THE COMBINING ABILITY OF SINGLE CROSSES AND THEIR REACTION TO MAIZE LETHAL NECROSIS VIRUS

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DECLARATION

DECLARATON BY THE STUDENT

This research thesis is my original work and has not been submitted anywhere for a degree in any other university.

Cheruiyot Susan Chepchumba.

AGR/PGB/03/12

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DECLARATION BY SUPERVISORS

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DEDICATION

This Work is dedicated to My Father HOSEA CHERUIYOT

To My Mother ROSELYNE CHERUIYOT

To My Sisters IRENE CHEROTICH and RUTH CHEPCHIRCHIR

To my brother EMMANUEL KIPKEMBOI

To my nephew VINCENT KIPKORIR

Your affection, Support, Guidance and Prayers made this a success

ABSTRACT

Maize is a key staple crop that is grown in most regions of Kenya and it is consumed in various forms by 80% of the population. Among the biotic constraints, maize lethal necrosis disease causes heavy yield losses thus compromising food security in the country. In 2011, Maize lethal Necrosis came about as a destructive disease in Kenya and many parts of Sub-Saharan Africa. MLN was discovered to be as a result of a dependent interaction between Maize Chlorotic Mottle Virus and Sugarcane Chlorotic Mottle Virus. MLND affects maize plants at all developmental phases from seedling to maturity. Although MLN has been extensively studied, it is still a persistent menace in many parts of Africa due to various hindrances in developing maize varieties that are tolerant to MLND which includes: assembling of germplasm that is resistant to the disease from various sources, using few sources that are resistant and association of the genotype and location. The trial focused on 120 single cross hybrids set up in an incomplete block design in the two sites. The single crosses were sourced from CIMMYT and KALRO formed through crossing resistant and susceptible inbred lines. Diallel crosses are used to investigate the gene action controlling grain yield. Data collection was on the basis MLN disease score, grain yield, days to pollen shed, days to silking, moisture content, plant height and ear height. The data was analyzed using GENSTAT statistical package (5^{th} edition). Generally, the study was able to show that MLN is still a major problem in Kenya with rising incidences and intensity in maize fields of farmers. MLN resistance levels in varieties that are grown locally need to be boosted. Severity scores for the single crosses were variable indicating the existing and potentially useful germplasm for improving MLN resistance in breeding programs. The study identified lines SC-MLN-15-56 in Naivasha and lines SC-MLN-15-3 and SC-MLN-15-23 which had disease severity scores of ≤ 3.0 which could be further improved to be used in disseminating resistant gene varieties that are susceptible to MLN. When analysis of variance for diallel cross was exploited both GCA and SCA were highly significant showing that grain yield is as a result of both additive and non-additive effects. GCA effects revealed CML498 as an ideal combiner for most characters and cross combinations involving CML498 as one of its parents recorded desirable SCA effects. Estimates of SCA showed cross CML395×CML505 as the most desirable cross which could be further improved in hybridization programs for developing hybrids that are efficient and MLN resistant. These would be given out as recommendations to program that mainly deal with development of hybrids that are resistant to MLN.

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LIST OF ABBREVIATIONS

CIMMYT	-	International Centre for Maize and Wheat Improvement
ELISA	-	Enzyme Linked Immune-Sorbent Assay
FAO	-	Food and Agricultural Organization.
KALRO	-	Kenya Agricultural and Livestock Research Organization
MCDV	-	Maize Chlorotic Dwarf Virus
MCMV	-	Maize Chlorotic Maize Virus
MDMV	-	Maize Dwarf Mosaic Virus
MLN	-	Maize Lethal Necrosis
SCMV	-	Sugar Cane Mosaic Virus
WSMV	-	Wheat Streak Mosaic Virus

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CHAPTER ONE

INTRODUCTION

1.1 World Maize Production

Maize (*Zea may*s L.) is one the important cereal crops and is places third in terms of production worldwide after wheat and rice (FAO, 2017)). In most countries in the world, maize is the only source of calories and protein for the poor and mostly used during weaning of infants. In many parts of Africa, corn is among the main essential food stuffs serving as a source of daily food and earnings to millions of small scale farmers (Zhang et al., 2012). About 600 million people eat on average 40 kg of maize per year (an increase of about 30% since 1961), reaching 80–141kg in Kenya, Lesotho, Malawi, South Africa, Zambia and Zimbabwe (Lumpkin and Armstrong, 2009). Maize is grown in the whole African continent as a major subsistence crop and in most countries maize adds up to about 56% of the entire crops harvested for food and approximately 30-60% of the total energy intake (Tefera et al., 2011).

1.2 Maize Production in Kenya and Its Constraints

Maize is mainly grown for food in the country (Muriithi and Mutinda, 2001), a widely consumed cereal by over 90% of the population (Muiru, 2008). A shortage of the commodity often leads to hunger. The key areas that produce maize lie in the Rift Valley region and include; Trans-Nzoia and Uasin- Gishu counties, although the cultivation is widespread. The bulk of the maize produced is consumed at household level. Kenya leads in maize consumption among East African countries with an annual consumption of 105 kg (Muiru, 2008) which tis about 30-42 bags annually. Among the crops grown in Kenya,

Maize production covers approximately29% of the entire crops grown by small scale farmers (Sibhatu and Qaim, 2017).

Rainfall failure or erratic rains is the principal cause of low yields since maize is produced mainly under rain fed conditions (Nyoro et al., 2004). In most parts of Kenya, smallholder farmers who cultivate maize are lack resources and can hardly get the farm inputs necessary for farming such as certified seeds. High costs and inadequate supply of certified seeds has led to under-utilization of improved germplasm resulting in low productivity (Kiptanui, 2013). Low soil fertility and weeds infestation also cause significant maize yield decline being estimated at 30-100% annually (Manyong et al., 2007; Karaya et al., 2012).

Disease causing pests which affect maize crop during cultivation and storage are the major biotic constraint limiting maize production in the country (Pingali and Pandey, 2001). Among the field pests, stem borers approximately lead to annual yield losses of about 12 to 15% while the larger grain borer may result in 100% yield loss in storage (Tefera et al., 2011). Foliar diseases range from fungal, bacterial and viral and include maize rust, maize smut, northern leaf blight (NLB), ear rots, gray leaf spots (GLS), maize streak disease and Maize Lethal Necrosis that was recently discovered (Mwangi, 1998, Wangai et al., 2012). In storage, infection of maize by fungi results in production of toxins such as aflatoxins which result in deaths when infected grains are consumed (Njuguna et al., 2001).

The disease is a threat to corn (*Zea mays*) cultivation in many regions (Mahuku et al., 2015a; Wangai et al., 2012; Adams et al., 2013; Adams et al., 2014; Lukanda et al., 2014; Mahuku et al., 2015b). MLN is due to a synergistic interaction between Maize chlorotic

mottle virus (MCMV) and any other virus in the potyvirus group including; Wheat streak mosaic virus (WSMV), Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV) (Niblett and Claflin, 1978; Uyemoto et al., 1980). In East Africa the primary cause of the disease is a combined infection with Maize chlorotic mottle virus and Sugarcane mosaic virus (Wangai et al., 2012; Adams et al., 2014; Lukanda et al., 2014; Mahuku et al., 2015). MLN causes stunted plant growth, premature death or aging, male sterility and failure to tassel, malformed ears or lack of ear formation, chlorotic mottling from the plant base, leaf necrosis from the margins to the midrib and rotten or minute maize cobs that hve fewer or no seeds at all (Niblett and Claflin, 1978; Wangai et al., 2012). This suite of symptoms leads to MLN being a devastating disease.

MLN is a disaster for sectors that rely on maize in Africa. Isabirye and Rwomushana (2015) projected an increase of incidence and distribution to other regions of East and Central Africa having the same weather conditions to the current hotspots and a significant southward movement to Southern African countries like Mozambique, Malawi, Angola, Namibia, Zimbabwe and Madagascar which are among the biggest maize producers (FAOSTAT, 2017). This threat of potential spread is the justification for drastic measures to find a solution to MLN. To safeguard maize production in East Africa and Sub-Saharan region, maize breeders have to come up with germplasm that is resistant to MLND.

Plate 1.1 : Experimental Field in Naivasha Showing Maize Plants Affected By MLN Disease (Source: Author, 2015).

1.3 Statement of the Problem

MLN is a threat to people's livelihoods, food security, and nutritional well-being. The disease is also a threat to the economic stability of countries in Africa. MLN is an issue for the sectors that produce maize in Kenya, East Africa and Sub-Saharan Africa. The magnitude of yield loss associated with the disease makes developing cultivars with disease resistance to be used by farmers in the region of great importance. In Kenya, MLN caused an estimated loss of $187 million equivalent to $364/ton (De Groote et al., 2016). The farmers are affected heavily because of their complete reliance on the crop for food production and income. Farmers in MLN areas have experienced a significant decrease in yield from 2011-2014 since when MLN was first reported in 2010 (Makone et al., 2014).

Plate 1.1: Maize Plants Affected By MLND in Bomet Experimental Site (Source: Author, 2015).

For a long time, the national maize production has not been in equilibrium with consumption rates. Kenya has been buying maize from Tanzania and Uganda and from as far as USA, South Africa and Malawi so as to reduce the gap between supply and demand of maize (Nyoro et al., 2004). The low maize yields are due to disease and pests, among other factors. There are various disease causing fungi, bacteria, nematodes, and viruses found in the country that infest on maize maize (Njuguna et al., 2001). Infection of maize by these diseases cause high yield losses (up to 100%) leading to a drop in food production and income from agricultural production. In the recent years, no extensive study has been undertaken to show the occurrence, spread and the prevalence of maize

lethal necrosis in the areas that cultivate maize, the last one having been done over a decade ago (Mwangi, 1998). There are many genotypes that have been introduced in the country and are not yet evaluated on how they react to the maize lethal necrosis disease. Changes in climate may also have led to creation of conditions conducive for the disease, resulting in dissemination of diseases to areas that were not affected before, and therefore changes in occurrence and distribution. New diseases that may not have been reported previously may have emerged, creating the need to screen the available maize germplasm for their reaction to these new diseases.

1.4 Justification

For maize seed business to grow in Kenya and the region, this disease must be controlled. As MLN is due to a dependent association of MCMV and viruses found in the potyvirus family (Maize dwarf mosaic virus (MDMV), Sugar cane mosaic virus (SCMV), Wheat streak mosaic virus (WSMV), studies is required to find out which of the Potyviruses are available in locations that disease occurs. Presently, SCMV has been examined, but there could be other group of viruses responsible for causing MLND. The rapid spread of the disease made farmers, government, research institutions like CIMMYT and KARI, research institutes in the US among others, as well as seed companies eager to find a solution to the above problem (CIMMYT, 2018).

Weather changes especially conditions of drought and rising temperatures is suitable for the growth and increasing number of vectors that disseminate MLN. This has been a major challenge to scientists who are trying to come up with a solution to MLND (Wangai et al., 2012). The emergence of the Maize Lethal Necrotic epidemic especially major maize farming areas is a serious problem to countries food security. A survey was

done in East African countries to study the distribution of MLN causing viruses suggested up to 94% incidence in randomly selected and symptomatic plants (Mahuku et al., 2015). Tanzanian samples collected at Arusha and Mwanza had 60% to 69% incidence with both viruses detected (Mahuku et al., 2015). The survey indicated wide distribution and high frequency of MLN viruses in East and Central Africa emphasizing the urgency of controlling MLN in the region.

In contrast to vector control methods which may require farmers to purchase pesticides for chemical control, use of MLN tolerant varieties is considered an effective way to manage diseases since it requires less input and hence is more cost effective and environmentally sustainable (Zambrano et al., 2014). This is especially true for small holder farmers in Sub Saharan Africa with little or insufficient capital to acquire chemicals for vector control.

Potyviruses are endemic to East Africa and were observed to cause crop loss of 18% to 46% (Louie, 1980). The introduction of MCMV and co-infection of maize with the endemic potyviruses to cause MLN represents a new threat to maize cultivation in countries in the African continent (Wangai et al., 2012). There is a great need come up with MLN resistance sources, mapping of genomic regions with MLN resistance and introgression of resistance genes into widely used susceptible inbred lines and hybrids in East Africa (Semagn et al., 2014).

Efforts to control MLN through resistance breeding have begun in individual East African countries and among international organizations such as International Maize and Wheat Improvement Center (CIMMYT). Together, the Kenya Agriculture and Livestock research Organization (KALRO) and CIMMYT have evaluated 25,000 accessions for

MLN resistance (Semagn et al., 2014). Majority of the evaluated lines have mild to moderate resistance (Semagn et al., 2014). The concept on the combining ability of the parents that are suitable is important for development of varieties that are adapted to many locations. The exploitation of the parental germplasm for its combining ability is able to show us what gene action is involved and helps in selection of parental lines that are superior and inbred lines having high specific combining ability.

The goal of the study was to evaluate combining abilities of single crosses and their reaction to MLN disease in Bomet and Naivasha under natural and artificial inoculation respectively. The project aimed to fill the knowledge gap concerning combining abilities of single crosses for control of MLN. In addition, the experimental design will clarify how cultural practices such as planting date may contribute to controlling of MLN in Kenya. Finally the study will develop germplasm which can be further improved and utilized in breeding programs to come up with varieties that are resistant.

1.5 Broad Objective

To determine the combining ability of single crosses and their reactions to maize lethal necrosis disease.

1.6 Specific Objectives

The specific objectives are;

i. To identify the response of single cross varieties to MLN disease grown under high disease pressure.

ii. To determine the combining ability of single cross varieties with potential use in maize improvement against MLND.

CHAPTER TWO

LITERATURE REVIEW

2.1 Evolution, Taxonomy and Genetics of Maize

Scientists believe that maize a domesticated variant of an earlier ancestral plant called teosinte (*zea mexicana*) (Galinat 1988).Domestication of maize is thought to have begun approximately 6000 years ago as suggested by pre-historical records and genetic analysis and was later introduced into Africa by Portuguese travelling from America to the west coast of Africa Matsuoka et al., 2002).

