis and stunted growth (Karanja *et al*,. 2020; Prasanna *et al*., 2020). Furthermore, 10 random competitive plants from P_1 and P_2, 15 plants from F_1, 20 plants from BC_1 and BC_2 and 30 plants from F_2 populations were measured for traits such as plant and ear height, number of plants and ears at harvest, number

of rotten ears, number of rows and kernels per row and field and grain weight as the trait compromised by the MCMV.

5.3 Data Analysis

All the generation means were subjected to analysis of variance to see if there were significant differences among the generations. Traits that showed significant variation were then subjected to individual scaling test (Mather and Jinks, 1982) and joint scaling test (Cavalli, 1952).

5.3.1 Estimation of genetic effects

Gene effects were estimated based on the method proposed by Hayman (1958) and Jinks and Jones (1958). According to the Hayman (1958) model, programmes were written in SAS for generation mean analysis to determine the inheritance of resistance to MLN, using the 6 populations for a six-parameter model. These analyses were done using the online statistical package of CCSHAU, Hariyana (Sheoran, 2016).

First regression model (Model 1) consisted of 3 parameters (m), (a) and (d). The second model (Model 2) consisted of the epistatic effects, (aa), (ad), (dd), in addition to the parameters in Model 1. Model 2 is used only if a significant additive or dominant effect is detected and to determine if significant epistatic effects exist that are contributing to the significance in Model 1. The models were weighted using reciprocals of the standard errors of the generation means to adjust the unequal population sizes of each generation (Jinks and Jones, 1958). The significance of difference from three-parameter model was estimated through t-test at the 0.05 and 0.01 levels of probability. The six parameter model of Hayman (1958), Mather and Jinks (1982) was used to estimate the variation present among generations by incorporating mean (m), additive effect (a), dominance effect (d), and the three digenic interaction components additive x additive (aa), dominance x dominance (dd) and additive x dominance (ad) as follows:

m = F2;

a = BC1P1 - BC1P2;

d = - 1/2P1 - 1/2P2 + F1 - 4F2 + 2BC1P1 + 2BC1P2;

aa = - 4F2 + 2BC1P1 + 2BC1P2;

ad = - 1/2P1 + 1/2P2 + BC1P1 - BC1P2;

dd = P1 + P2 + 2F1 + 4F2 - 4BC1P1 - 4BC1P2.

An estimate of number of genes (n) involved in the resistance to MCMV was obtained by the formula derived by Pochlman (1987).

$n= (Xp_1-Xp_2)^2/8[\sigma^2F_2- \sigma^2F_1)]$

Where XP1-XP2 were the mean scoring of parents and σ^2F_2 and σ^2F_1 were the variance of the respective generations.

5.3.2 Estimated broad and narrow sense heritability

For these parameters, following formulae were used:

$(h_{2b}) = [VF_2 - (VP_1 + VP_2 + VF_1) / 3] / VF_2$

$(h_{2n}) = [2VF_2 - (VBC_1P_1 + VBC_1P_2)] / VF_2$

Where, h2

b = broad sense heritability; H^2

n = narrow sense heritability (h^2), V =variance for P_1, P_2, F_1, F_2, BC_1P_1 and BC_2P_2 generations.

5.4 Results

5.4.1 Disease reaction among the parents, F_1, F_2, and backcrosses of three generations

Analysis of variance revealed that generations differed significantly in disease severity ($p<0.001$) for the three crosses. MCMV symptoms developed very clearly on the new leaves, 15 days after the first inoculation, and the disease developed continuously. The intensity of infections was very high in segregating and susceptible plants resulting in stunted growth with highly susceptible plants dead at 43 days after inoculation. MCMV rating recorded at 43dpi discriminated the parents, F_1, F_2 and backcross progenies as susceptible or resistant which corresponded with the AUDPC (Table 5.1). The resistant parents (MLN013, MLN018 and MLN019) recorded a score of <2.2 and showed few chlorosis and necrosis while the susceptible parent (CML539) recorded a score of 3.7 at 43dpi. The F_1 crosses recorded similar rating as the resistant parents while the F_2 varied significantly with crosses (CML539 x MLN018)$_2$ and (CML539 x MLN019)$_2$ close to F_1. The average rating for Backcross (BC_1) was >3.0, similar to the susceptible parent, while that of BC_2 scored as the resistant parent (Table 5.1). The means of generations for CML539/MLN013 and CML539/MLN019 were in the decreasing order of $P_1 > F_2 > BC_1P_1 > BC_1P_2 > P_2 > F_1$ and $P_1 > BC_1P_1 > F_2 > BC_1P_2 > F_1, > P_2$ respectively. However, the population mean distributions obtained for CML539 x MLN018 showed that P_1, BC_1P_1 and F_1 were skewed towards the MCMV susceptible parent (Table 5.1). In CML539/MNL018, means of generations were in increasing order of P_1 >

