Genetic analysis, combining ability and yield stability of maize genotypes under maize streak virus prone environments

By

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Thesis Abstract

Maize (*Zea mays* L.) is one of the most important food security crops in Tanzania. It is annually cultivated in an area of 2 million hectares representing 45% of the total area allocated to crop production. However, maize yields are very low in the country due to several biotic and abiotic stresses and socio-economic constraints. Among the biotic factors, maize streak disease caused by the maize streak virus (MSV) inflicts significant yield losses reaching up to 100%. Development of farmers' preferred, high yielding and MSV resistant cultivars is the best strategy to boost maize productivity in Tanzania. Therefore, the objectives of this study were to: (1) determine farmers' preferred traits of maize and production constraints limiting maize production in the northern areas of Tanzania, (2) determine agro-morphological diversity present among 80 local and introduced maize inbred lines under maize streak virus (MSV) prone environments of the northern zone of Tanzania, (3) assess the genetic diversity and genetic relationship among 79 maize inbred lines collected from five different origins using 30 polymorphic simple sequence repeat (SSR) markers, (4) determine combining ability and heterosis for grain yield and related traits and resistance to maize streak virus (MSV) among 10 elite maize inbred lines and their hybrid progenies, and (5) investigate the GXE interaction for grain yield and MSV resistance among newly developed maize hybrids in Tanzania using AMMI and GGE biplot methods.

A participatory rural appraisal (PRA) study was conducted in 2012 at Babati, Arumeru and Hai Districts in northern Tanzania. Data were collected involving 500 farmers using structured interviews and focused group discussions (FGD). Results showed that maize was the most important crop in the study areas and ranked first among other food crops. Grain yield potential, disease resistance and drought stress tolerance were farmers preferred traits with relative importance of 71.9, 70.0 and 69.9%, respectively. Through FGD farmers identified ear rot, MSV and common rust as most important diseases affecting maize production. High costs of production inputs and low price of maize were also among the challenges to maize production in the study areas. Knowledge of the farmers' preferences and production constraints is required by breeders to enhance the productivity of maize in the northern areas of Tanzania.

Eighty maize inbred lines were evaluated using ago-morphological traits. Field experiment was established during 2011/2012 at maize streak virus (MSV) prone environment of Ngaramtoni Research Farm of Selian Agricultural Research Institute in northern Tanzania using a 10 x 8 alpha lattice design with two replications. Analyses of variance on seven quantitative traits revealed highly significant ($P \leq 0.001$) variations among inbred lines. TL2012-42 and TLl2012-41 were identified as

superior lines with grain yields of 3.52 and 2.46 t/ha respectively. These genotypes showed low (< 30%) level of MSV reaction suggesting their suitability for hybrid breeding to achieve high grain yield and MSV resistance. Principal component analysis revealed 68.9% of the total variation explained by four principal components. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the inbred lines into nine clusters consistent with their heterotic patterns. The study identified the following inbred lines: TL2012-53 and TL2012-61 from cluster II and TL2012-20, TL2012-70, and TL2012-78 from cluster IV for breeding.

Genetic diversity and relationships of 79 maize inbred lines collected from five diverse sources were subjected to SSR analysis using 30 polymorphic markers. The mean numbers of observed and effective alleles were 4.70 and 2.40, respectively. The markers displayed high Shannon's information index of 0.96 and polymorphic information content (PIC) of 0.51. The mean values of observed and expected heterozygosity among lines were 0.136 and 0.508, respectively. A dendrogram constructed based on UPGMA clustered the inbred lines into three main genetic groups with varied sub-clusters. The principal coordinate analysis (PCA) explained 20.4% of the total genetic variation detected among inbred lines and separated them into two main clusters. Analysis of molecular variance (AMOVA) showed that 72% of the total variation was attributed to differences among inbred lines across locations, 26% of the total variation was due to inbred lines within sub-populations/locations and 2% was attributed to variation between the five geographic origins of inbred lines. The study identified inbred lines such as TL2012-20, TL2012-24 and TL2012-54 (from cluster I) and TL2012-25, TL2012-21 and TL2012-12 (from cluster III) showing genetic difference for hybrid breeding to exploit heterosis.

Ten selected inbred lines were crossed and 45 F_1 hybrids developed using a 10x10 half diallel mating design. Parents, F_1 hybrids and five standard checks were evaluated using a 6 x 10 lattice design with two replications at Ngramtoni, Inyala and Igomelo during 2012/13 and 2013/14. General combining ability (GCA) of parents, specific combining ability (SCA) of hybrids, heritability and heterosis of grain yield and related traits and MSV resistance were calculated. The mean squares of GCA and SCA effects showed significant differences for all the traits except days to 50% anthesis and silking. The SCA effect was important for all traits except for MSV, number of ears per plant and husk cover while the GCA effect was most important for resistance to MSV. Heritability estimates of traits were high associated with high GCA effects. Line TL2012-42 was a good general combiner for grain yield showing highly significant positive GCA effect of 0.695 while lines TL2012-41, TL2012-1 and TL2012-42 had significant negative GCA effects of -10.926, -10.792 and -10.748 respectively for MSV reaction. These inbred lines could be exploited in hybrid breeding to develop high yielding and

MSV resistant varieties. Hybrids TL2012-38/TL2012-55 and TL2012-25/TL2012-6 had highest negative significant SCA effect of -10.892 and -19.451, respectively for MSV reactions (desirable direction). Maximum mid-parent heterosis for grain yield was recorded for hybrid TL2012-7/TL2012-38 at 138 while TL2012-25/TL2012-26 had the lowest and negative heterosis of -38.2 for MSV reaction. Crosses TL2012-7/TL2012-42 and TL2012-7/TL2012-68 had significant positive SCA effects for grain yield which can be used for direct production as single cross hybrids or developed further as three way hybrids for large scale production.

Genotype by environment interaction (GXE) of grain yield and MSV resistance was investigated among newly developed maize hybrids in Tanzania. Forty five novel single cross hybrids and five standard check three-way cross hybrids were evaluated using a 5x10 alpha lattice design with two replications across six environments. The Additive Main Effects and Multiplicative Interaction (AMMI) and genotype, and genotype by environment (GGE) biplot models were used to assess the magnitude of GXE interaction of grain yield and reaction to MSV disease among test genotypes. Results from the AMMI analysis of variance revealed high (52.06%) contribution of the environmental effect on grain compared to genotypes and GXE interaction which, respectively accounted for 12.4% and 17.76% of the total variation on this trait among hybrids tested. Genotypes and GXE contributed to 12.4% and 17.76% of the total variation of hybrids of this trait, respectively. Genotypes explained 45.52% of the total variation of hybrids for MSV resistance while the contribution of environments was minimal (2.77%). Hybrid G43 was identified with relatively high mean grain yield of 6.70 t/ha with low MSV severity of 31.88% across environments. Experimental hybrids such as G10, G14 and G28 had high yield performance of 6.72, 6.00, and 6.23 t/ha, in that order across environments but with highly susceptible reaction to MSV. Conversely, hybrid G31 expressed low MSV infection but yielded the lowest at each environment. Hybrids such as G23 with low grain yields of 4.84 t/ha, G18 (5.14 t/ha), and G34 (1.94 t/ha) showed relatively low MSV infection levels which are useful genetic resources for resistance breeding. Experimental hybrids with high grain yield and MSV resistance selected in this study are good candidates for direct production or for future three-way hybrid development in Tanzania.

Overall, the current study selected valuable maize inbred lines with high combining ability for grain yield and related traits and MSV resistance. Also, new experimental maize hybrids were generated for direct production or further development of three-way hybrids.

Declaration

I, Lameck Makoye Nyaligwa, declare that

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

2   This thesis has not been submitted for any degree or examination at any other University.

3   This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4   This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

   (a) Their words have been re-written but the general information attributed to them has been referenced.

   (b)   Where their exact words have been used, then their writing has been placed in Italics and inside quotation marks, and referenced.

5   This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Lameck Makoye Nyaligwa

As the candidate's supervisor, I agree to the submission of this thesis:

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Prof. Shimelis Hussein (Supervisor)

Acknowledgements

Dedication

This thesis is dedicated to my late mother, Selina Sozi Madonange, who during her life time established a foundation for my education. I further dedicated this work to my daughter, Selina L. Makoye, for her love and support.

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Introduction to Thesis

Production and importance of maize

Maize (*Zea mays* L.) is one of the most important cereal crops in the world serving as human food, feed, industrial uses and bio-energy (M'mboyi et al., 2010; Ranum et al., 2014). In developing countries such as in sub-Saharan Africa maize is predominantly grown for food by smallholder farmers under rain-fed condition and with minimal production inputs such as fertilizers and crop protection chemicals (Carns et al., 2013). The leading maize producers of the world include the United States of America, China, Brazil and Mexico (Ranum et al., 2014). The current global mean maize yield stands at 4.9 tons per hectare.

Maize is the most important cereal food crop in sub-Saharan Africa (SSA). In the eastern and southern Africa maize occupies 53% of the total area allocated to cereal production (Erenstein et al., 2011; Carns et al., 2013). It accounts for 30-70% of calories consumed in SSA (Erenstein et al., 2011). About 69.6% of countries in the world with the highest per capita consumption of maize are located in SSA (Sibiya et al., 2013). Maize yields in SSA are very low by virtue of frequent droughts, low soil fertility, and diseases and pests (Vivek et al., 2010). In the region supply of maize grain is below the present demand due to low productivity and high population growth (Erenstein et al., 2011; Sibiya et al., 2013). The global demand for maize as food is projected to increase by 45% in 2020 and expected to double by 2050 (CIMMYT and IITA, 2010). During the past years maize production areas have grown by 72% in developing countries and 18% in developed countries. Despite the increase in areas of production, maize productivity is low without meeting the present and projected demand especially in developing countries. Therefore there is a need to increase maize production and productivity in SSA.

In Tanzania, maize is grown by 4.5 million smallholder farmers (Minot, 2010; Keya and Rubaihayo, 2013) accounting for > 90% of the total maize production (Minot, 2010; Lyimo et al., 2014, Magehema et al., 2014). Unlike paddy rice and sorghum which are grown in limited agro-ecologies, maize is produced across all 26 mainland regions of the country (Minot, 2010; Barreiro-Hurle, 2012). Westengen and Brysting (2014) reported that maize was produced on 58% of the total area allocated to cereal production in 2010. Maize growing belts in Tanzania include: Iringa, Ruvuma, and Rukwa in the Southern Highland Zone (SHZ); Tabora, Kigoma, and Kagera in the west; Manyara and Arusha in the north and Tanga and Morogoro in the east. Shinyanga and Mwanza represent the major maize producing regions in the Lake Zone. Maize accounts for >30% of the total food production and constitutes >75% of cereal consumption in the country (Seth et al., 2011; Magehema et al., 2014). According to Barreiro-Hurle (2012) maize is consumed in different

forms both in rural and urban areas but it is usually processed into flour to make the local food 'ugali'. Maize is contributing to >30% of the total gross domestic products (GDP) attributable from agricultural production. Overall, maize is a valuable crop with the greatest political will and acceptability due to its excellent share in trade and value addition in Tanzania (Barreiro-Hurle, 2012). Other important staple crops grown in the country include sorghum, millet, cassava, sweetpotato, banana, pulses (common bean and pigeon pea), rice, and wheat (Minot, 2010; Lyimo et al., 2014).

Productivity of maize in Tanzania is considerably low with mean grain yields varying from 1.2 to 1.6 t/ha (Mrutu et al., 2014; Magehema et al., 2014). However, trends of maize production have increased over the past 10 years (Manot, 2010; Rowhania et al., 2011) with varying yield levels across seasons (Figure A). Maize yield during 2000/2001 was 2000 million tons and increased to more than 2500 MTs in 2002/2003. Low yields (< 2500 MTs) were recorded in 2003/2004. After 2004 maize yields increased considerably above 3000 MTs. During 2009/2010 maize yield reduced significantly (< 300 MTs) (Figure A). The low yields of maize during 2003/2004 and 2009/2010 growing season were attributed to drought and various diseases and pests (Rowhania et al., 2011; ICID, 2011; Ahmed et al., 2012; FAOSTAT, 2013; Lyimo et al., 2014).
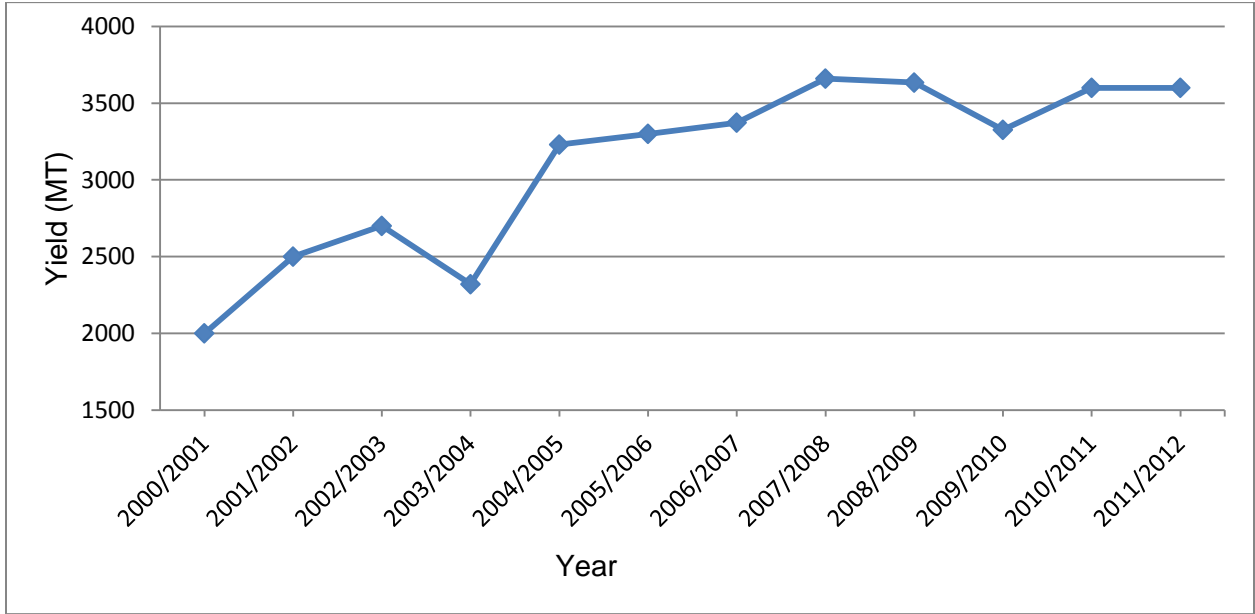
Figure A: Trends of maize production in Tanzania during 2000 to 2012 (source: FAOSTAT 2013)

The low and variable yield of maize in the country is attributed to biotic and abiotic stresses and socio-economic constraints. However, occurrence and magnitude of these factors vary among production areas and between seasons (Barreiro-Hurle, 2012). Low adoption rate of seeds of improved varieties by farmers has also been reported as a major factor for low productivity of maize in Tanzania (Ahmed et al., 2012; Lyimo et al., 2014; Magehema et al., 2014).

Among the biotic stresses, foliar diseases such as maize streak virus (MSV), maize lethal necrosis (MLN), grey leaf spot (GLS) (*Cercosporazeae maydis* Tehon & Daniel), rust (*Puccini sorgi* Schwein. and *P. polysora* Underw.) and northern corn leaf blight (*Exerohilum turcicum* Pass. Leornard & Snuggs) and ear rot (*Fusarium* and *Diplodia* spp.) inflict devastating yield losses in the country (Lyimo, 2006; Bucheyeki, 2012; Lyimo et al., 2013). Insect pests such as stem and grain borers, weevils and parasitic weeds cause significant yield losses in Tanzania. Abiotic constraints of maize production include recurrent drought, low soil fertility and salinity (Mmbaga and Lyamchai, 2001, Temu et al., 2011). The aforementioned stress factors are expected to increase due to global climate change affecting crop production and needing development of resilient crop varieties (Temu et al., 2011; Rowhania et al., 2011; Hellin et al., 2012).

Among biotic constraints, MSV is the most devastating disease of maize with yield losses reaching up to 100% on susceptible varieties. Recently maize lethal necrosis disease (MLN) becomes a menace to maize production in Tanzania, Kenya and other East African countries (Lyimo, 2006; Wangai et al., 2012; Lyimo et al., 2013; Kitenge et al., 2013; Adams et al., 2013). MLN is caused by a combined infection of sugarcane mosaic virus (SCMV) and maize chlorotic mottle virus (MCMV) (Uyemoto, 1983; Adams et al., 2013). In Tanzania, prevalence and severity of MLN is yet limited to the northern part of the country including areas of Lake Victoria to central regions in Singida, and Arusha, Manyara and Kilimanjaro in the north (Kitenge et al., 2013). Yield losses due to MLN disease are variable but reported to be low to 100% (Kitenge et al., 2013). Unlike MLN, MSV is common across the country and its epidemic occurrence, epidemiology and severity resulted significant crop damage and yield losses under many smallholder farmers' fields (Lyimo, 2006) (Figure B).

Figure B: Maize fields severely infected by MSV at Igomelo in Mbeya (1) and by MLN at Ngaramtoni in Arusha (2).

The symptoms and severity of both MSV and MLN are shown in Figure B (1 and 2). Breeding for resistance against MSV and MLN disease are essential to boost productivity and combat losses and to ensure food security both at household and national level. There are several control strategies to minimize losses caused by foliar diseases such as: use of resistant cultivars, biological control, phytosanitary measures, cultural practices such as early planting, crop rotation, mixed cropping, rouging off diseased plants and plant parts and use of insecticides to control leafhoppers which are principal vectors of MSV (Uyemoto, 1983; Ndhlela, 2012; Adams et al., 2013; Mengesha, 2013; Oppong, 2013). Development and use of resistant maize hybrids has been recognized as the cheapest, sustainable, and environmentally friendly control method of maize streak virus (Shepherd et al., 2010; Karavina et al., 2014). Good levels of resistance to MSV in high yielding commercial maize hybrid were reported (Shepherd et al., 2014). Maize germplasm with complete or partial resistance to MSV has been reported by various workers at the IITA-Nigeria, CIMMYT and South Africa.

Genetic gain for yield and stress tolerance could be realized through breeding (Aaron, 2013; Sharma et al., 2012). This is achieved through incorporation of desired attributes from chosen parents with high agronomic importance into a maize genotype via crosses and subsequent selection (Bello et al., 2012, Wilson et al., 2014). Complementary inbred lines are the most valuable germplasm for maize breeding. They carry desirable complementary genes and upon crossing they could provide hybrids with improved yield, disease resistance, and nutritional

qualities due to heterosis (Ferdous et al., 2011; Sharma et al., 2013; Aaron, 2013). Therefore, diversity assessment, genetic enhancement, inbred line development, combining ability tests, genotype x environment and stability analyses are important aspects for successful maize breeding and cultivar release.

Rationale of the study

The northern Tanzania is one of the major maize producing areas of the country. It is the second maize growing zone after the Southern Highlands. Most rural farming households dispose of their maize easily to Kenya where demand of maize is usually the highest in East Africa. However, outbreaks of MSV and MLN diseases severely curtail maize yields in northern Tanzania. The maize research program at Selian Agricultural Research Institute (SARI), located in the northern zone of Tanzania is mandated for maize research and development for the mid-altitude agro-ecologies of the country. This program has developed germplasm with broad genetic base which can be used to combat both MSV and MLN diseases through resistance breeding using conventional and molecular approaches. Maize productivity could be enhanced through effective breeding using locally adapted and introduced germplasm, having resistance genes for MSV and MLN, and agronomic attributes preferred by farmers'. This requires a well-designed hybrid cultivar development program. Therefore, development of high yielding, MSV and MLN resistant cultivars remains important for improving maize productivity and quality in Tanzania.

Research Objectives

The specific objectives of this study were to:

1) determine farmers' preferred traits of maize and production constraints limiting maize production in the northern areas of Tanzania.
2) determine agro-morphological diversity present among 80 local and introduced maize inbred lines under maize streak virus (MSV) prone environments of the northern zone of Tanzania
3) assess the genetic diversity and genetic relationship among 79 maize inbred lines collected from five different origins using 30 polymorphic simple sequences repeat (SSR) markers
4) determine combining ability and heterosis for grain yield and related traits and resistance to maize streak virus (MSV) among 10 elite maize inbred lines and their hybrid progenies
5) investigate the GXE interaction for grain yield and MSV resistance among newly developed maize hybrids in Tanzania using AMMI and GGE biplot methods

Research Hypotheses

The study was developed based on the following hypotheses:

1) Smallholder farmers in the northern Tanzania could identify their key maize production constraints which hamper successful production of maize in their areas.
2) There is abundant genetic diversity for both grain yield and resistance to MSV in the test genotypes assembled for breeding which can be detected using agro-morphological and simple sequence repeat DNA markers
3) There is high combining ability among genotypes helpful for hybrid breeding to exploit heterosis both for grain yield and disease resistance
4) There is high and stable yielding and MSV resistant new experimental hybrids that can be selected for release or furher breeding when tested across different environments in Tanznia.

Thesis Outline

This thesis consists of six distinct chapters (Table A) reflecting a number of activities related to the above-mentioned objectives. Chapters 2 to 6 are written in the form of discrete research chapters, each following the format of a stand-alone research paper. The referencing system used in the chapters of this thesis is based on the Journal of Crop Science system of referencing. This is the dominant thesis format adopted by the University of KwaZulu-Natal. As such, there is some unavoidable repetition of references and some introductory information between chapters.

Table A. Thesis structure

Chapter	Title
-	Introduction
1	A Review of the Literature
2	Key maize production constraints and identification of farmers' preferred traits in the mid-altitude maize agro-ecologies of northern Tanzania
3	Agro-morphological characterization of maize inbred lines under maize streak virus prone environment
4	Genetic diversity analysis of maize inbred lines collected from diverse origins using SSR markers
5	Combining ability and heterosis among maize genotypes for yield and yield components and resistance to maize streak virus disease
7	Genotype by environment interaction of grain yield and MSV resistance among novel maize hybrids in the mid-altitude agro-ecologies of Tanzania
8	An overview of research findings

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CHAPTER ONE:

A review of the literature

1.1 Introduction

This chapter presents a review of the literature in three different sections. The first section highlights importance of maize (*Zea mays* L.), origin and biology, production trends and constraints. The second section focuses on one of the most important production constraints that limit maize productivity in Tanzania – maize streak virus (MSV) and provides a detailed account on its economic importance, symptoms and epidemiology, distribution and control methods. The third section covers breeding maize for MSV resistance and improved grain yield, genotype by environment interaction, and the role of farmers in maize breeding and cultivar adoption.

1.2 General importance of maize

Maize is one of the most important cereal crops in the world serving as human food, feed and industrial uses such as in manufacturing corn starch and oil. Recently, maize is being widely used in the bio-fuel sector (Fischer et al., 2014; Ranum et al., 2014). The crop has wide adaptation and multiple uses (Sharma and Misra, 2011). Maize displays the highest productivity per unit area and occupies a relatively large global production area when compared to other cereals. Yields of maize in 2012 was estimated at 875 million tons (FAO, 2012; Edmeades, 2013), being higher than that of rice (690 M tons) and wheat (675 M tons). The United States, China, and Brazil are the major producers of maize accounting for 31, 24 and 8% of the world total production, respectively (FAO, 2012; Ranum et al., 2014). The global annual maize yields are two and three fold than that of rice and wheat, respectively (Edmeades, 2013; Fischer et al., 2014).

In sub-Saharan Africa (SSA), maize is the most preferred staple food crop serving over 900 million people (AGRA, 2014; Fischer et al., 2014; Ranum et al., 2014). It is produced in an area of 27 million ha representing 30% of the area under cereal production (Fischer et al., 2014; Kalinda et al., 2014) but yields remain low in SSA (Shiferaw et al., 2011; Westengen et al., 2014). In the region maize is predominantly grown by small-scale farmers under rain-fed condition with limited production inputs (Carns et al., 2013; AGRA, 2014). In SSA, the demand for maize is far beyond the level of production suggesting further production and productivity of this crop. The major factors attributing to low yields of maize in SSA include: unavailability of seeds of improved cultivars, diseases, pests, weeds, drought stress, low soil fertility, input unavailability and use, poor storage system and postharvest losses (Carns et al., 2013; Khan et al., 2014). It has been estimated that maize yield gaps in SSA are in excess of 100% across locations and farmers owing

to these constraints (Fischer et al., 2014). Therefore, use of improved cultivars and production inputs could boost maize productivity in the region.

In Tanzania, maize is the most important cereal food crop consumed by the entire population of apprximatetly 45 million people. Maize is grown across all eight agro-ecological zones (Mbirinyi et al., 2013). It occupies >2 million hectares representing 45% of the total land area allocated to annual crop production (Lyimo et al., 2014). In 2010, maize covered 58% of the total area allotted to cereal production (Westengen and Brysting, 2014) predominantly grown by smallholder farmers who contribute 85% of the total maize production (Minot, 2010; Lyimo et al., 2014). Maize accounts for 50 to 60% of the dietary food calories and proteins in Tanzania. It is an important commodity crop contributing to nearly 50% of the cash income by rural households. The crop plays an important role in the national economy contributing to close to 30% of GDP along other crops. Overall, maize is a key food security crop being maintained by the Strategic Grain Reserve (SGR) program in Tanzania to ensure sustainable food supply (Ashimogo, 2008). However, maize yields are considerably low under small-scale production systems (Minot, 2010; Magehema et al., 2014). The average national yields of maize have been fluctuating between 1.2 and 1.7 t/ha since 1990 due to a number of yield limiting factors such as diseases, drought, low soil fertility and poor adoption to improved agricultural technologies by farmers (Bucheyeki, 2012; Kathage et al., 2012; Westengen and Brysting, 2014; Mrutu et al., 2014).

1.3 Origin and biology of Maize

Maize has been classified as a monocot, determinate, and monoecious annual tall plant that belongs to the grass family Graminae or Poaceae in the tribe Maydeae with a chromosome number of 2n=2x=20 (Edward and Stevens, 2005). Maize is presumed to have been evolved from the wild grass relative, teosinte and is the only economically important species in the genus *Zea* (Edward and Stevens, 2005: Ranum et al., 2014). It is believed that maize originated from central Mexico some 7000 years ago. The rapid distribution of maize from its center of origin to other places of the world is attributed to the high level of genetic plasticity determining its wide adaptability and economic importance (Ranum et al., 2014). Maize has been a model crop and extensively studied in plant genetics, genetic engineering and plant breeding due to its considerable genetic diversity (Edward and Stevens, 2005; Prasanna, 2012).

1.4 Maize production constraints in Tanzania

The major maize production constraints in Tanzania include biotic stresses caused by maize streak virus (MSV), maize lethal necrosis (MLN) and abiotic stresses such as drought and poor soil fertility and socio-economic constraints (Mmbaga and Lyamchai, 2001; Lyimo, 2006; Mbirinyi et al., 2013; Bucheyeki, 2012; DFID, 2014). The low adoption rate of improved technologies by farmers is reported to be a major challenge to agricultural production and productivity in Tanzania and sub-Saharan Africa (Minot, 2010; Miti et al., 2011; Kathage et al., 2012; Ceccarelli, 2012; Lyimo et al., 2014). In Tanzania the adoption rate of hybrid maize seeds is estimated at 27% (Lyimo et al., 2014) while in Kenya it is > 70% (Keya and Rubaihayo, 2013). The low adoption rate of improved seeds of hybrid maize in Tanzania could be attributed to limited access, high price and poor performance of some cultivars when grown under farmers' field conditions (Bucheyeki, 2012). This has prompted farmers to use landrace varieties which are poor yielders and susceptible to various biotic and abiotic stresses (Bucheyeki, 2012). Among the biotic stress factors, maize streak virus is a number one production challenge severely limiting maize yields in Tanzania and sub-Sahara African countries (Bucheyeki, 2012; Lyimo et al., 2013; Shepherd et al., 2010; Karavina et al., 2014). Yield losses are often associated with cultivation of susceptible maize varieties or virulence shift of the virus (Shepherd et al., 2010; Karavina, 2014). This suggests the need for development of resistant cultivars for strategic control of this erratic but devastating disease of maize (Shepherd et al., 2010; Ruschhaupt et al., 2013).

1.4.1 Maize streak virus (MSV) disease
Origin, classification and mode of transmission of MSV

MSV is classified in the genus Mastrevirus of the family Geminiviridae (Tefera et al., 2011; Karavina et al., 2014; Karavina, 2014). It is naturally confined in African grasses (Owor et al., 2007). The disease is solely transmitted by leafhoppers (*Cicadulina mbila* Naude) (Oluwafemi et al., 2007; Shepherd et al., 2010). Several potential species in the genus *Cicadulina* including *C. mbila*, *C. storey*, *C. bipunctella*, *C. latens*, and *C. Parazeae* have been reported to transmit MSV disease (Oluwafemi et al., 2007). Of these vectors, *C. mbila* is the most important for transmission of MSV disease in Africa (Magenya et al., 2008, 2009; Shepherd et al., 2010).

Mechanism of MSV disease transmission

MSV disease is not transmitted mechanically, or via pollen grains or seeds. It is exclusively transmitted through its main vector, leafhoppers. Thus knowledge on the ecology and epidemiology of the disease and its vector is important. Leafhoppers readily move, feed and reproduce on most cereal crops and annual grass weeds (Antwerpent et al., 2011). Leafhoppers lay eggs at higher temperatures and during the wet season or on irrigated crops (Oluwafemi et al., 2007). The vector acquires MSV through feeding on the diseased maize plant and transmits the virus when feeding on the healthy plants. Studies have demonstrated that the virus once acquired by the leafhoppers remains in the insect's gut during the life span of the vector. MSV acquisition and transmission by the vectors is reportedly genetically inherited. Transmission of the virus has been attributed to a simple sex linked dominant gene present in the vector (Antwerpent et al., 2011). Differential transmission rate by the *Cicadulina* spp has been reported (Karavina, 2014).

Symptoms and epidemiology of MSV disease

Disease symptoms of MSV have been reported by many authors (Martin and Shepherd, 2009; Karavina, 2014). Maize streak symptoms are characterized by the development of chlorotic spots and streaks in longitudinal lines on maize leaves (Figure 1.1).The streaks on the leaves often fuse laterally, resulting in narrow broken chlorotic stripes, which extend over the entire length of the affected leaves (Mawere et al., 2006; Taiwo et al., 2006; Oluwafemi et al., 2008; Shepherd et al., 2010). The chlorosis is caused by failure of chloroplasts to develop in the tissue surrounding the vascular bundles, which results in reduced photosynthesis and increased respiration (Mawere et al., 2006). Severe chlorosis occurs in very susceptible maize cultivars, leading to stunted growth and premature death, poor ear formation, reduced seed set, and heavy yield losses (Shepherd et al., 2010).

Figure 1.1: Photos depicting the severity of infection by the maize streak virus disease at Igomelo in southern Tanzania

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The epidemic of MSV disease is often erratic and may not be predicted (Martin and Shepherd, 2009). Environmental and ecological factors favoring the vectors are the key components for the spread of the MSV disease in epidemic proportions. Presence of susceptible hosts at earliest growth stage is an important factor enhancing MSV disease epidemics (Martin and Shepherd, 2009). Further, the virulence of MSV and transmission ability of vectors is necessary conditions for disease epidemics and development. According to Martin Antwerpent et al., 2011 and Shepherd (2009), the complex biological interactions of viral strains with their multiple transmitting vectors, host species and environment are essential factors for MSV disease epidemics. In addition to maize, MSV has been reported to infect a wide range of other cultivated crop species such as wheat, oat, sugarcane, millet, rice, barley, rye and sorghum (Owor et al., 2007; Magenya et al., 2009; Shepherd et al., 2010).

Environmental factors favouring distribution of MSV and its vectors
According to Magenya et al. (2008), the distribution of leafhopper vector populations and the viral diseases they transmit are inherently influenced by agro-ecological factors. The influence of soil nutrients, altitude and temperature on the biology of maize streak virus (MSV) vector populations is discussed in previous papers (Magenya et al., 2008; Martin and Shepherd, 2009). These

environmental conditions have profound effect on the growth and survival of annual wild grass species which hosts both the disease and the vector, leafhoppers (Martin and Shepherd, 2009).

Yield loss due to MSV infection

Yield loss due to MSV infection in maize varies from 40-100% (Makenya et al., 2009; Antwerpent et al., 2011). Shepherd et al. (2010) reported that during high MSV epidemics, the disease can cause 100% yield losses. Also, extensive cultivation of susceptible maize varieties is considered to be the major cause for disease epidemics and subsequent yield loss (Oluwafemi et al., 2008). In Tanzania, yield losses due to MSV are common in areas encompassing Morogoro, Mbeya, Sumbawanga, Arusha and Manyara (Lyimo, 2006). Other authors reported total crop losses due to MSV infections (Mawere et al., 2006; Asea et al., 2009; Abalo et al., 2009; Gichuru et al., 2011). There are different options available to minimize losses incurred by MSV disease such as cultural practices, chemical control, biological control and host resistance (Pratt et al., 2003; Shepherd et al., 2010).

1.5 Integrated pest management (IPM) of MSV disease

1.5.1 Cultural method

Various cultural management controls have been suggested to minimize losses inflicted by maize streak virus disease (Oluwafemi et al., 2007; Shepherd et al., 2010). These include early planting, crop rotation and intercropping with non-host species. These are important MSV avoidance methods to reduce infection but may not be a sustainable option. During early crop stages the viral inoculum loads are to too low to cause infection (Shepherd et al., 2010). The population dynamics of the vector account for the occurrence and epidemics of maize streak, which in turn is influenced by rainfall, temperature, and availability of alternate host plants (Mawere et al., 2006). It has been studied that maize streak disease is very common in wet areas e.g. where irrigation is used for crop production. Wet environments facilitate the over-wintering of both the virus and the vectors. Previous studies reported that maize mono-cropping and the presence of wild grass species serve as hosts to the virus and vectors which facilitate the spread of MSV disease between crops (Mawere et al., 2006; Kwena, 2007). Cultural control strategies have been found to be effective when combined with the use MSV tolerant or resistant cultivars (Oluwafemi et al., 2007; Shepherd et al., 2010).

1.5.2 Chemical control method

Crop protection chemicals have been used to control various insect pests and diseases (Kwena, 2007). Insecticides such as carbofuran are reported to provide maximum MSV disease

management in maize through controlling leafhoppers (Karavina, 2014). However, use of insecticides as means of controlling MSV disease vary in their efficacy and economic feasibility (Kwena, 2007; Oluwafemi et al., 2007; Shepherd et al., 2010; Karavina, 2014). Correct timing of insecticide application, number of sprays, prevailing climatic conditions, and efficacy of the chemical group and the level of host resistance are important factors in determining the effectiveness of chemical control methods in crop plants (Kwena, 2007). Use of chemical control method in farmers' fields exposes them to health risks and can result in environmental pollution (Martin and Shepherd, 2009). Furthermore, insecticides are expensive for poor subsistence farmers and their application demands technical knowledge on time, method and rates of application (Karavina et al., 2014).

1.5.3 Biological control method

Biological control has been considered to be a viable tool where beneficial biological agents occur naturally or can be artificially developed (Kananji, 2007; Karavina, 2014) to infect or parasitize other crop pests (Kananji, 2007). For example, *Dinarmus basalis* (Rondani) was found to be a promising control agent against bruchids in beans (Schmale et al., 2002). There is little information regarding the use of biological control method to manage the MSV vector (Karavina, 2014). Therefore, further research is needed to identify natural enemies against leafhoppers, the potential vector for maize streak virus disease in cereals.

1.5.4. Host resistance

Development and use of resistant maize hybrids has been recognized as the cheapest, sustainable, and environmentally friendly control method of maize streak virus (Pratt et al., 2003; Taiwo et al., 2006; Niks et al., 2011; Sheperd et al., 2010; Karavina, 2014). Good level of resistance to MSV in high yielding commercial maize hybrid was reported (Lyimo, 2006; Karavina et al., 2014). Maize germplasm with complete or partial resistance to MSV has been reported by various workers at the IITA-Nigeria, CIMMYT and South Africa. Resistance in host plants is often associated with fleck like reactions or immunity to infection (Niks et al., 2011). MSV tolerant genetic stocks express a reduced disease development and comparatively better yield levels when compared to susceptible genotypes which displays progressive necrotic lesion with increased disease development (Kwena, 2007; Martin and Shpherd, 2009). A number of genotypes with MSV reactions ranging from fleck type to necrotic lesions have been observed in various studies under field conditions.