Maize (*Zea Mays*) is an annual plant that is found in the grass family (Gramineae) together with plants such as sugarcane, rye, barley, sorghum and wheat. It has an extensive fibrous system with the female (ear) and male (tassel) on separate places on the plant. (FAO, 1992). The female part of the plant is the cob or ear while the silks which are usually found along the top of the cob are long stigmas that grow from an egg found on the cob. The tassel is the male part of the plant that produces pollen and is found at the top of the plant which is approximately 30cm long and occurs in 8-16 rows (Hitchcock and Chase, 1971).

Pollination in maize takes place by wind which transfers tiny pollen grains to the silk either of the same plant or a different plant where they move down inside the silk to fertilize an egg which then develops to a kernel. Pollen grains that are shed are able to germinate for 10-30 minutes but under suitable environmental conditions they can retain their ability to germinate for a longer period of time (Coe, Nueffer & Hoisington, 1988)

2.2 Habitat and Cultivation of Maize

Maize crop is able to thrive in different areas with different climatic conditions; it can either be cultivated as an arid land or under irrigation (Agbonifo & Olufolaji, 2012). Maize is mainly planted at altitudes higher than 3000m and rainfall levels of about 250-5000mm per year (Twumasi-Afriyie et al., 2001). Generally maize is able to tolerate low water levels as compared to any other crop sorghum included (Beckingham, 2007). Maize is also able to grow in a diverse environment as compared to any other crop. Maize crop prefers warm soil temperatures (≥10) (OMAF, 1994). Higher temperatures may cause physiologic damage and reduced rates of photosynthesis for the crop. The crop also performs best in soils rich in essential nutrients with good drainage and PH levels of 5.5- 7.0.

2.3 Significance of Maize

In many parts of Africa, the crop is one of the most prominent and is cultivated in about 2.5 million hectares of land mainly by small holder farmers who produce million tonnes of grain (FAOSTAT, 2017). Maize constitutes 34% of all cereal crops and 8% of all the crops grown in the country. Maize an important food crop in Africa and over 80% of the cereal grown in this area is for consumption, while in Africa approximately 94% of the maize gown is used as food (Shiferaw et al., 2011).

Maize is a main crop grown as food in many parts of Africa and it constitutes a major section of diet to many people in this area. 15 million hectares is used to plant maize in this region in all major growing areas at about 2500 m above sea level (asl) (Twumasi-Afriyie et al., 2001). The crop constitutes a major part of the meals prepared by over 44 million people in Kenya. Over 70% of maize is produced for consumption mainly as

maize flour which makes up the main source of energy in the Kenyan diet. It is prepared as porridge for breakfast and stiff porridge (ugali) for lunch and dinner. Small holder farmers also depend on selling their maize surplus maize meet their basic needs. Maize also contributes to the country's economy through export earning; Kenya is named among the top 25 maize producers worldwide (FAO, 2015; http://www.fao.org/3/a-at481e.pdf; URL verified, 19/08/2015).

In Kenya, maize crop is cultivated in approximately two million hectares of land accounting for 3.6 metric tonnes in terms of production (MoA, 2013). Maize is grown in large scale more than the other cereals; wheat and rice but when it comes to production maize come in third with rice being first and wheat second. (MoA, 2013).

2.4 Challenges in Maize Production

Maize is faced by numerous problems during its cultivation and has not been produced extensively in the country as compared to wheat and rice. In areas having high potential, mainly the highlands, maize cultivation is at 6t/ha while in other areas production is at 1.6t/ha (FAOSTAT, 2010). Researchers have to put more effort so as to ensure a rise in maize production in the country (De Groote et al., 2005), since stagnation in planting maize is a common scenario in many parts of the country (Mbithi & Van Huylenbroeck, 2000, Ngoune et al., 2018). In 2012, the ministry of Agriculture in Kenya named the major factors that affect growing of maize as the following: maize lethal necrosis, maize streak virus, head smut, maize stem borers and Striga weed. Other factors include the using low quality seeds, increased precipitation during harvest that leads to rotting of grains and no available buyers for their produce (MoA, 2013). The study is on the maize lethal necrosis disease.

2.5 Maize Lethal Necrosis (MLN) Disease

The disease as a result of infection of maize crop with MCMV in association with any other virus that infects cereals in the family Potyviridae such as SCMV, MDMV or WSM (Niblett and Claflin, 1978; Uyemoto, Bockelman and Clafin, 1980, Family, 2012)). Viruses foundin other families, including Maize rayado fino virus (MRFV), family Tymoviridae, genus Marafivirus, Maize chlorotic dwarf virus (MCDV), family Secoviridae, genus Wakavirus and Maize mosaic virus (MMV), family Rhabdoviridae, genus Nucleorhabdovirus, can also result to interactions in association with MCMV. Non-living components such as arid conditions, poorly drained soils and high salinity also increases infection by MCMV to result to MLND (Redinbaugh and Zambrano, 2014).

2.5.1 History of MLN Disease

The virus MCMV is classified in the genus Machlomovirus in the family Tombusviridae. The virus was first discovered in maize in Peru (Castillo and Hebert, 1974) and later in the United States in Kansas associated with either MDMV or WSMV causing MLN disease (Niblett and Clafin, 1978; Uyemoto et al., 1980; Jiang, Wilkinson and Berry, 1990). It then moved to Nebraska (Doupnik, 1979, Fatma et al., 2016)). In USA, MCMV has not spread widely with reports in only three states of Kansas, Nebraska and in 1992 MCMV was reported in Hawaii (Jiang, Meinke, Wright, Wilkinson and Campbell, 1922). There are at about two strains of MCMV that are hereditary distinct and found in two different geographical locations, MCMV- P (Peru) and MCMV- K (Kansas) (Nyvall, 1999). In China it was discovered together with SCMV (Xie et al., 2011) and In East

Africa MCMV was first reported in Kenya in combination with SCMV (Wangai et al., 2012, Adams et al., 2017).

Table 2.1: Sequential Accounts of MCMV and Other Potyviruses.

Location	Year first reported	Potyvirus	Reference
Peru	1973	NR	Castilo & Herbert, 1974
United States, mainland	1976	WSMV/MDMV	Niblett & Claflin, 1978
Argentina	1982	NR	Teyssandier et al., 1981
Thailand	1982	NR	Klingkong &subabutra,1982
United States, Hawaii	1992	MDMV	Jiang et al., 1992
Colombia	1999	NR	Morales et al., 1999
China	2011	SCMV	Xie et al., 2011
Kenya	2012	SCMV	Wangai et al., 2012
Rwanda	2013	SCMV	Adams et al., 2014
DRC	2013	SCMV	Lukanda et al., 2014
Taiwan	2014	SCMV	Deng et al., 2014
Ethiopia	2015	SCMV	Mahuku et al., 2015

Potyviruses associated MCMV report: NR=not reported: DRC= Democratic Republic of Congo; (source: Mahuku et al., 2015).

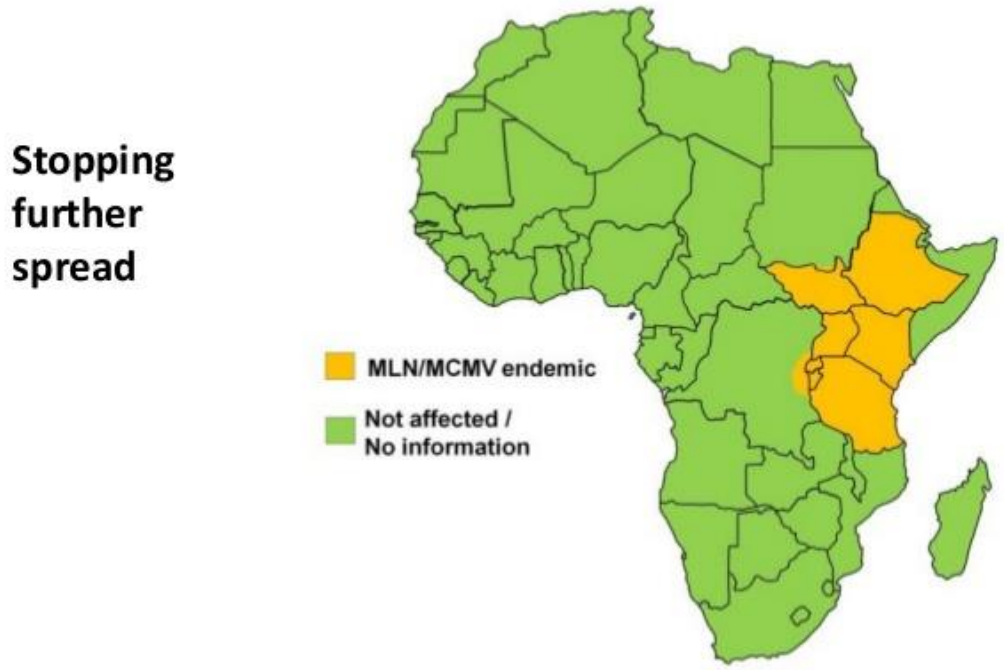
2.5.2 Occurrence and Frequency of Viruses Causing MLN in East Africa

In 2012-2014, a study to find out the spread of MCMV and SCMV was done on maize growing areas in East Africa. The survey was done on the basis of monitoring symptoms and diagnostic tests to assess if the virus is present in leaves using ELISA. (Jones et al., 2011). Occurrence of SCMV was lower as compared to MCMV incidence. In the western parts of Kenya, the incidence of MCMV increased in the year 2013-2014 which indicated rise in disease pressure. This was attributed to lack of skills in identifying MLND symptoms and lack of awareness about the disease by farmers. In Uganda, in the year 2013, symptomatic plants were collected and about 60% of the samples collected showed infection by MCMV, 23% were SCMV infected. Majority of the samples showed infection by both MCMV and SCMV with very few samples showing infection by Maize Streak Virus (MSV). In Tanzania (Arusha and Mwanza Districts), 60% of samples collected were infected with MCMV and 69% showed infection by SCMV, with majority of the plants collected having been virus infected.

In 2013-2014, a study was done in the main areas that grow maize in the country. Results indicate that out of the 2467 samples selected randomly, 60% of them were positive for MCMV and 28% were infected by SCMV and very few samples showing infection by SCMV only. In previous studies, MCMV was found in 40-80% of plants sampled in Kivu province of Democratic Republic of Congo (Lukanda et al., 2014).

Study findings show a wide spread of MCMV in East and Central Africa. Infestation prevalence was high in all those countries. The high occurrence of MCMV infection indicates that disease development can be caused by MCMV infection alone. It is also

possible that SCMV isolates that have not been detected, abiotic factors and other viruses makes the study of MLN in East Africa complicated. Surveys should be carried out in other Sub-Saharan Africa countries especially in areas where MLN/MCMV has not been suspected in order to be able to find out if MCMV is present or how far it has spread and to know other factors or disease causing organisms contribute to disease development. The information obtained will be very important in coming up with procedures on how to contain the disease and also in production of virus free germplasm.



(Source: FAOSTAT 2014).

Figure 2.1: Distribution of MLN in East Africa.

2.5.3 Causes of Maize Lethal Necrosis

MLN is as a result of a combined interaction of MCMV and any virus in the potyviridae group such as SCMV, MDMV and WSMV (Niblett and Claflin, 1978). In East Africa, although other members of the potyvirus were reported (Louie, 1980), MLN is said to be due to a combined infection of MCMV and SCMV (Wangai et al., 2012).

2.5.3.1Maize Chlorotic Mottle Virus

The virus is found in the Tombusviridae family and genus Machlomovirus. MCMV has 30 nm icosahedral virions enclosing a 4.4 kb an RNA genome (Scheets, 2004). The virus has six reading frames which overlap each other and are encoded by the viral genome, five of which are needed by the virus for division and movement within the plant. The virus is able to cause various symptoms which depend on: the stage of development the plant is at when the virus attacks it and the weather conditions at that time. Symptoms include: deformed and incompletely filled ears, severe mosaic and stunting, yellowing and necrosis, mild chlorotic mottling and premature death of the maize plant. In infections that occur naturally, yield loss of between 10-15% has been reported and up to 59% in maize plots inoculated artificially (Uyemoto et al., 1981).

The natural host of MCMV is the maize plant. Infection of other members of the Gramineae (Poaceae) with mechanical transmission has occurred. Recent studies have shown the presence of the virus in sugarcane (Wang et al., 2014) and sorghum (Huang et al., 2016). No dicotyledonous species has been found to be affected by the virus, including experiments with artificial transmission (Bockelman, Claflin and Uyemoto, 1982; Castillo & Hebert, 1974; Niblett and Claflin, 1978). The virus is spread by vectors which include chrysomelid beetles (Nault et al., 1978), corn thrips, corn root worm, and

corn flea beetle (Canabas et al., 2011). In East Africa, the commonly identified vector is maize thrips (*Frankliniella williamsi*) (Wangai et al., 2012; Mahuku et al., 2015).

2.5.3.2 Potyviruses

Potyviruses (genus Potyvirus; family Potyviridae) are RNA viruses disseminated by aphids with non-enveloped flexious virions having an average diameter of 10-17 nm and length of 720 to 850 nm. Potyviruses consists of 30% plant infecting viruses (Riechmann et al., 1992). Potyviruses are the most destructive virus group in crops (Shunkla et al., 1994, Revers and Garcia, 2015) causing diseases major economic importance worldwide (Kuntze, 1995; Shunkla et a.l, 1994; Mahuku et al., 2015). Maize infecting potyviruses include SCMV (Abbot & Tipet, 1966), MDMV (Williams and Alexander, 1965; Louie and Knoke, 1975), Johnson grass mosaic virus (JGMV), Sorghum mosaic virus (SrMV) (Shunkla, 1989) and Zea mosaic virus (ZeMV) (Seifers., et al, 2000). The related WSMV is classified in the genus Tritimovirus but for simplicity has been included with the potyviruses because of similarities in the resistance responses of maize to the virus.

Portyviruses were first identified in East Africa 1973 (Louie, 1980). SCMV, Maize streak virus (MSV) and Maize mosaic virus (MMV) were reported in samples collected from 28 districts in 8 surveyed provinces in Kenya (Louie, 1980). SCMV was reported in sugarcane, maize and sorghum. SCMV was found in 15.2% of sampled fields in Nyanza and 15.8% of sampled fields the Rift valley province and 19.6% in Western provinces of Kenya (Louie, 1980). These are areas where the initialcase of MLN was reported in Kenya by Wangai et al (2012).

