DEVELOPING MAIZE HYBRIDS RESISTANT TO MAIZE LETHAL NECROSIS DISEASE FROM DIVERSE MAIZE INBRED LINES IN TANZANIA

JACOB G. KIYYO

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EXTENDED ABSTRACT

One hundred maize genotypes of different categories were evaluated for Maize Lethal Necrosis Disease (MLND) resistance in three locations under natural infestation. Sixty inbred lines, thirty landraces and ten improved varieties were subjected to disease hot spot areas. Experiment was conducted at Ngaramtoni in Arusha municipality, Mlangarini in Arumeru District and Kiru six in Babati Rural Distriduring 2014 and 2015 seasons. The trial was laid down in Randomized Incomplete Alpha Lattice design and replicated three times. Breeding nursery was established in an un-replicated trial at Kirusix in 2014 off season. Single cross hybrids were developed using 6x6 full diallel fashion following Griffing's (1956) design I Model I. The parental materials used were drawn from diverse inbred lines. Evaluation trials were conducted in three locations (Ngaramtoni, Mlangarini and Kirusix). The trials were laid down in a Randomized Complete Block Design with three replications. All genotypes were evaluated for resistance against MLND and yield components. Analysis of variance showed significant differences among treatments at ($p \leq 0.001$). No genotype showed complete immunity against MLND. However, landraces showed some resistance scoring from 3-4 compared to inbred lines and improvedvarieties where in most of the locations they scored4-5.Nature of gene action and genetic parameters for disease resistance were studied in a diallel cross involving six maize inbreds. The adequacy of genetic model was determined through regression coefficient and covariance – variance (Wr-Vr) test to validate the data set. The data were analysed according to Hayman's analysis of variance and components of genetic variance were estimated. Additive genetic effects appeared to be more pronounced in the genetic control than non-additive. The parent CML 144 was found to be the best combiner with GCA of **(-0.556***)** while CML 503 and CML 444 were found to be among poor combiners with GCA of **0.62***** and **0.231****respectively.

The graphic analysis revealed thatallelic distributions were highly influenced by environment for some genotypes. Since there is high genetic variation among the genotypes studied, selection for the promising material can be done successfully.

DECLARATION

I, Jacob GidaleKiyyo, do hereby declare to the senate of Sokoine University of Agriculture that, the work presented here is my own original work and has not been submitted for a higher degree or any award in any other University.

.....……

Jacob G.Kiyyo Date

(MSc. Candidate)

The above declaration is confirmed by:

.....

Prof. P.M. Kusolwa. Date

(Supervior)

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DEDICATION

This work is dedicated to my parents, without them my future would have been in tragedy. When I remember my past is like dream but they shaped my path and directed to the right track. They are always my role model.

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
ARI	Agricultural Research Institute
ATC	Average Tester Coordinate
BPMV	Bean Pod Mottle Virus
CIMMYT	International Maize And Wheat Improvement Centre
CLND	Corn Lethal Necrosis Disease
CV	Coefficient of Variation
DAP	Di-Ammonium Phosphate
FAOSTA	Food And Agriculture Organization Statistics
GCA	General Combining Ability
GLS	Grey Leaf Spot
GXE	Genotype by Environmental interaction
ICRISAT	International Crop Research Institute for the Semi-Arid Tropics
ICW	Ilonga Composite White
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
KARI	Kenya Agricultural Research Institute
LSD	Least Significant Difference
MAS	Marker Assisted Selection
m.a.s.l	Meter Above Sea Level
MCDV	Maize Chlorotic Dwarf Virus
MCMV	Maize Chlorotic Mottle Virus
MDMV	Maize Dwarf Mosaic Virus
MET	Multi Environmental Trials

MIP	Maize Improvement Programme
MLND	Maize Lethal Necrosis Disease
mm	Millimeter
MMV	Maize Mosaic Virus
MSV	Maize Streak Virus
NMRP	National Maize Research Programme
OPV	Open Pollinated Variety
QTL	Quantitative Trait Loci
RCBD	Randomized Complete Block Design
RNA	Ribo-Nucleic Acid
SCA	Specific Combining Ability
SCMV	Sugar Cane Mosaic Virus
Sed	Standard error of difference
SE	Standard errors
SIMLESA	Sustainable Intensification of Maize –Legumes Systems for Eastern and Southern Africa
SSA	Sub- Saharan Africa
TMV-1	Tanzania Maize Variety-1
UCA	Ukiriguru Composite (A)
UH	Uyole Hybrid
USAID	United State Of America International Development Agency
WAE	Week After Emergence
WEMA	Water Efficient Maize For Africa
WSMV	Wheat Streak Mosaic Virus
ZARDEF	Zonal Agricultural Research Development Fund

CHAPTER ONE

1.0 INTRODUCTION

1.1 Back ground information

1.1.1 Maize origin and diversity

Maize (*Zea mays L.)* originated from the teosinte (*Zea mays L.spp Mexicana*) in the Western Hemisphere about 7,000 to 10,000 years ago. It was widely grown by native Americans, for example it was the first crop in North Dakota in the United States during 1600s and 1700s (Halluer and Carena, 2009). Similar to other crops species, maize arose from wild weedy species native to the area. It was brought to Europe by the early explorers and widely distributed to other parts of the world. Although the transition from wild species to modern cultivated species is similar to other crops in many aspects, maize had some different properties other than its origin. Maize is the cross- pollinated specie with unique and separate male (tassel) and female (ear) organs (Halluer and Carena, 2009).

1.1.2 Importance of Maize in Africa

The popularity of maize in Africa has been increasing to the extent of replacing traditional crops like sorghum and millet. (DeVries and Toenniessen, 2001). An estimate of 90% of the maize produced in Africa is consumed as food (Kanitila*et al*., 1998). Maize is the most important cereal crop in sub-Saharan Africa (SSA) and an important staple food for more than 1.2 billion people in SSA and Latin America. It is the staple food for more than 300 million people in Sub-Saharan Africa alone where it is grown predominantly by smallholder farmers under rain-fed conditions. The arable land under irrigation is approximately 5 % (Kanitila*et al*., 1998)

All parts of the crop can be used for food and non-food products. In industrialized countries, maize is largely used as livestock feed and as a raw material for industrial products. Maize accounts for 30−50% of low-income household expenditures in Eastern and Southern Africa. The grains are rich in vitamins A, C and E, carbohydrates, and essential minerals, and contain 9% protein. They are also rich in dietary fiber and calories which are a good source of energy [http//www.iita.org/maize].

1.2 Objectives

1.2.1 Overall objective

To develop maize hybrids resistant to Maize Lethal Necrosis (MLN) disease in Tanzania.

1.2.2 Specific objectives

i. To identify maize genotypes for Maize Lethal Necrosis Disease (MLND) resistance

ii. To develop and validate F_1 hybrids with resistance to MLND under natural disease pressure in Arusha and Manyara region of Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1Maize production in Tanzania

Maize is the 5th agricultural commodity in the United Republic of Tanzania by value of production during the period 2005-2010 accounting for 7.5 percent of total production value. Moreover, it represents close to five percent of total Agricultural imports in the country during the same period and is the main energy source in the diet accounting for 25 percent of total caloric intake (FAOSTAT, 2010). Maize provides 60 percent of dietary calories and more than 35 percent of utilizable protein to the Tanzanian population. Maize is produced for both human consumption and the market use, about 40 percent is sold, mostly locally (Nancy and Anna, 2015). Annual per capita consumption is 73 kg per person per year (DT-Maize, 2014). However, there is considerable geographical variation on consumptions. It accounts for 51% of total calories in the Southern Highlands and 32% in the Lake Zone. It makes up a larger share of calories than any other food category (Nancy and Anna, 2015).

It is grown almost in every part of the country by the small holder farmers who produced about 85% of the total maize production (Mbwaga, 1988). Almost all Agro- Ecology of Tanzania is suitable for maize production; Lake Zone, West Zone, Northern Zone, Central Zone, Eastern Zone, Southern Zone and Southern Highland. However, the Southern Highlands (Iringa, Mbeya and Rukwa) produce more than 50% of total National maize production (Mdadila, 1995).

2.1.1 Maize production constraints in Tanzania

Maize production in Tanzania is dominated by smallholder farmers. Despite the importance of maize as the main staple crop, average yields in farmers' fields are relatively low averaging to 1.2 metric tons per hectare compared to the estimated potential yields of 4–5 metric tons per hectare (WEMA,2010).While farmers are keen on increasing maize productivity, their efforts are hampered by a wide range of constraints. Low soil fertility, drought, and insect pests are among the primary constraints in maize production.

In 2012 a new maize disease known as Maize Lethal Necrosis Disease (MLND) was identified in Mwanza along Lake Victoria, Arusha, Manyara and west part of Kilimanjaro region in Northern Zone. Unlike other diseases MLND is devastating in nature and can cause complete crop loss. In Tanzania the infected maize plant samples were serologically tested in 2012 by the Ministry of Agriculture, Food Security and Cooperatives and showed positive results indicating the presence of Maize Chlorotic Mottle Virus (MCMV) and Sugar Cane Mosaic Virus (SCMV).

According to the survey conducted by CIMMYT in 2012, potential yield loss of more than 60% was reported in the affected areas. Infection rate and damage can be very high seriously affecting yields and sometimes causing complete crop loss (Wangai *et al*., 2012). Infected plants are frequently barren; ears formed may be small or deformed and set little or no kernels at all. So far the outbreak of the disease is a serious threat causing food shortage in the country especially in the Northern and Lake Zones. The plant can be affected at any growth stage from seedling to maturity.

Maize Lethal Necrosis Disease (MLND) is currently among the great threat to most of the African countries' food security including Tanzania since it affects the staple food crop (Maize). According to Wangai *et al*., (2012), it can cause 40-100% crop loss whereby crop can be affected at any stage of growth. Since MLND is a viral disease it has no cure. However, development of a resistant cultivar is among the best option to control the disease. Preliminary data from one season of screening in Kenya under natural disease pressure of forty three pre-commercial maize hybrids and seven commercial hybrids at Bomet, Chepkitwal and Naivasha, and 200 elite inbred lines at Naivasha show that MLN-resistant maize germplasm can be identified and developed as a long-term solution in controlling the disease (Wangai *et al*., 2012).

In Tanzania although the disease spreads in many parts of Northern, Lake and Southern highlands zones little is known about it. The target areas of study are Ngaramtoni and Mlangarini in Arusha and Kirusix in Manyara where the incidence of MLND is currently high.

2.1.2 Major Viral disease in Maize

Genes or major QTL for resistance to Maize Dwarf Mosaic Virus (MDMV), Wheat Streak Mosaic Virus (WSMV), Maize Mosaic Virus (MMV), Maize Streak Virus (MSV), High Plain Virus and Maize Chlorotic Dwarf Virus (MCDV) have been mapped in the maize genome (Redinbaugh *et al*., 2004). The viral pathogens are globally distributed with divergent host ranges, for instance currently there are at least eight viruses known to cause significant agronomic losses in maize worldwide (Table1).

Table 1: Distribution of Some Plants Virus restricted to grass family

Virus	Acronym	Virus Family	Coverage
Maize dwarf mosaic virus	MDMV	Potyviridae	World wide
Sugar cane mosaic virus	SCMV	Potyviridae	World wide
Wheat streak mosaic virus	WSMV	Potyviridae	World wide
Maize mosaic virus	MMV	Rhahdoviridae	Carribean
Maize streak virus	MSV	Geminiviridae	Africa
Maize Chlorotic dwarf virus	MCDV	Sesquiviridae	USA
Maize Chlorotic mottle virus	MCMV	Tombusviridae	USA
Maize rayadofino virus	MRFV	Marafivirida	Carribean
Maize rough dwarf virus	MRDV	Fijiviridae	Europe/Asia/Africa
Maize riocuarto virus	MRCV	Fijiviridae	South America
Maize streap virus	MStV	Tenuivirus	World wide

Source: CABI, 2014

2.1.3 Breeding for Disease Resistance

Generally, the methodology ofbreeding for disease resistance is the same as that used in breeding for any other trait. Although effort was spent on breeding disease resistant cultivars before 1900, the discovery of Mendel's work lighted the way for producing them scientifically (Curtis, 1973). Availability of suitable sources of resistance is a basic prerequisite for successful resistance breeding. In the beginning of resistance breeding, sources of resistance were to be selected among cultivated crops or their wild relatives.

Since plants encounter numerous beneficial and harmful organisms (pathogens) in their environment and use different strategies and mechanisms to cope with in order to survive and reproduce successfully, basal resistance is of great concern. Basal resistance is referring to the constitutive defence provided by pre-existing physical and chemical barriers in order to disable penetration of pathogen to the host-cell. Another aspect of basal resistance is the recognition of microbial surfaces by cell surface receptors that trigger immune response and offer broad-spectrum resistance

2.1.4 Types of Disease Resistance

Genetic resistance in plants is often divided into two major classes which are qualitative and quantitative resistance. Qualitative or major-generesistance is based on single major-effect resistance genes (R genes) and generally provides race-specific and high-level resistance. Quantitative resistance on the other hand, has a multi-genic basis and generally provides non-race-specific intermediate levels of resistance. Quantitative resistance is conferred by many genes with small effects. It is generally assumed to be non-race specific (though exceptions exist) and provides intermediate to high levels of resistance (Balint-Kurti and Johal, 2009). On the other hand, qualitative resistance is often associated with a rapid cell death called a hypersensitive response (HR) around the point of pathogen ingress. This is generally quickly overcome when deployed in the field, though there are exceptions (Peter and Gurmukh, 2009). The vast majority of genetic resistance used by maize breeders is quantitative. The major factor might be that maize is substantially more genetically diverse than wheat or rice, probably because it alone is an out-crossing species (Buckler et al., 2001; Cited by Peter and Gurmukh, 2009).

