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Evaluation of maize accessions for diversity, yield and tolerance to maize lethal necrosis disease in Tanzania

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EVALUATION OF MAIZE ACCESSIONS FOR DIVERSITY, YIELD AND TOLERANCE TO MAIZE LETHAL NECROSIS DISEASE IN TANZANIA

Mujuni Sospeter Kabululu

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of PhD in Life Science of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Maize (*Zea mays* L.*)* is among the most important crops in Tanzania with still low average yield of 1.2 metric tonnes per hectare as compared with potential yields of 4 to 5 metric tonnes per hectare. Low yield is due to a number of factors including pests and diseases. Recently, East Africa has been hard hit by a new deadly disease of maize called Maize Lethal Necrosis Disease (MLND). The disease started in Kenya in 2011 and later spread to other countries including Tanzania and it continues to spread fast in other countries. It is caused by a combination of two viruses i.e., *Maize chlorotic mottle virus* (MCMV) and any other Potyvirus, with *Sugarcane mosaic virus* (SCMV) reported for East Africa. The aim of this study was to evaluate genetic diversities of maize accessions (focusing more on landraces) with respect to their MLND resistance and optimum yield in Tanzania. Fifty one maize landraces from National Plant Genetic Resources Centre (NPGRC) in Tanzania, thirty four commercial varieties as checks for yield and thirteen elite lines from CIMMYT Kenya as checks for resistance against MLND were used. Three field experiments were conducted at Tengeru, Mlangarini and Selian in Tanzania for genetic diversity study and MLND evaluation, one field experiment was conducted at Naivasha Kenya for MLND evaluation. Molecular study was conducted at Nelson Mandela African Institution of Science and Technology in Tanzania. Significant ($p < 0.05$) variations were observed among the tested accessions and an OPV Situka 1 and a hybrid DH 04 were the best yielding varieties across Tengeru, Mlangarini and Selian with 116.01g and 115.90g per plant respectively. Landraces TZA 2793 and TZA 5170 were among the highest yielding accessions with 100.46g and 99.80g per plant respectively. The allele distribution and frequency associated with quantitative trait loci for SCMV resistance were detected with landraces and TZA 2793 as well as TZA 3544 expressed low MLND progression across Mlangarini and Naivasha. The results of significant genetic diversity and response against MLND of maize landraces tested in this study calls for further investigation to ascertain their utilization in breeding and crop improvement.

DECLARATION

I, **Mujuni Sospeter Kabululu** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

_____	_____
Mujuni Sospeter Kabululu	**Date**

The above declaration is confirmed by

	18/12/2017
Dr. Tileye Feyissa	**Date**
_____	_____
Prof. Patrick Alois Ndakidemi	**Date**

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CERTIFICATION

The undersigned certify that they have read the dissertation titled **Evaluation of Maize accessions for diversity, yield and tolerance to Maize Lethal Necrosis Disease in Tanzania** and recommend for examination in fulfillment of the requirements for the degree of PhD in Life Science and Bioengineering of the Nelson Mandela African Institution of Science and Technology.

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Date

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DEDICATION

I dedicate this dissertation to the Almighty God and also to my wife Grace D. John and my two little children Godlove Kabululu and Hannah Kabululu

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LIST OF ABBREVIATIONS AND SYMBOLS

'	Minutes
"	Seconds
^{o}	Degrees
%	Percent
µl	Microlitre
^{o}C	Degrees centigrade
AEC	Average Environment Coordination
AFLP	Amplified Fragment Length Polymorphisms
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ASI	Anthesis Silking Interval
AUDPC	Area Under Disease Progress Curve
BLUP	Best Linear Unbiased Prediction
CGIAR	Consultative Group on International Agricultural Research
CIMMYT	International Maize and Wheat Improvement Center
cm	Centimeter
CML	CIMMYT Maize Lines
COSTAT	Statistical Software
CTAB	Cetyl trimethylammonium bromide
CV	Coefficient of Variation
DNA	Deoxyribonucleic Acid
dNTPs	deoxyribonucleoside triphosphate
E	East
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
g	Grams
Genstat	General Statistics Software
GGE	Genotype plus Genotype by Environment interaction
h	Gene diversity

ha	Hectare
HCl	Hydrochloric Acid
I	Shannon – Weaver diversity index
IBPGR	International Board for Plant Genetic Resources
KALRO	Kenya Agricultural and Livestock Research Organization
kg	Kilograms
L.	Linnaeus (famous botanist)
LSD	Least Significant Difference
M	Molar mass
m.a.s.l	Meters above sea level
MCMV	Maize Chlorotic Mottle Virus
MDMV	Maize Dwarf Mosaic Virus
min	Minutes
ml	Millilitre
MLN	Maize Lethal Necrosis
MLND	Maize Lethal Necrosis Disease
mM	Millimolar
MT	Metric tones
N	Nitrogen
nm	Nanometre
NM-AIST	Nelson Mandela African Institution of Science and Technology
NPGRC	National Plant Genetic Resource Centre
NPK	Nitrogen, Phosphorus, Potasium
NTSYS pc	Numerical Taxonomy and Multivariate Analysis System
OPV	Open Pollinated Variety
p	Probability
PAST	Statistical Software
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase chain reaction

pH	Potential of Hydrogen to specify the acidity or alkalinity of solutions
PIC	Polymorphism Information Content
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
rAUDPC	Relative Area Under Disease Progress Curve
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
S	South
SCMV	Sugarcane Mosaic Virus
SSR	Simple Sequence Repeat
STATISTICA	Statistical Software
TBE	Tris/Borate/EDTA buffer
TE	Tris-EDTA buffer
TZA	The code identifying accessions collected in Tanzania
U	Unit, measure for Taq polymerase
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
UV	Ultra Violet
WSMV	Wheat Streak Mosaic Virus

CHAPTER ONE

General introduction

1.0 Introduction

1.1 Background information

Maize (*Zea mays* L.) is one of the most important crops in the world, because it is one of the main sources of human food, animal feed, and raw materials for industrial processes (Romay *et al.*, 2013). In Tanzania, maize is the major food and cash crop as it provides 60% of dietary calories and more than 50% of utilizable protein (Mwakalinga and Massawe, 2007). It is cultivated in all the agro-ecological zones of Tanzania with an average of 2 million hectares, which is about 45% of the cultivated area in the country (Katinila *et al*., 1998, Otunge *et al*., 2010). The economic importance of maize in Tanzania cannot be over emphasized since its short supply is normally equated to the national food insecurity (Katinila *et al*., 1998). However, despite the importance of maize, the general average yields are still very low with 1.2 MT per hectare as compared with the estimated potential yields of 4 to 5 MT per hectare (Moshi *et al.*, 1990; Otunge *et al.*, 2010). This has been attributed to many factors such as lack of quality agricultural inputs (seeds, fertilizer), drought, pests and diseases to mention a few. Plant diseases are a potential threat to global food security which has always been calling upon extensive research using a wide range of methodologies. Plant diseases can cause losses of 30% to 50% or even more for major crops (Ali and Yan, 2012). Maize for instance, is affected by more than 100 pathogens, where some of them cause diseases with severe impacts in different locations depending on various factors (Ali and Yan, 2012). Recently, East Africa has been hit hard by the deadly disease known as Maize Lethal Necrosis (MLN) which was not there before while potyviruses that form one of the causing pathogens used to exist. The disease is caused by a combination of *Maize Chlorotic Mottle Virus* (MCMV) and other Potyviruses such as *Sugar Cane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* and or *Wheat Streak Mosaic Virus*. Since the disease is now in few areas within the region and the spread is very fast, there is a need to screen a wide range of accessions for MLN disease resistance. Maize landraces are usually genetically heterogeneous populations which are adapted to the local environments and linked to the local farming systems as well as resistance to biotic and abiotic stresses (Tokatlidis and Vlachostergios, 2016). Characterization of genetically heterogeneous populations using

conventional and molecular tools has been the most potential and efficient ways of establishing diversities for different important traits including disease resistance (Prasanna, 2012)**.** This study involved evaluation of Tanzanian maize landraces' diversities and compared with commercial varieties (checks for yield and yield related parameters) as well as elite lines from CIMMYT (as checks for MLND resistance) and hence screen all those materials for yield and MLN disease resistance.

1.2 Research problem and justification

Maize lethal necrosis disease (MLND) presents an immediate threat to food security in Tanzania with concomitant long-term consequences. The disease infection rate can reaches 100% with yields being severely affected up to a complete loss of a maize crop (Adams *et al*., 2013; Wangai *et al.,* 2012b). The occurrence of Maize Lethal Necrosis Disease (MLND) in Eastern Africa has recently posed a serious challenge to maize production with potential negative impact on trade and food security (Mahuku *et al*., 2015). The control of MLND is complicated because it is caused by a combination of more than one virus (*Maize chlorotic mottle virus* - MCMV and *Sugarcane mosaic virus* - SCMV) which are difficult to separately identify them based on visual symptoms (Ali and Yan, 2012). The outcome following the synergistic effect through the combinational viral infection is a serious damage that eventually kills infected plants (Wangai *et al*., 2012a). In addition, maize plants appear to be susceptible to MLND at all growth stages from seedling to maturity (CGIAR, 2012). Stakeholders in all circles and capacities agree that the foremost priority is to identify MLND resistant maize varieties (CGIAR, 2012) as well as those with other relevant traits such as yield. Valuable natural resources of maize in terms of genetic diversity are considered to play a key role in breeding programs (Reif *et al*., 2006). It presents a wider range of traits that can be exploited to generate new novel varieties against maize production challenges such as MLND and low yield. A good understanding of genetic diversity within and among maize landraces ensures effective utilization in plant breeding program (Rao and Hodgkin 2002). However, only 2% of the world maize germplasm collection is being absorbed in breeding programs (Dowswell *et al.,* 1996) and an important part of maize germplasm is still in the hands of small farmers (Carvalho *et al.,* 2004). Landraces and local cultivars in maize present a population which is genetically diverse and have been under farmer's selection for many years in terms of adaptation, plant characteristics, yield, biotic and abiotic

stress tolerance or resistance (Wasala *et al.*, 2013). They have so far not efficiently being utilized because of unreliable information on agronomic as well as genetic potentials (Nass *et al*., 1993). This study aimed at evaluating the genetic diversity levels among maize landraces with other maize accessions for the purpose of screening these maize materials against MLND and low yield for potential use in breeding programs. The information obtained on maize accessions' diversity, resistance levels against MLND and yield are expected to contribute towards speedy deployment of MLND resistant materials and higher yielding varieties to generate improved maize productivity and food security.

1.3 Objectives

1.3.1 General objective

Evaluating the diversity of maize accessions for MLND resistance and optimum yield in Tanzania.

1.3.2 Specific objectives

1) To evaluate agronomic performance of local and improved maize varieties in Tanzania
2) To assess genetic diversity of maize landraces from Tanzania as compared with commercial varieties and elite lines through morphological characterization
3) To evaluate the response of maize accessions against maize lethal necrosis disease (MLND)
4) To assess genetic diversity of maize landraces in Tanzania using SSR markers for maize lethal necrosis disease resistance

1.4 Hypothesis

The following hypotheses were tested for drawing up inferences;

Ho. The selected Maize accessions have no agronomic potentials worth for breeding programs

Ho. The selected Maize accessions possess low morphological diversity for crop improvement

Ho. The selected Maize accessions possess no varied range of responses against MLND

Ho. The selected Maize accessions possess low molecular genetic diversity for crop improvement

CHAPTER TWO

Literature review

Genetic diversity of maize accessions for maize lethal necrosis disease resistance[1]

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Abstract

Maize is among the most preferred crop in Tanzania and other parts of the world. However, its production has been facing a number of challenges including Maize Lethal Necrosis Disease (MLND). The control of MLND in Eastern Africa is complicated as it is caused by a combination of more than one virus that is *Maize Chlorotic Mottle Virus* (MCMV) and *Sugarcane Mosaic Virus* (SCMV). Stakeholders agree that the priority is to identify MLND resistant maize varieties. Therefore, genetic diversity provides the source of traits required to breed against maize production challenges such as MLND. The study of genetic diversity in maize accessions often involves characterizing morphological plant characteristics as well as molecular marker techniques to study variation at DNA level. This review explores different literatures that address the importance of genetic diversity and the possibility of generating information towards obtaining potential materials against maize production challenges in general and MLND in particular.

Key words: Genetic diversity, Landraces, Maize, MLND, Molecular markers, Morphological characterization.

[1] Article published in Indian Journal of Agricultural Research *(Vol. 51, Issue 1, pp 17 - 24)*

2.1 Introduction

Maize (*Zea mays* L.) is one of the most important crops in the world, because it is one of the main sources of human food, animal feed, and raw materials for industrial processes (Romay *et al.*, 2013). In Tanzania, maize is the major food and cash crop where its short supply is normally equated to the national food insecurity (Katinila *et al*., 1998). However, despite its importance, the general average yields are still very low with 1.2 MT per hectare as compared with the estimated potential yields of 4 to 5 MT per hectare (Moshi *et al.*, 1990; Otunge *et al.*, 2010). Cultivation of maize is hampered by several diseases which cause serious grain loss (Anjichi, 2005; Pechanova and Pechan, 2015). Recently, East Africa has been hard hit by a deadly disease known as Maize Lethal Necrosis (MLN) which was not there before while potyviruses that form one of the causing pathogens used to exist (Wangai *et al*., 2012a; Wangai *et al*., 2012b; Adams *et al*., 2014; Gowda *et al*., 2015; Mahuku *et al*., 2015). The disease is caused by a combination of *Maize Chlorotic Mottle Virus* (MCMV) and other Potyviruses such as *Sugar cane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* or *Wheat Streak Mosaic Virus* (Scheets, 1998; De Groote *et al*., 2016; Isabirye and Rwomushana, 2016). There is a need to screen a wide range of accessions for MLN disease tolerant or resistant materials. Maize landraces (accessions) are usually genetically heterogeneous populations (Ignjatovic *et al*., 2013) which are typically selected by farmers for better adaptation to specific environments as well as resistance to biotic and abiotic stresses (Aci *et al*., 2013). Characterization of those genetically heterogeneous populations using conventional and molecular tools is the most efficient way of establishing diversities for different important traits including disease resistance (Anumalla *et al*., 2015; Prasanna, 2012). This means, developing improved varieties with required traits through plant breeding would very much depend on the availability of a wide and reliable crop genetic diversity (Abraha *et al*., 2014; Sharma *et al.*, 2015).

2.2 Maize Lethal Necrosis Disease (MLND)

MLND was first observed in areas of South Rift Valley region of Kenya in 2011 and spread to several other places of Kenya (Wangai *et al*., 2012b). Later, the disease was identified as MLN after serological and molecular tests were carried out on infected maize plants from Bomet County and Nakuru County in 2012 (Wangai *et al*., 2012b). The same year, the disease was also

reported to spread to neighbouring countries of Tanzania and Uganda (Makumbi and Wangai, 2013) and later it was found in Rwanda (Adams *et al.*, 2014). The control of the disease has been reported to be difficult due to the combination of more than one virus that do not separately result into any significant symptoms (DSMZ, 2014; Xia *et al*., 2016). MLND occurs as a result of a positive interaction between *Maize Chlorotic Mottle Virus* (MCMV) and any of the cereal viruses in the family, Potyvirideae, such as *Sugarcane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* (MDMV), or *Wheat Streak Mosaic Virus* (WSMV) (Adams *et al*., 2014; Makone *et al*., 2014; Liu *et al*., 2016). In Eastern Africa, the disease has been reported to be caused by a combination of MCMV and SCMV infection (Gowda *et al.*, 2015; Mezzalama *et al*., 2015; Kiruwa *et al*., 2016). The two viruses together inflict serious damage or even completely kill infected plants (Scheets, 1998; CGIAR, 2012) and farmers in the affected areas have been reported to experience extensive to total crop loss (Wangai *et al*., 2012b). The disease causing viruses are mainly transmitted by insects (Makone *et al*., 2014) from plant to plant or by wind from field to field over long distances (CGIAR, 2012; Mezzalama *et al*., 2015). MCMV is normally transmitted by thrips and beetles while SCMV is transmitted by aphids (CGIAR, 2012; Kiruwa *et al*., 2016). The viruses can as well be transmitted from one generation to another through seed which are infected thus enhancing the possibility for wide spread of MLND (Zhang *et al*., 2011). Infected plants show mild to severe mottling on the leaves (Gowda *et al*., 2015; De Groote *et al*., 2016; Kagoda *et al*., 2016), usually starting from the base of young leaves in the whorl and extending upward towards the leaf tips (Wangai *et al*., 2012b). Other symptoms include stunting and premature aging of the plants, dying (necrosis) of the leaf margins that progresses to the mid-rib and eventually the entire leaf (Wangai *et al*., 2012b; Gowda *et al*., 2015). Necrosis of young leaves in the whorl before expansion, leads to a symptom known as "dead heart" and eventually plants death (Kagoda *et al*., 2016). In addition, infected plants often bear barren ears which are small and deformed with little or no seed set (CGIAR, 2012; Gowda *et al*., 2015; Kagoda *et al*., 2016).

2.3 Genetic diversity of maize accessions

Genetic diversity refers to the heritable genetic variation that occurs within populations of particular organisms (Rao and Hodgkin, 2002). The diversity in plants provides an opportunity for developing new varieties and improved cultivars with desirable characteristics (Govindaraj *et al*., 2015; Saleh *et al*., 2016). A number of methodologies exist for the assessment of genetic diversity in maize, those are (i) morphological characterization (Ristic *et al*., 2014) (ii) biochemical characterization that uses electrophoresis to detect allelic variants of enzymes at gene level (Govindaraj *et al.,* 2015) (iii) pedigree that employ the extraction of genealogical information (Drinic *et al.,* 2012) and (iv) DNA molecular analyses (Sao *et al.,* 2015) such as Simple Sequence Repeats (SSR), Restriction Fragment Length Polymorphism (RFLP) (Mondini *et al*., 2009) etc.

2.3.1 Morphological diversity

The morphological characteristics (phenotype) express the genetic constitution (genotype) of a given organism, while in other words genetic constitution give rise to what we see (Liao *et al*., 2010; Uphoff *et al*., 2015). However, the expression of phenotype (morphological characteristics) is always affected by the impact of environment (Anumalla *et al*., 2015), the so called genotype x environment interaction. On the other hand, the impact by environmental interaction coupled with the expression of genetic constitution presents the advantage that can only be obtained with morphological markers (Durga *et al.,* 2015). The following equation shows the interaction;

$$P = f(G + E + (G \times E)),$$

where P stands for phenotype, G for genotype and E for environmental influences, and the interaction term G × E refers to their joint effects (Uphoff *et al*., 2015). Genetic variation (diversity) can be evaluated using morphological characterization (Mondini *et al*., 2009). Morphological characterization of maize is conducted through assessing plant characteristics that are given as the list of descriptors provided by the International Board for Plant Genetic Resources (IBPGR, 1991). The morphological traits that are used to evaluate maize genetic

diversity include (i). Vegetative data. (ii). Ear data. and (iii). Kernel data (Table 1). Collected data from the parameters (descriptors) as shown in Table 1 are further subjected to statistical analysis which, generate informative results that explain the behaviour of each crop accession as well as how accessions relate to each other. The results also help to identify promising accessions through potential traits evaluation. Therefore, analysis of variance (ANOVA) can be performed for all measured traits in order to test the significance of variation among accessions (Beyene *et al*., 2005). The standardized traits mean values can be used to perform principal component (PC) and cluster analyses (to calculate similarities or dissimilarities between accessions) using softwares such as NCSS 2000 (Jerry, 2000) or NTSYS pc 2.1 (Rahman *et al*., 2008). Further cluster analysis can be conducted on the Euclidean distance matrix with the unweighted pair group method based on arithmetic averages (UPGMA) (Beyene *et al*., 2005).

Table 1: Some descriptors used to evaluate genetic diversity through morphological characterization (IBPGR, 1991).

DESCRIPTOR NO.	ITEM	DESCRIPTION
	VEGETATIVE DATA	
4.1.1	Days to tasseling	Number of days from sowing to when 50% of the plants have shed pollen
4.1.2	Days to silking	Number of days from sowing to when silks have emerged on 50% of the plants
4.1.4	Plant height [cm]	From ground level to the base of the tassel. After milk stage
4.1.5	Ear height [cm]	From ground level to the node bearing the uppermost ear. After milk stage
4.1.6	Foliage	Rating of total leaf surface. After milk stage. Observed on at least 20 representative plants
4.1.7	Number of leaves above the uppermost ear including ear leaf	Counted on at least 20 representative plants. After milk stage
4.1.9	Stem colour	Indicate up to three stem colours in the order of frequency, Observed between the two topmost ears. At flowering
4.1.12	Sheath pubescence	At flowering
6.1.2	Leaf length	From ligule to apex. Measure the leaf which subtends the uppermost ear. After flowering
6.1.3	Leaf width [cm]	Mid-way along its length. Measured on the same leaf as 6.1.2
6.1.5	Leaf orientation	After flowering
6.1.6	Presence of leaf ligule	After flowering
	EAR DATA	
6.2.2	Ear length [cm]	Length
6.2.4	Ear diameter [cm]	Measured at the central part of the uppermost ear
4.2.3	Kernel row arrangement	Use the uppermost ear
4.2.4	Number of kernel rows	Count number of kernel rows in the central part of the uppermost
6.2.5	Cob diameter [cm]	Diameter at the center part of the uppermost ear cob
6.2.6	Rachis diameter [cm]	Diameter at the center of the inner part of the cob
6.2.8	Number of kernels per row	Count kernels of any row
6.2.9	Cob colour	Observe the colour of cob
6.2.10	Shape of uppermost ear	Observe shape
	KERNEL DATA	
4.3.1	Kernel type	Indicate up to three kernel types in the order of frequency
4.3.2	Kernel colour	Indicate up to three kernel colours in the order of frequency
4.3.3	1000 kernel weight [g]	Adjusted to 10% moisture content
6.3.1	Kernel length [mm]	Average of 10 consecutive kernels from one row in the middle of the uppermost ear, measured with calliper
6.3.2	Kernel width [mm]	Measured on the same 10 kernels as 6.3.1
6.3.3	Kernel thickness [mm]	Measured on the same 10 kernels as 6.3.1
6.3.4	Shape of upper surface of kernel	Observe shape
6.3.5	Pericarp colour	Observe colour
6.3.6	Aleurone colour	Observe colour
6.3.7	Endosperm colour	Observe colour

2.3.2 Diversity at molecular level

In the past few decades, analyzing genetic diversity based on phenotypic traits (morphological characterization) has been enhanced with the use of molecular (DNA) markers (Ristic *et al*., 2014). This is due to the fact that discrepancies encountered with morphological markers are checked by the use of molecular markers for the purpose of obtaining variation at the DNA level (Dubreuil *et al*., 2006). Evaluating genetic diversity of genetic resources at molecular level is essential due to the fact that morphological differences themselves are usually determined by a small number of genes and may not be representative of genetic divergence in the entire genome (Brown-Guedira *et al.*, 2000). However, the importance of these genetic resources and their potentials for selection has been constrained due to limited amount of important traits being characterized at molecular level (Rao and Hodgkin, 2002; Drinic *et al*., 2012; Prasanna, 2012; Sood *et al*., 2014). A number of reports on maize populations have been showing a considerable amount of variability on morphological and agronomic traits (Ihsan *et al.*, 2005) as well as at molecular level (Legesse *et al.*, 2006). This call for the efforts of ensuring that potential useful traits found in a wide range of plant genetic resources are made available to plant breeders for the purpose of crop improvement (Frese *et al*., 2012; Maxted *et al*., 2013).

i. Molecular marker techniques

Molecular marker techniques present the ability to detect variation at the DNA level through breeding programs and plant biotechnology (Anumalla *et al*., 2015). PCR based molecular marker techniques have made it possible for breeders and other scientists to estimate genetic diversity through the use of different molecular markers (Arif *et al*., 2010; Poczai *et al*., 2013). Genetic diversity results from the genetic variation among individuals and is expressed in DNA sequences (Bindroo and Moorthy, 2014; Osawaru *et al*., 2015). It can be categorized in terms of the number of different alleles existing in different populations, distribution of those alleles in the chromosomes, the impact these alleles have on performance and the general variability among different populations under various environmental conditions (Rao and Hodgkin, 2002; Mondini *et al*., 2009; Bindroo and Moorthy, 2014). Some of the techniques that have been applied in molecular studies include RFLPs (Mondini *et al*., 2009), RAPDs (Brown-Guedira *et al*., 2000; Mondini *et al*., 2009), AFLPs (Mondini *et al*., 2009), and SSRs (Kumari *et al*., 2005;

Beyene *et al*., 2005; Mondini *et al*., 2009; Aci *et al*., 2013; Abraha *et al*., 2014). However, these different marker techniques emphasize on different features (Abdel-Mawgood, 2012) and different aspects of genetic diversity (Matsuoka *et al*., 2002; Mondini *et al*., 2009). Therefore, different marker techniques may lead to different results and the range of variation produced can be different (Hodgkin *et al*., 2001). Among those several marker techniques, microsatellites (SSR) have been exploited in many ways (Ignjatovic-Micic *et al.*, 2013) and specifically for characterizing genetic diversity in Maize (Reif *et al*., 2006).

ii. Simple Sequence Repeats markers

These are microsatellites which are abundant and occur frequently and randomly (Ristic *et al*., 2014) in eukaryotic genomes that are examined (Matsuoka *et al*., 2002; Wan *et al*., 2004). Irrespective of the fact that microsatellites are time consuming and costly, they actually are advantageous in terms of ease of use, high levels of inherited variation, co-dominant, reliable and highly reproducible (Rao and Hodgkin, 2002; Mondini *et al*., 2009; Ristic *et al*., 2014). The following constitutes major steps towards executing molecular characterization procedures:

Extraction of DNA

Extraction of DNA from sample to be analyzed is the first step for molecular marker diversity analysis (Semagn *et al*., 2006). DNA is extracted from the leaf samples taken from young seedlings (Legesse *et al*., 2006; Semagn *et al*., 2006) using the CTAB procedure as explained by Saghai-Maroof *et al.* (1984). Sometimes modifications can be made depending on circumstances. Primers of a specific marker type such as SSR are selected on the basis of their genomic locations (Kumari *et al*., 2005).

Polymerase Chain reaction (PCR)

The first step with PCR is denaturation process or melting step which separate the two DNA strands (template DNA), this step requires very high temp 94 - 98 ^{0}C (Kumari *et al*., 2005; Legesse *et al*., 2006). Annealing step follows that allow primers to bind to the complementary sequences on the template DNA and the temperature here ranges from 40 - 60 ^{0}C (Matsuoka *et*

al., 2002; Kumari *et al*., 2005). The next step is elongation after the primers are bound, this requires a temperature of 72 ^{0}C (Matsuoka *et al*., 2002; Kumari *et al*., 2005).

Gel electrophoresis

Finally, the DNA sample is loaded into wells of agarose gel and then the gel is ran and scanned under UV light on transilluminator for interpreting the results (Yýlmaz *et al*., 2012). From the gel electrophoresis, polymorphism (variation) is expected to be determined (Senior *et al.*, 1998). Also similarity matrix is analysed by NTSYS-pc analytical package to generate hierarchical classification by the use of Unweighted Pair Group Method using Arithmetic Averages (UPGMA) (Kumari *et al*., 2005).