1.6. Breeding for MSV resistance

Source of resistance to MSV

Resistance to maize streak virus disease is an essential trait for breeding (Vivek et al., 2010; Niks et al., 2011). Different sources of resistance to MSV have been identified in maize (Mawere et al., 2006) such as inbred lines Tzi4 and CML202 at the IITA and CIMMYT, respectively (Gichuru et al., 2011; Ndhlela, 2012). Subsequently, IITA, CIMMYT and other national research systems in Africa, have produced a good number of breeding lines with varied levels of resistance to MSV ranging from highly resistant (HR), resistant (R), to moderately resistant (MR) reaction (Stevens, 2008). Among these Tzi3, Tzi4, Tzi15, and Tzi17 were the most resistant inbred lines which were released by IITA/Nigeria. The CIMMYT lines CML217-238, CML195-CML215, CML442 and the population ZM607 were resistant to maize streak virus disease (Olaoye et al., 2009; Gichuru et al., 2011; Ndhlela, 2012). In Tanzania, most disease resistant commercial maize varieties were developed and released in the early 1980s. These varieties succumbed to the MSV disease over time due to the emergence and outbreak of new strains. Therefore, new sources of resistance to MSV and other maize diseases should be identified to breed resistant maize hybrids in the country.

1.6.2 Screening for resistance to MSV disease

Both natural and artificial inoculation techniques are widely employed to screen and identify maize genotypes with disease resistance against MSV or other foliar pathogens (Leuschner and Buddenhaggen, 1980; Antwerpent et al., 2011). Natural infection is cost effective and applicable in places where MSV and other foliar diseases are prevalent (Leuschner and Buddenhaggen, 1980). Hot spot areas are important for evaluation of viral diseases like MSV which are obligate pathogens that need living vectors for transmission onto their host (Lagat et al., 2008). Preparation of viral pathogen inoculum for artificial inoculation is difficult and cannot be made from dead or diseased plant materials. Artificial inoculation for MSV infection using controlled colony of viruliferous insects is possible. However, this is a demanding and long selection process requiring establishment of mass rearing cages, catching of leafhoppers and testing for harboring and virus transmission ability, all needing a long term investment (Lagat et al., 2008). The Kenyan Agriculture Research Institute (KARI) based at Muguga has established an artificial MSV disease screening structure and various test results were reported (Lagat et al., 2008). Similar structure is being established at Selian Agricultural Research Institute (SARI) based at Arusha in Tanzania for future germplasm screening and development for MSV resistance.

MSV disease assessment

Yield loss due to MSV disease is associated with lesion development on the leaf surface of plants inhibiting photosynthesis (Wang et al., 2014). The MSV disease severity or infection rate is visually assessed (Bigirwa et al., 2003). This is commonly performed on both young and old leaves from early growth to grain filling stages (Wang et al., 2014). For MSV disease evaluation the method of Wang et al. (2014) has been adopted which was developed to assess corn leaf blight resistance. Wang et al. (2014) used a visual rating scale of 1-9 (expressed in percentage). A score of 1 indicated highly resistant (HR) reaction and denoted none to scattered lesions, covering less than 5% of the leaf area. A score of 3 indicated resistant (R) reaction and denoted by a few lesions on leaves covering 6 to 10% while 5 represented moderate resistant (MR) reaction; the plant had large, coalesced lesions on its leaves covering 11 to 30%. A score of 7 represented susceptible (S), and denoted large coalesced lesions covering 31 to 70% of the leaf area and a score of 9 implied highly susceptible (HS) reaction which denoted extensive, large coalesced lesions covering almost the entire leaf surface.

1.7 Breeding maize for grain yield and disease resistance

Genetic gain for yield and stress tolerance could be realized through breeding. These are achieved through incorporation of desired attributes from chosen parents with high agronomic importance into a maize genotype via crosses and subsequent selection (Bello et al., 2012; Aaron, 2013). Complementary inbred lines are the most valuable germplasm for maize breeding. They carry desirable complementary genes and upon crossing they could provide hybrids with improved yield, disease resistance, and nutritional qualities due to heterosis (Lamkey and Lorenz, 2014; Bello et al., 2012). Therefore, diversity assessment, genetic enhancement, inbred line development, combining ability tests, genotype x environment and stability analyses are important aspects for successful maize breeding and cultivar release.

1.7.1 Genetic diversity assessment

Development of maize hybrids with enhanced yield and stress tolerance requires novel and genetically unrelated inbred lines to exploit heterosis (Reid et al., 2012; Mengesha, 2013). Utilization of inbred lines in genetic and breeding studies requires proper knowledge of their genetic diversity and heterotic relationships (Wang et al., 2014). This necessitates efficient characterization or assessment of the genetic diversity present among breeding lines to design crosses, to assign inbred lines into heterotic groups, and to identify potential cultivars for release. Different approaches are available which have been widely used in genetic diversity assessment of

maize genotypes (Duan et al., 2007; Legesse et al., 2007). This review focused on genetic diversity analysis using agro-morphological and molecular markers.

Diversity assessment using agro-morphological traits

Genetic diversity assessment using agro-morphological traits involves extensive field evaluation of genotypes, data collection and analysis to select unique and superior genotypes. Agro-morphological traits are genetically controlled and traceable in the next generations (Mollin et al., 2013). Selection based on morphological traits allows identification of ideotypes under the existing farming practices but its efficiency is high for traits with the highest heritability. Morphological characterization is a first step in description and classification of maize germplasm (Shrestha, 2013) and remains useful in conventional breeding (Karanja et al., 2009). This method has been extensively used in identifying and grouping accessions with desirable characteristics such as earliness, disease resistance or other improved agronomic traits. However, morphological descriptors have limitations because phenotypic traits do not often express as expected due to the influence of the environment (Karanja et al., 2009; Prasanna, 2012; Reid et al., 2012).

Diversity assessment using molecular markers

Molecular characterization of genotypes is frequently used by maize breeders as an alternative method to select unique genotypes or lines for hybrid development (Mollin et al., 2013). Presently, molecular markers are becoming cost-effective and provide high throughput data because they are able to detect genetic variations at a DNA level (Mondini et al., 2009) and they are less influenced by environmental effects (Stevens, 2008). Assessment of genetic diversity is performed at molecular level using various techniques such as allozyme or DNA analysis (Flamingh et al., 2014). Different DNA markers are available for assessing genetic diversity in various crops including maize but their choice depends on the objectives and availability of resources. Some of the commonly used markers include random amplified polymorphism DNA (RAPD), simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP). The RAPD markers have been widely used in diversity analysis of maize owing to their cost effectiveness and rapid detection of polymorphism. Microsatellites or simple sequence repeats (SSRs) are among the molecular markers which have been widely used in genetic studies of maize (Legesse et al., 2007). They are composed of DNA sequence motif of 2-6 bases in length (Flamingh et al., 2014). They feature high level of reproducibility, accuracy, discrimination and polymorphism. They are also abundant, uniformly distributed, co-dominant, and rapidly produced by PCR and give outputs which are easy to interpret in a biological sense. Genetic markers are useful for assigning lines to heterotic groups and genetic finger printing

(Jambrovic et al., 2008; Mollin et al., 2013). The SNP markers are the most abundant molecular markers in the genome, and are widely dispersed throughout the genome with variable distribution among species. The SNPs are more prevalent in the non-coding regions of the genome (Mollin et al., 2013).

1.8 Mating designs and their application in maize breeding

Various mating designs are employed in maize breeding to recombine favorable traits from chosen parents and for genetic analyses (Khan et al., 2009; Nduwumuremyi et al., 2013). Overall mating designs are used to: (1) provide information on the genetic control of the character under investigation, (2) to generate breeding populations for selection and development of potential cultivars, (3) provide estimates of genetic gains and (4) provide information for evaluating the parents used in the breeding programs (Acquaah, 2012). Several mating designs are available but the choice depends mainly on breeding objectives and the amount of information needed (Kearsey and Pooni, 1996). Other factors that affect choices of mating designs include: reproduction system of the crop; types of crossing (artificial or natural), and presence of male sterility system (Acquaah, 2012; Nduwumuremyi et al., 2013). Breeders perform several crosses to induce and determine genetic variations and the gene actions involved (Nduwumuremyi et al., 2013). Breeders also perform progeny testing to identify superior parents as judged by the performance of their progeny. Often suitable maize inbred lines are selected based on combining ability effects which are responsible in controlling the trait of interest. Information on the estimates of combining ability effects and gene actions is vital for successful breeding (Panhwar et al., 2008). Analysis of data from appropriate mating and experimental designs using appropriate statistical tool can provide better estimates of information present between the parents used in the cross and their cross combinations (Acquaah, 2012).

The commonly used mating designs in maize breeding include: paired crosses or bi-parental mating design; top crosses, North Carolina design I, II and III, and diallel designs (Hallauer et al., 2010). All these mating designs have been used in maize breeding and the information generated through them varies between studies. In this review, however, only diallel mating design was discussed which is widely used in studying inheritance of traits and heterotic patterns of inbred lines in maize (Hallauer et al., 2010).

1.8.1 Diallel mating designs

A complete diallel mating design allows the selected parents to be crossed in all possible combinations generating all direct crosses, reciprocals and selfs (Schlegel, 2010). Diallel mating design utilizes both random and fixed models which reflect the actual status of the parents used. A random model involves crossing random parents which are obtained from a random population

resulting to random model analysis which estimates the general combining ability (GCA) and specific combining ability (SCA) variances. Diallel design may also involve crossing sets of parents which have fixed effects in order to estimate the GCA effects for each parent and the SCA effect for each pair of crosses as applied in the present study. In practice, however, application of the diallel design varies depending on whether all generated crosses are used plus their parents (selfs). For this reason, four types of diallel analyses have been established which include: 1) direct and reciprocal crosses and parents, 2) direct crosses and parents, 3) direct and reciprocal crosses without parents and 4) direct crosses only without parents. This review was focused on half-diallel mating design involving direct crosses (F1s) and parents only.

1.8.2 Half diallel mating design

Griffing (1956) established this design to compare the relative performance of parents against their respective progenies, especially for traits that have no maternal effects (Olfati et al., 2011). This type of analysis allows estimation of heterosis based on either mid-parents or better-parent values and has been widely used in maize breeding. With this design, fixed and random model effects are estimated straightforward. The mathematical models for analyzing combining abilities for fixed and random effects are given below following Griffing (1956) (Table 1.1):

Fixed effect model I: Method II

This method includes parents and F1's without reciprocals. The total number of genotypes used is given by $^1/_2\,\rho(\rho+1)$, where ρ is the number of parents used. Estimation of GCA and SCA for fixed model is:

$$Y_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{bc}\sum_k\sum_l \varepsilon_{ijkl}. \qquad \begin{cases} i,j = 1\ldots\rho \\ \quad k = 1\ldots b \\ \quad l = 1\ldots c \end{cases}$$

Where, μ is a population mean, g_i and $\overline{g}_j$ are general combining ability effects for ith and jth parents; s_{ij} is the specific combining ability effects of the cross between ith and jth parents such that $s_{ij} = s_{ji}$ and ε_{ijkl} is the experimental error due to environmental effect associated with ijklth. For restriction, $\sum g_i = 0$ and $\sum(S_{ij} + S_{ii}) = 0$. The mathematical equation for analysis of combining ability for random model, i.e., model II is: $Y_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{b}\sum_k b_k + \frac{1}{b}\sum_k(bv)_{ijk} + \frac{1}{bc}\sum_k\sum_l \varepsilon_{ijkl}.$

Table 1.1: Variance analysis of half diallel, Method II

Source of variation	df	SS	MS	Expected Mean Squares	
				Model I	Model II
GCA	$\rho - 1$	S_g	M_g	$\sigma^2 + (2+\rho)(\frac{1}{\rho-1})\sum g_i^2$	$\sigma^2 + \sigma_s^2 + (\rho+2)\sigma_g^2$
SCA	$\frac{1}{2}\rho(\rho-1)$	S_s	M_s	$\sigma^2 + (\frac{2}{\rho(\rho-1)})\sum_i\sum_j S_{ij}^2$	$\sigma^2 + \sigma_s^2$
Error term	m	S_e	M'_e	σ^2	σ^2

Source: Griffing (1956)

Where $S_g = \frac{1}{\rho+2}\sum_i[(X_{i.} + X_{ii})^2 - \frac{4}{\rho}X_{..}^2]$ and $S_s = \sum_{i \leq j}\sum X_{ij}^2 - \frac{1}{(\rho+2)}\sum_i(X_{i.} + X_{ii})^2 + \frac{2}{(\rho+1)(\rho+2)}X_{..}^2$ (Griffing, 1956).

1.9 Combining ability analysis

Estimates of combining ability are useful in determining breeding value of maize inbred lines and their progenies (Gichuru et al., 2011; Olfati et al., 2011; Aly et al., 2011). It has been widely used to select suitable parents in hybrid breeding programs (Machikowa et al., 2011). The concepts of general and specific combining ability were introduced by Sprague and Tatum (1942) and have been extensively applied in maize breeding. The general combining ability (GCA) is defined as the mean performance of a line when crossed to an array of other lines and the deviation of this value to the overall mean performance of all crosses is termed as GCA effects (Sprague and Tatum, 1942; Olfati et al., 2011). Whereas the specific combining ability (SCA) refers to the performance of a specific combination of inbred lines and its effects is estimated as the deviation from the mean performance of the lines involved in that particular cross (Olfati et al., 2011). In statistics GCA is the main effect of the lines while SCA is their interaction (Olfati et al., 2011). The variance of GCA measures the additive gene action whereas that of SCA measures the non-additive gene actions (Gichuru et al., 2011; Olfati et al., 2011). The relative importance of gene actions involved in the expression of the traits determines the type of breeding approaches to be adopted (Akinwale et al., 2014). The SCA effect is also important parameter that has been used to evaluate the usefulness of a cross to exploit heterosis (Sprague and Tatum, 1942; Griffing, 1956; Machikowa et al., 2011; Aly et al., 2011). Combining ability for grain yield and resistance to foliar diseases in maize have been previously reported (Gichuru et al., 2011).

1.10 Heterosis for grain yield and resistance to MSV

Plant breeders attempt to increase yields of maize through hybrid breeding to exploit heterosis or hybrid vigor (Abdel-Moneam et al., 2014). Heterosis is the biological phenomenon that has been exploited by breeders to increase crop productivity (Tuhina-Khatun et al., 2010; Thiemann et al., 2014). This biological or genetic parameter (hybrid vigor) has been known since the early 1900s and is defined as biological phenomenon in which the progeny (F1 hybrid) exhibits enhanced mean performance compared to its parents for a given trait (Ali et al., 2012; Ding et al., 2014; Thiemann et al., 2014). Three types of heterosis are known in hybrid breeding such as: mid-parent heterosis (MPH), better-parent heterosis (BPH) and heterosis calculated based on the standard check cultivar (Tuhina-Khatun et al., 2010; Ali et al., 2012; Rajesh et al., 2014; Abdel-Moneam et al., 2014).

Estimation of heterosis

The mid-parent (average) heterosis (MPH) is calculated as follows: $MPH = \frac{F_1 - MP}{MP} x100$; Where F1 is the mean performance of the F1 hybrid, and MP is the mean performance of the two parents, that is, $= \frac{P_1 + P_2}{2}$; while the better-parent heterosis (BPH) is calculated as the increase (+) or decrease (-) exhibited by the F1 hybrid over the better parent: $BPH = \frac{F_1 - BP}{BP}$; where BP is the mean performance of the better parent. The third type of heterosis is calculated as the increase or decrease of F1 hybrid compared to the standard check variety (cv); $Hcv = \frac{F_1 - CV}{CV} x\ 100$; where Hcv is heterosis calculated based on a standard check variety, cv is the mean performance of the check variety. All forms of heterosis are important in hybrid breeding systems and helpful to identify crosses with improved grain yield (Tuhina-Khatun et al., 2010). Rajesh et al. (2014) were able to identify crosses with high heterosis based on standard check.

1.11 Genotype by environment interaction and stability of grain yield and related traits and resistance to MSV disease

Changing environmental or growing conditions, expansion of maize production into new agro-ecologies, and unavailability of high yielding and stably performing maize varieties across different environments necessitate a rigorous analysis of the genotype by environment interaction (GXE). This is helpful for cultivar development and release (Delghani et al., 2009; Adu et al., 2013). Studies on GXE interaction have been conducted elsewhere in order to determine the stability in yield performance of new genotypes bred for growing in wider or specific target growing environment(s) (Hooyer, 2012; Kamutando et al., 2013). Selection of potential or superior

genotypes is not always adequate, especially when genotype by environment interaction is significant. The presence of GXE interaction frequently changes the genotype ranks in different environment making selection difficult (Beyene et al., 2011; Abuali et al., 2014). Evaluating candidate cultivars across seasons, and locations before large scale recommendation is important because the environment has great effect on the performance of the new cultivar (Beiragi et al., 2011; Arulselvi and Selvi 2010; Kamutando et al., 2013).

There are a number of statistical methods to assess the magnitude of GXE interaction (Yan et al., 2007; Bujak et al., 2014). Analysis of variance (ANOVA), stability parameters, and multivariate methods are the commonly used methods (Fan et al., 2007; Beiragi et al., 2011; Adu et al, 2013). Other approaches include the Additive Main Effect and Multiplicative Interaction (AMMI) and genotype main effect and genotype x environment interaction (GGE) biplot. The AMMI and GGE biplot analyses are widely used and considered to be powerful to estimate genotype by environment interaction and stability (Vargas and Crossa, 2000; Dagnachew et al., 2014).

AMMI analysis

The AMMI analysis partitions the effects of genotype (G) and environment (E) additive main effects and their interaction as a multiplicative interaction component separately and submits to principal component analysis for partitioning (Adu et al., 2013). The advantage of AMMI model is that the interaction can be modeled by only one or two principal component axis (Vargas and Crossa, 2000). Genotypes or environments with large interaction principal component (PC) scores (positive or negative) have high interaction while those with small scores are considered to be stable. Abuali et al. (2014) using AMMI biplot models were able to identify genotypes with large and small GXE interaction on grain yield of inbred lines and F1- hybrids in maize.

GGE biplot analysis

The genotype G and genotype x environment (GE) interaction biplot analysis provides visual interpretation of GXE interaction effects on each genotype evaluated. This model does partitioning of GGE through GGE biplot analysis into two principal components (Ezatollah et al., 2011; Tonk et al., 2011). GGE biplot has the ability to identify areas of adaptations of genotypes through its utility view of which won where pattern of multi-environmental yield trials. It is also a useful tool for visual identification of mega environments within a large target region, least discriminating and representing environments (Tonk et al., 2011; Reza and Ahmed, 2012). Grain yield performance and stability of genotypes are clearly examined by the average environment coordination (AEC) method (Yan, 2007; Tonk et al., 2011). The PC1 and PC2 determine the relative *per se* performance of genotypes and their yield stability, respectively. The longer the distance from AEC line in either direction the higher the unstability of the genotype and vice versa. GGE biplot has

also an ability to identify the ideal genotypes possessing the highest mean performance and stability (Ezatollah et al., 2011; Tonk et al., 2011; Reza and Ahmed, 2012).

1.12 The role of farmers in maize breeding

Adoption rate of high yielding and disease resistant maize varieties is low by many stallholder farmers in marginal ago-ecologies in sub-Saharan Africa (Bucheyeki, 2012; Ceccarelli, 2012). This is because the newly bred varieties are highly productive only in the favorable environments. Also growing these varieties require the use of production inputs such as fertilizers. Often the cost of improved seeds and production inputs are high and unfordable to smallholder farmers who do not have access to cash or credits (Ceccarelli, 2001; Miti et al., 2011). Consequently, these varieties were poorly accepted and adopted by farmers (Abakemal et al., 2013; Machida et al., 2014). Farmers have continued growing their landraces which are characterized by low productivity and susceptibility to disease and pests (Bucheyeki, 2012).

Participatory rural appraisal (PRA) offers rapid cost-effective strategy for developing and selecting farmer-preferred superior varieties for large scale production (Ceccarelli, 2012). PRA is among the few approaches that are usually applied to capture farmers' indigenous knowledge and has been extensively used in plant breeding (Dorward et al., 2007). PRA gives greater opportunity for conventional breeders to understand the farmers' potential constraints, perception and preferences and to include them in breeding programs which would enhance adoption rate of newly developed technologies (Thijssen et al., 2008; Kudi et al., 2011; Ceccarelli, 2012; Machida et al., 2014).

Various studies have shown that PRA is an active multi-disciplinary research approach that uses many different tools to facilitate detection and collection of farmers' preferences on particular traits in maize (Bellon, 2001; Witcombe, 2003). Through physical field visit (transect walk), Gichuru (2013) identified high incidence of MSV disease in Mwea village of Embu district in Kenya and through focused group discussions farmers in that study area were able differentiate the two commonly grown hybrids H513 and 614 based on their attributes. Preferred cultivars and traits of economic importance to farmers were also identified using focused group discussions and ranking in studies conducted by Abakemal et al. (2013) and Machida et al. (2014) in Ethiopia and Zimbabwe, respectively. PRA tools provide insights into farmers thoughts and a deeper understanding of the phenomena being studied, and have been extensively used in maize breeding (Bellon, 2001; Nkongolo et al., 2008). Matrix and pair-wise rankings are important tools in focus group discussions that aid scientists to assess and rank the relative importance of farmers' traits of economic importance, their preferences and production constraints (Bellon, 2001; Sibiya et al., 2013). A semi- structured interview is an important survey technique used to identify farmers'

ideas. It works best as a complement to other qualitative research such as focus group discussions (FGD) (Nkongolo et al., 2008). Most PRA studies have started to give positive results because farmers' views regarding development and utilization of a given maize variety is being highly considered in maize breeding (Ceccarelli et al., 2001; Ceccarelli, 2012; Machida et al., 2014). For example, in the current study, farmers' views were captured and incorporated in breeding, testing and selection of the most preferred high yielding and MSV resistant maize hybrids evaluated across six different environments.

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CHAPTER TWO:

Key maize production constraints and identification of farmers' preferred traits in the mid-altitude maize agro-ecologies of northern Tanzania

Abstract:

The objective of this study was to determine and rank farmers 'preferred traits of maize and their perceived constraints which limit maize production in the northern areas of Tanzania. The study was conducted in 2012, at 12 villages selected from Babati, Arumeru and Hai Districts. Participatory rural appraisal (PRA) and survey methods were used to collect data from 500 farmers sampled across the study areas. Of these, 180 farmers were interviewed and 320 participated in the focused group discussions (FGD). Data collected were summarized and analyzed using various analytical tools such as matrix and pair-wise ranking and SPSS program. The most preferred traits according to farmers' criteria, ranks and matrix mean scores were high yield (71.9%), disease resistance (70.0%), drought tolerance (69.9%), good grain milling quality (65.3%), grain palatability (60.7%), dense grain (59.0%) and early maturity (55.8%). Other important traits were large grain size (50.3%), intercropping suitability (49.7%), large cob size (48.5%) storage pests' resistance (48.1%) and multiple ears (39.4%). Major biotic constraints limiting maize production in the study area were maize streak virus (MSV) and cob rot diseases while the important abiotic constraints were drought and poor soil fertility. High costs of production inputs and low price of maize were also among the challenges to maize production in the study area. Knowledge of the farmers' preferences and production constraints is required by breeders to enhance the productivity of maize in the northern areas of Tanzania.

Keywords: *Farmers' traits preferences, focus group discussions, maize, PRA, survey.*

2.1 Introduction

Maize (Zea mays L.) is an important food security crop in sub-Saharan Africa (SSA) and the developing world (De Groote et al., 2013). It is produced in different parts of SSA under diverse climatic and ecological conditions owing to its widespread adoption and adaptation (Tiwari et al., 2009a, 2009b; Kudi et al., 2011; Prasanna, 2012; Ureta et al., 2013). The crop has become a major staple and cash crop for approximately three hundred millions smallholder farmers in SSA (Langyintuo and Setimela, 2009; Mbuya et al., 2011; Mather et al. 2013; Homann-Kee et al., 2013; Mathenge et al., 2014). It has also been providing about 30% of the daily calories for more than 4.5 billion people in 94 developing countries (Bolade, 2010; Ismaila et al., 2010; Oyewo, 2011). According to FAOSTAT (2007), the daily per capita consumption of maize is estimated to be 53.2g and its demand is projected to double globally by 2050 (CIMMYT and IITA, 2010). In Tanzania, maize is the primary staple food crop consumed by 42 million people (Sokoni, 2008; Msuya and Isinika, 2011; Kwayu et al., 2014). The livelihoods of the majority of farmers in the country are based largely on maize (Mateko, 2013; Kwayu et al., 2014; Lyimo et al., 2014). The crop has also been an important commodity for improving farmers' income and the national economy as a whole. It also accounts for about 30% of the total agricultural derived gross domestic product (GDP) and is sold throughout the country (Mateko, 2013; Kwayu et al., 2014). Maize is now considered to be as a focal or priority crop for speeding up agricultural development in Tanzania through the national 'Kilimo Kwanza' declaration (SAGCOT, 2011; Kwayu et al., 2014).

Despite the significant importance of maize in the SSA, its yield levels have remained low relative to the global mean of 4.5 t ha^{-1} (Joshi and Witcombe, 1996; Bellon and Reeves, 2002; Lunduka et al., 2012; Motsumi et al., 2012; Mueller et al., 2012; Cairns et al., 2013; Khonje et al., 2014; Whitfield et al., 2014). In Tanzania, the mean yields vary from 1.19 to 2.3 t ha^{-1} (Makurira et al., 2007; Magehema et al., 2014). In general, low productivity in developing countries is attributed to outbreaks of foliar diseases such as maize streak virus (MSV), grey leaf spot (GLS), and maize lethal necrotic (MLN) disease, unfavorable climatic conditions, poor or declined soil fertility, and socioeconomic constraints (e.g. low adoption to improved seed) (McGuire, 2008; Tiwari et al., 2009a; Temu et al., 2011; Lunduka et al., 2012; Sibiya et al., 2013; Khonje et al., 2014). High costs and the unavailability of production inputs reduce farmers' opportunity to use them, leading to low crop yields (Mukanga et al., 2011; Abera et al., 2013). There has also been a low adoption rate of some improved cultivars because they lack one or more of the critical traits of farmers' preference, and most perform poorly under typical farmers low input conditions (Witcombe et al., 2003; Thijssen et al., 2008; Amudavi et al., 2009; vom Brocke et al., 2010; Trouche et al., 2012; Gebretsadik et al., 2014). As a result, most of the farmers have continued using their own

landraces (Thijssen et al., 2008; van de Steeg et al., 2010) which are low yielding. Participatory will rapdly improve food security through improved adoption of farmers to newly imporved crops cultivars (Joshi et al., 2012).

Farmers should therefore be involved not only in identification of their key preferences, but also in developing, testing and selection of new crop cultivars to increase their adoption rate (Reece, 2007; Kudi et al., 2011; Ceccarelli, 2012; Trouche et al., 2012; van Herzele et al., 2013; Herrero et al., 2014). The use of formal participatory research appraisal (PRA) can facilitate detection and collection of farmers' information for research (Reece, 2007; Rusinamhodzi et al., 2012), preferably when different tools such as semi structured survey and FGD are used in combination (Witcombe et al., 2003; Ceccarelli, 2012). Participatory research appraisal is an active multi-disciplinary research approach that uses a wide range of techniques or tools such as matrix and pairwise ranking, focus group discussions, transect walks, seasonal calendars and historical times to extract information from farmers (Joshi et al., 1996; Bellon, 2001; Witcombe et al., 2003; Bellon and Hellin, 2011). This approach is powerful in data collection and flexible because it can be done in parallel with other survey techniques such as semi-structured interviews to determine the farmers' views regarding the use of a particular technology or product (Khan et al., 2008; De Groote et al., 2010; Herrero et al., 2014). A focused group discussions is a form of interactive qualitative research in which a group of people are asked about their perceptions, opinions, beliefs, and attitudes towards a product, service, concept, advertisement, or idea. The tool provides insights into farmers thoughts and a deeper understanding of the phenomena being studied, and has been extensively used in maize breeding (Bellon, 2001; Bellon and Reeves, 2002; Nkongolo et al., 2008; Gebretsadik et al., 2014; Whitfield et al., 2014).

Matrix and pairwise rankings are important tools in focus group discussions that aid scientists to assess and rank the relative importance of farmers' traits of economic importance, their preferences and production constraints. The tools can produce sound results if they are used in combination with some techniques, e.g., triangulation or probing (Amudavi et al., 2009; Bellon, 2001; Bellon and Reeves, 2002; Sibiya et al., 2013). A semi- structured interview is an important survey technique used to identify farmers' ideas. It works best as a complement to other qualitative research such as focus group discussions (FGD) (Bellon and Reeves, 2002; Nkongolo et al., 2008; Trouche et al., 2011).

Therefore, the objective of this study was to determine farmers' preferred traits of maize and production constraints limiting maize production in the northern areas of Tanzania.

2.2 Materials and Methods

2.2.1 Description of study areas

The study was conducted in three selected districts: Babati, Arumeru and Hai of northern Tanzania during 2012. The study sites represent the major maize producing agro-ecologies of the Manyara, Arusha and Kilimanajaro regions of northern Tanzania (Figure 2.1). The sites were selected based on the relative importance of maize in the livelihoods of smallholder farmers, and the prevalence of major maize diseases such as MSV and other constraints. The sites also host diverse farmers with various ethnic backgrounds, socio-economic circumstances and farming systems, making them suitable for conducting a regionally representative PRA study.
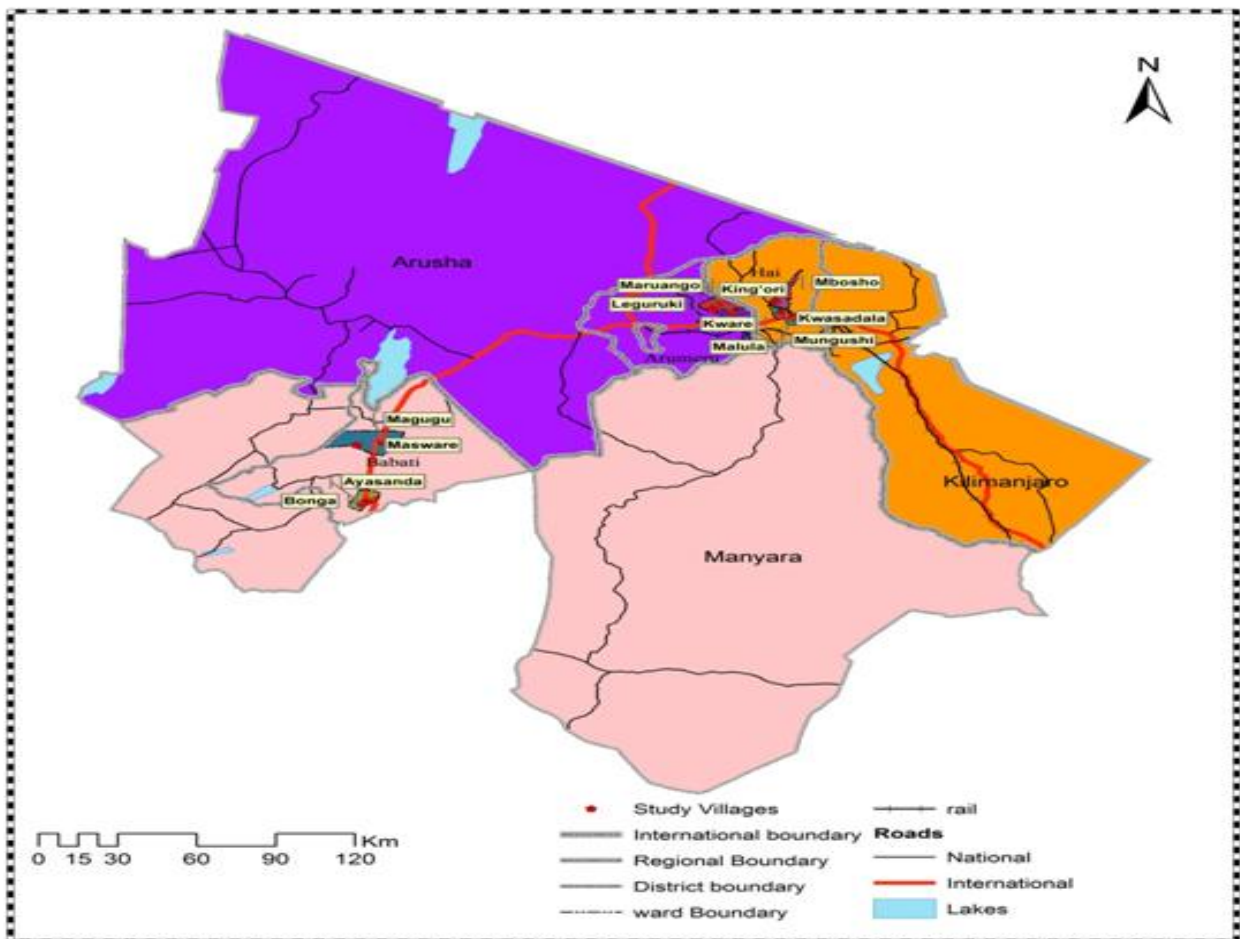
Figure 2.1: Map of northern Tanzania showing the study areas

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The soils of the study area are variable with volcanic ash soils at the high altitude volcanic plateaus and clay soils on the slopes. Most of the soils are fertile and farmers grow a range of food crops including coffee, banana, sweet potato, sesame, sunflower, beans, tea, vegetables, flowers, wheat, barley, sugarcane, maize, pigeon pea and sisal.

The study areas receive bimodal rains, which vary in intensity with altitudes. For example, in the Babati District, rainfall varies from 500 in the lowlands to 1200 mm year^{-1} in the highlands. Daily mean temperatures range from 22 to 25^{0}C. In the study areas, the short rainy season is from November to December, while the long season is from February to May. The short rains are adequate to give good crop harvests, especially on the slopes of mountains. The lowlands of northern Tanzania receive unreliable and poorly distributed rainfall and at times they are not suitable for crop production (Tanzania Metrological Agency, 2008).

2.2.2 Sampling

A multistage sampling procedure was used to identify the study sites. Three different administrative districts were selected (Table 2.1). Two wards were chosen per district to give a total of six wards, namely: Magugu and Ayasanda in the Babati District; King'ori and Leguruki in the Arumeru District and Masama Kusini and Masama Magharibi in the Hai District. From each ward, two villages were selected, providing a total of 12 villages for the study (Table 2.1). These villages include: Magugu, Masware, Ayasanda, Bonga, King'ori, Malula, Leguruki, Maruango, Kwasadala, Mungushi, Kware and Mbocho (Table 2.1). The study sites and corresponding altitude, geographical coordinate and population are summarized in Table 2.1. For semi-structured interviews, 15 female and male farmers were sampled per village, providing a total of 180 respondents (Table 2.2). For group discussions 320 famers (109 female and 211 male) participated, after sampling representative farmers across the twelve villages (Table 2.2).

Table 2.1 Districts, wards and villages selected for the study with corresponding altitude, geographical coordinates and population in northern Tanzania.

District	Ward	Village	Altitude (m)	Coordinates	Population
Babati	Magugu	Magugu	1177	$04^0$00'040''S and $035^0$46'120''E	32774
		Masware	1041	04^0 08'603''S and $035^0$95'974''E	
	Ayasanda	Ayasanda	1400	04^0 22'060''S and $035^0$43'817''E	12429
		Bonga	1433	04^0 62'060''S and $035^0$43'807''E	
Arumeru	King'ori	King'ori	1130	3^0 14'552''S and $036^0$77' 810''E	23280
		Malula	940	03^0 35'662''S and $037^0$01'136''E	
	Leguruki	Leguruki	1328	$03^0$15'422''S and $036^0$57'275''E	17637
		Maruango	1343	$03^0$14'667''S and $036^0$57'108''E	
Hai	Masama Kusini	Kwasadala	1018	$03^0$21'458''S and $037^0$19'846''E	13572
		Mungushi	1005	$03^0$17'908''S and 037^0 07'570''E	
	Masama Magharibi	Kware	1025	03^0 22'786''S and 036^0 39'352'E	13084
		Mbosho	1208	03^0 11'622''S and 036^0 58'417''E	

Source: The United Republic of Tanzania (URT) National Bureau of Statistics (2013)

Table 2.2 Farmers interviewed and participated during focus group discussions in three districts and corresponding wards and villages in northern Tanzania.

District	Ward	Village	Female	Male	Total
Survey respondents					
Babati	Magugu	Magugu	5	10	15
		Masware	9	6	15
	Ayasanda	Ayasanda	4	11	15
		Bonga	7	8	15
Arumeru	King'ori	King'ori	4	11	15
		Malula	7	8	15
	Leguruki	Leguruki	5	10	15
		Maruango	7	8	15
Hai	Masama Kusini	Kwasadala	4	11	15
		Mungushi	7	8	15
	Masama Magharibi	Kware	5	10	15
		Mbosho	3	12	15
Total			67	113	180
Group discussions					
Babati	Magugu	Magugu	11	15	26
		Masware	9	16	25
	Ayasanda	Ayasanda	4	23	27
		Bonga	7	20	27
Arumeru	King'ori	King'ori	9	18	27
		Malula	12	15	27
	Leguruki	Leguruki	9	18	27
		Maruango	10	17	27
Hai	Masama Kusini	Kwasadala	12	15	27
		Mungushi	9	18	27
	Masama Magharibi	Kware	10	17	27
		Mbosho	7	19	26
Total			109	211	320

2.2.3 Data collection and analysis

Data sources

Both primary and secondary data were collected. However, the primary data formed the core data used in this study. Primary data was collected through interview questionnaires of male and female farmers, key informants and focus group discussions. The questionnaires were developed and refined to suit collection of relevant information from target farmers. Four enumerators were selected from a government socio-economic and farming systems research unit, and trained on data collection from farmers. Data collected included farm size, mean yields of maize harvested by farmers, incidence and severity of maize diseases and insect pests, household characteristics and other important limitations or factors affecting maize production in their locality.