2.5.3.3 Synergism

MCMV acts in synergism with any cereal potyvirus to cause a disease with more damaging impacts (Niblett and Claflin, 1978; Uyemoto, Bockelman and Claflin, 1980; Uyemoto et al., 1981). In Hawaii, MMV and MCMV were observed to cause CLN (Nelson et al., 2011). MLN is linked to either MCMV or SCMV in East Africa.

When a potyvirus is present in aplant, it builds up MCMV particles concentration 5-10 times (Goldberg and Brakke, 1987; Scheets 1998). This increase in concentration is termed unilateral synergism and describes a phenomenon where the presence of one virus increases the concentration of the co-infecting virus resulting to more severe symptoms than when an individual virus infects alone (Goldberg and Brakke, 1987; Scheets 1998). The observed rise in concentration of MCMV in a plant infected by both MCMV and SCMV compared to the plant infected by MCMV alone is hypothesized to be due to the ability of the potyvirus to suppress regulatory systems that would normally limit MCMV concentrations in a cell allowing easy transmission of the MCMV and increasing the symptom severity (Goldberg and Brakke, 1987).

2.5.4 Host Range of MCMV

MCMV was known to infect maize only (Scheets 2004), later it was found also in sugar cane (Wang et al., 2014) and finger millet (Eleusine coracana) (Kusia et al., 2015) . Dicots are not infected by the virus, but it has a variety of hosts that includes at least 19 grass species (Bockelman et al., 1982). An experiment was done to investigate non-maize species that could act as hosts of MCMV in East Africa. (Redinbaugh et al., 2014). Fourteen species of grass that commonly grows in maize plantations were inoculated with extracts from maize leaves infected with MCMV by leaf rubbing. The leaves that were

not inoculated were checked to see if the virus is present using ELISA and Reverse Transcriptase – PCR at 7-10 weeks after inoculation.

About twelve species tolerated MCMV, and did not show any characteristics of being infected by MCMV as shown by ELISA and RT-PCR: Bermuda grass (*Cynodon dactylon*), Napier grass (*Pennisetum purpureum*), Common wild oat (*Avena fatua*), Pearl millet (*Pennisetum glaucum*), Brome grass (*Bromus inermis*), Sand love grass (*Eragrostis trichodes*), Wheatgrass (*Agropyron repens*), barnyard grass (*Echinochloa crus-galli*), Smooth crabgrass (*Digitaria ischaemum*), Nut grass (*Cyperus esculentus*), wheat (*Triticum aestivum*.), and oat (*Avena* (*Avena fatua*), Pearl millet (*Pennisetum glaucum*), Brome grass (*Bromus inermis*), Sand love grass (*Eragrostis trichodes*), Wheatgrass (*Agropyron repens*), barnyard grass (*Echinochloa crus-galli*), Smooth crabgrass (*Digitaria ischaemum*), Nut grass (*Cyperus esculentus*), wheat (*Triticum aestivum*.), and oat (*Avena sativa*).

Sorghum (*Sorghum bicolor*) was discovered as a highly resistant host of MCMV.

Proso millet (*Panicummili aceum*) showed mild symptoms while finger millet and Foxtail millet (*Setaria italica*) exhibited strong symptoms after inoculation with the virus; MCMV was found in all three species by ELISA and RT-PCR. Symptomatic, the samples of finger millet, sorghum, sugarcane, Napier grass, Kikuyu grass (*Pennisetum clandestinium*) collected in Uganda and Kenya tested positive for MCMV by ELISA.

These results show the possibility of MCMV could have a vast range of potential alternative hosts and which agrees with earlier reports by Bockelman and co-workers (1982). However, maize itself could be a habitat for MLN causing viruses since it is

planted all year round in most areas of eastern Africa. In addition, grasses should be analyzed further; in particular those that act as host of MCMV in experiments and those growing in and around maize fields needs to be done. Insect vectors also have to be analyzed to establish their ability to transmit MCMV from alternative hosts to maize.

2.5.5 Dissemination of MLN Viruses

MLN viruses affect maize plants at any stage; either as a seed or when mature. The MLN virus, the vector (usually insect vectors), and the host plant must combine in a suitable environment for infection to take place (Redinbaugh & Zambrano, 2014).

MCMV transmission can be mechanical through insect vectors such as maize thrips, maize rootworms, leaf beetles and leaf hoppers (Nault et al., 1978). The beetles are found mainly in the family Chrysomelidae include: the cereal leaf beetle (*Oulema melanopa*), the corn flea beetle (*Chaetocnema pulicaria*), the flea beetle (*Systena frontalis*), the Japanese beetle (*Popillia japonica*) (Nault et al., 1978).

Corn thrips species (*Frankliniella williamsi*) was found to spread MCMV in a semi-persistent manner (Cabanas, Watanabe, Higashi, & Bressan, 2013). The insects pass MCMV after acquiring them for three hours; latent period is not evident with both young and adult stages having the ability to pass the virus for about 6 days post acquiring them (Cabanas et al., 2013). The vectors responsible for MCMV in Africa still unknown, although thrips have been observed in all maize plantations, including farms infested with MCMV and MLN. It is possible that thrips and other vectors could be involved in MCMV spread within and between fields in the affected countries in Africa. Reports of corn thrips were first mentioned in East Africa in 2009 (Moritz, Brandt, Triapitsyn and

Subramanian, 2013), and survey that followed found them in various locations in Kenya, Uganda and DRC. Corn thrips has also been seen on many other plants such as baby corn, rice, sorghum and wheat, and were also regularly noted on onions (Moritz et al., 2013).

Dissemination of MCMV by seed is also possible although it occurs at very low rates (Jensen, Wysong, Ball & Higley, 1991). However, even at low rate, seed transmission can still cause disease because maize is only grown by means of seed and it leads to transfer of virus into new areas through seed (Mahuku et al., 2014). Soil and plant debris also is involved in the spread of the virus as the virus has the ability to live in plant debris (Nyvall, 1999). Continuous maize cultivation leads to an increase rate of the virus and vectors. SCMV is mainly transmitted by aphid vectors.

2.5.6 MLN Disease Symptoms

When MCMV infects a maize plant, it develops various symptoms depending on factors such as prevailing environmental conditions, plant age at infection time, variety of the plant, part of the plant infected and number of viruses that have infected the plant. (Wangai et al., 2012). Common symptoms range from chlorotic mottling of young leaves and extend towards the leaf tips, necrosis of leaf margins that extends to the midrib and eventually drying of the entire leaf, dead heart symptoms, dwarfing, pre-mature aging of plants and death of the plant (Uyemoto et al., 1980, 1981, Wangai et al., 2012).

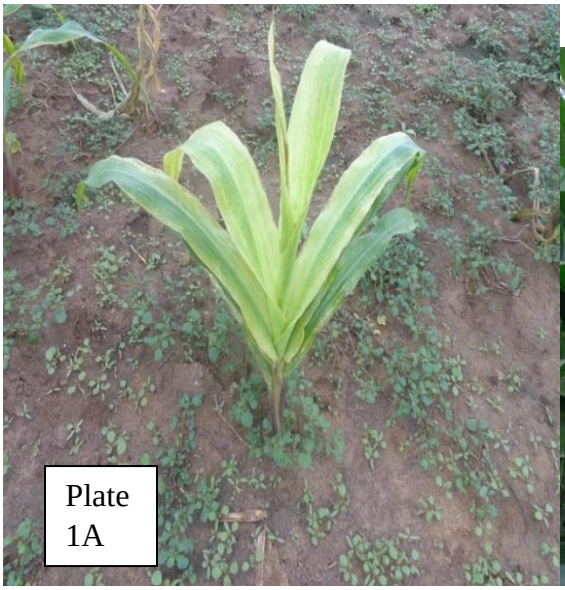


Plate 1A

Plate 1B

Plate IC

Plate 1 D

Plate 2.1: MLN Disease Symptoms: (Plate 1A) Chlorosis on Leaves, (Plate 1B) mottling and Necrosis of Leaves (Plate 1C) Necrosis Leading To Plant Death, (Plate 1D) Small Cobs that have Minimal or No Grains. (Author, 2015)

2.5.7 Disease Management for MLN

To be able to control MLND, manage efforts should be directed towards the vector, virus and a host that is not tolerant which must combine together in a suitable environment for disease to occur. The most suitable way to control MLN is to use a combined approach by integrating cultural practices together with vector control using chemical or biological control and use of varieties that are resistant to the disease. MCMV has been controlled in Hawaii by use of cultural practices with chemical control and use of species tolerant to the disease (Nelson, Brewbaker and Hu, 2011). Cultural practices used include rotation of maze for a period of about two seasons, with crops like potatoes, cassava, beans, onions and garlic. Crop rotation was found useful in parts of U.S.A in minimizing the rates of occurrence of MCMV (Phillips et al., 1982). Using seed that is certified control of weeds and destroying of infected material from the field also controls disease causing and vector populations (Fatma et al., 2016). Growing of maize is required at the start of the main rainy season, with use of fertilizer to increase vigor of the plant.

To be able to come up with commercial seed that is free from virus, cultural practices and control of vectors is necessary. However this method is not used by many small scale maize farmers in many parts of eastern Africa, where regular planting of maize is a common norm, and farmers don’t have knowledge and resources necessary for vector control and cultural management practices (Mahuku et al., 2015).

A sustainable method for MLND control is development of crops that are resistant to the virus then transfer of required genes into plants with stable and best agronomical characteristics (Nelson et al., 2011).

2.5.8 Breeding for MLN Disease Resistance

Efforts to come up with varieties that are resistant to MLN have been going on. During the old times, breeding used to involve mainly selection and backcross methods so as to pass resistant genes to germplasm in Africa (Storey and Howland, 1967). New techniques such as marker assisted selection are now being used in plant breeding to develop resistance without having an effect on yield (Pixley et al., 2006).

In Kenya, there are central screening stations where several lines are being screened for their resistance to or tolerance to MLND. Unfortunately, many temperate inbred and hybrid lines are showing high levels of susceptibility to the viruses (Wangai et al., 2012). Further reports by CYMMIT under 'CIMMYT Global Maize Program' on 2013 screening indicated that 122 of the screened 124 varieties had extremely high levels of susceptibility with additional screening of 62000 lines showing up to 90 % susceptibility (CYMMIT, 2018).As of 2017, only nine highly tolerant varieties namely Bazooka (UH5354), H12ML, H13ML, Meru HB607, WE5135, WE5136, WE5138, WE5139 and WE5140had been identified (CYMMIT, 2018; Makumbi and Wangai, 2012)

2.5.9 Combining Ability

The concept on better performance of parental lines is very important when coming up with varieties that can be grown in various climatic zones. The exploitation of combining abilities of parental germplasm shows what gene actions is involved and helps in the selection of parental lines showing high general combining ability and a high specific

combining ability for the hybrids. General combining ability (GCA) is how a genotype performs in a series of other hybrids while specific combining ability (SCA) refers to the better performance of a selected hybrid compared to other hybrids that come up as result of crossing different genotypes (Sprague and Tatum, 1942). GCA variance linked mainly with the additive part while SCA variance is related to the non-additive effects of genes which are mainly dominance and epistatic deviations (Hallauer and Miranda, 1988). The importance of GCA variance, additive variation indicates trait selection will be possible. Deviations of General Combining Ability can be positive or negative (Kearsey and Pooni, 1996). A deviation that is positive can either be favorable or unfavorable, but this depends on the character that is being selected. For example, positive GCA for yield is desirable as this shows high yielding variety. On the contrast, positive high values on any disease ratings would not be desirable. Negative GCA values on the anthesis date are required for selection of varieties that mature early. In addition, GCA and SCA effects stability is important in coming up with parental lines and subsequent hybrids that are stable in different environments (Dehghanpour and Ehdaie, 2013).

The effects of GCA and SCA have been identified for various agronomic characters in maize such as grain yield and hence the gene action that controls majority of the complex traits cannot be generalized. The effect of additive effects has been seen to determine resistance to several maize diseases including ear rots, NLB, GLS, PLS, common rust, head smut and MSV (Vivek et al., 2010). However, the significance of non-additive effects cannot be ignored since in cases where two parents show resistance that is average, they could pass the above average resistance when crossed with each other (Vivek et al., 2010).

CHAPTER THREE

THE RESPONSE OF SINGLE CROSSES TO INOCULATION WITH MAIZE LETHAL NECROSIS VIRUS.

Abstract

Majority of the small scale farmers in Kenya depend on subsistence farming with maize being the most important food and cash crop. A study was done in Naivasha and Bomet to screen for single crosses that are resistant to MLN grown under high disease pressure. Data collection was based on grain yield, disease severity and plant stand count. MLN disease intensity and MLN disease occurrence was based on monitoring of symptoms and carrying out diagnostic tests. Data on disease incidence and severity was recorded at 3 weeks interval after planting until the grain was fully filled in the cobs. GENSTAT (5^{th} edition) was used in data analysis. There was a significant difference on disease incidence and severity in the two areas studied. Infection was observed on all the genotypes studied in both locations but at varying levels. Naivasha showed highest disease occurrence at approximately above 40% while Bomet had the least occurrence. In Naivasha, the top performing line was SC-MLN-15-56 with a score of 2.3 while the most susceptible lines were: SC-MLN-15-1, SC-MLN-15-77, SC-MLN-15-94 and SC-MLN-15-101. In Bomet, the top performing line was SC-MLN-15-3 while the most susceptible genotypes were: SC-MLN-15-1, SC-MLN-15-35 and SC-MLN-15-59. MLN is still a major problem facing the maize growing sector in Kenya and many parts of Africa. Many of the commercial varieties are susceptible to MLND. More efforts have to be put in come up with varieties that are resistant to the disease and incorporate farmer desired

characteristics. The study will contribute useful germplasm that could be suitable for breeding for resistance to MLND.