2.4 Importance of landraces

Landraces are plant populations which are cultivated by local farmers that have historic origin, unique identity with no any formal crop improvement (Prasanna, 2012; Hagenblad *et al*., 2016). They are often endowed with diverse genetic inheritance (Zeven, 1998) as well as local adaption and strong connection to traditional farming systems (Camacho *et al*., 2005). They are typically selected by farmers for better adaptation to specific environment (Ristic *et al*., 204), yield potential, nutritional qualities and resistance/tolerance to biotic and abiotic stresses (Prasanna, 2012). However, domestication and selection of maize are said to cause reduced genetic diversity in the maize genome as compared with its progenitor population (Pineda-Hidalgo *et al*., 2013). The reduction in the genetic diversity of crops represents an increase in vulnerability to new pests and diseases (De Jaramillo, 2009; Ogwu *et al*., 2014). It has been reported that about 80% of African farmers grow only landraces since they are able to reuse the seeds in many seasons while on the other hand only 20% grow improved varieties together with landraces (Anjichi *et al*., 2005). In Mexico, landraces occupy more than 80% of the area under maize production (Mercer and Wainwright, 2007). In Tanzania, landraces and traditional cultivars attract more attention than commercial improved cultivars (Mitawa and Marandu, 1996). In addition, between 1974 to 2000, maize researchers under maize research program in Tanzania have managed to utilize potentials from maize germplasm sourced from within and outside the country to release a number of varieties (Kirway *et al*., 2000). Some of those were able to be adopted because they

possessed some traits prefered by the local communities besides being connected to the existing farming practices (Westengen *et al.,* 2014). The traits of those released varieties extends from plant characteristics, yield performance, disease resistance or tolerance to mention but a few. This gives an indication that maize germplasm present an opportunity for making genetic improvement against maize production challenges. Landraces in Mexico attract great attention to both farmers and researchers (Rodriguez *et al*., 1998). Tuxpeño maize for instance, is a Mexican landrace that was domesticated in the Oaxaca Chiapas region (Rodriguez *et al*., 1998; Prasanna, 2010) and it is a cultivar which is very productive in the fertile lowland and has been used in the breeding programmes (Rodriguez *et al*., 1998; Prasanna, 2010). Another example is Tuxpeño crema, a Mexican cultivar, which is characterized as a late maturing cultivar and resistant to tropical foliar diseases with white kernels, short and strong stalk (Rodriguez *et al*., 1998). Therefore, landraces are expected to be a very important source of new and unique alleles which have not yet been well exploited (Mercer and Wainwright, 2007; Olson *et al*., 2012). However, limited characterization data of landraces have caused difficulties to use, manage and conserve them (Rao and Hodgkin, 2002; Drinic *et al*., 2012; Prasanna, 2012; Sood *et al*., 2014). Another challenge with maize landraces is that many plant breeders limit their breeding research by using germplasm that contain narrow genetic base (Prasanna, 2012). Thus, there is an urgent need to establish efficient and well organized characterization of maize germplasm for creating comprehensive information that would be useful to generate strong maize varieties against production challenges (Saad and Rao, 2001; Drinic *et al*., 2012; Prasanna, 2012) including MLND in particular.

2.5 Conclusion

Maize continues to be the most preferred staple food and cash crop in Tanzania as well as in other parts of the world. However, maize is affected by many pathogens and some of them cause big negative impacts to its productivity. The outbreak of a new disease in East Africa called Maize Lethal Necrosis Disease (MLND) presents immediate threat to food security as well as uncertain long-term consequences. MLND infection rate reaches 100% and yields severely affected up to a complete loss of the crop. The International Maize and Wheat Improvement Center (CIMMYT) and Kenya Agriculture and Livestock Research Organization (KALRO) have

conducted joint studies from 2012 to 2014 with about 25,000 germplasm of elite inbred lines, Double Haploid lines, and hybrids against MLND under artificial inoculation and more than 95 percent of the tested materials were found to be completely susceptible. Stakeholders in maize research and development admit that expanding the range of maize germplasm for MLND screening is still needed to increase the possibility of acquiring materials with resistance or tolerance against the disease. Landraces and local cultivars in maize present a population which is genetically diverse and have been under farmer's selection for many years in terms of adaptation, plant characteristics, yield, biotic and abiotic stress tolerance or resistance. However, only 2% of the world maize germplasm collection is being absorbed in breeding programs and an important part of maize germplasm (landraces and local materials) are still in the hands of small farmers. Hence, good understanding of the performance on yield and resistance against MLND through screening a wide range of maize accessions ensure effective utilization of the genetic resources available for the fight against the current major challenges in maize production.

CHAPTER THREE

Evaluation of agronomic performance of local and improved maize varieties in Tanzania[2]

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Abstract

Maize (*Zea mays* L.) is one of the most important crops and yet it realizes very low average yield in Tanzania due to a number of production challenges including the use of poor varieties by most of the farmers. Availability of an efficient and well organized agronomic, morphological and genetic knowledge of maize germplasm make it easy to select the best recombinants and plan well for crosses in future breeding programs. The objective of this study was to evaluate the agronomic performance of 50 Tanzanian maize local varieties compared with 7 commercial varieties and 11 elite lines from CIMMYT in Nairobi. The genotypes were laid out in a randomized complete block design (RCBD) in three sites located in Arusha region of Tanzania in 2015. The data obtained was subjected to statistical analysis using COSTAT and STATISTICA analytical packages. These software identified significant variations among genotypes and their interactions with the environments. The GGE biplot analyses identified the best genotypes with respect to mean yield and stability. An open pollinated variety (OPV), Situka 1 and a hybrid DH 04 had generally the best performance in terms of grain yield and stability across all the 3 locations. A local cultivar TZA 2793 emerged to be the most promising landrace with overall appealing yield and stability performance. The results of this study may be a good source of new allelic diversity that could be used for developing different important elite maize materials.

Key words: *Maize, Tanzania, agronomic performance, OPV, hybrid, local cultivars, GGE biplot*

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3.1 Introduction

Maize (*Zea mays* L.) receives much of attention worldwide because of its importance as food and feed (Guruprasad *et al*., 2016; Dogan *et al.,* 2015). In Tanzania, maize is important as it is normally equated to the national food security (Katinila *et al*., 1998). However, the general average yield of maize is still very low with 1.2 MT per hectare (Makurira *et al.,* 2007) as compared with the potential estimated yield of 4 to 5 MT per hectare (Otunge *et al.*, 2010). The low yield is attributed to the use of poor varieties, abiotic and biotic constraints, low soil fertility, poor agronomic management and uncertain input supply (Lisuma *et al.,* 2006). There is a need to identify adapted maize breeding materials that possess traits with high potentials to be used by maize breeders to develop superior cultivars (Sharma *et al.,* 2015). Successful plant breeding requires a more careful choice of genotypes that would be used to develop desirable recombinants (Toshimenla *et al*., 2016). Only two percent of the world maize germplasm collection is being absorbed in breeding programs (Dowswell *et al.,* 1996) and an important part of maize germplasm is still in the hands of small-scale farmers (Carvalho *et al.,* 2004). Landraces and local cultivars in maize present a population which is genetically diverse and have been under farmer's selection for many years. Selection criteria include adaptation to local climatic conditions, plant characteristics, yield, biotic and abiotic stress tolerance or resistance (Wasala *et al.,* 2013). However, landraces are so far not efficiently being utilized because of unreliable information on agronomic as well as genetic potentials (Nass *et al*., 1993). The objective of this study was to evaluate the agronomic performance of local cultivars in comparison to improved maize varieties in Tanzania as well as elite lines from CIMMYT in order to identify potential breeding materials.

3.2 Materials and methods

In this study, 68 accessions (Table 2) were laid out in a randomized complete block design (RCBD) with three replications. The study was conducted in three sites namely Mlangarini (S 03^{o} 26' 12", E 036 ^{o} 47' 13.4" at 1128 m.a.s.l.); Tengeru (S 03^{o} 22' 30.2", E 036 ^{o} 48' 30.2" at 1237 m.a.s.l.) and Selian (S 03^{o} 21' 31.4", E 036 ^{o} 37' 51.9" at 1415 m.a.s.l.).

Table 2: Maize genotypes used in the evaluation of agronomic performances in Tanzania during April to September 2015.

SNo.	Accession name	Status	Source	SNo.	Accession name	Status	Source
1	CKDHL0500	Elite line	CIMMYT, Nairobi	35	TZA3167	Local cultivar	Genebank, Tanzania
2	CKDHL120552	Elite line	CIMMYT, Nairobi	36	TZA3171	Local cultivar	Genebank, Tanzania
3	CKSBL10205	Elite line	CIMMYT, Nairobi	37	TZA3181	Local cultivar	Genebank, Tanzania
4	CLRCY034-B	Elite line	CIMMYT, Nairobi	38	TZA3206	Local cultivar	Genebank, Tanzania
5	CLRCY039	Elite line	CIMMYT, Nairobi	39	TZA3310	Local cultivar	Genebank, Tanzania
6	CLYN261	Elite line	CIMMYT, Nairobi	40	TZA3536	Local cultivar	Genebank, Tanzania
7	CML440	Elite line	CIMMYT, Nairobi	41	TZA3544	Local cultivar	Genebank, Tanzania
8	CML442	Elite line	CIMMYT, Nairobi	42	TZA3585	Local cultivar	Genebank, Tanzania
9	CML443	Elite line	CIMMYT, Nairobi	43	TZA3614	Local cultivar	Genebank, Tanzania
10	CML544	Elite line	CIMMYT, Nairobi	44	TZA3837	Local cultivar	Genebank, Tanzania
11	TZMI730	Elite line	CIMMYT, Nairobi	45	TZA3914	Local cultivar	Genebank, Tanzania
12	DEKALB (DK 8031)	Hybrid	Agro Input shop	46	TZA3926	Local cultivar	Genebank, Tanzania
13	DH04	Hybrid	Agro Input shop	47	TZA3958	Local cultivar	Genebank, Tanzania
14	PIONEER (Phb 3253)	Hybrid	Agro Input shop	48	TZA3971	Local cultivar	Genebank, Tanzania
15	SC403	Hybrid	Agro Input shop	49	TZA4020	Local cultivar	Genebank, Tanzania
16	SITUKA1	OPV	Agro Input shop	50	TZA4164	Local cultivar	Genebank, Tanzania
17	SITUKAM1	OPV	Agro Input shop	51	TZA4203	Local cultivar	Genebank, Tanzania
18	TMV-1	OPV	Agro Input shop	52	TZA4320	Local cultivar	Genebank, Tanzania
19	TZA163	Local cultivar[b]	Genebank, Tanzania	53	TZA4351	Local cultivar	Genebank, Tanzania
20	TZA1723	Local cultivar	Genebank, Tanzania	54	TZA4574	Local cultivar	Genebank, Tanzania
21	TZA1724	Local cultivar	Genebank, Tanzania	55	TZA4667	Local cultivar	Genebank, Tanzania
22	TZA1745	Local cultivar	Genebank, Tanzania	56	TZA5102	Local cultivar	Genebank, Tanzania
23	TZA1753	Local cultivar	Genebank, Tanzania	57	TZA5129	Local cultivar	Genebank, Tanzania
24	TZA1757	Local cultivar	Genebank, Tanzania	58	TZA5138	Local cultivar	Genebank, Tanzania
25	TZA212	Local cultivar	Genebank, Tanzania	59	TZA5162	Local cultivar	Genebank, Tanzania
26	TZA2263	Local cultivar	Genebank, Tanzania	60	TZA5169	Local cultivar	Genebank, Tanzania
27	TZA2264	Local cultivar	Genebank, Tanzania	61	TZA5170	Local cultivar	Genebank, Tanzania
28	TZA2330	Local cultivar	Genebank, Tanzania	62	TZA5200	Local cultivar	Genebank, Tanzania
29	TZA2338	Local cultivar	Genebank, Tanzania	63	TZA5205	Local cultivar	Genebank, Tanzania
30	TZA2731	Local cultivar	Genebank, Tanzania	64	TZA5618	Local cultivar	Genebank, Tanzania
31	TZA2793	Local cultivar	Genebank, Tanzania	65	TZA5619	Local cultivar	Genebank, Tanzania
32	TZA2813	Local cultivar	Genebank, Tanzania	66	TZA599	Local cultivar	Genebank, Tanzania
33	TZA2843	Local cultivar	Genebank, Tanzania	67	TZA608	Local cultivar	Genebank, Tanzania
34	TZA2904	Local cultivar	Genebank, Tanzania	68	TZA93	Local cultivar	Genebank, Tanzania

[a] OPV = Open Pollinated Varieties

[b] Local cultivar = Local cultivated variety

Sowing was done in 4 rows at a spacing of 75 cm between rows and 30 cm within rows of 4 m long each. Two seeds were planted followed by thinning to one plant per hill in two weeks after sowing. At the time of sowing, the recommended dose of fertilizer (40:20:20 kg /ha NPK) was applied in the form of urea, single super phosphate and muriate of potash. Full dose of phosphorus, potash and 50% nitrogen was applied as basal dose and 50% nitrogen top dressed 30 days after sowing. The experiments were conducted during rainy season and irrigation was applied whenever necessary. Sowing at Mlangarini, Selian and Tengeru were both in April, 2015 while harvesting was done in September, 2015 for Mlangarini and October, 2015 for both Selian and Tengeru. Data were recorded on the two inner rows for days to 50% silking and anthesis (tasseling), ear diameter, ear length, number of kernel rows per ear, number of kernels per row, 1000 seed weight and grain yield per plant. The yield per plant data were collected by harvesting grains in all plants of the two inner rows and divided by the total number of plants harvested. Collected data were subjected to COSTAT and STATISTICA for analysis of variance and the correlation between parameters. Also, the analysis using GGE biplot was used to determine Genotype main effect and Genotype by Environment interaction where the variability factor was expected to be maize accessions (Genotype) and location (Environment). The Genotype plus Genotype by environment interaction (GGE biplot) analysis is a multivariate analytical technique that uses principal component analysis (PCA) to explore the relation between genotypes, environments and their interaction. It partition genotypes and genotype by environment interaction (G + GE) into principal components (Yan, 2001). Yield data were subjected to evaluation among the three groups, that is, landraces, improved varieties popularly grown in Tanzania and elite materials from CIMMYT, Nairobi, Kenya to see how they differ and perform in terms of yield.

3.3 Results and discussion

3.3.1 Analysis of variance of agronomic traits

In general, high significant ($p < 0.05$) difference among genotypes, locations as well as their interactions (Location x Genotype) were observed except for the interaction on ear length with environment which, was not significantly different (Table 3). OPV Situka 1 and hybrid DH04 emerged to be the best genotypes with respect to mean grain yield per plant with 116.01 g and

115.90 g, respectively across the three locations (Table 3). The least mean grain yield was obtained from a local cultivar TZA 2813 with 11.52 g per plant. The detailed table showing the performance of all the 68 accessions across all three locations is given in Appendix 1. The OPV maize cultivars are reported to be able to produce grain yield which is the same or a little more than hybrids (Omondi *et al.,* 2014) because they are in most cases domesticated and developed under localized marginal areas (Gudu *et al.,* 2005).

The local cultivar TZA 2813 took the longest time (100.67 days) to 50% silking while another local cultivar TZA 1724 was late maturing among all genotypes with 90.67 days to 50% anthesis (tasseling) (Table 3). On the other hand, a hybrid SC 403 was the earliest with both 63.67 days to 50% anthesis (tasseling) as well as 69.78 days to 50% silking. However, OPV's Situka 1 and Situka M1 were as well among the genotypes that took shorter time to 50% anthesis (tasseling) and silking. The number of days to anthesis or tasseling or even to silking signify maturity differences (Olaoye, 2009). The genotypes which took the shortest time to reach maturity had also high grain yield as compared with those that were late to reach maturity (Table 3). This is due to the fact that early maturing cultivars posses an opportunity to escape early occurring stresses and adapt to stresses that would occur at the end of the season (Salami *et al.,* 2007). Silking delay causes maize to become barren and it is associated with poor development of an ear during flowering (Edmeades *et al.,* 1993). This is also evidenced by the frequent occurrence of negative association between days to 50% flowering and grain yield (Bolafios and Edmeades, 1996). The current study showed that hybrid Pioneer (Phb 3253) with respect to yield related-parameters had the highest ear length, ear diameter and number of kernels per row while hybrid SC 403 had the highest 1000 seed weight (Table 3). The trend of improved varieties (hybrids) to perform better than landraces in terms of yield and other yield-related parameters had also been found by Wasala *et al.* (2013).

Table 3: Performance of maize local cultivars (TZA lines), OPV, improved varieties and elite lines from CYMMIT on grain yield and yield components.

Genotype name	Status	Yield per plant (g)	1000 Kernel weight (g)	Days to 50% Tasseling	Days to 50% Silking	Ear Length (cm)	Ear diameter (cm)	Number of Kernels per row	Number of Kernel rows
The first ten genotypes									
SITUKA1	OPV[a]	116.01±12.28	275.51±19.47	64.33±2.25	70.89±2.67	16.02±0.89	4.28±0.17	31.17±2.08	13.05±0.33
DH04	Hybrid	115.90±14.48	273.74±15.33	72.11±2.05	75.44±2.01	15.58±0.41	4.51±0.07	32.65±1.75	12.78±0.13
PIONEER	Hybrid	112.73±17.19	258.10±25.60	69.89±1.76	75.89±2.04	17.66±0.36	4.64±0.05	36.07±0.96	13.83±0.18
TMV-1	OPV	108.14±10.84	247.27±13.52	73.44±1.82	77.78±1.88	16.50±0.52	4.36±0.07	31.92±1.71	13.92±0.50
DEKALB	Hybrid	104.74±9.11	302.73±19.86	66.44±2.29	69.89±2.39	16.63±0.51	4.58±0.09	33.09±1.17	12.42±0.13
TZA2793	Local cultivar [b]	100.46±11.22	260.68±10.45	78.33±2.38	84.00±2.74	15.86±0.39	4.24±0.08	29.36±1.47	12.45±0.20
TZA5170	Local cultivar	99.80±14.55	285.36±26.12	77.44±2.46	83.00±2.71	15.14±0.54	4.38±0.09	26.77±1.55	11.78±0.26
SITUKAM1	OPV	99.41±14.94	301.99±13.68	64.11±2.19	71.56±1.89	15.95±0.33	4.43±0.08	30.30±1.16	13.19±0.22
TZA2263	Local cultivar	97.54±17.01	260.73±20.96	80.33±2.69	86.11±3.29	15.35±0.69	4.37±0.12	28.86±1.73	12.77±0.14
SC403	Hybrid	95.14±14.76	313.55±18.15	63.67±2.05	69.78±1.96	16.77±0.59	4.52±0.11	33.10±1.88	12.67±0.16
Ten genotypes in the middle									
TZA3544	Local cultivar	63.34±7.75	272.89±15.38	78.11±2.75	85.67±2.82	14.54±0.70	4.21±0.06	23.41±1.78	12.47±0.18
TZA3914	Local cultivar	61.16±7.01	271.82±15.96	79.33±2.95	82.89±3.28	14.83±0.78	4.26±0.11	22.73±1.59	12.25±0.40
TZA4574	Local cultivar	60.84±8.43	221.23±23.26	81.44±2.92	89.44±3.50	13.59±0.81	3.67±0.17	25.07±2.53	10.90±0.36
CLYN261	CIMMYT Line	60.46±10.82	232.81±24.03	84.56±2.75	85.89±2.78	13.01±0.55	3.65±0.13	18.96±1.82	13.01±0.21
TZA1723	Local cultivar	60.36±5.02	265.78±15.32	81.89±2.84	89.33±2.87	15.11±0.49	3.73±0.06	23.90±0.96	11.10±0.17
TZA5138	Local cultivar	60.13±11.55	213.58±11.39	76.78±2.02	83.33±2.35	14.18±0.48	4.14±0.08	25.37±1.66	13.52±0.41
TZA5162	Local cultivar	59.20±13.58	219.55±25.49	86.56±3.31	93.78±3.34	14.34±0.39	3.80±0.10	24.17±1.75	11.43±0.30
TZA2264	Local cultivar	58.28±11.09	250.20±14.40	78.44±2.26	84.11±2.73	14.03±0.43	4.37±0.11	26.81±1.28	13.05±0.27
TZA3167	Local cultivar	57.66±6.25	272.20±14.34	77.33±2.49	83.89±2.66	14.72±0.63	3.98±0.08	24.62±1.08	11.12±0.12
TZA3310	Local cultivar	57.39±7.29	238.47±13.35	82.72±2.52	90.72±2.39	13.83±0.36	3.87±0.08	24.57±1.95	11.09±0.33
The last ten genotypes									
TZA3181	Local cultivar	41.26±6.98	269.77±27.96	81.22±3.78	89.67±4.32	14.25±0.89	3.8±0.14	23.15±1.47	10.90±0.82
TZA93	Local cultivar	40.45±2.91	271.26±18.52	77.89±2.20	87.33±1.98	16.46±0.48	4.05±0.09	25.27±0.99	11.37±0.18
TZA5618	Local cultivar	36.69±5.03	219.87±24.64	85.78±2.99	94.44±3.31	15.41±0.67	4.01±0.08	21.50±1.33	11.99±0.26
CKSBL10205	CIMMYT Line	35.95±11.31	185.63±15.87	79.56±2.22	81.22±2.17	10.80±0.55	3.28±0.18	17.72±1.91	12.53±0.96
TZA1757	Local cultivar	35.72±8.24	284.61±24.16	85.11±3.46	93.00±3.73	14.59±0.64	4.23±0.17	23.35±1.31	11.61±0.40
CML544	CIMMYT Line	29.20±5.18	192.49±19.20	78.00±2.81	80.56±2.84	12.72±0.50	3.69±0.09	18.84±0.73	12.94±0.37
CML440	CIMMYT Line	29.03±4.80	174.27±10.31	72.11±1.98	76.22±2.52	11.48±0.30	3.36±0.11	17.88±0.75	12.82±0.37
TZA1724	Local cultivar	24.47±3.27	248.36±25.28	90.67±2.98	101.33±2.97	16.00±0.52	3.72±0.13	20.18±2.43	10.12±0.40
TZMI730	CIMMYT Line	21.14±3.85	204.93±21.38	87.78±1.88	91.00±2.10	10.32±0.73	3.34±0.19	15.44±2.45	12.01±0.63
TZA2813	Local cultivar	11.52±2.16	174.11±15.90	88.22±3.10	100.67±3.41	11.39±0.80	3.37±0.10	13.18±1.81	11.19±0.62
Grand mean		**62.94±1.48**	**249.59±2.59**	**78.98±0.40**	**85.70±0.45**	**14.52±0.10**	**4.01±0.02**	**24.59±0.26**	**12.21±0.06**
2 WAY ANOVA (F - Statistics)									
Location (L)		49.31***	155.64***	1342.61***	1321.48***	131.61***	129.77***	113.69***	26.93***
Genotypes (G)		6.59***	5.48***	30.65***	36.79***	6.92***	15.40***	10.86***	9.43***
Interaction (L x G)		1.45**	1.59***	1.41**	1.64***	1.12^{ns}	1.50**	1.60***	1.98***

[a] OPV = Open Pollinated Varieties; [b] Local cultivar = Local cultivated variety; Means in a column followed by the same alphabets are not significantly different; a letter followed by a dash and then a letter means to reduce a number of letters where that indicates a range of letters from the first to the last after the dash in alphabetical order. *** signify the statistical significance difference at a probability level of $p < 0.05$, ^{ns} stands for non significant difference

Note: Means of all parameters are ranked following the performance in terms of yield per plant.

3.3.2 Correlation between parameters

The Pearson's correlation analysis expressed significant ($p < 0.05$) relationships among eight parameters (traits) except for the relationship between number of kernel rows per an ear and 1000 kernel weight. Similar results of non-significant relationship was found by Zarei *et al*. (2012) thus supporting this study. The analysis showed a significant negative correlation between days to 50% anthesis and days to 50% silking with the rest of the parameters (Table 4). The number of days to anthesis and silking are very important because when the duration is long, it eventually causes yield and other related parameters to be low (Bolafios and Edmeades, 1996). The rest of the yield-related parameters which include ear length, ear diameter, number of kernels per row, number of kernel rows per ear, 1000 kernel weight and grain yield per plant were positively correlated to each other (Table 4). Correlation between traits and identification of fundamentally important traits which contribute to each other as well as to the ultimate goal is useful in selection during breeding programs.

Table 4: The Pearson correlation coefficient of eight parameters obtained from yield evaluation experiment of 68 maize accessions at three locations.

	Days to 50% tasseling	**Days to 50% silking**	**Ear length (cm)**	**Ear diameter (cm)**	**Number of kernels per row**	**Number of kernel rows per ear**	**1000 kernel weight (g)**	**Yield per plant (g)**
Days to 50% tasseling	1							
Days to 50% silking	0.944**	1						
Ear length (cm)	-0.485**	-0.440**	1					
Ear diameter (cm)	-0.430**	-0.404**	0.656**	1				
Number of kernels per row	-0.551**	-0.527**	0.721**	0.740**	1			
Number of kernel rows per ear	-0.262**	-0.320**	0.273**	0.524**	0.417**	1		
1000 kernel weight (g)	-0.285**	-0.241**	0.469**	0.532**	0.395**	0.033^{ns}	1	
Yield per plant (g)	-0.457**	-0.464**	0.524**	0.536**	0.622**	0.310**	0.401**	1

** signify the statistical significance difference at a probability level of $p < 0.05$, ^{ns} stands for non significant different

3.3.3 GGE biplot analysis

The first two principal components (PC1 and PC2) explained 91.2% of the total variance given by the 68 genotypes across the three locations (Fig. 1). PC1 accounted for 69.5% and PC2 21.7%, where PC1 explained genotype productivity and PC2 expressed genotype stability as explained by Yan *et al*. (2000). That means Genotypes having PC1 reading less than 0 are likely

to be high yielding and the vice versa is also true. On the other hand, genotypes with PC2 values close to zero are more stable (Hugos and Abay, 2013).

i. Which win where?

Fig. 1 displays a polygon which is obtained through joining points where genotypes are located furthest from origin of the biplot while other remaining genotypes are contained within the polygon (Yan and Tinker, 2006). The lines extending from the biplot origin toward points where perpendicular lines are formed gives out divisions of what is called sectors. The winning genotypes appear on the corners (vertex) of respective sectors. Thus, Pioneer (Phb 3253) emerged to be the best on yield at Selian experimental site while Situka 1 gave the best yield at both Tengeru and Mlangarini sites. In addition, TZA 2263, TZA 5162, TZA 2813, TZA 1724 and TZA 4320 were genotypes that expressed general adaptation with no specific location (Fig. 1).

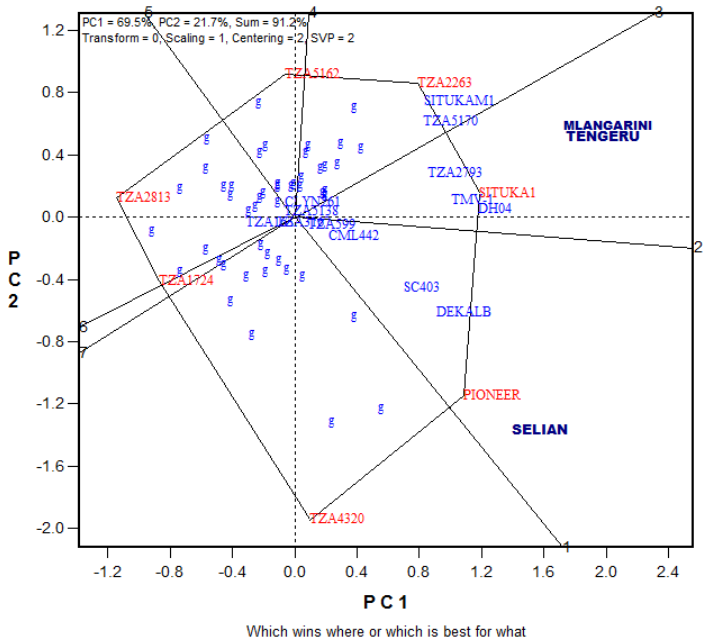

Figure 1: The "which win where" feature of the GGE biplot for maize grain yield of 68 genotypes in three locations, where g stands for genotypes name.

ii. Genotypes performance against an ideal genotype

In Fig. 2, the 68 maize genotypes were ranked in relation to mean yield as well as stability with reference to an ideal genotype. The ideal genotype is found at the center of the inner circle as a point along the single arrow Average Environment Coordination (AEC) x - axis (Fig. 2) (Yan and Tinker, 2006). The point (an ideal genotype) possesses the longest vector with zero value across the double arrow axis. Thus, any genotype found near to the point of an ideal genotype can be considered promising than others. Therefore, Situka 1 and hybrid DH 04 were found to be the most desirable genotypes than others because they had the highest yield and still were more stable. Genotypes TMV 1, TZA 2793 and SC 403 could still be considered desirable while hybrid Pioneer (Phb 3253) was among the higher yielding genotypes but less stable across environments. On the other hand, TZA 2813 gave the least overall mean yield and TZA 4320 was the most unstable genotype (Fig. 2).