To understand about potential constraints to maize production and farmers' preferred maize traits, various PRA tools were used including focused group discussions (FGD), transect walks, matrix scoring and pair-wise ranking. Farmers listed the maize varieties they grew and constraints to maize production, and ranked these constraints according to their relative importance. The facilitators used pictures and cards that had drawings representing various maize traits to assist farmers during discussions and in drawing conclusions. They also used checklists to stimulate and guide discussions among farmer groups. Gender balance was taken into account, especially during focus group discussions. Transect walks were used to collect information about the physical and biological characteristics of the study area; a group of six energetic men and women farmers were involved. During discussions, farmers were encouraged to express their opinions, using their own languages. The Agricultural and Livestock Development Officers (DALDOs), village extension workers and local leaders played a major role in conducting this study. The information collected from farmers was then enhanced by the contributions of the key informants who were assumed to have knowledge about the people and problems affecting maize productivity. The key informants included maize researchers, experienced farmers in the villages, local leaders and agricultural agents.

A matrix scoring was done and participating farmers placed their criteria. Each criterion was scored using scores of 1 to 8 to rank their importance traits: where 1 = worse, 2 = very poor, 3 = poor, 4 = average, 5 = satisfactory, 6 = good, 7 = and 8 = excellent.

Data analysis

Data collected were subjected to analysis using the SPSS computer package (SPSS, 2009). Relationships were explored through frequencies, descriptive statistics and analysis of variance (ANOVA) for data collected in each village followed by mean comparisons between villages.

2.3 Results

2.3.1 Administered survey

Household characteristics in the study areas

Table 2.3 summarizes the household characteristics of the study areas. Of the total number of farmers interviewed in the study area, 62.8% were male while 37.2% were female. Farmers aged between 31 and 50 years accounted for 56.7% in the Babati District, 70% in the Arumeru District and 61.7% in the Hai District. Farmers aged less or equal to 30 years and above 50 years varied significantly across the three districts. For example, 40% of farmers were below 30 years in Babati, 18.3% in Arumeru and 8.3% in Hai, while farmers aged 51 years of age and above accounted for 30% in Hai and ranged between 3 to 11.7% in the other two districts. Male farmers who were also heads of households accounted for 95% in Babati, 98.3% in Arumeru and 96.7% in Hai districts while female household heads ranged between 1.7 to 5% in all districts (Table 2.3). Family size of households ≥ 4 accounted to 70.0% in Babati, 65.0% Arumeru and 68.3% in Hai. Results on education background indicated that 76.7, 78.3 and 51.7% of farmers interviewed had primary education in Babati, Arumru and Hai, respectively. Farmers who reached secondary and tertiary education were at 25 and 20% in Hai, respectively and were comparably higher than in Arumeru and Babati. On average farmers who did not have any formal education accounted for 3.9% in all the districts (Table 2.3).

Table 2.3 Household characteristics of respondent farmers in three districts of northern Tanzania

Variable	District			Mean (%)
	Babati (N=60) (%)	Arumeru (N=60) (%)	Hai (N=60) (%)	
Gender				
Male	55	75	58.3	62.8
Female	45	25	41.7	37.2
Total	100	100	100	100
Age				
≤30 years	40	18.3	8.3	22.2
31-50	56.7	70	61.7	62.8
≥ 51 years	3.3	11.7	30	15
Total	100	100	100	100
Household head				
Male	95	98.3	96.7	96.7
Female	5	1.7	3.3	3.3
Total	100	100	100	100
Family size				
One member	1.6	3.3	0	1.6
Two members	6.7	8.3	11.7	8.9
Three members	21.7	23.4	20	21.7
≤ 4 members	70	65	68.3	67.8
Total	100	100	100	100
Formal education level				
Primary	76.7	78.3	51.7	68.9
Secondary	15	13.3	25	17.8
College	5	3.3	20	9.4
No education	3.3	5	3.3.	3.9
Total	100	100	100	100

2.3.2 Farming system

Seed source, area of maize production and utilization

The sources of maize seed for farmers in the study areas are presented in Table 2.4. About 29.4% of farmers obtained maize seed from their own fields, 27.2% from agro dealers and 26.7% from markets, while 4 to 8% acquired seeds from private seed companies and public research institutions. Maize was cultivated on farms ranging in size from 1 to 10 ha. The data showed that about 72.8% of farmers grew maize on small plots of land ranging in size between 1.5 and 3 ha. Only 3.3% of farmers grew maize on more than 10 ha (Table 2.4). In terms of production and utilization of maize in the study area, 76% of farmers produced 1.1 to 3 t ha^{-1} of maize. Levels of maize production ranged from 1 to 10 tons per hectare. About 75% of maize produced was directly consumed as food, while 21.1% was sold and approximately 4% of maize produced was used as feed for animals (Table 2.4).

Table 2.4 Source of maize seed, production area, yield and uses of produce in three districts of northern Tanzania.

Variable	Percent
Source of maize seed	
Farmers' own field	29.4
Private seed companies	4.4
Local market	27.2
Agro-dealers	26.7
Public research institutions	8.3
Total	100.0
Farm size (in ha) used for maize production	
≤1	10.6
1.5-3	72.8
3.5 -10	13.3
>10	3.3
Total	100.0
Maize production (in tones)	
≤ 1	6.7
1.1-3	76.1
3.1 -10	14.4
>10	2.8
Total	100.0
Use of maize	
Household food	76.1
For sale to earn cash	20.0
Feed for animals	3.9
Total	100.0

Production inputs and markets for maize

Table 2.5 summarizes the perceptions of farmers on the cost of inputs used in maize production and the availability of markets for their maize produced. About 67.8% of the interviewed farmers reported that the fertilizers were too expensive to use in maize production. Only 32.2% of farmers purchased fertilizers and used them in maize production (Table 2.5). Farmers in the study area reported various markets where they sold their maize. About 19.4% of the interviewed farmers reported that there was an established formal market for selling of their maize, while roughly 40% sold maize at local markets or sold their maize to buyers directly from the farm (Table 2.5).

Table 2.5 Cost of fertilizer and markets for maize produced in the three districts of northern Tanzania

Variable	Percent
Affordability of fertilizer for maize production	
Too expensive to use	67.8
Affordable	32.2
Total	100.0
Markets of maize grain produced	
Established formal markets	19.4
Local markets	40.6
Direct sales from the farm	40.0
Total	100.0

2.3.3 Focus group discussions (FGD)

2.3.4 Major crops grown in the study area

Through pair-wise ranking scores farmers with researchers identified the major crops grown in the study area (Table 2.6). Maize scored the highest (7.9) mean value across all the districts, followed by common bean (7.3), pigeon pea (6.5), sunflower (6.3) and sweet potato (5.7) (Table 2.6). Overall scores ranged from 1 to 5 based on their perceived importance. Ranks for all crops varied significantly between locations, however, maize was ranked first throughout the areas of study. Pigeon pea and sunflower equally ranked 4th and 3rd in the Babati and Hai Districts respectively, but they were also respectively second and fourth in the Arumeru District (Table 2.6). Some crops like rice, banana, coffee, sugarcane, sorghum and cotton were limited to one or two districts only (Table 2.6).

Table 2.6 Pair-wise ranking of major crops grown in the study areas using 42 focus group discussions across the three districts of northern Tanzania

District											
	Babati (N=11)			Arumeru (N=19)			Hai (N=12)			Overall	Overall
Crop	Mean	SD	Rank	Mean	SD	Rank	Mean	SD	Rank	Mean	Rank
Maize	8.0	0.0	1	8.0	0.0	1	7.8	0.0	1	7.9	1
Common bean	7.8	1.5	2	7.0	1.2	3	7.3	0.5	2	7.3	2
Pigeon pea	7.0	2.9	4	7.3	0.6	2	5.3	0.5	4	6.5	3
Sunflower	7.3	1.0	3	6.0	1.2	4	5.5	1.0	3	6.3	4
Sweet potato	6.3	1.3	7	5.5	0.6	5	5.3	1.2	4	5.7	5
Vegetable	5.5	1.5	9	3.8	-	6	4.5	4.6	6	4.6	6
Sesame	6.1	0.5	8	2.8	1.2	9	-	-	14	2.9	7
Ground nut	4.8	0.0	13	-	5.2	11	2.5	-	7	2.4	8
Banana	-	-	14	3.3	4.0	8	2.3	5.3	8	1.8	9
Cassava	5.1	2.6	10	-	3.5	12	-	-	15	1.7	10
Rice	6.6	6.3	5	-	0.0	10	0.5	4.1	9	1.3	11
Coffee	-	-	15	3.5	4.0	7	0.5	5.8	9	1.3	12
Sorghum	3.1	2.6	13	-	0.0	15	-	-	11	1.0	13
Cotton	5.1	4.9	10	-	-	14	-	-	12	0.9	14
Sugarcane	4.6	3.8	12	-	-	13	-	-	13	0.8	15
Mean	5.3			5.2			4.1				

With ANOVA	F-value									10.58	
	P-value									<0.001	
	LSD (0.05)									2.031	
	CV (%)									18.1	

SD = standard deviation

2.3.5 Maize varieties grown in the study area

Farmers grow a range of maize varieties across the different districts of Babati, Arumeru and Hai. The varieties differed significantly ($P \leq 0.001$) across the locations (Table 2.7). Farmers grew the hybrids, open pollinated varieties (OPVs) and landraces. However, maize hybrids were planted by 69.2% compared to the OPVs (23.1%) and landraces (7.7%). The hybrid 'Pannar 4M-19' was grown throughout the three districts and had an overall mean score of 9.3 followed by the PHB 3253 (8.4), Kitale 513 (7.4) and DK8031 (7.4). Other prominent hybrids in the study areas were SC 627 (6.8) and SC 407 (6.7) (Table 2.7). Situka, Kilima and TMV1 were the open pollinated varieties (OPVs) of maize, which had overall, mean scores of 5.3, 3.9 and 2.3 respectively. Kienyeji (landrace) scored nearly as high as SC 407 with mean score of 7.0 in the Babati; therefore it was a prominent variety in this district.

Table 2.7 List of maize varieties grown in the study areas according to 44 farmers who participated in focus in focus group discussions (FGD) across three districts of northern Tanzania

Variety	Type	District						Overall mean
		Babati (N=14)		Arumeru (N=13)		Hai (N=17)		
		Mean	SD	Mean	SD	Mean	SD	
Pannar 4M-19	Hybrid	10.0	2.0	8.8	1.3	9.3	2.1	9.3
PH 3253	Hybrid	8.5	2.1	9.3	2.4	7.5	1.9	8.4
DK 8031	Hybrid	9.3	2.6	6.5	2.5	6.5	2.5	7.4
Kitale 513	Hybrid	9.0	0.0	6.7	0.6	6.7	2.3	7.4
SC 627	Hybrid	6.0	0.8	6.5	3.1	7.8	1.3	6.8
SC 407	Hybrid	7.5	1.9	7.0	1.6	5.5	1.3	6.7
Situka	OPV	4.5	4.0	6.5	6.4	5.0	2.6	5.3
DK 8053	Hybrid	6.3	4.0	5.5	1.7	3.3	1.5	5.0
Kienyeji	Landrace	7.0	1.4	2.7	1.5	3.0	0.0	4.2
SC 403	Hybrid	5.5	1.9	4.0	5.2	2.5	1.3	4.0
Kilima	OPV	3.8	1.0	3.3	0.5	4.8	2.2	3.9
SC 513	Hybrid	4.0	3.6	4.3	2.9	2.0	1.4	3.4
TMV1	OPV	3.3	1.0	3.0	0.0	0.7	0.6	2.3
Mean		6.50		5.69		4.95		
With ANOVA:		F-value			18.08			
		P-value			<0.001			
		LSD (0.05)			1.62			
		CV (%)			22.5			

OPV=open pollinated variety; SD = standard deviation

2.3.6 Farmers-preferred traits of maize

Matrix score results for farmers-preferred traits of maize are presented in Table 2.8. Highest mean scores were given to high yield (71.9%), disease resistance (70.0%), and drought tolerance (69.9%) traits. Farmers also gave first, second and third overall rank scores to these traits respectively (Table 2.8). About 50% of farmers preferred traits studied ranked differently within and between the three districts. Grain palatability for example, was ranked 10th in the Babati, 4th and 5th in the Arumeru and Hai, respectively. The rest 50% of traits of economic importance to farmers ranked equally between the two districts or locations. For instance, grain milling quality ranked 4th in Babati and Hai districts while early maturity ranked 7th in the Arumeru and Hai. Drought tolerance, large cob size and multiple ears had the same ranks in the Babati and Arumeru (Table 2.8). Other identified traits were dense grain (59.0%), early maturity (55.8%), large grain size (50.3%) suitability for intercropping (49.7%), large cob size (48.5%), resistance to storage pests (48.1) and multiple ears per plant (39.4%) (Table 2.8). Standard deviations (SD) for farmers-preferred traits scores varied significantly within and between districts. They ranged from 4 to 21.3 in the Babati, 5 to 12.3 in the Arumeru and 4 to 15.1 in the Hai. Small standard deviations of 4.4, 5 and 4 were recorded to resistance to storage pests alone, while relatively large SD values of 21.3, 12.3 and 15.1 were respectively recorded to disease resistance, large cob size and intercropping suitability (Table 2.8).

Table 2.8 Average matrix ranking scores of farmers-preferred maize traits according to 90 farmers who participated in the focus group discussions (FGD) across the three districts of northern Tanzania

Preferred traits	District									Overall mean (%)	Overall rank
	Babati (N=27)			Arumeru (N=34)			Hai (N=29)				
	Mean %)	SD	Rank	Mean (%)	SD	Rank	Mean (%)	SD	Rank		
High yield	72.8	4.5	1	65.5	6.7	2	77.5	10.5	1	71.9	1
Disease resistance	68.5	21.3	2	67.0	7.9	1	74.5	9.7	3	70.0	2
Drought tolerance	67.7	16.1	3	64.5	5.4	3	77.5	7.2	1	69.9	3
Good milling quality	64.3	16.6	4	60.3	8.9	6	71.5	10.1	4	65.3	4
Grain palatability	51.3	7.8	10	63.8	5.9	4	67.0	6.2	5	60.7	5
Dense grain	53	16.2	8	62.5	6.2	5	61.5	5.7	6	59.0	6
Early maturity	58.5	12.5	5	52.0	6.4	7	57.0	11.8	7	55.8	7
Large grain size	56.3	8.8	6	46.5	11.2	8	48.0	3.4	9	50.3	8
Intercropping suitability	55.8	11.9	7	46.0	6.4	9	47.3	15.1	11	49.7	9
Large cob size	47.8	7.5	11	43.8	12.3	11	54.0	6.2	8	48.5	10
Storage pests resistance	52.5	4.4	9	44.8	5.0	10	47.0	4.0	12	48.1	11
Multiple ears	43.8	6.1	12	26.5	8.1	12	48.0	10.1	10	39.4	12
Mean	57.7			53.2			60.9				
With ANOVA:	F-value									13.47	
	P-value									<0.001	
	LSD (0.05)									9.37	
	CV (%)									7.8	

OPV=open pollinated variety; SD=standard deviation, CV = coefficient of Variation; LSD = Least significance difference

2.3.7 Biotic and abiotic constraints of maize production in Babati, Arumeru and Hai districts

Tables 2.9 and 2.10 present the pair-wise ranking on biotic and abiotic constraints affecting maize production in the study area. Cob rot diseases scored the highest within and between locations. It scored 4.3 in the Babati and 5.5 in the Arumeru and Hai Districts (Table 2.9). MSV ranked second after cob rot diseases with overall mean score of 4.5. Common rust and stalk borer scored slightly the same with overall mean scores of 3.7 and 3.6 respectively. In addition, ranks for many constraints considered varied slightly between locations. However, stalk borer was ranked throughout with 5th position (Table 2.9). The highly ranked abiotic constraints to maize production identified were drought (4.5), high cost of maize production inputs (4.3) and lack of improved cultivars of farmers' preference (3.8) (Table 2.10). Overall, mean scores for other reported abiotic constraints were as follows: poor soil fertility (3.0) low price of maize (2.9), low market access (1.2) and poor storage facilities (1.1) and (Table 2.10).

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Table 2.9 Ranks for major biotic constraints affecting maize production, as per 66 farmers who participated in focus group discussions (FGD) in the Babati, Arumeru and Hai Districts

Biotic constraints	District									Overall Rank	Overall Mean
	Babati (N=24)			Arumeru (N=19)			Hai (N=23)				
	Mean	SD	Rank	Mean	SD	Rank	Mean	SD	Rank		
Cob rot	4.3	1.3	2	5.5	2.2	1	5.5	1.0	2	5.1	1
Maize streak virus	5.5	0.8	1	3.4	2.5	3	5.3	1.0	1	4.5	2
Common rust	3.0	1.6	5	3.3	0.6	4	4.8	1.3	3	3.7	3
Stalk borer	3.8	1.3	4	2.5	1.4	6	4.5	0.8	4	3.6	4
Leaf blight	3.0	1.0	5	3.2	1.5	5	3.0	0.8	5	3.2	5
Grain borer	4.0	1.0	3	3.5	1.5	2	2.0	1.3	6	3.1	6
Grey leaf spot	2.8	1.3	7	1.5	2.1	8	1.5	1.3	7	1.9	7
Head smut	1.0	1.0	8	2.8	1.3	7	1.5	0.8	8	1.8	8
Mean	3.4			3			3.5				
With ANOVA:	F-value										7.32
	P-value										<0.001
	LSD (0.05)										1.161
	CV (%)										10.5

SD=standard deviation; CV = coefficient of Variation; LSD = Least significance difference

Table 2.10 Ranks for important abiotic and socio-economic constraints affecting maize production reported by 42 farmers during group discussions (FGD) in the Babati, Arumeru and Hai Districts

| Constraints | District | | | | | | | | | Overall Mean | Overall Rank |
| | Babati (N=11) | | | Arumeru (N=17) | | | Hai (N=14) | | | | |
	Mean	SD	Rank	Mean	SD	Rank	Mean	SD	Rank		
Drought	4.3	1.0	2	4.8	1.5	1	5.3	1.0	1	4.5	1
High cost of agro-inputs	4.3	1.0	1	4.5	1.3	2	4.3	0.5	2	4.3	2
Lack of improved cultivars	3.8	1.0	4	4.0	0.8	3	3.5	0.6	3	3.8	3
Poor soil fertility	4.3	0.5	3	1.8	1.0	6	3.0	1.4	4	3.0	4
Low price of maize	2.3	0.5	5	4.0	1.6	4	2.5	0.6	6	2.9	5
Low market access	1.0	0.8	7	2.0	0.8	5	0.5	1.0	7	1.2	6
Poor storage facilities	1.3	1.3	6	0.0	0.0	7	2.0	1.4	5	1.1	7
Mean	3.0			3.0			3.0				
With ANOVA:	F-value									23.5	
	P-value									<0.001	
	LSD(0.05)									1.31	
	CV (%)									6.2	

SD=standard deviation; CV = coefficient of Variation; LSD = Least significance difference

2.4 Discussion

Maize production in the northern areas of Tanzania is dominated by smallholder farmers (SHFs). Farmers identified maize as one of the major crops for their food security, income and livelihoods improvement. The high percent of male farmers (62.8%) compared to female farmers (37.2%) who are engaged in maize production (Table 2.3) reflects the high commercial values of maize in the study areas. In Africa, men tend to grow crops which are considered profitable and women grow other food crops that are less profitable but useful for home consumption (Kaaria et al., 2007). About 76% of maize produced in the study area is consumed while 20% is sold (Table 2.4). Findings like these were also reported by Mpogole et al. (2013) who conducted a study on the importance of maize to the SHFs in Tanzania. Ahmed et al. (2011) also reported that Tanzania earned a sizable income from exporting about five million tons of maize to other countries; thereby reflecting the importance of maize at a national level.

The use of matrix and pair wise ranking tools during group discussions (FGD) aided identification of most farmers–preferred traits, the predominately grown maize varieties and production constraints in the northern areas of Tanzania (Tables 2.7, 2.8 and 2.9). Farmers identified high grain yield, disease resistances, good grain milling quality and drought tolerance to be the most important traits in the study areas. These farmers-preferred traits were ranked number one, two and three respectively (Table 2.8). Results showed significant variation in ranks within and between locations for some traits under investigations, this indicates that the farmers' perceptions vary among themselves within and between locations. According to Abera et al. (2013), maize traits of preferences to farmers influence the direction of breeding research and have been widely used in cultivar development and selection. Temu et al. (2011) evaluated maize producing households in the Manyoni and Chamwino Districts in Tanzania, and found that high yield potential, disease resistance and drought tolerance were the most important traits for the farmers.

Farmers reported that cob rot and MSV diseases are important biotic constraints to maize production in the northern areas of Tanzania. High incidence of this disease is a result of many factors including environmental conditions which led to the increase of ear feeding insects and prolification of fungal and bacterial diseases. On the other hand, there has been no critical reasearch which has been done focusing on ear rot diseases therefore most of the released varieties might have been selected without taking much consideration on this disease. Whereas drought, high cost and limited supply of agricultural inputs (seed and fertilizer) were perceived as important abiotic constraints limiting maize production in the study area (Table 2.9). Several

PRA studies have reported similar constraints to maize production (Bamire et al., 2010; Onuk et al., 2010; Temu et al., 2011). This study suggests that to improve maize productivity in the study areas, farmers have to use maize varieties with improved resistance for MSV disease, insect pests (stalk borer in particular) and drought stresses.

High cost of maize production inputs and a limited supply of seed of improved varieties and fertilizers are potential barriers which restrict farmers' opportunities to use these important production inputs (Abera et al., 2013). Most farmers in developing countries face financial constraints that stopping them from buying seed of improved varieties and fertilizers for crop production (Miti et al., 2011). Key informants who participated in this study also added that some farmers face shortage of food in their stores especially during the cropping season. Hence, they spend their remained cash to purchase food rather than to buy fertilizers. To increase the usage of fertilizers and improved varieties by at least 20%, the Government of Tanzania has to review its policy on input supply and distribution, and to look at the possibility of subsidizing. It also has to consider the establishment of farmers' financial credit services to empower them with enough cash to access the inputs for increased their maize productivity and livelihoods. According to Druilhe and Barreiro-Hurle (2012), subsidization of agricultural inputs is one of the effective ways for improving agricultural productivity through increased farmers' access to fertilizer and other necessary inputs. Minot (2009) who conducted a study on fertilizer use in Tanzania and indicated that 63% of farmers could not use fertilizer due to the fact that the price of fertilizers was too costly and unaffordable while 20% respondents said that fertilizers were not available.

Despite the good number of farmers involved in the PRA study and their response on various issues petaining maize production, most of them failed to give the actual yield estimates from their fields because they account only the final crop yield harvested and not considering the maize easten as a green cob. This is a challenge that needs to be considered when conducting survey with farmers. Another challenge was the fact that female farmers could not talk with full freedom in presence of their husbands especially in some villages of Hai and Arumeru districts.

2.5 Conclusions

The current study identified the most important farmers-preferred traits and constraints that limit maize production in the study areas. Farmers reported that high grain yield, disease resistances, good grain milling quality and drought tolerance are the most preferred traits for maize in the

northern areas of Tanzania. Farmers' preferences play a role in the adoption process of new products or technologies and have been widely studied elsewhere. To enhance maize productivity, farmers-preferences need to be integrated from the initial stages of breeding and technology development for successful adoption by end-users. Both cob rot and maize streak virus (MSV) diseases were considered to be the most important biotic constraints to maize production in the study. About 67.8% of farmer respondents perceived that both fertilizers and improved seed were too expensive. Other constraints to maize production were infestation of stalk and grain borers, recurrent drought, and poor soil fertility. Knowing farmers' preferences and production constraints identified in the study area will be useful to maize breeders to enhance the productivity of maize in the northern areas of Tanzania.

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CHAPTER THREE:

Agro-morphological characterization of maize inbred lines under maize streak virus prone environment

Abstract:

Genetic diversity is one of the important success factors in crop breeding programs. The objective of this study was to determine the genetic diversity present among 80 maize inbred lines using ago-morphological traits to select promising parents for breeding. Field experiment was established during 2011/2012 at maize streak virus (MSV) prone environment of Ngaramtoni Research Farm of Selian Agricultural Research Institute in northern Tanzania using a 10 x 8 alpha lattice design with two replications. Qualitative and quantitative data on agro-morphological characters and MSV reaction were collected and analysed. Analyses of variance on seven quantitative traits revealed highly significant (P ≤ 0.001) variations among inbred lines. TL2012-42 and TLl2012-41 were identified as superior lines with grain yields of 3.52 and 2.46 t/ha respectively. These genotypes showed respectively low (21.80% and 26.20 %) level of MSV reaction suggesting their suitability for hybrid breeding to achieve high grain yield and MSV resistance. Principal component analysis captured 68.9% of the total variation explained by four principal components. The Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the inbred lines into nine clusters consistent with their heterotic patterns. Crosses between lines TL2012-53 and TL2012-61 from cluster II with TL2012-20, TL2012-70, and TL2012-78 from cluster IV may provide considerable level of heterosis or novel recombinants for further breeding in northern areas of Tanzania where maize productivity has substantially decreased in recent years due to biotic constraints such as MSV and maize lethal necrosis (MLN) diseases and other abiotic stresses.

Keywords: agro-morphological characterization, genetic diversity, inbred line, maize, maize streak virus.

3.1 Introduction

Maize (*Zea mays* L., 2n=2x=20) is the most important food security crop in sub-Saharan Africa. It is being produced on nearly hundred million hectares of land across 125 developing countries (FAOSTAT, 2010; Prasanna, 2012). In Tanzania, maize is one of the most important food security crops serving as a major source of food and income for majority of smallholder farmers (Barreiro-Hurle, 2012; Kage et al., 2013, Mrutu et al., 2014). In the country annual maize cultivation covers about two million hectares, representing more than 45% of the arable land available for crop production (Kage et al., 2013; Mrutu et al., 2014). However, maize yields are substantially low due to biotic stresses caused by maize streak virus (MSV), maize lethal necrosis (MLN) and other abiotic stresses (Bucheyeki, 2012; M'mboyi et al., 2011; DFID, 2014).

Maize streak virus is a number one biotic production challenge severely limiting potential yields in Tanzania and sub-Sahara African countries (Bucheyeki, 2012; Shepherd et al., 2010, M'mboyi et al., 2010; Prasanna, 2012; DFID, 2014; Karavina, 2014). Yield losses are often associated with cultivation of susceptible maize varieties or virulence shift of the virus (Shepherd et al., 2010). This suggests the need for development of resistant cultivars for strategic control of this erratic but devastating disease of maize (Shepherd et al., 2010; Ruschhaupt et al., 2013; Karavina et al., 2014).

Favourable genes that can contribute to high yield and disease stress tolerance may be available among maize inbred lines (Prasanna, 2012; Gichuru, 2013; Shepherd et al., 2014). However, the genes are scattered over a wide array of germplasm suggesting that critical characterization should be done in order to identify potential germplasm for breeding (Subramanian and Subbraman, 2010; Prasanna, 2012; Edmeades et al., 2013). Genetic diversity analysis among elite inbred lines is essential for hybrid breeding to exploit heterosis or hybrid vigour and for germplasm enhancement against biotic or abiotic stresses (Aghaee et al., 2010; Prasanna, 2012; Mengesha, 2013; Edmeades et al., 2013). Different methods are available to characterise and select parental inbred lines and to assign them into heterotic groups (Kundu and Pal, 2011; Song et al., 2013; Akinwale et al., 2014; Salazar-Salas et al., 2014). Among these methods are: characterisation using agro-morphological traits, pedigree analysis, genetic analysis with designed crosses, and genetic distance estimates using molecular markers (Glaszmann et a., 2010; Parasanna, 2012; Semagn et al., 2012; Fischer et al., 2014; Lopez-Morales et al., 2014).

Assessment of genetic diversity using morphological characterization is relatively a cheaper option where genomic tools are underdeveloped or not readily available such as in Tanzania

(Mbuya et al., 2012, Semagn et al., 2012 Khan et al., 2014). Morphological traits are genetically controlled and heritable (Shrestha, 2013; Molin et al. 2013) which remain useful in distinguishing genetic variability for selection (Aghaee et al., 2010; Molin et al., 2013; Shrestha, 2013). Elezi et al. (2013) studied morphological characterization of maize landraces and found significant variation in days to flowering, plant height, ear height, stem colour and tassel type. Shrestha (2013) reported significant variation (34.9%) in tassel branch number, ear height (29.8%) and plant height (20.1%) when studying agro-morphological characterization of maize inbred lines. Morphological characterization has been extensively used as an important tool to aid identification and selection of diverse parents suitable for hybrid combinations and in broadening the genetic base of breeding populations (Subramanian and Subbraman, 2010; Mbuya et al., 2012; Semagn et al., 2012; Parasanna, 2012; Fischer et al., 2014; Lopez-Morales et al., 2014).

The most commonly used agro-morphological descriptors are grain yield, plant height, ear height, cob length, days to 50% flowering, grain type and grain colour. Further a wide range of qualitative traits are available in phenotypic characterisation of maize germplasm (CIMMYT, 1991; Bode et al., 2012; Ranawat et al., 2013; Shrestha, 2013).

A number of statistical software is available for analysis of both quantitative and qualitative characters including XLSTAT, BMS, SAS, and GENSTAT (Shrestha, 2013; Osawaru et al., 2013). Statistical analysis tools have utilities to estimates genetic variation and compare differences between genotypes for a range of attributes (Malosetti et al., 2013; Crossa et al., 2014). They also conveniently cluster genetic resources into recognisable groups based on genetic dissimilarity or similarity using genetic distances. For instance, principal component analysis and hierarchical cluster analysis by Un-weighted Pair Group Method and Arithmetic Average (UPGMA) depict the relationships among inbred lines using phenotypic descriptors (Subramanian and Subbaraman, 2010; Lekgari and Dweikat, 2014).

The maize research program at Selian Agricultural Research Institute (SARI), located in the northern zone of Tanzania is mandated for maize research and development for the mid-altitude agro-ecologies of the country. During the past ten years, the program focused on maize breeding to enhance productivity through hybrid breeding and open pollinated variety (OPV) development. The program has developed suitable germplasm against abiotic stress particularly for low nitrogen and drought tolerance. Recently, the program has embarked on a dedicated MSV and MLN resistance breeding initiatives. Subsequently several genotypes were collected from local and exotic sources for effective genetic characterisation and breeding. The objective

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of this study was to determine agro-morphological diversity present among 80 local and introduced maize inbred lines under maize streak virus (MSV) prone environments of the northern zone of Tanzania. Promising parents will be used for hybrid breeding or to broaden the genetic base of resistance against biotic stresses such as MSV and MLN or abiotic stress factors.

3.2 Materials and methods

3.2.1 Study site

The study was carried at Ngaramtoni Research Farm of Selian Agricultural Research Institute situated in northern Tanzania (3º 18' S and 36º 36' E), during the 2011/2012 summer season. This site lies at an altitude of 1520 m above sea level and receives mean annual temperature and rainfall of 19.15ºC and 819 mm, respectively. The area is dominated by fine volcanic clay soils.

3.2.2 Plant material

The experimental material used in the present study comprised of 80 maize inbred lines collected from various sources. Of which, 27% of inbred lines were collected from the International Maize and Wheat Improvement Centre (CIMMYT)/Kenya; 25% from Selian Agricultural Research Institute (SARI) Tanzania, 16% from CIMMYT/Zimbabwe, 26% from the International Institute of Tropical Agriculture (IITA)/Nigeria and 6% from University of KwaZulu-Natal/South Africa. The list and details of maize inbred lines used in the study are presented in Table 3.1. Most of the exotic inbred lines are reported to be tolerant against the MSV disease. All the lines are stable and homozygous descended through controlled selfing.

Table 3.1 List of maize inbred lines used in the study

No	Name/designation/pedigree	Code	Source	No	Name/designation/pedigree	Code	Source
1	CML505	TL2012-1	CIMMYT/Kenya	41	CML390	TL2012-41	CIMMYT/Kenya
2	CML202-BB	TL2912-2	CIMMYT/Zimbabwe	42	SML125	TL2012-42	SARI/Tanzania
3	CML442	TL2012-3	CIMMYT/Kenya	43	KSO3-OB15-1	TL2012-43	SARI/Tanzania
4	MAS[MSR/312]-119-5-1-4-3-B	TL2012-4	CIMMYT/Zimbabwe	44	KSO3-OB15-188	TL2012-44	SARI/Tanzania
5	MAS[MSR/312]-119-5-1-4-1-B	TL2012-5	CIMMYT/Zimbabwe	45	A-LINE	TL2012-45	SARI/Tanzania
6	CML488	TL2012-6	CIMMYT/Kenya	46	KSO3-OB15-53	TL2012-46	SARI/Tanzania
7	MAS[MSR/312]-119-5-1-4-1-BB	TL2012-7	CIMMYT/Zimbabwe	47	KSO3-OB15-45	TL2012-47	SARI/Tanzania
8	TZEE-W Pop x LD S6 (Set B) Inb.23	TL2012-8	IITA/Nigeria	48	KSO3-OB15-83	TL2012-48	SARI/Tanzania
9	TZE-W Pop STR Co S6 Inb.136-3-3	TL2012-9	IITA/Nigeria	49	KSO3-OB15-85	TL2012-49	SARI/Tanzania
10	P100C6-200-1-1-H-H-B-B-B-B-B	TL2012-10	CIMMYT/Kenya	50	TS6GF2-38-1-3-3-1-BBB	TL2012-50	SARI/Tanzania
11	CML440	TL2012-11	CIMMYT/Kenya	51	KSO3-OB15-92	TL2012-51	SARI/Tanzania
12	ZM523A-16-2-1-1-B*5-B	TL2012-12	CIMMYT/Kenya	52	KSO3-OB15-111	TL2012-52	SARI/Tanzania
13	CML538	TL2012-13	CIMMYT/Kenya	53	TZE-W Pop X 1368 STR S7 Inb.13	TL2012-53	IITAI/Nigeria
14	MAS[202/312]-20-1-1-4-1-BB	TL2012-14	CIMMYT/Zimbabwe	54	09MAK 17-36	TL2012-54	UKZN/South Africa
15	CML312	TL2012-15	CIMMYT/Kenya	55	09MAK 1-77	TL2012-55	UKZN/South Africa
16	CML206	TL2012-16	CIMMYT/Kenya	56	09MAK 17-15	TL2012-56	UKZN/South Africa
17	V547-1-VL0835	TL2012-17	SARI/Tanzania	57	09MAK 17-5	TL2012-57	UKZN/South Africa
18	CML489	TL2012-18	CIMMYT/Kenya	58	CML443	TL2012-58	CIMMYT/Kenya
19	CML539	TL2012-19	CIMMYT/Kenya	59	MAS[202/312]-20-1-1-4-2-B	TL2012-59	CIMMYT/Zimbabwe
20	CML78	TL2012-20	CIMMYT/Kenya	60	MAS[206/312]-159-2-3-4-1-B	TL2012-60	CIMMYT/Zimbabwe
21	MAS[MSR/312]-119-5-1-1-1-BB	TL2012-21	CIMMYT/Zimbabwe	61	WEC STR S7 Inb.12	TL2012-61	IITA/Nigeria
22	MAS[202/312]-20-11-2-1-BB	TL2012-22	CIMMYT/Zimbabwe	62	MAS[MSR/312]-117-2-2-1-B*4	TL2012-62	CIMMYT/Zimbabwe
23	MAS[MSR/312]-119-5-1-4-2-B	TL2012-23	CIMMYT/Zimbabwe	63	TZE-W Pop STR Co S6 Inb.143-3-3	TL2012-63	IITA/Nigeria
24	MAS[MSR/312]-119-5-1-3-2-B	TL2012-24	CIMMYT/Zimbabwe	64	CML395	TL2012-64	CIMMYT/Kenya
25	MAS[MSR/312]-119-5-1-1-3-B	TL2012-25	IITA./Nigeria	65	WEC STR S7 Inb.9	TL2012-65	IITA/Nigeria
26	TZE-W Pop x 1368 STR S7 Inb.6	TL2012-26	IITA/Nigeria	66	CML444	TL2012-66	CIMMYT/Kenya
27	TZEE-W SR BC5 x 1368 STR S6 Inb.33	TL2012-27	IITA/Nigeria	67	CML204	TL2012-67	CIMMYT/Kenya
28	TZEE-W Pop x LD S6 (Set A) Inb.26	TL2012-28	IITA/Nigeria	68	CML509	TL2012-68	CIMMYT/Kenya
29	TZE-W Pop x LD S6 Inb.3	TL2012-29	IITA/Nigeria	69	CML202	TL2012-69	CIMMYT/Kenya
30	KAT2/2-92-1-1-2	TL2012-30	SARI/Tanzania	70	TZEE-W SR BC5 X 1368 STR S7 Inb.76	TL2012-70	IITA/Nigeria
31	TUX5-50-1-2-6-1	TL2012-31	SARI/Tanzania	71	TZEE-W SR BC5 X 1368 STR S7 Inb.85	TL2012-71	IITA/Nigeria
32	KIL4-78-2-3-2	TL2012-32	SARI/Tanzania	72	TZEE-W SR BC5 X 1368 STR S7 Inb.91	TL2012-72	IITA//Nigeria
33	MV501-6-86-3-1-1	TL2012-33	SARI/Tanzania	73	TZEE-W SR BC5 X 1368 STR S7 Inb.100	TL2012-73	IITA/Nigeria
34	TZE-W Pop X LD S6 Inb.4	TL2012-34	IITA/Nigeria	74	CML206-BB	TL2012-74	CIMMYT/Zimbabwe
35	WEC STR S8 Inb.4	TL2012-35	IITA/Nigeria	75	TZEE-W Pop X LD S6 (Set A) Inb.41	TL2012-75	IITA/Nigeria
36	TZE-W Pop X 1368 STR S7 Inb.2	TL2012-36	IITA/Nigeria	76	MAS[MSR/312]-119-5-1-4-3-BB	TL2012-76	CIMMYT/Zimbabwe
37	KAT 12-1-4-2-1	TL2012-37	SARI/Tanzania	77	TZEE-W Pop Co S6 Inb.35-2-3	TL2012-77	IITA/Nigeria
38	P43-1-1-1-BBB	TL2012-38	SARI/Tanzania	78	TZEE-W Pop Co S6 Inb.96-2-2	TL2012-78	IITA/Nigeria
39	F-LINE	TL2012-39	SARI/Tanzania	79	TZEE-W SR BC5 X 1368 STR S7 Inb.80	TL2012-79	IITA/Nigeria
40	CML197	TL2012-40	CIMMYT/Kenya	80	KSO3-OB15-12	TL2012-80	SARI/Tanzania

CIMMYT= International Maize and Wheat Improvement Centre, IITA= International Institute of Tropical Agriculture, SARI= Selian Agricultural Research Institute

3.2.3 Experimental design and MSV infection

An 8 x 10 alpha lattice design with two replications was used for the study. Plants were established involving two row plots of 5 m length with an inter-row spacing of 0.75 m and intra-row spacing of 0.3 m. Diammonium Phosphate (DAP) was applied at a rate of 150 kg/ha at planting and 150 kg/ha of Urea was top dressed at knee height. Other trial management practices were based on the recommendation of the location. The MSV infection was studied at Ngaramtoni Research Farm. This site is a hotspot for foliar diseases of maize including MSV, MLN, and gray leaf spot; consequently, screening using natural disease epidemic was followed for this study. Disease screening through natural epidemics can provide adequate assessment among inbred lines (Shepherd et al., 2010; Benardo et al., 2013). During this experiment, the disease pressure was very high that facilitated collection of adequate data (Figure 3.1). Also a susceptible genotype, UH615, was planted at border rows to increase inter-plot infection of MSV disease among tested inbred lines.