Qualitative resistance typically confers a high level of resistance which is usually race-specific and is based on single dominant or recessive genes. In contrast, quantitative resistance in plants is typically partial and race-nonspecific in phenotype oligogenic or polygenic in inheritance and is conditioned by additive or partially dominant genes (Randall*et al*., 2006). In addition, gene by gene (epistasis) and gene by environment interactions play an important role in the phenotypic expression of QTLs complicating fine mapping and cloning approaches (Ali and Yan, 2012).

The evolution of resistance genes is a dynamic process involving duplication, deletions, sequence exchange, mutations, diversified selection, recombination, gene conversion and retro-element insertion, while the cluster arrangement of resistance genes seems to arise by gene conversion, gene duplication, unequal crossing-over, ectopic recombination or diversifying selection (Friedman and Baker, 2007; Ribas*et al*., 2011).

2.1.5 Plants Resistance to Viral Pathogens

Viruses are obligate intracellular microscopic entities that require host factors for replication and spread. A virus is defined as a nucleoprotein that multiplies only in living cells and has the ability to cause disease (Agrios, 2005). In contrast with other pathogens that cause diseases by consuming or killing host cells with toxins, viruses cause diseases by utilizing the host cellular machinery and disrupting plant cellular process (Agrios, 2005). Most viruses require vectors to spread and move from plant to plant. The vast majority of vectors transmitting viruses are arthropods and a few are transmitted by fungi or nematodes (Agrios, 2005; Lapierre and Signoret, 2004)

Viral diseases are of special importance in crop production due to the high losses in yield and quality. It is also important to note that there are no direct counter measures available to fight them. When infected, no curative methods can be applied to recover healthy plants; and only preventive control is efficient in impeding viral epidemics thus breeding for virus resistance is therefore of special interest.

2.1.6Genetic Mechanism for Viral Resistance in Maize

Plants have evolved in an environment rich with microorganisms that are eager to capitalize on the plants' biosynthetic and energy-producing capabilities. There are approximately 450 species of plant-pathogenic viruses, which cause a range of diseases (Jennifer *et al.,* 2005). Depending on the virus, characterization of the genetic basis of virus resistance in maize has had relatively modest success. Characterized virus resistance in maize is primarily dominant and monogenic or oligogenic, such as resistance to the potyviruses MDMV, SCMV, or tritimovirus WSMV (Ding *et al*., 2012), but it can also be polygenic or quantitative as resistance to MCDV or MMV (Jones *et al*., 2004).

The study of classical Mendelian segregation ratios and QTL analysis provided insights into type of resistance, the mode of action, and the genetic location.However the number of genes involved in resistance and their mode of action has varied across germplasm and experimentscomplicating the analysis and interpretation of the results (Jones *et al*., 2007; PokornyandPorubova, 2006). This variation has been attributed to the use of diverse maize genetic sources, virus isolates or strains, different classification systems for resistant and susceptible plants, and the presence

of genes that modify the activity of resistance loci as well as to the presence of disease escapes and environmental effects(Jones *et al*., 2007; Jones *et al*., 2011).

2.2 Breeding for Viral Disease Resistance

The principles of breeding for viral resistance do not differ with any other breeding methods for biotic and abiotic stress resistance. According to Johnson and Jellis (1992), host resistance is the major means of controlling plant viruses. Dominant resistant alleles are strongly associated with virus localizing mechanisms normally involving local lesions (Johnson and Jellis, 1992). Breeding methods thus differ depending on the mode of pollination (self or cross) and the type of propagation (vegetative or generative). The resulting cultivars can be grouped into four major categories: (a) lines propagated by self-pollination (b) population propagated by cross pollination (c) hybrid propagated by controlled crossings and (d) clonal propagated varieties. Breeding methods differ between these categories.

Plants have developed genetic mechanisms to suppress virus multiplication and/or spread into other parts of the plant. The use of genetic resistance is considered the most economically and environmentally sustainable approach to control viral disease (Gomez *et al., 2009*; Redinbaugh and Pratt, 2009). Incompletely dominant and recessive alleles allow the spread of the virus but inhibit multiplication or symptoms development. Fully recessive alleles may be associated with complete immunity (Johnson and Jellis, 1992).Breeding for virus resistance was successful in the past years using conventional breeding methods since many virus resistant cultivars have been delivered for a wide range of crops (Carole*et al*., 2011).

Quantitative and qualitative types of resistance to virus diseases in plants have been reported, but in the vast majority of cases, virus resistance has been conferred by a single gene (Gomez *et al*., 2009). The hypersensitive response (HR) mediated by *R* genes is similar to that described for other pathogens, but in many cases virus resistance is not associated with HR (Kang *et al*., 2005).

Maize virus resistance conferred by single dominant genes have been associated with the suppression of systemic virus movement rather than programmed cell death (Redinbaugh and Pratt, 2009). The fact that the virus replicated in protoplasts of the resistant cultivar supported the hypothesis that resistance was conferred by a transient lack of movement.

2.2.1 Maker Assisted Selection in Breeding for Disease Resistance

Genome mapping provide Molecular Markers for many resistance loci (major gene or quantitative trait loci) that are to be introgressed into cultivar for instance through backcrossing breeding scheme. Molecular mapping also derived much information on the genomic architecture polygenic and quantitative resistance. However Marker Assisted Selection (MAS) for such complex trait is difficult so the combination of quantitative resistance factors from multi allelic origin commonly relies on sophisticated phenotyping procedures (Carole *et al.,* 2011).

2.3 Maize Lethal Necrosis Disease Overview

2.3.1 The ecology and Distribution

Maize Chlorotic Mottle Virus which is a core virus particle for the Maize Lethal Necrosis to occur was first identified in Peru in 1973 (Castillo and Hebert, 1974) and

subsequently reported in the USA in Kansas and Nebraska and the parts of Latin America (Niblett and Claflin, 1978). The virus is now distributed across the globe, for example in Asia (China) was reported in 2010, North and Central America and Africa. Although one of its component viruses, Sugar Cane Mosaic Virus (SCMV) was not new in most of the African countries including Tanzania, Maize Chlorotic Mottle Virus (MCMV) is a new viral strain in Africa (Louie *et al*., 1980). The virus is now present in many parts of East, Central and Southern African countries. As with all viral diseases in plants, a vector transmits the MLN viruses from plant to plant and field to field. MCMV is carried by thrips and beetles and SCMV by aphids (Nault*et al*., 1978 and Jiang *et al*., 1992).Transmission of MCMV via seed from infected plants is normally very low about 0.04% as reported by (Jensen *et al*., 1991).

It is also evident that most of plant viruses survive in wide range of environment including those hosted by grass family.

2.3.2 Characterization of Maize Chlorotic Mottle Virus (MCMV) Associated with MLND

Maize Chlorotic Mottle Virus (MCMV) is an icosahedral plant virus, 30nm in diameter composed of a single 25kDa capsid protein subunit and 4.4-kb single stranded positive sense Genomic RNA (Lommel *et al.*, 2002).The smaller double stranded RNA corresponds to a 1.1kb sub genomic messenger RNA that is homologous to the 3'- terminal region of MCMV genomic RNA and encodes the viral capsid protein (Lommel*et al.*, 2002). According to Sharma and Misra, (2011), majority of the plants virus have the positive stranded RNA genome, (+) RNA

compatible with the protein translation apparatus of the host. The viruses have no known physiological functions and enzymatic activities and their entry into the host cell depends on the vectors or wounds (Narayanasany, 2008). The plants viruses therefore have to accomplish four main steps for successful infection of the plant, (a) entry into the plant cells (b) replication in the primarily infected cells (c) cell-to-cell movement through plasmodesmata and (d) long-distance movement through vascular system (Narayanasany, 2008).

2.3.3 Host Range of MLND Virus

The host range of the disease is restricted to *Poaceae*family with maize as the main natural host. Use of tolerant or resistant varieties ultimately would be the most effective means of managing the MLND. Superior resistance to MCMV is widely available in the tropical maize stocks and they provide the best control of the disease. According to Nelson *etal.,* (2011), trial performed in Hawaii in 2011 found many tropical inbred and varieties to be highly resistant to MCMV. It was reported that 30 out of 40 inbreds (75%) of the Hawaii showed positive to resistance. However no complete immunity was observed. Most of the temperate climate inbred lines and hybrids are highly susceptible to the virus. The level of MCMV resistance widely varies among pure lines tested in Hawaii suggesting that it is a quantitative trait (Nelson *et al.,* 2011). Preliminary studies on the inheritance pattern suggest a polygenic control of the disease with resistance partially dominant; this encourages the production of hybrids only if both parents are resistant to the pathogen.

2.3.4 Transmission and Symptoms of MLND

As with all viral diseases in plants, a carrier known as a “vector” transmits the MLN viruses from plant to plant and field to field.Maize chlorotic mottle Virus (MCMV) causes a variety of symptoms in maize depending upon genotype, age of infection and environmental conditions. They range from a relatively mild chlorotic mottle to severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and/or shortened, malformed, partially filled ears (Castillo and Herbert, 1974; Niblett and Caflin, 1978). When MCMV co-infects maize with anypotyvirus, the infected plants in the field show a diverse range of symptoms. Diseased plants develop symptoms characteristic of virus diseases. There is Chlorotic mottling of the leaves, usually starting from the base of the young leaves in the whorl and extending upwards toward the leaf tips. The leaves can experience necrosis at the leaf margins that progress to the mid-rib resulting in drying of the whole leaf. If there is necrosis of young leaves in the whorl before expansion, then 'dead heart' symptoms will be visible. Other symptoms include premature aging of the plants and mild to severe leaf mottling. Severely affected plants form small cobs with little or no grain set. The entire crop can frequently be killed before tasseling (Niblett and Claflin, 1978; Wangai*et al*., 2012).

2.4 Reaction of Viral combination in the host plant

Viral interactions in the host plant may either be antagonistic or synergistic depending on the nature of virus and host involved.

2.4.1 Synergism reaction

Co-infection of plants by two or more unrelated viruses may result in disease synergism with symptoms more severe than the additive effects of each virus individually (Drake, 2007).

Corn lethal necrosis disease (CLND) is a field example of synergism caused by double infection of maize with the Machlomovirus*MaizeChloroticmottlevirus*(MCMV) and one of several maize infecting potyviruses. Separately, each virus induces systemic chlorosis but not necrosis. In contrast, infection of maize with both viruses causes extensive necrosis, stunting, and premature death of infected plants (Drake, 2007). In the second class of interaction, co-infection of a host plant with two unrelated viruses elicits disease symptoms that are more severe than the sum of those induced in either single infection (Prus*et al*, 1997). Interestingly, a large number of reported plant viral synergisms involve a member of the potyvirus group of plant viruses as one of the synergistic pair. Several of these potyvirus-associated synergisms have been examined in some detail: First is the Corn lethal necrosis caused by co-infection with Maize Chlorotic Mottle virus (MCMV) and maize dwarf mosaic virus (MDMV) potyvirus.

The second is the interaction of bean pod mottle virus (BPMV) with soybean mosaic virus (SMV), potyvirus (Prus*et al.*,1997). In each of these potyvirus-associated synergisms, the level of the non-potyvirus in the synergistic pair MCMV and BPMV increases 5-10 fold in co-infected plants, while the level of the potyvirus is

unchanged from that seen in singly infected plants. The increase in the non-potyvirus accumulation is correlated with the increased symptom severity typical of the co-infection. However, it is not clear that the positive correlation between non-potyviral accumulation and symptom severity reflects a cause and effect relationship and in fact, the basis of synergistic disease is not understood (Prusset *al.*, 1997).

2.4.2 Antagonistic Reaction

Often the multiplication of one virus interferes with the subsequent replication or movement of another virus in the same host and renders the plant resistance to the second of the two invading viruses (Prusset *al.*, 1997). According to (Zhanget *al.,* 2001), sharing the same host population implies competition, and this imposes an increased constraint on the survival of both viruses. It was shown that, in order to ensure virus survival in a mixed infection, the basic reproductive number should exceed a critical value which is larger than unity (R0 >Rc> 1). Increased virulence (equivalent to disease severity) in dually infected plants decreases the opportunities for both viruses to coexist, while increased virus transmission from dually infected plants increases such opportunities (Zhanget *al*., 2001). Doubly inoculated plants with the lowest WSMV levels also had the lowest MCMV concentrations, but the concentrations of MCMV and WSMV in the most heavily infected plants did not directly correlate. These results suggest that there are genes in both MCMV and WSMV which directly or indirectly affect the replication and/or spread of the other virus in CLN (Sheets, 1997)

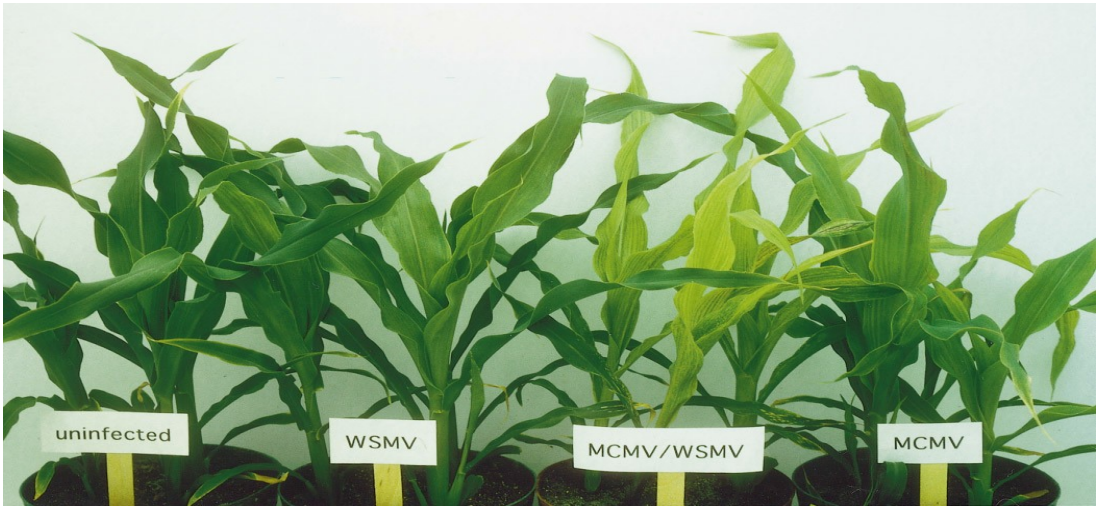

Figure 1: Illustration of un- infected, singly and co-infected maize plants for comparing the synergism effects.