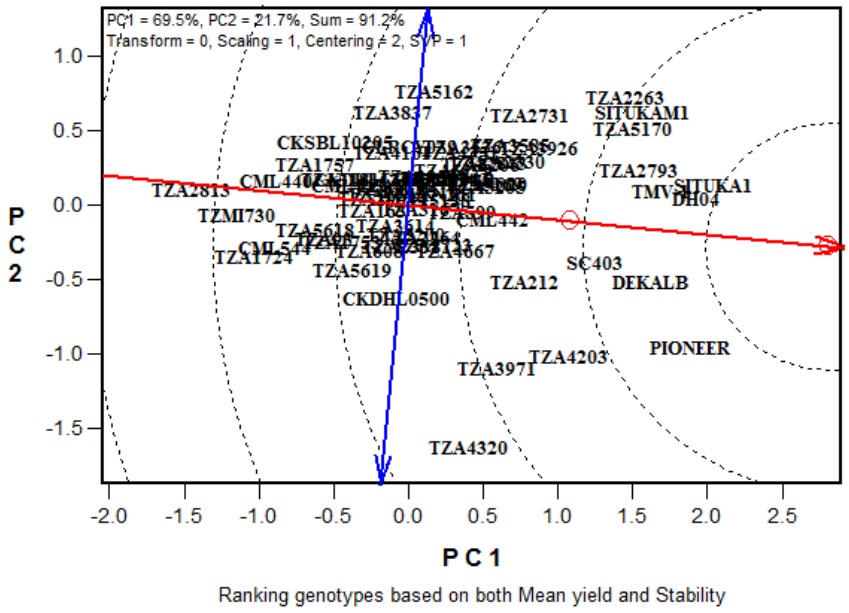

Figure 2: The average environment coordination (AEC) graphic that rank genotypes relative to an ideal genotype (the center of the concentric circles) through a GGE biplot for maize grain yield of 68 genotypes at three locations.

iii. Test environment evaluation

Fig. 3 presents the test-environment evaluation of the three locations where the line that joins biplot origin and any location, corresponds to the standard deviation derived from the mean of the genotypes in a certain environment (Yan and Tinker, 2006). It shows Tengeru to be more discriminative than the others. This means that Tengeru was able to provide much information about differences among genotypes than other locations. In line with explanation by Mehari *et al*. (2015), Tengeru was more strongly correlated to Mlangarini as the angle between them was small, while the angle between both Tangeru and Mlangarini with Selian was bigger and hence not closely related to them. Therefore, the biplot identified Tengeru to be the test environment that is close to an ideal environment which is eventually defined to be both discriminating and representative (Yan *et al.*, 2007).

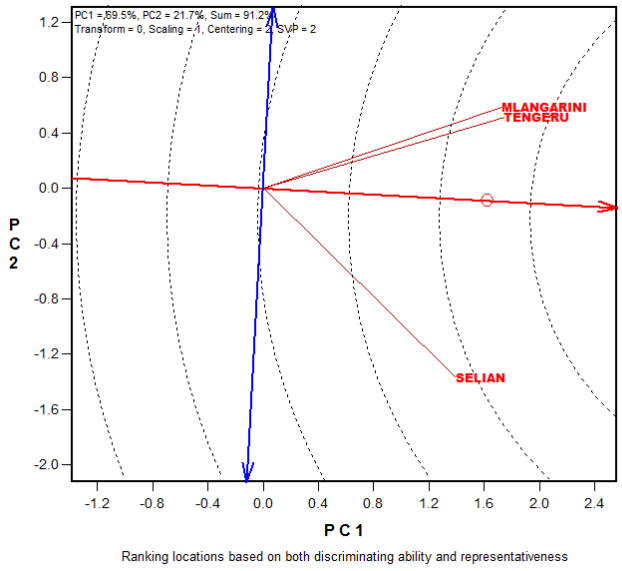

Figure 3: The GGE biplot displaying the relation among experimental locations, their discriminating ability and representativeness through evaluation of 68 genotypes.

3.4 Conclusion

The significant differences expressed among the 68 genotypes and the interactions among those genotypes and environments suggest that genotypes were different (variable) from each other and they responded differently in different environments. This variability is desirable for use in breeding programs to develop improved maize varieties required by the farmers for specific traits and even for specific location. Successful plant breeding then requires careful selection of genotypes that possess desirable traits for best combinations. The correlations between traits are also another important knowledge that helps on predicting the required performance in terms of a certain trait. For example, the negative correlation between yield and flowering parameters help to have an indirect selection of higher yielding genotypes through flowering behaviour. The determination of best genotypes also requires knowing the location with high discriminative power, meaning the location which is able to provide much information about differences among genotypes. This study showed that an OPV Situka 1 and a hybrid DH 04 were generally the best performing genotypes in terms of grain yield and stability as well as for other yield-related parameters across all the three locations. However, TZA 2793 was a local cultivar that expressed promising performance for yield. The maize landraces have always been considered less productive than other improved varieties, but they present an important source of genetic variability that can be exploited during a search for genes against biotic and abiotic stresses. Therefore, identifying potential traits as well as genotypes possessing those important traits make it possible to plan for crosses and obtain required characteristics for future breeding programs.

CHAPTER FOUR

Genetic diversity of maize landraces from Tanzania as compared with commercial improved varieties and elite lines through morphological characterization[3]

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Abstract

Maize production challenges require well-known genetic diversity that are well adapted to geographic sites to ensure effective improvement. This study aimed at conducting morphological evaluation for 50 maize landraces from Tanzania compared with 7 commercial varieties and 11 elite lines from CIMMYT in Kenya. The experiments were laid out in randomized complete block design (RCBD) at three locations in Arusha region, Tanzania. Data were collected on 19 quantitative and 12 qualitative traits that were subjected to analysis of variance, descriptive and multivariate statistics. Significant variations ($p < 0.05$) were observed for all traits while higher contribution for accessions variability were found with yield, a thousand kernel weight, flowering traits, kernel, ear and vegetative plant characteristics. Commercial varieties were characterized by significant yield (107.4g per plant) and yield related parameters namely a thousand seed weight, number of rows per an ear, ear diameter and ear length. Also early days to tasseling and silking of 67.7 and 73 days, respectively. CIMMYT elite lines were characterized by significant low plant and ear height of 138.9cm and 50.6cm, respectively as well as flint kernel type. Landraces were more diverse in every trait evaluated with significant long anthesis-silking interval of 7.5 days and large ear height of 95.9cm. Some landraces such as TZA 2793 and TZA 5170 expressed significant traits that would be tapped for further crop improvement. Other landraces clustered themselves irregularly in terms of their collection sites within their

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major group due to selection and exchange of seeds. Thus, farmers as custodians of landraces are supposed to be involved in a systematic selection and breeding.

Key words: Genetic diversity, Maize, Landraces, Morphological characterization, Improved varieties

4.1 Introduction

Maize (*Zea mays* L.) sustains a huge population in the world (Romay *et al.*, 2013) and is even equated to the national food security in Tanzania (Katinila *et al*., 1998). However, its average yield is still very low with 1.2 MT per hectare in Tanzania as compared with the estimated potential yields of 4 to 5 MT per hectare (Moshi *et al.*, 1990; Otunge *et al.*, 2010). Low yield has been attributed to factors such as lack of quality inputs (eg seeds, fertilizer), drought, pests and diseases. Nevertheless, maize is a crop which is potentially diverse in terms of phenotypic and genetic characters (Whitt *et al*., 2002). The genetic variation of maize constitute a very important package for breeding (Prasanna, 2010; Yao *et al*., 2007) which requires the availability of desirable characters for maize crop improvements (Ristic *et al.*, 2013). However, for the past decades, breeding in maize have been concentrated in short breeding programs that uses inbred lines, elite lines and breeder materials (Cömertpay, 2012; Yao *et al.*, 2007). These materials are in most cases uniform such that for a long time they have caused the existence of a narrow based genetic background (Shiri *et al*., 2014; Yao *et al*., 2007). The narrow based genetic background has always been coupled with genetic erosion and habitat alteration that resulted in an increased sensitivity to new pathogenic races as well as decreased resistance and tolerance to environmental extremes (Prasanna, 2010). Germplasm are heterogeneous in nature and are open pollinated with a wide range of adaptability to an extensive range of environmental variability (Rahman *et al.*, 2008). Maize landraces are reported to be genetically heterogeneous populations which have been selected by farmers for environmental adaptability (Aci *et al*., 2013; Ignjatovic *et al*., 2013). They can also be used to explore for resistance and tolerance against biotic and abiotic environmental stress factors (Molin *et al.*, 2013). Salami *et al*. (2015) found significant morphological variation with Benin local and improved maize varieties on all traits with distinctive potential highlight on early maturity and sensitivity to maize streak virus. Traits such as plant growth, tassel characteristics and yield had a significant contribution to phenotypic

variation between maize landraces that were assessed by Ristic *et al*. (2014). Significant amount of variability was observed by Rahman *et al*. (2008) from the morphological traits evaluated in maize population from Pakistan. Italian maize landraces showed significant morphological variation on earliness, plant architecture traits, tassel, ear and kernel characteristics (Hartings *et al*., 2008). Asare *et al*. (2016) suggested that maize landraces presents a significant genetic diversity reserve of important morphological characteristics on phenology, plant growth, grain yield, and leaf photosynthesis that reflects farmer preferences and worth for maize crop improvement.

In Tanzania, Nestory and Reuben (2016) evaluated maize landraces from northern part of the country and obtained high traits variability that serve as an opportunity for enhancing genetic improvement to maize germplasm required by the community. On the other hand, Bucheyeki (2012) evaluated maize landraces from Tanzania and identified potential sources of northern leaf blight disease resistance. However, Tanzania still holds a vast majority of germplasm that remain marginally exploited and are of great importance for food, adaptability, resistance to pests and diseases as well as for quality attributes (Ngwediagi *et al*., 2009).

The general decreasing trend in maize production and yield in Tanzania is caused by recurrent abiotic and biotic stresses (Bucheyeki, 2012) and recently Tanzania and East Africa in general has been hit hard by another new deadly disease called Maize Lethal Necrosis disease (Kabululu *et al*., 2017; Kiruwa *et al*., 2016; Wangai *et al*., 2012b). Thus detailed characterization of landraces and other germplasm is required to establish a gene pool for crop improvement (Drinic *et al*., 2012; Obeng-Antwi, 2012; Saad and Rao, 2001). The objective of this study was therefore to evaluate the genetic diversity of maize accessions from Tanzania through morphological characterization in order to establish the existing genetic diversity worth for maize crop improvement.

4.2 Materials and methods

4.2.1 Seed materials

The 68 accessions used in this study included 50 landraces collected in Tanzania, seven improved commercial varieties in Tanzania and eleven elite lines from CIMMYT in Nairobi,

Kenya (Table 5). Seeds of the 50 landraces of maize were obtained from the National Plant Genetic Resources Centre (NPGRC) in Arusha, Tanzania and were collected from different parts of the country (Fig. 4). The sampling of the collected maize accessions was done considering a wide distribution over the country.

Table 5: List of maize accessions and their source as used in the genetic diversity evaluation study in Tanzania.

SNo.	Accession	Source	SNo.	Accession	Source
1	CKDHL0500	CIMMYT	35	TZA3171	Kigoma
2	CKDHL120552	CIMMYT	36	TZA3181	Kigoma
3	CKSBL10205	CIMMYT	37	TZA3206	Tabora
4	CLRCY034-B	CIMMYT	38	TZA3310	Tabora
5	CLRCY039	CIMMYT	39	TZA3536	Morogoro
6	CLYN261	CIMMYT	40	TZA3544	Morogoro
7	CML440	CIMMYT	41	TZA3585	Mtwara
8	CML442	CIMMYT	42	TZA3614	Mtwara
9	CML443	CIMMYT	43	TZA3837	Mtwara
10	CML544	CIMMYT	44	TZA3914	Mara
11	DEKALB (DK8031)	Commercial variety	45	TZA3926	Mara
12	DH04	Commercial variety	46	TZA3958	Mara
13	PIONEER (Phb 3253)	Commercial variety	47	TZA3971	Mara
14	SC403	Commercial variety	48	TZA4020	Mwanza
15	SITUKA1	Commercial variety	49	TZA4164	Kagera
16	SITUKAM1	Commercial variety	50	TZA4203	Mwanza
17	TMV-1	Commercial variety	51	TZA4320	Kagera
18	TZA163	Mtwara	52	TZA4351	Kagera
19	TZA1723	Njombe	53	TZA4574	Mwanza
20	TZA1724	Njombe	54	TZA4667	Mwanza
21	TZA1745	Njombe	55	TZA5102	Tanga
22	TZA1753	Mbeya	56	TZA5129	Tanga
23	TZA1757	Mbeya	57	TZA5138	Tanga
24	TZA212	Mbeya	58	TZA5162	Tanga
25	TZA2263	Lindi	59	TZA5169	Tanga
26	TZA2264	Lindi	60	TZA5170	Tanga
27	TZA2330	Lindi	61	TZA5200	Tanga
28	TZA2338	Mtwara	62	TZA5205	Tanga
29	TZA2731	Morogoro	63	TZA5618	Manyara
30	TZA2793	Morogoro	64	TZA5619	Manyara
31	TZA2813	Tanga	65	TZA599	Singida
32	TZA2843	Tanga	66	TZA608	Singida
33	TZA2904	Ruvuma	67	TZA93	Rukwa
34	TZA3167	Kigoma	68	TZMI730	CIMMYT

Also seven improved commercial maize varieties were obtained from the agro-input shops in Arusha and CIMMYT inbred lines were sourced from CIMMYT in Nairobi, Kenya. Both improved commercial maize varieties and CIMMYT inbred lines were used in this study as checks.

4.2.2 Field location and experimental design

Morphological characterization experiment was laid out in a randomized complete block design with three replications. The experiment was conducted in three locations in Arusha region as follows; Mlangarini at S 03^{o} 26' 12", E 036 ^{o} 47' 13.4" with elevation of 1128 m.a.s.l.; Tengeru at S 03^{o} 22' 30.2", E 036 ^{o} 48' 30.2" with elevation of 1237 m.a.s.l and Selian at S 03^{o} 21' 31.4", E 036 ^{o} 37' 51.9" with elevation of 1415 m.a.s.l. Maize accessions were planted in rows per each plot at spacing of 75 cm between rows and 30 cm within rows. Each row was 4 meters long and with an approximation of 44,444 plants population per hectare. The seeds were planted during the rainy season in 2015 where irrigation was applied whenever necessary to provide an optimum growing condition as possible. Two seeds were planted per hill followed by thinning to one plant per hill in two weeks after sowing. At planting, NPK (40:20:20) was applied at a rate of 100 kg N per hectare and top dressing with Urea (46%) were applied later at a rate of 100 kg N per hectare. Data collection generally included: vegetative, ear and kernel characteristics (Table 6). The data were collected according to the descriptor list by IBPGR (1991). Yield per plant data were collected by harvesting grains on the two inner rows and divided by the total number of plants harvested.

4.2.3 Data analysis

Descriptive statistics on means, minimum, maximum, standard errors and coefficient of variation using STATISTICA 8.0 were obtained for quantitative traits while one-way analysis of variance (ANOVA) was applied for significance test on morphological differences among maize accessions. Box and whisker plot was also used to show variability within quantitative characters. Cluster analysis was performed based on average linkage method through Genstat discovery edition 4 to generate similarity and dissimilarity among accessions and eventually comparing between groups of accessions clustered together. Principle component analysis was

done to identify traits with significant contribution to the overall variation within each principle component.

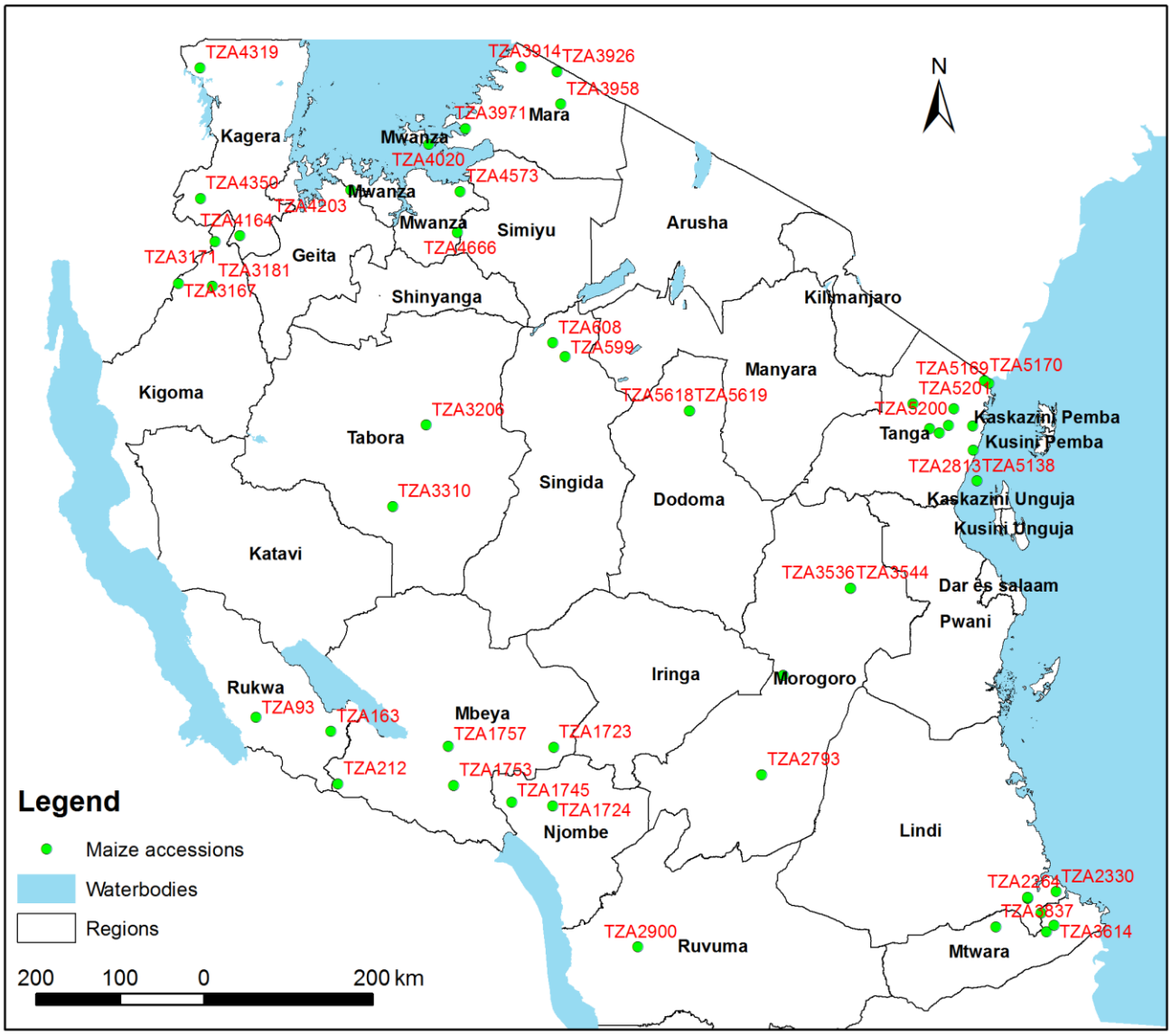

Figure 4: Map of Tanzania showing the collection sites of the 50 maize landraces

Table 6: Some descriptors used to evaluate genetic diversity through morphological characterization (IBPGR, 1991).

ITEM		DATA COLLECTION PROCEDURE
Vegetative data	**Abbreviation**	
Days to tasseling	D50T	Number of days from sowing to when 50% plants shed pollen
Days to silking	D50S	Number of days from sowing to 50% plants with silks
Anthesis-Silking Interval	ASI	Difference between days to silking and anthesis stages
Plant height (cm)	PH	Ground level to the base of the tassel. After milk stage
Ear height (cm)	EH	Ground to the node at the uppermost ear. After milk stage
Foliage	FG	Rating of total leaf surface. After milk stage, on 20 plants
Number of leaves above the uppermost ear	NLAUME	Counted on at least 20 plants. After milk stage
Stem colour	SC	Observed between the two topmost ears. At flowering
Sheath pubescence	SP	The hairy condition of leaf base encasing the stem of a plant
Leaf length	LL	From ligule to apex of the uppermost ear leaf . After flowering
Leaf width (cm)	LW	Mid-way along its length. Measured on the same leaf
Ear data		
Ear length (cm)	EL	Measured from the base to the tip of the uppermost ear
Ear diameter (cm)	ED	Measured at the central part of the uppermost ear
Kernel row arrangement	KRA	Pattern and arrangement of rows of the uppermost ear
Number of kernel rows	NKR	Counting kernel rows in the central part of the uppermost ear
Cob diameter (cm)	CD	Mid-way of cob length
Rachis diameter (cm)	RD	Diameter of the inner part of the cob
Number of kernels per row	NK_R	Count number of kernel in a single row of the uppermost ear
Cob colour	CC	Rating colours of the cobs
Shape of uppermost ear	SOUME	Determining the shape through observation
Kernel data		
Kernel type	KT	Indicate up to three kernel types in the order of frequency
Kernel colour	KC	Indicate up to three kernel colours in the order of frequency
1000 kernel weight (g)	1000KW	Adjusted to 10% moisture content
Kernel length (mm)	KL	Average of 10 kernels from the row in the middle
Kernel width (mm)	KW	Measured on the same 10 kernels
Kernel thickness (mm)	KTH	Measured on the same 10 kernels
Shape of upper surface of kernel	SOUSOK	Determining the shape through observation
Endosperm colour	EC	Colour of the tissue inside the seeds
Yield per plant (g)	Y_P	Grain yield per plant in grams

4.3 Results

4.3.1 Variability in quantitative characters

The descriptive statistics and analysis of variance of the 19 quantitative traits revealed a significant ($p < 0.05$) variation of all the traits among landraces (Table 7). Significant variation was observed in commercial varieties except for leaf width, cob diameter, rachis diameter, kernel width, kernel thickness and yield per plant. The CIMMYT elite lines had also significant traits variability except for only ear diameter and cob diameter. Significant coefficient of variation was observed with anthesis-silking interval as well as with yield per plant. The box and Whisker plot farther displayed the performance and variability among the groups of maize accessions, where the overall grain yield per plant was higher with commercial varieties, but high variability is seen

with landraces portrayed by the range of non outliers values (Fig. 5A). This was also observed with the weight of a thousand seed weight (Fig. 5B) where commercial varieties generated heavier seeds than other groups, but also the variability of this trait in landraces is more than other groups. Landraces showed high variation in number of kernel rows per ear while commercial varieties exhibited higher variability for number of kernels per row than other groups (Fig. 5D and E). For flowering behaviour, commercial varieties flowered earlier as compared with other groups that had almost the same median value. Landraces showed high variation in flowering as well as for plant height when compared with other groups (Fig. 5C and F).

Table 7: Mean values with standard errors, minimum and maximum (range), and coefficient of variation of the 19 quantitative morphological descriptors generated from the performance of the three groups of accessions.

	Landraces			**Commercial varieties**			**CIMMYT lines**		
[a]Descriptor	**Mean ± SE**	**Range**	**CV**	**Mean ± SE**	**Range**	**CV**	**Mean ± SE**	**Range**	**CV**
D50T	80.2±0.4***	61.0-94.7	6.6	67.7±0.9***	62.3-75.0	6.0	80.6±0.9***	70.0-91.7	6.5
D50S	87.7±0.5***	74.0-108.3	7.4	73±0.7***	69.0-79.7	4.5	84.6±0.9***	73.7-94.3	6.2
ASI	7.5±0.2***	2.0-15.7	32.7	5.3±0.4***	2.0-7.7	32.5	4.0±0.4***	0.3-8.0	52.7
NLAUME	6.5±0.0***	5.6-7.7	5.4	6.7±0.1***	6.1-7.1	4.5	6.6±0.1***	5.2-7.6	10.3
PH (cm)	205.4±1.8***	162.1-275.0	11.0	211.8±3.4***	187.3-239.1	7.3	138.9±3.6***	96.6-172.9	15.0
EH (cm)	95.9±1.4***	59.6-156.8	18.1	78.7±2.4***	61.9-100.2	14.1	50.6±1.6***	29.8-65.6	18.7
LL (cm)	80.1±0.4***	69.2-95.7	6.2	83.2±0.8***	74.2-90.2	4.7	70.7±1.3***	59.1-85.5	10.8
LW (cm)	9.1±0.1***	7.5-12.0	7.5	9.7±0.1[ns]	9.1-11.1	5.3	8.7±0.2***	7.0-11.0	12.4
EL (cm)	14.6±0.1***	10.9-18.4	8.8	16.4±0.2***	15.1-18.2	4.5	12.7±0.3***	9.4-20.0	15.4
ED (cm)	4.8±0.0***	3.6-6.2	9.1	5.2±0.1***	4.3-6.1	7.4	4.4±0.1[ns]	3.3-5.4	12.9
CD (cm)	3.0±0.0***	2.3-4.0	10.6	3.1±0.1[ns]	2.8-3.8	8.1	2.8±0.1[ns]	2.3-3.6	10.6
RD (cm)	1.6±0.0***	1.2-3.6	14.5	1.6±0.0[ns]	1.5-1.8	4.4	1.4±0.0***	1.1-1.7	11.8
NK_R	24.7±0.3***	12.6-33.3	15.1	32.6±0.4***	29.6-37.0	6.3	19.1±0.5***	14.2-26.5	14.6
NKR	12.0±0.1***	9.1-14.7	9.0	13.1±0.1***	12.3-14.8	5.2	12.6±0.2***	9.0-14.8	9.0
KL (cm)	1.0±0.0***	0.8-1.2	6.7	1.1±0.0***	1.0-1.3	6.3	0.9±0.0***	0.7-1.1	11.3
KW (cm)	1.0±0.0***	0.8-1.4	7.4	1.0±0.0[ns]	0.9-1.2	6.6	0.8±0.0***	0.7-0.9	5.5
KTH (cm)	0.5±0.0***	0.4-0.9	12.8	0.5±0.0[ns]	0.4-0.5	4.8	0.6±0.0***	0.5-0.8	11.1
1000KW (g)	256.2±2.7***	160.0-340.0	12.9	281.8±6.5***	237.0-334.4	10.5	199.1±4.4***	148.2-257.3	12.6
Y/_P (g)	60.6±1.7***	9.8-133.7	34.3	107.4±4.8[ns]	50.1-136.6	20.4	45.2±3.0***	11.5-85.9	38.4

[a] abbreviation of the descriptors are defined and explained in Table 2
*** stands for significant difference at P <0.05
[ns] not significant different

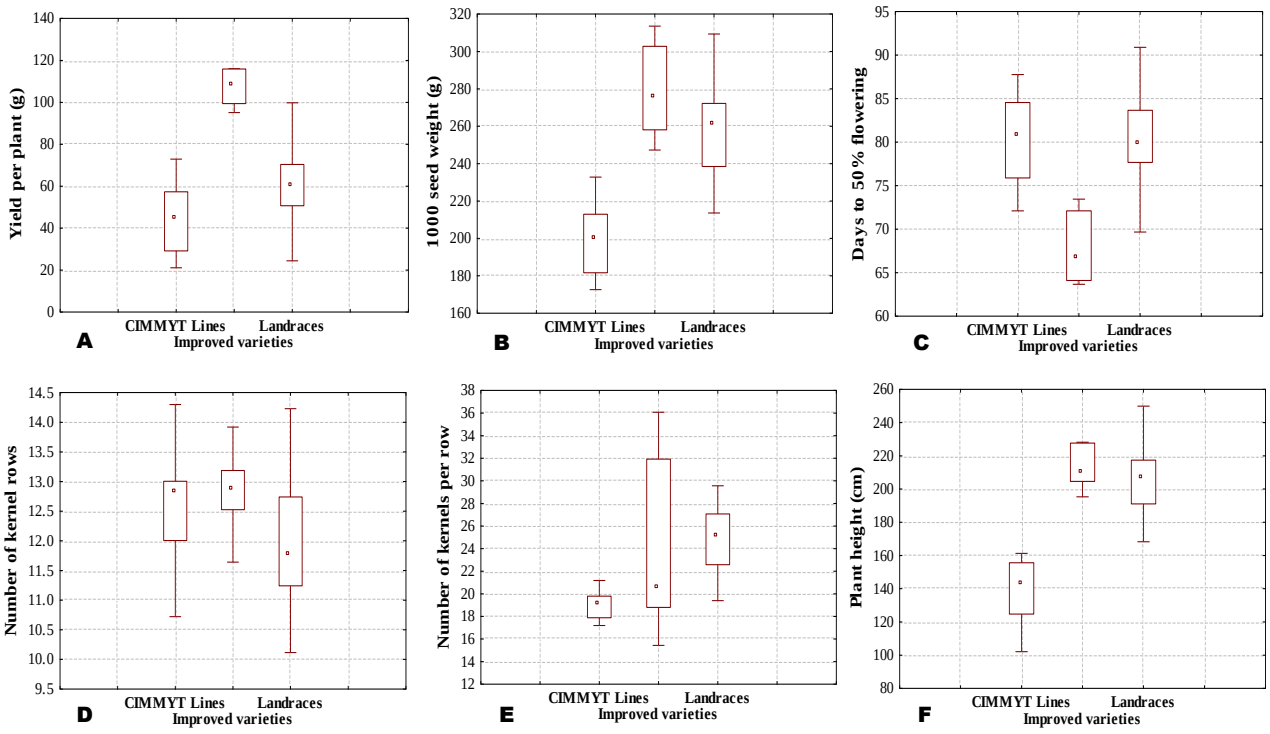

Figure 5: Box plots displaying variability in morphological descriptors given by the three different groups of CIMMYT inbred lines, commercial/Improved varieties and landraces from Tanzania. Small empty boxes inside big boxes represent median, big vertical boxes show a range of values falling between 25% and 75% and the vertical lines cover the range of non-outliers.