Figure 3.1 Maize inbred lines showing MSV infection during the study at Ngarmtoni Research Farm of Selian Agricultural Research Institute of northern Tanzania

3.2.4 Data collection

Thirty four morphological characters consisting of seven quantitative and 27 qualitative traits were collected for assessment of genetic diversity of 80 maize inbred lines. The quantitative data were measured in metric unit systems and the qualitative characters were collected according to the descriptors of CIMMYT (1991) as indicated in Table 3.2. The MSV disease severity progress was scored using the area under disease progress curve (AUDPC) approach. AUDPC is frequently used to combine multiple observations of disease progress into a single value (Craven and Fourie, 2011; Simko and Pipho, 2012). Disease severity progress over time was calculated using the following formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})(t_{i+1} - t_i)}{2}$$; where n is the number of observations, ti days after planting

for the ith disease assessment and yi disease severity.

Table 3.2 Quantitative and qualitative agro-morphological characters and their descriptions assessed in the study

Characters	Description
Quantitative	
Grain yield [[YLD]	Calculated using field weight at 12.5% moisture content
Days to 50% tasseling (male flowering) [ADF]	Number of days from sowing to when 50% of the plants have shed pollen per plot
Days to 50% silking (female flowering) [DSL]	Number of days from sowing to when silks have emerged on 50% of the plants per plot
Plant height [PHT]	Measured from ground level to the base of the tassel in cm, after milk stage of the plant growth
Ear height [EHT]	Measured from ground level to the node bearing the uppermost ear in cm, after milk stage
Number of tassel branches [NTB]	Measured at milk stage, 1 = primary, 2 = primary-secondary, 3 = primary-secondary-tertiary tassel types
Ear diameter [EDM]	Measured at central part of the uppermost ear in cm
MSV reaction	Scored using a scale of 1-5, 1 = resistant and 5 = susceptible
Qualitative	
Anthocyanin colouration of glume of cob	1 = absent, 2 = weak, 3 = strong, 4 = very strong
Angle between blade and stem	1 = small (<25 degree), 2 = medium (25-75 degree) and 3 = large >75 degree
Width of blade	1= narrow, 2 = medium, 3 = wide
Attitude of blade	1 = straight, 2 = slightly curved, 3 =curved, 4 = strongly curved
Stem degree of zigzag	1 = straight, 2 = slightly curved, 3 = curved, 4 = strongly curved
Anthocyanin coloration of base of glume	1 = present, 2 = absent
Anthocyanin colouration of glume without base	1 = absent, 2 = weak, 3 = strong, 4 = very strong
Anthocyanin colouration of sheath of stem	1 =weak, 2 = strong, 3 = absent, 4 = present
Anthocyanin colouration of brace root	1 = absent, 2 = weak, 3 = strong, 4 = very strong
Anthocyanin colouration of internodes	1 = absent, 2 = weak, 3 = strong, 4 = very strong
Anthocyanin coloration of anthers	Was taken in the middle 3rd of the main axis, 1 = present, 2 = absent
Anthocyanin color of silks	1 = absent, 2 = present

Intensity of anthocyanin colouration of silks	1 = weak, 2 =strong, 3 = very strong
Density of spikelet of tassel	It was taken in the middle 3rd of the main axis 1 = dense, 2 = medium,,3 = lax
Angle between main axis and lateral branch of tassel	1 = small (<25 degree), 2 = medium (25-75 degree), 3 = large (>75 degree)
Attitude of lateral branches of tassel	1 = straight, 2 = slightly curved, 3 = curved, 4 = strongly curved
Number of primary lateral branches of tassel	1= few, 2 = medium, 3 = many
Length of main axis above lower branch of tassel	Measured in cm
Length of main axis above upper branch of tassel	Measured in cm
Length of peduncle of the ear	Measured in cm or classified as 1 = short, 2 = medium, 3 = long or 4 = very long
Length of husks off the tip of the ear	Measured in cm upper side of the ear, 1 = short, 2 = medium, 3 = long, or 4 = very long
Length of ear	1 = small, 2 = medium, 3 = long, 4 = very long
Number of ear rows	Number of kernel rows in the central part of the uppermost ear
Shape of the ear	1 = conicacal, 2 = conical-cylindrical, 3 = cylindrical
Type of grain	1 = flint, 2 = flint-like, 3 = intermediate, 4 = dent, 5 = dent-like
Colour of grain	1 = white, 2 =yellow, 3 = purple, 4 = variegated, 5 = brown, 6 = orange, 7 = mottled, 8 = white cap, 9 = red
Anthocyanin colouration of dorsal side of grain	1 = absent, 2= present

3.2.5 Data analysis

A total of 34 agro-morphological traits were assessed in the study. Seven were quantitative and 27 qualitative traits (Table 3.2). The quantitative data were subjected to analysis of variance to test significant differences between lines using the Breeding Management Systems (BMS) software version 2.1 (MacLaren, 2014). Principal component and cluster analyses were done to determine influential components and traits relationships. The cluster analysis was done using un-weighted pair group method with arithmetic mean (UPGMA) to yield a dendrogram depicting the morphological relatedness of inbred lines. The XLSTAT software developed by Addinsoft (2010) for Microsoft Excel was used in both analyses. Principal component analysis was performed using seven quantitative data while clustering of inbred lines was done using 27 qualitative characters. The XLSTAT program has utilities to calculate the similarity matrix using the Euclidean genetic distances between lines (Cana et al., 2011).

3.3 Results
3.3.1 Analysis of variance of quantitative traits

Analysis of variance on seven morphological characteristics was done using the BMS statistical analysis tool version 2.1 (MacLaren, 2014). Results showed significant differences between inbred lines for all traits evaluated except EDM (Table 3.3). The mean, minimum, and maximum values of inbred lines for each trait are shown in Table 3.4.

Table 3.3 Mean square and significant differences of eight traits of 80 maize inbred lines

		YLD		ADF		DSL		PHT	
Source of variation	DF	MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replications	1	0.208	0.208	0.23	0.20	3.025	1.44	26.41	21.37
Genotypes	79	0.185	3.74***	38.2	34.07***	42.94	20.47***	2355.1	1905.9***
Blocks	18	0.632	0.19	14.59	13.01	10.85	5.17	346.92	280.75
Error	61	9.88	0.13	1.12		2.10		1.236	
		EHT		EDM		NTB		MSV	
		MS	F-value	MS	F-value	MS	F-value	MS	F-value
Replications	1	0.16	0.00	5.776	2.49	2.525	0.26	0.29	0.58
Genotypes	79	1431.9	32.63***	1.754	0.76ns	18.75	1.94***	2.72	5.46***
Blocks	18	3.598	0.19	3.95	1.70	7.314	0.76	1.90	3.82
Error	61	9.88		2.32		9.693		0.50	

***= Significantly different at p- <0.001, ns =non-significant, DF= Degrees of freedom, MS= Mean squares, YLD= Yield (t/ha), ADF= Days to 50% anthesis, DSL= Days to 50 % silking, PHT= Plant height (cm), EHT= Ear height (cm), EDM= Ear diameter (cm), NTB= Number of tassel branches, and MSV= Maize streak virus

3.3.2 Mean performance of inbred lines

The summary statistics of mean performance for 80 inbred lines studied are given in Table 3.4. Grain yield (YLD) among inbred lines varied significantly from 0.02 t/ha to 3.52 t/ha, with a grand mean of 0.93 t/ha. The highest grain yielder genotypes were TL2012-42 and TL2012-17 at 3.52 and 2.76 t/ha, respectively. These were followed by TLl2012-41 and TL2012-26 with grain yields of 2.46 and 2.08 t/ha; respectively. These genotypes had also low (< 40%) reaction levels of MSV except for TL2012-17 which had susceptible reaction (Table 3.4). Lines: TL2012-25, TL2012-24, TL2012-55 and TL2012-68 showed low MSV reactions of 29.80%, 35.40%, 34.60, and 27.20, respectively; hence were considered as resistant genotypes. Lines: TL2012-2 and TL2012-23 had low susceptibility to MSV at 27.20% and 28.00%, respectively except their poor yields. YLD had the highest coefficient of variation (CV) of 73.6% as compared to other variables such as ADF and DSL which recorded a coefficient of variation of about 6% only. The high percentage of coefficient of variation in YLD might be attributed to poor performances and adaptability of test inbred lines.

Inbred lines showed significant differences for ADF and DSL. The number of days of pollen shedding (50% anthesis) was the highest at 77.8 for TL2012-9 followed by 76.1, 74.8 and 74.2 for TL2012-66, TL2012-58 and TL2012-69, respectively. Highest days of silk emergence were 75.8 displayed by TL2012-57, followed by 75.6 and 75.2 days by lines TL2012-4 and TL2012-63, respectively (Table 3.4). For this reason, most breeders have been classifying their study materials into different maturity groups to facilitate development of cultivars for specific environments.

PHT varied significantly among inbred lines, the shortest inbred line was TL2012-65 that showed a height of 81.3 cm while the tallest was TL2012-42 at 222.4 cm. The overall mean plant height of inbred lines was 151.6 cm. Likewise EHT, varied from 10.6 cm (TL2012-65) to 126.3 cm (TL2012-38) with a grand mean of 72.7 cm. The number of tassel branches showed the greatest variation at 82.5%. The largest branch numbers were 19.9 and 19.6 displayed by TL2012-29 and TL2012-31 respectively, while the lowest was 1.9 expressed by TL2012-54 (Table 3.4). EDM was not significantly different among inbred lines but varied from 0.48 cm for TL2012-6 to 8.6cm for TL2012-80, with an overall mean of 2.4 cm (Table 3.4).

Table 3.4 Mean performance of 80 maize inbred lines for eight quantitative traits

S/N	Genotype	YLD	ADF	DSL	PHT	EHT	NTB	EDM	MSV
1	TL2012-42	3.52	68.80	68.30	152.90	110.30	14.30	2.37	21.80
2	TL2012-17	2.76	65.50	64.90	129.00	65.60	7.90	1.89	95.40
3	TL2012-41	2.46	68.70	68.30	150.90	91.20	16.10	2.19	26.20
4	TL2012-26	2.25	66.40	66.10	135.80	72.60	12.10	2.11	41.20
5	TL2012-25	2.19	66.40	66.00	135.20	66.80	17.50	3.40	29.80
6	TL2012-55	1.97	70.50	70.20	165.70	85.30	10.40	3.49	34.60
7	TL2012-49	1.90	69.70	69.30	158.30	48.40	13.80	3.31	92.20
8	TL2012-68	1.89	72.50	72.60	183.30	31.90	10.30	1.66	27.20
9	TL2012-48	1.70	69.60	69.20	158.20	91.30	10.00	2.40	72.60
10	TL2012-30	1.69	67.00	66.70	141.80	90.30	12.70	3.28	85.60
11	TL2012-15	1.64	64.60	63.40	124.10	60.90	10.10	2.07	53.40
12	TL2012-38	1.63	68.50	67.50	148.20	126.30	16.50	2.21	87.80
13	TL2012-27	1.58	66.60	66.40	138.20	69.30	15.50	1.50	69.80
14	TL2012-43	1.57	68.80	68.50	153.20	68.70	8.80	2.16	73.40
15	TL2012-24	1.48	66.40	65.70	134.80	63.10	13.90	1.94	35.40
16	TL2012-11	1.45	64.10	62.40	119.40	83.40	10.10	1.85	36.20
17	TL2012-54	1.39	70.40	70.10	162.30	115.50	13.40	2.78	30.60
18	TL2012-44	1.28	69.00	68.50	153.70	71.30	11.00	2.30	87.60
19	TL2012-61	1.27	71.20	71.40	172.30	58.70	4.40	2.64	64.80
20	TL2012-16	1.23	64.70	64.70	128.10	51.00	8.40	2.14	96.40
21	TL2012-76	1.18	74.20	75.00	209.10	68.90	12.80	2.71	53.60
22	TL2012-46	1.12	69.20	68.70	154.00	66.10	11.40	1.63	83.20
23	TL2012-31	1.12	67.20	66.80	141.90	110.10	19.60	2.39	69.00
24	TL2012-52	1.12	69.80	69.50	160.10	92.60	17.20	3.20	73.60
25	TL2012-57	1.11	70.80	70.40	167.30	73.70	16.40	2.56	72.00
26	TL2012-47	1.10	69.50	69.20	157.30	89.00	13.60	1.28	94.80
27	TL2012-7	1.07	60.70	61.00	110.60	79.00	13.30	0.53	67.00
28	TL2012-14	1.02	64.60	63.00	122.00	28.50	7.50	1.85	46.60
29	TL2012-65	0.96	71.80	72.00	176.40	19.60	10.90	2.15	43.80
30	TL2012-78	0.96	76.10	75.30	215.30	68.90	13.30	2.56	68.60
31	TL2012-19	0.95	65.70	65.20	130.40	65.60	10.00	1.71	102.60
32	TL2012-29	0.93	66.90	66.60	140.80	67.40	12.90	1.27	91.40
33	TL2012-35	0.89	68.30	67.00	143.60	84.20	17.70	2.02	76.80
34	TL2012-36	0.88	68.30	67.20	147.00	117.10	12.20	2.65	102.00
35	TL2012-72	0.86	73.20	73.70	186.80	72.10	10.90	2.05	38.80
36	TL2012-62	0.86	71.30	71.40	174.70	33.70	1.90	4.06	72.00
37	TL2012-22	0.79	66.20	65.50	131.00	70.30	12.00	1.48	47.60
38	TL2012-50	0.78	69.70	69.30	158.80	85.80	14.60	2.77	87.00
39	TL2012-37	0.78	68.30	67.30	147.80	117.00	14.60	2.61	63.00
40	TL2012-59	0.77	71.10	71.00	170.30	36.30	11.80	3.74	62.20
41	TL2012-45	0.73	69.10	68.60	153.80	108.60	10.40	1.66	71.00
42	TL2012-63	0.70	71.40	71.60	174.80	38.90	4.00	1.83	41.60
43	TL2012-67	0.69	72.40	72.50	183.20	36.10	11.30	2.47	73.00
44	TL2012-51	0.68	69.70	69.50	159.60	86.00	10.50	1.52	84.40
45	TL2012-23	0.65	66.30	65.70	133.30	70.50	11.40	2.48	28.80
46	TL2012-60	0.63	71.10	71.00	170.90	59.10	7.60	7.31	53.80
47	TL2012-4	0.63	59.80	60.40	98.30	96.10	13.00	2.02	50.20
48	TL2012-73	0.61	73.50	73.70	194.30	71.40	16.70	2.95	57.00
49	TL2012-1	0.60	59.20	57.80	104.00	81.30	14.80	1.23	37.00
50	TL2012-3	0.59	59.40	59.80	91.00	54.00	15.30	1.43	107.00
51	TL2012-71	0.59	72.90	73.00	186.80	79.60	13.90	2.15	73.80
52	TL2012-77	0.58	74.80	75.20	211.00	73.60	13.90	2.75	50.80
53	TL2012-32	0.58	67.90	66.90	142.40	114.90	10.90	1.97	76.40
54	TL2012-70	0.55	72.70	72.90	186.70	78.60	14.80	0.57	88.00
55	TL2012-28	0.54	66.60	66.50	138.70	54.50	16.70	2.12	65.60
56	TL2012-20	0.54	65.80	65.30	130.60	55.60	5.40	2.24	50.40
57	TL2012-10	0.53	63.90	62.40	114.60	40.90	13.10	2.32	103.40
58	TL2012-80	0.50	77.80	75.80	222.40	63.60	8.30	8.60	60.80
59	TL2012-56	0.50	70.60	70.20	166.40	72.60	11.70	2.95	58.60
60	TL2012-40	0.49	68.70	68.30	150.10	87.10	11.20	2.65	42.00
61	TL2012-33	0.49	67.90	66.90	143.00	88.80	11.40	3.17	86.20
62	TL2012-79	0.49	77.10	75.60	217.20	60.70	9.10	2.26	80.00
63	TL2012-75	0.46	74.00	74.80	203.90	69.00	12.90	2.38	50.80
64	TL2012-34	0.42	68.10	67.00	143.60	61.90	13.30	2.55	99.40
65	TL2012-12	0.39	64.40	62.40	119.40	55.10	10.00	1.96	87.00
66	TL2012-53	0.38	70.40	70.10	160.80	80.90	14.30	2.29	55.60

67	TL2012-64	0.36	71.40	72.00	176.20	29.10	10.10	3.81	63.80
68	TL2012-6	0.33	60.40	60.60	108.20	44.00	10.80	0.48	77.00
69	TL2012-74	0.33	73.60	73.90	194.40	94.20	13.70	2.31	57.20
70	TL2012-21	0.28	66.10	65.50	130.90	60.40	8.60	2.01	40.20
71	TL2012-58	0.27	70.90	70.90	169.10	58.50	9.80	1.51	72.20
72	TL2012-13	0.27	64.40	63.00	120.00	25.20	5.70	1.83	57.80
73	TL2012-8	0.26	62.80	61.60	111.40	30.40	12.60	1.28	94.80
74	TL2012-9	0.24	63.70	61.60	112.90	93.50	16.30	2.37	39.80
75	TL2012-69	0.23	72.60	72.60	185.70	51.90	14.30	2.08	64.40
76	TL2012-18	0.14	65.60	65.10	130.10	77.30	11.50	1.33	77.60
77	TL2012-5	0.08	60.30	60.60	108.20	40.40	8.60	1.58	54.80
78	TL2912-2	0.07	59.20	59.40	88.00	83.60	13.80	2.12	26.60
79	TL2012-66	0.06	72.00	72.20	176.40	129.40	16.60	2.83	40.80
80	TL2012-39	0.04	68.50	67.70	148.50	117.50	19.90	4.04	79.60
Mean		0.93	68.40	67.90	151.60	72.70	12.10	2.38	64.46
Minimum		0.040	59.200	57.800	81.300	19.600	1.900	0.480	21.800
Maximum		3.52	77.80	67.80	222.40	125.30	19.90	8.60	107.00
LSD (0.05)		0.326	2.401	2.71	15.8	11.57	2.368	1.4	0.8
%CV		73.6	6.1	6.4	20.3	34.1	28.3	48.7	34.2

YLD= Yield (t/ha), ADF= Days to 50% anthesis, DSL= Days to 50 % silking, PHT= Plant height (cm), EHT= Ear height (cm), EDM= Ear diameter (cm), TB = Number of tassel branches, and MSV= Maize streak virus

3.3.3 Variation of qualitative characters

Variation was observed among inbred lines in all the qualitative characters considered in the study except for anthocyanin colouration of glumes of cob (Table 3.5). Distribution of the average angle leaf inclination classified the studied inbred lines into three distinct groups, those with small angle constituted 52.5%, medium angle size were 45% and those with large angle were 2.5% of the total number of inbred line. The size of blade width varied between narrow (42.5%) and medium (57.5%) among the inbred lines. About 96.2% of genotypes had slightly curved blades or leaves while only 3.8% had straight attitude. Close to 70% of stems for most inbred lines were straight but 23.8% and 6.2% were slightly and strongly curved sideways in their appearance, respectively. The anthocyanin colouration on the base of glumes varied among inbred lines, however, 46.2% of the inbred lines showed no colouration at the base side of their glumes. With regards to anthocyanin colouration, 8.8% had weak colouration, 28.8% medium and 16.2% strong colouration at the base of their glumes. The anthocyanin colouration of glumes *per se* without base distributed differed among the inbred lines, with 15% showing no colouration of glumes. About 46.3% and 26.2% had weak and medium colouration, respectively; while 2.5% had glumes with strong colouration (Table 3.5). Regarding the anthocyanin colouration of sheath of stem, more than 60% of inbred lines had none colouration on their leaf sheath of stems. However, 33.7% of them demonstrated weak and 2.5% strong colouration of their leaf sheath of stems. The anthocyanin colouration of brace root differed significantly among germplasm with 35% showing absent, however, weak and strong anthocyanin colouration were 52.5% and 12.5%, respectively. Similarly, anthocyanin colouration of the

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internodes of stem was showen by 51.3% of germplasm, while weak and medium colouration constituted 30% and 16.2% of the inbred lines, respectively. Strong internodes colouration was observed by 2.5% of germplam with anthocyaninin colouration on their internodes.

The distribution of anthocyanin colouration of anthers differed substantially between the study materials although 38.8% of them showed absent. The proportions of plants with weak, medium and strong colouration of their anthers were 18.8%, 30.0% and 12.4%, respectively (Table 3.5). Silks exhibited absence of anthocyanin colouration by 67.5% of the entries while 32.5% of inbred lines recorded anthocyanin colouration on their silks. The intensity of silks anthocyanin colouration varied significantly from 15.0% (weak), 43.8% (medium), 20.0% (strong), and 21.2% of very strongly colouration. Density of spikelets of tassel showed 12.5% (lax) and 87.5% (medium).

The distribution of average angel between main axis and lateral branch of tassel ranged from small to large angle. The proportion of the small angle size was 61.2%, 37.5% for medium size and 1.3% for large angles. The altitudes of these lateral branches of tassels were also not uniformly distributed among the inbred lines because over 68% was slightly curved, 2.7% was curved while 28.8% was not curved. Similarly, tassel size or the number of primary lateral branches of tassel differed from a few (56.5%), medium (41.2%) to many (2.5%) (Table 3.5).

Over 90% and 80% of inbred lines used exhibited medium length of the main axis above lower and upper branch of tassel respectively. Inbred lines with short length of main axis above lower branch of tassel were only 7.5% while those with long length of main axis above upper branch of tassel were 16.2% (Table 3.5).

Some characters such as length of peduncle of the ear, length of ear, number of ear rows and length of husks off the tip of the ear showed binary distribution system because they had only two classes. The length of peduncle of the ear among inbred lines was 37.5% short and 62.5% medium; number of ear rows for most inbred lines was few (12.5%) and medium (87.5%). Likewise inbred lines with short length of husks off the tip of the ear were 7.5% and 92.5% medium. About 73.8% of inbred lines described short ear length, 23.7% medium and 2.5% had long ear length (Table 3.5). The shapes of ears of inbred lines studied were variable. The most dominating shape was cylindrical accounted for 92.5% while the conical and intermediate shapes had relatively low proportions of 2.5% and 5.0%, respectively (Table 3.5). The type of grain ranged from flint (2.5%), to flint-like (46.3%) to intermediate (17.5%) to 21.3% dent-like and to 12.4% dent type grain. About 64.2% of grains of these inbred lines was white in colour,

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34.5% yellow and 1.3% was orange in colour. The dorsal side of grain showed significant variation with 58.8% being yellow, 3.8% orange and 37.5% intermediate (Table 3.5).

Table 3.5 Classification of 80 maize lines using 27 qualitative morphological traits

Trait	Class	Percent
Anthocyanin coloration of glumes of cob	Absent	100
Angle between blade and stem	Small	52.5
	medium	45
	Large	2.5
Width of blade	Narrow	42.5
	medium	57.5
Attitude of blade	Straight	3.8
	slightly curved	96.2
Stem degree of zigzag	Straight	70.0
	slightly zigzag	23.8
	strongly zigzag	6.2
Anthocyanin coloration on base of glumes	Absent	46.2
	Weak	8.8
	medium	28.8
	Strong	16.2
Anthocyanin colouration of glume without base	Absent	15
	Weak	46.3
	medium	26.5
	Strong	2.5
Anthocyanin colouration of sheath of stem	Absent	63.8
	Weak	33.7
	Strong	2.5
Anthocyanin colouration of brace root	Absent	35
	Weak	52.5
	Strong	12.5
Anthocyanin colouration of internodes	Absent	51.3
	Weak	30
	medium	16.2
	Strong	2.5
Anthocyanin coloration of anthers	Absent	38.8
	Weak	18.8
	medium	30.0
	Strong	12.4
Anthocyanin color of silks	Absent	67.5
	Present	32.5
Intensity of anthocyanin colouration of silks	Weak	15
	Medium	43.8
	Strong	20.0
	very strong	21.2
Density of spikelet of tassel	Lax	12.5
	Medium	87.5
Angle between main axis and lateral branch of tassel	Small	61.2
	Medium	37.5
	Large	1.3
Attitude of lateral branches of tassel	straight	28.8
	slightly curved	68.5
	Curved	2.7
Number of primary lateral branches of tassel	Few	56.3
	Medium	41.2
	Many	2.5

Length of main axis above lower branch of tassel	Short	7.5
	Medium	92.5
Length of main axis above upper branch of tassel	Medium	83.8
	Long	16.3
Length of peduncle of the ear	Short	37.5
	Medium	62.5
Length of husks off the tip of the ear	Short	7.5
	Medium	92.5
Length of ear	Short	73.8
	Medium	23.8
	Long	2.5
Number of ear rows	few	12.5
	Medium	87.5
Shape of the ear	Conical	2.5
	Intermediate	5.0
	Cylindrical	92.5
Type of grain	Flint	2.5
	flint-like	46.3
	Intermediate	17.5
	dent-like	21.3
	Dent	12.4
Colour of grain	White	64.2
	Yellow	34.5
	Orange	1.3
Anthocyanin colouration of dorsal side of grain	Yellow	58.8
	Yellow-orange	37.4
	Orange	3.8

3.3.4 Cluster analysis

Cluster analysis was done based on 27 qualitative phenotypic traits which grouped the study materials into nine different clusters (Figure 3.2). The clusters are designated as I, II, III, IV, V, VI, VII, VIII and IX. Clusters with largest number of inbred lines were IV, VI, and III, consisting of 30, 18 and 14 inbred lines, respectively (Table 3.6). Clusters III, IV and VI represented 77.5% of inbred lines (Table 3.6). The dendrogram (Figure 3.1) reflects the pattern of genetic relationship between inbred lines. Overall, crosses involving inbred lines TL2012-53 and TL2012-61 (cluster II) with TL2012-20, TL2012-70, TL2012-78 (cluster IV) may provide considerable heterosis or novel recombinants for further breeding. Other uniquely identified inbred lines were TL2012-1 and TL2012-11 from cluster VIII and lines TL2012-5, TL2012-6, TL202-55 and TL2012-56 from cluster III (Figure 3.2).
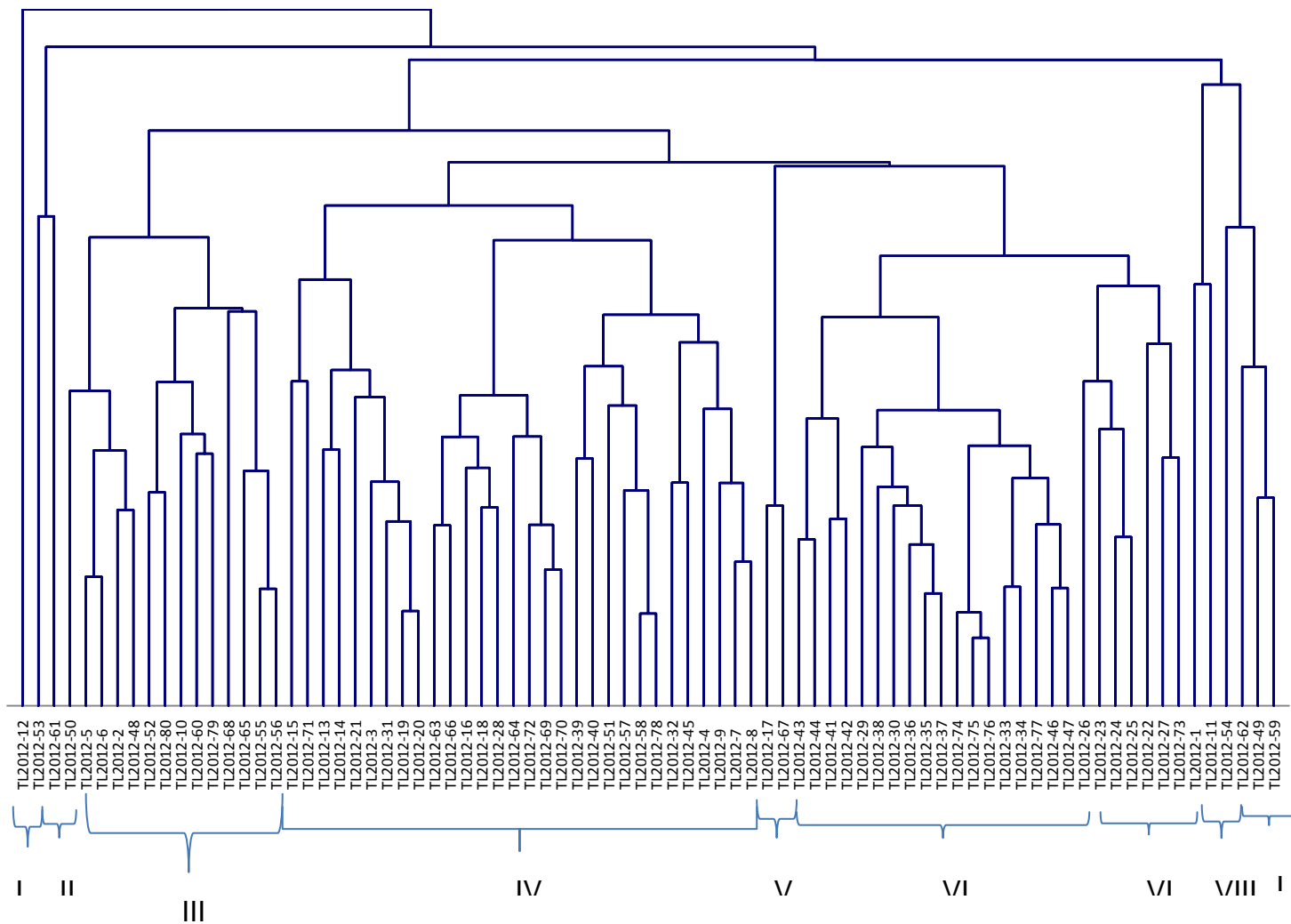
Figure 3.2: Dendrogram based on 27 qualitative phenotypic traits showing genetic relationship of 80 maize inbred lines when evaluated at MSV prone environment in northern Tanzania

Table 3.6 Nine clusters identified in the study with their corresponding number and codes of genotypes.

Clusters	No of genotypes	Codes of genotypes*						
I	1	TL2012-12						
II	2	TL2012-53	TL2012-61					
III	14	TL2012-50	TL2012-5	TL2012-6	TL2012-2	TL2012-48	TL2012-52	TL2012-80
		TL2012-10	TL2012-60	TL2012-79	TL2012-68	TL2012-65	TL2012-55	TL2012-56
IV	30	TL2012-15	TL2012-71	TL2012-13	TL2012-14	TL2012-21	TL2012-3	TL2012-31
		TL2012-19	TL2012-20	TL2012-63	TL2012-66	TL2012-16	TL2012-18	TL2012-28
		TL2012-64	TL2012-72	TL2012-69	TL2012-70	TL2012-39	TL2012-40	TL2012-51
		TL2012-57	TL2012-58	TL2012-78	TL2012-32	TL2012-45	TL2012-4	TL2012-9
		TL2012-7	TL2012-8					
V	2	TL2012-17	TL2012-67					
V1	18	TL2012-43	TL2012-44	TL2012-41	TL2012-42	TL2012-29	TL2012-38	TL2012-30
		TL2012-36	TL2012-35	TL2012-37	TL2012-74	TL2012-75	TL2012-76	TL2012-33
		TL2012-34	TL2012-77	TL2012-46	TL2012-47			
VII	7	TL2012-26	TL2012-23	TL2012-24	TL2012-25	TL2012-22	TL2012-27	TL2012-73
VIII	2	TL2012-1	TL2012-11					
IX	4	TL2012-54	TL2012-62	TL2012-49	TL2012-59			

* See Table 3.1 for codes of genotypes;

Principal components analysis

Principal component analysis revealed four important principal components (PCs) with eigenvalues greater than 1. These components explained 67.9% of the total variation among 80 inbred lines (Table 3.7). The first principal components explained 28.9% of the total variation with eigenvalue of 3.181. The eigenvectors with significant contribution to this component were YLD (0.395), PHT (0.485), EHT (0.465), EDM (0.960) and NTB (0.385). The second principal component had eigenvector of 1.945 accounting for 17.7% of the total variation. Eigenvector loading with significant contribution to this component was ADF (0.680) and DSL (0.678). PC3 and PC4 had eigenvalues of 1.210 and 1.129, respectively. The two explained about 10% of the total variation. The most contributing eigenvector loadings to these components were EDM and MSV; they contributed 0.250 and -0.635, respectively (Table 3.7).

Table 3.7 Principal components, eigenvalues, proportion of total variance, and cumulative variance and eigenvector loadings of 8 characters used level of probability

	Principal Components			
	PC1	PC2	PC3	PC4
Explained variance(Eigenvalue)	3.181	1.945	1.210	1.129
Proportion of total variance (%)	28.917	17.682	11.001	10.260
Cumulative variance (%)	28.917	46.599	57.600	67.860
Traits[+]			[a]Eigenvector loadings	
YLD	**0.395**	0.118	0.293	-0.053
ADF	-0.126	**0.680**	0.093	-0.005
DSL	-0.121	**0.678**	0.120	-0.014
PHT	**0.485**	0.052	-0.247	0.103
EHT	**0.465**	0.093	-0.277	0.119
EDM	**0.296**	0.010	**0.250**	0.096
NTB	**0.385**	0.127	-0.159	0.057
MSV	0.164	0.114	-0.233	**-0.537**

[+]Refer Table 3.4 for variable name;   [a]Bold eigenvector loadings are significant at ≤ 0.05

3.4 Discussion

The present study characterized 80 maize inbred lines using agro-morphological traits in MSV prone environment in Tanzania. Results showed highly significant differences among inbred lines (Table 3.3) for most agro-morphological attributes. This suggests the presence of high level of genetic diversity among the inbred lines for breeding. Detailed analysis of genetic diversity permits efficient utilization of the available maize germplasm to enhance yield and stress tolerance for breeding (Subramanian and Subbaraman, 2010; Shrestha, 2013; Oleyede-Kamiyo et al., 2014). The significant variations observed for YLD, ADF, SDL, PHT, EHT, and NTB (Table 3.3) among inbred lines are attributed to differences in their genetic background. Also environment significantly plays an important role in affecting the performance of quantitative traits (Yadav and Singh, 2010; Alam et al., 2013; Charles et al., 2013; Singh et al., 2014). DNA markers are robust in genetic characterisation studies which are less influenced by environmental effects (Semagn et al., 2012; Yadav et al., 2013). Previous studies reported significant variation in quantitative traits like grain yield, ear height, plant height and earliness in maize (Abrha et al., 2013; Charles et al., 2013). In this study grain yields varied significantly among the studied maize inbred lines. The highest yielding genotypes were TL2012-42 and TLl2012-41 at 3.52 and 2.76 t/ha, respectively (Table 3.4). These inbred lines were also associated with low reaction to MSV suggesting their potential for developing new cultivars with improved grain yield and resistance to MSV disease.