Source: Kay Sheets, (1997)

2.5 Breeding efforts for Maize Crop in Tanzania

Breeding efforts in the 1960s resulted in the release of Ukiriguru Composite (A) (UCA) and Ilonga Composite White (ICW). The government launched a Maize Project in 1974, with assistance from the U.S. Agency for International Development (USAID) to promote maize production in pursuit of food self-sufficiency (Lyimo*et al*., 2014). The National Maize Research Program (NMRP) was launched with the broad objective of developing cultivars suitable for the major maize producing areas (Nkonya*et al*., 1998). Since the mid-1970 to the mid-1990, about 15 improved maize varieties (hybrids and OPVs) have been released by the NMRP. Although Ukiriguru composite no longer exist, Ilonga Composite White (ICW) was changed to Tanzania Maize Variety 1 (TMV1) at ARI-Ilonga to overcome the water logging and Maize Streak problems. The former Ilonga Composite have long stems and susceptible to Maize Streak Virus (Mbiza, A.B.C, Personal communication, 2015).

2.5.1 Breeding efforts for disease Resistant Cultivars in Tanzania.

The Maize Improvement Programme (MIP) at Uyole Agricultural Research Institute commenced a massive screening and evaluation of both local and exotic commercial and pre-commercial maize varieties and inbred lines for Gray Leaf Spot (GLS) tolerance (Lyimo, 2006). Evaluation of the GLS-tolerant germplasm both on-station and on-farm in these districts confirmed the superiority of several potential new maize hybrids one of which was officially released during the 2000/2001 season under the name UH615. A further GLS-tolerant hybrid, UH6303, was approved for release in late 2004. In addition to their disease resistance, farmers like these varieties because they are high yielding compared to their local varieties. Other MSV-tolerant (but not GLS-tolerant) OPVs (Open Pollinated Varieties) were also available, e.g. Staha and TMV1developed at ARI-Ilonga but variety trials conducted in Mbarali district had found that this tolerance was inadequate under the very early and severe MSV disease occurring in irrigated areas where vectors and disease are maintained year-round.

A good balanced maize improvement program must involve development of improved source of germplasm, development of new superior inbreds and improvement of established inbreds, through recurrent selection for population improvement and pedigree method in developing improved inbred lines. Germplasm used come from within Tanzania, CIMMYT-Kenya, CIMMYT-Zimbabwe, CIMMYT-Mexico, IITA-Nigeria and other NARS breeding programs (Kitenge, 2010).

According to the Zonal Agricultural Research Development Funds (ZARDEF) Annual Progress Report (2010), the germplasm collected in 2005 were planted for evaluation. After several selfings and selection few inbred lines are starting to stabilize. This is at S_5 stage of development at ARI Selian. Several new varieties were developed using our maize inbred lines crossed with acquired ones to develop three–way hybrids. Eight varieties were developed and tested for tolerance/resistance to Grey Leaf Spot (GLS) and the results were promising.

Besides the fact that there are number of Maize breeding initiatives in Tanzania, for example drought, Nitrogen stress and disease and other pest tolerance there is no pronounced efforts in combating MLND. Though screening and selection of parental materials are in place, there is no published information on the progress.

2.5.2 Prevention and Control strategies for mitigation MLND epidemic.

2.5.3 Agronomic control

In the short-term farmers are advised to uproot and remove affected plants , opting for crop rotation or grow alternative crops, conscious to specific season and planting timely to avoid spread of the disease, apply good agronomic practices and chemical spraying of vector under specific circumstances (De-Groote *et al*, 2002). A plant health inspectorate organization can test for *Maize Chlorotic mottle virus* (MCMV) in all seed coming into the country including the material for breeding. Domestic regulation can be put in place to prevent the movement of maize products from affected areas to disease-free regions. The public can be informed about the disease through press releases, posters, brochures, sensitization workshops and radio

programmes. Good agronomic practices, field hygiene and destroying affected plants are among the short term solutions available to farmers

2.5.4 IPM control

Another best approach for the management of MLND is to deploy integrated pest management practices encompassing cultural control such as timely planting crops, crop rotation and crop diversification, vector control using seed treatment followed by broad-spectrum foliar sprays, and host-plant resistance. Vector control should target soil borne and early season vectors and combine long residual and fast-acting control agents to achieve faster knockdown and longer protection. Application of seed dressing in combination with foliar sprays can reduce the early stage infestation. In Hawaii producers of maize seed spray regularly after planting to control insects that spread the virus the result is promising (Nelson *et al*., 2011).

2.5.5Cultural Control and Sanitary Measures

Crop rotation can effectively control MCMV. Producers are advised to practice crop rotation for at least two seasons with alternative non-cereal crops such as potatoes, sweet potatoes, cassava, beans, bulb onions, spring onions, vegetables and garlic. Planting different crops each season will diversify farm enterprises as extra benefits for farmer. Manure and basal/top dressing fertilizers can be applied to boost plant vigour. It is necessary to use good field sanitation methods, including weed control measures to eliminate alternate hosts for potential vectors (Wangai*et al*., 2012). Infected foliar material should be removed from the field to reduce pathogen and vector populations.

2.5.6Movement Control

This is based on the regulation set by governments to impose quarantine on the movement of maize seeds or any other material that can habour viral particles from theaffected areas within or outside the country. Enforcing such regulations can be challenging but, alongside increased awareness by the farming community, they can help to reduce the spread of the disease.

2.5.7Host-Plant Resistance

Use of tolerant or resistant varieties ultimately would be the most effective means of managing MLND. Superior resistance to MCMV is widely available in tropical maize seed stocks and provides the best control for this disease. In Kenya, varieties are being screened for resistance/tolerance by KARI and CIMMYT in two sites Naivasha and Bomet. Preliminary data gave hope to control the disease. Since MLND is due to the co-infection of two viruses which are MCMV and any other *Potyvirus*, resistance against any one of the viruses would substantially reduce the damage. Results of a trial of elite CIMMYT inbred lines under artificial SCMV inoculation showed several highly-resistant lines (Makumbi and Wangai, 2012). In Tanzania few inbred lines developed at Selian Agricultural Research Institute and the double haploid lines from CIMMYT were tested in Ngaramtoni and Babati under natural infestation during 2014. The results show some moderately resistant lines emanating from the lot (Kitenge, K. Personal communication, 2015). In the long run, deployment of varieties that are resistant to both MCMV and SCMV will be the best means of managing MLND.

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CHAPTER THREE

3.0 Identification of new sources of resistance for Maize Lethal Necrosis Disease (MLND) from the diverse maize germplasm.

3.1 ABSTRACT

Maize Lethal Necrosis Disease (MLND) infestation on farmers' fields is one of the major factors responsible for low maize yields in Northern Zone of Tanzania. It is estimated that 40-100% crop loss is associated with the disease. Identification of the new sources of resistance to MLND would provide options towards MLND control. A total of one hundred maize genotypes of different genetic background were screened under natural epiphytotic in Arusha and Manyara during 2015 cropping season. Out of these genotypes sixty were inbred lines collected from CIMMYT and Selian Agricultural Research Institute, thirty landraces collected from farmers in Arumeru, Karatu, Mbulu and Babati rural and ten improved varieties (both OPV and hybrids) commonly used in the Northern zone. The best performing inbred lines were CML144 and CML 312 in most locations. However most landraces showed the promising results than inbred lines and improved varieties. Thus superior genotypes were identified among the accessions which can therefore be used in the development of maize varieties adapted for areas prone to MLND infestation.

Key words:*MLND, Landraces, Natural epiphytotic, inbred lines.*

3.2 INTRODUCTION

Crop plant diseases caused by various pathogens such as viruses, bacteria, oomycetes and fungi pose major challenges to global crop production and food security. Global climate change is predicting to further increase the negative impact of biotic stresses. Higher temperatures and erratic weather pattern are likely to change the geographical pathogen distribution. This in turn might decrease the effectiveness of existing resistance genes in crop varieties by promoting more aggressive races of pathogens (Garrett *et al*., 2006; Milus*et al*., 2009: cited by Kumar *et al*., 2014).Due to these facts boththraditional and modern approach in breeding for disease resistance in crops are inevitable. The utilization of disease nurseries in hot spots areas (places where the disease is severe and commonly found) is the easiest approach to screen for virus resistance in maize. This approach was used as an initial step to combat MLND epidemics in Kenya and Tanzania. The disease emerged as a serious concern for the farming communities of Eastern Africa, especially in Kenya, Tanzania and Uganda in early 2012. In Tanzania the current maize production status is estimated between 1.2 to 2.0 tones/ha. However the production is expected to be below these figures due to the MLN epidemic (CIMMYT, 2012).

Maize Lethal Necrosis disease is a complex disease that consists of not just one virus, but at least two viruses of *Potyviridae* family to infect the plant. The disease usually requires a combination of Sugarcane Mosaic Virus and maize Chlorotic mottle virus (MCMV) to infect a single plant. Traditional methods of plant breeding have been employed in an attempt to transfer resistance to MLN or MCMV into commercially viable germplasm for decades. However, little is known about local germplasm in Tanzania.

3.3 Materials and Methods

3.3.1 Experimental Materials

Sixty maize inbred lines were collected from the International Maize and Wheat Improvement Center (CIMMYT) and Selian Agricultural Research Institute. Some of these lines were screened both in screen house using artificial inoculation and in the field under natural disease pressure in Naivasha, Bomet and Chepkitwal in Kenya. Some of these materials were also tested in Tanzania at Ngaramtoni in the field under natural infestation. Thirty entries were landraces collected from farmers in Arumeru, Karatu, Mbulu and Babati, while ten entries were the maize varieties both hybrids and Open Pollinated Varieties (OPV's) commonly used in the Northern Zone of Tanzania to make the total of one hundred genotypes for screening. (APPENDIX 1).

3.3.2 Description of Study Areas

The study was conducted in the Northern Zone of Tanzania which covers Arusha, Kilimanjaro, Manyara and some parts of Tanga based on Agricultural Research Mandate Areas. The Zone is characterized by several farming systems and Agro-ecological sub-zones with elevation varying from 900 masl to more than 2500 masl. The rainfall is mono-modal type where the estimated annual average ranges between 1000mm to 1500mm. the crops grown are maize often intercropped with beans, wheat, rice, pigeon pea, coffee, banana, sugar cane, sunflower sesame and wide range of horticultural crops. The study was therefore conducted at Ngaramtoni ($3^0$18′S and $36^0$38′E), Mlangarini ($3^0$13'S and $36^0$86'E) and Kirusix in Babati-Manyara ($4^0$13'Sand $35^0$45'E).

3.3.3Research Methodology

One hundred maize genotypes were screened at Ngaramtoni, Kirusix and Mlangarini where the incidence of MLND is very high. Different classes of genotypes were screened in order to identify the new source of resistance to MLND. The experiment was laid down in an Alpha Lattice Randomized Incomplete Block Design and replicated three times in each location during 2015 season. Each entry was planted in one row plot of 5m long. DAP fertilizer was applied at a rate of 10grams per hill. Urea was applied 3 weeks after emergence and repeated after 8 weeks for top dressing. Inter and intra row spacing was kept to 0.75m by 0.30m respectively. Three spreader rows of CML 503 entry were planted as boarder rows. This is the highly susceptible line used as check. Pesticides were not applied to allow vectors to infest the field**.**

3.3.4 Data Collected

Data collected includes disease scores, days to 50% flowering and grain weight. Disease scores were rated on scale of 1-5 disease severity according to Shekha and Kumar, (2012) as follows: 1= Resistant (No Symptoms), 2=Moderately Resistant, 3= Moderately Susceptible, 4= Susceptible, 5= Highly Susceptible (plant dead completely). The disease scores were recorded three weeks after emergence (3WAE), six weeks after emergence (6WAE), ten weeks after emergence (10WAE) and fifteen weeks after emergence (15WAE).

3.3.5 Data Analysis

Data collected was subjected to analysis of variance (ANOVA) using the GenStat computer software, 13th edition after square root transformation. Data transformation is necessary in this study in order to normalize for the Analysis of variance to work. Treatment means separation was done using Fisher's Protected LSD at 5% level of significance.