3.1 Introduction

Maize is a significant crop planted for subsistence in Kenya although it is now used as a source of fodder (Murdoch et al., 2013). As a result, large amounts are needed to feed the population that is increasing daily and also as food for livestock. There little increase in production level nationwide, but that is because of expansion of areas used for maize cultivation some of which are not suitable for agricultural production (Olwade & Smale 2012). Production countrywide however is still low at 1-2t/ ha against a potential of 6t/ha (Jaetzold et al., 2006). This is due to poor soils, harsh weather conditions, pest and diseases (MOA, 2013). Depletion of the ozone layers and greenhouse gases has altered climatic trends leading to poor distribution of precipitation leading to arid conditions. While most of these are general challenges, the aim of this study was to understand maize lethal necrosis disease as an important maize production constraint in Naivasha and Bomet counties.

MLN is causes serious effects to maize many countries in the region including Kenya. It was first reported in September 2011 in Longisa Bomet County. By 2012, characteristics of disease similar to MLN were observed in a number of areas in central, Nyanza and Rift valley regions of Kenya (Wangai et al., 2012). MLN is still a common problem facing areas that produce maize in the country (Magenya et al., 2009) leading to low yield (Murdoch et al., 2003). Although the problem being researched extensively, commercial varieties are still affected by the disease.

Weather changes and high temperature favors and allows increase in number of insects that transmit MLN. This creates a challenge to scientists grappling with the disease (Wangai et al., 2012). The outbreak of MLN is a serious hindrance to food security in the country. To effectively manage MLN disease, there is need to identify MLN resistant varieties. This study was carried done to screen for MLN resistant maize varieties grown under high disease pressure in Naivasha and Bomet counties.

3.2 Materials and Methods

3.2.1 Experimental Site

There were two experimental sites. One under artificial inoculation at Naivasha {Latitude: 0°43.0002′ S Longitude: 36°26.1546′ E, 1915m above sea level(asl) }and one under natural inoculation at Bomet[latitude 01°05′S, longitude 35°52′E, 1827 m above sea level (asl)].

3.2.2 Materials and Experimental Design

The trials comprised of 120 single cross hybrids set up in an incomplete block design in the two sites. The single crosses were formed using tolerant and susceptible inbred lines sources from CIMMYT AND KALRO. The trial in Naivasha was artificially inoculated twice as in indicated in 3.2.4 below.

3.2.3 Collection and Maintenance of Virus Isolates

The presence of SCMV and MCMV in the leaf samples was confirmed using ELISA and then transferred to H614 which is a susceptible hybrid. In different green houses, the leaf samples which were infected were collected , chopped, weighed and grinded using a blender in cold 0.1M potassium phosphate at buffer pH 7.0(ratio 1:10). The extract was then passed through a cheese cloth to remove any debris. The extract from the two

viruses was then mixed and carborandum was added to decanted sap extract at the rate of 0.7 g/ l of Inoculum and stirred to ensure even distribution of carborandum. The susceptible plants were inoculated at 3-4 leaf stage in the green house by applying sap onto the leaves with fingers. For Inoculum production, two separate sealed green houses for SCMV and MCMV were maintained. ELISA was then conducted 3 weeks pre-inoculation on leaf samples collected randomly from the different green houses to confirm purity of the Inoculum.

3.2.4 Artificial Field Inoculation

For even disease pressure in the fields the mixture of MLN viruses were mixed at a ratio of 4:1 and inoculated at 5th - 6th week post planting using a motorized pump (Solo 423 Mist Blower, 12 L capacity). The Inoculum spray was delivered at a rate of 120L/Ha using an open nozzle (2-inche diameter). Symptoms appeared 10 days after inoculation and ELISA was done to confirm presence of MLN viruses in the field trials.

3.3 Data Recording

3.3.1 Disease Severity

Data was collected on plant stand count and grain yield, Data collection on disease severity was based on symptom observation in the susceptible control and rated as described by (Gowda et al., 2015); 1- non-symptomatic leaves, 2- mild symptoms on 20-40% leaf area, 3= moderate symptoms on 40-60% leaf area, 5= severe symptoms on 75% or more leaf area, plants severely stunted, drying/dead. Resistance was classified as follows;

1.0 –Symptomless, immune

1.2- 1.4 – Highly resistant

1.5-2.4– Resistant

2.5- 2.9– Moderately resistant

3.0- 5.0– Susceptible

Occurrence of MLN was based on symptoms of disease and diagnostic tests carried out to identify the disease and the respective data recorded three weeks after planting and after every three weeks until the grains were fully formed in the maize cobs.

3.3.2 Disease Rating System

The disease rating system was visual and started two weeks post inoculation. It was conducted after every 14 days until 42 days post inoculation. Disease score was given on row basis. A minimum of 3 ratings were collected.

3.4 Data Analysis

The data was analyzed using GENSTAT (15^{th} edition). Data severity was evaluated using a scale of 1 to 5 is used by KALRO/CIMMYT where 1=no symptoms observed, 2= fine Chlorotic streaks on upper leaves, 3= Chlorotic mottling throughout the plant, 4= excessive Chlorotic mottling and dead heart and 5= complete plant necrosis. The average severity per treatment combination was determined. All data was subjected to Analysis of Variance using GENSTAT statistical package to determine the effects of the different treatments.

3.5 Results

3.5.1 Identification of MLN Resistant Single Cross Varieties Grown Under High Disease Pressure

In each site of the study there were two reps and each rep had 120 plants. Generally, the mean value for MLND severity in Bomet county was 3.077(0.336) and in Naivasha was 3.533(0.325), Table 1. The statistical results suggested that maize varieties in Naivasha were far much affected by Maize lethal necrosis as compared to Maize plants varieties in Bomet, when analysis of variance (ANOVA) was exploited during the analysis at 5% significance level, P-value≤0.05 Table 3.1. The single crosses showed different severity levels across the two sites. Some maize plants were seriously affected by MLN disease while others were moderately affected. Therefore, variability of the infection of Maize lethal necrosis disease across the two sites was probably due to different climatic changes, especially temperatures and other abiotic factors that may have favored Maize lethal necrosis infection in the different maize varieties.

Table 3.1: Summary Statistics of Disease Score for Maize Lethal Necrosis In Each Site

Site	Mean	SD	Sample (n)	P-value
Bomet	3.0769	0.336	240	
Naivasha	3.533	0.325	240	0.05

From descriptive analysis, the results showed that 1(0.42%) maize plant had slightly been affected, 160(67.51%) Maize plants had moderate infection and 76(32.07%) maize plants had severe infection from Maize lethal necrosis in Bomet while in Naivasha 33(13.87%)

maize plants experienced moderate impact and 205(86.13%) maize plants had severe infection from Maize lethal necrosis disease, figure 3.2.

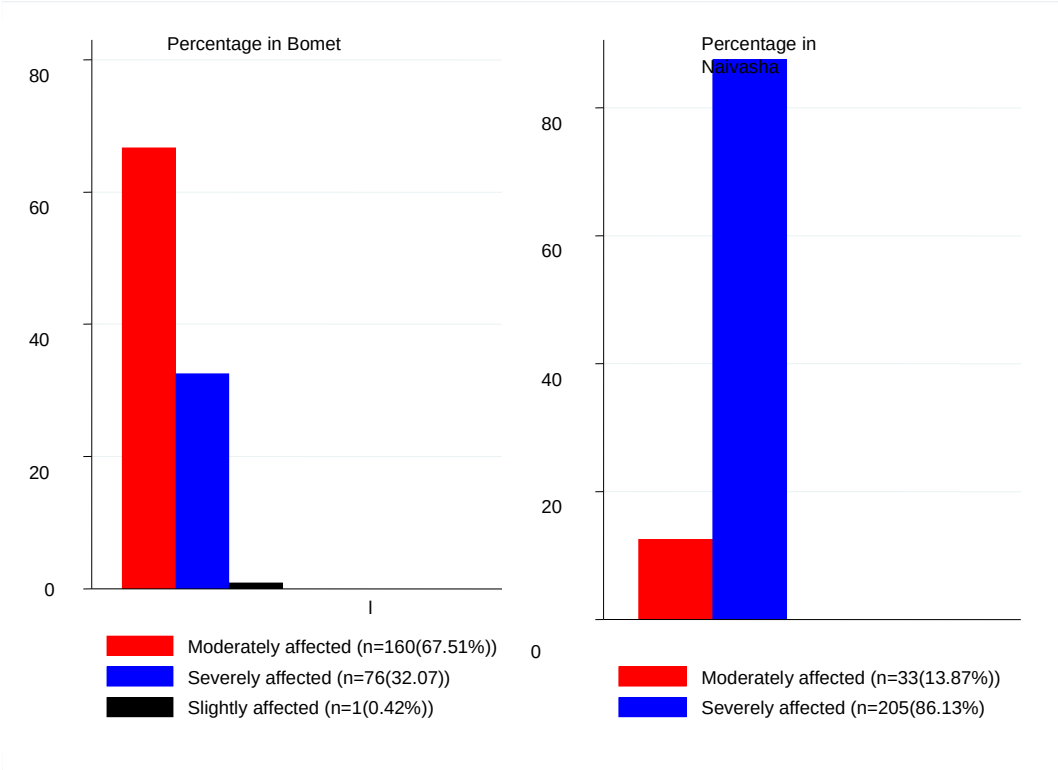


Figure 3.1: Distribution of Maize Lethal Necrosis Disease in Maize Varieties by Phenotypic Ranking

For the two sites, the results presented shows that there was no statistical significant difference in their means values at 95% confidence interval, P-value=0.896, Table3.2.

Table3.2: Summary Statistics for Disease Score 0f Maize Lethal Necrosis Disease in the Two Sites.

Site	Mean	Sd	Sample	P-value
Bomet	3.077	0.337	240	
Naivasha	3.533	0.325	240	0.896

Table 3.3: Means of MLN Scores and Yield of 120 Inbreds Screened In 2015 in Naivasha

GENOTYPE	DISEASE SCORE(NAIVASHA)	DISEASE REACTION	YIELD (t/ha)
SC-MLN-15-1	4	S	0.63
SC-MLN-15-2	3.5	S	1.07
SC-MLN-15-3	3.3	S	1.41
SC-MLN-15-4	3.5	S	0.71
SC-MLN-15-5	3.8	S	0.5
SC-MLN-15-6	3	S	1.02
SC-MLN-15-7	3.8	S	1.33
SC-MLN-15-8	3	S	1
SC-MLN-15-9	3.8	S	0.53
SC-MLN-15-10	3.8	S	1.19
SC-MLN-15-11	3.8	S	0.65
SC-MLN-15-12	3.8	S	0.73
SC-MLN-15-13	3.8	S	0.5
SC-MLN-15-14	3.8	S	0.79
SC-MLN-15-15	3	S	1.54
SC-MLN-15-16	3.3	S	0.95
SC-MLN-15-17	3.6	S	1.14
SC-MLN-15-18	3.8	S	0.59
SC-MLN-15-19	3.3	S	1.11
SC-MLN-15-20	3.3	S	1.26
SC-MLN-15-21	3.5	S	0.47
SC-MLN-15-22	4	S	0.58

SC-MLN-15-23	3.8	S	0.57
SC-MLN-15-24	3.8	S	0.52
SC-MLN-15-25	3.5	S	0.46
SC-MLN-15-26	3	S	1.12
SC-MLN-15-27	3.5	S	0.61
SC-MLN-15-28	3.8	S	0.72
SC-MLN-15-29	3.8	S	0.77
SC-MLN-15-30	3.5	S	0.87
SC-MLN-15-31	3.5	S	0.36
SC-MLN-15-32	3.8	S	0.72
SC-MLN-15-33	3.5	S	0.27
SC-MLN-15-34	3.8	S	0.49
SC-MLN-15-35	3.5	S	0.62
SC-MLN-15-36	3.8	S	0.89
SC-MLN-15-37	3.3	S	1.28
SC-MLN-15-38	3.5	S	0.59
SC-MLN-15-39	3.8	S	0.6
SC-MLN-15-40	3.5	S	0.73
SC-MLN-15-41	3.8	S	0.92
SC-MLN-15-42	3.5	S	0.87
SC-MLN-15-43	3.5	S	0.62
SC-MLN-15-44	3.8	S	0.78
SC-MLN-15-45	3.5	S	0.72
SC-MLN-15-46	3.5	S	0.76
SC-MLN-15-47	3.5	S	0.77
SC-MLN-15-48	3.5	S	0.91
SC-MLN-15-49	3.3	S	0.84
SC-MLN-15-50	3.8	S	0.64
SC-MLN-15-51	3.8	S	0.71
SC-MLN-15-52	3.5	S	0.71
SC-MLN-15-53	3.8	S	0.49
SC-MLN-15-54	3.8	S	0.82
SC-MLN-15-55	3.5	S	0.95
SC-MLN-15-56	2.5	MR	0.72
SC-MLN-15-57	3.5	S	0.98
SC-MLN-15-58	3.3	S	0.84
SC-MLN-15-59	3.5	S	0.94
SC-MLN-15-60	3.8	S	0.46
SC-MLN-15-61	3.8	S	0.37
SC-MLN-15-62	3.5	S	0.45
SC-MLN-15-63	3.5	S	0.43
SC-MLN-15-64	3.5	S	0.57
SC-MLN-15-65	3	S	0.78

SC-MLN-15-66	3.5	S	0.67
SC-MLN-15-67	3.5	S	0.95
SC-MLN-15-68	3.5	S	0.79
SC-MLN-15-69	3	S	1.14
SC-MLN-15-70	3.5	S	1.05
SC-MLN-15-71	3.8	S	0.42
SC-MLN-15-72	3.3	S	1.18
SC-MLN-15-73	3.3	S	0.68
SC-MLN-15-74	3.5	S	0.69
SC-MLN-15-75	3.8	S	0.75
SC-MLN-15-76	3.8	S	0.48
SC-MLN-15-77	4	S	0.22
SC-MLN-15-78	3.5	S	0.62
SC-MLN-15-79	3.5	S	0.67
SC-MLN-15-80	3.5	S	0.89
SC-MLN-15-81	3	S	1.59
SC-MLN-15-82	3.5	S	1.01
SC-MLN-15-83	3.5	S	1.04
SC-MLN-15-84	3.8	S	0.8
SC-MLN-15-85	3.5	S	0.9
SC-MLN-15-86	3.8	S	0.4
SC-MLN-15-87	3.8	S	0.74
SC-MLN-15-88	3.8	S	0.91
SC-MLN-15-89	3.5	S	0.31
SC-MLN-15-90	3.5	S	0.82
SC-MLN-15-91	3.8	S	0.65
SC-MLN-15-92	3.3	S	0.91
SC-MLN-15-93	3.8	S	0.59
SC-MLN-15-94	4	S	0.4
SC-MLN-15-95	3.8	S	0.33
SC-MLN-15-96	3.3	S	0.82
SC-MLN-15-97	3.5	S	0.97
SC-MLN-15-98	3.5	S	1.07
SC-MLN-15-99	3.5	S	0.49
SC-MLN-15-100	3.5	S	0.89
SC-MLN-15-101	4	S	0.39
SC-MLN-15-102	3.5	S	1.45
SC-MLN-15-103	3.3	S	0.64
SC-MLN-15-104	3.5	S	0.75
SC-MLN-15-105	3.8	S	0.88
SC-MLN-15-106	3.5	S	0.97
SC-MLN-15-107	3.3	S	1.12
SC-MLN-15-108	3.5	S	0.4

SC-MLN-15-109	3.8	S	0.45
SC-MLN-15-110	3.8	S	0.52
SC-MLN-15-111	3.3	S	0.98
SC-MLN-15-112	3.5	S	0.81
SC-MLN-15-113	3.5	S	0.71
SC-MLN-15-114	3.8	S	0.59
SC-MLN-15-115	3.5	S	0.39
SC-MLN-15-116	3.5	S	0.74
SC-MLN-15-117	3.5	S	0.61
SC-MLN-15-118	3.5	S	0.88
SC-MLN-15-119	3.5	S	0.78
SC-MLN-15-120	3.5	S	0.64
Mean	**3.6**		**0.76**
CV	**0.064**		**0.074**

Naivasha site

MLND severity was high in Naivasha and this can be attributed to artificial inoculation done in this site. The mean MLN disease score in Naivasha was 3.5. The mean scores were variable and significantly different at $p \leq 0.05$. The disease score ranged from 2.5 -4. SC-MLN-15-56 had the least disease score of 2.5. SC-MLN-15-1, SC-MLN-15-22, SC-MLN-15-77, SC-MLN-15-94 and SC-MLN-15-101 were severely affected with a mean score of 4.