BC_1P_1 > F_1 > P_2 > F_2 >BC_1P_2. The MCMV susceptible parent CML539 had the highest AUDPC value of 357 compared to that of the resistant parents of MLN018, MLN019, and MLN013 of 223.8, 204.6 and 217.4, respectively. The F_1, F_2, BC_1 and BC_2 had an AUDPC of 175.5 to 343.3 which were intermediate between the parents. Symptoms among individual F_2 plants varied greatly and showed continuous variation in crosses CML539 x MLN019 and CML539 x MLN013, while some plants showed transgressive segregation for resistance for cross CML539 x MLN018 (Table 5.1).

Table 5.1: Scoring, means and Area Under the Disease Progress Curve (AUDPC) of MCMV on parents and their progenies in the screen house at KALRO-Kabete, Nairobi

Generation	**dpi8**	**dpi15**	**dpi22**	**dpi28**	**dpi36**	**dpi43**	**Mean**	**AUDPC**
CML539 (P1)	1	1.1	2.3	3.2	2.6	3.7	2.5	357
MLN018 (P2)	1	1	1.4	2	2	2.1	1.6	223.8
CML539 x MLN018 (F1)	1.1	1.3	1.5	2.2	2.2	2.2	1.8	232.1
CML539 x MLN018 (F2)	1	1.1	1.3	1.7	1.8	1.8	1.5	187.3
CML539 x	1.1	1.5	1.1	1.3	1.7	1.9	1.4	175.3
CML539 x	1.2	1.4	1.5	1.9	2.5	2.6	1.8	234.3
MLN019 (P2)	1	1	1.2	1.3	2.1	2.2	1.5	204.6
CML539 x MLN019 (F1)	1.1	1.1	1.4	1.8	2.1	2.3	1.6	199.4
CML539 x MLN019 (F2	1.1	1.3	2.1	2.5	2.7	2.7	2.1	270
CML539 x MLN019/CML539	1.1	1.3	1.8	2.4	2.8	3.2	2.1	275.7
CML539 x MLN019/MLN019	1.1	1.1	1.7	2	2.4	2.5	1.8	243.6
MLN013(P2)	1.1	1.1	1.4	2	2	2	1.6	217.4
MLN013 x CML539 (F1)	1.1	1.3	1.6	1.9	2	2	1.6	217.5
MLN013 x CML539 (F2)	1.1	1.7	2.8	3.1	3.2	3.3	2.5	348.3
MLN013 x CML539/CML539	1	1.2	2.3	2.8	3.3	4	2.4	345.2
MLN013 x CML539/MLN013	1.2	1.7	2	2.3	2.2	2.2	2	233.7
Mean	1.1	1.3	1.9	2.3	2.6	2.8	2	267.2
s.e.	0.22	0.49	0.69	0.62	0.65	0.71	0.44	62.7
Lsd	0.3	0.6	0.7	0.7	0.7	0.7	0.6	69.4
cv%	20.6	37.6	36.6	27.2	25	25.8	22.3	23.5
Significant level (P)	0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