3.4 RESULTS

3.4.1 Combined Analysis of Variance across Locations

The analysis of variance for the disease scores was done based on the results recorded on 15th week after emergence. This is the time where most of the genotypes attained the physiological maturity and the impact of new infection cannot cause significant crop loss.The Analysis of variance showed significant differences ($p \leq 0.05$) among genotypes for disease scores, 50% flowering and the days to maturity except for the Anthesissilking interval (ASI). The effects of location were also significant for all the variables studied. The locations x treatment interactions were significant ($p \leq 0.05$) for disease scores and days to maturity (Table: 2)

Table 2: ANOVA Summary for 100 screened genotypes for MLND across locations (Means square given)

SV	df	Disease Scores	ASI	50% Flowering	Days to Maturity
Treatments	99	2.497*	9.44	54.96*	126.18*
Replication	2	0.068	27.51	0.04	0.77
Location	2	5.201*	194.04*	141.53*	54.91*
Treatment x Location	198	0.870*	0.71	35.98	73.98*
Residue	598	0.654	15.59	31.76	51.29
Total	899				

*= P≤ 0.05 Level of significance

3.4.2 The promising Genotypes across Locations

Disease severity scores were done using standard disease severity scores according to Shekha and Kumar (2012) as previously explained. The scoring was done on the 3rd week, 6th week, 10th week and 15th week after seed emergence. 15th week is the

period when all the genotypes attained physiological maturity thus there will be no new infection causing crop loss.Although MLND infection can occur at any growth stage thecritical period was observed when crops approach flowering stage and during flowering.

Out of hundred genotypes screened no one has shown complete immunity against MLND in all three locations three locations.However, CML144 and CML 312 are the only two inbred linesshowed some levels of immunity. On the other hand, most landraces showed some degrees of tolerance to disease than inbred lines.

Hence, in the top ten promising genotypes two were inbred lines and eight landraces(Table3) and the entire table for all the genotypes is presented in (Appendix1).

The disease severity also differs among the locations where the study was conducted. Ngaramtoni showed higher disease severity compared to the rest of the locations (Fig.2). This is among the areas where the disease was first reported in the Northern Zone in 2012.

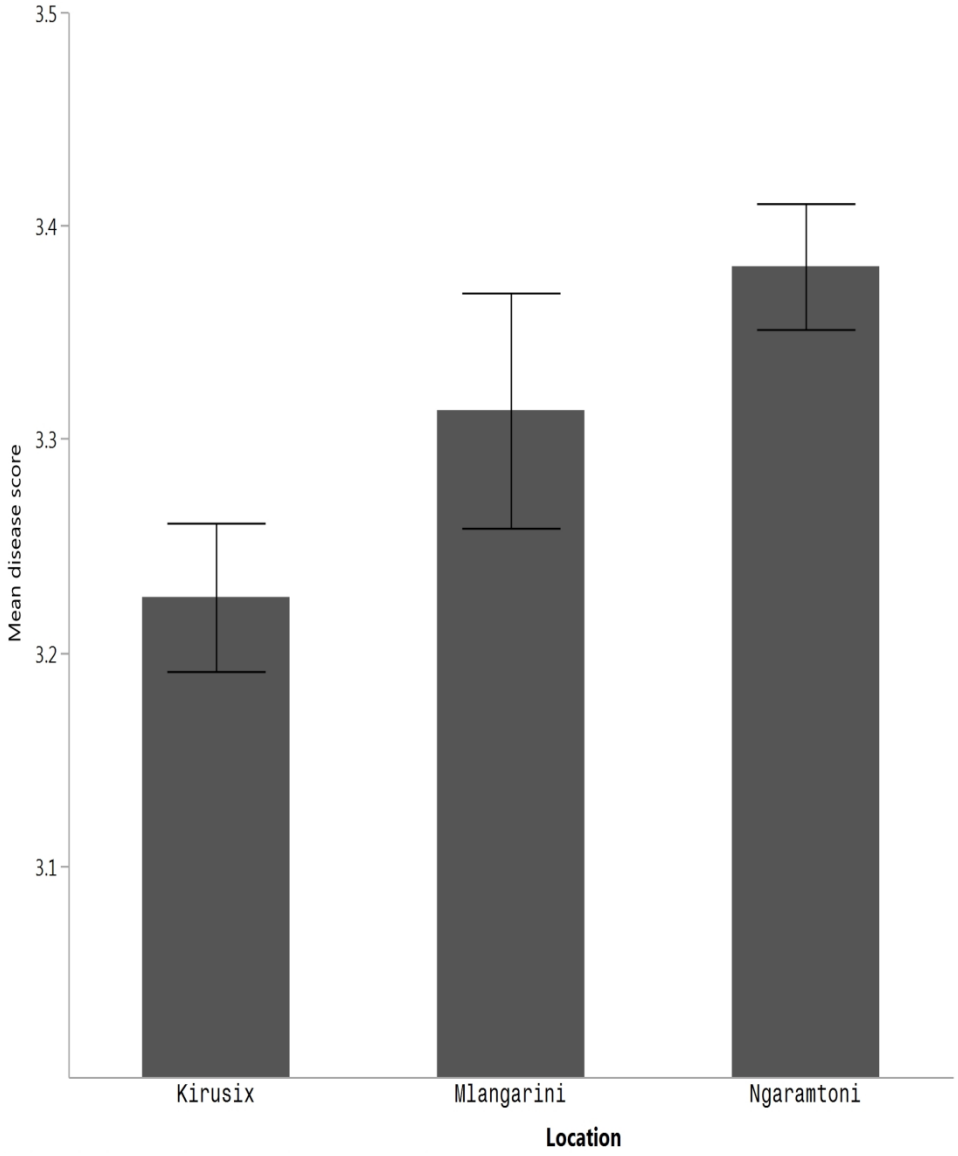

Figure 2: Disease severity scores using standard scale (1-5) for one hundred screened maize genotypes in three locations during 2015 cropping seasons (statistics given in Table:2)

Table 3: The top ten promising genotypes from the combined analysis across locations based on the disease scores indicating the level of damage for one hundred screened genotypes

Entry	Genotype	Mean disease Scores	Genotype Category	Ratings
1	CML 144	2.333 m	Inbred line	MR
2	LMBL06	3 lm	Landraces	MS
3	LAR03	3.222 kl	Landraces	MS
4	LMBL03	3.222 kl	Landraces	MS
5	LKRT08	3.222 kl	Landraces	MS
6	CML312	3.333 jkl	Inbred line	MS
7	LKRT05	3.333 j-l	Landraces	MS
8	LMBL02	3.333 j-l	Landraces	MS
9	LMBL04	3.333 j-l	Landraces	MS
10	LKRT04	3.444 i-l	Landraces	MS
	Grand Mean	4.11		
	LSD	0.7848		
	SE	0.8088		
	F-Value	3.82		
	P-Value	0.001		
	CV (%)	19.7		

Means within column followed with the same letters shows no significant difference based on Fisher's Protected LSD test at 5% probability level. MR= moderately resistant, S=Susceptible, HS= highly susceptible, WAE=Week after emergence.

3.4.3 The top ten promising genotypes in each Location

From the ANOVA (Table2) there is also Location X Genotype interactions, therefore in each location the top ten outstanding genotypes were determined based on the screening results.

InMlangarini for instance most of the genotypes were promising which indicate low disease severity compared to other location where the study were conducted. The top ten were landraces and one inbred line CML 144(Table4). All of these genotypes

showed moderate resistance based on the individual mean disease scores. The complete list is presented in Appendix 2.

Table 4: The top ten promising genotypes for Mlangarini based on the disease scores indicating the level of damage for one hundred screened genotypes

ENTRY	GENOTYPE	3WAE	6WAE	10WAE	15WAE	Individual Mean Disease Scores	Ratings
1	CML 144	1.7	1.7	2.3	2.3	2	MR
2	LMBL06	1.7	2	2	2.3	2	MR
3	LMBL08	1.7	2	2	2.3	2	MR
4	LMBL05	2.3	2.3	1.3	2.3	2.05	MR
5	LBBT05	2	1.7	2	3	2.175	MR
6	LKRT05	1.7	2	2.7	2.3	2.175	MR
7	LKRT08	2	2	2	2.7	2.175	MR
8	LMBL12	1.7	2.3	2	2.7	2.175	MR
9	LMBL01	1.7	2.3	2.3	2.7	2.25	MR
10	LMBL03	2	2.7	2.3	2.3	2.325	MR
Grand Mean		2.739	3	3.53	3.988		
LSD		1.38	1.26	1.17	1.27		
SE		0.856	0.784	0.726	0.156		
SED		0.6	0.641	0.5928	0.645		
F-Values		2.75	2.06	0.71	0.68		
P-Values		<0.01	<0.01	0.01	0.01		
CV (%)		31.3	26.1	20.6	19.9		

MR=moderately resistant, WAE= week after emergence

In Kirusixonly one genotype (CML144), this is an inbred line showed moderate resistance. Another inbred line, CML444 showed moderate susceptibility in this location while it was rated as susceptible in the rest of the locations. The landraces are still the best candidates in this location as in other locations (Table 5 and Appendix 3).

Table 5: The top ten promising genotypes for Kirusix based on the disease scores indicating the level of damage for one hundred screened genotypes

ENTRY	GENOTYPE	3WAE	6WAE	10WAE	15WAE	Individual Means disease scores	Rating
1	CML 144	1.7	1	2	2.3	1.75	MR
2	LKRT08	2	2	2.7	3.3	2.5	MS
3	LMBL04	2	2.3	3	2.7	2.5	MS
4	CML 444	2	2.3	2.7	3.3	2.58	MS
5	LAR03	2.3	3	2.3	3	2.65	MS
6	LKRT06	1.3	2.3	3	4	2.65	MS
7	LKRT05	1.7	2.7	2.3	4	2.68	MS
8	LMBL10	2	2.7	2.7	3.3	2.68	MS
9	LMBL12	1.7	2.3	2.7	4	2.68	MS
10	LAR04	2	2.3	3.3	3.3	2.73	MS
	Grand Mean	2.218	3.043	3.526	4.115		
	LSD,0.05	1.311	1.208	1.76	1.33		
	F-Values	0.89	2.25	0.83	1.32		
	P-Values	0.8	0.01	0.03	0.05		
	SE	0.815	0.751	1.091	0.827		
	SED	0.665	0.897	0.897	0.675		
	CV (%)	36.7	24.6	30.9	20.1		

MR= moderately resistant, MS= moderately susceptible, WAE= Weeks after emergence

ForNgaramtoni three inbred lines: CML 144, CML312 and DHL32 are among the top ten candidates. However CML 144 showed moderate resistance as in other locations while the rest showed moderate susceptibility to the MLND including landraces (Table6 and Appendix 4).

Table 6: The top ten promising genotypes for Ngaramtoni based on the disease scores indicating the level of damage for one hundred screened genotypes

ENTRY	GENOTYPE	3WAE	6WAE	10WAE	15WAE	Individual Means	Rating
1	CML 144	1.3	2	2.3	2.3	1.975	MR
2	SEEDCO627	1.3	3	3	3.3	2.65	MS
3	LMBL04	2.3	1.3	3	4.3	2.725	MS
4	LKRT07	1.7	2.3	3.7	3.3	2.75	MS
5	LMBL01	1.7	2.3	3	4	2.75	MS
6	DHL 32	2.3	2	3.3	3.7	2.825	MS
7	LMBL02	1.7	3	3.3	3.3	2.825	MS
8	CML312	2.3	2.7	3.3	3.3	2.9	MS
9	LBBT03	2	3	3	3.7	2.925	MS
10	LKRT04	2.3	3.3	3.3	3	2.975	MS
	Grand Mean	2.41	2.97	3.89	4.25		
	LSD at 5%	1.1	1.2	1.2	1.3		
	SE±	0.67	0.75	0.84	0.79		
	F-Values	1.48	1.77	0.99	1.27		
	P-Values	0.011	0.01	0.03	0.05		
	SED	0.54	0.6	0.68	0.65		
	CV (%)	27.6	25.2	21.5	18.7		

MR= moderately resistant, Ms= moderately susceptible, WAE= weeks after emergence

3.5 Discussion

The study was undertaken to screen different maize genotypes for their reaction to Maize Lethal Necrosis Disease in some areas of Arusha and Manyara in Northern Zone of Tanzania where maize is among the chief food and cash crop. The incidence of MLND was reported in 2012 and subsequent years thereafter. From the ANOVA results there is significant difference (P= 0.05) among genotypes (treatments) in terms of disease reactions and days to 50% flowering, thus significant genetic

variation exist among these genotypes. On the other hand, mean square for ASI didn't show significant difference among the treatments. Moreover, treatments by locations interactions were significant at 5% level of probability for the disease reaction and days to maturity (Table: 2).This indicates that the environmental factors are also among the important aspects in disease progression.

All the genotypes screened were observed to be infected with the disease with varied levels of severity. However, among the top ten genotypes showed moderate susceptibility, nine of them were landraces collected from farmers. These landraces therefore can be used in the maize breeding program to develop inbred lines with moderate resistance to MLND infection.

It is thus evident that new sources for MLND resistance can be exploited from landraces and some inbred lines as exhibited by the performance of these materials under natural infestation. This study therefore enlighten for further research, showing that, if large number of diverse germplasm were screened probably we can have potential breeding materials emanating from our germplasm. However both conventional and molecular breeding approaches can be followed to utilize R-gene and QTL that are mostly present in cultivated varieties and their wild relatives to confer durable resistance.