Variation in the amount and distribution of rainfall has significant effect on yield variability among smallholder farmers in Tanzania (Bello et al., 2012). For instance the annual rainfall at the Selian Agricultural Research Institution where this study was conducted declined over the past three years from 830 to 400 mm (Bello et al., 2012; Ruane et al., 2013). Therefore, variation in ADF and DSL reported in this study will help breeders in developing cultivars for early maturity at different agro-ecologies. The number of days to 50% anthesis and silk emergence were 77.8 and 75.8, respectively, suggesting that the inbred lines are generally early maturing suitable for growing in those environments which receive relatively low rainfall.

Plant height is an important character that will influence grain yield and dry matter production (Bello et al., 2012; Zheng and Liu, 2013). The present result revealed highly significant variability in plant height (Table 3.3). The tallest inbred line was TL2012-42 at 222.4 cm and the shortest was TL2012-65 at 81.3 cm. Shorter plant height is desirable for lodging resistance (Abrha et al., 2013). Nazir et al., (2010) reported that plant height was positively correlated with

days to flowering. Internodes formation stop during floral initiation consequently early flowering maize varieties are usually shorter in plant heights.

Previous studies showed highly significant variability in ear height in maize genotypes (Nazir et al., 2010). Ear height has been described to be one of the most important selection criteria in maize breeding especially for root and stock lodging resistance and increased grain yield (Nazir et al., 2010; Bello et al., 2012; Zheng and Liu, 2013). High ear position could be susceptible to root and stock lodging, therefore most breeders usually prefer selecting for lower ear position in maize (Bello et al., 2012).

Mean NTB varied from 1.9 (TL2012-54) to 19.9 (TL2012-29) (Table 3.4). Tassel size is positively correlated with pollen production and consequently of seed set therefore has great implications in breeding programmes. Few tassel branches implies less pollen production this in turn can affect controlled pollination process in breeding programmes. For example, if a pollen parent has low pollen production ability it will not sufficiently pollinate the desired number of female parents therefore lowering the desirable number of crosses to be generated. However a desirable female parent could have a lesser number of tassel branches to avoid assimilates being invested in excessive pollen production than grain yield (Bello et al., 2012).

Principal component analysis

The principal component analysis measures important characters which have significant contributions to the total explained variation (Sinha and Mishra, 2013). The first few principal components with eigenvalues of >1 are often of most important in reflecting the variation pattern among study materials and differentiation of their associated characters (Sinha and Mishra, 2013). In this study, the first four principal components (PCs) captured about 67.9% of the total variation hence were considered as the most important components. This result was comparable to that of Lopez-Morales (2014) who reported 54% of the total variation which was attributed to three components, when studying the morphological diversity of native maize in the humid tropics of Puebla, Mexico.

Variability of inbred lines based on qualitative characters

The results presented in Table 3.5 reflect the variability of studied inbred lines based on 27 qualitative characters used. Knowledge on these variables can be useful for several applications including site-specific crop management in precision agriculture (Shrestha, 2013). The present result shows that about 40% and 50% of these inbred lines had narrow leaf width and small average angle of inclination (< 25^0), respectively. Narrow leaves and small leaf angle of

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inclination are associated with low interception to sun light thereby reduced photosynthesis and subsequently of decreased yields (Torres et al., 2011). Inbred lines with small angle of inclination (< 25^0) and narrow leaves may not be grown in places where light is limiting. Plants with large leaf width and angle of inclination are preferable. Because they are efficient in light interception, provide good ground surface cover to minimize loss of moisture due to evaporation and to supress weeds. In the current result, 70% of the lines had upright or straight stems desirable for mechanical harvesting. Genotypes with curved stems do not show uniformity and good physical appearance. Often qualitative morphological characters are used to differentiate morphotypes. However, these traits may not have direct contributions to yield. Some characters such as length of peduncle of the ear, length of ear, number of ear rows and length of husks off the tip of the ear, shapes of ears, grain type and colour have direct implications for breeding and end users preferences.

Genetic relationships among 80 maize inbred lines used in the study

The classification and expression of inbred lines using dendrogram provide visual assessment of genetically unrelated individuals for use in maize breeding. Inbred lines within the same cluster are genetically related in one or several traits and should not be sampled for cross formation. The UPGMA cluster analysis (Figure 3.2) generated a dendrogram of 80 inbred lines germplasm using 27 morphological qualitative data revealing nine different clusters. This suggests that the tested lines showed considerable genetic diversity. Similar result was reported by Azad et al. (2012). The major clusters identified in this study were III, IV and VI, consisting of 77.5% of the inbred lines evaluated (Table 3.6). Crosses involving parents belonging to the maximum divergent clusters are expected to manifest maximum heterosis and also wide genetic variability on agronomic traits. Thus, cross combinations of genotypes TL2012-53 and TL2012-61 (from cluster II) with TL2012-20, TL2012-70, and TL2012-78 (cluster IV) may provide considerable degree of heterosis or novel recombinants for further breeding and genetic analysis. Also inbred lines such as TL2012-1 and TL2012-11 from cluster VIII and TL2012-5, TL2012-6, TL2012-55 and TL2012-56 (cluster III) could be considered as potential parents for breeding owing to their genetic divergence.

Limitations of morphological descriptors

Assessment of genetic diversity using morphological characterization is relatively a cheaper option where genomic tools are underdeveloped or not readily available such as in Tanzania (Mbuya et al., 2012; Semagn et al., 2012; Khan et al., 2014). In conventional breeding, this method has been extensively used as an important tool to aid identification and selection of

diverse parents suitable for hybrid combinations (Subramanian and Subbraman, 2010; Mbuya et al., 2012; Semagn et al., 2012; Parasanna, 2012; Fischer et al., 2014; Lopez-Morales et al., 2014) despite its limitations. Morphological characterization is greatly limited by several factors such as seasons and growth stage and results can be unrealistic especially when working with quantitative traits because they are influenced by environments. Therefore in order to increase the efficiency of genetic diversity characterization molecular characterization techniques should compliment the weakness of the conventional approach.

3.5 Conclusions

Genetic diversity was studied among 80 inbred lines using agro-morphological characters. The high level of genetic diversity identified in this study will permit efficient utilization of the inbred lines in maize breeding programs for increased productivity. The study is of particularly important in the northern areas of Tanzania, where maize productivity has substantially decreased in recent years due to biotic constraints such as MSV and the maize lethal necrosis (MLN) diseases, and other random stresses. This study has identified high yielding lines TL2012-42 (3.52 t/ha), and TLl2012-41 (2.46 t/ha), and TL2012-26 (2.08 t/ha) which had low reaction to MSV disease. These lines can be used in developing high yielding and MSV resistant maize hybrids. The UPGMA cluster analysis grouped inbred lines into nine divergent clusters. Crosses made from most divergent parents are expected to manifest maximum heterosis in yield and generate more variability. In summary, the study identified unique inbred lines such as TL2012-53 and TL2012-61 (from cluster II), TL2012-20, TL2012-70, and TL2012-78 (cluster IV), TL2012-1 and TL2012-11 (cluster VIII) and TL2012-5, TL2012-6, TL2012-55 and TL2012-56 from cluster III for breeding.

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CHAPTER FOUR:

Genetic diversity analysis of maize inbred lines collected from diverse origins using SSR markers

Abstract:

Understanding the genetic diversity and genetic relationships among diverse genetic resources is important in crop improvement programmes and for strategic conservation of genetic resources. The objective of this study was to assess the genetic diversity and genetic relationships among 79 maize inbred lines collected from five origins using 30 SSR markers. The mean numbers of observed and effective alleles were 4.70 and 2.40, respectively. The markers displayed high Shannon's information index of 0.96 and polymorphic information content (PIC) of 0.51. The mean values of observed and expected heterozygosity among lines were 0.136 and 0.508, respectively. A dendrogram constructed based on UPGMA clustered the inbred lines into three main genetic groups with sub-clusters especially in group II. The principal coordinate analysis (PCA) explained 20.4% of the total genetic variation detected among inbred lines and separated them into two main clusters. The present study demonstrated that SSR markers successfully detected the genetic diversity present in the maize inbred lines of varied origins. Analysis of molecular variance (AMOVA) showed that 72% of the total variation was attributed to differences among inbred lines over all locations, 26% of the total variation was due to inbred lines within subpopulations/locations and 2% was attributed to variation between the five geographic origins of inbred lines. The study identified inbred lines such as TL2012-20, TL2012-24 and TL2012-54 (from cluster I) and TL2012-25, TL2012-21 and TL2012-12 (from cluster III) showing clear genetic difference for hybrid breeding of maize to exploit heterosis.

Keywords: genetic diversity, diverse origins, inbred line, Maize, SSR markers.

4.1 Introduction

Maize (*Zea mays* L.) is one of the most important cereal food crops in the world. It is a monocot, C4 plant, predominantly cross pollinated and cultivated in diverse agro-ecologies (Prasanna, 2012; Li et al., 2014). Maize belongs to the tribe *Maydeae* of the grass family *Poaceae* (*Gramineae*) and consists of chromosome number of 2n=20 (Kumar et al., 2012) whose genetic length is estimated to be 1500cm (Li et al., 2014). Due to cross pollination, maize portrays the highest phenotypic and genetic variability (Parasanna, 2012; Mollin et al., 2013), which determine its wide geographical adaptations (Handi et al., 2013; Li et al., 2014). Global demand for maize is high due to fast increasing population (Linehan et al., 2013) and utilization of maize in various dynamics such as bio-energy production (Miranowski et al., 2011; Ranum et al., 2014) signalling for increased productivity and production (Amar et a., 2011). In view of this, most breeders worldwide, especially in recent years are using molecular technology in assessing genetic diversity and relationships among the available germplasm for improved selection of suitable parental materials for breeding. Thus knowledge on germplasm diversity is fundamentally important for germplasm improvement and ultimately for hybrid breeding (Li et al., 2008; Phumichai et al., 2008; Hu et al., 2009; Singh et al., 2013). It also facilitates accurate classification of breeding materials into specific heterotic groups (Choukan et al., 2006; Bidhendi et al., 2012; Kanyamasoro et al., 2012). Genetic diversity has also been utilized in enhancement of biotic and abiotic stress tolerance and to improve traits such as quality, maturity, and yield potential in maize (Baranek et al., 2006; Choukan et al., 2006; Xie et al., 2010; He et al., 2012; Ramu et al., 2013; Xu et al., 2013). Genetic diversity also offers opportunity for general genetic enhancements in various crops because it provides insight on genetic base for sustained genetic improvement and conservation (Lee et al., 2010; Yadavi and Singh, 2010; He et al., 2012; Semagn et al., 2012; Nikhou et al., 2013; Ramu et al., 2013).

Genetic diversity is defined as the result of variations in DNA sequences which exist within and among crop species (Pagnotta et al., 2009; Huang et al., 2010; Lamia et al., 2010; Wang et al., 2013). This variation is substantial because each of the individual plants in a given crop species has unique DNA sequence (Pagnotta et al., 2009). In conventional breeding, genetic diversity and genetic relationships among maize inbred lines are usually assessed based on morphological data and pedigree records of inbred lines (Baranek et al., 2006; Lee et al., 2010). However, the uses of these descriptors present several limitations because they are confounded by the influence of environment, hence, they often do not portray the exact genetic background or estimates of the germplasm under study (Shetaha et al., 2009; Lee et al., 2010).

This necessitates the use of molecular markers based technology in accurate characterisation of maize inbred lines. Markers are not influenced by factors such as environments, growing seasons or growth stage of the crop (Yao et al., 2007; Wu et al., 2014; Semagn et al., 2012). Inbred lines are important, they form key primary input in maize breeding programmes (Zou et al., 2010; Abera et al., 2012) because they possess numerous attributes or genes for disease resistance and traits of economic importance (Wu et al., 2010b: Chen et al., 2011; Ali et al., 2012; CGIAR, 2012). Successful exploitation of inbred lines in any breeding program requires accurate characterization using molecular and phenotypic markers (Goodman et al., 2008; Yadavi and Singh, 2010; Amar et al., 2011; Kage et al., 2012; Prasanna, 2012; Xu et al., 2013).

Molecular markers have been widely used to determine the genetic diversity present in major crops such as wheat, maize, rice and common beans (Yao et al., 2007; Shetaha et al., 2009; He et al., 2012; Simko et al., 2012; Wang et al., 2013; Zaccardell et al., 2013). The most common molecular markers used to assess the genetic diversity in maize include restriction fragment length polymorphism (RFLP), random amplified polymorphic (RAPD), microsatellite or simple sequence repeats (SSRs), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism SNP (Semagn et al., 2012; Sharma et al., 2010; Molin et al., 2013). The SSR markers are known for their dominant inheritance, locus specificity, extensive genome coverage and simple detection of locus using labeled primers (Wu et al., 2010a, 2010b; Daniel et al., 2012; Xu et al., 2013). Over 400 CIMMYT maize lines have been genetically characterized using SSR markers (Xie et al., 2007; Semagn et al., 2012; Zeid et al., 2012). However, despite the importance of the markers and their wide application in a range of crop species, yet the use of this technology in some countries like Tanzania is limited. In Tanzania, conventional plant breeding relies on phenotypic characterisation. Most breeders still use poorly characterized inbred lines in their breeding programms when developing cultivars. The consequence of which, has been reflected by low farmers' acceptability of newly bred released varieties and have showed significant susceptibility to various crop diseases including the maize streak virus (MSV) caused by *Geminivirus*, leaf blight caused by *Exserohilum turcicum* Pass Leonard & Suggs, grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) and common leaf rust (*Puccinia sorghi* Schr) (Lamia et al., 2010; Parasanna, 2012). Therefore, the objective of this study was to assess the genetic diversity and genetic relationship among 79 maize inbred lines collected from five different origins using 30 polymorphic simple sequences repeat (SSR) markers.

4.2 Materials and methods

4.2.1 Genetic materials and DNA sampling

The experimental materials used in the current are listed in Table 3.1 in the previous chapter except genotype KSO3-OB15-12. About 26.6% of which were collected from CIMMYT/Kenya; 22.8% from Selian Agricultural Research Institute (SARI)-Tanzania; 19.0% from CIMMYT/Zimbabwe; 26.6% from IITA/Nigeria and 5.1% from the University of Kwa-Zulu Natal/South Africa (Table 3.1). These materials were planted in northern Tanzania at Ngaramtoni research site of SARI in 2012 to collect leaf samples. Leaf samples of 5-6 cm long were randomly harvested from 10 young maize plants of about 3 to 4 weeks old and bulked in the 50 cm^3 centrifugal falcon tubes. Sampling was done using a single use 25K size carbon steel (surgical blades) sterilized by GAMMA radiation using GY while DNA extraction was done using the solvent method procedures as described below.

4.2.2 DNA extraction

The leaf samples were stored overnight at -80°C for DNA extraction. About 100mg freeze-dried leaf samples were grinded into fine powder using GenoGrinder-2000 (SPEX Sample Prep, LLC, NJ,USA) at a speed of 500 strokes per minute by shaking for 4 minutes. The samples were grinded for an additional 2 minutes after the addition of 600 µl of freshly prepared modified CTAB DNA extraction buffer. This served to disperse or homogenize the powdered tissue with the extraction buffer. The samples were incubated at 65°C water bath for 30 minutes with continuous gentle shaking. The tubes were then removed and cooled for 5-10 min in a fume hood and subjected to centrifugation at 3500 rpm for 10 min at 15°C. The supernatant was transferred into fresh microtubes and 400 µl chloroform: isoamylalcohol (24:1) was added into the side of the tubes and mixed gently. Samples were shaken for up to 30 minutes at room temperature. The corrosive chloroform was removed carefully using pipette to avoid destruction of DNA in the samples. The aqueous layer was transferred to fresh strip tubes and the chloroform: isoamylalcohol was washed repeatedly to produce a clean DNA solution.

To each sample 300 µl of isopropanol was added and mixed very gently for DNA precipitation while keeping the tubes in the -20°C freezer overnight. The samples were then centrifuged at 3500 rpm for 30 min and the supernatant was discarded to obtain the DNA pellet. Each DNA pellet was further washed with 70% ethanol and centrifuged for 15 min. The supernatant was discarded and traces of ethanol were removed by air dry the DNA pellet for about 15-20min. The DNA was suspended in 150 µl of 10mM Tris-HCl ph 8.3 and the samples were incubated for about 45 min at 45°C water bath with gentle tapping every 10 min. Each samples was

treated with 3 µl RNAse and the RNAse was spanned down with centrifuge (3500 rpm for 1-2 min). The DNA was incubated at 37˚C water bath for 3 hours and finally the DNA extracted was stored in 4˚C fridge for further use.

4.2.3 Genotyping and DNA fragments analysis

The study used 30 SSR markers to genotype 79 maize inbred lines. Markers were selected from the maize genome database (http://www.agron.missouri.edu) based on their degree of polymorphisms and distribution among the maize genome. Genotyping was done using a standard PCR protocol for maize SSR markers (CIMMYT, 2005). The SSR analysis involved preparation of the cocktail mix composed of PCR products, highly deionized (Hi-Di) formamide and GENESCAN 500 internal lane size standard (LIZ-500) labeled with N, N, N', N'-tetramethyl-6-carboxyrhodamine (TAMARA) (Perkin Elmer-Applied Biosystems). The PCR mix was prepared in a total reaction volume of 15µl containing 2µl (50 ng) genomic DNA, 2.5mM magnesium chloride (MgCl2), 0.4mM of dNTPs, 50 ng of each forward and revers primers, 2µl of 1 x reaction buffer, 0.1ml Taq DNA polymerase and sterile water to bring volume to 15 ul. Samples containing 1.2 µl of the PCR products, 1.0 ml (Hi-Di) formamide and 12 µl of LIZ-500 internal lane size standard was denatured at 95^0C for 3 minutes and placed on ice for 5 minutes. DNA samples were electrophoresed on an ABI-3730 automatic DNA sequencer (Applied Biosystems, USA) equipped with GENESCAN 672 software v. 1.2 (PE-Applied Biosystems). The 2 µl of each DNA sample was loaded in the polymerase chain reaction (PCR) and the resultant PCR fragments were resolved on the genetic analyzer, the ABI 3730. A total of 2362 data points was captured out of the expected 2370 data points using the Genscan®software giving an overall success rate of 99.7%.

4.2.4 Data analysis
Genetic diversity analysis

Genetic diversity of 79 maize inbred lines was analyzed using GenAlex version 6.5 (Peakall and Smouse, 2007) software program. The x^2 test was also performed to determine if the allelic frequencies among the 30 SSR markers used were significant. The genetic diversity parameters considered in this study were: the total number of alleles per locus (N_a), the number of effective alleles per locus (N_e), observed and expected heterozygosity denoted by (H_o) and (H_e), respectively. Other genetic parameters estimated were: total gene diversity (H_t), polymorphic information content (PIC), Shonnan's Information Index (I) and fixation index (F) (Nei's, 1978).

The polymorphic information content was estimated according to Smith et al. (1997):$PIC = \sum_{i=1}^{N} \rho i^2$, where *pi* is the frequency of the ith allele.

Genetic distance and cluster analysis

To examine the degree of population differentiation among the study material, the Nei's unbiased genetic distance (Nei, 1978) was estimated using the GenAlex while the genetic relationships or relatedness of 79 sampled inbred lines were estimated using neighbour-joining algorithm using the unweighted pair group method (UPGMA) in DARwin 5.0 software (Perrier and Jacquemoud-Collet, 2006). A dendrogram for 79 inbred lines was then generated based on the dissimilarity matrix to visualize pattern of clusters within and among inbred lines. Further, a principal coordinate analysis was also performed to complement clustering or grouping patterns revealed by the dendrogram. The genetic structure was investigated as described by Nei's (1978) analysis

Analysis of molecular variance (AMOVA)

Analysis of Molecular Variance (AMOVA) was performed to estimate population genetic structure and differentiation among and within the sets of inbred lines based on their geographic locations of origin. AMOVA uses the estimated fixation indexes such as F_{ST}, F_{IS}, and F_{IT} to compare the genetic structure among and within populations. It has potential to apportion the total molecular variances into different sources or populations which attributed to the variations and differentiate the study materials into various clusters for easy management and utilization. The AMOVA procedures were done using GenALex and the effect of spatial separation on genetic structure was tested by the Mantel test (Mantel and Valand, 1970) on genetics matrices (Nei, 1978) between populations.

4.3 Results

4.3.1 Summary statistics of the SSR markers

Polymorphism among the 79 maize inbred lines was investigated using 30 SSR markers. These markers, revealed a total of 140 alleles. The observed number of alleles (N_a) varied from 2 (when using markers umc2250, umc1266 and phi062) to 11 (phi96100) with a mean of 4.7; and the effective numbers of alleles (N_e) detected varied from 1.0 (umc 1266) to 4.7 (phi031) with a mean of 2.4 per locus (Table 4.1). Over 50% of the total (140) numbers of observed alleles in this study was detected by 43.3% of the markers used suggesting the existence of significant polymorphism among the markers. The results further showed that the observed heterozygosity

(H_o) varied from zero (umc2250) to 0.861(phi031) with a mean of 13.6%, being lower than that of expected heterozygosity (50.8%). The expected heterozygosity also varied slightly similar to the observed heterozygosity with values ranging between 0.013 (1266) and 0.793 (phi031). This analysis also showed that the polymorphic information content (PIC) values for all markers, ranged from 0.013 (umc1266) to 0.788 (phi063) (Table 4.1). Again, 46.7% of all loci used manifested PIC values greater than the overall mean of 50.5% indicating that most of the markers used had high polymorphic information content. The most polymorphic loci were phi063, phi96100 and phi063; providing PIC values of 0.788, 0.775 and 0.753, respectively. The results of the χ^2 test showed significant differences in major allele frequencies at all loci for all sets of inbred lines. The total genetic diversity (H_t) varied from 0.026 (umc1266) to 1.648 (phi031) with high mean of 0.641. The Shannon's information index (I) also varied significantly from 0.039 (umc1266) to 1.711 (phi031), with high mean of 0.962. This reflects high genetic differences among the inbred lines evaluated. The fixation index level (F) which measures the level of inbreeding among and within inbred lines varied significantly from -0.093 (phi031) to 1.0 (umc2250) with substantial mean of *F at* 72%, indicating the presence of appreciable levels of homozygosity among the study materials (Table 4.1).

Table 4.1 Genetic parameters of the 30 SSR markers used in the study of 79 maize inbred lines collected from five sources

Marker	N_a	N_e	I	H_t	H_o	H_e	F	PIC
umc 130	4	1.7	1.370	0.026	0.329	0.418	0.207	0.415
phi 014	4	2.3	1.711	0.140	0.190	0.564	0.661	0.560
phi 029	4	2.1	1.454	0.218	0.076	0.519	0.853	0.516
phi 031	7	4.7	0.642	0.230	0.861	0.793	-0.093	0.788
phi 041	5	3.6	1.009	0.345	0.167	0.728	0.770	0.723
phi 046	4	1.5	0.605	0.401	0.089	0.315	0.717	0.313
phi 056	8	2.5	1.371	0.417	0.403	0.607	0.332	0.603
phi 062	2	1.2	1.551	0.424	0.038	0.193	0.802	0.192
phi 063	6	4.1	1.706	0.444	0.165	0.758	0.782	0.753
phi 069	3	2.7	0.825	0.472	0.101	0.635	0.839	0.631
phi 072	5	3.2	0.947	0.520	0.139	0.692	0.798	0.688
phi 075	4	2.0	1.047	0.571	0.076	0.501	0.848	0.498
phi 084	4	1.8	0.818	0.574	0.038	0.437	0.913	0.434
phi 093	4	3.0	0.341	0.592	0.076	0.674	0.887	0.670
phi 112	4	2.0	1.301	0.636	0.025	0.498	0.949	0.495
phi 114	5	3.5	1.362	0.648	0.127	0.720	0.823	0.716
phi 96100	11	4.4	0.781	0.732	0.101	0.776	0.869	0.771
phi 102228	3	1.3	0.039	0.744	0.089	0.258	0.654	0.256
phi 108411	3	1.9	0.906	0.746	0.091	0.483	0.811	0.480
phi 227562	6	2.7	0.269	0.750	0.247	0.639	0.612	0.635
phi 299852	9	3.7	0.808	0.827	0.139	0.732	0.809	0.727
phi 308707	5	3.6	1.292	0.838	0.114	0.729	0.843	0.724
phi 331888	4	2.4	0.965	0.842	0.051	0.589	0.913	0.585
phi 374118	5	2.3	1.278	0.867	0.076	0.575	0.867	0.572
umc 1266	2	1.0	0.980	0.872	0.013	0.013	-0.006	0.013
umc 1304	4	1.6	1.242	0.882	0.051	0.368	0.862	0.366
umc 1367	5	1.2	0.491	0.890	0.051	0.168	0.697	0.167
umc 1917	5	1.5	0.739	0.918	0.101	0.325	0.687	0.323
umc 2047	3	1.6	0.609	1.005	0.051	0.396	0.871	0.394
umc 2250	2	1.2	0.412	1.648	0.000	0.141	1.000	0.140
Overall mean	4.7	2.4	0.962	0.641	0.136	0.508	0.719	0.505
SE	0.4	0.2	0.078	0.317	0.030	0.039	0.049	0.210

N_a = number of observed alleles, N_e = Number of effective alleles, **I** = Shannon's Information Index, H_t = Total gene diversity, H_o = Observed heterozygosity, H_e = Average gene diversity within genotypes, **F**= Fixation index, **PIC**= Polymorphic information content.

4.3.2 Cluster and principal component analyses

The dendrogram discriminated and clustered the genotypes into three major clusters (clusters I, II and III) with few sub-clusters in cluster II (Figure 4.1). The distribution of the inbred lines into these three main clusters was not homogeneous. Cluster I consisted of 12 inbred lines that is 15.2% of the total number of all genotypes evaluated, and were genetically diverse within the cluster. Most of inbred lines contained in this cluster were from SARI/Tanzania, CIMMYT/Kenya, CIMMYT/Zimbabwe and UKZN/South Africa in order of magnitude but no genotype was found from IITA/Nigeria entries. Cluster II encompassed 36 (45.6%) of the total inbred lines studied. This cluster was broadly divided into two sub-clusters (IIA and IIB) (Figure 4.2). The IIA consisted of 22 (27.8%) genotypes of the total study materials but it was further subdivided into two small clusters IIA-1 and IIA-2, respectively. Each of these sub-clusters IIA-1 and IIA-2 consisted of 11 genotypes, which were relatively similar. Sub-cluster IIB consisted of 14 (17.2%) inbred lines, which were fairly diverse. Most inbred lines in cluster II were from IITA/Nigeria, CIMMYT/Kenya and SARI/Tanzania only. Cluster III comprised of highly variable, 31 inbred lines (Figure 4.2). Most of these inbred lines in this cluster were from SARI/Tanzania, IITA and CIMMYT/Zimbabwe, IITA/Nigeria, and SARI/Tanzania in order of magnitude. CIMMYT/Kenya and UKZN/South Africa had few genotypes in this cluster. Moreover, a principal coordinate analysis (PCA) constructed to examine genetic clustering of all 79 inbred lines using the genetic distances (Figure 4.2) explained a total of 20.4% of genetic variation of the data. It also discriminated the 79 inbred lines into two major clusters only.
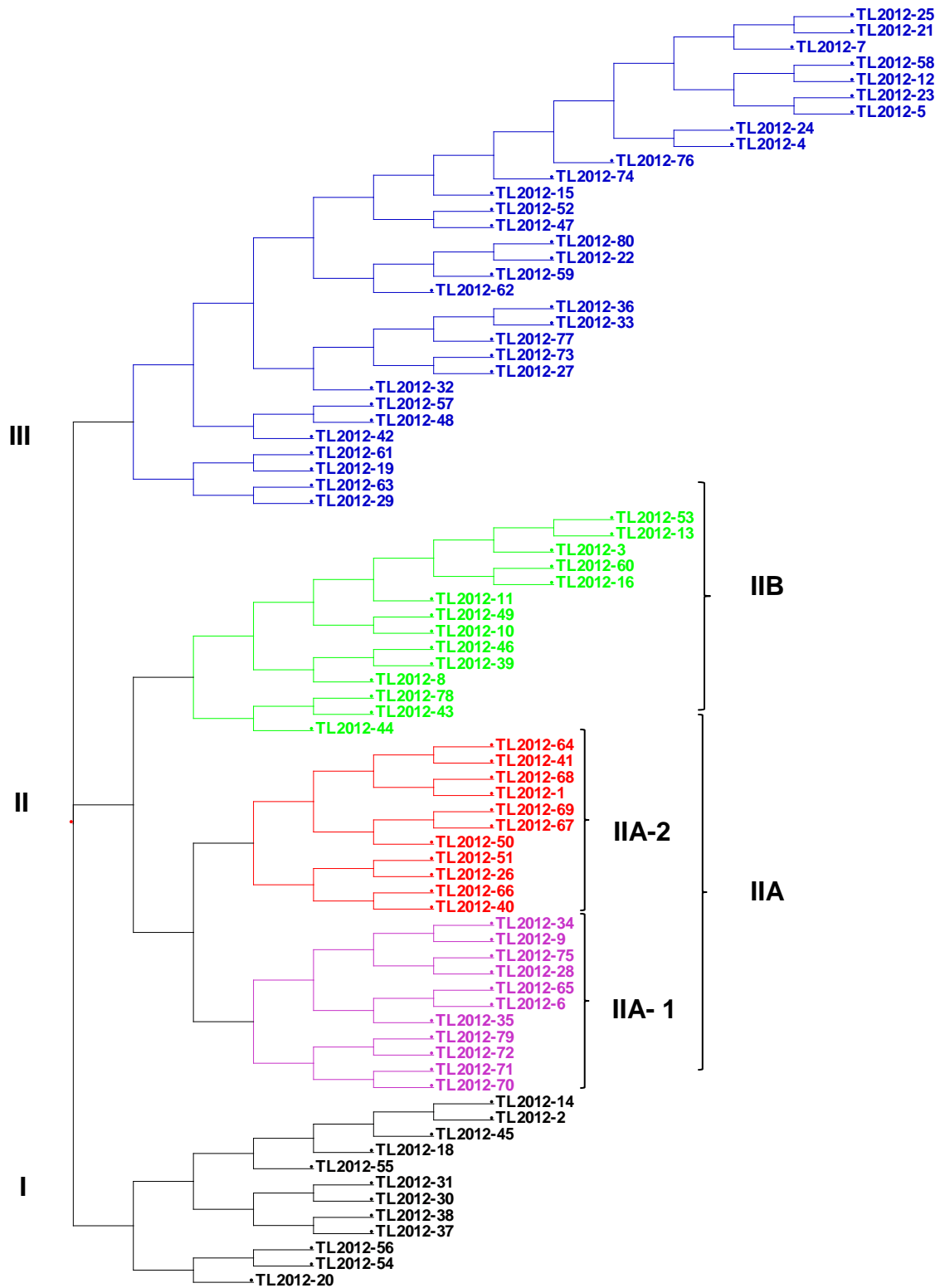
Figure 4.1: Dendrogram showing the genetic relationship of 79 maize inbred lines using 30 SSR markers
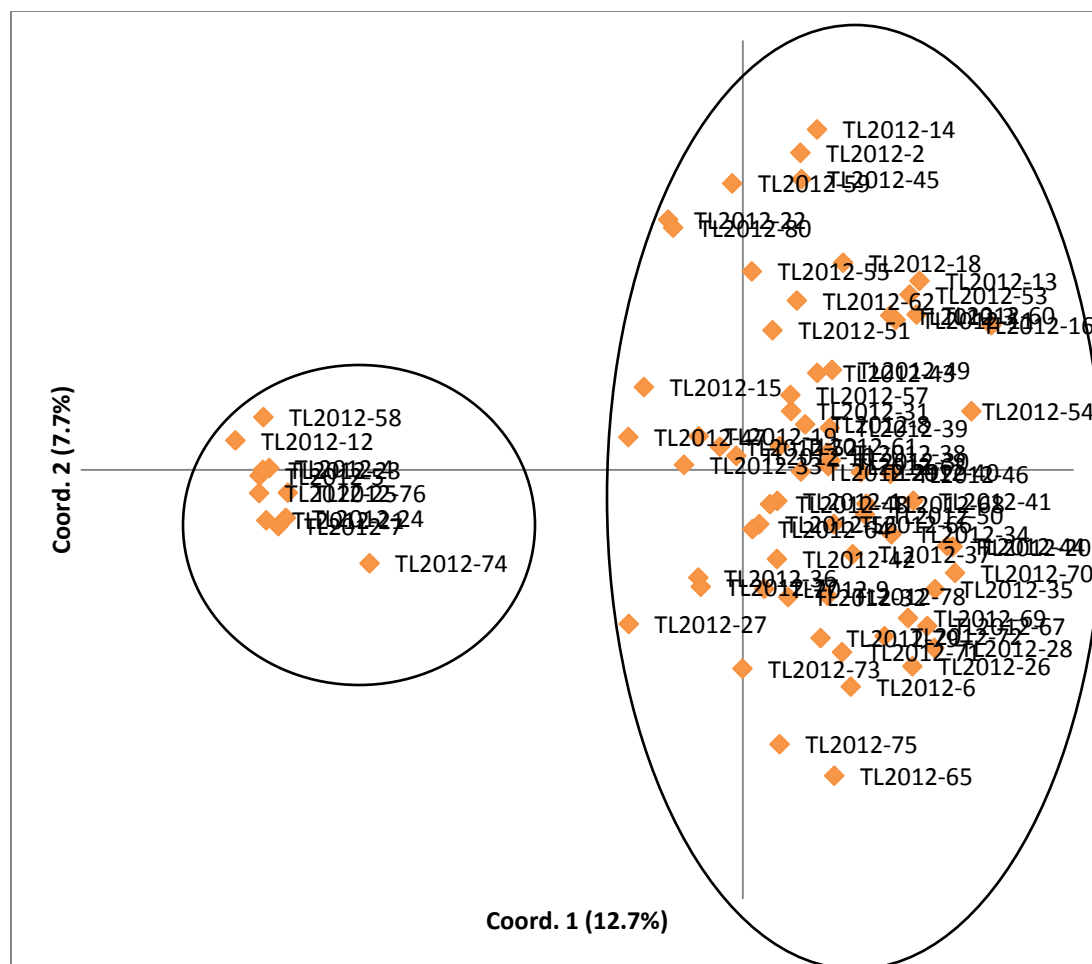
Figure 4.2: Principal coordinate analysis showing genetic grouping of 79 maize inbred lines assessed by 30 SSR markers

4.3.3 Genetic variability/structure of inbred lines based on their regions of origins

Analysis of molecular variance (AMOVA) revealed highest percentage of polymorphic information of the SSR markers in all of the five populations. The inbred lines from SARI/Tanzania and IITA/Nigeria showed highest (96.7%) of polymorphism and those from UKZN/South Africa showed the least (Table 4.2). The overall mean was 92.7% indicating that 7.3% of polymorphism was lost or missing.

Shannon's information index fluctuated among all populations and had a mean value of 0.82. The lowest value of 0.61 was recorded by the inbred line populations from South Africa (UKZN) and highest value of 0.92 from IITA, Nigeria (Table 4.2). The average values of observed (N_a) and effective (N_e) number of detected alleles across all populations were 3.26 and 2.20,

respectively. The lowest values 2.1 and 1.8 for the same parameters respectively were recorded by the inbred line populations from South Africa. Similarly, the highest values of these parameters (Na and Ne) were found to be 3.8 and 2.4, recorded in IITA, Nigeria respectively. Both observed heterozygosity (H_o) and expected heterozygosity (H_e) were 3% and 5% respectively across all populations, with overall mean values of 0.136 and 0.48. Their respective lowest values were 0.12 and 0.45, recorded in CIMMYT/Kenya and South Africa. The highest value of H_o = 0.15 was recorded by IITA/Nigeria, and *He* was the highest (0.50) among inbred lines collected from Tanzania and IITA/Nigeria (Table 4.2). The F- statistics or fixation indexes estimates of genetic differentiation (F_{ST} = 0.022), coefficient of inbreeding among inbred lines within regions of origin/subgroups (F_{IS} = 0.733) and heterozygosity indicator of individual inbred lines over the total population (F_{IT} = 0.738) were highly significant (P<0.001).