5.4.2 Gene action for MCMV resistance

Results of the model tests indicated that the observed variation in MCMV for each cross consisted of additive, dominance, and epistatic components (Table 5.2). All gene effects were significant for CML539 x MLN019, while in CML539 x MLN013 additive x dominance was the only significant epistatic gene effect. In CML539 x MLN018 all gene effects were significant except dominance x dominance gene effect (Table 5.2). The sign of the effect reflects the relationship between the mid-parent and the means of the F_1 and F_2 generations, indicating which parent was contributing to the additive variation (Mather and Jinks, 1982). Significant additive and dominance effects ($P<0.01$) were detected with Model 1 in all the three crosses, which measures only additive and dominance effects. The F_1 results of the Model 1 test indicated that the observed variation in MCMV for each cross consisted of additive, dominance, and epistatic components (Table 5.2). This model indicates that the additive gene effects were important in the inheritance of MCMV resistance in parent MLN013 and MLN019 and dominance and additive x dominance in parent MLN018 (Table 5.2). In Model 1, the epistatic effects are included in the additive and dominance effects. Model 2 was then fitted to estimate the epistatic effects, as well as the remaining additive and dominance effects. The epistatic effect of additive x dominance (ad) was significantly ($p<0.05$) different from zero, while additive x additive and dominance x dominance (were not significant in CML539 x MLN013, supporting the conclusion from the scaling test.

Table 5.2: Estimates of the additive, dominance, and epistatic effects in the generation means for MLN in 6 populations of three crosses of maize

Population/Effects	**Estimates**	**Standard error**	**t-value**
CML539 X MNL013			
Model I			
F_2 Means (m)	2.6	0.035	7.0^{**}
Additive (a)	2.3	0.035	63.3^{**}
Dominance (d)	2.5	0.035	63.3^{**}
Model II			
F2 Means (m)	2.6	0.29	8.23^{**}
Additive (a)	2.51	0.04	60.02^{**}
Dominance (d)	1.54	1.07	3.35^{**}
Additive x Additive (aa)	0.12	0.29	0.66^{ns}
Additive x dominance (ad)	-3.59	0.25	12.11^{**}
Dominance x dominance (dd)	1.15	0.62	1.64^{ns}
CML539 x MLN018			
Model I			
F2 Means (m)	3.06	0.035	80.22^{**}
Additive (a)	2.52	0.037	64.89**
Dominance (d)	1.82	0.061	29.72^{**}
Model II			
F2 Means (m)	1.68	0.27	6.24^{**}
Additive (a)	2.78	0.03	93.50^{**}
Dominance (d)	4.5	0.88	4.61^{**}
Additive x Additive (aa)	1.43	0.26	3.25^{**}
Additive x dominance (ad)	-5.15	0.2	-10.50^{**}
Dominance x dominance (dd)	-1.22	0.53	-1.67^{ns}
CML539 x MLN019			
Model I			
F2 Means (m)	3.12	0.034	83.75^{**}
Additive (a)	2.01	0.44	45.75^{**}
Dominance (d)	2.1	0.074	26.83^{**}
Model II			
F2 Means (m)	7.93	0.236	16.58^{**}
Additive (a)	1.99	0.039	51.02^{**}
Dominance (d)	-7.57	0.01	8.31^{**}
Additive x Additive (aa)	-3.86	0.232	13.64^{**}

Additive x dominance (ad)	-2.39	0.19	10.68**
Dominance x dominance (dd)	6.59	0.502	11.61**

***Significant at 0.01 probability level*

In Model 1, the epistatic effects are included in the additive and dominance effects. Model 2 was then fitted to estimate the epistatic effects, as well as the remaining additive and dominance effects. The epistatic effects of additive x dominance type were significantly ($p<0.05$) different from zero, while additive x additive and (additive x dominance were not significantly different from zero in the CML539 x MLN013 cross. Only additive x additive and additive x dominance were significantly different from zero in the CML539 x MLN018 cross, while all the three epistasis types were present in the CML539 x MLN019.