Given the diversity of strategies that pathogen use and their ability to rapidly adapt to new cultivar and environment it would be unreasonable to rely only on conventional breeding techniques. According to Thakur (2007), of the several

control measures available host plant resistance has been a strong choice for economic and effective management of plant diseases.Although many exciting insights have emerged from recent research on plant defense signaling, our overall understanding of the process is still fragmentary.For example, we still know very little about the structural basis of Maize Lethal necrosis virus recognition. The majority of described virus resistance genes characterized for dominant alleles in plants fall into the class of R genes (Gururani*et al*., 2012). However, not all characterized dominant virus resistance genes correspond to this resistance mechanism. It is not clear if a single gene or a cluster of genes are responsible for the multiple virus-resistance loci found in some maize chromosomes. From the results the level of virulence varies from one location to another probably due to difference in weather and strain variability for example the Ngaramtoni has more virulence than other locations. This information is not known yet since the disease is new in Africa and Tanzania in particular

3.6 Conclusion

The present study followed classical methods for genetic analysis of disease resistance that begins with the identification of resistant and susceptible genotypes, the determination of the mode of inheritance of the resistance, reciprocal effects in the parental lines and stability parameters based on genotypes by environmental interactions. The study also revealed that Genetic X Environmental interactions were important factor that influence the disease development among the screened genotypes. For instance CML 312 showed moderate susceptibility in Ngaramtoni but it was susceptible in Mlangarini and Kirusix. Likewise CML444 which was initially

known as susceptible inbred line performed better in Kirusix but highly susceptible in the rest of the locations

On top of that, it has also been found that landraces are better adapted against MLND than either improved varieties or inbred lines. This is because of the wide genetic diversity present in landraces than the improved genotypes.

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CHAPTER FOUR

4.0 Genetic Studies and validating single cross maize hybrids resistant to Maize Lethal Necrosis Disease in the Northern Zone of Tanzania.

4.1 ABSTRACT

The outbreak of MLND posed great challenge in Maize production, since the disease is new in the region and no known study has been done in Africa so far. Although the disease have been in other parts of the world for years, but most of these area where the disease was reported have different climatic conditions with most of the tropical regions and Sub-Saharan Africa in particular.

Nature of gene action and genetic parameters for disease reactions are very important attributes in developing resistant cultivars since it provide the sustainable, economically justifiable and environmentally friend means of controlling plant diseases.

In the present studya full diallel cross involving six genetically divergent maize inbred lines was performed to develop resistant cultivar against MLND. The field experiment was conducted in Kirusix using six inbred lines 2014.The evaluation trials were conducted under MLND hot spot areas in Mlangarini, Ngaramtoni and Kirusix during 2015 cropping season. The experiment was laid down in a Randomized Complete Block Design (RCBD) with three replications per location.Data collected were transformed using square root transformation. Data analysis was carried out according to Griffing (1956) design I model II (random model). The general combing ability (GCA) and specific combining abilities marked

significant differences among genotypes across all locations. GCA washighly significant at (P≤0.001) than SCA in all locations with mean squares of (5.551***), (1.61***) and (4.527***)for Mlangarini, Kirusix and Ngaramtoni respectively. The graphical analysis using Wr-Vr were performed in order to obtain the allelic constitutions of the arrays in different locations and the genotypes stability and suitability across locations was done using GGEbiplot analysis.

Key words:*Diallelcross,GCA, SCA, additive genetic effects, non-additive genetic effects, broad sense heritability, narrow sense heritability.*

4.2 INTRODUCTION

Maize is a natural host for more than 50 viruses and an experimental host for about 30 more (Lapierre and Signoret, 2004), but only some cause diseases that seriously affect yield (Ali and Yan, 2012; Redinbaugh and Pratt, 2009). Among the most damaging are members of the *Potyviridae*and*Maize Chlorotic mottle virus* (MCMV), which form the devastating complex known as maize lethal necrosis virus (Uyemoto*et al*., 1980; Wangai*et al*., 2012).Although plants have evolved passive and active defense mechanisms that are responsible for the suppression of virus multiplication and spreads, such mechanisms require interaction of plant and viral factors to confer plant resistance or susceptibility (Gomez*et al*., 2009). This information can be gathered through appropriate breeding technique.

Therefore, in this study full diallel cross was used in order to gather important genetic information of the parental materials to combat maize lethal necrosis virus regime. This technique has been extensively used and hailed by plant breeders as a long over-due methodology for rationalizing the genetic study of continuous variation (Jawahar, 2006). The strength of the diallel technique is that, additional information such as reciprocal effects, maternal and paternal effects and allelic distribution can be obtained quite in early generation (F_1 itself), thus useful to define breeding strategy without losing much time. The genetic material evaluated in diallel experiments includes random individuals from a population and progenies obtained by crossing those individuals in all possible combinations. Moreover diallel design is a useful tool of obtaining combining ability of the parents used in the cross. Combining ability has a prime importance in plant breeding since it provides

information for the selection of parents and also provides information regarding the nature and magnitude of involved gene action. The knowledge of genetic structure and mode of inheritance of different characters help breeders to employ suitable breeding methodology for their improvement (Kiani*et al.,* 2007). Genetic diversity and combining ability of lines are important to obtain high heterosis values in the development of maize hybrids. However, it is also important to consider the behavior *per se* of the line and the SCA for successful hybrids development.

Based on parental variance (Vr) and parent-offspring co-variance (Wr) relationships in diallel cross progenies, a two way representation or distribution of parental arrays along regression line were also studied. This two directional figure depiction is often known as "**Wr-Vr graph"** or Wr-Vr graphical approach whereby the allelic constitutions and the order of dominance or recessive can studied from parent-offspring regression.

Besides Wr-Vr graphical analysis the GGE biplot analysis was also used to identify the location suitable for specific developed single cross hybrids and stability parameters those materials.For selection purposes, the ideal genotypes are those with low mean for disease score and high stability in wide ranges of environment. In the biplot figure, they are close to the origin and have the shortest vector from the average tester coordinate (ATC). (Ezatollah *et al*., 2012).

4.3 Materials and Methods

4.3.1 Experimental Materials

The experimental materials used in this study were thirty hybrids developed from six maize inbred lines (CKH 10767, CKH 114272, CML312, CML444, CML503 and CML144) and 2 local checks (SeedCo527 and Selian 308). These hybrids were concurrently evaluated with their parents for MLND and yield in the MLND hot spot areas at Mlangarini, Kirusix and Ngaramtoni.

4.4 Methodology

4.4.1 Development of single Cross Hybrids

None replicated trial was established at Kirusix secondary school garden under irrigation system during 2014 off season for developing single cross hybrids. Crossings were performed in 6x6 full diallel fashions according to Griffings (1956) Design I model I using sixheterotically divergent parents (Table:7), whereby thirty hybrids including reciprocals were obtained. In order to increase genetic variation, resistant, moderately resistant and susceptible inbred lines were used in the ratio of 3:2:1. Both ear and tassel bagging were done prior to flowering in order to avoid unintended cross pollination. In the breeding nursery sowing dates were adjusted to facilitate synchronization in flowering in order to obtain sufficient crosses.

Table 7: Parental Information

Genotype	Origin	Description
CKH 10767	CIMMYT	Moderately resistant
CKH 114272	CIMMYT	Moderately resistant
CML312	CIMMYT	Resistant
CML444	CIMMYT	Susceptible
CML 503	CIMMYT	Highly susceptible (Tester)
CML 144	CIMMYT	Resistant

4.4.2 Multi- location Evaluation trials For the Hybrids and their parents

Evaluation trials were laid down in a Randomized Complete Block Design (RCBD) with three replications. Each entry was planted in one row plot of 5m long. DAP fertilizer was applied at rate of 10grams per hill. Urea was applied 3 weeks after emergence and repeated after 8 weeks for top dressing. Inter and intra row spacing was kept to 0.75m by 0.30m. Three spreader rows of CML 503 entry were planted as boarder rows. This CML 503 is the highly susceptible check. Pesticides were not applied to allow movement of insect vectorsin the field**.** The F_1's hybrids were evaluated for general and specific combining ability for disease resistance in three locations at Nagaramtoni, Mlangarini and Kirusix. These sites are among the areas currently facing high MLND disease regime. Parental lines were concurrently evaluated together with the developed hybrids in order to study their genetic information.

4.4.3 Data collected

Data collected include disease scores, days to 50% flowering and grain weight for the ten plants randomly selected in each row. These plants we tagged three weeks

after emergence. Disease severity scores were rated on scale of 1-5 according to Shekha and Kumar, (2012) as follows: 1= Resistant (No Symptoms), 2=Moderately Resistant, 3= Moderately Susceptible, 4= Susceptible, 5= Highly Susceptible (plant dead completely). The disease scores were recorded three weeks after emergence (3WAE), six weeks after emergence (6WAE), ten weeks after emergence (10WAE) and fifteen weeks after emergence (15WAE). The score intervals were chosen based on crop development. For the period of ten weeks after emergence more than 75% of the crops attain flowering because this the important scoring period.

4.4.4 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using both Window stat version 9.2 and R-statistics computer software.

4.5 RESULTS

4.5.1 ANOVA for MLND Response across Locations

Analysis of variance showed highly significant differences among genotypes for MLND response indicating the presence of sufficient genetic variation among the treatments (Table: 8). All parents and hybrids were highly significant in terms of disease response across all three locations (P≤0.001).

This trend revealed that the material used in this study have genetic broad base. On the other hand, there is highly significant differences among parents (P≤0.001) at Mlangarini and Kirusix but not significant at Ngaramtoni. This is because Ngaramtoni was found to have more virulence than other locations where this was conducted, thus no parent showed significant resistance here.

Moreover both direct and reciprocals crosses for F_1s were highly significant in disease resistance (P≤0.001) at different levels in different locations indicating that maternal effects were important in controlling the disease resistance among the studied genotypes.

Table 8: ANOVA Summary for disease response among thirty maize hybrids (direct andreciprocals cross) and their parents across locations (Means square given).

Locations		**Mlangarini**	**Kiru6**	**Ngaramtoni**
Source of Variations	**df**		**Probability levels**	
Replicates	2	0.6340	0.3964	0.5444
Treatments	35	5.678***	2.911***	0.072***
Parents	5	0.002***	0.018***	0.1613
Hybrids	29	5.648***	5.375***	0.00042***
Parent Vs. Hybrids	1	0.011*	0.1904	0.8021
F1's	14	7.030***	0.005**	0.00063***
Reciprocals	14	3.898***	6.541***	0.010*
Error	70			
Total	107			

Level of Significance: *≤ 0.05, **≤0.01, ***≤0.001

4.5.2 Combining Ability analysis

In this study both GCA and SCA were determined in respect to resistance response against Maize Lethal Necrosis Virus in three different locations. The ANOVA shows that GCA was highly significant (P≤0.001) than SCA (Table: 9).This indicates preponderance of additive genetic effects in controlling the disease.

Table 9: ANOVA Summary for GCA, SCA and Reciprocalcrosses for parents and hybrids across three locations based on disease response (Means square given)

Locations		Mlanagrini	Kiru6	Ngaramtoni
Source of Variations	**df**	**Probability**		
GCA	5	5.551***	1.61***	4.527***
SCA	15	0.011*	0.0472*	0.1227
Reciprocal	15	0.4127	0.0775*	0.4919
Error	70			

Probability levels: *≤0.05, ***≤0.001

Few parents showed highly significant GCA to different magnitude of positive and negative in all Environments where this study was conducted. Generally CML 144, CML 503 and CML 444 were the best combiners. However, only CML 144 showed highly negative GCA which imply that generally itwas the best combiner for disease resistance whereas CML 503 and CML 444were susceptible line.Moreover, the SCA ability was not significant either for crosses or reciprocals for the CML 144 while CML 503 shows some degree of dominance when used as female with CML312 (CML 503xCML312) and when used as male with CML444 (CML444xCML503). This means that CML 503 may be used as a susceptible tester, though may not be an appropriate breeding material for disease resistance. The estimate of narrow sense heritability (h^2) was relatively low ranging from 0.491 to 0.675than the broad sense

heritability (H^2) which ranged from 0.666 to 0.854. The predictability ratio was found to range from 0.737 to 0.791. According to Patel *et al.,* (2014) the predictability ratio approaching unity indicates the preponderance of additive genetic effects. (Table: 10).

Table 10: GCA summary for parents and components of Genetic variance for crossesbased on disease response in three locations.