Naivasha site had a mean yield of 0.76t/ha. The highest yielding crosses were SC-MLN-15-81(1.59t/ha) and SC-MLN-15-15(1.54t/ha). The lowest yielding cross was SCMLN-15-77(0.22t/ha).

Table 3.4: Means of MLN Disease Score and Yields of 120 Inbreds Screened In 2015 in Bomet

Genotype	Disease score	**Disease reaction**	**Yield**
SC-MLN-15-1	3.7	S	0.74
SC-MLN-15-2	3	S	1.49
SC-MLN-15-3	2.3	S	1.38
SC-MLN-15-4	3.2	S	0.8
SC-MLN-15-5	2.7	S	1.23
SC-MLN-15-6	3	S	1.58
SC-MLN-15-7	3.1	S	1.34
SC-MLN-15-8	2.9	S	2.64
SC-MLN-15-9	3.1	S	2.26
SC-MLN-15-10	3	S	2.04
SC-MLN-15-11	3.5	S	3.41
SC-MLN-15-12	3.1	S	1.75
SC-MLN-15-13	3.1	S	2.77
SC-MLN-15-14	2.8	S	2.14
SC-MLN-15-15	2.9	S	1.68
SC-MLN-15-16	3	S	1.21
SC-MLN-15-17	3.1	S	0.81
SC-MLN-15-18	2.9	S	1.19
SC-MLN-15-19	3	S	1.13
SC-MLN-15-20	3.4	S	1.16
SC-MLN-15-21	2.8	S	1.01
SC-MLN-15-22	3.1	S	0.4
SC-MLN-15-23	2.5	S	0.48
SC-MLN-15-24	2.9	S	0.15
SC-MLN-15-25	2.9	S	0.63
SC-MLN-15-26	3.5	S	1.61
SC-MLN-15-27	3.4	S	1.43
SC-MLN-15-28	3.2	S	2.45
SC-MLN-15-29	2.7	S	2.42
SC-MLN-15-30	3.3	S	3.53
SC-MLN-15-31	3.8	S	1.44
SC-MLN-15-32	3.3	S	2.22
SC-MLN-15-33	3	S	2.17
SC-MLN-15-34	3	S	1.11

SC-MLN-15-35	3.7	S	1.79
SC-MLN-15-36	2.9	S	1.68
SC-MLN-15-37	2.9	S	1.99
SC-MLN-15-38	3.1	S	1.46
SC-MLN-15-39	3.3	S	0.77
SC-MLN-15-40	3.3	S	1.89
SC-MLN-15-41	2.9	S	1.62
SC-MLN-15-42	3.3	S	1.2
SC-MLN-15-43	2.8	S	0.89
SC-MLN-15-44	3.3	S	1.85
SC-MLN-15-45	3	S	2.93
SC-MLN-15-46	2.8	S	1.74
SC-MLN-15-47	3.2	S	1.73
SC-MLN-15-48	3	S	2.16
SC-MLN-15-49	2.8	S	1.42
SC-MLN-15-50	3.5	S	0.98
SC-MLN-15-51	3.2	S	1.64
SC-MLN-15-52	3	S	2.07
SC-MLN-15-53	2.8	S	1.81
SC-MLN-15-54	2.8	S	1.7
SC-MLN-15-55	2.9	S	0.89
SC-MLN-15-56	3.2	S	1.5
SC-MLN-15-57	3.3	S	1.63
SC-MLN-15-58	3.1	S	1.46
SC-MLN-15-59	3.7	S	0
SC-MLN-15-60	3	S	0
SC-MLN-15-61	2.8	S	2.22
SC-MLN-15-62	2.9	S	3.76
SC-MLN-15-63	2.7	S	3.08
SC-MLN-15-64	3	S	2.41
SC-MLN-15-65	3.4	S	1.66
SC-MLN-15-66	3.2	S	3.37
SC-MLN-15-67	2.8	S	2.66
SC-MLN-15-68	2.9	S	3.07
SC-MLN-15-69	2.9	S	1.83
SC-MLN-15-70	3.3	S	3.26
SC-MLN-15-71	3	S	2.84
SC-MLN-15-72	3.5	S	1.55
SC-MLN-15-73	3	S	1.29
SC-MLN-15-74	2.9	S	3.24
SC-MLN-15-75	3.2	S	2.08

SC-MLN-15-76	3.5	S	1.24
SC-MLN-15-77	3.2	S	2.83
SC-MLN-15-78	3.1	S	2.22
SC-MLN-15-79	3.2	S	1.77
SC-MLN-15-80	2.9	S	3.21
SC-MLN-15-81	2.9	S	2.08
SC-MLN-15-82	3	S	2.24
SC-MLN-15-83	3.3	S	2.29
SC-MLN-15-84	3.5	S	2.56
SC-MLN-15-85	2.9	S	2.18
SC-MLN-15-86	3.5	S	2.41
SC-MLN-15-87	3.4	S	3.03
SC-MLN-15-88	3	S	2.17
SC-MLN-15-89	2.9	S	2.17
SC-MLN-15-90	3	S	0.93
SC-MLN-15-91	3.3	S	2.28
SC-MLN-15-92	3.2	S	2.67
SC-MLN-15-93	3.4	S	0.75
SC-MLN-15-94	2.9	S	1.43
SC-MLN-15-95	3.2	S	1.88
SC-MLN-15-96	2.9	S	1.75
SC-MLN-15-97	3.3	S	1.37
SC-MLN-15-98	2.9	S	1.46
SC-MLN-15-99	2.8	S	2.37
SC-MLN-15-100	2.9	S	2.48
SC-MLN-15-101	3.3	S	2.83
SC-MLN-15-102	2.9	S	2.35
SC-MLN-15-103	3	S	1.36
SC-MLN-15-104	3.3	S	1.71
SC-MLN-15-105	3.3	S	1.44
SC-MLN-15-106	2.7	S	1.39
SC-MLN-15-107	3	S	1.27
SC-MLN-15-108	3.2	S	1.16
SC-MLN-15-109	3.3	S	2.19
SC-MLN-15-110	3	S	1.66
SC-MLN-15-111	3.1	S	1.3
SC-MLN-15-112 (check 1)	2.9	S	1.12
SC-MLN-15-113 (check 2)	3.1	S	1.61

SC-MLN-15-114 (check 3)	3.1	S	1.44
SC-MLN-15-115(check4)	3.4	S	1.03
SC-MLN-15-116(check5)	3.2	S	0.95
SC-MLN-15-117(check6)	3.1	S	1.64
SC-MLN-15-118(check7)	2.9	S	1.08
SC-MLN-15-119 (check 8)	3.3	S	0.94
SC-MLN-15-120(check9)	3.2	S	0.94
Mean	3.1		1.77
Cv	0.064		0.59

Bomet site

In Bomet infection was also observed in all genotypes but at varying levels. Single crosses with least disease scores include: SC-MLN-15-3(2.3) and SC-MLN-15-23(2.5). The most affected genotypes were: SC-MLN-15-1(3.7), SC-MLN-15-31(3.8) and SC-MLN-15-35(3.7). The mean yield in Bomet was 1.77t/ha. The highest yielding crosses include: SC-MLN-15-30(3.53t/ha), SC-MLN-15-11(3.42t/ha) and SC-MLN-15-66(3.37t/ha). The most affected were SC-MLN-15-60 and SC-MLN-15-61 and did not have any yield at all.

Discussion

The study was carried out to screen genotypes for resistance to MLN disease. The disease was distributed in all counties surveyed and was not limited to a particular region. All genotypes studied were also infected with MLND although at varying levels. Variation in response to MLN was observed in the single crosses which show existing and suitable

germplasm for improving MLND susceptible varieties. Earlier studies had identified MLND as an important disease across the country affecting all the major maize growing areas in the region (Wangai et al., 2012). The disease incidence was high in both locations. This may be an indication that despite the efforts being put by researchers to manage MLND, true resistance has not been found. However severity for MLND was high in Naivasha compared to Bomet and this is because of artificial inoculation that was done in Naivasha.

In Naivasha site there were 240 plants. The mean value for MLN disease score in Naivasha was 3.5. When Analysis of variance was exploited it showed that average squares for genotypes were highly significant. The scores for disease severity for the single crosses were significantly different at $p \leq 0.05$ in Naivasha. The average disease severity scores of the single crosses ranged from 2.5-4(table). The single cross with the least disease score was SC-MLN-15-56 with a disease score of 2.5. SC-MLN-15-1, SC-MLN-15-22, SC-MLN-15-77, SC-MLN-15-94 and SC-MLN-15-101 were severely affected with disease scores 0f 4. Majority of the single crosses in Naivasha were susceptible with scores ranging from 3- 5.

The highest yielding crosses in Naivasha were SC-MLN-15-81 and SC-MLN-15-15 with yields of 1.59t/ha and 1.54 t/ha respectively. SCMLN-15-56 which had the least disease score ha a yield of 0.72t/ha. The single crosses with the highest disease scores; SC-MLN-15-1, SC-MLN-15-22, SC-MLN-15-77, SC-MLN-15-94 and SC-MLN-15-101 had yields of 0.63t/ha, 0.58t/ha, 0.22t/ha, 0.40t/ha and 0.39t/ha respectively.

The lowest yielding single cross in Naivasha was SC-MLN-15-77 with a yield of 0.22t/ha.

In Bomet there were240 single crosses and infection was observed on all genotypes studied but at varying levels.10% of the single crosses in Bomet were slightly affected by the disease, 67% were moderately affected while 32% were severely affected. There was significant genetic variation in the single crosses at $p \leq 0.05$.

The single cross with the least severity score in Bomet was SC-MLN-15-3 with a disease score of 2.3 and SC-MLN-15-23 with a disease score of 2.5. The most severely affected genotypes include: SC-MLN-15-1(3.7), SC-MLN-15-11(3.5), SC-MLN-15-31(3.8), SC-MLN-15-35(3.7), SC-MLN-15-50(3.5), SC-MLN-15-72(3.5) and SC-MLN-15-86(3.5).

The mean yield (t/ha) in Bomet was 1.77(t/ha). The highest yielding crosses in Bomet were SC-MLN-15-11, SC-MLN-15-30 and SC-MLN-15-66 with yields of 3.41t/ha, 3.53t/ha and 3.37t/ha.

SC-MLN-14-60 and SC-MLN-15-61 dis not have any yield.

Other single crosses with low yields include SC-MLN-15-24 and SC-MLN-15-22 with yields of 0.15t/ha and 0.40t/ha

Disease score and maize yield were negatively correlated. The absence correlation between grain yield and MLN disease resistance is important because it indicates both of these characteristics can be improved together. Other studies using different germplasm under MLND pressure reported similar results. (Betran et al., 2003)

CHAPTER FOUR

COMBINING ABILITY OF SINGLE CROSSES

Abstract

The use of diallel crosses to establish high yielding combinations is a usual practice in maize (*Zea mays L*) breeding programs. The type of gene involves and genetic characteristics for tolerance to disease is a crucial trait in coming up with tolerant varieties. This gives a method of controlling disease that is friendly to the environment, cheap and sustainable. The objective of this study was to identify the performance of 16 maize inbred lines derived from CIMMYT and KALRO as to their general (GCA) and specific (SCA) combining abilities using a complete diallel scheme with the aim of coming up with tolerant genotypes against Maize Lethal Necrosis Disease in MLN hot spot areas in Bomet and Naivasha in 2015 cropping season. The experimental material included 120 single crosses 16 parental lines and two four local grown varieties as checks. The experiment was set in an incomplete block design with two replications in each site. The general combining ability (GCA) and specific combining ability (SCA) effects of genotypes in all sites differed significantly in their response to MLND. When combined analysis of variance for diallel cross was performed, it revealed GCA and SCA values that were highly significant showing that additive and non- additive effects are important for grain yield. The best general combiners for grain yield were CKH10767, CML312 and CML540. These lines would serve as a source of germplasm to increase hybrid grain yield in the country.