4.3.2 Qualitative characters

The qualitative data of the 68 accessions were categorized into three groups and they showed landraces to be more diverse as compared to the commercial varieties and CIMMYT lines (Table 8). This means the landraces were distributed in classes of all the 12 qualitative traits within the accessions while for the other groups the accessions distribution was not in all classes of each trait. The percentage distribution of accessions at each trait differentiated the three groups of accessions in terms of foliage (rating of total leaf surface), where landraces were mostly characterized as intermediate (44.9%), commercial varieties were large (63.5%) while CIMMYT

Table 8: The 12 qualitative morphological descriptors with their percentage (%) frequency accessions distribution as generated from the performance of three different groups within 68 maize accessions used in this study.

Morphological descriptor	Descriptor	Landraces	Commercial Varieties	CIMMYT Lines
Stem colour	Green	95.11	100.00	100.00
	Purple	4.89	0.00	0.00
Sheath pubescence	Sparse	1.11	4.76	3.03
	Intermediate	94.22	85.71	82.83
	Dense	4.67	9.52	14.14
Foliage	Small	18.67	12.70	70.71
	Intermediate	44.89	23.81	26.26
	Large	36.44	63.49	3.03
Tassel type	Primary	3.56	0.00	23.23
	Primary-secondary	96.44	100.00	76.77
Tassel size	Small	4.44	6.35	70.71
	Medium	48.44	49.21	25.25
	Large	47.11	44.44	4.04
Cob colour	White	96.00	100.00	100.00
	Red	3.33	0.00	0.00
	Purple	0.67	0.00	0.00
Shape of uppermost ear	Cylindrical	0.67	0.00	3.03
	Cylindrical-conical	6.22	0.00	17.17
	Conical	93.11	100.00	79.80
Shape of upper surface of Kernel	Shrunken	3.33	41.27	9.09
	Indented	23.11	33.33	12.12
	Level	1.33	25.40	26.26
	Rounded	71.78	77.78	3.03
	Pointed	0.44	0.00	0.00
Kernel row arrangement	Regular	94.67	71.43	89.90
	Irregular	4.00	0.00	10.10
	Straight	1.33	28.57	0.00
Kernel type	Semi-floury	5.78	20.63	7.07
	Dent	55.78	53.97	28.28
	Semi-dent	32.89	14.29	19.19
	Semi-flint	5.56	11.11	41.41
	Flint	0.00	0.00	4.04
Kernel colour	White	84.44	100.00	67.68
	Yellow	9.33	0.00	28.28
	Purple	0.44	0.00	0.00
	Variegated	2.67	0.00	2.02
	White cap	1.33	0.00	2.02
	Red	1.78	0.00	0.00
Endosperm colour	White	94.44	100.00	78.79
	Pale yellow	0.44	0.00	0.00
	Yellow	4.67	0.00	21.21
	White cap	0.44	0.00	0.00

lines were small (70.7%). With regard to tassel size, landraces and commercial varieties were medium with 48.4% and 49.2%, respectively, while CIMMYT lines were small with 70.7%. On

shape of upper surface of kernel, landraces and CIMMYT lines were characterized as rounded with 71.8% and 49.5%, respectively while commercial varieties were mostly shrunken with 41.3%. The kernel type characterized landraces and commercial varieties to be dent with 55.8% and 54.0%, respectively while CIMMYT lines were mostly semi-flint with 41.4%. The rest of traits characterized the groups similar though with different percent accessions distribution, that is stem colour as all green, sheath pubescence as intermediate, tassel type as primary-secondary, cob colour as white, upper most ear shape as conical, kernel row arrangement as regular, kernel and endosperm colour as white.

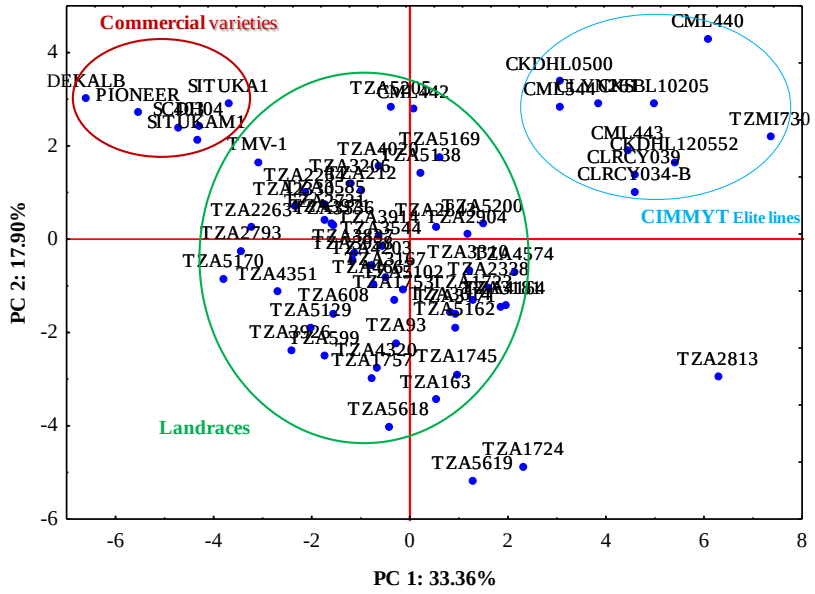

Figure 6: Principal component analysis distributing the 68 accessions into the first two components as performed through 25 morphological traits.

4.3.3 Principal components analysis (PCA)

The analyses of principal components for the 25 morphological traits are shown in Table 9. The first six components expressed 78.36% of the total variation and each had an eigenvalue of more than one. The first principal component (PC1) in particular accounted for 33.36% of the total morphological variation of the studied traits. Morphological traits that highly contributed to the PC1 include leaf length, ear length, ear diameter, number of kernels per row, kernel length, 1000 kernel weight, and yield per plant. Principal component two (PC2) accounted for 17.91% of the total variation and was highly influenced by days to 50% tasseling and silking, anthesis silking interval, plant height, ear height, kernel width and thickness. Morphological traits that had high contributions to Principal component three (PC3), which accounted for 8.51% variations, were number of leaves above uppermost ear, cob diameter, rachis diameter and number of kernel rows. The fourth component (PC4) was influenced by shape of uppermost ear, kernel colour, leaf width and contributed 7.94% of the total variation. The fifth component had variability contribution of 5.76% as caused by kernel row arrangement and endosperm colour. The sixth component contributed 4.89% variation given by shape of upper surface of kernel and kernel type. The PCA plot further characterized the three groups of accessions differently with specific traits discriminating them on a plotted plane (Fig. 6). The commercial varieties were grouped on the upper left hand side quadrant, CIMMYT lines grouped themselves on the upper right hand side quadrant. Landraces were mostly scattered along the origin of the plane and to all the quadrants.

Table 9: Proportions of variability contributions given by the 25 morphological traits in different principle components.

Trait	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Days to 50% tasseling	0.177	**-0.299**	0.282	0.020	0.159	-0.162
Days to 50% silking	0.149	**-0.369**	0.220	-0.007	0.113	-0.161
Anthesis Silking Interval	-0.003	**-0.374**	-0.059	-0.079	-0.072	-0.079
Shape of uppermost ear	-0.042	-0.092	-0.107	**0.478**	-0.421	-0.040
Shape of upper surface of kernel	0.180	-0.169	-0.057	-0.124	-0.166	**0.325**
Kernel row arrangement	-0.068	0.116	-0.071	-0.287	**0.478**	-0.202
Kernel type	0.177	0.054	0.201	0.091	-0.067	**0.508**
Kernel colour	0.029	0.067	-0.212	**0.278**	0.220	0.172
Endosperm colour	0.088	0.062	0.064	0.359	**0.407**	0.378
Number of leaves above uppermost ear	-0.003	0.062	**0.401**	0.309	-0.315	-0.194
Plant height (cm)	-0.247	**-0.286**	0.033	0.110	0.045	-0.027
Ear height (cm)	-0.156	**-0.378**	0.059	0.020	0.074	-0.092
Leaf length (cm)	**-0.231**	-0.177	0.173	0.193	0.215	-0.076
Leaf width (cm)	-0.184	0.003	0.117	**0.393**	0.259	-0.047
Ear length (cm)	**-0.270**	-0.095	-0.185	0.033	0.113	0.189
Ear diameter (cm)	**-0.324**	-0.006	0.149	-0.094	-0.049	0.050
Cob diameter (cm)	-0.258	-0.027	**0.312**	-0.186	-0.077	0.184
Rachis diameter (cm)	-0.179	-0.028	**0.370**	-0.247	-0.082	0.324
Number of kernels per row	**-0.307**	0.104	-0.100	0.057	-0.037	0.007
Number of kernel rows	-0.099	0.281	**0.416**	-0.070	0.093	0.000
Kernel length (cm)	**-0.320**	0.026	-0.100	-0.043	-0.064	-0.117
Kernel width (cm)	-0.198	**-0.277**	-0.183	-0.033	-0.094	0.193
Kernel thickness (cm)	0.164	**-0.313**	-0.001	0.009	0.171	0.197
1000 Kernel weight (g)	**-0.262**	-0.100	-0.180	-0.115	-0.054	0.201
Yield per plant (g)	**-0.279**	0.166	-0.014	0.152	0.055	0.005
Eigenvalues	**8.34**	**4.48**	**2.13**	**1.99**	**1.44**	**1.22**
Total variance (%)	**33.36**	**17.91**	**8.51**	**7.94**	**5.76**	**4.89**
Cumulative total variance (%)	**33.36**	**51.27**	**59.77**	**67.71**	**73.47**	**78.36**

4.3.4 Cluster analysis

The dendrogram of the 68 maize accessions evaluated based on average linkage analysis is presented in Fig. 7. The combined analysis was generated from the 19 quantitative and 12 qualitative traits. The dendrogram at a distance of 0.945, clustered the accessions into four different groups following their similarity and dissimilarity distances (Fig. 7). Cluster I was

comprised of all the seven commercial varieties and two landraces (TZA 2793 and TZA 5170), cluster II had one CIMMYT line (CML 442) and two landraces (TZA 3206 and TZA 5169), cluster III grouped the rest of 46 landraces while 10 CIMMYT lines were grouped into cluster IV.

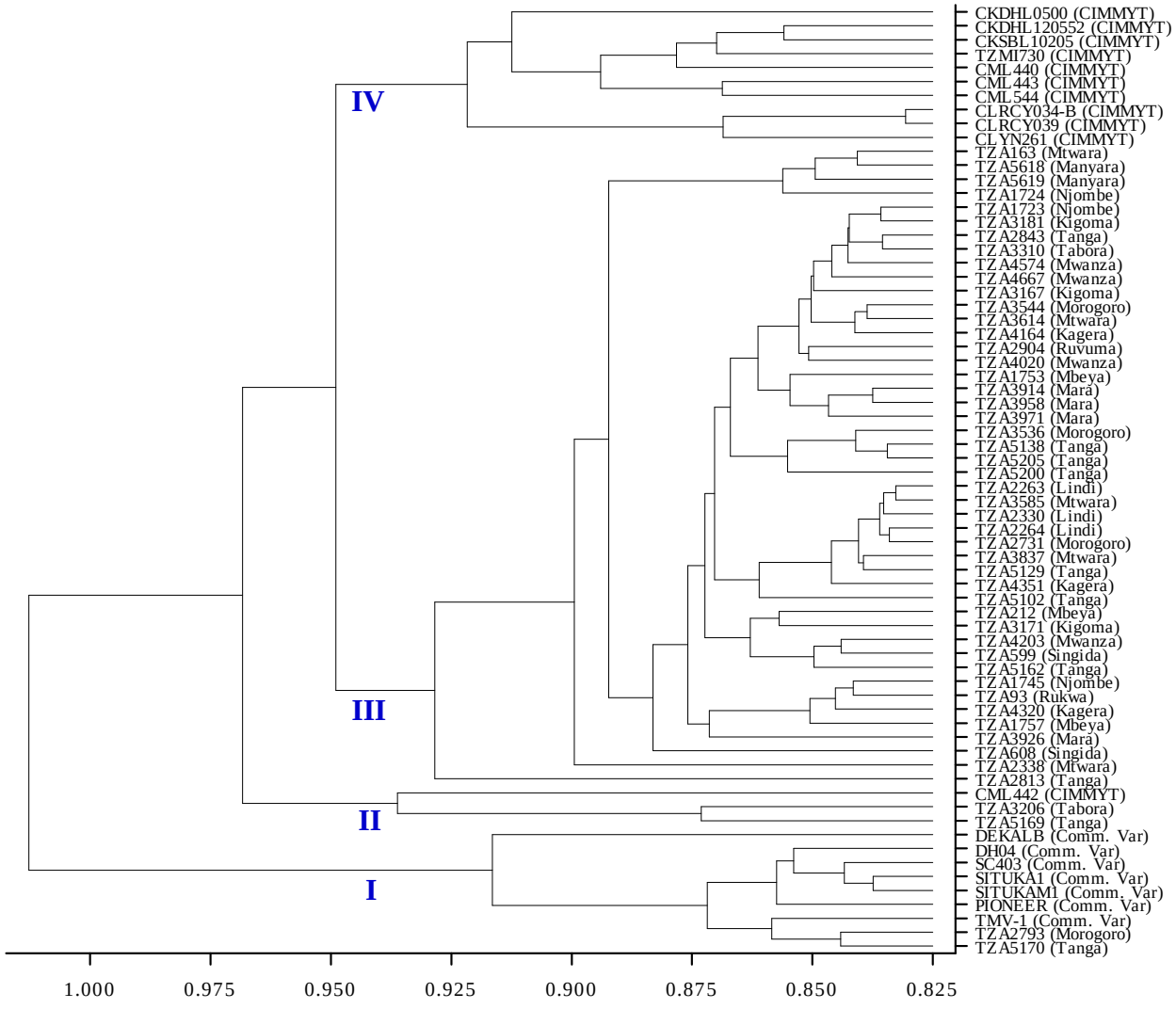

Figure 7: Dendrogram for cluster analyses based on Euclidean genetic distance with average linkage of the 25 morphological characters generated from the performance of 68 maize accessions. Words in brackets show the source of seeds/collection site.

4.4 Discussion

4.4.1 Quantitative morphological traits

Maize is reported to be among the crops with high genetic diversity in terms of morphological as well as genetic variability (Hartings *et al.,* 2008). The maize accessions used in this study expressed a huge amount of variability in terms of quantitative characteristics. Landraces were found to be more variable than commercial varieties and the CIMMYT lines. Significant coefficient of variation among all evaluated traits in this study includes anthesis-silking interval and yield per plant. This was also reported by Sharma *et al.* (2010) in that significant genetic variation among maize landrace populations were found through yield related traits and flowering characteristics. The flowering behaviour might define the maturity differences among accessions (Olaoye, 2009) and they can also be connected to the yielding ability that early maturing accessions could generate high grain yield while those which are late maturing produce low yield (Lafitte *et al.,* 1997). A wide range of variation in flowering behaviours could signify the potential variability within accessions that would help on developing genotypes adaptable to different areas with different characteristics (Cömertpay, 2012). The existence of wide variability among the 68 accessions evaluated were further strengthened by box plot and Whisker, where landraces had more variability as compared with other groups despite their general low yield and other related parameters. Moreover, the principal component analysis identified quantitative morphological traits in different components that highly contributed to the total variation expressed by the accessions under study. The traits include 1000 kernel weight, plant height, ear height, yield per plant and days to 50% silking. That means, traits with high values in principal components present the potential characteristics for discriminating and identifying important accessions. The traits could also be used to characterize several maize landrace populations and discover potential candidates as parents for generating elite materials. Moreover, the principal component analysis expressed the distinction of the three groups of accessions used in this study with the traits contributing to their discriminating behaviours. Commercial varieties were discriminately identified by high yield, a thousand seed weight, number of rows per ear, ear diameter, ear length and early days to tasseling and silking. CIMMYT elite lines were characterized by significant low plant and ear height. Landraces were scattered along all

quadrants in a PCA plane, which signify them to be more diverse than the rest of the accessions involved in this study.

4.4.2 Qualitative morphological traits

The qualitative traits observed in this study explained distribution of accessions within each trait which differ among landraces, commercial varieties and CIMMYT lines. Only landraces had accessions distributed in each trait and not for commercial varieties and CIMMYT lines. The frequency distribution in percentage of accessions within traits differentiated the three groups in terms of foliage, tassel size, shape of upper surface of kernel and kernel type. Other traits like stem colour, sheath pubescence, tassel type, cob colour, shape of upper most ear, kernel row arrangement, kernel colour and endosperm colour characterized the three groups similarly though with different percent distribution. The former traits were able to discriminate between and within the three groups while the later identified differences just within each group. Traits that had higher percentage distribution of accessions towards one class within a trait include stem colour (green), sheath pubescence (intermediate), tassel type (primary-secondary), cob colour (white), shape of uppermost ear (conical), shape of kernel upper surface (rounded), kernel row arrangement (regular), kernel colour (white) and endosperm colour (white). The defined trait classes with high percentage accessions distribution might reflect farmers' preferences through successive selection (Ntundu *et al.*, 2006; Louette and Smale, 2000). In addition to the influence of farmers in shaping the structure of maize population, other factors such as species biology, geographical positioning, climatic settings, agricultural systems, biodiversity and local traditions have also an impact on population structuring (Prasanna, 2010; Pressoir and Berthaud, 2004).

4.4.3 Cluster analysis

The clustering indicated that the groups of accessions (landraces, commercial varieties and CIMMYT lines) were quite different from each other, though admixtures were observed. The grouping of the accessions mostly reflected individual performance and type of accessions. The commercial varieties and the two landraces in Cluster I were characteristically isolated due to distinct performance in high yield per plant, 1000 kernel weight, number of kernels per row, leaf length, plant height and few number of days to 50% tasseling and silking (Table 7). On the other

hand, landraces were more diverse in performance especially those that were grouped in cluster III. They had no specific unique behaviour except for ear height and the lengthy to anthesis-silking interval which explain their variability in terms of flowering time. CIMMYT elite lines in cluster IV had unique characteristics of low yield per plant, small plant sizes, mostly semi flint kernel type and many days to tasseling (Table 7). The mixed cluster II with one CIMMYT line and two landraces occurred due to the very unique characteristics that isolated them from the specific groups they were supposed to be. For example, CML 442 had a very high grain yield per plant as compared with the rest of CIMMYT lines. It also had intermediate sheath pubescence as compared with sparse pubescence that the rest of CIMMYT lines had (data not shown). On the other hand, TZA 5169 had the red kernel and cob colour, while TZA 3206 had yellow kernel colour and purplish cob colour different from the rest of the landraces (data not shown). The big cluster III of 46 landraces lack consistent originality grouping in terms of collection sites (Fig. 7). This observation was similar to the finding by Sun *et al*. (2016) who observed geographically close populations of Chinese sweetgum in different clusters. This implies that the landraces involved are comprised of a heterogeneous group that would have occurred through repeated exchange and selection of germplasm executed by farmers. The results of exchange and selection create the occurrence of irregular pattern of clustering (Ntundu *et al.,* 2006). Other reports also relate heterogeneity groupings with socio-economic factors, cultural, biological (open pollination) and migration of maize germplasm from one region to another (Hartings *et al.,* 2008; N'Da *et al.,* 2015; Cömertpay *et al.,* 2012). The findings of Ashimogo and Rukulantile (2000) explained that 35.4% of farmers in three regions studied in Tanzania use maize seeds they acquired from their neighbours and 60.1% grow their own farm-saved seeds. Furthermore, the clustering displayed some unique placement of accessions collected from Tanga to different clusters. Accession TZA 5170 from Tanga together with accession TZA 2793 from Morogoro were found in cluster I along with commercial varieties, also accession TZA 5169 from Tanga was grouped with other unique accession of TZA 3206 from Tabora region and CIMMYT line CML 442 in cluster II. In a mixed cluster III accessions TZA 2813, TZA 5162, TZA 5102 and TZA 5200 from Tanga were isolated and exerts higher distances (dissimilarities) with other accessions. This suggests a source of high variation is found in Tanga region.

4.5 Conclusion

This study revealed significant range of genetic diversity in the 68 maize accessions evaluated. This might provide a source of variation required for breeding programs to hold back the genetic vulnerability as a result of the recurrent outbreak of new strains of pest and diseases. It also offers an opportunity to widen the genetic background of the available maize germplasm because the materials that are currently at disposal for several breeding programs are composed of narrow genetic base. The traits that expressed high contribution towards total variability across the three groups of maize accessions used in this study include quantitative characteristics such as a 1000 kernel weight, plant height, ear height and yield per plant. The qualitative traits that had a significant contribution include foliage, tassel size, shape of upper surface of kernel and kernel type. All these traits might serve the purposes of generally discriminating among several populations through morphological characterization. However, each group was characterized specifically from other groups with specific traits and might as well signify the potentials expressed by each group. Cluster analysis identified the potential of landraces towards contributing a wealth of genetic resource for future breeding. Two landraces, TZA 2793 and TZA 5170, were grouped together with commercial varieties. The rest of the landraces possessed a wide range of variability in different traits that form a significant gene pool. That means the accessions might have strong contributions for producing superior varieties when used for introgressing promising traits. The cluster analysis also disclosed the expression of landraces lacking regular pattern in clustering within their major group. This elucidate the fact that farmers select cultivars based on their preferences and also exchange seed crop materials with fellow farmers even from very distant regions. Farmers play a significant role in shaping the structure of landrace population existing in a certain area. This calls for systematic involvement of farmers in breeding and selection process through participatory breeding in order to have an organized process of population structuring.

CHAPTER FIVE

Evaluating the response of maize accessions against maize lethal necrosis disease (MLND) in east Africa[4]

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Abstract

Maize lethal necrosis (MLN) is a viral disease that has severely affected maize crop and has put the east African community in jeopardy since maize is a crop that supports millions of people. The disease started in Kenya and spread to Tanzania, Uganda, South Sudan, Rwanda, DR Congo, Ethiopia and it continues to spread fast in other countries as well. It is caused by a combination of two viruses of *Maize chlorotic mottle virus* (MCMV) and any of the Potyvirus, but *Sugarcane mosaic virus* (SCMV) in particular for east Africa. Selection and production of genetically resistant varieties is the main solution of choice to this problem. Therefore, the objective of this study was to screen and select resistant maize genotypes. Field experiment with artificial inoculation was done at Naivasha in Kenya whereas natural field infestation was done at Mlangarini in Tanzania to screen for resistant local cultivars from Tanzania and to compare with elite lines from CIMMYT, Kenya. Data collected were subjected to Meta-R. Version 5.0, Genstat discovery edition 4 and STASTICA softwares for analyses. There were significant difference on mean disease occurrence and relative area under disease progress curve (rAUDPC) at Naivasha and no significant difference were found for Mlangarini. Accessions TZA2793 and TZA3544 had the lowest rAUDPC in both locations and they had both no significant difference with resistant check varieties. In addition, the cluster analysis grouped a landrace TZA2793 together with moderately resistant and resistant check varieties and hence considered promising for further improvements. Heritability at Naivasha was much higher (0.86) as compared to Mlangarini (0.18) and across locations (0.30). Significant negative correlation was found on

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MLND scores with sheath pubescence, kernel type and endosperm colour while significant positive correlations were found with anthesis silking interval, tassel size, ear damage and kernel type.

Key words: *Maize, Tanzania, disease resistance, landrace, rAUDPC, MLND, east Africa, virus*

5.1 Introduction

Maize lethal necrosis (MLN) is a viral disease that adversely affects maize crops in East Africa and other parts of the world (Mahuku *et al.*, 2015). In East Africa, it started in Kenya in 2011 as unknown disease and later was identified as *maize lethal necrosis disease* (MLND) after serological and molecular tests were carried out on infected maize plants from Bomet County and Nakuru County in 2012 (Wangai *et al*., 2012b). In 2012, the disease was also reported to have spread in other areas of Kenya, neighbouring countries of Tanzania and Uganda and later to South Sudan, Rwanda, DR Congo, Ethiopia and it continues to spread fast into other countries. The emergence of MLND in Eastern Africa has added more challenges to the maize farming communities and other maize based-stakeholders (CGIAR, 2012). The control of this disease is difficult due to the fact that it is caused by a combination of more than one virus (Mahuku *et al*., 2015; DSMZ, 2014). MLND occurs as a result of a positive interaction between *Maize chlorotic mottle virus* (MCMV) and any of the cereal viruses in the family, Potyvirideae, such as *Sugarcane mosaic virus* (SCMV), *Maize dwarf mosaic virus* (MDMV), or *Wheat streak mosaic virus* (WSMV) (CIMMYT, 2013, Adams *et al*., 2014). Specifically in East Africa, MLND is caused by MCMV and SCMV. These two viruses together inflict serious damage or even completely kill infected plants. For instance, *Maize chlorotic mottle virus* (MCMV) alone has been reported to be able to cause significant losses (Mahuku *et al*., 2015). Farmers in the affected areas have been reported to experience extensive to total crop loss (Adams *et al*., 2013; Wangai *et al*., 2012b). The disease causing viruses are mainly transmitted from plant to plant by some insect vectors and they may also be carried by wind from field to field over long distances while spreading the disease (CGIAR, 2012). MCMV is normally transmitted by thrips and beetles while SCMV is transmitted by aphids (Kiruwa *et al*., 2016). Infected plants show mild to severe mottling on the leaves, usually starting from the base of young leaves in the whorl and extending

upward towards the leaf tips. Also, stunting and premature aging of the plants, dying (necrosis) of the leaf margins that progresses to the mid-rib and eventually the entire leaf. In addition, necrosis of young leaves in the whorl before expansion has been reported leading to a symptom known as "dead heart" and eventually plant death (Wangai *et al*., 2012b).