Table 4.2 Genetic diversity of 79 inbred lines among five regions of origins

Regions of origin	N	Na	Ne	I	Ho	He	%Polymorphism
				Genetic parameters			
CIMMYT/Kenya	20	3.6	2.3	0.88	0.12	0.49	93.3
IITA/Nigeria	22	3.8	2.4	0.92	0.15	0.50	96.7
South Africa	4	2.1	1.8	0.61	0.14	0.45	86.7
Tanzania	17	3.5	2.3	0.89	0.14	0.50	96.7
CIMMYT/Zimbabwe	16	3.3	2.2	0.81	0.13	0.47	90
Mean	15.8	3.26	2.2	0.82	0.136	0.48	92.7
SE	0.51	0.12	0.08	0.034	0.015	0.018	1.9

N = Population size, **N$_a$** = number of observed alleles, **N$_e$** = Number of effective alleles, **I** = Shannon's Information Index, **H$_o$** = Observed heterozygosity, **H$_e$** = Average gene diversity within genotypes, **F**= Fixation index, **PIC**= Polymorphic information content.

4.3.4 Analysis of molecular variances (AMOVA) and fixation index estimates

The analysis of molecular variance (AMOVA) partitioned the total molecular variances within and among the sets of inbred lines evaluated based on their geographic regions of origin. About 72% of the total genetic variation was attributed to variation among inbred lines within regions of origins, while 26% of the total variation was explained by variation within inbred lines and 2% of total genetic variation was explained by variation between regions of origins (Table 4.3). This

gives an indication that the origins of inbred lines had little contribution to the total molecular variances detected and that the highest variation explained by variation among the inbred lines.

Table 4.3 Analysis of molecular variance (AMOVA) among 79 maize inbred lines assembled from five geographic origins using 30 SSR markers

Source of variation	DF	SS	MS	EVAR	Variation (%)
Among geographic origins/regions	4	73.1	18.3	0.2	2
Among individual lines within regions	74	971	13.1	5.6	72
Within individual lines	79	160	2	2	26
Total	157	1204.1	33.4	7.7	100

DF= Degree of freedom, SS= sum of squares, MS= mean sum of squares, EVAR= estimated variance,

4.3.5 Genetic correlation of inbred lines among their regions of origins

The pair-wise correlation coefficient estimates of some selected parameters showed that genetic differentiation, F_{ST}, ranged from 0.03 (IITA/Nigeria and CIMMYT/Kenya) to approximately 0.08 (UKZN/South Africa and CIMMYT/Zimbabwe) (Table 4.4, above diagonal within brackets). The low variability of F_{ST} imply that there is high frequency of identical alleles among inbred lines between regions of origins, hence, low genetic differentiation of inbred lines among regions. Gene flow (Nm) or gene migration coefficient varied considerably among the regions of origins of the inbred lines with high value of 1.79 in SARI/Tanzania and CIMMYT/Zimbabwe to 6.0 in Tanzania and CIMMYT/Kenya (Table 4.4, above diagonal) showing that allele or gene transfer between inbred lines among regions of origins was high, this could be caused by exchange of genetic materials. The genetic distance (GD) of inbred lines among regions of origins were small, ranging from 0.03 (UKZN/South Africa and IITA/Nigeria) to 0.08 in CIMMYT/Zimbabwe and SARI/Tanzania), (lower diagonal within brackets) suggesting that there is substantial genetic relationships of inbred lines despite the fact that they originated from different regions. Similarly, the genetic identity (GI) also varied from 0.92 to 0.97 (lower diagonal). This concludes that most of the inbred lines obtained from different sources were closely related.

Table 4.4 Pair-wise estimates of gene flow (Nm) (above diagonal, without brackets), genetic differentiation, F_{ST} (above diagonal); genetic distance GD (lower diagonal without brackets) and genetic identity, GI, (lower diagonal)

Regions of origins	CIMMYT/ Kenya	IITA/Nigeria	UKZN/South Africa	SARI/Tanzania	CIMMYT/ Zimbabwe
CIMMYT/Kenya		4.593(0.030)	5.641(0.058)	6.003(0.028)	2.270(0.053)
IITA/Nigeria	0.963(0.038)		5.767(0.048)	4.361(0.031)	4.061(0.027)
UKZN/South Africa	0.954(0.047)	0.971(0.030)		4.288(0.055)	2.114(0.075)
SARI/Tanzania	0.970(0.031)	0.957(0.044)	0.962(0.039)		1.785(0.055)
CIMMYT/Zimbabwe	0.924(0.079)	0.974(0.026)	0.928(0.074)	0.920(0.084)	

Nm = gene flow = 0.25 (1-F_{ST})/F_{ST}, CIMMYT= International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, UKZN = University of KwaZulu Natal and SARI= Selian Agricultural Research Institute

4.4 Discussion

Genetic diversity among 79 maize inbred lines as revealed by SSR markers

The thirty SSR markers used in this study revealed a total of 140 observed numbers of alleles, with an average of 4.7 (Table 4.1). This result is in agreement to the report of Gichuru (2013), who reported a total number of observed alleles at 135 with a mean of 4.8 during a characterization study of 40 maize inbred lines for their resistance to MSV using 28 SSR markers. Mora et al. (2013) also reported a mean of observed number alleles (N_a = 4.8) when analyzing the genetic structure of a Brazilian popcorn germplasm using SSR markers. The large mean of the expected heterozygosity (H_e = 0.508), gene diversity (H_t = 0.641), effective number of allele per locus (Ne = 2.41) and PIC (0.505) detected by the 30 SSR markers in this study reflects the high level of genetic diversity present among the maize inbred lines used. Similar results were also reported by Rupp et al. (2009) in their study of genetic structure and diversity among sweet corn (*su1*-germplasm) progenies using SSR markers. They identified mean heterozygosity (H_e) 0.509 and effective number of alleles (N_e = 2.16) per locus which are consistent to the present findings. In general, the high level of genetic diversity identified in the current work will assist maize breeders to set out their breeding objectives and to select potential parents to be used in their breeding programmes. Xu et al. (2013) reported that genetic diversity if well managed can be used to enhance biotic and abiotic stress tolerance in any crop

species. Also, Kanagarasu et al. (2013) added that successful adaptation to certain agro-climatic conditions and improvement of any crop species depends on the availability of genetic diversity within the available breeding material. Hence, molecular characterization of the available germplasm would facilitate development of high yielding maize varieties and will improve the limitations present in conventional breeding systems. Information about the genetic diversity and relationships among diverse genetic resources is very valuable in crop improvement programmes and for strategic conservation of genetic resources (Abera et al., 2012; Kage et al., 2013; Wu et al., 2014).

Polymorphism and discrimination power of SSR markers

The polymorphism information content (PIC) demonstrates the informativeness of the SSR loci used. The PIC values ranged from 0.013 to 0.788, with an overall mean value of 50.5% indicating that the chosen markers have high levels of polymorphism. The average PIC value determined in this investigation agrees with the earlier findings reported by Oppong et al. (2014), who showed an average PIC value of 0.504 in bulk genetic characterization of Ghanaian maize landraces using microsatellite markers. Moreover, some markers showed significant discrimination than others in this study, for example, umc1367 and phi 041 both detected the same number of alleles (N_a = 5), but they had different PIC values (0.167) and (0.723), respectively. This implies that marker phi041 has higher discriminatory capability or is more informative than umc 1367 (Table 4.1). Discriminatory power of a locus depends on many factors such as rate of amplification, length of repeat and detection ability of loci. Missing values in the dataset could also affect differently the polymorphism and hence discrimination ability of markers.

 Cluster analysis and relationships of inbred lines

The dendrogram (Figure 4.1) grouped the inbred lines into three major clusters (I, II and III) using the UPGMA algorithm on genetic distance, which is an indication that substantial genetic variability exist among the study materials. Grouping of individual inbred lines into different clusters is very important because it facilitates formation of various heterotic groups which are useful in hybrid breeding. For example, the first five genotypes coded as TL2012-29, TL2012-63, TL2012-19, TL2012-61 and TL2012-42 in cluster III fall in the same heterotic group (genetically similar) relative to genotypes TL2012-25, TL2012-21, TL2012-53, TL2012-12 and TL2012-23, also in cluster III. Therefore any cross made between the two groups will produce superior yield but not crosses made from within groups. This is the potential of assessing genetic diversity and grouping individual germplasm into different specific heterotic groups.

Classification of inbred lines into different or genetically dissimilar (heterotic) groups facilitates exploitation of heterosis in maize and has been widely studied (Abera et al., 2012; Bidhendi et al., 2012; Kanyamasoro et al., 2012; Wu et al., 2014). Cluster analysis with the dendrogram showed that the inbred line coded by TL2012-20 from cluster I (Figure 4.1) was identified with highest difference in genetic diversity relative to TL2012-25, TL2012-21, TL2012-53, TL2012-12 and TL2012-23 all from cluster III, which means any possible crosses made between this line with other lines would produce significant measurable yield due to hybrid vigour or heterosis.

Genetic structure and differentiation of 79 inbred lines based on geographic regions of origins

Analysis of molecular variance (AMOVA) was performed to estimate or quantify variations within and among the sets of populations based on their geographic origins. The results showed that percentage of polymorphism among the populations varied with a mean of 92.7%. The F statistics showed low but significant genetic differentiation, F_{ST} = 0.022 relatively similar to 0.017 reported by van Heerwaarden et al. (2010). This implies that genetic differentiation among the inbred lines does exist but at low level. The pair-wise correlation estimates of F_{ST} ranged between 0.03 and 0.08, while those of Nm ranged from 1.79 to 6.0 (Table 4.5) indicating very little genetic isolation. Kashiani et al. (2012) reported strong genetic isolation when characterizing tropical sweet corn inbred lines using microsatellite markers. They reported mean values of 0.96 and 0.01 of genetic differentiation (F_{ST}) and gene flow (Nm), respectively. The low level of genetic differentiation or high gene flow of the inbred lines with respect to their geographic regions of origins is attributed to the exchange of genetic materials between CIMMYT and member countries for germplasm evaluation, breeding and release.

4.5 Conclusions

This study demonstrated the presence of genetic diversity among maize inbred lines adapted to the mid altitude maize growing conditions of northern Tanzania and exotic inbred lines using 30 SSR markers. The microsatellites were found to be informative because they revealed the total genetic variations that existed among the inbred lines studied. The variations were mostly due to differences between inbred lines, showing the presence of varied heterotic groups, which help in selection of best parents for further breeding. Overall, inbred lines such as TL2012-20, TL2012-24 and TL2012-54 (from cluster I) and TL2012-25, TL2012-21 and TL2012-12 (from cluster III) were identified from varied genetic groups showing clear genetic differences useful in hybrid breeding of maize to exploit heterosis. Although the use of molecular markers offers good

identification and selection of best genotypes in breeding programs yet challenges such as low availability and access to analytical tools is low in some countries like Tanzania. This may reduce research progress and its expected impacts.

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CHAPTER FIVE:

Combining ability and heterosis among maize genotypes for yield and yield components and resistance to maize streak virus disease

Abstract:

Combining ability analysis of maize (*Zea mays* L.) inbred lines and their hybrids are essential to develop novel recombinants or hybrid varieties to exploit heterosis. The objective of this study was to determine combining ability and heterosis for grain yield and related traits and resistance to maize streak virus (MSV) among 10 elite maize inbred lines and their hybrids when tested across six environments in Tanzania. Ten inbred lines were crossed and 45 F_1 hybrids developed using a 10x10 half diallel mating design. Parents, F_1 hybrids and five standard checks were evaluated using a 6 x 10 lattice design with two replications at Ngramtoni, Inyala and Igomelo during 2012/13 and 2013/14. General combining ability (GCA) of parents, specific combining ability (SCA) of hybrids, heritability and heterosis of grain yield and related traits and MSV resistance were calculated. The mean squares of GCA and SCA effects showed significant differences for all the traits except days to 50% anthesis and silking. The SCA effect was important for all traits except for MSV, number of eras per plant and husk cover while the GCA effect was most important for resistance to MSV. Heritability estimates of traits were high associated with high GCA effects. Line TL2012-42 was a good general combiner for grain yield showing highly significant positive GCA effect of 0.695 t/ha whileTL2012-41, TL2012-1 and TL2012-42 had significant negative GCA effects of -10.926%, -10.792% and -10.748%, respectively for MSV reaction. These inbred lines could be exploited in hybrid breeding to develop high yielding and MSV resistant varieties. Hybrid TL2012-7/TL2012-38 had highest positive SCA effect of 4.803 t/ha while TL2012-38/TL2012-55 and TL2012-25/TL2012-26 had negative significant SCA effect of -10.892 and -19.451%, respectively for MSV reaction which were in a desirable direction. Maximum mid-parent heterosis for grain yield was recorded in hybrid TL2012-7/TL2012-38 at 138% while TL2012-25/TL2012-26 had the lowest and negative heterosis of -38.2% for MSV reaction. Crosses TL2012-7/TL2012-42 and TL2012-7/TL2012-68 had significant positive SCA effects for grain yield which can be used for direct production as single cross hybrids or developed further as three way hybrids for large scale production.

Keywords: *Combining ability, Diallel analysis, GCA, Heterosis, inbred lines, maize, maize streak virus, SCA.*

5.1 Introduction

Maize (*Zea mays* L.), is one of the most important cereal crops with the highest yield potential per unit area. It is grown throughout the world (Langyintuo et al., 2010; Ranum et al., 2014) with the United States, China and Brazil contributing to 31, 24 and 8% of the world total production, respectively (M'mboyi et al., 2010; FAO, 2012; Edmeades, 2013). It is the most important staple food crop supporting the livelihoods of more than 1.2 billion people in sub-Saharan Africa (Cains et al., 2013). In Tanzania, maize is one of the food security crops consumed by approximately 45 million people. In the country, it is grown in all the seven agro-ecological zones covering a total area of two million hectares. Maize occupies about 45% of the total land area allocated to food crops in Tanzania. It is a major source of income for smallholder farmers who constitute the majority of Tanzanian population (M'mboyi et al., 2010; Kage et al., 2013). Also, it substantially contributes to the national economy due to its outstanding share in the global trade (FAO, 2012) owing to the growing demand for food, feed, bio-energy production and other industrial uses (M' mboyi et al., 2010; Lineham et al., 2014; Ranum et al., 2014).

Despite the significance of maize in Tanzania and other many countries of sub-Saharan Africa region, its yield has remained low (<2.2 t/ha) (Temu et al., 2011, Barreiro-Hurle, 2012). Low yield in Tanzania is attributed to stress factors such as foliar leaf diseases (MSV, MLN, GLS, NLB, and common rust), random stresses and poor soil fertility (Bucheyeki, 2012; Meseka et al., 2013). This requires genetic improvement of maize germplasm to identify novel genotypes with high grain yield and resistant to multiple disease and pests (Williams et al., 2011; Ali et al., 2012; Mengesha, 2013; Sibiya et al., 2011; Ding et al., 2014; Mrutu et al., 2014).

Establishing the genetic relationships among maize inbred lines and their crosses is crucial in hybrid breeding programs (Sher et al., 2012; Khalid et al., 2013; Li et al., 2013). Parents with significant general combining ability (GCA) effect and crosses with high specific combining ability (SCA) effects are selected for breeding (Balestre et al., 2011). The variance for the GCA effect is associated with additive genetic effect while that of SCA is related with non-additive genetic effect arising largely from dominance and epistasis (Falconer and Mackay, 1996; Khalid et al., 2013). Diallel mating design has been widely used in plant breeding programmmes to determine general and specific combining ability effects (Griffing, 1956; Musila et al., 2010; Lou et al., 2011; Ze-su et al., 2012; Ketthaisong et al., 2014). The diallel analysis is useful to select superior parents for hybrid formation (Mostafavi et al., 2012), identify experimental hybrids, and to assign inbred lines into new heterotic groups (Blank et al., 2012; Ze-su et al., 2012; Fan et al.,

2014; Ketthaisong et al., 2014; Mrutu et al., 2014). Data from diallel can be easily analyzed and interpreted in genetic concept (Blank et al., 2012; Lv et al., 2012; Mostafavi et al., 2012).

Heterosis or hybrid vigour predicts the value of a hybrid variety relative to its parental inbred lines (Goff and Zhang, 2013; Singh et al., 2014). Heterosis is often associated with improved performance of traits of economic importance (Ali et al., 2012; Ding et al., 2014). Two types of heterosis are known: mid-parent or better-parent heterosis. Mid-parent heterosis is an increase in a given character of the hybrid compared to the mean of the parents. Better-parent heterosis is an increase in the character of the hybrid compared to that of the better-parent for the character (Falconer and Mackay, 1996; Marcon et al., 2013; Goff and Zhang, 2013; Singh et al., 2014). Heterosis is regarded as the driving factor that contributed to a remarkable success of the commercial hybrid maize industry in the world (Stuber, 1992; Marcon et al., 2013). Overall, information on genetic variability and genetic relationship among inbred lines and their crosses remains important for hybrid maize development in Tanzania or other target production environments (Prasanna, 2012; Welsh and McMillan, 2012; Oloyede-Kamiyo et al., 2014).

Among the biotic stresses, MSV is the most destructive viral disease of maize which can cause yield losses reaching up to 100% in susceptible varieties (Martin and Shepherd, 2009; Karavina, 2014). The MSV disease is cosmopolitan than any other maize diseases in the world (Olaoye, 2009; Gichuru et al., 2011). Diseased plant manifests pronounced continuous parallel chlorotic streaks on leaves, with severe stunting which usually fails to produce complete cobs or seed set. According to Martin and Shepherd (2009), mildly infected maize plants show 25% less seed set than healthy plants.

MSV is found in all places in Africa where maize is growing due to the presence of its vectors, the leaf hoppers (*Cicadulina mbila*, Naunde). The epidemiology of this disease is erratic; but it is influenced mostly by high temperatures, moisture and availability and population build up of its vectors (Martin and Shepherd, 2009; Antwerpent et al., 2011; Oppong, 2013). High incidences of the MSV disease has been reported in Kenya, Tanzania, Uganda, Zambia, Mozambique, South Africa, Nigeria, Cameroon and in the Island of La Reunion (Olaoye, 2009; Shepherd et al., 2010; Gichuru et al., 2011; Karavina, 2014). Globally MSV is ranked the third devastating foliar diseases of maize after NLB and GLS (Martin and Shepherd, 2009).

Various integrated management options are recommended to minimize the damaging effects of the MSV disease under farmers' field conditions. These include: 1) the use of cultural practices such as early planting as an avoidance mechanism. However, this does not always hold

effective because of delayed rains during the cropping season, 2) the use of insecticides such as carbofuran to control the vector. This option is associated with high costs to resource poor farmers and has negative impact to the environment and users and 3) development and use of MSV resistant cultivars. This is probably the most effective, economic and environmentally friendly method of minimizing epidemics (Shepherd et al., 2010; Gichuru et al., 2011).

Breeding for resistance to MSV disease has been actively pursued by CIMMYT and a number of national breeding programs for the past 30 years including Tanzania. In Tanzania, studies on MSV resistance commenced some 10 years ago focusing open pollinated varieties only. However, these varieties are low yielders and most of them succumbed to MSV disease.

Genes conferring resistance for the MSV disease and their inheritance have been identified in maize germplasm (Gichuru et al., 2011). Mafu (2013) working on CIMMYT inbred lines identified CML505 and CML509 to be resistant to MSV disease using single nucleotide polymorphisms (SNPs) DNA markers. These genetic resources can be exploited by breeders to develop cultivars with enhanced resistance to MSV disease. Therefore there is a need to embark on development of high yielding and MSV resistant maize hybrids in order to enhance maize productivity in MSV disease prone areas of northern Tanzania using newly identified sources of resistance. The objective of this study was to determine combining ability and heterosis for grain yield and related traits and resistance to maize streak virus (MSV) among 10 elite maize inbred lines and their hybrid progenies when tested across six environments in Tanzania. Good combiners may be used for direct production or their genes incorporated in resistance breeding programs to minimize yield losses incurred by the MSV disease in Tanzania.

5.2 Materials and methods

5.2.1 Plant material, mating design and trial management

The study used ten inbred lines (Table 5.1) selected in the preceding study. Lines were crossed using a half-diallel mating design to generate 45 F1 hybrids. Hybrids, inbred parents and five standard check three-way hybrids largely grown in Tanzania were evaluated at Ngramtoni, Inyala and Igomelo during 2012/13 and 2013/14 representing six environments. A 6 x 11 unbalanced lattice design with two replications was used to evaluate 60 entries. Each plot consisted of 2 rows of 5.0 m length, with 75 cm and 30 cm spacing between and within rows respectively. Seedlings were thinned after two weeks keeping a healthy and vigorous plant per hill. Di-amonim phosphate (DAP) of 150kg ha^{-1} P_2O_5 was used at planting and the same rate of

Calcium Amonium Nitrates (CAN) was top dressed six weeks after planting. Trials in the first season were established in December across all sites while in the second season it was done in December for Ngaramtoni, January for Igomelo and February for Krishna trials at Babati district. Trials were conducted during the main cropping season under rain fed conditions. Supplemental irrigation was applied as required. The present study sites are known hot spot areas of MSV infection allowing reliable scoring of MSV reaction under natural disease infection.

Table 5.1 List of parental inbred lines used in the study, their yield potential and MSV reaction

SN	Pedigree	Code	Origin [a]	YP[+] (t/ha)	Reaction type
1	09MAK1-77	TL2012-55	UKZN/South Africa	1.97	Resistant
2	V457-1-VLO835	TL2012-17	SARI/Tanzania	2.76	Susceptible
3	CML390	TL2012-41	CIMMYT/Kenya	2.46	Moderate resistance
4	CML505	TL2012-1	CIMMYT/Kenya	0.60	Resistant
5	WPopX1368 STR S7 Inb.6	TL2012-26	IITA/Nigeria	2.08	Moderate resistance
6	MAS[MSR/312]-119-5-1-1-3-B	TL2012-25	CIMMYT/Zimbabwe	2.19	Resistant
7	P43-1-1-1-BBB	TL2012-38	SARI/Tanzania	1.63	Moderate resistance
8	SML125	TL2012-42	SARI/Tanzania	3.52	Resistant
9	CML509	TL2012-68	CIMMYT/Kenya	1.89	Resistant
10	MAS[MSR/312]-119-5-1-4-1-BB	TL2012-7	CIMMYT/Zimbabwe	1.07	Moderate resistance

[a]SARI = Selian Agricultural Research Institute; CIMMYT= International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture; UKZN=University of KwaZulu-Natal; [b]See YP= Yield potential of inbred lines from Table 3.4.

5.2.2 Data collection

Data collected included grain yield, MSV disease severity, maize lethal necrosis (MLN) and other agronomic traits of economic importance. Grain yield (YLD) per plot was measured at 12.5% moisture content and later converted into t/ha. The MSV incidence was collected as percentage of diseased plants per plot while disease severity was measured using a visual scale of 1-5, where 1 indicates as highly resistant and 5 highly susceptible (Kyetere et al., 1999). Disease assessment was taken from two weeks after germination and continued in the interval of 8 days up to flowering. Days to 50% anthesis (DA) and silking (DSL) were obtained by counting the number of days from planting to when 50% of the plants in each experimental plot attained anthesis or silking. Plant height (PHT) was measured from the ground level to the

first tassel branch and expressed in centimetres (cm); ear height (EHT) was measured as a distance from ground level to the upper most ears bearing node and expressed in centimetres (cm). The last two variables were determined from 10 randomly selected and tagged plants from each experimental plot and measured at 50% anthesis. Also the number of eras per plant (EPT) was counted while husk cover (HSC) was assessed using a visual scale of 1-5; where 1 designated very short husks and 5 very long as the best husk cover of cob

5.2.3 Data analysis

Data were subjected to a half-diallel analyses following model I method II described by Griffings (1956) using SASprogram version 9.3 (SAS Institute, 2012). The total sums of squares were partitioned into replication, environment, genotypes and genotypes x environment interaction. The sum of squares of genotypes were further portioned into general and specific combining ability effects using the following model: $Y_{ij} = \mu + gi + gj + Sij + (1/r)\sum_k \varepsilon ijk$. Where $i = j = 1, ..., p; k = 1, ..., r,$; μ = population mean. The term Y_{ij} = observed entry mean of the ith and j[th] genotypes gi = the general combing ability effects of ith parent and gj= the general combining ability of the jth parent. The Sij= the specific combining ability of the cross between i[th] and j[th] parents, such that slj=slji, and $\sum_k \varepsilon ijk$= the environmental effects associated with the observation ijk[th]

Estimation of general combining ability (GCA) and specific combining ability (SCA) effects, heritability and mid-parent heterosis

The general combing ability (GCA) of inbred lines and specific combining ability (SCA) among the crosses were estimated using a general linear model (GLM) procedure ofs SAS software version 9.3 (SAS Institute, 2003) whereas their relative importance, measured by GCA to SCA ratio was calculated according to Baker (1978) as follows $\frac{2MS_{GCA}}{2MS_{GCA}+MS_{SCA}}$; where MS_{GCA} and MS_{SCA} were the mean squares for GCA and SCA, respectively. The mid parent-heterosis (MPH) for grain yield and MSV reaction was also estimated based on the method described by Falconer and Mackay (1966): $MPH = \left[\frac{F1-MP}{MP}\right] X100$; where F1=Mean performance of F1 hybrid, MP = (P1+P2)/2 in which P1 and P2 are the means of the inbred lines involved the cross. The narrow sense heritability was calculated according to the formula proposed by Hallauer et al (2010); $h^2{}_n = \left[\frac{GCA}{GCA+SCA+error}\right] X100$; where $h^2{}_n$ = narrow sense heritability, GCA and SCA are general and specific combining ability, respectively.

5.3 Results

5.3.1 Analysis of variance

The combined analysis of variance (ANOVA) revealed significant differences among crosses and inbred parents (Table 5.2). The GCA and SCA effects for grain yield and MSV reaction were highly significant (Table 5.2). Records of mean squares (MS) of GCA and SCA for most of traits studied suggests that both additive and dominance effects were important. The relative importance of GCA and SCA, measured by Baker's ratio showed that about 51.1% of SCA effects determined grain yield (YLD) expression while GCA effects contributed approximately 48.9% only (Table 5.2; Figure 5.1). About 97.7% of GCA effects determined the expression of MSV reaction among study materials whereas the effects attributed to SCA was only 2.3%. In contrast, the influence due to dominance gene effects on ear height was at 98.3% while an additive gen effect was only 1.7%. This result also showed that plant attributes such as plant height (PHT), ears per plant (EPT) and husk cover of cob (HSC) were mainly controlled by additive gene effects compared to dominance effects as reflected by their respective ratios of 62.3%, 79.6% and 70.8%, respectively. However, dominance gene influence was prevailed more than 50% on days to anthesis and silking. Preponderance of GCA variance demonstrates the role of additive gene effects while the predominance of SCA denotes the high influence of dominance gene action. The narrow sense heritability varied significantly among the experimental materials for the traits considered. Heritability was the highest (95%) for MSV reaction, followed by EPT (61.3%). However, grain yield and other agronomic traits demonstrated low heritability below 50% (Table 5.2).

Table 5.2 Combined analysis of variance of grain yield, yield components and MSV reaction of maize genotypes evaluated across six environments.

Source of Variation	DF	Mean squares							
		YLD	MSV	PHT	EHT	EPT	HSC	DA	DSL
Replication (within E)	6	0.16	31.02	4396.86	35.17	0.46	0.45	1.32	3.29
Environments (E)	5	360.79***	545.70***	19321.83**	1361.13***	11.01***	9.37***	567.57***	539.90***
Genotype (G)	54	30.51***	4416.22***	17010.05***	4661.01***	1.79***	4.23***	71.58***	112.69***
GXE interaction	270	3.41***	157907.67***	5986.716*	1269.41***	0.71***	2.20***	36.40***	37.22***
GCA	9	16.14***	20802.20***	14835.02**	32.85***	2.87***	4.56***	30.60ns	40.51ns
SCA	45	33.75***	1001.23***	17921.590***	3896.76***	1.47***	3.77***	79.80***	118.30***
Error	324	0.71	88.65	4942.79	1219.92	0.34	0.8667	3.09367	5.68906
Total	982	450.86	39814.06	4259407	19075.19	10.99	30.29	790.36	857.6
h^2_n (%)	-	31.9	95.0	39.4	0.6	61.3	49.6	27.0	24.6
GCA/SCA (Baker ratio) %	-	48.9	97.7	62.3	1.7	79.6	70.8	43.4	40.6

*, **, and *** denote significance differences at P≤0.05, P≤ 0.01; and P≤0.001, respectively; DF=Degree of freedom; YLD= Grain yield (t/ha); MSV=MSV disease reaction in %; PHT=Plant height in cm; EHT= Ear height in cm; EPT= Number of ears per plant; HSC= Husk cover of cob; DA= Days to 50% anthesis; DSL= Days to 50% silking; h^2_n is narrow sense heritability
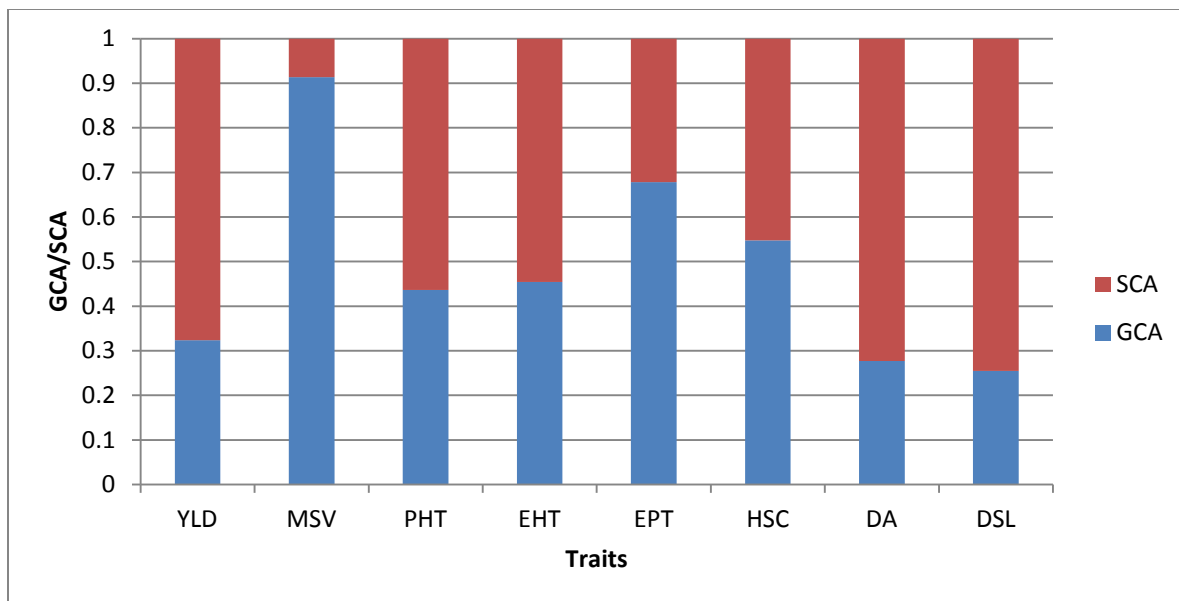
Figure 5.1: The ratio of GCA to SCA effect in percentage of yield, yield components and MSV reaction.

5.3.2 Mean performance of yield, yield components and MSV reaction of 10 parents, 45 hybrids and five standard check maize genotypes

The mean performances of 45 F1 hybrids, the 10 parents and five standard check maize genotypes for yield, yield components and MSV reaction when evaluated across six environments is presented in Table 5.3. Grain yield varied significantly among genotypes from 1.92 to 7.75 t/ha. The grand mean yield of entries was 4.45 t/ha. Hybrid TL2012-68/TL2012-42 yielded the highest at 7.73 t/ha, followed by TL2012-41/TL2012-17, TL2012-42/TL2012-1, and TL2012-42/TL2012-17; yielding 6.78, 6.16 and 6.06 t/ha, respectively. The lowest yielder hybrid was TL2012-25/TL2012-26 at 1.92 t/ha. However, this hybrid showed lowest (21.53%) reaction to MSV disease (Table 5.2). Interestingly, the parents of this hybrid, i.e., TL2012-25 and TL2012-26 showed low MSV reactions of 28.20% and 39.90%, respectively suggesting their value for resistance breeding. Other hybrids with relatively low MSV disease reaction (<40%) were TL2012-68/TL2012-41, TL2012-1/TL2012-41, TL2012-42/TL2012-41, TL2012-26/TL2012-41, TL2012-68/TL2012-42, TL2012-42/TL2012-26, TL2012-25/TL2012-1, TL2012-68/TL2012-26, TL2012-42/TL2012-25, TL2012-25/TL2012-41, TL2012-26/TL2012-1, and TL2012-42/TL2012-55.

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In this study hybrid TL2012-68/TL2012-1 was the tallest with plant height of 345.08 cm while the shortest was TL2012-68/TL2012-25 at 104.53 cm. The inbred parents of this hybrid are TL2012-68 and TL2012-1 showing plant height of 197.42 cm and 180.38 cm, respectively (Table 5.2). Ear height (EHT) also varied significantly among 45 single cross hybrids (Table 5.2). The maximum height was 157.17 cm recorded to TL2012-1/TL2012-41 and the lowest was 83.2 cm displayed by TL2012-7/TL2012-25. Other hybrids with relatively high ear heights were TL2012-42/TL2012-1 (156.5 cm), TL2012-38/TL2012-55 (134.92 cm) and TL2012-68/TL2012-55 and TL2012-7/TL2012-68 with the same height of 130 cm. Ear prolificacy is an important selection criterion of genotypes. In this study, hybrid TL2012-1/TL2012-17 had the highest number of ears per plant (3.5), followed by TL2012-7/TL2012-17 (2.83), TL2012-38/TL2012-17 (2.75) and TL2012-7/TL2012-42 (2.71). The hybrid with lowest number of eras per plant was TL2012-26/1 with 1.67. Husk cover of cob ranged from one for hybrid TL2012-17/TL2012-55 to four recorded to TL2012-7/TL2012-55. Other hybrids with good husk cover of cobs were TL2012-1/TL2012-55, TL2012-42/TL2012-26 and TL2012-26/TL2012-41 with values of 3.33, 3.29, and 2.88, respectively. Hybrid TL2012-38/TL2012-41 had shortest days to 50% flowering of male inflorescence at 65.75 days followed by TL2012-42/TL2012-55 (68.07), TL2012-38/TL2012-1 (68.97) and 2012-41/TL2012-55 (69.78). The late flowering hybrid was TL2012-26/TL2012-17 which took 76.82 days to flower. Days to 50% silking (formation of female inflorescence) was the shortest (< 70 days) for TL2012-68/TL2012-42, TL2012-38/TL2012-17, TL2012-25/TL2012-1, TL2012-42/TL2012-55, TL2012-38/TL2012-1, TL2012-1/TL2012-41, and TL2012-41/TL2012-55.