5.5 Discussion

The results indicate that the 43 days post inoculation and AUDPC are the best in discriminating geneotypes for MCMV resistance. The susceptible parent, CML539 that served as a check showed conspicuous chlorosis and necrosis and a high score of 3.7 indicating susceptibility. The tolerant parents MLN013, MLN018 and MLN019 and exhibited reistance in form of mild symptoms indicative of partial resitance to MCMV (Beyene *et al*., 2017, Karanja *et al*., 2018). However, the exact number of MCMV isolates or mutants in the country is not known (Wamaitha *et al*., 2018). Therefore, some maize lines can show resistance in one ecological zone but would be susceptible to the virus in another as it has been reported with maize streak virus (Bosque-Perez, 2000). There is therefore the need to understand the distribution of MCMV strains across the six maize agro-ecologies and season. The unique segregation pattern and expression of MCMV resistance observed among the three generations were evaluated. All the F_1, F_2, BCP_1 and BCP_2 in cross MLN539 x MLN013 and CML539 x MLN019 had scores closer to that of P_1 and P_2 indicating that co-dominance and partial dominance-controlled resistance in the tolerant parents MLN013 and MLN019. This indicates that the heterozygotes between resistance and susceptible lines reacted to infestation in a manner intermediate between the parents (Story and Howland 1967). The AUDPCs of the F_1, F_2, and $BC_{1:1}$ and $BC_{1:2}$ crosses were intermediate but closer to the AUDPC of the tolerant parents MLN013, MLN019 and MLN018 than the susceptible CML539, further showing that there was an improvement in resistance among the crosses arising from MCMV superior alleles donated by the resistant parent sources. There are more than one quantitative trait loci (QTL), two or more major gene pairs, with the possible

involvement of minor and modified genes that control MCMV in maize germplasm. Using mapping approaches based on recombination frequency or genomic positions of SNP on the B73 physical map, MCMV QTLs were identified on chromosomes 3 (Oh1VI), 5 (N211), 6 (KS23-5 and KS23-6), and 10 (DR and Oh1VI) (Jones *et al*., 2018). In CML539/MLN018, the segregation pattern of the six generations means scores and AUDPC were lower or deviated little from the resistant parent MLN018, hence indicating complete dominance over the susceptible parent. Improvement of MCMV resistance observed in the F_2 and BC of CML539x MLN018, suggests that the MLN018 is a superior donor for MCMV resistance. MCMV resistance in the tolerant lines MLN013 and MLN019 were observed to be controlled by additive, additive x additive, and additive x dominance gene actions, while in MLN018 was controlled by genes expressed in a complete dominant manner as different levels of magnitude between populations were observed. The results also deviated from the theoretical Mendelian segregation, which attributes the MCMV resistance to modifying genes, thus variations of symptoms observed among the segregating populations.

5.6 Conclusion

The analysis revealed that the nature of inheritance of MCMV resistance could be population-specific. Although various types of gene effects, namely additive, dominance and epistasis (i.e. additive x additive, additive x dominance, and dominance x dominance) were observed in this study, the general tendency was for additive genetic component to be of predominant importance. Therefore, appropriate breeding methods are to be adopted for the improvement of each population. The present study identified MLN013, MLN018 and MLN019 as useful sources of MCMV resistance. These lines can be used by breeders in short and medium-term in creating inbred lines and hybrid tolerance to MLN. This would reduce the spread of MCMV and consequently the MLN and the yield losses which are associated with them. The resistance genes can further be utilized by breeders for long-term breeding through gene/QTL pyramiding, recurrent selection to attain higher levels of MCMV resistance.

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CHAPTER 6

Overview of research findings

6.1 Introduction

This study focused on identifying resistant maize germplasm MLN and its causative viruses for integration in breeding programs. Therefore, investigations were pursued in order to understand the economic importance of MLN, SCMV, and MCMV, search for suitable resistance germplasm towards developing a viable strategy for generating MLN resistant hybrids with farmers' preference in Kenya. Target maize germplasm should be carefully selected to avoid the disruption of existing heterotic groups to achieve a high level of MLN resistance in maize hybrids. As a result, the germplasm used in this study was of diverse genetic background and was characterized based on its reaction and performance to assess its utility for improving hybrids for MLN resistance. This was also to determine its potential not only on disease severity but in developing productive and stable maize hybrids as keys for new maize hybrids by seed companies and farmers. The work accomplished by this study is reported in the four research chapters. This overview gives a summary of the main findings and recommendations for further research.