The control of the MLN disease relies on several management packages. However, generating varieties that are genetically resistant is important and economically feasible (Saleh *et al*., 2016; Mahuku *et al*., 2015; Wisser *et al*., 2006). Thus, there is an urgent need to find germplasm with resistance traits and eventually establish genomic regions (markers) or map linked to MLN disease resistance. The identified candidates with those markers would then be crossed through breeding with MLND susceptible varieties carrying other desirable traits such as yield to obtain potential varieties against MLND (Semagn *et al*., 2015; Ragimekula *et al*., 2013; Ali and Yan, 2012). So far, there have been initiatives by several local and international institutions to evaluate as much number of germplasm against MLND as possible, but most of the materials ended up being susceptible. For instance, International Maize and Wheat Improvement Center (CIMMYT) and Kenya Agriculture and Livestock Research Organization (KALRO) have conducted joint studies from 2012 to 2014 with about 25,000 germplasm of elite inbred lines, Double Haploid lines, and hybrids against MLND under artificial inoculation and more than 95 percent of the tested materials were found to be completely susceptible (Gowda *et al*., 2015). In this regard, Gowda *et al*. (2015) found 14 promising lines from 615 inbred lines that expressed MLND resistance and could be used as potential candidates for further improvements. Also, some few promising lines were identified with improved response to MCMV alone and they require further characterization to determine whether they have some level of resistance or tolerance to MLND (Mahuku *et al*., 2015). Generally there is a believe that there are so far no maize materials which are completely resistant to the disease and hence further screening of more germplasm is justified (Mahuku *et al*., 2015). Thus, a need arises to widen the source of germplasm with more diverse nature so as to increase the chance of finding more resistant sources against these 2 disease causing viruses and all kinds of strains associated with the viruses. High diversity in maize germplasm presents the opportunity to have a wide range of genetic variations that would enable it to produce sources of traits for survival against extreme

conditions like MLND. Maize landraces provide the most significant diverse sources of traits that are tolerant and / or resistant against pest and diseases (George *et al*., 2004). Furthermore, maize genes within its genome are reported to be distant to an extent that any significant correlation in germplasm gives an indication of relationship between genetic variation and phenotypic trait variation (Wisser *et al*., 2011). Therefore, phenotypic variation is an important aspect in plant breeding as it presents a significant component in MLND screening because it expresses the combined effects of all genes (Ragimekula *et al*., 2013). In addition, Benson *et al*. (2015) pointed out that some genes conferring disease resistance are linked to phenotypic variations and other growth process. The aim of this study, therefore was to evaluate the phenotypic response of maize landraces, commercial varieties and CIMMYT lines against *maize lethal necrosis disease* (MLND).

5.2 Materials and methods

5.2.1 Natural MLND field infestation experiment (Mlangarini, Tanzania)

i. Materials and field layout

The experiment had 50 landraces, 7 commercial varieties from Tanzania and 11 inbred lines from CIMMYT Kenya (Table 10). A MLND hot spot site at Mlangarini located at S 03° 26' 12", E 036 ° 47' 13.4" and an elevation of 1128 meters above sea level was selected for establishing a natural field MLND infestation in Tanzania. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Maize accessions were planted in rows of 4 meters long at spacing of 75 cm between rows and 30 cm within rows. The experiment was planted during rainy season and irrigated whenever it was necessary as well as other agronomic management practices to make sure that the plants faced no stress for a complete expression of their response to the disease. Two seeds were planted per hill followed by thinning to one plant two weeks after sowing. At planting, NPK (40:20:20) were applied at a rate of 100 kg N per hectare and top dressing was applied with Urea (46%) at a rate of 100 kg N per hectare.

Table 10: List of maize accessions and their sources as used in the screening experiments for resistance against maize lethal necrosis disease (MLND) in Kenya and Tanzania.

NAIVASHA, KENYA						MLANGARINI, TANZANIA					
SNo.	**Acc name**	**Source**	**SNo.**	**Acc name [c]**	**Source**	**SNo.**	**Acc name**	**Source**	**SNo.**	**Acc name**	**Source**
1	CML395[S]	CML[a]	35	TZA4203	GBTZ	1	CKDHL0500[MR]	CML	35	TZA3167	GBTZ
2	CML494[R]	CML	36	TZA4320	GBTZ	2	CKDHL120552[MR]	CML	36	TZA3171	GBTZ
3	TZA163	GBTZ[b]	37	TZA4351	GBTZ	3	CKSBL10205[MR]	CML	37	TZA3181	GBTZ
4	TZA1723	GBTZ	38	TZA4574	GBTZ	4	CLRCY034B[R]	CML	38	TZA3206	GBTZ
5	TZA1724	GBTZ	39	TZA4667	GBTZ	5	CLRCY039[R]	CML	39	TZA3310	GBTZ
6	TZA1745	GBTZ	40	TZA5102	GBTZ	6	CLYN261[R]	CML	40	TZA3536	GBTZ
7	TZA1753	GBTZ	41	TZA5129	GBTZ	7	CML440[MR]	CML	41	TZA3544	GBTZ
8	TZA1757	GBTZ	42	TZA5138	GBTZ	8	CML442[S]	CML	42	TZA3585	GBTZ
9	TZA212	GBTZ	43	TZA5162	GBTZ	9	CML443[MR]	CML	43	TZA3614	GBTZ
10	TZA2263	GBTZ	44	TZA5169	GBTZ	10	CML544[MR]	CML	44	TZA3837	GBTZ
11	TZA2264	GBTZ	45	TZA5170	GBTZ	11	DEKALB	CMMV	45	TZA3914	GBTZ
12	TZA2330	GBTZ	46	TZA5200	GBTZ	12	DH04	CMMV	46	TZA3926	GBTZ
13	TZA2338	GBTZ	47	TZA5201	GBTZ	13	PIONEER	CMMV	47	TZA3958	GBTZ
14	TZA2731	GBTZ	48	TZA5618	GBTZ	14	SC403	CMMV	48	TZA3971	GBTZ
15	TZA2793	GBTZ	49	TZA5619	GBTZ	15	SITUKA M1	CMMV	49	TZA4020	GBTZ
16	TZA2813	GBTZ	50	TZA599	GBTZ	16	SITUKA1	CMMV	50	TZA4164	GBTZ
17	TZA2843	GBTZ	51	TZA608	GBTZ	17	TZMI730[MR]	CML	51	TZA4203	GBTZ
18	TZA2904	GBTZ	52	TZA93	GBTZ	18	TMV1	CMMV	52	TZA4320	GBTZ
19	TZA3167	GBTZ				19	TZA163	GBTZ	53	TZA4351	GBTZ
20	TZA3171	GBTZ				20	TZA1723	GBTZ	54	TZA4574	GBTZ
21	TZA3181	GBTZ				21	TZA1724	GBTZ	55	TZA4667	GBTZ
22	TZA3206	GBTZ				22	TZA1745	GBTZ	56	TZA5102	GBTZ
23	TZA3310	GBTZ				23	TZA1753	GBTZ	57	TZA5129	GBTZ
24	TZA3536	GBTZ				24	TZA1757	GBTZ	58	TZA5138	GBTZ
25	TZA3544	GBTZ				25	TZA212	GBTZ	59	TZA5162	GBTZ
26	TZA3585	GBTZ				26	TZA2263	GBTZ	60	TZA5169	GBTZ
27	TZA3614	GBTZ				27	TZA2264	GBTZ	61	TZA5170	GBTZ
28	TZA3837	GBTZ				28	TZA2330	GBTZ	62	TZA5200	GBTZ
29	TZA3914	GBTZ				29	TZA2338	GBTZ	63	TZA5205	GBTZ
30	TZA3926	GBTZ				30	TZA2731	GBTZ	64	TZA5618	GBTZ
31	TZA3958	GBTZ				31	TZA2793	GBTZ	65	TZA5619	GBTZ
32	TZA3971	GBTZ				32	TZA2813	GBTZ	66	TZA599	GBTZ
33	TZA4020	GBTZ				33	TZA2843	GBTZ	67	TZA608	GBTZ
34	TZA4164	GBTZ				34	TZA2904	GBTZ	68	TZA93	GBTZ

[R] stands for resistant check variety; [MR] stands for moderate resistant check variety; [S] stands for susceptible check variety; [a] **CML** stands for CIMMYT Lines; [b] **GBTZ** stands for Genebank Tanzania; [c] **Acc name** stands for Accession name.

ii. Data collection

Standardized screening protocol for MLND that was developed under the collaboration between USDA-ARS/Ohio State University, the Monsanto Company and KALRO was used to assess different resistance levels shown by the maize materials used in this study. The disease occurrence at each plot for every maize accession was scored using a 1 to 5 scale. The following is the description of each scoring scale;

1 = no MLN symptoms
2 = fine chlorotic streaks on lower leaves
3 = chlorotic mottling throughout plant
4 = excessive chlorotic mottling and dead heart
5 = complete plant necrosis

Along with MLND score data, the following characterization data were recorded; days to 50% tasseling, days to 50% silking, anthesis-silking interval, tassel size, foliage, plant height, ear height, leaf length, leaf width, number of leaves above uppermost ear, leaf pubescence, sheath pubescence, ear damage, ear length, ear diameter, cob colour, shape of uppermost ear, number of kernel rows, number of kernels per row, kernel length, kernel width, kernel thickness, kernel colour, endosperm colour, shape of upper surface of kernel, kernel row arrangement, 1000 kernel weight, kernel type, yield per plant.

5.2.2 Artificial inoculation experiment (Naivasha, Kenya)

i. Field layout

The experiment was laid out in an alpha lattice design with two replications. Two maize seeds per hill were sown in a 3 meters row and later thinned to one seed per hill. Naivasha is located at latitude 0° 43' S, longitude 36° 26' E, 1896 meters above sea level in Kenya. All other required agronomic managements were applied.

ii. Materials and artificial inoculation

The experiment involved 50 landraces from Tanzania and 2 inbred lines from CIMMYT Kenya (Table 10). The optimum viral ratio of combination between MCMV and SCMV was (1:4) for uniform MLND stress on plants. Inoculum production was prepared in two separate screen houses. Each viral inoculum was prepared by taking 1.0 g of infected leaf samples (viral presence confirmed by ELISA) and mixed with 20 ml of cold 0.1M Potasium phosphate buffer at pH 7.0. The mixture was homogenized using a blender and then sieved with cheese cloth to remove debris. Then 1.0 g carborundum was added to 1 litre of inoculum extract. The first plant inoculation in the field was done at 4 - 6 leaf stage using motorized mist blower (Solo 423). The second inoculation was done one week later after the first in order to make sure that maximum MLND pressure is inflicted on plants.

iii. Data collection

Data scoring on disease severity were taken using a 1 - 5 scale as described above. The MLND ratings on maize accession's response were recorded at 14, 28, 42 and 56 days after inoculation.

iv. Data analysis

The means estimated from the collected data of the response of maize accessions against MLND were predicted and adjusted towards the true population means using the statistical software Meta-R.Version 5.0. The analysis produced what is called best linear unbiased prediction (BLUP) for each accession and also coefficient of variations, genotypic variations, residual variations and heritability.

The response of maize accessions to MLND together with other morphological characters was related to each other through Pearson's correlation coefficient using STATISTICA 8.0 analytical software. Cluster analysis was performed based on group average method through Genstat discovery edition 4 to obtain clusters based on similarity and dissimilarity of accessions due to their response performance on MLND resistance. The disease severity was also measured using area under the disease progress curve (AUDPC) values which were obtained through an equation by Forbes *et al*. (2014) as follows;

$$AUDPC = \sum_{i=1}^{n-1} [(y_i + y_{i+1})/2] * [(t_{i+1} - t_i)]$$

where; *Yi* is the percentage (or disease assessment score) of affected foliage at the i^{th} reading, *ti* is the time (days) of each reading at the ith observation and n is the total number of readings or observation. Moreover, "t" stands for days after planting, emergence or inoculation. The AUDPC produce an estimation of the amount of disease occurring on crop plants across the growing season. The relative area under the disease progress curve (rAUDPC) was calculated to take care of the interaction between genotypes and environments using the following formula;

rAUDPC = Specific AUDPC/((Days to the last reading- Days to the first reading)*100)

Both AUDPC and rAUDPC was calculated using Microsoft Excel spreadsheet program.

5.3 Results

5.3.1 Area under disease progress curve

The relative area under disease progress curve (rAUDPC) extended from 0.20 to 0.36 for Mlangarini experiment while at Naivasha rAUDPC was from 0.66 to 0.90 (Table 11). There was a significant ($p < 0.05$) variation with the disease progress curve at Naivasha while no significant difference was observed on plant response to MLND at Mlangarini. The average rAUDPC for Mlangarini was 0.25 and Naivasha was 0.76. Accessions DH04, TZA2263, CML442, DEKALB, TZA3544, TZA2904, TZA2793, SITUKA M1, and SITUKA1 expressed resistance at Mlangarini because they had the least rAUDPC and were not significantly different to the resistant check varieties (Table 11). On the other hand, accessions TZA3914, TZA4203, TZA3926, TZA1724, TZA3544, TZA5200, TZA2793, TZA2330, TZA2813, TZA3536, and TZA3167 expressed resistance at Naivasha as they had the least rAUDPC and were not significantly different from the resistant check variety (Table 11). Accessions CKDHL120552, SITUKA1, SITUKA M1, TZA2793 at Mlangarini and accession CML 494 at Naivasha had the least rAUDPC. The most susceptible accessions were TZMI730 and TZA2338 for Mlangarini closely followed by the susceptible check CML395 for Naivasha. Accessions TZA2793 and TZA3544 expressed the lowest rAUDPC at both locations.

Table 11: The selected 20 accessions at each location of experiment with the lowest relative area under disease progress curve (rAUDPC).

MLANGARINI		NAIVASHA	
Accession name	**rAUDPC**	**Accession name**	**rAUDPC**
Twelve accessions with the lowest rAUDPC			
CKDHL120552	0.20e	CML494	0.66j
SITUKA1	0.20e	TZA3167	0.68ij
SITUKA M1	0.20e	TZA3536	0.68ij
TZA2793	0.20e	TZA2813	0.68ij
TZA2904	0.21e	TZA2330	0.68hij
TZA3544	0.22de	TZA2793	0.68hij
DEKALB	0.22de	TZA5200	0.68hij
CML442	0.22de	TZA3544	0.68hij
TZA2263	0.22de	TZA1724	0.69ghij
DH04	0.22de	TZA3926	0.69ghij
CLYN261	0.22cde	TZA4203	0.70fghij
CLRCY039	0.22cde	TZA3914	0.70fghij
Ten accessions with the highest rAUDPC			
TZMI730	0.36a	TZA2338	0.90a
TZA5200	0.33ab	CML395	0.88ab
TZA2813	0.32abc	TZA3614	0.87abc
TZA5138	0.31abcd	TZA2843	0.85abcd
TZA163	0.30abcde	TZA5102	0.85abcd
TZA3837	0.29abcde	TZA2904	0.85abcd
TZA2843	0.29abcde	TZA3181	0.84abcde
TZA599	0.29abcde	TZA5619	0.84abcde
TZA1723	0.29abcde	TZA2264	0.83abcdef
TZA2338	0.28abcde	TZA5138	0.83abcdefg
Grand mean	**0.25**	**Grand mean**	**0.76**
F-statistics	**1.29ns**	**F-statistics**	**2.39 ****
CV (%)	**19.53**	**CV (%)**	**7.35**
LSD	**0.078**	**LSD**	**0.112**

5.3.2 Means, variances and heritability

There was significant difference on maize accessions tested for MLND resistance at Naivasha, but no significant differences were observed at Mlangarini as well as across the two locations (Table 12). The mean MLND score at Naivasha was higher with a mean of 4.22 ranging from 2.31 to 4.89 while at Mlangarini the mean score was lower at 1.47 and across the two locations with 2.87. The genotypic variance recorded was high with 0.55 at Naivasha than at Mlangarini

and across environments. The residual variance was low (0.18) at Naivasha as compared with Mlangarini and across the locations. The heritability at Naivasha was much higher (0.86) as compared with Mlangarini (0.18) and across locations (0.30). The general coefficient of variation was higher at Mlangarini (29.91%) than at Naivasha and across the two environments.

Table 12: Response of maize accessions to MLND under artificial inoculation at Naivasha in Kenya and natural infestation at Mlangarini in Tanzania both on a 1–5 scale.

Environment	Mean (range)	Genotypic variance	Residual variance	Heritability	LSD	CV (%)
Naivasha	4.22 (2.31 - 4.89)**	0.55	0.18	0.86	0.85	10.03
Mlangarini	1.47 (1.39 - 1.71)ns	0.02	0.19	0.18	0.88	29.91
Across environments	2.87 (2.47 - 3.07)ns	0.07	0.19	0.30	0.61	10.65

5.3.3 Cluster analysis

The dendrogram which was generated from the 57 maize accessions analyzed based on group average is presented in Fig. 8. The analysis involved combining MLND scores collected from the two locations of Mlangarini in Tanzania and Naivasha in Kenya. The obtained dendrogram grouped the accessions into four different clusters based on their similarity and dissimilarity distances. Cluster I included two landraces, TZA 2813 and TZA 5200, cluster II grouped the 47 landraces, cluster III had just TZMI 730 and cluster IV contained 7 accessions of CKDHL0500, CKSBL10205, CKDHL120552, TZA2793, CLRCY039, CLYCN261, CLRCY034.

5.3.4 Correlation matrix

The Pearson correlation matrix presented the interrelationship between MLND scores and other morphological characters collected during this study (Table 13). There was a significant correlation between MLND scores and anthesis-silking interval, sheath pubescence, foliage, tassel size, ear damage, kernel type, endosperm colour, plant height, ear height, leaf length, ear length, ear diameter, number of kernels per row, kernel length, kernel width and 1000 kernel weight.

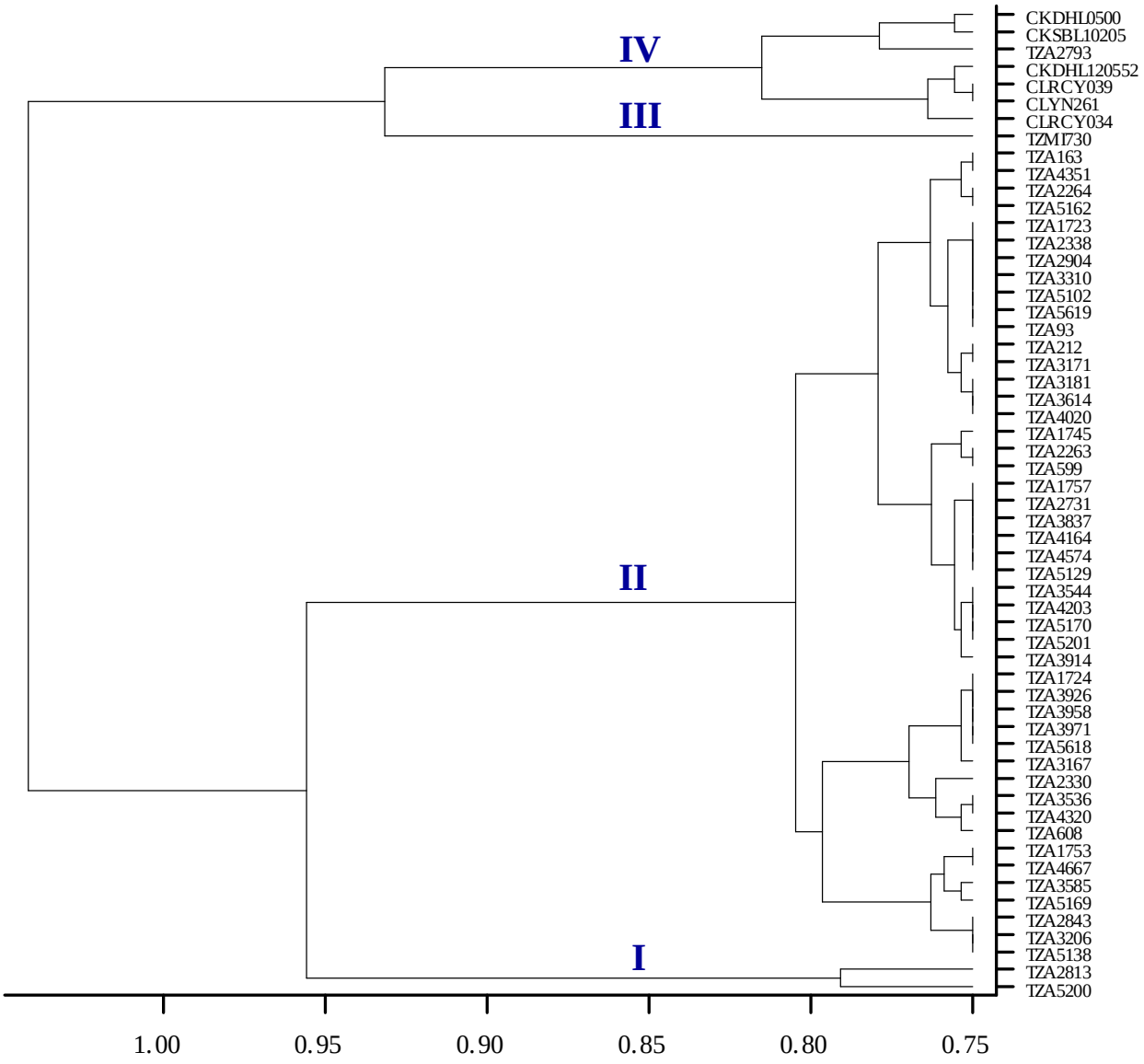

Figure 8: Dendrogram for cluster analyses based on Euclidean genetic distance with group average of the 57 accessions generated from their performance against MLND in two experiments at Naivasha, Kenya and Mlangarini, Tanzania.

Table 13: The Pearson correlation coefficient of 29 parameters in relation to the maize lethal necrosis diseases (MLND) scores obtained from a disease screening experiment of 68 maize accessions, probability significance at $p < 0.05$.

	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.00																													
2	0.93*	1.00																												
3	0.30*	0.62*	1.00																											
4	-0.18	-0.18	-0.09	1.00																										
5	0.07	0.16	0.28*	0.14	1.00																									
6	-0.11	0.00	0.25	-0.07	0.13	1.00																								
7	-0.08	0.12	0.49*	-0.04	0.24	0.65*	1.00																							
8	-0.38*	-0.36*	-0.11	0.26	0.00	0.00	0.01	1.00																						
9	0.04	0.24	0.54*	-0.18	0.38*	0.35*	0.48*	-0.11	1.00																					
10	-0.32*	-0.37*	-0.28*	0.01	0.00	0.16	-0.01	0.04	0.03	1.00																				
11	0.14	0.24	0.32*	-0.03	0.2	-0.26*	0.02	0.13	0.05	-0.16	1.00																			
12	0.05	0.00	-0.11	0.18	0.00	-0.27*	-0.24	-0.03	-0.27*	-0.57*	0.09	1.00																		
13	0.07	-0.02	-0.22	0.02	-0.32*	-0.40*	-0.44*	-0.18	-0.32*	0.09	0.19	0.09	1.00																	
14	-0.15	-0.15	-0.06	0.13	-0.12	-0.03	0.01	0.70*	-0.09	-0.15	0.09	-0.03	-0.07	1.00																
15	0.03	-0.09	-0.30*	-0.08	-0.35*	-0.09	-0.29*	-0.06	-0.12	0.07	-0.13	-0.04	0.36*	0.19	1.00															
16	0.18	0.06	-0.23	-0.10	-0.13	-0.05	-0.19	-0.16	-0.29*	0.16	-0.18	-0.35*	0.14	-0.01	-0.02	1.00														
17	0.05	0.21	0.46*	-0.08	0.29*	0.65*	0.76*	-0.12	0.58*	0.14	-0.25	-0.31*	-0.44*	-0.06	-0.20	-0.12	1.00													
18	0.25	0.41*	0.56*	-0.11	0.39*	0.61*	0.71*	-0.12	0.58*	-0.03	-0.13	-0.19	-0.49*	-0.07	-0.28*	-0.18	0.92*	1.00												
19	0.13	0.18	0.19	-0.24	-0.06	0.58*	0.62*	-0.08	0.28*	0.07	-0.36*	-0.21	-0.35*	0.05	-0.03	0.10	0.75*	0.68*	1.00											
20	-0.02	-0.07	-0.13	0.04	-0.27*	0.27*	0.22	0.31*	0.02	0.18	-0.38*	-0.32*	-0.12	0.21	0.20	0.12	0.31*	0.21	0.41*	1.00										
21	-0.21	-0.06	0.30*	-0.03	0.2	0.59*	0.66*	0.01	0.57*	0.27*	-0.10	-0.16	-0.28*	0.01	-0.05	-0.41*	0.74*	0.65*	0.54*	0.25	1.00									
22	-0.30*	-0.24	0.04	-0.05	0.24	0.49*	0.53*	-0.06	0.38*	0.24	-0.32*	-0.04	-0.48*	-0.21	-0.26	-0.07	0.57*	0.46*	0.50*	0.27*	0.59*	1.00								
23	-0.55*	-0.47*	-0.05	-0.01	0.13	0.59*	0.49*	0.16	0.30*	0.39	-0.30*	-0.10	-0.36*	0.04	-0.16	-0.16	0.51*	0.38*	0.39*	0.24	0.66*	0.67*	1.00							
24	-0.11	-0.25	-0.44*	-0.13	-0.26	-0.05	-0.24	-0.13	-0.19	0.00	-0.27*	0.30*	0.06	-0.14	0.08	0.20	-0.19	-0.22	0.12	0.21	-0.12	0.40*	0.16	1.00						
25	-0.48*	-0.36*	0.08	0.05	0.31*	0.55*	0.59*	0.06	0.39*	0.32	-0.31*	-0.12	-0.55*	-0.11	-0.28*	-0.14	0.63*	0.51*	0.42*	0.22	0.62*	0.83*	0.78*	0.06	1.00					
26	-0.13	0.06	0.45*	0.01	0.38*	0.40*	0.61*	0.00	0.49*	0.25	0.03	-0.32*	-0.34*	-0.07	-0.20	-0.32*	0.67*	0.61*	0.35*	0.08	0.68*	0.52*	0.32*	-0.42*	0.58*	1.00				
27	0.50*	0.57*	0.43*	-0.04	-0.05	-0.19	0.02	-0.16	0.19	-0.31*	0.23	-0.07	0.14	0.03	0.13	-0.18	0.08	0.16	0.06	-0.03	0.08	-0.31*	-0.63*	-0.42*	-0.42*	0.30*	1.00			
28	-0.37*	-0.25	0.12	0.16	0.38*	0.33*	0.49*	0.11	0.46*	0.32*	-0.12	-0.09	-0.41*	-0.14	-0.17	-0.31*	0.49*	0.40*	0.25	0.05	0.64*	0.65*	0.54*	-0.21	0.75*	0.66*	-0.07	1.00		
29	-0.47*	-0.49*	-0.29*	0.05	-0.19	0.51*	0.26	0.12	0.01	0.31*	-0.54*	-0.06	-0.21	0.06	0.04	0.02	0.37*	0.19	0.39*	0.41*	0.46*	0.53*	0.78*	0.28*	0.57*	0.09	-0.53*	0.39*	1.00	
30	-0.15	0.07	0.50*	-0.28*	0.20	0.39*	0.58*	0.19	0.45*	-0.04	0.20	-0.25	-0.54*	0.08	-0.33*	-0.19	0.36*	0.41*	0.29*	-0.01	0.30*	0.31*	0.35*	-0.17	0.36*	0.42*	-0.08	0.29*	0.02	1.00

[a] ***Numbers in columns and rows heads are described below;***

1. Days to 50% tasseling
2. Days to 50% silking
3. Anthesis-Silking Interval
4. Sheath pubescence
5. Leaf pubescence
6. Foliage
7. Tassel size
8. Cob colour
9. Ear damage
10. Shape of uppermost ear
11. Shape of upper surface of kernel
12. Kernel row arrangement
13. Kernel type
14. Kernel colour
15. Endosperm colour
16. Number of leaves above uppermost ear
17. Plant height
18. Ear height
19. Leaf length
20. Leaf width
21. Ear length
22. Ear diameter
23. Number of kernels per row
24. Number of kernel row
25. Kernel length
26. Kernel width
27. Kernel thickness
28. 1000 kernel weight
29. Yield per plant
30. MLND score

Significant negative correlation was found with sheath pubescence (-0.28), kernel type (-0.54) and endosperm colour (-0.33). The significant correlations with high values included anthesis-silking interval (0.50), tassel size (0.58), ear damage (0.45) and kernel type (0.54).