4.1 Introduction

Grain yield of maize is a complex trait. Grain yield incorporates various factors that are quantitatively passed to the next generation (Živanovic et al., 2006). The major role of selection in maize is to be able to come up with cross breeds, that have better genetic ability for yield and other farmer desired characteristics which surpass the hybrids that are available commercially (Secanski et al., 2010, Cvarkovik et al., 2009). Diallel crosses have been widely used in plant breeding to investigate combining abilities of the parental lines in order to identify superior parents for use in hybrid development programs. The diallel mating design has also been used, in genetic research to find out how important traits are inherited among a set of genotypes and gene effects in action (Malik et al., 2005).

The idea of general and specific combining ability was brought about by SPRAGUE and TATUM (1942). General combining ability (GCA) is how a line performs in a series of hybrid combinations and specific combining ability (SCA) is the value of a line in consideration in a specific cross.

There are four experimental methods and two models that were proposed (Griffing, 1956) to be used in the investigation of GCA and SCA in a diallel mating design. The variation for GCA is associated with additive genetic action while that of SCA is mainly associated with non-additive genetic effects, which involve dominance and epistasis (Falconer & Mackay, 1996). The notion of combining ability in maize has been researched by several maize breeders (Beck et al., 1990; Crossa et al., 1990; Vasal et al., 1992; Kang et al., 1995 Kim and Ayala, 1996; Xingming et al., 2001; Betran et al., 2002; Revilla et al., 2002; Bhatnagar et al., 2004; Glover et al., 2005). In this study, 16 inbred lines were

mated in a complete diallel scheme adopted from Griffings method 1V to find out the gene action controlling grain yield

4.2 Materials and Methods

4.2.1 Germplasm Sources

The experimental material included 16 inbred lines of maize: CKH10767, CKH114272, CML312, CML444, CML503, CML 144, CML442, CML395, CML505, CML498, CML539, CML540, CML562, CML578, CML 034, and CML494. The inbreds were formed using resistant and susceptible inbred lines sources from CIMMYT AND KALRO and were chosen because they were diverse genetically and their response to disease. These lines were crossed in 2015 in a diallel mating design to form 120 F1 hybrids

4.2.2 Experimental Design

The original diallel was evaluated in an incomplete block design. Each set was replicated twice in each location. The Experiment was done in two locations in Kenya, in 2015 in Bomet and Naivasha. These are locations currently having high MLND occurrences. The experimental plot comprised of two rows of 2.5m at spacing of 75cm inters rows and 125cm between hills. Leaves were trimmed at 4-6 leaf stage and quality cultural practices to ensure high maize production was observed in the two locations.

4.2.3 Data Collection

The parental lines were evaluated so as to understand their genetic make -up on : disease scores, plant height(cm) measured from the base of the plant to the base of the tassel, anthesis date(50% pollen shed), silking date(50% silk emergence),ear height measured from the base of the plant to the node bearing the top ear and moisture content. Data for

grain yield was recorded on a plot basis at all locations as shown by Magorokosho et al., 2009 were shelled to determine percent moisture. Grain yield adjusted to 12.5% moisture was computed from ear weight and grain weight based on the following formulae:

Grain Yield (t ha-1) = [Grain weight (kg plot-1) x 10 x (100 – MC)/ (100 – 12.5)/ (Plot area)],

Where:

MC = measured grain moisture content

4.2.4 Statistical Analysis

Analysis of variance (ANOVA) to detect differences among the single crosses was performed using GENSTAT (5^{th} edition). The random effects included; the genotypes, locations and replicates. The parental GCA effects and the crosses SCA together with their average squares were evaluated using a half diallel adopted from Griffings 4 model 11 (random parental effects) (Griffing 1956). This was done using diallel GENSTAT program.

The statistical model for the combined diallel analysis in the two locations 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 lth environment (Hallauer and Miranda, 1988). GCA and SCA effects were tested using a t- test. The standard errors of the GCA and SCA effects were determine using the square root of GCA and SCA variances (Griffing,

1956). The relative importance of GCA and SCA was determined using Bakers ratio (1978): $2\sigma^2$GCA/

$$2\sigma GCA + \sigma^2 \text{sca}$$

Where;

GCA=Σxi.2/ (p-2) -4x..2/ [p(p-2)]

SCA=ΣΣi<jxij2 –Σxi.2/ (p-2) +2.2/[(p-1)(p-2)]

GCA = general combining ability; SCA = specific combining ability; xi. = mean of *i* th parent; x.. = overall mean of all crosses;

4.3 Results

The mean square of all the traits studied was revealed by Analysis of Variance. GCA effects showed mean squares that were highly significant for all the characteristics under study while mean squares for SCA was also significant for most traits except for ear height.

Table 4.1: Combined Analysis Of Variance For Different Traits In A Diallel Cross of Maize.

Source of variation	Df	Grain yield per plot (t/ha)	Days to 50% pollen shedding	Days to 50% silking	Moisture content (%)	Plant height(cm)	Ear height (cm)
			Mean squares				
Locations	1	0.53**	0.13**	0.13**	0.22**	0.49**	0.44**
GCA	15	0.04**	0.75**	0.44**	0.32**	0.70**	0.89**
SCA	120	0.05**	0.18**	0.19**	0.24*	0.45**	0.58**
GCA x Locations	15	0.02**	0.28**	0.87**	0.30**	0.46**	0.38**
SCA x Locations	120	0.03	0.30**	0.30**	0.45**	0.14	0.11
σ^2s/ σ^2g		0.021	0.353	0.127	0.065	0.080	0.075

***, ** significant at 5% & 1 % level respectively.**

Table 4.2: GCA Estimates Of Effects For Different Traits Of Inbred Lines In Maize.

Parents	Pedigree	Grain Yield (t/ha)	Pollen shed	Silking	Moisture content (%)	Plant height (cm)	Ear height (cm)
1	CKH10767	0.29*	1.56**	-1.64**	-0.22*	-0.14	-4.77**
2	CKH114272	-0.03	-0.45**	-0.12	0.76**	4.27**	-6.69**
3	CML312	-0.04	0.06	0.35**	0.44**	6.04**	5.74**
4	CML444	0.26*	-0.1	-0.56**	0.19*	1.98**	-0.70**
5	CML503	-0.14	-0.21*	1.14**	-0.11	-1.01**	4.97**
6	CML144	-0.02	-1.37**	-0.97**	-0.05	8.56**	2.35**
7	CML442	0.05	1.76**	-1.64**	0.58**	1.62**	-3.98**
8	CML395	0.1	2.14**	1.43**	-0.16*	10.46**	1.66**
9	CML505	0.25*	-0.02	-0.81**	-0.66**	15.02**	9.64**
10	CML498	0.43**	-4.00**	-2.41**	-1.48**	-34.37**	-9.62**
11	CML539	0.11	0.06	0.05	0.08	9.64**	0.10*
12	CML540	0.17	0.09	0.08	1.13**	0.1	-1.37**
13	CML562	0.29*	0.44**	-1.01**	-1.01**	0.15	-0.1
14	CML578	-0.03	-0.70**	-0.67**	-0.14	5.27	-0.21*
15	CML034	-0.04	1.62**	0.06	-0.22*	1.76**	0.58**
16	CML494	0.26*	-1.48**	0.1	0.05	-0.21	6.04**

***, ** significant at 5% & 1 % level respectively**

Table 4.3: SCA Estimates of Effects of Selected Crosses in Maize

Cross	Grain yield	Pollen shed	Silking	Moisture content	Plant height	Ear placement
CKH10767×CML503	0.31*	1.16**	1.10*	-0.39*	32.34**	0.27
CKH10767×CML505	0.34*	-2.22**	-1.65**	-1.74**	22.3**	16.8**
CKH14272×CML144	0.32*	-0.85**	0.10	0.28*	14.78**	6.51**
CML312×CML444	0.42*	-2.35**	-3.13**	-0.29*	35.85**	-4.27**
CML444 × CML395	0.37*	-1.80**	-3.12**	-2.49**	32.00**	7.79**
CML444 ×CML498	0.35*	-1.68**	1.56**	-2.63**	15.23**	-0.20
CML503×CML442	0.53**	6.53**	2.06**	-0.30**	-21.57**	-11.85**
CML503×CML395	0.34*	-1.12**	-1.82**	0.33**	-4.07**	0.38**
CML144×CML494	0.31*	-0.25**	0.20*	-0.90**	11.48**	13.03**
CML498×CML540	0.46**	-1.80**	-1.25**	-2.03**	-2.05**	-2.29**
CML395×CML505	0.54**	-1.35**	-1.59**	-4.49**	-5.3**	-20.07**
CML395×CML498	0.30*	-0.24**	2.59**	-3.68**	5.55**	-1.91**
CML505×CML539	0.16**	0.07*	0.08	0.10	12.38**	0.14
CML498×CML540	0.55**	0.26	0.29	0.37	7.22**	0.48
CML539×CML562	0.31*	1.16**	1.10*	-0.39*	32.34**	0.27
CML540×CML578	0.34*	-2.22**	-1.65**	-1.74**	22.3**	16.8**

***, ** significant at 5% & 1 % level respectively**

Results

Analysis of variance for GCA showed significant mean square values for all the traits that were studied. SCA mean squares were also highly significant majority of the traits apart from plant and ear height. For days to pollen shed, the highest GCA values were observed in CKH10767, CML442 AND CML395. The best specific combinations were CML503× CML442 (6.53) followed by CML540×CML578 (1.16) and CKH10767× CML503 (1.16). When early maturity in plant is desired, negative values for GCA and SCA effects would be desirable which were estimated in CKH114272, CML503, CML444, CML144, CML505, CML498, CML578 and CML494. Same study outcomes

have been reported for other lines by Mungoma and pollak (1988) and Revilla et al (1999).

The highest GCA effects for plant height were observed for CML539 (9.64) followed by CML144 (8.56) followed by CML312 (6.04). Good specific combinations were observed in in hybrids CML539×CML505 (35.85) followed by CML539×CML562 (32.34). Significant GCA and SCA mean squares have also been presented by Revilla et al 1999 for plant height in some lines of maize.

For ear height, the GCA effects were highly significant as compared to SCA effects. The prevalence of GCA effects shows that the difference among the crosses was mainly caused by additive and not the non- additive gene effects and to be able to improve ear height selection would be necessary and effective. Inbreds CML505, CML 494 and CML312 came up as the best general combiners with GCA values of 9.64, 6.04 and 5.74 respectively. The SCA was highest for cross CML505× CML539 (16.8), CKH10767×CML505 (16.8) and CML494×CML034 (16.8).

The inbreds with high GCA values for moisture at harvest was CML 540(1.13) and CKH10767 (0.76). The best specific combinations were observed in CML498× CML540 and CK114272×CML144.

For silking, high GCA effects were observed in CML 395(1.43) and CML503 (1.14). The best specific combinations were observed in CML505×CML539 (2.59) and CML503× CML442 (2.06).

For grain yield, high estimates of GCA were observed in CML498 (0.43), CKH10767 (0.29), CML562 (0.29) and CML492 (0.26). The highest SCA effects were observed in

CML395× CML498, CML503× CML442 and CML562×CML539. Mungoma and Pollack also reported high GCA and SCA effects for grain yield in their study.

CHAPTER FIVE

GENERAL DISCUSSION

Maize lethal necrosis disease is not only as a result of infection by either SCMV or MCMV, but it also includes their synergistic interaction which simultaneously cause high yield loss and endangers the food security presently in eastern Africa (Ali and Yan 2012). The genetic characteristic of SCMV and other Potyviruses has been researched comprehensively in maize with varied germplasm (as reviewed by Redinbaugh and Pratt 2009). The genetics and inheritance of resistance to MLND is not yet known and is expected to be very complex which may be due to combination of two viruses.

This study was carried out to screen genotypes for MLN resistance. The disease was distributed in all the counties surveyed and was not restricted to any particular ecological zone. All the genotypes were also infected with MLND although at varying levels. Earlier studies had identified MLND as an important disease across the country affecting major maize growing areas (Wangai et al., 2012). The occurrence of MLN was high in both locations. This may be an indication that despite the effort being put by researchers to manage MLND, true resistance has not been found. However severity for MLND was high in Naivasha compared to Bomet and this may be due to artificial inoculation that was done in Naivasha.

Variation in response to MLN was seen in all the genotypes showing presence of suitable germplasm for developing varieties resistant to MNLD.

Disease score and maize yield were negatively correlated. The absence of association between grain yield and MLN disease resistance is important because it indicates both of

these characteristics can be improved together. Several surveys using different germplasm under MLND pressure came up with the same results. (Betran et al., 2003)

Combined analysis of variance for diallel cross showed highly significant values for both GCA and SCA for grain yield showing that both additive and non- additive effects play a role in yield of crop this is a similar finding to (Dehghanpour and Ehdaie 2013, Estakhr and Heidan 2012, Gafish et al.,2012, Haddadi et al.,2015.).

The GCA to SCA ratio was 0.60 which shows the importance of the non-additive gene action in the inheritance of grain yield and this is similar to other findings (Srdic et al., 2007, Unay et al., 2004). The intermediate contribution of SCA to hybrid difference indicates that it would be difficult to ascertain hybrid performance based on GCA effects alone. Therefore it will be necessary to test parental lines in combination with multiple testers to identify superior hybrids (Gichuru 2013). The GCA and SCA effects interacted together with location which shows the effect of the environment on grain yield and similar results has been reported in other studies (Badu-Apraku and Oyenkunle, 2012, Badu Apraku et al., 2013, Gakunga et al., 2012). This would lead to complication during selection because of genotype and location interaction effects stressing the importance to examine inbreds in environments that are not similar to ensure consistent performance of hybrids in terms of stability and productivity.

The lines with high GCA for grain yield were: CK10767 AND CML540 would contribute to favorable genes for the coming up with new varieties as a result of GCA. The large grain yield GCA effect of these lines shows their values as testers in selection for yield. Some parents such as CML034, CML442 and CML578 showed no significant GCA effects but positive and significant SCA effects when crossing. This behavior is as a

result of complementary gene effects or nicking effects (Dehghanpour and Ehdaie, 2013). CML395 × CML505 was the most desirable cross combination followed by CML144 × CML498 and CML395× CML498 Combinations involving CML503 as one of the parental lines showed suitable SCA effects for most of the characteristics that were studied.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Maize is affected by a host of different pathogens among them MLND which causes significant yield losses. During the study period all locations showed MLND infestation. The different maize genotypes reacted differently to MLNS. Naivasha had the highest incidence and most severe disease scores. The disease was also present in Bomet though at different level of severity between the different genotypes evaluated. The disease continues to affect maize causing significant yield losses in the country. The maize germplasm available to farmers including hybrids are mostly susceptible to the disease. There is a likelihood that the status of the disease can change to epidemic levels especially with climate change. From this study, it is evident that different maize genotypes are involved in the development of MLN disease and therefore it is important to evaluate the genotypes available in Kenya and screen them for MLN resistance. From the study, high variability was also seen in the 120 maize genotypes showing the existing and suitable germplasm for improving MLN resistance in local varieties.