The hypotheses tested in the study include:

1. The studied maize inbreds have a great genetic divergence that can be exploited for breeding for SCMV, MCMV and MLN resistance and improved grain yield and performance in the MLN infected fields.
2. MLN resistance is dependant on SCVM and MCMV resistance. The yield loss associated with MLN, SCMV, and MCMV is dependant at the time of inoculation, disease incidences, and severity.
3. Hybrids with high MLN resistance are high-yielding and have stable yield performance and wide adaptation. The selected inbred lines have stable performance over MLN, which calls for their use in developing stable and highly adapted F1 hybrids.
4. Inheritance of MCMV resistance is controlled by a complex mechanism that can be introgressed into susceptible backgrounds through backcrossing and progressive selection

6.2 Summary of the major findings

6.2.1 Response of selected maize germplasm to MLN and its causative viruses

Maize lines adapted to tropical and sub-tropical environments and with a high level of resistance to SCMV, MCMV and MLN were identified. Based on disease severity, Area Under the Disease Progress Curve (AUDPC), and ELISA analysis, MLN001 and MLN006 expressed a high level of resistance to MCMV, while MLN042 and MLN041 were identified as the most promising sources of resistance against SCMV. Inbred lines MLN0019 (CKDHL120918) and MLN013 (CML584) were identified as the best performers under MLN infestation. These inbred lines are important donors for use in developing MLN resistance varieties, MLN management, and enhancing maize productivity in the MLN endemic regions. The inbred lines can also be used in further research to understand the mode of gene action for MLN resistance and synergistic interaction between SCMV and MCMV.

6.2.2 Effect of maize lethal necrosis and its causative viruses on the growth and yield of maize as influenced by varietal tolerance /susceptible levels and plant stages at time of inoculation

This study revealed that; (i) MCMV tolerant maize hybrids are less affected by MLN, and (ii) dual inoculation with MCMV and SCMV exacerbates symptom expression and yield loss in MCMV susceptible hybrids. Disease severity scores, grain yield and its attributes significantly varies at V_3, V_7 and V_T inoculation stages, with the greatest effect observed at V_3 stage and increased number of rotten cobs observed V_T.

Among the attributes affected include plant height, ear height, days to 50% anthesis, number of ears at harvest, rotten ears and grain yield.

Hybrid CKLMLN146038 was the least affected across the three treatments recording the lowest yield loss of 17.0%, 29.7% and 31.5% in MCMV, MLN and SCMV, respectively. Susceptible varieties were significantly affected, recording a yield reduction of more than 80% under SCMV, MCMV, and MLN. Percentage of yield loss was proportional to the percentage of disease incidence, severity and effect on yield attributes which varied from variety and season. In the farmers' fields, the infestation may commence at any stage depending on the inoculum load in the soil, residual crop, and vectors.

6.2.3 Half-Diallel cross analysis of MLN tolerance maize inbred lines

Breeding efforts to increase resistance to SCMV, MCMV and MLN require a good choice of parents. The inbred lines MLN001, MLN019, OH001 and OH002 had good GCA for both

MLN disease resistance and high grain yield qualifying them as suitable candidates for use in breeding new productive MLN resistant hybrids. MLN013 and MLN019 were identified as reliable donors for MLN tolerance and should be exploited in developing MLN tolerant varieties. The highest yielding were CML312 x CML442, CML444 x MLN019, CML442 x CML444, MLN018 x MLN019, MLN018 x OH002, CML444 x MLN018 and MLN008 x MLN013 > 20% recording yield advantage above the mean of commercial checks.Genotype and genotype ×environment (GGE) biplots revealed positive associations among testing location, signifying the suitability of all the locations for earliness and yield. Inbred lines CML312, CML442, MLN013, OH001 and MLN 019 are identified as good combiners for yield and earliness.

6.2.4 Determining inheritance of maize chlorotic mottle virus (MCMV) resistance in tropical germplasm

Knowledge on the mode of inheritance of MCMV resistance is fundamental in developing a viable breeding strategy. The segregating generations (F_2, BCP_1, BCP_2) were developed from three maize F_1 crosses which were derived from MCMV resistant (P_2: MLN013, MLN019, MLN018) and susceptible (P_1:CML539) inbred lines. The 43 days post inoculation and AUDPC were identified as the best in discriminating genotypes for MCMV resistance. All the F_1, F_2, BCP_1 and BCP_2 in cross MLN539 x MLN013 and CML539 x MLN019 had scores closer to that of P_1 and P_2, indicating that co-dominance and partial dominance-controlled resistance in the tolerant parents MLN013 and MLN019.