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Table 5.3 Mean performance of parents, F1 hybrids and standard checks of grain yield, yield components and MSV reaction of maize when evaluated across six environments

	Genotypes					Traits			
SN	Parents	YLD	MSV	PHT	EHT	EPT	HSC	DA	DSL
1	TL2012-42	2.83	20.32	202.17	107.25	3.00	2.13	72.00	79.17
2	TL2012-41	1.79	33.73	196.33	89.28	2.46	1.42	70.73	84.17
3	TL2012-25	1.72	28.20	191.67	73.90	1.92	2.00	70.04	73.42
4	TL2012-17	1.71	78.40	213.33	61.64	2.50	1.00	67.26	73.00
5	TL2012-26	1.70	39.90	148.26	76.10	1.29	1.25	67.25	80.25
6	TL2012-55	1.61	44.00	208.92	86.00	1.63	3.84	72.35	78.00
7	TL2012-68	1.61	27.27	197.42	56.58	1.63	1.42	72.68	68.83
8	TL2012-1	1.56	33.18	180.38	81.08	1.58	2.00	65.9	75.67
9	TL2012-7	1.36	83.24	227.25	82.03	1.63	3.00	64.39	71.58
10	TL2012-38	1.06	67.33	109.8	116.58	2.33	2.54	72.00	76.17
	Hybrids								
1	TL2012-68/TL2012-42	7.73	32.31	158.41	114.14	2.50	2.04	70.62	68.33
2	TL2012-41/TL2012-17	6.78	64.48	210.08	94.83	2.54	2.38	72.88	73.08
3	TL2012-42/TL2012-1	6.16	41.06	191.92	156.5	1.75	2.42	74.32	74.08
4	TL2012-42/TL2012-17	6.06	71.80	210.00	100.03	2.42	2.30	72.41	72.00
5	TL2012-26/TL2012-1	5.99	38.10	184.00	101.85	1.67	2.41	71.75	72.5
6	TL2012-38/TL2012-17	5.95	89.66	193.67	101.18	2.75	2.54	72.30	68.58
7	TL2012-42/TL2012-41	5.94	27.51	190.25	99.32	2.17	1.88	72.88	70.33
8	TL2012-17/TL2012-55	5.78	73.24	179.67	117.08	2.38	1.00	71.56	77.58
9	TL2012-26/TL2012-17	5.74	55.66	228.83	104.33	2.54	2.58	76.82	74.25
10	TL2012-7/TL2012-38	5.65	76.57	202.58	123.89	2.50	1.63	73.24	70.33
11	TL2012-25/TL2012-17	5.45	73.20	128.35	94.43	2.33	1.46	74.06	72.00
12	TL2012-7/TL2012-42	5.42	68.59	191.33	113.67	2.71	2.46	74.69	72.83
13	TL2012-38/TL2012-41	5.34	61.55	231.83	87.35	1.67	2.27	67.75	70.08
14	TL2012-26/TL2012-41	5.28	28.66	188.33	104.38	2.17	2.88	74.96	73.25
15	TL2012-38/TL2012-26	5.24	73.54	188.00	101.79	1.83	2.58	71.52	70.75
16	TL2012-41/TL2012-55	5.20	51.16	194.25	103.55	2.17	2.18	69.78	69.83
17	TL2012-68/TL2012-1	5.20	41.44	345.08	91.60	2.33	1.58	71.77	72.50
18	TL2012-68/TL2012-55	5.14	47.14	194.92	130.73	1.96	2.67	72.71	73.50
19	TL2012-1/TL2012-41	5.08	25.33	230.5	157.17	2.21	2.75	72.31	69.58
20	TL2012-42/TL2012-25	5.03	34.50	147.81	109.19	2.28	2.38	75.77	72.58
21	TL2012-26/TL2012-55	5.02	63.38	253.00	110.00	2.04	2.67	72.29	71.33
22	TL2012-42/TL2012-38	4.98	55.79	199.08	120.17	2.42	2.63	74.23	74.92

No	Name	YLD	MSV	PHT	EHT	EPT	HSC	DA	DSL
23	TL2012-68/TL2012-17	4.97	64.48	171.50	93.11	2.42	2.25	72.56	71.00
24	TL2012-25/TL2012-1	4.93	33.41	162.33	113.38	2.00	2.08	70.52	68.67
25	TL2012-25/TL2012-41	4.92	37.23	162.92	98.39	2.13	2.02	72.93	74.58
26	TL2012-68/TL2012-41	4.92	24.33	190.58	113.28	2.38	2.68	72.75	73.50
27	TL2012-25/TL2012-55	4.91	60.46	170.92	110.08	2.50	2.08	71.83	72.67
28	TL2012-68/TL2012-25	4.91	43.22	104.53	108.25	2.33	2.70	73.37	75.17
29	TL2012-38/TL2012-25	4.89	70.63	198.25	112.88	2.43	2.00	72.36	75.58
30	TL2012-1/TL2012-55	4.88	43.48	193.42	123.78	2.25	3.33	71.92	72.00
31	TL2012-7/TL2012-1	4.87	50.05	184.67	84.17	2.38	2.21	72.43	74.25
32	TL2012-42/TL2012-26	4.85	32.54	244.50	116.58	1.92	3.29	71.54	72.42
33	TL2012-38/TL2012-55	4.81	61.23	188.50	134.92	2.38	2.04	73.53	75.08
34	TL2012-7/TL2012-55	4.80	76.33	180.00	120.25	2.13	4.00	76.71	71.92
35	TL2012-7/TL2012-41	4.79	67.91	128.08	106.62	2.21	2.78	72.74	72.83
36	TL2012-42/TL2012-55	4.73	39.23	180.00	102.94	2.29	2.13	68.07	68.83
37	TL2012-38/TL2012-1	4.69	65.93	186.75	93.58	2.25	2.08	68.97	69.50
38	TL2012-7/TL2012-17	4.57	82.53	206.33	129.32	2.83	2.53	71.01	70.17
39	TL2012-7/TL2012-68	4.55	51.65	178.17	130.17	2.17	2.79	74.72	72.42
40	TL2012-7/TL2012-26	4.39	72.54	190.42	93.22	2.36	2.50	75.25	73.67
41	TL2012-7/TL2012-25	4.30	79.29	125.59	83.20	2.33	2.28	72.04	74.25
42	TL2012-68/TL2012-38	4.24	70.51	203.25	99.64	2.58	2.67	71.09	70.08
43	TL2012-1/TL2012-17	2.38	51.24	192.00	125.42	3.50	2.33	73.10	70.42
44	TL2012-68/TL2012-26	2.03	33.46	159.58	84.41	2.58	1.88	72.08	70.75
45	TL2012-25/TL2012-26	1.92	21.53	136.59	109.53	1.96	1.88	73.20	71.58
	Standard checks								
1	PANNAR 4M-19	5.60	61.23	188.50	134.92	2.38	2.04	73.53	75.08
2	H308	5.06	76.33	180.00	120.25	2.13	4.00	76.71	71.92
3	H208	4.98	67.91	128.08	106.62	2.21	2.78	72.74	72.83
4	SC627	4.50	39.23	180.00	102.94	2.29	2.13	68.07	68.83
5	UH615	5.30	82.53	186.75	93.58	2.25	2.08	68.97	69.50
	Grand mean	4.45	52.43	188.44	188.3	104.63	2.310	72.00	72.83
	Minimum	1.06	20.21	104.53	56.58	1.29	1.00	64.39	68.33
	Maximum	7.73	89.66	345.08	157.17	3.50	4.00	76.82	84.17
	SE	0.843	9.415	70.305	0.581	5.732	0.931	1.759	2.385
	CV (%)	19.14	17.96	37.34	25.83	5.48	40.39	2.44	3.28

YLD= Grain yield (t/ha), MSV=Disease reaction in %, PHT =Plant height in cm, EHT= Ear height in cm, EPT= Number of ears per plant, HSC= Husk cover of cob, DA= Days to 50% anthesis and DSL= Days to 50% silking SE=Standard error; CV=coefficient of variation

5.3.3 Estimates of GCA and SCA effects

Estimates of GCA effects

The GCA effects of grain yield were generally not significant different among inbred lines (Table 5.4). However, inbred lines like TL2012-42 showed significantly high positive (p < 0.001) GCA effects of 0.695 while TL2012-26 and TL2012-25 had significant but negative GCA effects of -0.46 and -0.38, respectively (Table 5.4). Inbred lines were highly significant different (p <0.001) with variable GCA effects for MSV disease resistance. About 60% of these lines demonstrated significant and negative GCA effects while the remaining 40% showed positive GCA effects. The lines with negative general combining ability effects were TL2012-41(-10.926%), TL2012-1 (-10.792%), and TL2012-42 (-10.748%), followed by TL2012-68 (-9.533%), TL2012-26 (-7.182%) and Tl2012-25 (-4.045%). Lines those with positive GCA effect estimates were TL2012-55, TL2012-17, TL2012-38 and TL2012-7 (Table 5.4). Positive and negative GCA effects have great implication in breeding because their interpretation is determined by the trait under consideration. In disease resistance breeding significant and negative GCA effects are desirable.

Lines TL2012-17 and TL2012-26 exhibited highly significant (p <0.001) GCA effects estimates of 0.346 and -0.225 for ears per plant, respectively and were considered as best general combiners. The first line has a tendency to increase the number of ears per plant in its hybrid combinations while the second line was a poor general combiner due to its negative contribution on the number ears per plant implying their relative significance for breeding. Significant positive or negative GCA effects were observed to husk cover of cob. TL2012-55 and TL2012-7 revealed positive GCA effects of 0.266 and 0.290, respectively while TL2012-17 and TL2012-25 had negative estimates of -0.292 and -0.241, respectively. Ninety percent of the inbred lines evaluated did not show significant differences of GCA effects on days to 50% anthesis and silking (Table 5.4).whileTL2012-1 and TL2012-68 showed significant negative GCA effects of -0.955 and -0.906 to days to 50% anthesis (DA) and silking (DSL), respectively (Table 5.4).

Table 5.4 Estimates of GCA effects of ten parental inbred lines used in the study

Lines	YIELD	MSV	PHT	EHT	EPT	HSC	DA	DSL
TL2012-55	0.010ns	2.822*	1.299ns	7.143***	-0.104ns	0.266**	-0.179ns	0.561ns
TL2012-17	0.260ns	17.356***	16.448**	-4.653*	0.346***	-0.292**	0.143ns	-0.306ns
TL2012-41	0.326ns	-10.926***	-9.694ns	-1.373ns	-0.066ns	-0.006ns	-0.281ns	0.611ns
TL2012-1	-0.104ns	-10.792***	5.265ns	6.0627**	-0.083ns	-0.015ns	-0.955*	-0.597ns
TL2012-26	-0.463**	-7.182***	-9.716ns	-6.572***	-0.225***	0.064ns	0.414ns	0.561ns
TL2012-25	-0.378*	-4.047**	-13.076*	-5.466**	-0.054ns	-0.241*	0.359ns	0.534ns
TL2012-38	0.008ns	16.161***	-4.115ns	2.408ns	0.039ns	-0.031ns	-0.554ns	-0.406ns
TL2012-42	0.695***	-10.748***	15.128*	7.188***	0.069ns	0.035ns	0.40ns	0.036ns
TL2012-68	-0.147ns	-9.533***	3.439ns	-4.599*	0.013ns	-0.070ns	0.183ns	-0.906*
TL2012-7	-0.206ns	17.757***	-4.978ns	-0.138ns	0.063ns	0.290**	0.470ns	-0.089ns

*, **, and *** denote significance differences at P≤0.05, P≤ 0.01; and P≤0.001, respectively; DF=Degree of freedom; YLD= Grain yield (t/ha); MSV=MSV disease reaction in %; PHT=Plant height in cm; EHT= Ear height in cm; EPT= Number of ears per plant; HSC= Husk cover of cob; DA= Days to 50% anthesis; DSL= Days to 50% silking

Estimates of SCA effects

The SCA effects are presented in Table 5.5. About 45% of the crosses showed significant SCA effects for YLD. The crosses between inbred line TL2012-7 with TL2012-55, TL2012-17, TL2012-41, TL2012-1, TL2012-26, TL2012-25, TL2012-38, TL2012-42, and TL2012-68 manifested significant (p<0.001) positive SCA effects of 3.412, 3.325, 3.536, 3.413, 2.437, 2.406, 4.803, 3.501 and 3.003 respectively. Other crosses with positive SCA effects were TL2012-41/TL2012-17 (1.517), TL2012-26/TL2012-17 (1.261) TL2012-26/TL2012-1 (1.877), and TL2012-68/TL2012-42 (2.504) (Table 5.5). According to Fan et al. (2014) hybrid with significant positive SCA effects contributes substantially and directly to the increased performance of traits under consideration. Therefore these hybrids involving inbred parent TL2012-7 have high or good specific combining ability. Conversely, hybrids TL2012-1/TL2012-17 (-2.457), TL2012-25/TL2012-26 (-1.912) and TL2012-68/TL2012-26 (-2.041) had significant but negative SCA effects hence were considered to have low or poor specific combining ability for grain yield (Table 5.5).

The SCA effects for MSV disease reaction showed significant difference among the 45 hybrids evaluated across six environments. TL2012-38/TL2012-55 and TL2012-25/TL2012-26 had significant negative SGA effects of -10.892 and -19.451 respectively implying their suitability to suppress MSV infection On the other hand, TL2012-26/TL2012-55 (14.592), TL2012-25/TL2012-55 (9.440), TL2012-7/TL2012-55 (17.428), TL2012-42/TL2012-17 (12.078), TL2012-42/TL2012-1 (9.485), TL2012-38/TL2012-26 (11.450), TL2012-7/TL2012-25 (28.377), and TL2012-68/TL2012-38 (10.768) were poor combiners for MSV resistance due to their positive SGA effects (Table 5.5).

With regards to plant height, five hybrids: L2012-42/TL2012-17, TL2012-7/TL2012-17, TL2012-1/TL2012-41, TL2012-7/TL2012-1 and TL2012-7/TL2012-68 displayed significant high SCA estimates at 119.981, 126.518, 63.902, 86.493 and 69.850, respectively which is not desirable for breeding for short plant stature (Table 5.5).

About 33.3% of the hybrids had considerable differences on SCA effects for ear height. Of which ten hybrids exhibited positive while five had negatively significant SCA effects (Table 5.5). Hybrids with positive SCA effects were TL2012-38/TL2012-55 (18.5757), TL2012-68/TL2012-55 (21.392) and TL2012-7/TL2012-55 (41.531) which all had one male parent in common. The hybrids with negative SCA effects were TL2012-42/TL2012-55 (-18.179), TL2012-68/TL2012-1 (-16.653), TL2012-38/TL2012-41 (-13.289), TL2012-38/TL2012-1 (-21.678), and TL2012-38/TL2012-41 (-20.475) which are not preferred due to small ear heights.

Seven hybrids out of the 45 showed significant differences with regards to their SCA effects on the number of ears per plant (EPT). The hybrids with significant positive estimates were TL2012-1/TL2012-17 (0.962), TL2012-7/TL2012-17 (0.617), TL2012-7/TL2012-1 (0.646), TL2012-68/TL2012-26 (0.520), and TL2012-7/TL2012-26 (0.922) while those with negative estimates were TL2012-38/TL2012-41 (-0.581) and TL2012-42/TL2012-1 (-0.511). Nine hybrids evaluated showed marked differences of SCA effects for husk covers of cob. Six hybrids had positive SCA effects and the remaining three had negative values. The hybrids with significant positive SCA estimates were TL2012-1/TL2012-55 (1.0747), TL2012-7/TL2012-41 (1.071), and TL2012-42/TL2012-26 (0.866) whereas others had negative estimates including TL2012-17/TL2012-55 (-1.301), TL2012-68/TL2012-1 (-0.675) and TL2012-7/TL2012-38 (-1.238).

Both positive and negative SCA effects were observed for days to anthesis (DA) and silking (DSL). Eight hybrids had significantly high SCA effects for DA, of which only two hybrids viz. TL2012-42/TL2012-55 (-4.406) and TL2012-38/TL2012-41 (-3.666) revealed negative SCA effects suggesting that they were good specific combiners. The remaining six hybrids which exhibited positive and significant SCA effects were considered as poor combiners. These hybrids were TL2012-26/TL2012-17 (4.008), TL2012-1/TL2012-41 (2.574), TL2012-42/TL2012-1 (2.620), TL2012-7/TL2012-1 (5.1), TL2012-7/TL2012-26 (7.94) and TL2012-42/TL2012-25 (2.756) (Table 5.5).

Thirteen hybrids had significant SCA effects for DSL. Seven of which were considered as best combiners because they had negative SCA effects for days to 50% silking. These hybrids are TL2012-42/TL2012-55 (-4.277), TL2012-38/TL2012-17 (-3.219), TL2012-38/TL2012-41 (-2.636), TL2012-42/TL2012-41 (-2.827), TL2012-7/TL2012-41 (-10.633), TL2012-68/TL2012-42 (-3.311) and TL2012-7/TL2012-42 (-6.208). The hybrids with positive estimates of SCA effects were TL2012-17/TL2012-55 (4.814), TL2012-41/TL2012-55 (3.852), and TL2012-7/TL2012-55 (5.433), TL2012-1/TL2012-41 (2.944), TL2012-25/TL2012-1 (3.786) and TL2012-7/TL2012-26 (5.933) (Table 5.5).

Table 5.5 Estimates of the SCA effects of 45 single cross hybrids for yield and yield components when evaluated across six environments.

No.	Cross	YLD	MSV	PHT	EHT	EPT	HSC	DA	DSL
1	TL2012-17/TL2012-55	0.832ns	-0.080ns	-1.190ns	7.796ns	-0.1421ns	-1.301***	-0.657ns	4.814***
2	TL2012-41/TL2012-55	0.188ns	6.119ns	6.785ns	-9.009ns	0.0621ns	-0.403ns	-2.016ns	3.853**
3	TL2012-1/TL2012-55	0.294ns	-1.698ns	9.909ns	3.788ns	0.1621ns	0.747*	0.799ns	-0.477ns
4	TL2012-26/TL2012-55	0.794ns	14.592**	-1.109ns	2.639ns	0.095ns	0.010ns	-0.195ns	-2.303ns
5	TL2012-25/TL2012-55	0.602ns	9.440*	11.916ns	1.617ns	0.383ns	-0.268ns	-0.607ns	-0.944ns
6	TL2012-38/TL2012-55	0.115ns	-10.892**	-0.461ns	18.576***	0.165ns	-0.520ns	2.006ns	2.414ns
7	TL2012-42/TL2012-55	-0.655ns	-5.984ns	-30.287ns	-18.179**	0.051ns	-0.502ns	-4.406***	-4.277**
8	TL2012-68/TL2012-55	0.598ns	0.709ns	30.569ns	21.392***	-0.225ns	0.136ns	0.453ns	1.331ns
9	TL2012-7/TL2012-55	3.412***	17.428*	51.602ns	41.531***	0.333ns	0.134ns	3.709ns	5.433**
10	TL2012-41/TL2012-17	1.517***	4.940ns	-8.947ns	-5.930ns	-0.013ns	0.346ns	0.762ns	0.264ns
11	TL2012-1/TL2012-17	-2.457***	-8.436ns	16.594ns	17.217**	0.962***	0.305ns	1.661ns	1.194ns
12	TL2012-26/TL2012-17	1.261*	-7.629ns	-11.924ns	8.768ns	0.145ns	0.476ns	4.008***	1.481ns
13	TL2012-25/TL2012-17	0.886ns	7.678ns	-8.899ns	-2.237ns	-0.234ns	-0.336ns	1.305ns	0.744ns
14	TL2012-38/TL2012-17	1.008ns	3.029ns	-11.609ns	-3.362ns	0.089ns	0.538ns	0.460ns	-3.219**
15	TL2012-42/TL2012-17	0.425ns	12.078**	119.981***	-9.300ns	-0.274ns	0.230ns	-0.386ns	-0.244ns
16	TL2012-68/TL2012-17	0.180ns	3.547ns	-18.496ns	-4.428ns	-0.217ns	0.277ns	-0.018ns	-0.302ns
17	TL2012-7/TL2012-17	3.325***	3.724ns	126.518***	63.16***	0.617*	0.952ns	3.422ns	3.050ns
18	TL2012-1/TL2012-41	0.178ns	-6.070ns	63.902***	45.687***	0.083ns	0.4357ns	1.293ns	2.944*
19	TL2012-26/TL2012-41	0.740ns	-6.347ns	24.967ns	5.530ns	0.183ns	0.498ns	2.574*	-0.436ns
20	TL2012-25/TL2012-41	0.297ns	-0.015ns	0.743ns	-1.558ns	-0.030ns	-0.063ns	0.604ns	0.923ns
21	TL2012-38/TL2012-41	0.332ns	3.203ns	-17.384ns	-20.475***	-0.581**	-0.023ns	-3.666**	-2.636*
22	TL2012-42/TL2012-41	0.238ns	-3.931ns	-36.044ns	-13.289*	-0.111ns	-0.481ns	0.513ns	-2.827*

23	TL2012-68/TL2012-41	0.061ns	-8.329ns	3.3123ns	12.466*	0.154ns	0.416ns	0.597ns	1.2808ns
24	TL2012-7/TL2012-41	3.536***	5.501ns	21.459ns	9.293ns	-0.379ns	1.0708*	1.265ns	-10.633***
25	TL2012-26/TL2012-1	1.877***	2.9614ns	9.175ns	-4.431ns	-0.3015ns	0.043ns	0.039ns	0.022ns
26	TL2012-25/TL2012-1	0.738ns	-3.966ns	7.701ns	5.997ns	-0.138ns	0.004ns	-1.139ns	3.786**
27	TL2012-38/TL2012-1	0.110ns	7.444ns	-10.01ns	-21.678***	0.019ns	-0.206ns	-1.776ns	2.011ns
28	TL2012-42/TL2012-1	0.886ns	9.485*	30.581ns	36.459***	-0.5112**	0.062ns	2.61983*	2.131ns
29	TL2012-68/TL2012-1	0.777ns	8.654ns	-13.73ns	-16.653**	0.129ns	-0.675*	0.2873ns	1.489ns
30	TL2012-7/TL2012-1	3.413***	-11.683ns	86.493**	16.098ns	0.6457*	-0.088ns	5.100**	1.925ns
31	TL2012-25/TL2012-26	-1.912***	-19.451***	9.266ns	14.773*	-0.038ns	-0.275ns	0.1748ns	2.027ns
32	TL2012-38/TL2012-26	1.014ns	11.450**	7.055ns	-0.835ns	-0.256ns	0.223ns	-0.595ns	1.919ns
33	TL2012-42/TL2012-26	-0.059ns	-2.642ns	7.396ns	9.176ns	-0.203ns	0.866**	-1.524ns	-0.694ns
34	TL2012-68/TL2012-26	-2.041***	-2.940ns	-9.082ns	-11.210ns	0.520**	-0.454ns	-0.765ns	1.419ns
35	TL2012-7/TL2012-26	2.437**	7.702ns	58.912ns	10.683ns	0.922***	1.024*	7.944***	5.933**
36	TL2012-38/TL2012-25	0.580ns	6.298ns	26.914ns	9.143ns	0.173ns	-0.055ns	0.301ns	2.939ns
37	TL2012-42/TL2012-25	0.039ns	-2.918ns	6.588ns	0.6798ns	-0.015ns	0.254ns	2.756*	-0.502ns
38	TL2012-68/TL2012-25	0.758ns	4.583ns	8.111ns	11.526ns	0.099ns	0.676*	0.573ns	3.022ns
39	TL2012-7/TL2012-25	2.406**	28.387***	30.561ns	3.972ns	0.299ns	-0.247ns	1.889ns	1.458ns
40	TL2012-42/TL2012-38	-0.396ns	-2.734ns	-8.206ns	3.780ns	0.0329ns	0.294ns	2.127ns	2.772ns
41	TL2012-68/TL2012-38	-0.295ns	10.768*	-1.184ns	-4.957ns	0.256ns	0.433ns	-0.788ns	-1.119ns
42	TL2012-7/TL2012-38	4.803***	7.636ns	42.913ns	9.855ns	0.143ns	-1.237**	0.217ns	6.150ns
43	TL2012-68/TL2012-42	2.504***	-0.524ns	-3.176ns	4.763ns	0.143ns	-0.258ns	-2.218ns	-3.311**
44	TL2012-7/TL2012-42	3.501***	19.770ns	74.314ns	13.743ns	-0.285ns	0.078ns	2.622ns	-6.208**
45	TL2012-7/TL2012-68	3.003***	-2.907ns	66.850*	69.130***	0.492ns	1.023*	1.7458ns	2.767ns

*, **, and *** denote significance differences at P≤0.05, P≤ 0.01; and P≤0.001, respectively; DF=Degree of freedom; YLD= Grain yield (t/ha); MSV=MSV disease reaction in %; PHT=Plant height in cm; EHT= Ear height in cm; EPT= Number of ears per plant; HSC= Husk cover of cob; DA= Days to 50% anthesis; DSL= Days to 50% silking

5.3.4 Heterosis

The mid-parent heterosis was calculated following Falconer and Mackay (1966) and summarized for grain yield and MSV reaction considering the best 21 F1 maize hybrids only (Figures 5.2 and 5.3). The MPH for grain yield varied from 8% for TL2012-42/TL2012-1 to 138% for TL2012-7/TL2012-38 among the top yielding genotypes. Some 47.62% of these hybrids had heterosis above 50% (Figure 5.2). Hybrids such as TL2012-68/TL2012-42, TL2012-41/TL2012-17 and TL2012-7/TL2012-38 exhibited MPH above 50% and had also positive significant SCA effects for grain yield of 2.50, 1.52 and 4.80 t/ha, respectively (Table 5.5). Hybrid TL2012-7/TL2012-38, showed significantly high SCA effect among all hybrids (Table 5.5) which also demonstrated the highest MPH of 138% (Figure 5.2). Hybrids such as TL2012-42/TL2012-1, TL2012-1/TL2012-41, TL2012-42/TL2012-25, and TL2012-68/TL2012-1 had poor (< 20%) MPH and revealed non-significant SCA effects (Table 5.5). As expected heterosis and SCA effects are positively correlated.

Heterosis for MSV disease reaction varied from -38.2% for hybrid TL2012-25/TL2012-26 to 67.6% for TL2012-68/TL2012-25 amongst 18 best selected genotypes (Figure 5.4). Hybrids which exhibited negative heterosis in a desirable direction for MSV reaction were TL2012-68/TL2012-41, TL2012-26/TL2012-41, and TL2012-1/TL2012-41 (Table 5.5). These hybrids had one common male parent, TL2012-41, (Figure 5.3). Negative heterosis for MSV reaction is desirable and hence all genotypes with negative heterosis are considered for breeding because they are presumably resistant to MSV. In contrast, hybrids TL2012-68/TL2012-25, TL2012-42/TL2012-1, TL2012-42/TL2012-25, TL2012-68/TL2012-42 and TL2012-68/TL2012-55 revealed substantial mid-parent hetersosis above 35% (Figure 5.3) but with non-significant SCA effects except TL2012-68/TL2012-42 (Table 5.5).
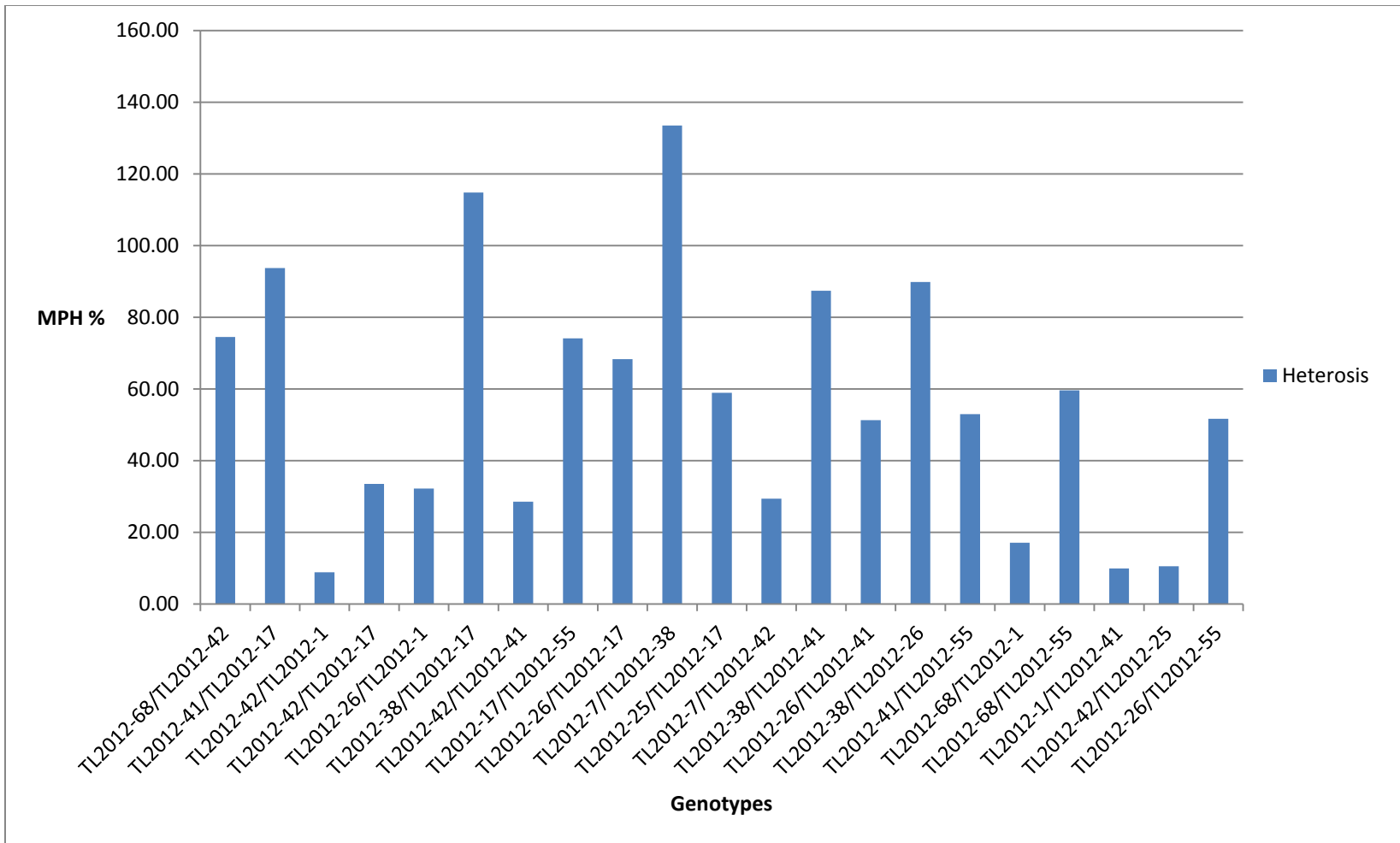
 Figure 5.2: The magnitude of mid-parent heterosis (%) of grain yield among 21 best selected maize hybrids when evaluated across six MSV prone environments
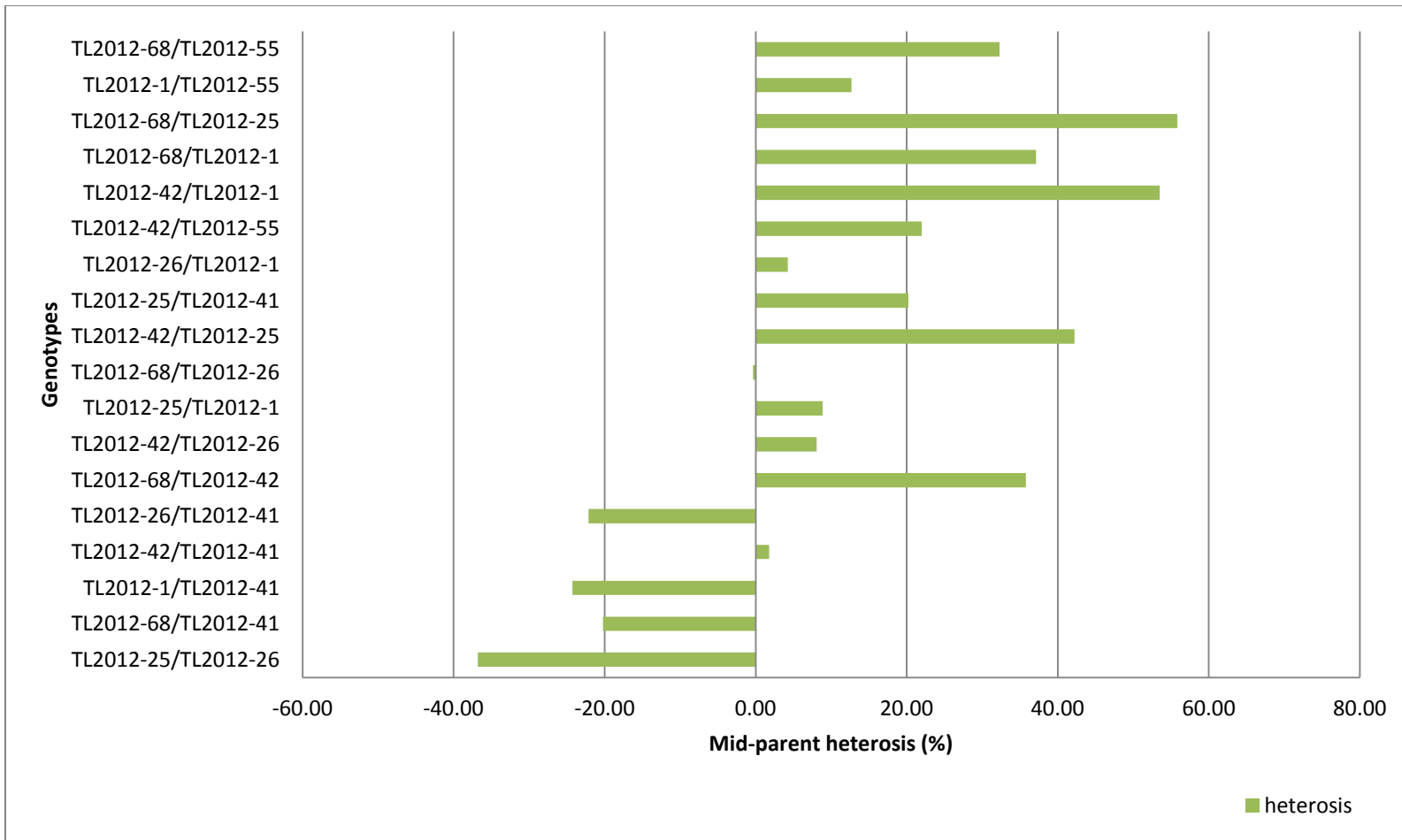
Figure 5.3: The magnitude of mid-parent heterosis (%) of MSV reaction of 18 best selected maize hybrids when evaluated across six MSV prone environments

5.4 Discussion

Genetic variability of parental inbred lines and their respective F1 hybrids

Assessment of general and specific combining ability in maize is important for yield enhancement and stress tolerance (Khalid et al., 2013; Aly, 2013; Ali et al., 2014). Better utilization of parental inbred lines and their progenies in breeding depends on their general combing ability (GCA) and specific combing ability (SCA), respectively (Lou et al., 2011; Singh et al., 2014). In the current study, ten parental inbred lines were crossed using a half diallel design to determine estimates both GCA of the parents and SCA for their respective 45 cross progenies for MSV resistance, grain yield and other agronomic traits. Analysis of variance revealed that the mean squares of these two genetic parameters were significantly different (P<0.001) for most attributes studied. This implies that the importance of additive and non additive types of gene actions was prevailed among the experimental materials. Detection of genetic variability for GCA and SCA may facilitate selection of novel genetic materials (best combiners) for genetic improvement in maize because the success of any crop improvement is dependent on the amount of genetic variability available in the breeding materials (Ali et al., 2011; Bello, 2012). The results were in general agreement with previous reports (Nzuve et al., 2013; Ali et al., 2014; Moradi, 2014). The GCA/SCA ratio ranged from 1.7% for ear height to 97.7% for MSV resistance implying that both GCA and SCA were involved in genetic control of the traits. Similar results were reported by Zare-Kohan and Heidari (2014) in wheat. This ratio measures the relative importance of the two genetic parameters in the control of the traits' expressions; when the ratio approached unity it shows high predictability of GCA alone (Baker, 1978; Abdel-Moneam et al., 2014; Karaya et al., 2014). GCA/SCA ratio was high (97.7%) on MSV reaction suggesting that additive gene action was important for this trait and selection-based strategies of accumulating additive gene effects would be appropriate and effective. High Baker's ratio on MSV resistance was also reported by Mutengwa et al. (2012) who reported that GCA to SCA ratio was 83% in dwarf maize germplasm. Narrow sense heritability is directly related to the proportion of additive gene effects (Zare-Kohan and Heidari, 2014). The high narrow sense heritability of 97.7% on MSV resistance suggests that it is possible to obtain genetic gains through selection for MSV resistance from F1 maize hybrids since; some parents were able to transmit favourable genes to their subsequent cross progenies (Abrha et al., 2013). Recurrent and backcross breeding can therefore be used to develop maize varieties with resistant to MSV disease. High narrow sense heritability can also imply that selection for resistance to MSV can be effective.

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Estimates of GCA effects among inbred lines

The GCA effect is a good measure of additive gene action and it varies among the parental inbred lines. The line TL2012-42 was the best general combiner for grain yield. Hybrid combinations involving this parent produced significant mean yield performance suggesting that it transmits greatest favourable genes to its progenies (Badu-Apraku and Oyekunle, 2012). Therefore, deployment of this inbred line in breeding would result in increased grain yield of maize in Tanzania. Lines TL2012-41 and TL2012-1 were identified with significant negative GCA for resistance to MSV implying that they are good combiners for MSV resistance. Their cross progenies have a tendency to reduce infection for MSV thus they should be selected for use in resistance breeding programmes. Selection procedures such as recurrent and backcross breeding will maximize resistance genes. TL2012-42 and TL2012-55 were considered as best general combiners for PHT and EHT due to their significant positive GCA effects, respectively and can be selected for increasing yield in their hybrid combinations. Tall maize genotypes with high ear positioning or placement may offer opportunity for more ears to develop on the nodes below and ultimately increasing final yield (Estakhr and Heidari, 2012; Ali et al., 2012) even though they may be susceptible to lodging (Amiruzzaman et al., 2010; Estakhr and Heidari, 2012). Number of ears per plant is an indicator for increased grain yield while genotypes with long tipped off husk covers of maize cob provide maximum protection of the ear against birds' damage, fungal infection and early germination of kernel when moisture and conditions suitable for germination occurred in the field, the reason why breeders prefer genotypes with long husk covers of cob. Therefore lines TL2012-17 and parents TL2012-55 are best candidates for breeding towards these two traits and increased final grain yield. These results are consistently similar with those reported by Paven et al. (2011) and Abrha et al. (2013). Of all inbred lines, TL2012-1 and TL2012-68 were selected with negative significant GCA effects for reduced days to anthesis and silking, respectively. Breeding for early maturity is important especially in recent decades due to undesirable effects of climate change on the amount and distribution of rainfall.