5.4 Discussion

The standardized area under the disease progress curve (rAUDPC) was utilized to assess the cumulative disease progression within the growing process of the crops as explained by Luitel *et al*. (2016). The rAUDPC was higher at Naivasha (0.76) than at Mlangarini (0.26) because the extent of disease pressure at Naivasha was higher due to artificial inoculation. The inoculation procedure was done in such a way that maximum pathogen inoculum was applied to the plants. The inoculum application was carefully done twice at Naivasha while maize accessions were subjected to the natural disease pressure at Mlangarini with the extent of general occurring disease progression being low. The rAUDPC evaluated how the genetic differences among the accessions would determine the way they react to the extent of disease development on plants (Skelsey and Newton, 2014). The accessions with the lowest rAUDPC values were considered to be resistant to the MLN disease and vice versa (Safavi *et al*., 2010). The tolerance performance is on the other hand determined by comparing the performance of accessions with known susceptible and resistant check materials (Massa *et al*., 2015). Therefore, accessions that were able to show the least disease progression trend in both locations could be considered promising for resistance against MLND. In this case, accessions TZA2793 and TZA3544 may be considered for further screening to ascertain their resistance characteristics against MLND. The artificial inoculation of MLND pathogens at Naivasha realized the highest level of disease expression and the highest level of response by the accessions. The occurrence of the disease at Naivasha enhanced high heritability (0.86) as compared with the low heritability (0.18) at Mlangarini. This is due to the fact that uniform environmental conditions ensure high level of heritability while variable environments lower the level of heritability (Brunda *et al*., 2014). The amount of inoculum applied on the plants at Naivasha was actually uniform such that genetic variation of the accessions in this study exerted an influence responsible for the variation in response against MLND. On the other hand, the Mlangarini situation was different because the amount of pathogens occurred naturally with no regular inoculum pressure distributed on plants.

Heritability measures phenotypic variance as caused by genetic variation, it provides prediction for plant breeding strategies. Heritability also determines the extent that a particular trait is transmitted to the next generation. It gives an opportunity for obtaining choices through selection process towards crop improvement (Bello *et al*., 2012b).

The correlation between sheath pubescence and the occurrence of MLN disease was significantly negative. That means, an increase of pubescence is associated with lowering the disease (enhance resistance) to the crop plants. The resistance might directly be the close link of the pubescent marker and the gene controlling resistance or else the indirect link through the prevention of the insect pests carrying the pathogen causing the disease. Mmbaga *et al*. (1996) found the presence of dense pubescence to be linked to the resistance of rust on common bean and the pubescence trait was merely inherited. Endosperm colour is another character which was negatively correlated to the occurrence of MLN disease. The descriptor for endosperm colour used for scoring, ranged from white, cream, pale yellow, yellow, orange and white cap. A large descriptor number of endosperm colour indicated presence of more coloured endosperm such as yellow or orange, while the lower number were associated with whitish colour. Therefore, as the colour becomes more yellow or orange, it is associated with resistance and the white endosperm relate to susceptibility. Endosperm colour arises from the colour of the nutritious materials covering an embryo. Scott (1989) studied the link between endosperm colour and potyvirus resistance and found that the gene for yellow endosperm colour was linked to the resistance on potyviral disease. Also the promising inbred lines resistant to MLND that had been developed so far have yellow endosperm colour (Semagn *et al*., 2015). Anthesis-silking interval (ASI) was significantly and positively correlated to the occurrence of the MLN disease, which means the longer the ASI the higher the chance for possible susceptibility, while short ASI indicated the possible resistance. Ngugi *et al*. (2013) established that ASI was significant as well as positively correlated with stress susceptible index. ASI is to a large extent determined by the variation in number of days to silking where a strong association of their Quantitative Trait Loci (QTLs) confirms the close link of the two traits (Gemenet *et al*., 2010). This implies that short ASI depends on the short duration of the silking from the planting date (Magorokosho *et al*., 2003) and a genotype with short days to silking would have a high chance of being able to tolerate or

resist growing stresses like diseases and drought. Another positive relationship was for MLND scores and tassel size, where large size of tassels associated with susceptibility and small tassels with resistance. Kernel type was also significantly correlated with the disease scores such that flint kernel type was found to associate with resistance traits. The indication of kernel type being correlated to disease resistance traits was also found by Marcon *et al*. (1996), but they found that dent kernel types had the relative resistance potential against high plains virus while sweet corn (sweet kernel type) expressed high susceptibility.

The cluster analysis grouped the accessions into groups depending on their performance against MLND in the two experiments that were set according to different ways of disease occurrence. That is, the disease established through artificial inoculation in the field (Naivasha, Kenya) with the highest pathogenic pressure and the natural disease occurrence in the field (Mlangarini, Tanzania) with low disease pressure. The clusters brought a landrace TZA2793 grouped together with the check varieties that are moderately resistant CKDHL0500, CKSBL10205, CKDHL120552 and resistant CLRCY039, CLYCN261, CLRCY034. The landrace could be considered for other tests to confirm its potential for MLND resistance. Cluster III had only TZMI 730 which was as well a check variety for moderate resistance but it isolated itself from the rest of the check varieties because it performed worse under natural infestation than even the landraces. The performance of TZMI 730 could have been caused by its adaptability and the exposure to a different environment which could not be verified for the rest of the CIMMYT lines that had the same source as itself. As expected, the rest of the 49 landrace accessions were grouped together in cluster II except for two accessions from Tanga regions TZA 2813 and TZA 5200 that had grouped themselves in cluster I. The two accessions isolated themselves from the other 47 accessions because they uniquely performed well at Naivasha and completely different at Mlangarini with worse performance. The way accessions distribute and group themselves on dendrogram reflects their performance (Lavanya *et al*., 2008). However, some of the accessions may be named differently and yet they originated from the same geographic region (Biniam *et al*., 2015). This also means that the current set of accessions especially landraces presents the existence of duplications from farmers who embrace a varied system of naming and managing their local materials (Tairo *et al*., 2008).

5.5 Conclusion

The objective of this study was to evaluate the response of maize landraces from Tanzania against *maize lethal necrosis disease* (MLND) in comparison with commercial varieties and CIMMYT lines as checks. There was a significant variation in disease progression at Naivasha and no significant difference on disease progression was observed on plant response to MLND at Mlangarini. The artificial inoculation of MLND pathogens at Naivasha enhanced high heritability of 0.86 as compared to the low heritability (0.18) at Mlangarini. This was due to the fact that uniform disease pressure at Naivasha ensured high level of heritability while variable pathogenic distribution lowered the level of heritability at Mlangarini. The phenotypic variance (response against MLND) at Naivasha as caused by genetic variation was higher as compared with Mlangarini. The average relative area under disease progress curve (rAUDPC) was higher at Naivasha (0.76) than at Mlangarini (0.26) due to the extent of disease pressure at Naivasha being higher as compared with Mlangarini. The two locations presented the two different conditions that enabled evaluation of the response of accessions under investigation to the extreme situations. There is also a possibility that the two locations were infected with different strains of the virus causing MLND that is *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV) although not confirmed. Regarding, the assessment of accessions' performance on MLND progression over time, accessions TZA2793 and TZA3544 had the lowest rAUDPC in both locations and may be considered stable and promising for resistance characteristics against the disease. Phenotypic variations are important components to consider because they are linked to some genes related to disease resistance. Consequently, Pearson correlation matrix showed sheath pubescence, kernel type and endosperm colour to be negatively correlated with MLN disease scores. Also anthesis-silking interval, tassel size, ear damage and kernel type were positively correlated with MLND scores. Cluster analysis associated TZA2793 with moderate and resistant variety checks and grouped them in the same cluster.

CHAPTER SIX

Analyses of genetic diversity of maize landraces in Tanzania using SSR markers for maize lethal necrosis disease resistance[5]

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Abstract

Maize is a staple crop as well as cash crop which is very important for millions of people in sub-Saharan Africa and other parts of the world. Its production has been adversely confounded by several diseases that cause significant yield loss. A new disease known as *maize lethal necrosis* (MLN) has recently hit East Africa and added even more challenges in maize production. Breeding for genetically resistant varieties is the most preferred and reliable solution against MLN disease. Genetic diversity provides key information that aid in identifying important traits against maize production constraints. The aim of this study was to evaluate the genetic diversity of maize landraces from Tanzania and other accessions using SSR markers for resistance against *maize lethal necrosis disease*. A total of 63 alleles with an average of 3.15 per locus were detected using 20 simple sequence repeats (SSR) loci distributed in 9 chromosomes of maize. The polymorphism information content (PIC) ranged from 0.17 to 0.79, with an average of 0.52. Analysis of molecular variance showed that more than half of the total variation (71%) was accounted for the variation within accessions and the rest (29%) was divided to among accessions (17%) and among populations (12%). Cluster and principal coordinate analysis clearly clustered and isolated CIMMYT inbred lines from landraces and commercial varieties because they were obtained from the same source with similar breeding programs different from the other groups. The distribution and frequencies of alleles in SSR markers phi029 on

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chromosomal bin location 3.04 and phi062 on bin location 10.04 previously identified to be associated with quantitative trait loci (QTL) for *Sugarcane mosaic virus* (SCMV) resistance were calculated and found more with landraces. Thus, the obtained genetic details associated with resistance against SCMV provided an insight towards establishing the utility function of the occurring genetic diversity within studied accessions for MLND resistance.

Key words*: Maize, landraces, SCMV, MCMV, MLND, SSR markers, genetic diversity, Tanzania*

6.1 Introduction

Maize (*Zea mays* L.) which was domesticated about 9000 years ago in Mexico (Warburton *et al*., 2008) belongs to the Poaceae family (Abdellatif and Khidr, 2010). It is the third most important cereal crop in the world after rice and wheat (Al-Badeiry *et al*., 2014, Legesse *et al*., 2007). In Tanzania, maize is the major food as well as cash crop where its supply is normally equated to the national food security (Kabululu *et al*., 2017; Katinila *et al*., 1998). However, the production of maize has been challenged by a number of diseases which cause serious grain yield loss (Anjichi, 2005; Pechanova and Pechan, 2015). In 2011, a devastating disease called *maize lethal necrosis disease* (MLND) emerged in East Africa through Kenya (Wangai *et al*., 2012b). The disease was found to be caused by synergistic interaction between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV). The two viruses together inflict serious damage to an extent that farmers in the affected areas have been reported to experience extensive to total crop loss (Mahuku *et al*., 2015; Adams *et al*., 2014; Wangai *et al*., 2012b). The control of the disease depends on several methods, while generating varieties that are genetically resistant being important and economically feasible (Saleh *et al*., 2016; Mahuku *et al*., 2015; Wisser *et al*., 2006). Thus, there is a strong need to discover germplasm with resistance traits and eventually establishing genomic regions (markers) map linked to MLN disease resistance. The identified candidates with those markers would then be crossed through breeding with MLND susceptible varieties carrying other desirable traits such as yield to obtain potential varieties against MLND (Semagn *et al*., 2015; Ragimekula *et al*., 2013; Ali and Yan, 2012).

Genetic diversity in maize germplasm helps to have reliable information to facilitate breeding programs for genetic improvement and reduction of genetic vulnerability to pests and diseases (Lee, 1998; Abdellatif and Khidr, 2010). It is the basis for survival and adaptation for plants (Rao and Hodgkin, 2002) which provides adequate variation that underscores the possibility for further manipulation to attain a certain goal (Rahman *et al*., 2008). Different germplasm such as landraces, cultivated and new elite materials have always been screened to look for the plant materials that would express desirable traits such as yield and resistance to diseases (ESA, 2010). Furthermore, genetic diversity at molecular level using markers is critical as a resource to find new alleles for important traits through potential sources of resistances and tolerances to biotic and abiotic stresses (Taba *et al*., 2004; Rauf *et al*., 2010). It can be categorized in terms of the number of different alleles existing in different populations, distribution of those alleles in the chromosomes, the impact they have on performance and the general variability among different populations under various environmental conditions (Rao and Hodgkin, 2002; Mondini *et al*., 2009; Bindroo and Moorthy, 2014). A molecular marker is a variant of DNA or a protein which can be detected and whose inheritance can be monitored reliably (Jones *et al*., 1997). Polymerase chain reaction (PCR) based molecular marker techniques have made it possible for breeders and other scientists to make genetic diversity estimates as generated through different molecular markers (Arif *et al*., 2010; Poczai *et al*., 2013). Some of the PCR based molecular techniques that have been applied in molecular studies include RAPDs (Brown-Guedira *et al*., 2000; Mondini *et al*., 2009), AFLPs (Mondini *et al*., 2009), Simple Sequence Repeats (SSRs) (Kumari *et al*., 2005; Beyene *et al*., 2005; Mondini *et al*., 2009; Aci *et al*., 2013; Abraha *et al*., 2014). However, these different marker techniques emphasize on different features (Abdel-Mawgood, 2012) and different aspects of genetic diversity (Matsuoka *et al*., 2002; Mondini *et al*., 2009). SSRs have been the marker system of choice for population genetic studies, because it combines many desirable properties including co-dominance, robustness, hypervariability, rapid and simple assays, and it is uniformly dispersed in plant genomes (Powell *et al*., 1996; Beyene *et al*., 2005; Prasanna and Hoisington, 2003).

George *et al*. (2004) screened 102 inbred lines using 76 SSR markers for downy mildew resistance and the frequencies of alleles in SSR loci linked to previously identified quantitative

trait loci (QTL) were calculated. The association of alleles with disease and pest resistance identified five inbred lines through allelic patterns (George *et al*., 2004). Resistance to MLND requires a good understanding of the genetic structure of the viruses that cause the disease so as to enhance efficient generation of resistant germplasm. Apparently, the genetics of resistance to *Sugarcane mosaic virus* (SCMV) as one of the two MLND causing viruses has been investigated very well, where the major quantitative trait loci (QTL) against SCMV has repeatedly been detected on the short arm of chromosome 3 and on the long arm of chromosome 10 (Mahuku *et al*., 2015; Zhang *et al*., 2003). The markers linked to the SCMV resistance QTLs can be used in the marker assisted selection to attain germplasm improvement towards obtaining solution against MLND. Utilization of the underlying variation emphasizes the use of landraces and wild relatives of crop plants because they carry desirable genes that grant resistance to pests and diseases (Beyene *et al*., 2005). However, landraces and wild relatives present a group of germplasm which are less exploited with limited agronomic and genetic data that renders difficulties to use them (Hoisington *et al*., 1999; Warburton *et al*., 2002; Molin *et al*., 2013). The objective of this study was to evaluate the genetic diversity of maize landraces from Tanzania and other accessions through the use of SSR markers as relates to the resistance against *maize lethal necrosis disease*.

6.2 Materials and methods

6.2.1 Plant materials

A total of 96 accessions were used for this study comprising of 51 landraces from Tanzania, 11 elite lines from CIMMYT, Kenya and 34 commercial varieties in Tanzania (Table 14). The landraces from Tanzania were collected from different regions representing all agroecological zones and were kept at the national plant genetic resource center (NPGRC) in Arusha. The elite lines from CIMMYT, Kenya were included in this study as checks for resistance, moderately resistance and susceptibility to MLND (Table 14). In order to enhance genetic variability, commercial varieties were also included as they have potential for other agronomic traits.

6.2.2 Extraction of DNA

Fifteen seeds were planted in greenhouse for each accession and at 3 - 4 leaf stage, an approximate 0.75 g healthy young leaf tissue was taken from 15 bulked plants. The total genomic DNA from the leaf samples was extracted using CTAB procedure by Dellaporta *et al*. (1983) with slight modification. DNA pellets were washed once with 70% ethanol and then air dried for 1 hour before dissolving them in 100 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The concentration of DNA samples was determined through 0.8% agarose in 0.5xTBE buffer (Tris base + Boric acid + 0.5M EDTA), containing a gel staining solution of EZ – Vision (Amresco, fountain parkway solon, OH USA).

6.2.3 Simple Sequence Repeats markers

Thirty SSR primers shown in Table 15 were selected as of the previous study (Warburton *et al*., 2002) and from the public Maize GDB (http://www.agron.missouri.edu/ssr_probes/ssr.htm**)** based on their good polymorphism information content (data not shown) and coverage of 10 chromosomes.

6.2.4 Polymerase Chain Reaction and gel electrophoresis

The reactions were carried out in a thermal cycler with 10 µl reaction mixture consisting of 20 ng template DNA with a volume of 2µl, then 0.5 µl of 1 mM dNTPs, 2 µl of 2.5 mM SSR primers (forward and reverse), 0.05 µl of 5 U/ µl Taq polymerase, 2.5 µl of 10x Taq buffer and 0.95 µl of autoclaved distilled water. PCR reactions were performed in a BIO-RAD thermocycler with the following conditions: an initial denaturation at 95°C for 5 min, followed by 35 cycles of 1min at 95°C denaturation, 1min annealing (52 to 60°C depending on the requirement of specific primer), and 1min extension at 72°C and the final extension step of 72°C for 5 min was performed. The amplified products were separated by electrophoresis in 2.5% agarose in 0.5xTBE buffer (Tris base + Boric acid + 0.5M EDTA), containing a gel staining solution of EZ – Vision (Amresco, fountain parkway solon, OH USA). The gels were photographed under UV light in BIO-RAD Gel Doc EZ Imager and the images were transferred to a computer for scoring and documentation. A 100 base pairs DNA ladder was used as standard molecular weight.

Table 14: List of maize accessions and their sources as used in the evaluation of genetic diversity for maize lethal necrosis disease resistance.

SNo.	Accession name	Status	Collection source	Population/ zone	SNo.	Accession name	Status	Collection source	Population/ zone
1	TZA597	Landrace	Singida	Central zone	49	TZA2731	Landrace	Morogoro	Eastern zone
2	TZA599	Landrace	Singida	Central zone	50	TZA2793	Landrace	Morogoro	Eastern zone
3	TZA608	Landrace	Singida	Central zone	51	TZA2813	Landrace	Tanga	Eastern zone
4	CKDHL0500 [MR]	Elite line	CIMMYT	CIMMYT	52	TZA2843	Landrace	Tanga	Eastern zone
5	CKDHL120552[MR]	Elite line	CIMMYT	CIMMYT	53	TZA3536	Landrace	Morogoro	Eastern zone
6	CKSBL10205 [MR]	Elite line	CIMMYT	CIMMYT	54	TZA3544	Landrace	Morogoro	Eastern zone
7	CLRCY034 [R]	Elite line	CIMMYT	CIMMYT	55	TZA5101	Landrace	Tanga	Eastern zone
8	CLRCY039 [R]	Elite line	CIMMYT	CIMMYT	56	TZA5102	Landrace	Tanga	Eastern zone
9	CLYN261 [R]	Elite line	CIMMYT	CIMMYT	57	TZA5129	Landrace	Tanga	Eastern zone
10	CML440 [MR]	Elite line	CIMMYT	CIMMYT	58	TZA5138	Landrace	Tanga	Eastern zone
11	CML442 [S]	Elite line	CIMMYT	CIMMYT	59	TZA5162	Landrace	Tanga	Eastern zone
12	CML443 [MR]	Elite line	CIMMYT	CIMMYT	60	TZA5169	Landrace	Tanga	Eastern zone
13	CML544 [MR]	Elite line	CIMMYT	CIMMYT	61	TZA5170	Landrace	Tanga	Eastern zone
14	TZMI730	Elite line	CIMMYT	CIMMYT	62	TZA5186	Landrace	Tanga	Eastern zone
15	CML445	Comm Var	Comm Var	Comm Var	63	TZA5200	Landrace	Tanga	Eastern zone
16	CML444	Comm Var	Comm Var	Comm Var	64	TZA3914	Landrace	Mara	Lake zone
17	CML489	Comm Var	Comm Var	Comm Var	65	TZA3926	Landrace	Mara	Lake zone
18	DH04	Comm Var	Comm Var	Comm Var	66	TZA3958	Landrace	Mara	Lake zone
19	DK8031	Comm Var	Comm Var	Comm Var	67	TZA3971	Landrace	Mara	Lake zone
20	Phb3253	Comm Var	Comm Var	Comm Var	68	TZA4020	Landrace	Mwanza	Lake zone
21	SC403	Comm Var	Comm Var	Comm Var	69	TZA4203	Landrace	Mwanza	Lake zone
22	SITUKA 1	Comm Var	Comm Var	Comm Var	70	TZA4320	Landrace	Kagera	Lake zone
23	SITUKA M1	Comm Var	Comm Var	Comm Var	71	TZA4351	Landrace	Kagera	Lake zone
24	TMV-1	Comm Var	Comm Var	Comm Var	72	TZA4574	Landrace	Mwanza	Lake zone
25	DKC9089518	Comm Var	Comm Var	Comm Var	73	TZA4667	Landrace	Mwanza	Lake zone
26	H519	Comm Var	Comm Var	Comm Var	74	TZA5618	Landrace	Manyara	Nothern zone
27	H614D	Comm Var	Comm Var	Comm Var	75	TZA5619	Landrace	Manyara	Nothern zone
28	KH600-95A	Comm Var	Comm Var	Comm Var	76	TZA5620	Landrace	Manyara	Nothern zone
29	P2859W	Comm Var	Comm Var	Comm Var	77	TZA1723	Landrace	Njombe	Southern Highland
30	SC513	Comm Var	Comm Var	Comm Var	78	TZA1724	Landrace	Njombe	Southern Highland
31	STAHA	Comm Var	Comm Var	Comm Var	79	TZA1745	Landrace	Njombe	Southern Highland
32	TZH536	Comm Var	Comm Var	Comm Var	80	TZA1757	Landrace	Mbeya	Southern Highland
33	DK8053	Comm Var	Comm Var	Comm Var	81	TZA212	Landrace	Mbeya	Southern Highland
34	TZM523	Comm Var	Comm Var	Comm Var	82	TZA2910	Landrace	Ruvuma	Southern Highland
35	ZAMS606	Comm Var	Comm Var	Comm Var	83	TZA93	Landrace	Rukwa	Southern Highland
36	H628	Comm Var	Comm Var	Comm Var	84	TZA163	Landrace	Mtwara	Southern zone
37	KILIMA	Comm Var	Comm Var	Comm Var	85	TZA2263	Landrace	Lindi	Southern zone
38	PAN15	Comm Var	Comm Var	Comm Var	86	TZA2264	Landrace	Lindi	Southern zone
39	PAN3M-01	Comm Var	Comm Var	Comm Var	87	TZA2330	Landrace	Lindi	Southern zone
40	PAN4M-19	Comm Var	Comm Var	Comm Var	88	TZA2338	Landrace	Mtwara	Southern zone
41	PAN4M-21	Comm Var	Comm Var	Comm Var	89	TZA3585	Landrace	Mtwara	Southern zone
42	Phb30G19	Comm Var	Comm Var	Comm Var	90	TZA3614	Landrace	Mtwara	Southern zone
43	SC627	Comm Var	Comm Var	Comm Var	91	TZA3837	Landrace	Mtwara	Southern zone
44	LUBANGO	Comm Var	Comm Var	Comm Var	92	TZA3167	Landrace	Kigoma	Western zone
45	TZH538	Comm Var	Comm Var	Comm Var	93	TZA3171	Landrace	Kigoma	Western zone
46	DK9089	Comm Var	Comm Var	Comm Var	94	TZA3181	Landrace	Kigoma	Western zone
47	CZL0616/CZL097	Comm Var	Comm Var	Comm Var	95	TZA3206	Landrace	Tabora	Western zone
48	H625	Comm Var	Comm Var	Comm Var	96	TZA3310	Landrace	Tabora	Western zone

R stands for resistant check variety; **MR** stands for moderate resistant check variety; **S** stands for susceptible check variety; [a] **Comm Var** stands for Commercial variety; [b] **SNo.** stands for Serial number

Table 15: The 30 primers used in this study with their names, repeat units, bin location and sequence details.