In CML539/MLN018, the segregation pattern of the six generations means scores and AUDPC were lower or deviated little from the resistant parent MLN018, hence indicating of complete dominance over the susceptible parent. Improvement of MCMV resistance observed in the F_2 and BC of CML539x MLN018, suggests that the MLN018 is a superior donor for MCMV resistance.

There are more than one quantitative trait loci (QTL), two or more major gene pairs, with the possible involvement of minor and modified genes that control MCMV in maize germplasm. MCMV resistance in the tolerant lines MLN013 and MLN019 were observed to be controlled by additive, additive x additive and additive x dominance gene actions while in MLN018 was controlled by genes expressed in a complete dominant manner as different levels of magnitude between populations were observed.

6.3 Implication of the findings in breeding for resistance to MLN and its causative viruses

6.3.1 Response of selected maize germplasm to MLN and its causative viruses

MLN continues to be the most important production constraint affecting maize productivity. The study on screening maize lines for SCMV, MCMV, and MLN demonstrated presences of disease resistance available and adapted to the local environment. The identified lines bring additional sources of tolerance/resistance to breeding programmes focusing on MLN resistance. These lines have to be tested in hybrid combinations and under MLN artificial and natural infestation across the six maize ecologies of Kenya for efficiency of resistance. Through the use of doubled haploid technology, improved lines with better sources if resistance can be developed. Other technologies such as gene editing can be deployed to enhance the levels of resistance in current farmers' preferred commercial hybrids susceptible to the disease. Disease severity expression varied among the tested maize lines. The disease symptoms were expressed early in SCMV (5 to 6 days) and MLN (10-12 days), where disease progression is slow symptom development in MCMV (14-15 days). The optimal time for symptom assessment would be between 35 and 56 days after inoculation for SCMV, MCMV and MLN. The scoring at flowering (56 days post-inoculation) is important for it indicates the influence of the disease on yield potential. At this stage the disease is known to cause delayed anthesis, male sterility, poorly filled and high number of rotten grains. The high correlation between AUDPC, 35dpi and 56dpi scores show that the two scores eliminate the need for several ratings when evaluating large numbers of germplasm both in the field and screen house. Depending on the biotic and abiotic factors, delayed symptoms are observed in some maize germplasm e.g. Unlike MCMV and MLN diseases, SCMV was not found to have a great impact on days to flowering.

6.3.2 Effect of maize lethal necrosis and its causative viruses (maize chlorotic mottle virus and sugar cane mosaic virus) on the growth and yield of maize as influenced by varietal tolerance/susceptible levels and plant stage at time of inoculation

A high MLN incidence and severity continue to be observed in the major maize growing regions of Kenya, calling for an urgent need for resistant varieties. Disease incidences and severity and their impact on grain yield attributes and grain yield depend on the variety, time of infestation, and season. In the susceptible varieties, >90% of yield is lost due to SCMV and MLN and 85% to MCMV with early infestation. Other factors highly affected by the

early inception of the disease include plant and ear height due to reduced internodes, delayed flowering, male sterility and poor synchronization of anthesis and silking. Late occurrence of the disease affects the cobs resulting to pre-mature drying of the husk and poorly formed grains that are rotten, which attract fungal growth and mycotoxin contamination. The use of these resistant maize varieties offers many advantages, especially if grown with integrated pest management practices, as they are less affected. However, the yield potential of these hybrids is not guaranteed since the virus present in the infected fields and prevailing weather conditions may result to varied damage. Multi-seasonal testing for MLN tolerant varieties is required before settling on a suitable hybrid for release and registration. This is after recording an average of 70% yield loss in the pre-commercial MLN tolerant variety WE5135 and pre-mature drying of the husk cover in WE5104. Commencement of late infestation highly depends on the inoculum load in the soil, residual crop and vectors, season break and crop rotation. However, this need to be investigated further to increase the gains in yield in the MLN prone fields. It should be noted that time of planting is largely in Kenya is dependent on the onset of rainfall. Breeders need to screen their new crosses for SCMV and MCMV which to discard susceptible crosses at an early stage before conducting the expensive multi-location testing and nomination for registration. This is in addition to high yields, maturity and palatability traits that enhance fast adoption of new varieties by Kenyan farmers.