Parents	Mlangarini	Kiru6	Ngaramtoni
CKH10767	0.046	-0.130	0.046
CKH114272	-0.176*	-0.130	0.046
CML144	-0.593***	-0.546***	-0.565***
CML312	-0.343***	-0.046	-0.231*
CML444	0.463***	0.231**	0.296**
CML503	0.602***	0.620***	0.407**
s^2g	0.208	0.146	0.113
s^2 r	0.002	0.034	-0.002
s^2 e	0.088	0.102	0.156
s^2 a	0.417	0.292	0.226
s^2 D	0.11	0.085	0.081
s^2 p	0.617	0.513	0.461
h^2	0.675	0.569	0.491
H^2	0.854	0.734	0.666
GCA/SCA Ratio	1.894	1.726	1.403
Predictability Ratio	0.791	0.775	0.737

Probability levels: *≤0.05, **≤0.01 and ***≤0.001 respectively

s^2g = Genotypic Variance
s^2 r= Variance due to reciprocal effects
s^2 e= Variance due to Environmental effects
s^2 a= Additive genetic variance
s^2 D= Dominance genetic Variance
s^2 p= Phenotypic Variance
h^2 = Narrow sense heritability
H^2 =Broad sense heritability

Table 11: SCA Summary of fifteen single cross hybridsfor disease response for Mlangarini, Kirusix and Ngaramtoni

Crosses	Mlangarini	Kiru6	Ngaramtoni
CKH10767 x CKH114272	-0.10	0.16	0.04
CKH10767 x CML144	0.15	-0.09	-0.35
CKH10767 x CML312	-0.27	-0.26	-0.35
CKH10767 x CML444	0.26	-0.04	0.29
CKH10767 x CML503	0.12	0.41	0.34
CKH114272 x CML144	-0.13	0.24	-0.02
CKH114272 x CML312	-0.05	-0.09	-0.02
CKH114272 x CML444	0.48*	-0.04	0.12
CKH114272 x CML503	-0.16	-0.09	0.18
CML144 x CML312	-0.13	0.32	-0.24
CML144 x CML444	-0.10	-0.45*	-0.10
CML144 x CML503	0.43*	-0.01	0.12
CML312 x CML444	0.48*	0.38	0.73**
CML312 x CML503	0.01	0.32	-0.05
CML444 x CML503	-0.13	-0.29	-0.57*

Probability levels: *≤0.05 and **≤0.01

4.5.3Graphical Analysis (Wr-Vr) and GGEbiplotfor a Diallel analysis of MLND resistance

Graphical analysis of the experimental data recorded was done in order to get information about allelic constitutions of the parents used in the diallel cross. In the present study, regression coefficient values (b) for MLND reaction did not differ significantly from unity for Mlangarini and Kirusix(b=0.856 and b=1.082) respectively indicating the absence of epistasis. The regression line also crossed Wr-axis at the positive part which imply the presence of incomplete or partial dominance (Fig.3&5). Nevertheless,regression coefficient is significantly less than unity (b=0.512) and the correlation coefficient is weak (r=0.668) for disease resistance at

Ngaramtoni (Fig.4). This can be handled by analyzing one array at a time and eliminate the one cause deviation. The graphical analysis also provides the allelic distribution of parents within the limiting parabola. From the graph, the genotypes closer to the interception have higher dominant alleles while farthest genotypes from the intercept have more frequency of recessive alleles (Figure 3, 4 and 5).

More over the GGEbiplot analysis were also done for crosses, reciprocals and their parents. This is an effective method which is based on principal component analysis (PCA) to fully explore multi-environment trials (METs). It allows visual examination of the relationships among the test environments, genotypes and the genotype-by-environment interactions (GxE interaction). In this study GGEbiplot is based on "which/what won where". It simply implies which genotype was suitable in particular location. The more closely the genotype to location in a given angle the more suitable it becomes and reverses are also true.For example, P5xP1 relatively performs better in Kirusix while P5xP3 and P3xP5 perform better in Mlangarini and Ngaramtoni respectively. The genotypes concentrated at the center have indifferent performance in all locations thus considered as stable ones. These includes P6xP5, P2xP6, and P5xP2 and most crosses involving P6 (Fig: 6).For selection purposes, the ideal genotypes are those with low mean for disease score and high stability.

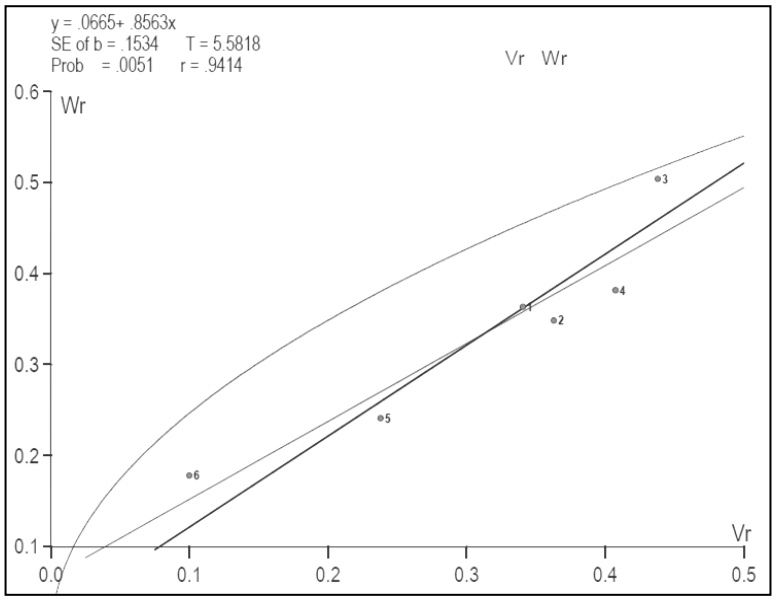

Figure 3:Wr-Vr graph for MLND response at Mlangarini showing the allelic distribution of 6 parents.

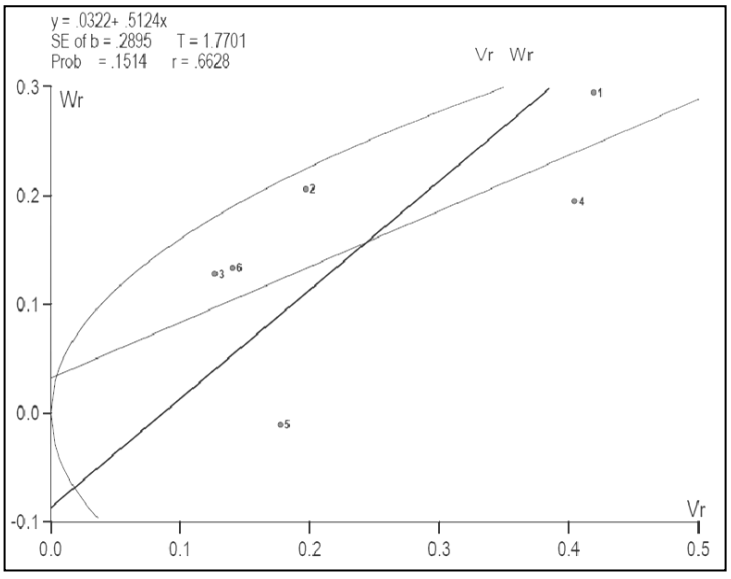

Figure 4: Wr-Vr graph for MLND response at Ngaramtoni showing the allelic distribution of 6-parents.

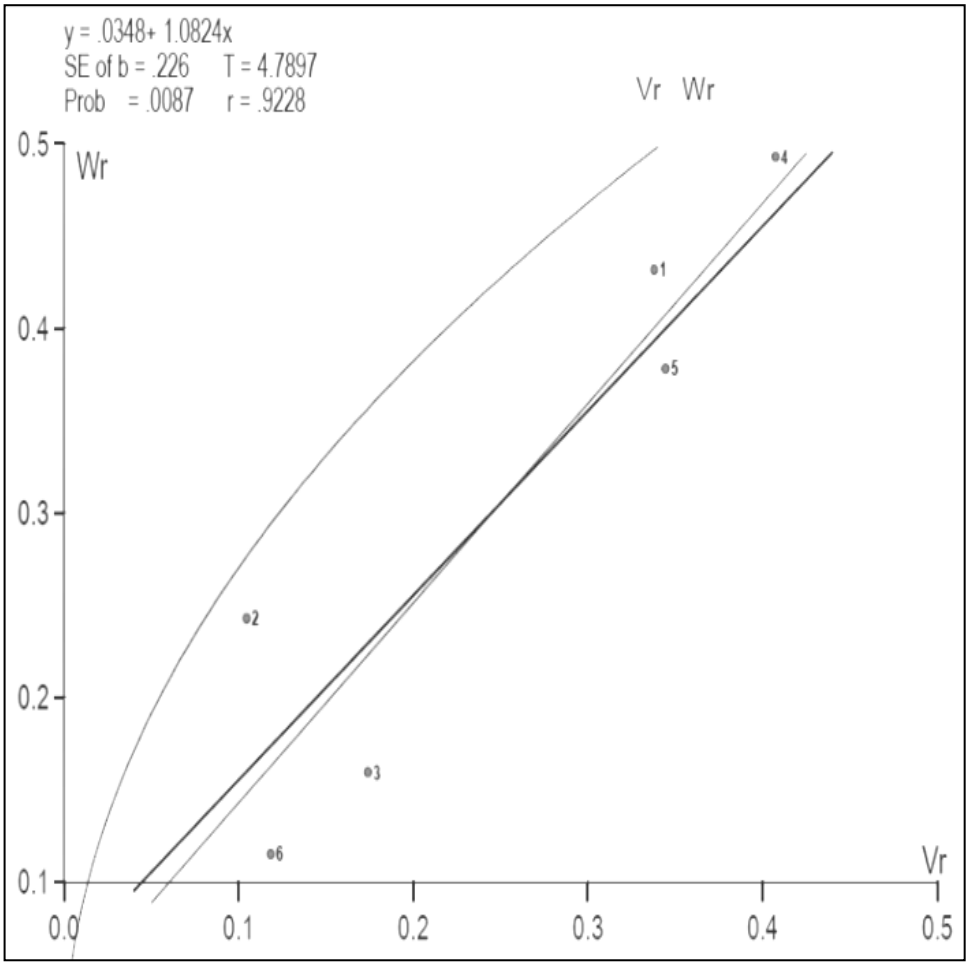

Figure 5: Wr-Vr graph for MLND response at Kiru6 showing the allelic distribution of 6-parents. Parent 1, 4 and 5 have more recessive alleles, while 6, 3 and 2 have more dominant alleles.

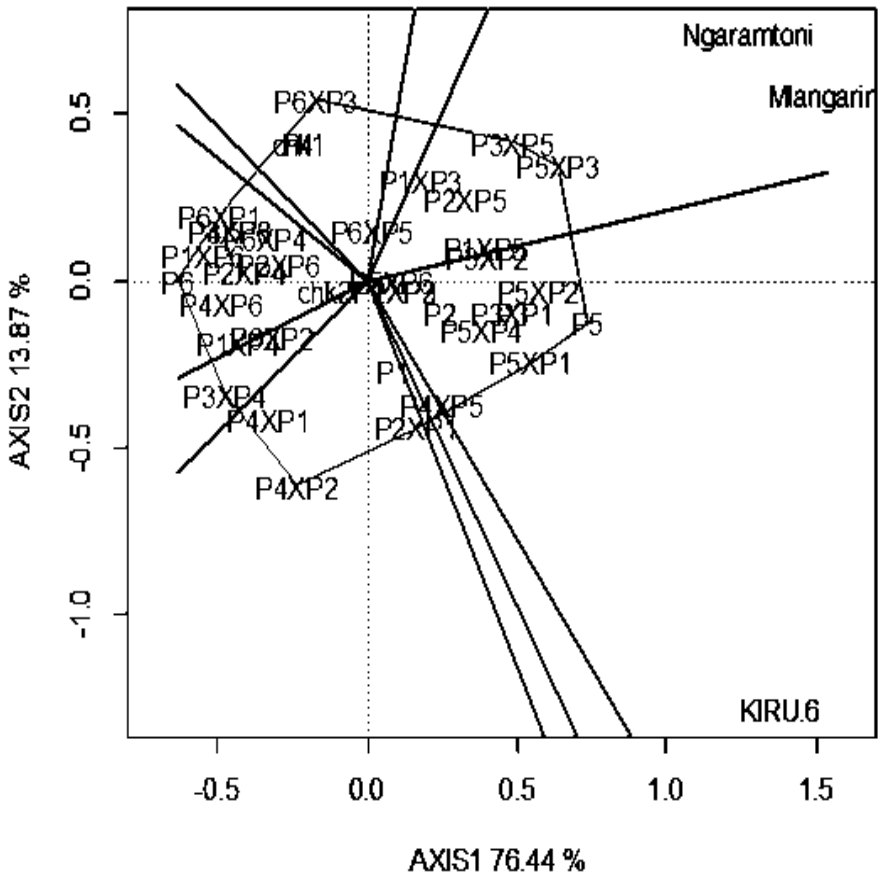

Figure 6: GGEbiplot for full diallelcross and their parents based on MLND response in three locations (Ngaramtoni, Mlangarini and Kirusix).

4.5.4Heterosis Compared to Mid (MP) and Better (BP) Parents.

Heterosis is the deviation in performance among homozygous parents and their resulting off-springs. The heterotic effects of F_1 are normally estimated as percentages over mid-parent using the following formula:

Mid Parent heterosis$= \frac{\text{F1}-\text{mid parent}}{\text{Mid parent}} \text{X}100$

Better parent heterosis $= \frac{\text{F1}-\text{Better parent}}{\text{Better parent}} \text{X}100$, whereby the mid parent is obtained as $\frac{\text{P1}-\text{P2}}{2}$

Significant differences were observed among 30 F_1 hybrids for disease response. The heterosis for the yield would have been very important information in this study. However, due to excessive missing variables on yield, only heterosis for disease reaction was reported. Someof the crosses showed significant differences in heterosis includes: CML503x CML 312 (-26.67*), CML 503XCML 144 (30.43**), CML 312X CML 444 (36.36**) and CML 444 x CKH 10767 (25.0**). (Table: 12)

Since disease is undesirable phenomenon therefore heterosis with negative means will be favoured in the selection of best crosses.

Table 12: Estimation of Heterosis relative to Mid-parent (MP) and better parents for disease response in three locations.