The findings of the study also noted that, combining ability and environment interactions poses difficulty in selection because of effects of the genotype associating with the location. Hence a genotype may be stable in one environment and not another environment therefore there is need to test hybrids in dissimilar environments.

6.2 Recommendations

1. MLND was found to be prevalent in the two counties. The disease infected all genotypes evaluated in the field. More efforts are needed to develop management strategies to minimize losses that may be associated with the disease.

2. Lines SC-MLN-15-3 and SC-MLN-15-56 had low severity scores and were more tolerant to MLND. These two varieties may be good sources for tolerance to MLND and should be incorporated in breeding programs.

3. Lines SC-MLN-15-81 had the heist yield in Naivasha while lines SC-MLN-15-11, CS-MLN-15-30, SC-MLN-5-66 were high Yielding in Bomet despite disease pressure. These lines would contribute useful germplasm to increase production of hybrid grain in Kenya.

4. GCA and SCA associated significantly with the environment for grain yield hence there is need to test inbred lines and dissimilar environment for stable performance and productivity of hybrids.

5. CML498, CKH10767, CML562 and CML492 were favorable general combiners for yield of grain. These lines could be utilized as testers for selection of high yielding varieties in hybridization programs.

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APPENDICES

Appendix I – Data Collection Sheet

Sheet #							
Date/Time							
Name of area							
District/LGA							
State							
Agro-ecology							
Latitude							
Longitude							
Altitude (m)							
Summary							
Plant (#)	Symptoms	Severity score	P#	Photo ID	Symptoms	Severity. Score	Variety
1			1				
2			2				
3			3				
4			4				
5			5				
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							

19
20

Abbreviations for describing symptoms:

Prefix: m – mild; o – moderate; s – severe; Suffix: m – mosaic; mo – mottling;; st – stunting; d – deformation; de – death

Severity rating criteria:

1. No symptoms seen; plants are disease free

2. mosaic mottling of leaves /branches of a plant (25% of the plant exhibiting symptoms)

3. necrosis or puckering of leaf veins clearing symptoms are on 50% of the plant

4. Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant) but no stunting of deformation

5. Severe mosaic/mottling/yellowing/necrosis and severe stunting (entire plant) deformation and death of the infected plants

Appendix II- Disease and Yield (T/HA) Score (Naivasha)

	Disease Scores			Yield T/Ha	
No.	**Entry Code**	**D.Score**	**No.**	**Entry Code**	**YIELD/HA**
1	SC-MLN-15-6	3	1	SC-MLN-15-15	1.922
2	SC-MLN-15-7	3	2	SC-MLN-15-81	1.583
3	SC-MLN-15-8	3	3	SC-MLN-15-102	1.447
4	SC-MLN-15-15	3	4	SC-MLN-15-3	1.407
5	SC-MLN-15-26	3	5	SC-MLN-15-7	1.328
6	SC-MLN-15-65	3	6	SC-MLN-15-37	1.277
7	SC-MLN-15-69	3	7	SC-MLN-15-20	1.259
8	SC-MLN-15-81	3	8	SC-MLN-15-72	1.18
9	SC-MLN-15-3	3.25	9	SC-MLN-15-17	1.14
10	SC-MLN-15-16	3.25	10	SC-MLN-15-69	1.14
11	SC-MLN-15-17	3.25	11	SC-MLN-15-26	1.118
12	SC-MLN-15-19	3.25	12	SC-MLN-15-107	1.118
13	SC-MLN-15-20	3.25	13	SC-MLN-15-19	1.106
14	SC-MLN-15-37	3.25	14	SC-MLN-15-2	1.072
15	SC-MLN-15-49	3.25	15	SC-MLN-15-98	1.067
16	SC-MLN-15-58	3.25	16	SC-MLN-15-70	1.05
17	SC-MLN-15-72	3.25	17	SC-MLN-15-83	1.044
18	SC-MLN-15-73	3.25	18	SC-MLN-15-65	1.038
19	SC-MLN-15-92	3.25	19	SC-MLN-15-6	1.016
20	SC-MLN-15-96	3.25	20	SC-MLN-15-82	1.01
21	SC-MLN-15-103	3.25	21	SC-MLN-15-8	0.999
22	SC-MLN-15-107	3.25	22	SC-MLN-15-111	0.981
23	SC-MLN-15-111	3.25	23	SC-MLN-15-57	0.976
24	SC-MLN-15-2	3.5	24	SC-MLN-15-97	0.97
25	SC-MLN-15-4	3.5	25	SC-MLN-15-106	0.964
26	SC-MLN-15-21	3.5	26	SC-MLN-15-16	0.947
27	SC-MLN-15-25	3.5	27	SC-MLN-15-55	0.947

28	SC-MLN-15-27	3.5	28	SC-MLN-15-67	0.947
29	SC-MLN-15-30	3.5	29	SC-MLN-15-59	0.936
30	SC-MLN-15-31	3.5	30	SC-MLN-15-48	0.919
31	SC-MLN-15-33	3.5	31	SC-MLN-15-10	0.913
32	SC-MLN-15-35	3.5	32	SC-MLN-15-88	0.908
33	SC-MLN-15-38	3.5	33	SC-MLN-15-92	0.908
34	SC-MLN-15-40	3.5	34	SC-MLN-15-80	0.896
35	SC-MLN-15-42	3.5	35	SC-MLN-15-36	0.891
36	SC-MLN-15-43	3.5	36	SC-MLN-15-100	0.891
37	SC-MLN-15-45	3.5	37	SC-MLN-15-105	0.879
38	SC-MLN-15-46	3.5	38	SC-MLN-15-42	0.874
39	SC-MLN-15-47	3.5	39	SC-MLN-15-30	0.862
40	SC-MLN-15-48	3.5	40	SC-MLN-15-58	0.84
41	SC-MLN-15-52	3.5	41	SC-MLN-15-49	0.823
42	SC-MLN-15-55	3.5	42	SC-MLN-15-90	0.823
43	SC-MLN-15-56	3.5	43	SC-MLN-15-96	0.823
44	SC-MLN-15-57	3.5	44	SC-MLN-15-54	0.811
45	SC-MLN-15-59	3.5	45	SC-MLN-15-84	0.8
46	SC-MLN-15-62	3.5	46	SC-MLN-15-14	0.794
47	SC-MLN-15-63	3.5	47	SC-MLN-15-68	0.794
48	SC-MLN-15-64	3.5	48	SC-MLN-15-44	0.777
49	SC-MLN-15-66	3.5	49	SC-MLN-15-29	0.772
50	SC-MLN-15-67	3.5	50	SC-MLN-15-47	0.766
51	SC-MLN-15-68	3.5	51	SC-MLN-15-46	0.755
52	SC-MLN-15-70	3.5	52	SC-MLN-15-75	0.749
53	SC-MLN-15-74	3.5	53	SC-MLN-15-104	0.749
54	SC-MLN-15-78	3.5	54	SC-MLN-15-87	0.743
55	SC-MLN-15-79	3.5	55	SC-MLN-15-12	0.732
56	SC-MLN-15-80	3.5	56	SC-MLN-15-40	0.726
57	SC-MLN-15-82	3.5	57	SC-MLN-15-45	0.721
58	SC-MLN-15-83	3.5	58	SC-MLN-15-28	0.715

59	SC-MLN-15-85	3.5	59	SC-MLN-15-32	0.715
60	SC-MLN-15-89	3.5	60	SC-MLN-15-56	0.715
61	SC-MLN-15-90	3.5	61	SC-MLN-15-4	0.709
62	SC-MLN-15-97	3.5	62	SC-MLN-15-52	0.709
63	SC-MLN-15-98	3.5	63	SC-MLN-15-51	0.703
64	SC-MLN-15-99	3.5	64	SC-MLN-15-74	0.692
65	SC-MLN-15-100	3.5	65	SC-MLN-15-73	0.675
66	SC-MLN-15-102	3.5	66	SC-MLN-15-79	0.669
67	SC-MLN-15-104	3.5	67	SC-MLN-15-66	0.664
68	SC-MLN-15-106	3.5	68	SC-MLN-15-11	0.647
69	SC-MLN-15-108	3.5	69	SC-MLN-15-91	0.647
70	CHECK 1	3.5	70	SC-MLN-15-50	0.641
71	CHECK 2	3.5	71	SC-MLN-15-41	0.635
72	CHECK 4	3.5	72	SC-MLN-15-85	0.635
73	CHECK 5	3.5	73	SC-MLN-15-103	0.635
74	CHECK 6	3.5	74	SC-MLN-15-1	0.624
75	CHECK 7	3.5	75	SC-MLN-15-78	0.618
76	CHECK 8	3.5	76	SC-MLN-15-27	0.613
77	CHECK 9	3.5	77	SC-MLN-15-35	0.613
78	SC-MLN-15-1	3.75	78	SC-MLN-15-43	0.613
79	SC-MLN-15-5	3.75	79	SC-MLN-15-39	0.601
80	SC-MLN-15-9	3.75	80	SC-MLN-15-18	0.596
81	SC-MLN-15-10	3.75	81	SC-MLN-15-38	0.596
82	SC-MLN-15-11	3.75	82	SC-MLN-15-93	0.596
83	SC-MLN-15-12	3.75	83	SC-MLN-15-22	0.573
84	SC-MLN-15-13	3.75	84	SC-MLN-15-64	0.567
85	SC-MLN-15-14	3.75	85	SC-MLN-15-23	0.562
86	SC-MLN-15-18	3.75	86	SC-MLN-15-9	0.528
87	SC-MLN-15-23	3.75	87	SC-MLN-15-110	0.522
88	SC-MLN-15-24	3.75	88	SC-MLN-15-24	0.516
89	SC-MLN-15-28	3.75	89	SC-MLN-15-5	0.499

90	SC-MLN-15-29	3.75	90	SC-MLN-15-13	0.499
91	SC-MLN-15-32	3.75	91	SC-MLN-15-34	0.494
92	SC-MLN-15-34	3.75	92	SC-MLN-15-99	0.488
93	SC-MLN-15-36	3.75	93	SC-MLN-15-53	0.482
94	SC-MLN-15-39	3.75	94	SC-MLN-15-76	0.477
95	SC-MLN-15-41	3.75	95	SC-MLN-15-21	0.465
96	SC-MLN-15-44	3.75	96	SC-MLN-15-25	0.46
97	SC-MLN-15-50	3.75	97	SC-MLN-15-60	0.46
98	SC-MLN-15-51	3.75	98	SC-MLN-15-109	0.454
99	SC-MLN-15-53	3.75	99	SC-MLN-15-62	0.448
100	SC-MLN-15-54	3.75	100	SC-MLN-15-63	0.431
101	SC-MLN-15-60	3.75	101	SC-MLN-15-71	0.42
102	SC-MLN-15-61	3.75	102	SC-MLN-15-86	0.397
103	SC-MLN-15-71	3.75	103	SC-MLN-15-94	0.397
104	SC-MLN-15-75	3.75	104	SC-MLN-15-108	0.397
105	SC-MLN-15-76	3.75	105	SC-MLN-15-101	0.386
106	SC-MLN-15-84	3.75	106	SC-MLN-15-61	0.374
107	SC-MLN-15-86	3.75	107	SC-MLN-15-31	0.357
108	SC-MLN-15-87	3.75	108	SC-MLN-15-95	0.323
109	SC-MLN-15-88	3.75	109	SC-MLN-15-89	0.306
110	SC-MLN-15-91	3.75	110	SC-MLN-15-33	0.267
111	SC-MLN-15-93	3.75	111	SC-MLN-15-77	0.221
112	SC-MLN-15-95	3.75	112	CHECK 1	0.811
113	SC-MLN-15-105	3.75	113	CHECK 2	0.709
114	SC-MLN-15-109	3.75	114	CHECK 3	0.584
115	SC-MLN-15-110	3.75	115	CHECK 4	0.397
116	CHECK 3	3.75	116	CHECK 5	0.743
117	SC-MLN-15-22	4	117	CHECK 6	0.601
118	SC-MLN-15-77	4	118	CHECK 7	0.874
119	SC-MLN-15-94	4	119	CHECK 8	0.777
120	SC-MLN-15-101	4	120	CHECK 9	0.635

Appendix III: Disease Score for Bomet

REP	PLOT No.	ENTRY. No.	ENTRY CODE	D. SCORE	YIELD SCORE(T/HA)
1	1	102	SC-MLN-15-102	2.8	0.75
1	2	105	SC-MLN-15-105	3	1.04
1	3	111	SC-MLN-15-111	3.2	0.98
1	4	82	SC-MLN-15-82	3	0.47
1	5	7	SC-MLN-15-7	3	1.03
1	6	21	SC-MLN-15-21	2.8	0.41
1	7	17	SC-MLN-15-17	3.2	1.17
1	8	87	SC-MLN-15-87	3.8	0.33
1	9	47	SC-MLN-15-47	3.2	0.60
1	10	13	SC-MLN-15-13	3.2	0.36
1	11	75	SC-MLN-15-75	3.5	0.31
1	12	95	SC-MLN-15-95	3.5	0.74
1	13	97	SC-MLN-15-97	3.8	1.21
1	14	12	SC-MLN-15-12	3	0.65
1	15	53	SC-MLN-15-53	2.8	0.75
1	16	29	SC-MLN-15-29	2.5	0.61
1	17	85	SC-MLN-15-85	2.8	0.99
1	18	25	SC-MLN-15-25	3	1.30
1	19	44	SC-MLN-15-44	3	0.23
1	20	40	SC-MLN-15-40	3.5	0.65
1	21	80	SC-MLN-15-80	2.8	0.58
1	22	94	SC-MLN-15-94	2.8	1.52
1	23	108	SC-MLN-15-108	3.2	0.67
1	24	104	SC-MLN-15-104	3	1.10
1	25	43	SC-MLN-15-43	2.8	0.68
1	26	3	SC-MLN-15-3	2.5	0.52