Serial No.	Primer name	Repeat [a]	Bin [b]	Sequence [c]
1	phi011	Tri	1.09	**For [e]:** TGTTGCTCGGTCACCATACC **Rev [f]:** GCACACACACAGGACGACAGT
2	phi015	Tetra	8.09	**For:** GCAACGTACCGTACCTTTCCGA **Rev:** ACGCTGCATTCAATTACCGGGAAG
3	phi029	Comp. [d]	3.04	**For:** TTGTCTTTCTTCCTCCACAAGCAGCGAA **Rev:** ATTTCCAGTTGCCACCGACGAAGAACTT
4	phi031	Tetra	6.04	**For:** GCAACAGGTTACATGAGCTGACGA **Rev:** CCAGCGTGCTGTTCCAGTAGTT
5	phi062	Tri	10.04	**For:** CCAACCCGCTAGGCTACTTCAA **Rev:** ATGCCATGCGTTCGCTCTGTATC
6	phi065	Penta	9.03	**For:** AGGGACAAATACGTGGAGACACAG **Rev:** CGATCTGCACAAAGTGGAGTAGTC
7	phi072	Tetra	4.01	**For:** ACCGTGCATGATTAATTTCTCCAGCCTT **Rev:** GACAGCGCGCAAATGGATTGAACT
8	phi083	Tetra	2.04	**For:** CAAACATCAGCCAGAGACAAGGAC **Rev:** ATTCATCGACGCGTCACAGTCTACT
9	phi100175	Tetra	8.06	**For:** TATCTGACGAATCCCATTCCC **Rev:** TACGTAACGGACGGACGG
10	phi102228	Tetra	3.04	**For:** ATTCCGACGCAATCAACA **Rev:** TTCATCTCCTCCAGGAGCCTT
11	phi109642	Tetra	2.00	**For:** CTCTCTTTCCTTCCGACTTTCC **Rev:** GAGCGAGCGAGAGAGATCG
12	phi233376	Tri	8.03	**For:** CCGGCAGTCGATTACTCC **Rev:** CGAGACCAAGAGAACCCTCA
13	phi229852	Tri	6.08	**For:** GATGTGGGTGCTACGAGCC **Rev:** AGATCTCGGAGCTCGGCTA
14	phi420701	Tri	8.01	**For:** GATGTTTCAAAACCACCCAGA **Rev:** ATGGCACGAATAGCAACAGG
15	phi453121	Tri	3.00	**For:** ACCTTGCCTGTCCTTCTTTCT **Rev:** CAAGCAAGACTTTTGATCAGCC
16	phi96342	Tetra	10.02	**For:** GTAATCCCACGTCCTATCAGCC **Rev:** TCCAACTTGAACGAACTCCTC
17	umc1109	Tri	4.10	**For:** GCAACACAGGACCAAATCATCTCT **Rev:** GTTCGGTCCGTAGAAGAACTCTCA
18	umc1122	Tri	1.06	**For:** CACAACTCCATCAGAGGACAGAGA **Rev:** CTGCTACGACATACGCAAGGC
19	umc1136	Tri	3.10	**For:** CTGCATACAGACATCCAACCAAAG **Rev:** CTCTCGTCTCATCACCTTTCCCT
20	umc1143	Penta	6.00	**For:** CGTGGTGGGATGCTATCCTTT **Rev:** GACACTAGCAATGTTCAAAACCCC
21	umc1152	Tetra	10.01	**For:** CCGAAGATAACCAAACAATAATAGTAGG **Rev:** ACTGTACGCCTCCCCTTCTC
22	umc1153	Tri	5.09	**For:** CAGCATCTATAGCTTGCTTGCATT **Rev:** TGGGTTTTGTTTGTTTGTTTGTTG
23	umc1279	Tri	9.00	**For:** CAATCCAATCCGTTGCAGGTC **Rev:** GATGAGCTTGACGACGCCTG
24	umc1304	Tetra	8.02	**For:** GCCAACTAGAACTACTGCTGCTCC **Rev:** CATGCAGCTCTCCAAATTAAATCC
25	umc1545	Tetra	7.00	**For:** GAAAACTGCATCAACAACAAGCTG **Rev:** ATTGGTTGGTTCTTGCTTCCATTA
26	phi006	Tri	4.11	**For:** AGGCGGCGTGCTGAACACCT **Rev:** CGCTTCATCTCCCGTGACAATG
27	phi034	Tri	7.02	**For:** TAGCGACAGGATGGCCTCTTCT **Rev:** GGGGAGCACGCCTTCGTTCT
28	phi063	Tetra	10.02	**For:** GGCGGCGGTGCTGGTAG **Rev:** CAGCTAGCCGCTAGATATACGCT
29	phi064	Tetra	1.11	**For:** CCGAATTGAAATAGCTGCGAGAACCT **Rev:** ACAATGAACGGTGGTTATCAACACGC
30	phi227562	Tri	1.12	**For:** TGATAAAGCTCAGCCACAAGG **Rev:** ATCTCGGCTACGGCCAGA

[a] **Repeat** indicates the repeat units of a primer
[b] **Bin** indicates chromosomal location
[c] **Sequence**, the first sequence represents the Forward and the second Reverse
[d] **Comp.** represents a compound repeat, containing more than one repeat type
[e] **For** stands for Forward and [f] **Rev** stands for Reverse

6.2.5 Data analysis

Band profiles were binary coded 1 for presence or 0 for absence within each locus. The discriminatory power of each locus generated by all accessions, were established by the polymorphism information content (PIC) given by the following formula;

$$PIC = 1-\Sigma fi^2$$

where fi is the frequency of the ith allele (Smith *et al*. 1997). Nei's genetic distances between groups of population, Nei's gene diversity and Shannon's information index were analysed using POPGENE software version 1.31 (Nei and Li 1979). The analysis of the molecular variance (AMOVA) among the populations, among accession and within accessions were done by GenAlEx 6.5 Excel package software. Cluster analysis was performed on the genetic dissimilarity matrix using unweighted pair group arithmetic average method (UPGMA). The dendrogram and principal coordinate analysis were generated using the PAST statistical software.

6.3 Results

6.3.1 Simple Sequence Repeats polymorphism

Among the thirty primers utilized in this study, only twenty were selected for further analysis (Table 16) while the other ten that did not amplify or produced monomorphic bands were excluded. The allelic diversity measured at each SSR locus is presented in Table 16. A total of 63 alleles with an average of 3.15 per locus were detected using 20 simple sequence repeat (SSR) loci distributed on 9 chromosomes of maize. The number of alleles ranged from five (phi109642 and phi065) to two (phi011, umc1122, phi031, phi420701, phi062) . The polymorphism information content (PIC) ranged from 0.17 (phi031) to 0.79 (phi065), with an average of 0.52. Eleven primers had the PIC greater than the overall average of 0.52 while the other 9 primers had the PIC less than the average (Table 16).

Table 16: The selected 20 primers with their number of alleles, bin location, repeat units and polymorphism information content (PIC).

Primer	Repeat [a]	Bin [b]	Total number of alleles	PIC [c]
phi011	Tri	1.09	2	0.50
umc1122	Tri	1.06	2	0.49
phi083	Tetra	2.04	3	0.56
phi109642	Tetra	2.00	5	0.63
phi029	Comp. [d]	3.04	3	0.26
phi453121	Tri	3.00	4	0.72
umc1136	Tri	3.10	3	0.50
phi006	Tri	4.11	3	0.56
umc1153	Tri	5.09	3	0.53
phi031	Tetra	6.04	2	0.17
umc1143	Penta	6.00	3	0.54
phi015	Tetra	8.09	3	0.66
phi233376	Tri	8.03	3	0.42
phi420701	Tri	8.01	2	0.32
umc1304	Tetra	8.02	4	0.65
phi065	Penta	9.03	5	0.79
umc1279	Tri	9.00	3	0.61
phi062	Tri	10.04	2	0.44
phi96342	Tetra	10.02	3	0.54
umc1152	Tetra	10.01	3	0.49
Average/Total			**63**	**0.52**

[a] **Repeat** indicates the repeat unit of a primer
[b] **Bin** indicates chromosomal location
[c] **PIC** stands for Polymorphism Information Content
[d] **Comp.** represents a compound repeat, containing more than one repeat type

6.3.2 Analysis of molecular variance (AMOVA)

The summary of AMOVA as given in Table 17 was done to estimate the population genetic variation among and within the groups of accessions used in this study. The source of variation included among populations of accessions, among accessions and within accessions. There were highly significant differences ($p < 0.05$) of molecular variance within and among the accessions used in this study (Table 17). The 71% of the total genetic variation was significantly ($p = 0.001$) observed within accessions while 17% of the total variance was found among accessions and 12% was generated among populations at $p = 0.03$ and 0.005 respectively (Table 17 and Fig. 9).

Table 17: Analysis of molecular variance (AMOVA) for the 96 accessions used in this study.

Source	Degrees of freedom	Sum of Squares	Mean Square	Estimated Variance	Molecular variance (%)	Probability value
Among populations	2	4.146	2.073	0.032	12	0.001
Among accessions	93	25.833	0.278	0.045	17	0.030
Within accessions	96	18.000	0.188	0.188	71	0.005
Total	**191**	**47.979**		**0.265**	**100**	

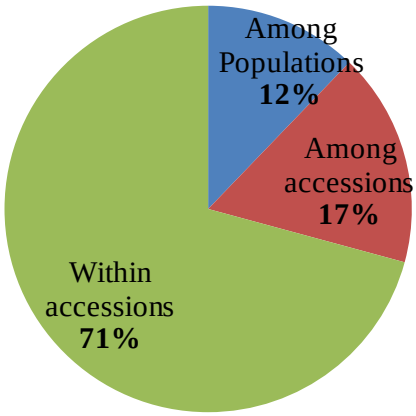

Figure 9: Pie chart showing the distribution of genetic variation generated by 96 accessions divided into three groups viz landraces, commercial varieties and CIMMYT inbred lines.

6.3.3 Genetic relationship between groups of accessions

The observed Nei's pairwise population's differentiation (between landraces, commercial varieties and CIMMYT inbred lines) were shown in Table 18. The highest pairwise differentiation between populations (0.347), which explains the lowest similarity was between landraces and CIMMYT inbred lines. On the other hand, the lowest differentiation (0.086) which corresponds to the highest similarity was between landraces and commercial varieties.

Table 18: Pairwise genetic differentiation among the three populations, Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Populations	CIMMYT lines	Commercial varieties	Landraces
CIMMYT lines	****	0.834	0.707
Commercial varieties	0.181	****	0.918
Landraces	0.347	0.086	****

6.3.4 Genetic variation and diversity

The Nei's gene diversity (h) and Shannon's information index (I) analysis for the three groups of accessions are presented in Table 19. The Nei's gene diversity ranged from 0.212 (CIMMYT lines) to 0.475 (commercial varieties) with an average of 0.379. On the other hand, the Shannon index was highest in commercial varieties (0.667) and the lowest in CIMMYT lines (0.325) while the average was 0.544.

Table 19: Genetic diversity indices for the three groups of accessions.

Populations	Nei's gene diversity (h)	Shannon's Information index (I)
CIMMYT lines	0.212	0.325
Commercial varieties	0.475	0.667
Landraces	0.449	0.640
Average	**0.379**	**0.544**

6.3.5 Cluster and principal coordinate analyses

The cluster analysis was used to show more associations in terms of the genetic diversity among different groups of maize accessions through classical dendograms using Ward's algorithm method. The 96 accessions were clustered into five major groups (Fig. 11). The first (I) group consisted of 26 accessions with subgroup "a" containing a mixture of landraces and commercial varieties, while subgroup "b" contained only commercial varieties. The second (II) cluster consisted of only 14 landraces. Cluster three (III) contained 25 accessions which were mixed up with landraces, commercial varieties and just one accession (CKSBL10205) from CIMMYT in subgroup "b". The fourth (IV) group contained 18 accessions that were again a mixture of landraces and commercial varieties both in subgroup "a" and "b". The last group (V) clustered 13

accessions from CIMMYT inbred lines. However, within those 13 CIMMYT inbred lines , CML 489, CML 444, CML 445 were among the commercial varieties used in this study. On the other hand, the first two principal coordinates (Coordinate 1 and Coordinate 2), expressed 20.4% and 12.9% of the total variation in the SSR data, respectively (Fig. 10). The CIMMYT inbred lines clearly isolated themselves in the lower right quadrant, these lines clustered in one group different from the rest of other accessions. The other two groups (landraces and commercial varieties) were mixed and scattered along the four quadrants of the scatter plot plane (Fig. 10).

3.3.6 Genetic diversity associated with MLND resistance

The distribution and frequencies of alleles in two SSR loci phi029 (on chromosome 3) and phi062 (on chromosome 10) that are associated with QTLs against SCMV (Zhang *et al*., 2003) were calculated for a group of accessions *viz* landraces, commercial varieties and CIMMYT inbred lines. The three alleles detected at phi029 locus had frequencies of 0.07, 0.36 and 0.07, respectively in landraces (Table 20). On the other hand, only one allele was detected at phi029 locus with frequencies of 0.15 and 0.35 for CIMMYT inbred lines and commercial varieties respectively. Furthermore, two alleles were detected at phi062 locus with frequencies of both 0.33 in landraces. The frequencies of 0.25 and 0.08 were observed in CIMMYT inbred lines and commercial varieties only in one allele at phi062 locus respectively (Table 20). The frequencies of all alleles detected at both phi029 and phi062 were not significantly ($p > 0.05$) different between accessions (Table 20).

Table 20: Allelic distribution and frequency of the three groups of accessions along with the two loci associated with SCMV resistance QTL's.

Locus		**phi029**				**phi062**		
Allele		**Allele 1**	**Allele 2**	**Allele 3**	**Total**	**Allele 1**	**Allele 2**	**Total**
Landraces	*Amplicons*	4	20	4		4	4	
	Frequency	0.07	0.36	0.07	**0.51**	0.33	0.33	**0.67**
CIMMYT lines	*Amplicons*	0	8	0		0	3	
	Frequency	0.00	0.15	0.00	**0.15**	0.00	0.25	**0.25**
Commercial varieties	*Amplicons*	0	19	0		0	1	
	Frequency	0.00	0.35	0.00	**0.35**	0.00	0.08	**0.08**
Total					**1.00**			**1.00**
F - Statistics		**3.103^{ns}**				**5.571^{ns}**		

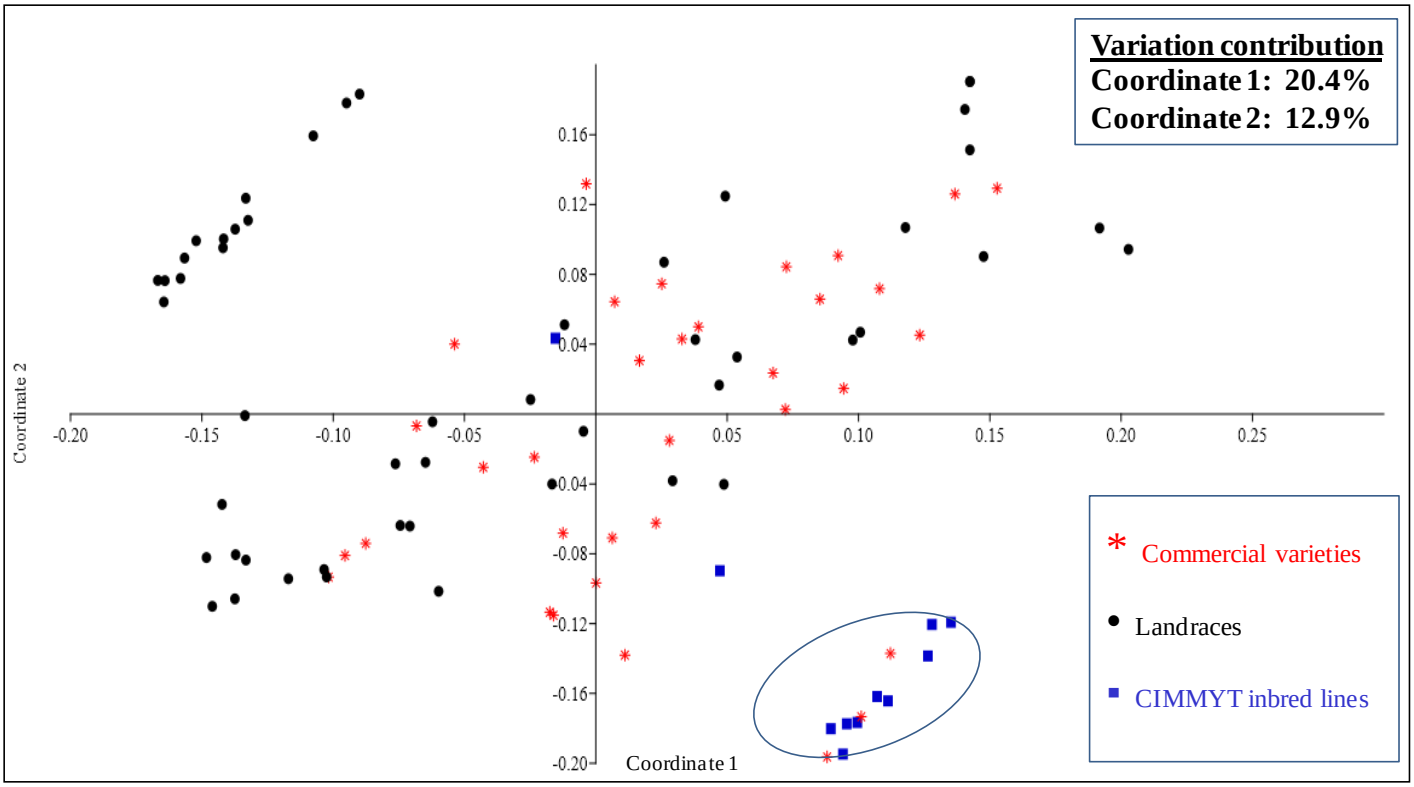

Figure 10: Relationships among the 96 accessions displayed by principal coordinate analysis using SSR data

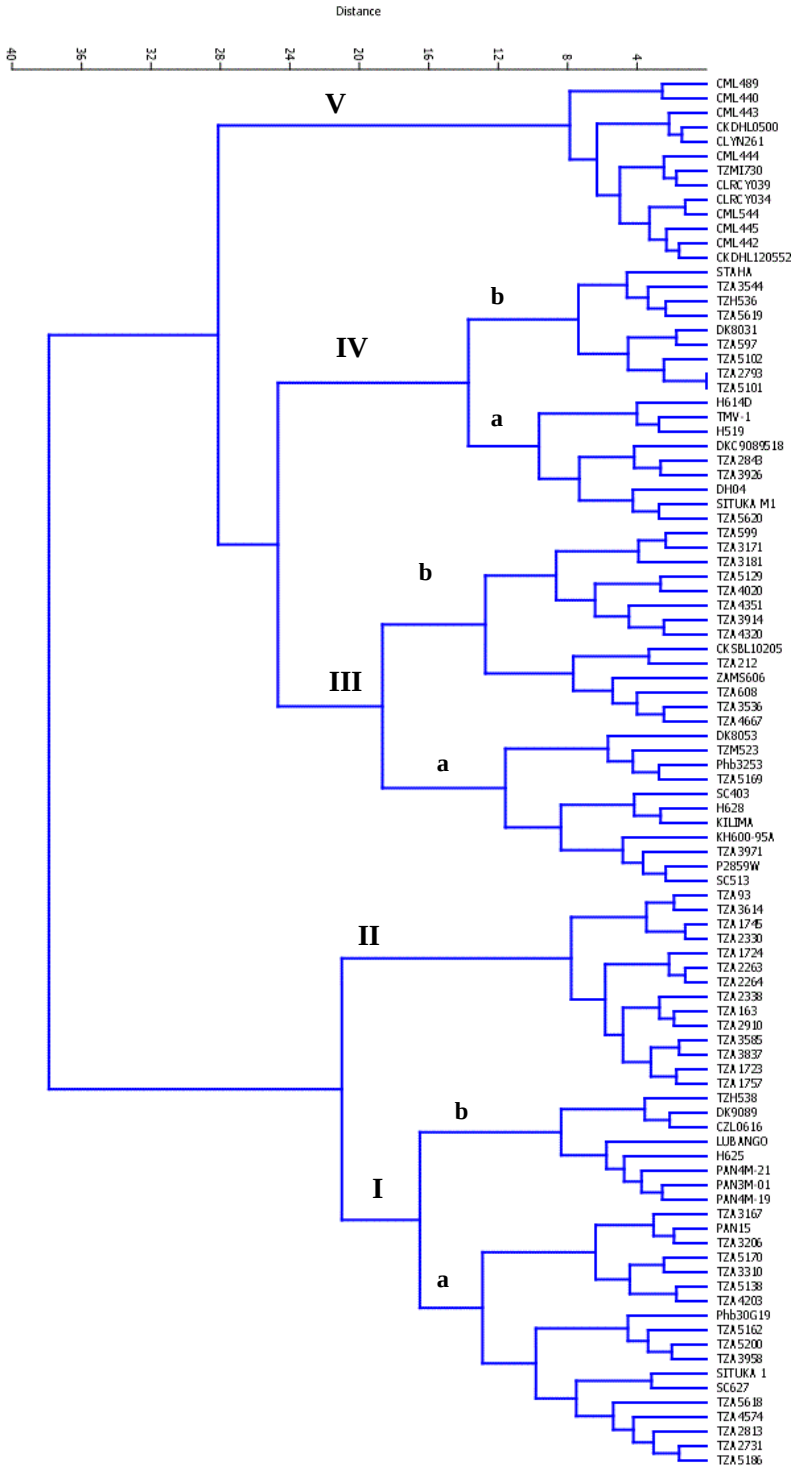

Figure 11: Dendrogram for cluster analyses based on Ward's algorithm method for genetic distance among the 96 accessions analyzed using SSR.

6.4 Discussion

It is very important to have the details and knowledge about genetic diversity and genetic relationships within, between and among germplasm for crop improvement programs. The variation between germplasm revealed at DNA level through molecular markers grant an opportunity to have an effective means for germplasm utilization. In this study SSR markers were used to study genetic diversity among different groups of maize accessions *viz* landraces, commercial varieties and CIMMYT inbred lines as relates to MLND resistance. A total of 63 alleles with polymorphic bands were observed in 96 accessions evaluated in this study using 20 SSR markers. All these 20 SSR loci expressed polymorphism with an average of 3.15 alleles per primer. Other studies reported allelic detection that was either lower or higher than what was obtained in this study. For instance, lower number of alleles per locus of 2.87 and 2.9 with higher number of SSR markers of 30 and 47 than in the current study were obtained by Lopes *et al*. (2015) and Mollin *et al*. (2013), respectively. On the other hand, Reid *et al*. (2011) and Enoki *et al*. (2002) generated higher number of alleles per locus that is 3.62 and 7.3, respectively than in the current study using 105 and 60 SSR markers. Legesse *et al.* (2007) stated that genetic diversity is the most important aspect that limits the number of alleles detected at each SSR locus. On the other hand, Terra *et al*. (2011) gave a reason for different levels of allele number detection being the types of gel used during electrophoresis process. The use of agarose gels is associated with low number of alleles, while polyacrylamide gels provide a very high detection power for even small fragments (Mollin *et al*., 2013; Terra *et al*., 2011; Legesse *et al.*, 2007). In this study, we employed agarose gel for running electrophoresis as used by Lopes *et al*. (2015) and Mollin *et al*. (2013). Reid *et al*. (2011) and Enoki *et al*. (2002) on the other hand, detected the amplification using polyacrylamide gels. Furthermore, polymorphism information content (PIC) expresses the ability of the SSR markers to detect variations within germplasm as caused by the number of alleles per locus and the distribution of those alleles (Nyaligwa *et al*., 2015). The PIC in the current study ranged from 0.17 to 0.79, with an average of 0.52 which was as well reported by Terra *et al*. (2011). Also Nyaligwa *et al*. (2015) obtained an average of 0.51 PIC. Legesse *et al*. (2007) reported 0.55 and Lopes *et al*. (2015) obtained a PIC of 0.41. PIC indicates the discriminatory power of primers which is associated with the variation occurring at the chromosome region of the accessions evaluated. Therefore, expression of lower PIC indicate

that the chromosomal region marked with a certain primer is conserved in a group of germplasm under study (Terra *et al*., 2011). On the other hand, loci that generate higher PIC indicate their ability to discriminate between germplasm and hence chromosomal region is variable between individuals within a group of germplasm (Lopes *et al*., 2015). The PIC in this study provided a room for discrimination and expression of genetic diversity within germplasm.

Analysis of molecular variance in this study showed that 71% of the total variation was accounted for by the variation within accessions and the rest (29%) was divided between among accessions (17%) and among populations (12%). This is explained by the characteristics of cross pollination expressed by plant species like maize which exhibit enormous heterogeneity that may cause SSR variability within as well as among accessions (Hu *et al*., 2007). For self-pollinating plant species, considerable genetic diversity is found among cultivars while little is found within a cultivar (Jensen *et al*., 2006). The low level of variation among populations indicate the occurrence of gene flow which is generally caused by seed mixing and pollen contamination (Nie *et al*., 2014; Louette *et al*., 1997). Thus, accessions used in this study represent the three groups from different sources and improvement status but also there is a certain level of interaction between them. This was evidenced by the general low level of differentiation between the three groups, where the highest 0.347 pairwise genetic distance in this study was between landraces and CIMMYT inbred lines which was actually still low. The exchange and movement of seeds between research institutions, breeding programs, seed companies, as well as between farmers still occur at regional level and in Africa at large (Bøhn *et al*., 2016). The Nei's gene diversity within each group was highest in commercial varieties and closely followed by landraces and lastly was the CIMMYT lines. The commercial varieties were comprised of varieties from different companies and different breeding programs, it also included the OPVs that were selected and developed before being commercialized. The landraces that formed the largest group of accessions had also higher diversity as expected and they were collected from different regions in Tanzania where at each region the diversity is constituted within regions' independent selection for specific desired traits (Beyene *et al*., 2006). However, instead of landraces possessing the highest gene diversity than the rest of the group as expected due to their nature of heterogeneity, they had the diversity slightly lower than the commercial varieties

probably because of seed mixing done by farmers through exchange as their strategy to improve crop performance (Louette *et al*., 1997). As for the CIMMYT inbred lines having the lowest gene diversity, this might be because the accessions in this group were obtained from the same source with possibly similar breeding programs. This finding is supported by Lu *et al*. (2009) who characterized 394 CIMMYT global maize and could not differentiate them into groups. The cluster analysis generated through UPGMA using Ward's algorithm method grouped the accessions into five clusters. However, the clustering of accessions was not independent to the designated group of accessions as were collected from different sources. It was only the CIMMYT lines that exhibited a unique pattern of clustering on dendrogram as well as on scatter plot with principal coordinates analysis. The grouping may express associations related to their pedigree records (Legesse *et al*., 2007), but also could be due to the effect of selection, drift and mutation (Warburton *et al*., 2002).

The extent of *Sugarcane mosaic virus* (SCMV) resistance that might give an indication for MLND resistance was examined with accessions in this study. The accessions were examined following the presence of alleles associated with QTL's on resistance to SCMV. The distribution and frequencies of alleles in two SSR loci phi029 (on chromosome 3 bin 3.04) and phi062 (on chromosome 10 bin 10.04) that are associated with those QTL's against SCMV (Zhang *et al*., 2003) were calculated for a group of accessions *viz* landraces, commercial varieties and CIMMYT inbred lines. Those two loci are located on genes and QTLs that are clustered in chromosomal regions associated with multiple viral pathogen resistance and in particular *Sugarcane mosaic virus* (SCMV) resistance (Wiser *et al*., 2006; Zhang *et al*., 2003). Therefore, it was worth contemplating that these loci provide a room for promising alleles that link to resistance traits against *Sugarcane mosaic virus* (SCMV) as well as other viral pathogens and diseases. The detection and distribution of all alleles at both loci were observed in landraces while in other groups of accessions only one allele at each locus was detected. Also the detection frequency was high in landraces as compared with the other groups. The associations with alleles attached to the loci within the chromosomal region of the QTLs for SCMV resistance are expected to enhance adaptability to a varying level of stresses caused by the virus. There was also no significant difference in allele frequency and distribution between both landraces and

commercial varieties with CIMMYT inbred lines which had phenotypically resistant accessions to MLND. However, SCMV constitute just a part of a synergistic interaction with *Maize chlorotic mottle virus* (MCMV) which is the major pathogen in triggering *maize lethal necrosis* (MLN) disease (Mahuku *et al*., 2015). Furthermore, little is understood about MCMV's genetics for resistance in maize, though promising tolerant genotypes have been grown and identified (Mahuku *et al*., 2015; Redinbaugh and Zambrano, 2014; Nelson *et al*., 2011). In addition, prevention or resistance to one virus that synergistically interact with another is anticipated to reduce the extent of the occurring disease. Thus establishment of genetic details associated with resistance against SCMV provided an insight towards understanding the utility function of the occurring genetic diversity of the studied accessions for MLND resistance.

6.5 Conclusion

Maize supports millions of people in sub-Saharan Africa and other parts of the world as an important staple food as well as a cash crop. However, maize production is challenged by a number of diseases which cause significant yield loss. Recently, maize production in East Africa has been brought into even more challenge due to an outbreak of MLND which is reported to cause up to complete loss of the crop in the field. The method to control the disease which is most reliable and economically feasible is through breeding for genetically resistant varieties. Successful maize breeding requires the availability of reliable genetic diversity to help in the identification and estimation of the level of expected heterosis from germplasm as well as the level of variability during breeding. Therefore, good understanding of genetic diversity within and among maize accessions ensures effective utilization of the genetic resource available for the fight against the current major challenge (MLND) in maize production. A total of 63alleles and a mean of 3.15 alleles per locus observed with the 20 SSR markers as well as the average PIC of 0.52 provided a room for discriminating and realizing genetic diversity within accessions. More than half of the total molecular variation (71%) was recorded within accessions and the rest (29%) was divided among accessions and among populations. That means accessions used in this study had high diversity within themselves. The Nei's gene diversity was highest with commercial varieties and closely followed by landraces and lastly was the CIMMYT lines. The locus phi029 on chromosomal bin location at 3.04 and phi062 on bin 10.04 are the loci

previously identified to be associated with quantitative trait loci (QTL) responsible for *Sugarcane mosaic virus* (SCMV) resistance. The distribution and frequencies of alleles in those two SSR loci were calculated and found higher with landraces than the rest of the group. However, SCMV constitute just a part of a synergistic interaction with *Maize chlorotic mottle virus* (MCMV) which is the major pathogen in triggering for maize lethal (MLN) disease. Therefore, establishment of the genetic details associated with resistance against SCMV provided just an insight towards understanding the utility function of the occurring genetic diversity within studied accessions for MLND resistance.