F_1	Mlangarini			Kirusix			Ngaramtoni		
	Mean	**MP**	**BP**	**Mean**	**MP**	**BP**	**Mean**	**MP**	**BP**
CKH10767 x CKH114272	3.67	-4.35	-8.33	4.67	27.27	27.27	4.33	4.00	0.00
CKH10767 x CML144	3.67	10.00	-8.33	3.67	10.00	0.00	3.33	-16.67	-23.08
CKH10767 x CML312	3.67	0.00	-8.33	4.00	14.29	9.09	3.67	-8.33	-15.38
CKH10767 x CML444	4.67	16.67	16.67	4.33	0.00	-13.33	5.00	15.38	15.38
CKH10767 x CML503	4.67	3.70	-6.67	5.00	15.38	0.00	5.00	7.14	0.00
CKH114272 x CKH10767	4.00	4.35	0.00	3.33	-9.09	-9.09	4.33	4.00	0.00
CKH114272 x CML144	3.33	5.26	-9.09	4.00	20.00	9.09	3.67	-4.35	-8.33
CKH114272 x CML312	3.67	4.76	0.00	4.00	14.29	9.09	4.33	13.04	8.33
CKH114272 x CML444	5.00	**30.43****	**25.00***	4.67	7.69	-6.67	4.33	4.00	0.00
CKH114272 x CML503	4.00	-7.69	**-20.00***	4.67	7.69	-6.67	5.00	11.11	0.00
CML144 x CKH10767	3.67	10.00	-8.33	3.00	10.00	-18.18	3.33	-16.67	-23.08
CML144x CKH114272	3.00	-5.26	-18.18	3.33	0.00	-9.09	3.67	-4.35	-8.33
CML144 x CML312	3.00	0.00	-10.00	3.67	15.79	10.00	3.00	-18.18	-18.18
CML144 x CML444	4.00	20.00	0.00	3.33	16.67	-33.33	3.33	-16.67	-23.08
CML144 x CML503	4.00	4.35	**-20.00***	4.00	0.00	-20.00	4.00	-7.69	-20.00
CML312 x CKH10767	3.33	-9.09	-16.67	3.33	-4.76	-9.09	3.67	-8.33	-15.38
CML312 x CKH114272	3.33	-4.76	-9.09	3.67	4.76	0.00	3.67	-4.35	-8.33
CML312 x CML144	3.00	0.00	-10.00	4.00	26.32	20.00	3.33	-9.09	-9.09
CML312 x CML444	5.00	**36.36****	**25.00***	4.33	4.00	-13.33	5.00	25.00	15.38
CML312 x CML503	4.33	4.00	-13.33	5.00	20.00	0.00	5.00	15.38	0.00
CML444 x CKH10767	5.00	**25.00****	**25.00***	4.00	-7.69	-20.00	4.67	7.69	7.69
CML444 x CKH114272	4.67	**21.74***	16.67	3.67	15.38	-26.67	5.00	20.00	15.38
CML444 x CML144	3.67	10.00	-8.33	3.33	16.67	-33.33	4.33	8.33	0.00
CML444 x CML312	4.33	18.18	8.33	5.00	20.00	0.00	5.00	25.00	15.38
CML444 x CML503	5.00	11.11	0.00	4.67	-6.67	-6.67	4.67	0.00	-6.67
CML503 x CKH10767	5.00	11.11	0.00	5.00	15.38	0.00	5.00	7.14	0.00
CML503 x CKH114272	4.67	7.69	-6.67	4.33	0.00	-13.33	4.67	3.70	-6.67
CML503 x CML144	5.00	**30.43****	0.00	4.33	8.33	-13.33	4.33	0.00	-13.33
CML503 x CML312	4.33	4.00	-13.33	5.00	20.00	0.00	3.67	-15.38	**-26.67***
CML503 x CML444	5.00	11.11	0.00	4.67	-6.67	-6.67	4.00	-14.29	-20.00

Probability Levels *≤0.05 and **≤ 0.01

4.6 Discussion

Although chemical control provides effective protection, their application is compromised by environmental effects and by the emergence of resistant pathogen strains. In addition chemical control for plant diseases is beyond the resource poor farmers. For this reason host resistance is the effective choice of controlling disease resistance. In order to deploy the host crop resistance, the information on combining ability is an important means of developing resistant cultivar. Combining ability describes the breeding values of parental lines to produce hybrids or composite.

Thus the present study focused to obtain information on relative importance of GCA and SCA as well as reciprocal effects for disease resistance in genetically divergent maize inbred lines. The information regarding general combining ability of the parents is of prime importance because it helps in successful prediction of genetic potential which would give desirable individuals in subsequent segregating populations. While the specific combining ability is associated with non-additive gene effects that are non-fixable in nature and useful for commercial exploitation as hybrids, the exploitation GCA provide suitable composite for the trait of interest.

The significant mean square for GCA and SCA effects specific for the set of crosses used in this study suggested that both additive and non-additive genetic were important in MLND resistance with the former more pronounced than the later. Greater mean square for GCA effects indicates that additive effects were more important than non-additive effects. Highly significant negative GCA effects were found from resistant inbreds CML144 and CML312. However, the magnitude of resistance was subject to the environment.Thisinformationwas obtained using diallel cross. This design has been widely used for obtainingsystematic approach for the

detection of suitable parents and crosses for investigated characters. In addition, diallel analysis gives plant breeders the opportunity to choose the efficient selection method by allowing them to estimate several genetic parameters (Unay*et al*., 2004). Heterosisranges were also calculated based on the comparison of mid-parental (MP) value and better parent (BP). Higher mean are desirable for some agronomic traits such as yield and yield components but for the disease response selection are always sought for highly negative and lower means since disease is undesirable phenomena its value should be kept lower.

The components of GxE were also among the important parameters included in this study as they play significant roles in detecting suitability of individual genotype(s) in particular set of environment and the aspect of stability using GGEbiplot tool.Derera *et al*., (2007) also found that disease development was highly affected by the environment indicating that incidence and severity may differ between locations and seasons, and between seasons within location.

The studies of allelic constitutions of the genotypes were of great interest for the appropriate selection. This information provides the distribution of array on regression line of Wr-Vr either closer to the intercept or away from it.The closer parents to the origin are said to be more dominant while those away from origin constituting recessive alleles. However, this approach is only relevant if the assumptions given by Hayman (1954) are adequate. These includes (a) Diploid segregation, (b) No difference between reciprocal crosses, (c) Independent action of

non-allelic genes, and in the diallel cross (d) No multiple allelism (e) Homozygous parents (f) Genes independently distributed between the parents.

4.7 Conclusion

The inbred lines used in this study were selected from a diverse set of germplasm to find new alleles for MDMV and SCMV resistance. Disease scores estimates of SCA variances were generally lower than GCA variances, with the exception of few parents and crosses. The relatively higher heritability estimates in some locations indicates that improvement of resistant line can be possible. Since GCA is generally high and narrow sense heritability is low in most crosses gene pyramiding can be employed to develop the disease resistant composite cultivars. Based on high *per se* performance and higher negative GCAeffects, the parent CML 144 and CML 312 were considered as best general combiners. On the other hand, CML 503 and CML444 had significantly higher positive values in all locations showing higher degree of susceptibility. Since they showed the consistent results across locations they can be maintain as broad base testers in the future breeding programmes for selection of resistant material.

4.8 Recommendations

Although the utilization of disease nurseries in hot spots (places where the disease is severe and commonly found) is the easiest approach to screen for virus resistance in maize compare to the use artificial inoculation, the main challenge of using technique is that it cannot assure the presence of pathogen or vector to transmit, therefore establishment of efficient screening facility is highly recommended.

Also since little is known about MLND epidemiology and vectors spreading the virus intensive studies involving multidisciplinary approach is necessary. The disease occur when there is a combination of two viruses MCMV and any other *potyvirus* infecting grass family such as SCMV, MSV and WSV. However the genome study of the virus and the nature of association are quite limited, thus there is a need of using molecular approach explorer more information. Although the results of this study given som lights, further evaluation of these materials and other germplasm to multiple locations are advisable.Lastly, but very importantly, mass screening of the local materials is very important. In this study for example, most of the landraces collected from farmers relatively performed better than the improved materials. This gave the indication that our local material can provide new sources of resistance for the current and future breeding programmes.

4.9 References

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APPENDICES

Appendix 1: MLND mean scores across locations for different classes of genotypes.

Entry	Genotype	Mean scores	Genotype category	Ratings
1	CML 144	2.333 m	Inbred line	MR
2	LMBL06	3 lm	Landraces	MS
3	LAR03	3.222 kl	Landraces	MS
4	LMBL03	3.222 kl	Landraces	MS
5	LKRT08	3.222 kl	Landraces	MS
6	CML312	3.333 jkl	Inbred line	MS
7	LKRT05	3.333 j-l	Landraces	MS
8	LMBL02	3.333 j-l	Landraces	MS
9	LMBL04	3.333 j-l	Landraces	MS
10	LKRT04	3.444 i-l	Landraces	MS
11	LKRT07	3.444 i-l	Landraces	MS
12	LMBL05	3.444 i-l	Landraces	MS
13	LMBL08	3.444 i-l	Landraces	MS
14	LAR04	3.556 h-l	Landraces	S
15	LKRT01	3.556 h-l	Landraces	S
16	LKRT02	3.556 h-l	Landraces	S
17	LKRT03	3.556 h-l	Landraces	S
18	LMBL01	3.556 h-l	Landraces	S
19	LBBT01	3.556 h-l	Landraces	S
20	LBBT05	3.556 h-l	Landraces	S
21	LMBL07	3.667 g-l	Landraces	S
22	LMBL09	3.667 g-l	Landraces	S
23	LMBL11	3.667 g-l	Landraces	S
24	LMBL12	3.667 g-l	Landraces	S
25	LBBT02	3.667 g-l	Landraces	S
26	LBBT03	3.667 g-l	Landraces	S
27	LBBT04	3.667 g-l	Landraces	S
28	LMBL10	3.667 g-l	Landraces	S
29	CML 444	3.778 f-k	Inbred line	S
30	LAR02	3.778 f-k	Landraces	S
31	LMBL13	3.778 f-k	Landraces	S
32	SEEDCO627	3.778 f-k	Certified local checks	S
33	STUKA-M1	3.778 f-k	Certified local checks	S
34	TAN250	3.778 f-k	Certified local checks	S

Appendix 1: MLND scores across locations (cont...)

35	TAN254	3.778 f-k	Certified local checks	S
36	TZE1	3.889 a-d	Inbred line	S
37	KS03-OB15-125	3.889 a-k	Inbred line	S
38	CKH 101509	3.889 e-k	Inbred line	S
39	SAH306	3.889 e-k	Certified local checks	S
40	CKH10767	3.9e-k e-k	Inbred line	S
41	CKH114272	4 d-j	Inbred line	S
42	LKRT06	4 d-j	Landraces	S
43	SEEDCO514	4 d-j	Certified local checks	S
44	TMV-1	4 f-k	Certified local checks	S
45	CML 536	4.111 ab	Inbred line	S
46	LAR01	4.111 ab	Landraces	
47	DHL 33	4.111 c-i	Inbred line	S
48	CML539	4.111c-i	Inbred line	S
49	P100C6-200-1-1-B ***	4.222 a-h	Inbred line	S
50	LAPOSTA SEQ(C7-F64-2-6-1-B-B-#	4.222 a-h	Inbred line	S
51	CML78	4.222 b-h	Inbred line	S
52	CML 159	4.222 b-h	Inbred line	S
53	LAPOSTA SEQ.C7	4.333 a-g	Inbred line	S
54	LAPOSTA SEQ (C7-F64-1-1-1-1-2-B-B-B-B)	4.333 a-g	Inbred line	S
55	CML 511	4.333 a-g	Inbred line	S
56	CML 202	4.333 a-g	Inbred line	S
57	CML 449	4.333 a-g	Inbred line	S
58	DTPW C9-F92-2-1-1-1BB	4.333 a-g	Inbred line	S
59	DHL 32	4.333 a-g	Inbred line	S
60	CML440	4.333 a-g	Inbred line	S
61	TZE1-83	4.333 a-g	Inbred line	S
62	MERUHB405	4.333 a-g	Certified local checks	S
63	CKL 05017	4.444 a-f	Inbred line	S
64	CL-02510-B	4.444 a-f	Inbred line	S
65	CML 395	4.444 a-f	Inbred line	S
66	DTPW SEQ C9-F115-1-4-1-1-B-B-#	4.444 a-f	Inbred line	S
67	LAPOSTA-SEQ C7-F180-3-1-1-BB#	4.444 a-f	Inbred line	S
68	CML 489	4.444 a-f	Inbred line	S
69	CML 307	4.444 a-f	Inbred line	S
70	CML 254	4.444 a-f	Inbred line	S

Appendix 1: MLND scores across locations (cont...)