6.3.3 Half-Diallel Cross Analysis of MLN Tolerant Maize Inbred Lines

Breeding for disease resistance offers a solution in enhancing crop productivity and yield stability. However, the selection of suitable inbred parents with desired attributes is vital in availing farmers' preferred products. In achieving MLN resistant lines and hybrids, the general and specific combining power and performance of the resultant crosses was assessed. Other than yield, plant height, ear heights, days to anthesis, days to silking and MLN severity are also of important. Combining ability for yield and MLN resistance identified lines MLN001, MLN019, OH001 and OH002 had good GCA for both MLN disease resistance and high grain yield. The MLN disease severity ratings on the maize hybrids derived from these inbred lines ranged from 2.0 to 4.0. The lines and hybrids were also found to be highly adapted to the tropical environments. Inbred lines CML312, CML442, MLN013, OH001 and MLN 019 were identified to be good combiners for yield and earliness. Among the top-performing and MLN resistant crosses included CML444 x MLN019, CML442 x CML444, MLN018 x MLN019, MLN018 x OH002, CML444 x MLN018 and MLN008 x MLN013

recording >20% yield advantage over the commercial checks used. The contribution of SCMV resistance in MLN resistance crosses was evident. CML442 and CML444 had been earlier identified to be SCMV resistant, OH002 and MLN008 MCMV resistant while MLN019 and MLN013 as highly resistant to SCMV and MLN. MLN013 and MLN019 should have been identified as donor for MLN tolerance and should be exploited in developing MLN tolerant varieties, but more sources would be needed. However, gene editing technologies should be applied in converting CML312 and CML442 to MLN resistance as they imaged as the best combiners for yields.

6.3.4 Determining inheritance of maize chlorotic mottle virus (MCMV) resistance in tropical maize germplasm

The high level of resistance in the crosses in this study could be attributed to the mode of resistance identified in MCMV. Using 43 days post-inoculation score and AUDPC the resistance to MCMV could mainly be controlled by additive and dominance gene action with no-additive gene playing some role. There are more than one quantitative trait loci (QTL), two or more major gene pairs, with the possible involvement of minor and modified genes that control MCMV in maize germplasm. This means that it is possible to use susceptible parents crossed with resistant parents (MLN013, MLN018 and MLN019) to produce MCMV resistant hybrids. The MCMV resistant genes from these parents can be incorporated through backcross or recurrent selection followed by double haploid induction. This is to avoid pedigree breeding which laborious and time-consuming and especially where heritability is low. However, backcross breeding might be time-consuming and costly, especially where undesirable genes closely linked to MCMV resistance are transferred along with the resistance genes. However, the exact number of MCMV isolates or mutants and their distribution need to be known (Wamaitha *et al*., 2018) to avoid developing lines or hybrids that would turn susceptible in some ecologies. Mapping out the quantitative trait loci linked to MCMV resistance in MLN013, MLN018 and MLN019 would allow the use of molecular marker-assisted selection to improve the resistance.

6.4 Conclusions

The study has identified maize inbred lines for use in the short, medium and long term in breeding new, improved lines and hybrids with resistance to MLN. It has also established for the first time the importance of incorporating SCMV and MCMV resistance in breeding for MLN resistance. SCMV and MCMV are equally important in affecting MLN on maize

output and productivity, according to the findings of this study. Infestation pressure of SCMV, MCMV, and subsequently MLN, as well as projected yield loss, are heavily influenced by maize growing farmers' practices in Kenya, such as seed selection and quality, time of planting, and vector pressure and control method. Although various types of gene effects, namely additive, dominance and epistasis (i.e., additive x additive, additive x dominance and dominance x dominance) this study observed that additive genetic component is predominant in control of MCMV resistance.

6.5 Recommendations

MNL001, MLN013, MLN018, and MLN019 and their derived crosses need to be evaluated across maize agro-ecologies and seasons to understand the number of MCMV strains and distribution of MLN by breeding programs across East and Central Africa. The presence and impact of SCMV and MCMV viruses in the field will aid in the development of more robust MLN control measures

References

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