CHAPTER SEVEN

General discussion, conclusion and recommendations

7.1 General discussion

Maize continues to be the most preferred staple food as well as a cash crop in Tanzania and in other parts of the world (Romay *et al.*, 2013). However, maize is affected by many pathogens and some of them cause significant yield loss (Ali and Yan, 2012). The outbreak of a new disease in East Africa, MLND, presents immediate concerns as well as uncertain long-term consequences (Kabululu *et al.*, 2017). MLND infection rate reaches 100% and yields severely affected up to a complete loss of the crop (Adams *et al*., 2013). Genetic diversity (morphological and molecular) study provides key information that may help in identifying important traits against several production constraints (Enoki *et al*., 2002). Hence, good understanding of genetic diversity within and among maize accessions ensures effective utilization of the genetic resource available for the fight against the current major challenge (MLND) in maize production. A total of 51 maize landraces from Tanzania were evaluated in different experiments along with 34 commercial varieties and 13 elite lines from CIMMYT Kenya to study their potential genetic diversity and how the diversity responds against MLND. The significant differences expressed among those accessions and their interactions with environments suggest that the accessions were different (variable) from each other and they could respond differently in different environments and stresses. This variability is what we desire to use in breeding programs to establish improved maize varieties required by the community for specific traits and even for specific location (Kabululu *et al*., 2017). Successful plant breeding then requires careful choices of genotypes that posses desirable traits for best combinations. The correlations between traits are also another important knowledge that helps on predicting the required performance in terms of certain traits. For example, the negative correlation between yield and flowering parameters help to have an indirect selection of higher yielding genotypes through flowering character.

Results in this current study showed that an OPV Situka 1 and a hybrid DH 04 were generally the best performing genotypes in terms of grain yield and stability as well as for other related parameters across all the three locations. However, TZA 2793 was a local cultivar that expressed

promising performance for yield. The maize landraces have always been considered less productive than other improved varieties, but they present an important source of genetic variability that can be exploited to search for genes against biotic and abiotic stresses. The flowering character defines the maturity differences among accessions (Olaoye, 2009) and it can also be connected to the yielding ability that early maturing accessions could generate high grain yield while those which are late maturing produce low yield (Lafitte *et al.*, 1997). However, the opposite also holds true under different circumstances where late maturing varieties produce high yield due to their ability to have longer periods to accumulate maximum assimilates into grains than the early maturing varieties (Wang *et al*., 2011; Bello *et al*., 2012a). The analysis in this study showed a significant negative correlation between both days to 50% anthesis and days to 50% silking to all the yield related parameters. A wide range of variation in flowering characters could signify the potential variability within accessions that helps on developing genotypes adaptable to different areas with different characteristics (Cömertpay, 2012).

Quantitative morphological traits that highly contributed to the total variation expressed by the accessions under study included 1000 kernel weight, plant height, ear height, yield per plant and days to 50% silking. These traits could be used to characterize several maize accession populations and discover potential candidates as parents for generating elite materials. The study identified distinction of the three groups of accessions used in this study where commercial varieties were discriminately identified by high yield related parameters and early flowering characteristics. CIMMYT elite lines were characterized by significant small plant structures and landraces were extensively diverse. For qualitative morphological traits, the percent frequency distribution of accessions within traits differentiated the three groups in terms of foliage, tassel size, shape of upper surface of kernel and kernel type. Other traits of stem colour, sheath pubescence, tassel type, cob colour, shape of upper most ear, kernel row arrangement, kernel colour and endosperm colour characterized the three groups similarly though with different percent distribution. The former traits were able to discriminate between and within the three groups while the later identified differences just within each group. Traits that had higher percentage distribution of accessions towards one class within a trait include stem colour (green), sheath pubescence (intermediate), tassel type (primary-secondary), cob colour (white), shape of

uppermost ear (conical), shape of kernel upper surface (rounded), kernel row arrangement (regular), kernel colour (white) and endosperm colour (white). This trend of accessions being distributed towards a certain class of trait reflects farmers' or consumers' preferences through successive selection (Ntundu *et al.*, 2006; Louette and Smale, 2000). On the other hand, the grouping of the accessions through cluster analysis reflected individual performance and type of accessions. Most of the landraces lacked consistent grouping in terms of collection sites. This was related to the finding by Sun *et al*. (2016) who observed geographically close populations of Chinese sweetgum in different clusters. The implication in this finding is that the landraces involved in this study were comprised of a heterogeneous group that occurred through repeated exchange and selection of germplasm executed by farmers (Ntundu *et al.*, 2006).

Having the distinct and diverse groups of accessions in this study, tests were made to see how this diversity results into responding against MLND. The standardized relative area under the disease progress curve (rAUDPC) was utilized to assess the cumulative disease progression within the growing process of the crop plants as explained by Luitel *et al*. (2016). The rAUDPC was higher at Naivasha than at Mlangarini because the extent of disease pressure at Naivasha was high, due to the artificial inoculation which generated maximum pathogen inoculum applied on plants. On the other hand, natural disease pressure at Mlangarini generated low disease pressure. The rAUDPC evaluated how the genetic differences among the accessions determine the way they react on the extent of disease development on plants (Skelsey and Newton, 2014). The accessions with the lowest rAUDPC were considered to be resistant to the disease and vice versa (Safavi *et al*., 2010). Also, the resistant performance is determined by comparing the accessions with the performance of the established susceptible and resistant check materials (Massa *et al*., 2015). Therefore, accessions that were able to show the least disease progression trend in both locations could be considered promising for resistance indication against MLND. In this study, accessions TZA2793 and TZA3544 had among the lowest rAUDPC, but across the two locations and may be considered for further screening to ascertain their resistance characteristics against MLND. The occurrence of the disease at Naivasha enhanced high heritability (0.86) as compared with the low heritability (0.18) at Mlangarini. This was due to the fact that uniform environmental conditions ensure high level of heritability while variable

environments lower the level of heritability (Brunda *et al*., 2014). The amount of inoculum applied on the plants at Naivasha was actually uniform such that genetic variation of the accessions in this study exerted an influence responsible for the variation in response against MLND. On the other hand, the Mlangarini situation was different because the amount of pathogens occurred naturally with no regular inoculum pressure distributed on plants. Heritability measures phenotypic variance as caused by genetic variation, it provides prediction for plant breeding strategies.

The correlation between sheath pubescence and the occurrence of MLN disease was significantly negative. That means, an increase of pubescence is associated with lowering the disease (enhance resistance) to the crop plants. The resistance might directly be the close link of the pubescent marker and the gene controlling resistance or else the indirect link through the prevention of the insect pests carrying the pathogen causing the disease. Mmbaga *et al*. (1996) found the presence of dense pubescence to be linked to the resistance of rust on common bean and the pubescence trait was merely inherited. Endosperm colour is another character which was negatively correlated to the occurrence of MLN disease. The descriptor for endosperm colour used for scoring, ranged from white, cream, pale yellow, yellow, orange and white cap. The higher number for endosperm colour signified for more coloured endosperm such as yellow or orange, while the lower number associated with whitish colour. Therefore, as the colour become more yellow or orange, it is associated with resistance, and the white endosperm is related to susceptibility. Scott (1989) studied the link between endosperm colour and potyvirus resistance and found that the gene for yellow endosperm colour was linked to the resistance on potyviral disease. The promising inbred lines resistant to MLND that had been developed so far have yellow endosperm colour (Semagn *et al*., 2015). Anthesis-silking interval (ASI) was significant and positively correlated to the occurrence of the MLN disease, which means the longer the ASI the higher the chance for possible susceptibility, while short ASI indicated the possible resistance. Ngugi *et al*. (2013) established the facts that relate to this finding, where Anthesis-Silking Interval was significant and positively correlated with stress susceptible index. ASI is to a large extent determined by the variation in silking number of days where a strong association of their Quantitative Trait Loci (QTLs) confirms the close link of the two traits (Gemenet *et al*.,

2010). This imply that short ASI depends on the short duration of the silking from the planting date (Magorokosho *et al*., 2003) and a genotype with short days to silking have a chance to be able to tolerate or resist growing stresses like diseases and drought. Kernel type was also significantly correlated with the disease scores such that flint kernel type was found to associate with resistance traits. The indication of kernel type being correlated to disease resistance traits was also found by Marçon *et al*. (1996), but here however, they learned that dent kernel types had the relative resistance potential against high plains virus while sweet corn (sweet kernel type) expressed high susceptibility. On testing the maize accessions in this study, the cluster analysis brought a landrace TZA2793 grouped together with the check varieties that are moderately resistant CKDHL0500, CKSBL10205, CKDHL120552 and resistant CLRCY039, CLYCN261, CLRCY034. The landrace could be considered for other tests to confirm its potential for MLND resistance.

It is very important to have the details and knowledge about genetic diversity and genetic relationships within, between and among germplasm for crop improvement programs. The variation between germplasm revealed at DNA level using molecular markers grant an opportunity to have effective means for germplasm utilization. In this study we used SSR markers to study genetic diversity among different groups of maize accessions *viz* landraces, commercial varieties and CIMMYT inbred lines as related to MLND resistance. A total of 63 alleles were observed within 96 accessions evaluated in this study using 20 SSR markers. All these 20 SSR loci expressed polymorphism with an average number of 3.15 alleles per primer. The Polymorphism Information Content ranged from 0.17 to 0.79, with an average of 0.52 was comparable to that reported by Terra *et al*. (2011). Analysis of molecular variance in this study showed that more than half of the total variation (71%) was accounted for the variation within accessions where 17% variations was attributed to among accessions and 12% of variation to among populations. This is explained by the characteristics of cross pollination expressed by plant species like maize which exhibit enormous heterogeneity that may cause SSR variability within as well as among accessions (Hu *et al*., 2007). The low level of variations among populations indicates the occurrence of gene flow that is generally caused by seed mixing and pollen contamination (Nie *et al*., 2014; Louette *et al*., 1997). Thus, accessions used in this study

represent the three groups from different sources and improvement status but also a certain level of interaction between them. This was evidenced by the general low level of differentiation among the three groups, where the highest 0.347 pairwise genetic distance in this study between landraces and CIMMYT inbred lines were actually still low. The exchange and movement of seeds between research institutions, breeding programs, seed companies, as well as between farmers still occur at regional level and in Africa at large (Bøhn *et al*., 2016). The cluster analysis generated through UPGMA using Ward's similarity matrix grouped the accessions with no independency to the designated group of accessions collected from different sources. Only CIMMYT lines grouped themselves together. The grouping may express associations related to their pedigree records (Legesse *et al*., 2007), but also could be due to the effect of selection, drift and mutation (Warburton *et al*., 2002).

The extent of *Sugarcane mosaic virus* (SCMV) resistance that might give an indication for MLND resistance was examined with accessions in this study. The accessions were examined following the presence of alleles associated with QTL's on resistance to SCMV. The distribution and frequencies of alleles in two SSR loci phi029 and phi062 were calculated for the accessions evaluated in this study. Those SSR loci phi029 on chromosome 3 bin 3.04 and phi062 on chromosome 10 bin 10.04 are associated with QTL's against SCMV (Zhang *et al*., 2003). The loci are located on genes and QTLs that are clustered in chromosomal regions associated with multiple viral pathogen resistance and in particular *Sugarcane mosaic virus* (SCMV) resistance (Wiser *et al*., 2006; Zhang *et al*., 2003). Therefore, it was worth contemplating that these loci provide a room for promising alleles that link to resistance traits against *Sugarcane mosaic virus* (SCMV) as well as other viral pathogens and diseases. The detection and distribution of all alleles at both loci were observed in landraces while in other groups of accessions only one allele at each locus was detected. The detection frequency was high in landraces as compared with the other groups. The association with alleles attached to the loci within the chromosomal region of the QTLs for SCMV resistance is expected to enhance adaptability to a varying level of stresses caused by the virus. However, SCMV constitute just a part of a synergistic interaction with *Maize chlorotic mottle virus* (MCMV) which is little understood about its genetics for resistance in maize (Mahuku *et al*., 2015; Redinbaugh *et al*., 2014; Nelson *et al*., 2011). Thus,

establishment of genetic details associated with resistance against SCMV provided an insight towards understanding the utility function of the occurring genetic diversity for MLND resistance.

7.2 Conclusion

Maize genome harbors potential amount of morphological and molecular diversity that can be sourced and invested for maize crop improvements. Therefore, good understanding of genetic diversity within and among maize accessions ensures effective utilization of the genetic resource available for resistance against the current major challenge (MLND) in maize production. The results in this study have revealed a significant range of genetic diversity in maize accessions evaluated. This might provide a source of variation required for breeding programs to hold back the genetic vulnerability as a result of recurrent outbreaks of new strains of pest and diseases. It also offers an opportunity to widen the genetic background of the available maize germplasm because the materials that are currently at disposal for several breeding programs are composed of narrow genetic base. Agronomic evaluation revealed that TZA 2793 is the most promising accession on yield. Cluster analysis also identified two landraces, TZA 2793 and TZA 5170 grouped with commercial varieties that are mostly higher yielding. The cluster analysis also disclosed the expression of landraces lacking regular pattern in clustering within their major group. This elucidate the fact that farmers select cultivars based on their preferences and also exchange seed crop materials with fellow farmers even from very distant regions. Farmers play a significant role in shaping the structure of landrace population existing in a certain area.

The assessment of accessions' performance on MLND progression over time, showed accessions TZA2793 and TZA3544 to have the lowest rAUDPC in both locations and may be considered stable and promising for resistance characteristics against the disease. Pearson correlation matrix showed sheath pubescence, kernel type and endosperm colour to be negatively correlated with MLN disease scores while anthesis-silking interval, and kernel type were positively correlated with MLND scores. Cluster analysis associated TZA2793 with moderate and resistant variety checks and grouped them in the same cluster. Molecular genetic diversity study revealed that 71% of the total molecular variation was recorded within accessions and the rest (29%) was

divided among accessions with 17% and 12% among populations. That means accessions used in this study had the high level of diversity within themselves.

The distribution and frequencies of alleles in phi029 and phi062 loci previously identified to be associated with quantitative trait loci (QTL) responsible for *Sugarcane mosaic virus* (SCMV) resistance were found to be higher with landraces. Thus, the established genetic detail associated with resistance against SCMV provides an insight towards understanding the utility function of the occurring genetic diversity for MLND resistance.

7.3 Recommendations

Lack of regular clustering pattern in terms of regions of collection calls for systematic involvement of farmers in breeding and selection processes through participatory breeding in order to have an organized process of population structuring.

The significant range of genetic diversity revealed in maize landraces evaluated in this study showed the need to establish extensive molecular and morphological genetic details of a vast germplasm materials kept in the Genebanks, research institutions and farmers in order to have the required allelic information for crop improvements.

Accessions TZA 2793, TZA 5170 and TZA 3544 expressed promising performance on yield and response against MLND, hence could further be investigated to utilize their potentials for future and imminent maize crop improvement especially resistance against MLND.

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APPENDICES

Appendix 1: Mean values on grain yield and yield components of all maize accessions used on yield evaluation study

Genotype name	Yield per plant (g)	1000 Kernel weight (g)	Days to 50% Tasseling	Days to 50% Silking	Ear Length (cm)	Ear diameter (cm)	Number of Kernels/ row	Kernel rows Number
SITUKA1	116.01±12.28	275.51±19.47	64.33±2.25	70.89±2.67	16.02±0.89	4.28±0.17	31.17±2.08	13.05±0.33
DH04	115.90±14.48	273.74±15.33	72.11±2.05	75.44±2.01	15.58±0.41	4.51±0.07	32.65±1.75	12.78±0.13
PIONEER	112.73±17.19	258.10±25.60	69.89±1.76	75.89±2.04	17.66±0.36	4.64±0.05	36.07±0.96	13.83±0.18
TMV-1	108.14±10.84	247.27±13.52	73.44±1.82	77.78±1.88	16.50±0.52	4.36±0.07	31.92±1.71	13.92±0.50
DEKALB	104.74±9.11	302.73±19.86	66.44±2.29	69.89±2.39	16.63±0.51	4.58±0.09	33.09±1.17	12.42±0.13
TZA2793	100.46±11.22	260.68±10.45	78.33±2.38	84.00±2.74	15.86±0.39	4.24±0.08	29.36±1.47	12.45±0.20
TZA5170	99.80±14.55	285.36±26.12	77.44±2.46	83.00±2.71	15.14±0.54	4.38±0.09	26.77±1.55	11.78±0.26
SITUKAM1	99.41±14.94	301.99±13.68	64.11±2.19	71.56±1.89	15.95±0.33	4.43±0.08	30.30±1.16	13.19±0.22
TZA2263	97.54±17.01	260.73±20.96	80.33±2.69	86.11±3.29	15.35±0.69	4.37±0.12	28.86±1.73	12.77±0.14
SC403	95.14±14.76	313.55±18.15	63.67±2.05	69.78±1.96	16.77±0.59	4.52±0.11	33.10±1.88	12.67±0.16
TZA4203	88.19±8.48	250.60±16.64	82.00±2.79	89.11±2.82	16.20±0.55	3.92±0.08	29.06±0.90	12.09±0.11
TZA3926	79.99±15.50	309.37±22.85	77.67±3.01	87.44±3.86	16.31±0.86	4.20±0.13	25.33±1.69	11.30±0.24
TZA212	79.88±10.22	260.34±15.24	74.78±2.25	80.33±2.36	15.82±0.20	3.97±0.05	29.56±0.63	12.41±0.17
TZA2731	78.56±14.39	256.75±19.40	79.67±3.76	87.00±4.27	14.05±1.12	4.21±0.16	27.26±2.60	13.21±0.50
TZA3585	74.75±12.80	256.48±25.25	77.11±3.32	81.78±3.80	14.38±0.82	4.18±0.12	29.35±1.80	12.75±0.30
TZA3971	74.20±11.58	274.02±9.55	73.78±2.48	79.00±3.14	14.99±0.54	4.12±0.08	26.25±1.07	12.34±0.12
TZA2330	74.04±11.67	246.04±15.90	79.33±2.46	86.33±2.81	14.75±0.61	4.46±0.09	27.61±1.45	13.85±0.30
CML442	72.93±6.68	212.98±16.96	75.89±2.10	81.67±2.09	14.87±0.34	3.91±0.08	25.22±0.79	13.31±0.23
TZA5205	70.85±8.15	226.52±20.09	71.11±1.96	76.22±2.08	14.06±0.53	4.22±0.07	26.07±1.04	14.23±0.23
TZA4020	70.80±6.73	259.01±9.81	73.67±2.11	77.67±2.32	14.35±0.41	4.06±0.06	28.11±1.15	11.69±0.22
TZA2843	70.41±9.17	236.78±17.97	78.00±2.68	85.11±2.93	13.30±0.58	3.96±0.11	25.57±1.26	11.34±0.16
TZA5169	70.07±8.79	236.43±14.19	71.44±2.52	77.33±2.79	14.66±0.96	3.72±0.12	27.89±2.66	11.35±0.49
TZA4320	69.15±29.26	269.92±15.70	84.50±3.65	93.44±3.29	15.31±1.10	4.20±0.14	24.61±3.12	12.00±0.33
TZA3206	69.09±8.53	296.91±19.14	70.44±2.66	76.11±2.79	14.09±0.59	4.06±0.08	26.05±1.41	11.43±0.25
TZA599	66.91±9.82	273.42±24.13	83.56±2.51	92.11±2.49	16.61±0.71	4.21±0.12	27.08±2.20	11.24±0.29
TZA3536	65.42±9.49	235.32±13.75	78.67±2.27	86.67±3.53	15.49±0.33	4.30±0.07	28.86±1.23	14.10±0.22
TZA4667	65.02±4.37	279.22±11.68	78.22±2.95	85.67±3.23	14.28±0.44	4.04±0.10	27.23±0.63	11.02±0.11
TZA4351	64.43±10.84	266.86±13.19	83.44±3.25	92.00±4.38	14.54±0.71	4.54±0.10	24.76±1.48	13.41±0.40
TZA5129	63.95±8.69	267.91±20.21	83.67±2.82	91.22±2.23	15.15±0.41	4.28±0.09	25.20±1.41	12.74±0.19
TZA3544	63.34±7.75	272.89±15.38	78.11±2.75	85.67±2.82	14.54±0.70	4.21±0.06	23.41±1.78	12.47±0.18
TZA3914	61.16±7.01	271.82±15.96	79.33±2.95	82.89±3.28	14.83±0.78	4.26±0.11	22.73±1.59	12.25±0.40
TZA4574	60.84±8.43	221.23±23.26	81.44±2.92	89.44±3.50	13.59±0.81	3.67±0.17	25.07±2.53	10.90±0.36
CLYN261	60.46±10.82	232.81±24.03	84.56±2.75	85.89±2.78	13.01±0.55	3.65±0.13	18.96±1.82	13.01±0.21
TZA1723	60.36±5.02	265.78±15.32	81.89±2.84	89.33±2.87	15.11±0.49	3.73±0.06	23.90±0.96	11.10±0.17
TZA5138	60.13±11.55	213.58±11.39	76.78±2.02	83.33±2.35	14.18±0.48	4.14±0.08	25.37±1.66	13.52±0.41
TZA5162	59.20±13.58	219.55±25.49	86.56±3.31	93.78±3.34	14.34±0.39	3.80±0.10	24.17±1.75	11.43±0.30
TZA2264	58.28±11.09	250.20±14.40	78.44±2.26	84.11±2.73	14.03±0.43	4.37±0.11	26.81±1.28	13.05±0.27
TZA3167	57.66±6.25	272.20±14.34	77.33±2.49	83.89±2.66	14.72±0.63	3.98±0.08	24.62±1.08	11.12±0.12
TZA3310	57.39±7.29	238.47±13.35	82.72±2.52	90.72±2.39	13.83±0.36	3.87±0.08	24.57±1.95	11.09±0.33
CLRCY034-B	57.32±8.99	172.64±14.64	87.00±3.02	92.00±2.44	13.25±0.13	3.37±0.08	19.19±1.05	12.92±0.40
TZA3958	57.06±9.71	296.41±25.21	73.44±1.50	79.56±1.36	14.72±0.63	4.30±0.08	22.29±2.39	11.77±0.39
TZA5200	54.98±6.40	218.86±16.17	80.44±3.52	90.11±3.20	13.36±0.52	3.96±0.12	25.22±1.25	13.03±0.23
TZA2338	53.92±7.27	265.96±15.04	84.67±3.24	91.56±2.90	13.72±0.72	3.78±0.10	22.45±2.28	11.73±0.32
CLRCY039	53.25±9.46	181.62±14.14	83.56±1.82	87.67±1.91	13.42±0.44	3.48±0.19	17.20±1.14	12.76±0.22
TZA5102	53.00±9.74	271.66±18.64	88.00±2.77	93.78±3.04	13.56±0.54	4.26±0.10	20.06±1.81	13.47±0.41
TZA3614	52.32±8.08	241.38±18.31	84.11±2.74	93.00±3.57	13.81±0.37	3.83±0.07	22.44±1.23	12.50±0.22
TZA3171	51.67±5.62	276.78±21.54	78.56±2.43	86.89±2.19	14.56±0.62	3.91±0.10	22.58±0.61	10.90±0.28
TZA4164	51.39±8.76	239.93±21.74	80.72±3.28	90.44±3.66	12.69±0.80	3.93±0.11	20.55±1.82	11.44±0.34
TZA3837	50.72±10.34	254.11±17.02	83.89±2.83	90.89±3.45	13.68±0.86	4.28±0.14	22.66±2.04	13.16±0.20
TZA1745	50.65±8.04	266.43±21.68	85.67±3.08	94.56±3.30	15.16±0.50	3.83±0.12	23.91±1.35	10.36±0.23
CKDHL0500	50.54±4.18	229.46±31.53	82.44±2.21	87.67±3.04	12.79±0.31	3.89±0.07	21.18±0.55	14.30±0.24
TZA163	49.02±8.86	254.16±13.35	83.56±2.60	94.56±3.25	15.32±0.62	3.80±0.10	21.95±2.19	11.59±0.32
TZA2904	48.99±5.08	279.01±22.62	69.67±4.59	78.56±3.10	15.22±0.42	3.67±0.11	22.23±1.20	10.84±0.29
TZA608	48.64±7.16	295.61±12.53	79.89±3.08	87.67±2.58	15.37±0.44	4.33±0.07	27.62±1.98	11.58±0.38
TZA5619	44.60±8.04	218.18±33.93	90.89±3.84	102.33±4.39	14.30±1.42	3.74±0.22	19.41±3.51	10.63±0.93
CKDHL120552	43.85±6.31	199.49±13.85	80.56±2.93	87.56±4.10	11.21±0.90	3.29±0.21	18.80±3.06	10.72±1.11
CML443	43.57±7.29	203.42±15.03	74.67±2.56	79.00±2.29	15.95±2.26	3.19±0.10	19.80±0.99	11.64±0.60
TZA1753	41.70±5.45	228.84±24.48	78.22±3.33	84.89±3.52	15.06±0.75	4.24±0.12	22.43±1.34	12.65±0.30
TZA3181	41.26±6.98	269.77±27.96	81.22±3.78	89.67±4.32	14.25±0.89	3.80±0.14	23.15±1.47	10.90±0.82
TZA93	40.45±2.91	271.26±18.52	77.89±2.20	87.33±1.98	16.46±0.48	4.05±0.09	25.27±0.99	11.37±0.18
TZA5618	36.69±5.03	219.87±24.64	85.78±2.99	94.44±3.31	15.41±0.67	4.01±0.08	21.50±1.33	11.99±0.26
CKSBL10205	35.95±11.31	185.63±15.87	79.56±2.22	81.22±2.17	10.80±0.55	3.28±0.18	17.72±1.91	12.53±0.96
TZA1757	35.72±8.24	284.61±24.16	85.11±3.46	93.00±3.73	14.59±0.64	4.23±0.17	23.35±1.31	11.61±0.40
CML544	29.20±5.18	192.49±19.20	78.00±2.81	80.56±2.84	12.72±0.50	3.69±0.09	18.84±0.73	12.94±0.37
CML440	29.03±4.80	174.27±10.31	72.11±1.98	76.22±2.52	11.48±0.30	3.36±0.11	17.88±0.75	12.82±0.37
TZA1724	24.47±3.27	248.36±25.28	90.67±2.98	101.33±2.97	16.00±0.52	3.72±0.13	20.18±2.43	10.12±0.40
TZMI730	21.14±3.85	204.93±21.38	87.78±1.88	91.00±2.10	10.32±0.73	3.34±0.19	15.44±2.45	12.01±0.63
TZA2813	11.52±2.16	174.11±15.90	88.22±3.10	100.67±3.41	11.39±0.80	3.37±0.10	13.18±1.81	11.19±0.62
Grand mean	**62.94±1.48**	**249.59±2.59**	**78.98±0.40**	**85.70±0.45**	**14.52±0.10**	**4.01±0.02**	**24.59±0.26**	**12.21±0.06**
Location (L)	49.31***	155.64***	1342.61***	1321.48***	131.61***	129.77***	113.69***	26.93***
Genotypes (G)	6.59***	5.48***	30.65***	36.79***	6.92***	15.40***	10.86***	9.43***
Interaction (L x G)	1.45**	1.59***	1.41**	1.64***	1.12^{ns}	1.50**	1.60***	1.98***