71	CKL 105	4.444 a-f	Inbred line	S
72	CML444	4.444 a-f	Inbred line	S
73	ZM525	4.444 a-f	Certified local checks	S
74	CML 539	4.556 a-e	Inbred line	S
75	DTPW	4.556 a-e	Inbred line	HS
76	ZCL 03007	4.556 a-e	Inbred line	HS
77	CZL 0003	4.556 a-e	Inbred line	HS
78	CML 488	4.556 a-e	Inbred line	HS
79	DTPW –C9-F104-5-4-1-1-B-B-#	4.556 a-e	Inbred line	HS
80	CKL 178	4.556 a-e	Inbred line	HS
81	DHL 31	4.556 a-e	Inbred line	HS
82	SAL09MAK1-8-6	4.556 a-e	Inbred line	HS
83	SELIAN308	4.556 a-e	Certified local checks	HS
84	CML 445	4.667 a-d	Inbred line	HS
85	CML204	4.667 a-d	Inbred line	HS
86	CML 149	4.667 a-d	Inbred line	HS
87	CML 179	4.667 a-d	Inbred line	HS
88	DHL 34	4.667 a-d	Inbred line	HS
89	DHL 35	4.667 a-d	Inbred line	HS
90	KS03-OB15-45	4.667 a-d	Inbred line	HS
91	CZK 0004	4.778 abc	Inbred line	HS
92	CML390	4.778 a-g	Inbred line	HS
93	CKL 163	4.778 a-g	Inbred line	HS
94	La Posta	4.889 ab	Inbred line	HS
95	DHL 29	4.889 ab	Inbred line	HS
96	DHL 30	4.889 ab	Inbred line	HS
97	CML 539	4.889 ab	Inbred line	HS
98	CKL05003	4.889ab	Inbred line	HS
99	INTA B-B-41-B-7-1B-B-B-B	5.00 a	Inbred line	HS
100	CML 0503	5.00 a	Inbred line	HS
Grand	**Mean**	**4.11**		
LSD		**0.78488**		
CV (%)		**19.7**		

MR=moderately resistant, MS=moderately susceptible, S=Susceptible and HS= highly susceptible

Appendix 2: Mean scores at Mlangarini

ENTRY	GENOTYPE	3WAE	6WAE	10WAE	15WAE
1	CKH10767	3	3	4	3.7
2	CKH114272	2.7	2.7	3.7	4
3	CKH 101509	2.3	2.7	3.7	3.7
4	CKL05003	3.3	3.7	4.7	5
5	La Posta	3.7	3.7	4.7	5
6	CKL 05017	4	4	5	5
7	LAPOSTA SEQ.C7	2.7	3	3.3	4.3
8	CML 539	3.7	4	4.7	5
9	CML78	3.7	3.7	4.7	4.7
10	CML 159	3	3.3	4.7	4.7
11	CML312	1.7	2	2.7	3
12	CML 444	2	2.3	3.3	3.7
13	DTPW	4	4	4.7	5
14	CL-02510-B	3.7	3.7	4.3	5
15	LAPOSTA SEQ (C7-F64-1-1-1-1-2-B-B-B-B)	3	3.3	4	4
16	CML 144	1.7	1.7	2.3	2.3
17	CML 395	2.3	3	3.7	4.3
18	CML 511	2.3	2.7	3.3	3.7
19	P100C6-200-1-1-B ***	2.3	2.3	3.3	4
20	CML539	2.7	3	4.3	5
21	CML 445	3.7	3.7	4.7	5
22	CML204	4.3	4.3	5	5
23	ZCL 03007	4	4	4.3	4.7
24	CML 202	3.7	4	5	5
25	CZL 0003	3.7	3.7	4	4
26	LAPOSTA SEQ(C7-F64-2-6-1-B-B-#	3.3	3.3	4.3	4.3
27	DTPW SEQ C9-F115-1-4-1-1-B-B-#	4	4	4.7	5
28	CML 488	2.7	3	4	5

Appendix 2*: Mean scores at Mlangarini (cont...)*

29	DTPW –C9-F104-5-4-1-1-B-B-#	4	4	4.7	4.7
30	LAPOSTA-SEQ C7-F180-3-1-1-BB#	4	4.3	5	5
31	CML 449	2.7	3	4	
32	DTPW C9-F92-2-1-1-1BB	3.7	4	4.3	4.7
33	INTA B-B-41-B-7-1B-B-B-B	3.7	4	4.7	5
34	CML 0503	4	4.3	4.7	5
35	CML 489	3	3	4	4.7
36	CML 307	3	3.7	4.3	4.7
37	CML 149	3	3.7	4.3	5
38	CML 254	3.3	4	5	5
39	CML 179	3	3.3	4	5
40	CZK 0004	3.3	3.3	4.3	5
41	CKL 105	2.7	3.3	4	5
42	CKL 163	3	3.3	4	4.7
43	CKL 178	3.3	3.3	4.3	5
44	DHL 29	3.7	4.3	4.7	5
45	DHL 30	4	4	4.7	5
46	DHL 31	3	3.7	4	5
47	DHL 32	3	3.7	4.3	4.7
48	DHL 33	3	3	4	5
49	DHL 34	3.3	3.3	4.7	5
50	DHL 35	3.3	3.3	4.3	5
51	CML440	2.3	2.3	3	3.7
52	CML 536	2.3	2.3	3.3	3.7
53	CML 539	3	3.3	4.7	5
54	CML390	2.7	2.7	4	4.3
55	SAL09MAK1-8-6	2.3	2.3	3.7	4.3
56	KS03-OB15-125	3	3.3	4	4.3
57	TZE1	2.7	2.7	3.3	3.7
58	CML444	2.3	3	4	4.3

Appendix 2: Mean scores forMlangarini (Cont...)

59	KS03-OB15-45	4	4	4.3	4.7
60	TZE1-83	2.3	2.3	3.3	4.3
61	LAR01	2	2	3	3
62	LAR02	2	2	2.3	3.3
63	LAR03	2.3	3	2	2.3
64	LAR04	2	2.3	2.7	3
65	LKRT01	2.3	3	3	3.3
66	LKRT02	2	2.3	2.7	2.7
67	LKRT03	2.3	2.3	2.3	3
68	LKRT04	2.3	3	2.7	3.3
69	LKRT05	1.7	2	2.7	2.3
70	LKRT06	2	2	2.7	3.7
71	LKRT07	2.3	2.7	2	2.7
72	LKRT08	2	2	2	2.7
73	LMBL01	1.7	2.3	2.3	2.7
74	LMBL02	2	2.7	2.7	3
75	LMBL03	2	2.7	2.3	2.3
76	LMBL04	2	2.3	2.3	3
77	LMBL05	2.3	2.3	1.3	2.3
78	LMBL06	1.7	2	2	2.3
79	LMBL07	2.7	2.7	2.7	2.7
80	LMBL08	1.7	2	2	2.3
81	LMBL09	1.7	2.3	2.7	3
82	LMBL10	2	2.3	2.3	3.7
83	LMBL11	2.3	2.7	2	2.7
84	LMBL12	1.7	2.3	2	2.7
85	LMBL13	2.7	2.7	2.7	3
86	LBBT01	2	2	2.7	2.7
87	LBBT02	2.7	2.3	2	3.7
88	LBBT03	2.3	2.3	2.7	3.3

Appendix 2: Mean scores forMlangarini (Cont...)

89	LBBT04	1.7	2.7	2.3	2.7
90	LBBT05	2	1.7	2	3
91	SEEDCO627	2.3	2.7	3.7	3.7
92	SEEDCO514	2	2	2.7	3.7
93	SAH306	2.7	3.3	3	3.7
94	TMV-1	2	3	3.3	3.7
95	STUKA-M1	2	3	3	3.3
96	TAN250	2	2.3	2.3	3.7
97	TAN254	2.7	3	3.3	4.3
98	MERUHB405	3	3	3.3	4
99	ZM525	3	3	4	4.7
100	SELIAN308	2.7	4	4.3	4.7
LSD		**1.38**	**1.26**	**1.17**	**1.27**
SE		**0.856**	**0.784**	**0.726**	**0.156**
CV (%)		**31.3**	**26.1**	**20.6**	**19.9**

Appendix 3: Mean MLND Scores for Ngaramtoni (Arusha).

ENTRY	GENOTYPE	3WAE	6WAE	10WAE	15WAE
1	CKH10767	2.7	3.7	3.7	4
2	CKH114272	3	2.7	3.3	3.7
3	CKH 101509	2.7	3.3	3.3	3.7
4	CKL05003	2.7	3	4	5
5	La Posta	2.3	4	4.3	5
6	CKL 05017	2.3	3.3	4.7	4.3
7	LAPOSTA SEQ.C7	2.7	2.7	4	4.7
8	CML 539	2.7	2.3	3.7	4.7
9	CML78	2.7	3.3	4	4.7
10	CML 159	2.7	4	4	4
11	CML312	2.3	2.7	3.3	3.3
12	CML 444	2.7	2.7	4	4.3
13	DTPW	3.7	3.3	4.3	4.3
14	CL-02510-B	3	3	4.3	4.3
15	LAPOSTA SEQ (C7-F64-1-1-1-1-2-B-B-B-B)	2.7	3.3	4.3	4.7
16	CML 144	1.3	2	2.3	2.3
17	CML 395	2.3	4	4	4.3
18	CML 511	3	3	4.3	4.7
19	P100C6-200-1-1-B ***	2.3	3	4	5
20	CML539	3	3	4.3	3.7
21	CML 445	2.7	3.3	4.7	4.7
22	CML204	3	3	4.3	4.3
23	ZCL 03007	2.7	3.7	4.7	5
24	CML 202	2.7	3.7	3	4
25	CZL 0003	2.3	3	4	4.7
26	LAPOSTA SEQ(C7-F64-2-6-1-B-B-#	2.7	3	3.7	4.3
27	DTPW SEQ C9-F115-1-4-1-1-B-B-#	2.3	3	4	4.3

Appendix 3: Mean MLND Scores for Ngaramtoni (cont...)

28	CML 488	2.7	3.3	3.7	4.7
29	DTPW –C9-F104-5-4-1-1-B-B-#	2.7	3	4	4.7
30	LAPOSTA-SEQ C7-F180-3-1-1-BB#	2.3	3	4.3	4.3
31	CML 449	2.7	2.3	4	4.3
32	DTPW C9-F92-2-1-1-1BB	2.7	2.3	3.3	3.7
33	INTA B-B-41-B-7-1B-B-B-B	2.3	3.7	4.3	5
34	CML 0503	2.3	3	4.3	5
35	CML 489	3	3.7	4	4
36	CML 307	2	3.7	4	4.7
37	CML 149	2.7	2.3	3.7	4.7
38	CML 254	2	2.7	3.7	4.7
39	CML 179	2.3	3.3	3.7	4.3
40	CZK 0004	3	3	4.7	5
41	CKL 105	2.7	3.3	3.7	4
42	CKL 163	2.3	2.7	4	5
43	CKL 178	2.3	2.7	3.3	4.7
44	DHL 29	4	4.3	4.3	5
45	DHL 30	2	3	4.7	5
46	DHL 31	3.3	3.7	4.3	4.7
47	DHL 32	2.3	2	3.3	3.7
48	DHL 33	3	3	3.3	3.7
49	DHL 34	2.3	3.7	4.3	5
50	DHL 35	2.7	3	4	4.7
51	CML440	2.3	2.7	4.3	4.3
52	CML 536	2.7	3.7	3.7	3.7
53	CML 539	2.3	3.7	4.7	5
54	CML390	2.3	2.7	4.3	5
55	SAL09MAK1-8-6	2.3	2.3	3.7	4.3
56	KS03-OB15-125	2.7	3	3.3	3.7

Appendix 3: Mean MLND Scores for Ngaramtoni (cont...)

57	TZE1	2.3	2	3.7	4
58	CML444	2.3	3.7	4.3	4.3
59	KS03-OB15-45	2.7	2.7	4.3	4.3
60	TZE1-83	2.3	3	4	4
61	LAR01	2.3	2.3	4.3	5
62	LAR02	2	2.3	3.7	4
63	LAR03	1.7	3	4	4.3
64	LAR04	1.7	2.7	3.7	4.3
65	LKRT01	2.7	2.7	3.7	4
66	LKRT02	2.7	2.3	4	4
67	LKRT03	2	2.3	4.3	3.7
68	LKRT04	2.3	3.3	3.3	3
69	LKRT05	2	2.7	4.3	3.7
70	LKRT06	1.3	3	3.3	4.3
71	LKRT07	1.7	2.3	3.7	3.3
72	LKRT08	1.3	3.3	4	3.7
73	LMBL01	1.7	2.3	3	4
74	LMBL02	1.7	3	3.3	3.3
75	LMBL03	2.3	3	3.7	3.7
76	LMBL04	2.3	1.3	3	4.3
77	LMBL05	2.3	3.7	4.3	4.3
78	LMBL06	1.3	3.3	3.7	3.7
79	LMBL07	2.3	2.7	3.7	4.3
80	LMBL08	2	4	4	3.7
81	LMBL09	2.3	3.3	4	4.3
82	LMBL10	2.7	3	3.3	4
83	LMBL11	2.7	2	3.3	4.7
84	LMBL12	2	3	3.7	4.3
85	LMBL13	2.7	3	4	4

Appendix 3: Mean MLND Scores for Ngaramtoni (cont...)

86	LBBT01	2.3	3	3.7	4
87	LBBT02	2	2.7	4.3	4.3
88	LBBT03	2	3	3	3.7
89	LBBT04	1.7	3.3	4	4
90	LBBT05	2.3	2.3	4.3	4.3
91	SEEDCO627	1.3	3	3	3.3
92	SEEDCO514	3	3	4	3.7
93	SAH306	2.3	2.7	4	4.7
94	TMV-1	2.3	2	3.7	4.3
95	STUKA-M1	2.3	2.3	3.7	4.3
96	TAN250	2.3	3.3	4	3.7
97	TAN254	2.3	2.7	3.7	4.3
98	MERUHB405	3	2.7	4.3	4.3
99	ZM525	2.7	3.7	3.7	3.7
100	SELIAN308	2	2.7	4.7	5
	LSD0.05	**1.1**	**1.2**	**1.3**	**1.3**
	SE	**0.66**	**0.75**	**0.84**	**0.79**
	CV (%)	**27.6**	**25.2**	**21.5**	**18.7**