1	27	46	SC-MLN-15-46	3	0.56
1	28	101	SC-MLN-15-101	3	0.74
1	29	57	SC-MLN-15-57	3.5	0.30
1	30	28	SC-MLN-15-28	2.8	0.35
1	31	90	SC-MLN-15-90	3	0.57
1	32	5	SC-MLN-15-5	2.8	0.50
1	33	9	SC-MLN-15-9	3	1.37
1	34	59	SC-MLN-15-59	3.8	0.68
1	35	18	SC-MLN-15-18	3	0.17
1	36	65	SC-MLN-15-65	2.8	0.75
1	37	62	SC-MLN-15-62	2.8	0.22
1	38	73	SC-MLN-15-73	3	0.48
1	39	107	SC-MLN-15-107	3	0.86
1	40	110	SC-MLN-15-110	3	1.20
1	41	35	SC-MLN-15-35	3.5	0.51
1	42	117	SC-MLN-15-117	3	1.40
1	43	23	SC-MLN-15-23	2.5	0.28
1	44	20	SC-MLN-15-20	3.5	0.51
1	45	45	SC-MLN-15-45	3	0.54
1	46	76	SC-MLN-15-76	3.5	0.40
1	47	71	SC-MLN-15-71	3.5	0.35
1	48	86	SC-MLN-15-86	3.8	0.58
1	49	30	SC-MLN-15-30	3	0.30
1	50	99	SC-MLN-15-99	2.8	0.71
1	51	15	SC-MLN-15-15	3	0.41
1	52	116	SC-MLN-15-116	2.8	0.34
1	53	78	SC-MLN-15-78	3	0.99
1	54	91	SC-MLN-15-91	3.5	0.42
1	55	63	SC-MLN-15-63	2.5	0.23
1	56	68	SC-MLN-15-68	2.8	0.75
1	57	19	SC-MLN-15-19	3	0.22

1	58	106	SC-MLN-15-106	2.8	1.03
1	59	120	SC-MLN-15-120	2.8	0.32
1	60	112	SC-MLN-15-112	3	0.56
1	61	4	SC-MLN-15-4	3.5	0.96
1	62	115	SC-MLN-15-115	3.8	1.07
1	63	93	SC-MLN-15-93	3.8	0.44
1	64	60	SC-MLN-15-60	3.2	0.33
1	65	88	SC-MLN-15-88	3	1.68
1	66	10	SC-MLN-15-10	3	0.73
1	67	38	SC-MLN-15-38	3.2	0.27
1	68	52	SC-MLN-15-52	3	0.64
1	69	58	SC-MLN-15-58	3	1.21
1	70	6	SC-MLN-15-6	3	1.13
1	71	67	SC-MLN-15-67	2.8	0.71
1	72	79	SC-MLN-15-79	2.8	0.84
1	73	61	SC-MLN-15-61	2.5	0.89
1	74	42	SC-MLN-15-42	3.5	1.28
1	75	72	SC-MLN-15-72	3.5	1.04
1	76	66	SC-MLN-15-66	3.2	0.70
1	77	1	SC-MLM-15-1	3.8	0.90
1	78	39	SC-MLN-15-39	3.8	1.45
1	79	54	SC-MLN-15-54	2.8	0.58
1	80	31	SC-MLN-15-31	3	0.69
1	81	37	SC-MLN-15-37	3	1.01
1	82	64	SC-MLN-15-64	3	1.91
1	83	32	SC-MLN-15-32	3	0.25
1	84	50	SC-MLN-15-50	3.8	0.57
1	85	22	SC-MLN-15-22	3	0.84
1	86	34	SC-MLN-15-34	3.2	0.42
1	87	92	SC-MLN-15-92	3.5	0.66
1	88	49	SC-MLN-15-49	3	0.47

1	89	14	SC-MLN-15-14	2.8	0.75
1	90	81	SC-MLN-15-81	2.8	1.17
1	91	103	SC-MLN-15-103	3	0.95
1	92	83	SC-MLN-15-83	3	1.09
1	93	26	SC-MLN-15-26	3	0.73
1	94	69	SC-MLN-15-69	2.8	0.69
1	95	84	SC-MLN-15-84	3	1.17
1	96	118	SC-MLN-15-118	2.8	0.12
1	97	109	SC-MLN-15-109	3.5	0.83
1	98	51	SC-MLN-15-51	3.2	0.75
1	99	98	SC-MLN-15-98	3	0.11
1	100	11	SC-MLN-15-11	3.5	0.60
1	101	100	SC-MLN-15-100	3	0.71
1	102	2	SC-MLN-15-2	3	0.76
1	103	48	SC-MLN-15-48	2.8	1.06
1	104	74	SC-MLN-15-74	3	0.69
1	105	96	SC-MLN-15-96	3	0.37
1	106	8	SC-MLN-15-8	2.8	0.82
1	107	27	SC-MLN-15-27	3	1.55
1	108	119	SC-MLN-15-119	3	1.12
1	109	33	SC-MLN-15-33	3.5	1.10
1	110	89	SC-MLN-15-89	3	1.33
1	111	24	SC-MLN-15-24	3	0.18
1	112	70	SC-MLN-15-70	3	0.50
1	113	55	SC-MLN-15-55	2.8	0.99
1	114	36	SC-MLN-15-36	2.8	0.36
1	115	41	SC-MLN-15-41	3	0.82
1	116	114	SC-MLN-15-114	3	1.91
1	117	56	SC-MLN-15-56	2.8	1.44
1	118	16	SC-MLN-15-16	3.2	0.34
1	119	77	SC-MLN-15-77	3.2	1.12

1	120	113	SC-MLN-15-113	3.2	0.18
2	1	89	SC-MLN-15-89	2.8	0.87
2	2	67	SC-MLN-15-67	2.8	0.57
2	3	74	SC-MLN-15-74	2.8	0.65
2	4	106	SC-MLN-15-106	2.5	0.78
2	5	71	SC-MLN-15-71	2.5	0.64
2	6	4	SC-MLN-15-4	2.8	0.99
2	7	64	SC-MLN-15-64	3	1.78
2	8	75	SC-MLN-15-75	2.8	0.71
2	9	2	SC-MLN-15-2	3	1.00
2	10	5	SC-MLN-15-5	2.5	1.33
2	11	81	SC-MLN-15-81	3	1.02
2	12	3	SC-MLN-15-3	2	0.47
2	13	115	SC-MLN-15-115	3	1.09
2	14	62	SC-MLN-15-62	3	1.27
2	15	24	SC-MLN-15-24	2.8	1.50
2	16	18	SC-MLN-15-18	2.8	0.87
2	17	57	SC-MLN-15-57	3	0.93
2	18	60	SC-MLN-15-60	2.8	0.52
2	19	23	SC-MLN-15-23	2.5	0.81
2	20	112	SC-MLN-15-112	2.8	1.75
2	21	46	SC-MLN-15-46	2.5	0.18
2	22	99	SC-MLN-15-99	3	0.47
2	23	86	SC-MLN-15-86	3.2	1.65
2	24	34	SC-MLN-15-34	2.8	0.84
2	25	19	SC-MLN-15-19	3	1.15
2	26	15	SC-MLN-15-15	2.8	0.73
2	27	95	SC-MLN-15-95	2.8	0.58
2	28	96	SC-MLN-15-96	2.8	0.42
2	29	20	SC-MLN-15-20	3.2	1.18
2	30	10	SC-MLN-15-10	3	0.58

2	31	58	SC-MLN-15-58	3.2	0.89
2	32	11	SC-MLN-15-11	3.5	0.56
2	33	21	SC-MLN-15-21	2.8	0.39
2	34	49	SC-MLN-15-49	2.5	0.51
2	35	53	SC-MLN-15-53	2.8	0.37
2	36	13	SC-MLN-15-13	3	0.17
2	37	82	SC-MLN-15-82	3	0.34
2	38	87	SC-MLN-15-87	3	1.40
2	39	41	SC-MLN-15-41	2.8	0.85
2	40	37	SC-MLN-15-37	2.8	0.87
2	41	33	SC-MLN-15-33	2.8	0.36
2	42	36	SC-MLN-15-36	3	1.34
2	43	113	SC-MLN-15-113	3	0.31
2	44	50	SC-MLN-15-50	3.5	0.73
2	45	39	SC-MLN-15-39	2.8	0.26
2	46	88	SC-MLN-15-88	3	0.75
2	47	30	SC-MLN-15-30	3.5	0.41
2	48	92	SC-MLN-15-92	2.8	0.98
2	49	93	SC-MLN-15-93	3	0.67
2	50	76	SC-MLN-15-76	3.5	1.18
2	51	110	SC-MLN-15-110	3	0.64
2	52	97	SC-MLN-15-97	2.8	0.83
2	53	17	SC-MLN-15-17	3	0.43
2	54	101	SC-MLN-15-101	3.5	0.65
2	55	68	SC-MLN-15-68	3	0.83
2	56	119	SC-MLN-15-19	3.5	1.26
2	57	32	SC-MLN-15-32	3.5	0.31
2	58	14	SC-MLN-15-14	2.8	0.95
2	59	107	SC-MLN-15-107	3	0.73
2	60	103	SC-MLN-15-103	3	1.53
2	61	29	SC-MLN-15-29	2.8	0.74

2	62	45	SC-MLN-15-45		3	0.94
2	63	27	SC-MLN-15-29		3.8	0.60
2	64	118	SC-MLN-15-118		3	0.79
2	65	54	SC-MLN-15-54	*		0.53
2	66	56	SC-MLN-15-56		3.5	0.42
2	67	84	SC-MLN-15-84		4	0.25
2	68	9	SC-MLN-15-9		3.2	1.10
2	69	61	SC-MLN-15-61		3	*
2	70	70	SC-MLN-15-70		3.5	0.75
2	71	59	SC-MLN-15-59		3.5	0.52
2	72	100	SC-MLN-15-100		2.8	0.73
2	73	63	SC-MLN-15-63		2.8	0.43
2	74	104	SC-MLN-15-104		3.5	0.23
2	75	35	SC-MLN-15-35		3.8	1.82
2	76	72	SC-MLN-15-72		3.5	0.51
2	77	28	SC-MLN-15-28		3.5	0.70
2	78	52	SC-MLN-15-52		3	1.11
2	79	80	SC-MLN-15-80		3	1.16
2	80	105	SC-MLN-15-105		3.5	0.78
2	81	55	SC-MLN-15-55		3	0.78
2	82	43	SC-MLN-15-43		2.8	0.22
2	83	111	SC-MLN-15-111		3	0.32
2	84	73	SC-MLN-15-73		3	0.62
2	85	69	SC-MLN-15-69		3	0.25
2	86	85	SC-MLN-15-85		3	0.54
2	87	117	SC-MLN-15-117		3.2	0.68
2	88	16	SC-MLN-15-16		2.8	0.37
2	89	47	SC-MLN-15-47		3.2	0.47
2	90	6	SC-MLN-15-6		3	0.75
2	91	79	SC-MLN-15-79		3.5	0.75
2	92	120	SC-MLN-15-120		3	0.61

2	93	25	SC-MLN-15-25	2.8	0.87
2	94	77	SC-MLN-15-77	3.2	1.54
2	95	22	SC-MLN-15-22	3.2	1.09
2	96	66	SC-MLN-15-66	3.2	1.03
2	97	8	SC-MLN-15-8	3	0.76
2	98	98	SC-MLN-15-98	2.8	0.54
2	99	48	SC-MLN-15-48	3	0.40
2	100	94	SC-MLN-15-94	3	0.27
2	101	109	SC-MLN-15-109	3	1.13
2	102	7	SC-MLN-15-7	3.2	0.77
2	103	83	SC-MLN-15-83	3.5	0.32
2	104	12	SC-MLN-15-12	3.2	0.68
2	105	40	SC-MLN-15-40	3	1.21
2	106	90	SC-MLN-15-90	3	0.44
2	107	38	SC-MLN-15-38	3	0.60
2	108	51	SC-MLN-15-51	3.2	0.64
2	109	114	SC-MLN-15-114	3.2	1.61
2	110	78	SC-MLN-15-78	3.2	0.57
2	111	44	SC-MLN-15-44	3.5	0.78
2	112	91	SC-MLN-15-91	3	0.49
2	113	102	SC-MLN-15-102	3	0.54
2	114	42	SC-MLN-15-42	3	0.58
2	115	65	SC-MLN-15-65	4	0.79
2	116	116	SC-MLN-15-116	3.5	0.83
2	117	1	SC-MLN-15-1	3.5	0.93
2	118	26	SC-MLN-15-26	4	0.91
2	119	108	SC-MLN-15-108	*	0.89
2	120	31	SC-MLN-15-31	4.5	0.59

Appendix IV: Combined Analysis Of Variance for Disease Score (Bomet)

Source	d.f.	s.s.	m.s.	v.r.	F pr.	
REP	1	0.0038	0.0038	0.04	0.851	
Variety	120	14.4801	0.1207	1.14	0.237	NS
Residual	116	12.259	0.1057			
Total	237	26.7429	0.1128			
	LSD	0.5257		5%	0.05	

Coefficient of variation and standard error of a single unit

%cv	se
10.57	0.3251

Appendix V: Combined Analysis for Yield (Bomet)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	1	10.76312	10.76312	193.72	0.046
REP.Variety	237	181.4037	0.76542	13.78	0.209
Residual	1	0.05556	0.05556		
Total	239	192.2223			
	LSD	0.387			
	Variate: Yield_T_Ha				
	d.f.	s.e.	cv%		
	1	0.236	13.4		

Appendix VI: Combined Analysis for Yield (T/HA) (Naivasha)

Variate: YIELD_T_HA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0.0601	0.0601	0.44	
REP.*Units* stratum					
ENTRY	119	18.9705	0.1594	1.17	0.198
Residual	118	16.0896	0.1364		
Total	238	33.7256			
l.s.d.	0.7312				
C.V.	48.6				

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