Estimates of SCA effects and mean performance of 45 F1 hybrids

Hybrid performance can be predicted mostly on the basis of SCA of progenies (Mutengwa et al., 2012). TL2012-68/TL2012-42 and TL2012-7/TL2012-42 had positive significant SCA effects for grain yield suggesting that these hybrids can be directly released as single cross hybrids or developed further as three way hybrids before being released for production in Tanzania or similar agro-ecologies in sub-Saharan Africa. The role of three way hybrids is to improve seed production because single cross hybrids are usually poor seed producers because the female

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parent is an inbred line (MacRobert et al., 2014). These results were in harmony with previous studies (Amiruzzman et al., 2010; Ali et al., 2012; Kamara et al., 2014).

For MSV reaction, inbred lines TL2012-25 and TL2012-26 were identified as good combiners useful in MSV resistance breeding of maize. Similar result was reported by Mutengwa et al (2012) when studying genetic analysis of resistance to MSV in dwarf maize germplasm. Hung and Holland (2012) also reported similar findings in their diallel analysis of resistance to Fusarium ear rot and Fumonisin contamination in maize. Tall maize genotypes are important not only for increase grain yield but also for high biomass production for silage production. Hybrids TL2012-42/TL2012-17 (119.981 cm) and TL2012-7/TL2012-17 (126.52 cm) showed good specific combining ability for plant height and can be released for silage production in intensive livestock production agro-systems like in Arusha and Manyara regions. These results were consistent with those reported by Bertoia and Aulicino (2014) and Ertiro et al. (2013).

Hybrids with large number of ears per plant and long husk cover off the tip of the cob are desirable for increased yield and cob protection from several disease and pests.Therefore hybrids such as TL2012-1/TL2012-17 (0.962) and TL2012-1/TL2012-55 (1.075) had significantly high positive SCA effects and were considered as best combiners for ears per plant and husk covers, respectively. TL2012-42/TL2012-55 (-4.406 days) and TL2012-42/TL2012-41 (-2.636 days), were good specific combiners for DA and DSL, respectively and can be grown in places like in Monduli, Simanjiro and Hai districts in Tanzania that receive short rains. Akinwale et al. (2014) reported similar findings when studying heterotic grouping of tropical early maturing lines based on combing ability.

Heterosis

Maize exhibits great potential for heterotic expressions to which selection of superior genotypes can be made (Ali et al., 2012; Goff and Zhang, 2013; Marcon et al., 2013; Shen et al., 2014). Analysis of mid-parent heterosis for grain yield and MSV reaction in the current study showed significant variations among selected F1 hybrids. This suggests the positive role of non-additive gene effects in the expressions of heterosis (Abdel-Moneam et al., 2014). Mid-parent heterosis in the current study ranged from 8 to 138%. Crosses TL2012-7/TL2012-38 and TL2012-38/TL2012-17 manifested the highest MPH of 118% and 138% respectively than other hybrids tested for grain yield (Figure 5.2). These hybrids can be selected for increased grain yield. This result was in line with that of Oppong (2013) who reported MPH for grain yield between -2.40 to 111.48% for TZE117/LA276 and CML442/LA80, respectively. Drinic et al. (2012) also reported that the values of mid-parent heterosis for grain yield of some lines evaluated were 136.72 and 144.46% when studying heterosis of maize hybrids. However, this result disagrees the reports

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of Abdel-Moneam et al. (2014) who found the highest mid-parent heterosis of 295.73%. Differences in heterosis are common among maize populations because they differ significantly in many aspects including potential yield, genetic diversity, levels of combining ability and production environments (Offermann and Peterhansel, 2014). The relative low levels of MPH of the test materials could be greatly caused by MSV infection stresses since they were evaluated under high MSV disease prevalence. For MSV resistance, hybrids with negative mid-parent heterosis are desirable because they have potential to reduce damage caused by the disease. Hybrid combinations involving a common male parent TL2012-41 with TL2012-1, TL2012-68 and TL2012-26 manifested negative MPH ranging from 0.0 to -38.2% with TL2012-25/TL2012-26 exhibiting highest negative value. This implies that these hybrids are considered to be resistant against MSV disease. Therefore these crosses can be selected for production in MSV stricken areas of Tanzania. This result was in harmony with that of Mengesha (2013) who found 0.0 to -25% MPH for corn leaf blight resistance in Ethiopia.

5.5 Conclusions

Combining ability analysis of maize (*Zea mays* L.) inbred lines and their hybrids are essential to develop novel recombinants or hybrid varieties to exploit heterosis. Estimates of both GCA and SCA have provided important information about the value of inbred lines and hybrids. This will facilitate development of new hybrids to enhance maize production and productivity in the northern Tanzania or throughout the country at large. The significant variation of GCA and SCA effects revealed that considerable genetic variations exist among genotypes. This information will be used to develop hybrids with high heterosis for yield and MSV resistance in Tanzania. Inbred line TL2012-42 had significant positive GCA effects for yield, and selected as good general combiner while lines TL2012-41 (-10.926), TL2012-1 (-10.792), and TL2012-42 (-10.748) were good general combiners expressing low MSV reactions. These lines will be exploited in maize breeding program for developing cultivars with improved grain yield and MSV resistance. In addition, crosses such as TL2012-7/TL2012-42, and TL2012-7/TL2012-68 had significant (P <0.001) positive SCA effects for grain yield suggesting that these hybrids have good specific combining ability for yield and can be selected for use as single cross hybrids or developed further as three way hybrids before being released for large-scale production. While TL2012-38/TL2012-55 (-10.892%) and TL2012-25/TL2012-26 (-19.451%) had negative significant SCA effects for MSV reaction and were selected with good specific combining ability. Heterosis for yield and MSV resistance revealed considerable genetic variation among hybrids. Maximum heterosis for grain yield at 138% was displayed by TL2012-7/TL2012-38 while TL2012-25/TL2012-26 had lowest desirable negative heterosis of -38.2% for MSV resistance.

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CHAPTER SIX:

Genotype by environment interaction of grain yield and MSV resistance among novel maize hybrids in the mid-altitude agro-ecologies of Tanzania

Abstract:

Maize (*Zea mays* L.) is among the main food security crops grown in a wide range of environments in Tanzania. In the country, grain yields of maize are considerably affected by genotype x environment interaction (GXE) and MSV disease. The objective of this study was to investigate the GXE interaction for grain yield and MSV resistance among newly developed maize hybrids in Tanzania. Forty five novel single cross hybrids and five standard check three-way cross hybrids were evaluated using a 5 x10 alpha lattice design with two replications across six environments. The Additive Main Effects and Multiplicative Interaction (AMMI) and genotype, and genotype by environment (GGE) biplot models were used to assess the magnitude of GXE interaction of grain yield and reaction to MSV disease among test materials. Results from the AMMI analysis of variance revealed a significant contribution of the environmental effect on grain yield accounting to 52.06% of the total variation among hybrids. Genotypes and GXE contributed to 12.4% and 17.76% of the total variation of hybrids of this trait, respectively. Genotypes explained 45.52% of the total variation of hybrids for MSV resistance while the contribution of environments was minimal (2.77%). Hybrid G43 was identified with relatively high mean grain yield of 6.70 t/ha with low MSV severity of 31.88% across environments. Experimental hybrids such as G10, G14 and G28 had high yield performance of 6.72, 6.00, and 6.23 t/ha, in that order across environments but with highly susceptible reaction to MSV. Conversely, hybrid G31 expressed low MSV infection but yielded the lowest at each environment. Hybrids such as G23 with low grain yields of 4.84 t/ha, G18 (5.14 t/ha), and G34 (1.94 t/ha) showed relatively low MSV infection levels which are useful genetic resources for resistance breeding. Experimental hybrids with high grain yield and MSV resistance selected in this study are good candidates for direct production or for future three-way hybrid development in Tanzania.

Keywords: AMMI analysis, GXE interaction, GGE biplot, Hybrid, Maize, MSV.

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6.1 Introduction

Maize (*Zea mays* L.) is the most important cereal crop grown throughout the world for food, livestock feed and other industrial uses. In Tanzania, maize is a key food security crop supporting approximately 45 million people (Barreiro-Hurle, 2012; Kage et al., 2013). The crop is commonly grown in a wide range of environmental conditions covering 45% of the total cultivated land. Maize contributes to about 50% of cash income in the rural areas of Tanzania (Barreiro-Hurle, 2012; Kage et al., 2013; Mrutu et al., 2014). Smallholder farmers are the key maize producers and account for about 85% of the total maize production in Tanzania. These farming systems predominantly depend on rain-fed agriculture and often achieve low yields due to several production constraints (Barreiro-Hurle, 2013; Mrutu et al., 2014) including the influence of genotype by environment (GXE) interaction (Adu et al., 2013; Fischer et al., 2014). GXE interaction is defined as the differential response or ranking of genotypes when grown across environments, i.e., across locations or multiple seasons (Kamutando et al., 2013; Mohamed et al., 2013; Mustapha and Bakari, 2014).

The main causes of GxE are differences among genotypes, environmental factors such as biotic and abiotic entities and their interaction (Dari, 2011; Rashidi et al., 2013). Previous studies have reported that GXE interaction is greatly exacerbated by the outbreak of crop stresses such as drought or diseases thereby causing significant reduction in yield stability of genotypes (Bazinger et al., 2006; Kassa et al., 2013; Mengesha, 2013; Carns et al., 2013; Badu-Apraku et al., 2014). GxE interaction reduces selection efficiency (Comstock and Moll, 1963; Badu-Apraku et al., 2014) and complicates cultivar recommendations, especially when crossover interaction and rank differences occur (Mengesha, 2013; Rashidi et al., 2013; Dagnachew et al., 2014) leading to minimal selection responses (Grishkevic and Yanai, 2013; Badu-Apraku et al., 2014). Thus rigorous data collection and analysis across representative test environments is important in order to understand GXE effects (Nyoka et al., 2012; Trouche et al., 2014).

Breeding for high yielding and stable cultivars is important in maize based farming communities such as in Tanzania (Liu et al., 2011; Badu-Apraku et al., 2014, Bujak et al., 2014). Yield stability assessment is especially important in the northern parts of the country due to the complex and poor performing farming systems and high infestations of foliar viral diseases such as maize streak virus (MSV) and maize lethal necrosis (MLN) (Liu et al., 2011; Dagnachew et al., 2014).

There is little information that reported the effect of GXE interaction and stability of newly developed maize cultivars when grown under maize streak virus stressed conditions. GxE

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studies will facilitate identification of possible adaptation areas of test genotypes for large scale production or further evaluation (Liu et al., 2011; Adu et al., 2013; Bujak et al., 2014). Genotypes with relatively high mean grain yield across environments and minimum GXE interaction are best candidates for wide-area production (Lopez et al., 2012: Mengesha 2013). Stable genotypes often show superiority in yield, quality and other desirable agronomic characteristics across target test or production environments (Adu et al., 2013; Kamutando et al., 2013; Mengesha, 2013). Identification of cultivars with consistently high mean yield performance across environments (over time and locations) would bring a good return on investment by maize growers who uses production inputs such as fertilizers and agro-chemicals (Hans, 2010).

Different statistical or stability models are available to estimate the magnitude of GXE interaction (Khalil et al., 2011; Jalala, 2011; Bujak et al., 2014; Badu-Apraku et al., 2012). The commonest and widely used statistical models include the Additive Main Effects and Multiplicative Interaction (AMMI) and the Genotype main effect and Genotype by Environment interaction effects (GGE) (Jandong et al., 2011; Mukherjee et al., 2013; Munawar et al., 2013; Shiri, 2013). These statistical tools are powerful to determine the pattern of genotypic responses across environments and have been widely used by plant breeders (Balestre et al., 2009; Habliza, 2010; Dehghani et al, 2009; Oliveira et al., 2010; Dagnachew et al., 2014). The AMMI and GGE biplot analyses models capture GXE interaction sum of squares and separate the main and interaction effects (Badu-Apraku et al., 2012). They also allow sensible biological interpretations of the data (Gurmu et al., 2009; Beyene et al., 2012; Kato et al., 2013; Rad et al., 2013; Rashidi et al., 2013). GGE biplot is based on environment centred principal component analysis (PCA) whereas AMMI uses double centered PCAs (Gordon-Mendoza et al., 2010; Oliveira et al., 2011; Farshadfar et al., 2013). Both analyses are suitable especially in delineating suitable mega-environments for production (Balestre et al., 2009; Oliveira et al., 2011). GGE biplot analysis provides a more complete visual evaluation of the data by simultaneously representing mean performance and stability (Yan et al., 2007). It also displays the won where pattern of the data leading to the identification of high yielding and stable genotype (Araus et al., 2008; Rad et al., 2013).

Multi environmental trials and subsequent data collection and analysis involving experimental hybrids is helpful to identify genotypes with high and stable yield performance and to select test environments (Kandus et al., 2010). Therefore, the objective of this study was to investigate the GXE interaction for grain yield and MSV resistance among newly developed maize hybrids in Tanzania using AMMI and GGE biplot methods.

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6.2 Materials and methods

6.2.1 Study sites

The study was conducted across six different environments, consisting of three locations and two seasons in Tanzania (Table 6.1 and Figure 6.1). The three locations are known hot spots for MSV disease. The locations vary significantly in soils, mean annual temperatures, amount and distribution of rainfall. Variation in growing environments allows testing of the newly developed hybrids for GXE interaction and their stability towards yield and MSV resistance.
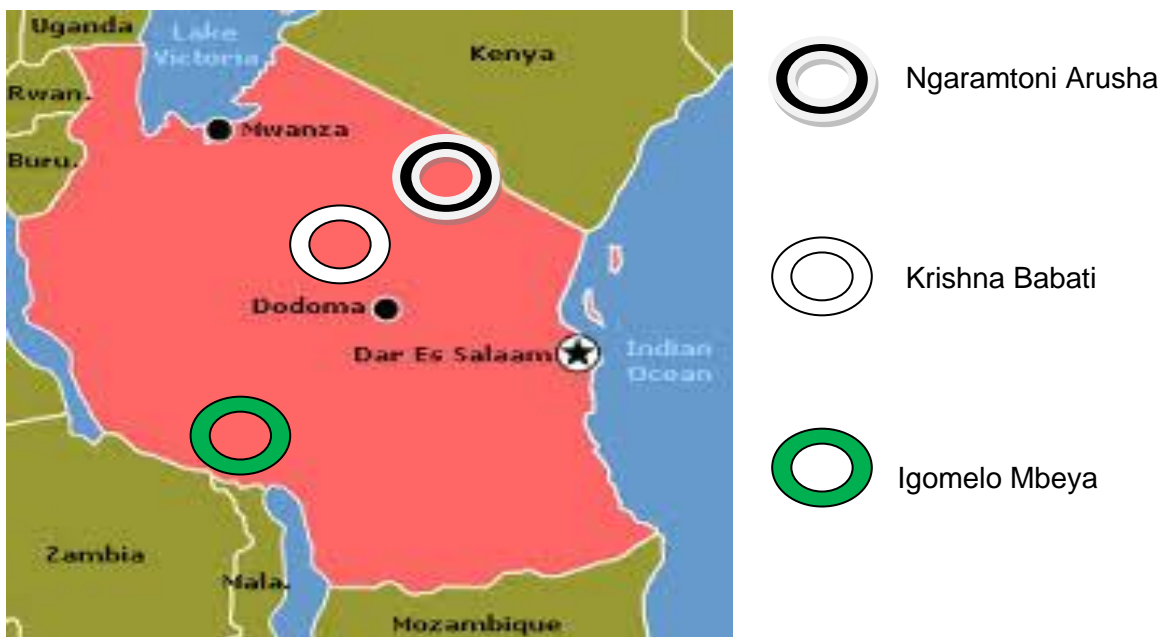
Figure 6.1: Map of Tanzania showing sites of the study

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Table 6.1 Descriptions of the six environments used for the study

| Environment Codes | Site | Season | District | Region | Geographic position | | | Mean annual rainfall (mm) | Temperature (^{0}C) | | Soil type |
					Longitude	Latitude	Elevation (masl)		Min	Max	
E1	Ngaramtoni	2012/13	Arusha	Arusha	3^0 18'S	$36^0$34'E	1520	214	17	29	Clay silt loam
E2	Ngaramtoni	2013/24						400	14	30	
E3	Krishna	2012/13	Babati	Manyara	$4^{.0}$ 22'S	35^0 77'E	1100	650	25	30	Red clay loam
E4	Krishna	2013/14						650	25	30	
E5	Igomelo	2012/13	Mbarali	Mbeya	$8^0$46'S	$34^0$23'E	1118	450	25	31.8	Red sandy loam
E6	Igomelo	2012/14						650	23.7	30	

Source: Environmental Benchmark (2012); East African Community Figures and Facts Report (2014)

6.2.2 Plant material and experimental design

The study used a total of 50 maize hybrids consisting of 45 newly developed and five standard check three-way hybrids (Table 6.2). The standard checks are commonly growing three-way hybrids which were obtained from the local markets. The 50 hybrids were field planted in a 5 x10 lattice design with two replications across the six environments. The plot consisted of 2 rows of 5.0 m length. The spacing between rows were 75 cm and between plants of 30 cm. A healthy and vigorous seedling was established per hill. A 150 kg ha^{-1} Di-amonim phosphate (DAP) fertilizer was applied during planting while an equal amount of Calcium Amonium Nitrates (CAN) was top dressed at six weeks after planting. The trials were conducted under rain-fed conditions with supplemental irrigation when required.

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Table 6.2 Fifty maize hybrids used in the study showing pedigree and sources of parents used in cross formation.

No	GID	Genotype/hybrid	Pedigree	Origin of parents/hybrids
1	G1	TL2012-68/TL2012-55	CML509/09MAK1-77	CIMMYT/Kenya X UKZN/South Africa
2	G2	TL2012-42/TL2012-55	SML125/09MAK1-77	SARI/Tanzania X UKZN/South Africa
3	G3	TL2012-42/TL2012-25	SML125/MAS[MSR/312]-119-5-1-1-3-B	SARI/Tanzania X CIMMYT/Zimbabwe
4	G4	TL2012-26/TL2012-1	TZE-W Pop x 1368 STR S7 Inb.6/CML505	IITA/Nigera X CIMMYT/Kenya
5	G5	TL2012-38/TL2012-1	P43-1-1-1-BBB/CML505	SARI/Tanzania X CIMMYT/Kenya
6	G6	TL2012-42/TL2012-41	SML125/ CML390	SARI/Tanzania X CIMMYT/Kenya
7	G7	TL2012-1/TL2012-17	CML505/CML505	CIMMYT/Kenya X CIMMYT/Kenya
8	G8	TL2012-68/TL2012-38	CML509/P43-1-1-1-BBB	CIMMYT/Kenya X SARI/Tanzania
9	G9	TL2012-68/TL2012-26	CML509/TZE-W Pop x 1368 STR S7 Inb.6	CIMMYT/Kenya X IITA/Nigeria
10	G10	TL2012-41/TL2012-17	CML390/CML505	CIMMYT/Kenya X CIMMYT/Kenya
11	G11	TL2012-7/TL2012-26	MAS[MSR/312]-119-5-1-4-1-BB/TZE-W Pop x 1368 STR S7 Inb.6	CIMMYT/Zimbabwe X IITA/Nigeria
12	G12	TL2012-42/TL2012-1	SML125/CML505	SARI/Tanzania X CIMMYT/Kenya
13	G13	TL2012-41/TL2012-55	CML390/09MAK1-77	CIMMYT/Kenya X UKZN/South Africa
14	G14	TL2012-42/TL2012-26	SML125/TZE-W Pop x 1368 STR S7 Inb.6	SARI/Tanzania X IITA/Nigeria
15	G15	TL2012-68/TL2012-17	CML509/CML505	CIMMYT/Kenya X CIMMYT/Kenya
16	G16	TL2012-7/TL2012-68	MAS[MSR/312]-119-5-1-4-1-BB/CML509	CIMMYT/Zimbabwe X CIMMYT/Kenya
17	G17	TL2012-26/TL2012-55	TZE-W Pop x 1368 STR S7 Inb.6/09MAK1-77	IITA/Nigera X UKZN/South Africa
18	G18	TL2012-42/TL2012-38	SML125/P43-1-1-1-BBB	SARI/Tanzania X SARI/Tanzania
19	G19	TL2012-7/TL2012-1	MAS[MSR/312]-119-5-1-4-1-BB/CML505	CIMMYT/Zimbabwe X CIMMYT/Kenya
20	G20	TL2012-38/TL2012-26	P43-1-1-1-BBB/TZE-W Pop x 1368 STR S7 Inb.6	SARI/Tanzania X IITA/Nigeria
21	G21	TL2012-7/TL2012-25	MAS[MSR/312]-119-5-1-4-1-BB/MAS[MSR/312]-119-5-1-1-3-B	CIMMYT/Zimbabwe X CIMMYT/Zimbabwe
22	G22	TL2012-26/TL2012-17	TZE-W Pop x 1368 STR S7 Inb.6/CML505	IITA/Nigera X CIMMYT/Kenya
23	G23	TL2012-68/TL2012-42	CML509/SML125	CIMMYT/Kenya X SARI/Tanzania
24	G24	TL2012-17/TL2012-55	CML505/09MAK1-77	CIMMYT/Kenya X UKZN/South Africa
25	G25	TL2012-25/TL2012-17	MAS[MSR/312]-119-5-1-1-3-B/CML505	CIMMYT/Zimbabwe X CIMMYT/Kenya
26	G26	TL2012-38/TL2012-25	P43-1-1-1-BBB/MAS[MSR/312]-119-5-1-1-3-B	SARI/Tanzania X CIMMYT/Zimbabwe
27	G27	TL2012-25/TL2012-55	MAS[MSR/312]-119-5-1-1-3-B/09MAK1-77	CIMMYT/Zimbabwe X UKZN/South Africa
28	G28	TL2012-42/TL2012-17	SML125/CML505	SARI/Tanzania X CIMMYT/Kenya

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29	G29	TL2012-68/TL2012-1	CML509/CML505	CIMMYT/Kenya X CIMMYT/Kenya
30	G30	TL2012-1/TL2012-41	CML505/ CML390	CIMMYT/Kenya X CIMMYT/Kenya
31	G31	TL2012-25/TL2012-41	MAS[MSR/312]-119-5-1-1-3-B/ CML390	CIMMYT/Zimbabwe X CIMMYT/Kenya
32	G32	TL2012-38/TL2012-17	P43-1-1-1-BBB/CML505	SARI/Tanzania X CIMMYT/Kenya
33	G33	TL2012-25/TL2012-26	MAS[MSR/312]-119-5-1-1-3-B/TZE-W Pop x 1368 STR S7 Inb.6	CIMMYT/Zimbabwe X IITA/Nigeria
34	G34	TL2012-7/TL2012-38	MAS[MSR/312]-119-5-1-4-1-BB/P43-1-1-1-BBB	CIMMYT/Zimbabwe X SARI/Tanzania
35	G35	TL2012-68/TL2012-25	CML509/MAS[MSR/312]-119-5-1-1-3-B	CIMMYT/Kenya X CIMMYT/Zimbabwe
36	G36	TL2012-26/TL2012-41	TZE-W Pop x 1368 STR S7 Inb.6/ CML390	IITA/Nigera X CIMMYT/Kenya
37	G37	TL2012-7/TL2012-55	MAS[MSR/312]-119-5-1-4-1-BB/09MAK1-77	CIMMYT/Zimbabwe X UKZN/South Africa
38	G38	TL2012-38/TL2012-41	P43-1-1-1-BBB/ CML390	SARI/Tanzania X CIMMYT/Kenya
39	G39	TL2012-1/TL2012-55	CML505/09MAK1-77	CIMMYT/Kenya X UKZN/South Africa
40	G40	TL2012-25/TL2012-1	MAS[MSR/312]-119-5-1-1-3-B/CML505	CIMMYT/Zimbabwe X CIMMYT/Kenya
41	G41	TL2012-7/TL2012-41	MAS[MSR/312]-119-5-1-4-1-BB/ CML390	CIMMYT/Zimbabwe X CIMMYT/Kenya
42	G42	TL2012-7/TL2012-42	MAS[MSR/312]-119-5-1-4-1-BB/SML125	CIMMYT/Zimbabwe X SARI/Tanzania
43	G43	TL2012-68/TL2012-41	CML509/ CML390	CIMMYT/Kenya X CIMMYT/Kenya
44	G44	TL2012-38/TL2012-55	P43-1-1-1-BBB/09MAK1-77	SARI/Tanzania X UKZN/South Africa
45	G45	TL2012-7/TL2012-17	MAS[MSR/312]-119-5-1-4-1-BB/CML505	CIMMYT/Zimbabwe X CIMMYT/Kenya
		Standard checks		
46	G46	SC627		SEEDCO
47	G47	UH615		ARI- Uyole
48	G48	SARI H308		ARI-Selian, ASA
49	G49	SARI H208		ARI-Selian, ASA
50	G50	PANNAR 4M-19		Pannar seed company

GID = Genotype identifier, ASA= Agricultural Seed Agency; ARI = Agricultural Research Institute

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6.2.3 Data collection and analysis

Data collected included grain yield (t/ha) and MSV reaction expressed in %. Data were first subjected to the combined analysis of variance (ANOVA) using the SAS standard GLM procedure (SAS Institute, 2012). This was followed by AMMI and GGE biplot analyses. The breeding view statistic utility in the BMS software was used for AMMI and GGE biplot analyses (McLaren, 2014).

MMI analysis

The AMMI analysis combines the significant results of analysis of variance with principal component analysis. It first fits the additive main effects such as genotypes and environmental main effects using the analysis of variance (ANOVA) procedure followed by the multiplicative effects of GXE interaction using the principal component analysis (PCA). The formula for AMMI model analysis was as follows: $Yij = \mu + gi + \beta j + \sum_{n=1}^{N} \lambda nYm\delta jn + \varepsilon ij$; where Yij is the mean performance of genotype i[th] in environment j[th;] μ is the grand mean; $gi + \beta j$ are the genotype and environmental deviation means from the grand mean, respectively; λn is the square root of eigenvalue of the PC analysis axis n; γm and δjn are principal component scores (eigenvectors) for axis n of the genotype i[th] and j[th] environment, respectively. The term n in the model is the number of principal component retained in the model and εij = the error term. The environment and genotype PCA scores are expressed as the unit vector multiplied by the square root of λn, that is the environment PCA score =(√ λn)Yjn and genotype PCA score = (√ λn)Ym.

AMMI stability value (ASV)

ASV is an important parameter that measures the relative stability of each genotype in each environment and across environments (Dagnachew et al. 2014). This parameter was calculated according to the formula suggested by Purchase (1997). The ASV is the distance of interaction principal component IPCA from coordinate point to the origin in a two dimensional plot of IPCA 1 against IPCA 2 scores in the AMMI model. Because the IPCA1 contributes more to the GXE interaction sum of squares then a weighted value has to be estimated for each genotype and environment according to the relative contributions of the first two IPCAs. The following formula was used in the calculation of AMMI stability value (ASV). $ASV = \sqrt{[(\frac{SSIPCA1}{SSIPCA2})(\text{IPCA2 csore})2 + (\text{IPCA2 csore})2]}$; Where $\frac{SSIPCA1}{SSIPCA2}$, represents the weight assigned to the first interaction principal component score due to its high contributions in the GXE model. The larger the ASV value in either direction positive or negative the more specifically adapted the genotype to a certain

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environment. Smaller ASV indicates a more stable genotype across environments (Purchase, 1997; Dagnachew et al., 2014).

GGE biplot analysis

The GGE-biplots (Yang et al., 2009) were generated using the first two symmetrical scaled principal components; PC1 and PC2 for an average tester coordinate (ATC) or average environment coordination (AEA) view biplots. To compare yield performance, adaptability and stability among genotypes a scatter diagram with two lines crossing each other at the original centre of the biplot was generated. The abscissa (i.e., x-axis) represented yield performance while the vertical line (y-axis) represented the level of variability from zero (i.e., centre of origin). Genotypes located in right hand side of this vertical line were considered to be adapted to high yielding environments while those which located on the left hand side of this line top or below the centre of origin were considered to be adapted to low yielding environments and any genotype situated far from the origin (y=0), was considered unstable in its performance. To visualize a correlation between environments and their discriminating ability a vector view biplos were generated while to identify mega environments and winning genotypes in each mega environments a polygon view was also generated. GGE biplot was also used to identify ideal test environments or genotypes using average environment coordination (AEA) view with concentric circles. All graphic summaries were done using the GENSTAT analytical software version 14. The applications of GGE biplot analysis have been described by other researchers (Yan and Tinker, 2006; Yan et al., 2007).

6.3 Results and discussion

6.3.1 Analysis of variance

Across site and combined analyses of variance for grain yield and MSV resistance showed significant (p <0.05) differences among genotypes and their interaction with the environment (Table 6.3). The preliminary analysis of variance detected the presence of GXE interaction and allowed to assess the magnitude of GXE interaction among the maize hybrids.

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Table 6.3 Combined analysis of variance (ANOVA) for grain yield and MSV reaction

Source of variation	DF	GYD				MSV			
		SS	MS	F Value	Pr > F	SS	MS	F Value	Pr > F
Replication	1	0.00	0.00	0	0.9792	1187.8	1187.789	1.66	0.1982
Block	18	15.73	0.87	0.9	0.578	12636.4	702.0206	0.98	0.4792
Genotype (G)	49	486.12	9.92	10.23	<.0001	247804.109	5057.227	7.08	<.0001
Environment (E)	5	2059.62	411.92	424.59	<.0001	8258.4	1651.686	2.31	0.0441
GXE interaction	245	679.14	2.77	2.86	<.0001	281115.918	1147.412	1.61	<.0001
Error	281	272.62	0.97			200637.629	714.0129		
Total	599	3578.13	426.46			760138.784	10460.2		

DF = Degrees of freedom, GYD= Grain yield (t/ha), MSV= Maize streak virus disease severity scores (%), SS = Sum of squares, MS =Mean squares

6.3.2 AMMI analysis

Results from AMMI analysis revealed that both genotypes and environments were highly significantly different (P<0.001) for grain yield and MSV reaction (Table 6.4). Several previous studies conducted on GXE interaction in many crops reported significant variations of genotype, environment and their interactions (Mohamed et al., 2013; Adu et al., 2013, Grada and Ciulca, 2013; Kamutando et al, 2013). The first and second interaction principal components (IPCA 1 and 2) were highly significant (p< 0.001) for all traits. This was in general agreement with Mohamed et al. (2013). However, only IPCA2 exerted significant effect on MSV reaction (Table 6.3). Both results indicated the existence of GXE interaction among genotypes for these traits. The IPCs explained 67.72% of the total GXE interaction effect on grain yield and 60.43% on MSV disease reaction, which were in agreement with that of Mohamed et al., 2013. The first principal component (IPCA 1) explained 40.97and 37.79% of the total variation of grain yield and MSV reaction among the hybrids while the second component (IPCA2) explained 26.73 and 22.64% of the variation, in that order (Table 6.3). Therefore, there was a differential yield performance among the 45 newly developed F1 maize hybrids owing to considerable GXE interaction. Akter et al. (2014) reported similar results in a study of AMMI biplot analysis for stability of grain yield in hybrid rice.

Genotype and GXE interaction contributed to 12.4 and 17.76% of the total explained variation of grain yield, respectively. This was relatively less when compared to the environmental contribution at 52.06%. This suggests that the environment contributed greatly to the observed GXE interaction of the test genotypes. The environmental effects in the current study explained

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52.06% of the total variation among genotypes on grain yield while 12.4% and 17.2% were due to genotype and GXE interaction, respectively. Mohamed et al. (2013) reported 9.48 and 15.5% were due to genotype and GEI respectively, typically the same results as what is presented in this study. Also, Nzuve et al (2013) reported that 64.5% of total variation was attributable to environments. Their result was comparable to this result although it is slightly higher in respect to what has been reported in this study.

Table 6.4 AMMI analysis of variance for grain yield and MSV severity of 50 maize genotypes tested across six environments in Tanzania

| Source of variation | DF | GYD | | | | MSV | | | |
		SS	MS	% TSS	%GEI	SS	MS	%TSS	%GEI
Genotypes (G)	49	249.1	5.08***	12.4		99157	2023.6***	42.52	
Environments (E)	5	1043.9	208.77***	52.06		1829	365.8ns	2.77	
GXE interactions	245	356.1	1.45	17.76		66109	269.8	28.35	
IPCA 1	53	145.9	2.75***		40.97	24984	471.4***		37.79
IPCA 2	51	95.2	1.87***		26.73	14970	293.5*		22.64
Residuals	141	115	0.82			26155	185.5		
Total	543	2005.2	220.74			233204	3609.6		

DF= Degree of freedom, SS=Sum of squares, TSS= Total sum of squares, MS= Mean squares; GYD= Grain yield (t/ha), MSV= Maize streak virus disease severity (%)

6.3.3 Mean grain yields of genotypes

The mean grain yields of the 45 experimental maize hybrids coded G1 to G45 across the six environments (E1-E6) are indicated in Table 6.5. Genotype, G10 had the highest overall mean grain yield of 6.72 t/ha and was ranked first across all environments. The same hybrid performed substantially high in E2, E3, and E6, with mean grain yield of 8.62, 7.37 and 7.54 t/ha, in that order where it ranked the second. The AMMI stability values (ASV) among experimental materials ranged from -3.714 to 2.587 displayed by G6 and G45, respectively (Table 6.5 and Figure 6.2). Other genotypes with significant negative ASVs were G3, G11, G26 and G50 while those with significant positive values were G28 and G37 (Table 6.5; Figure 6.2). Genotypes with large ASV are considered to be variable. Genotypes such as G12, G18, G19, G35, and G41 had relatively low ASV values of 0.157, 0.065, 0.038, 0.132 and 0.120, respectively and were considered stable. Dagnachew et al. (2014) reported similar results in a

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study of additive main effects and multiplicative interaction (AMMI) and genotype by environment interaction (GGE) biplot analyses aid selection of high yielding and adapted finger millet varieties. G43 ranked the second in yield performance across all environment with a mean of 6.70 t/ha and found to be most adapted in E5 and E6 as reflected by its relatively high ASV value of 0.788. It produced a maximum grain yield of 8.04 and 7.65 t/ha at E5 and E6, respectively (Table 6.5). G28 and G15 were also among the best yielding genotypes identified in this study; they respectively yielded 6.23 and 6.07 t/ha across all environments and were ranked the 3rd and 4th. G28 performed the highest (8.62 t/ha) in E3 but attained second and third positions in E5 and E1 providing mean yields of 6.84 and 9.91 t/ha, respectively. The hybrid G15 displayed grain yield of 8.44 and 6.92 t/ha at E2 and E3, respectively and consistently ranked second in these environments. Genotype G31 yielded lowest (1.95 t/ha across all the environments except in E6 (Table 6.5). G18 was the second yielder in E1 with substantial yield of 9.58 t/ha but performed poorly in other environments. G25 and G26 were generally poor yielders across other environments but in E4 and E2 with maximum mean yields of 5.65 and 8.88 t/ha, respectively (Table 6.5).

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Table 6.5 Mean grain yield (t/ha) of newly developed 45 F1 maize hybrids (G1-G45) with five standard checks (G46-G50) evaluated across six environments (E1-E5) with ranks, ASV and IPCA scores

GID	Genotype	Mean	Rank	E1	E2	E3	E4	E5	E6	ASV	IPCAg[1]	IPCAg[2]
G1	TL2012-17/TL2012-55	5.70	8	7.82	8.00	5.19	4.50	3.03	5.65	0.557	-0.135	-0.553
G2	TL2012-41/TL2012-55	5.18	21	9.41	7.19	4.15	2.66	3.03	4.64	0.130	0.343	-0.359
G3	TL2012-1/TL2012-55	4.88	37	8.77	7.99	3.31	1.49	4.46	3.23	-0.654	0.373	-0.118
G4	TL2012-26/TL2012-55	5.04	25	8.77	7.32	3.33	3.13	4.01	3.66	0.490	0.101	-0.493
G5	TL2012-25/TL2012-55	4.90	32	8.33	7.29	3.40	3.06	3.04	4.30	0.543	0.119	-0.546
G6	TL2012-38/TL2012-55	4.73	38	5.02	4.88	5.37	3.94	4.94	4.25	-3.714	-1.113	0.100
G7	TL2012-42/TL2012-55	4.69	39	7.11	7.60	4.70	1.24	3.04	4.47	0.261	0.187	0.124
G8	TL2012-68/TL2012-55	5.26	16	9.46	7.47	3.98	3.00	3.46	4.18	0.365	0.307	-0.445
G9	TL2012-7/TL2012-55	4.68	40	8.73	6.75	5.27	0.29	3.07	3.98	0.717	0.521	0.404
G10	TL2012-41/TL2012-17	6.72	1	9.50	8.62	7.37	2.96	4.33	7.54	0.343	0.216	0.287
G11	TL2012-1/TL2012-17	2.42	48	3.31	2.75	2.69	0.43	2.50	2.84	-1.401	-0.843	0.293
G12	TL2012-26/TL2012-17	5.60	11	9.16	6.89	5.29	2.82	3.95	5.49	0.157	0.093	-0.031
G13	TL2012-25/TL2012-17	5.43	13	8.49	6.91	5.45	4.48	3.87	3.35	0.452	-0.148	-0.444
G14	TL2012-38/TL2012-17	6.00	5	9.78	7.77	5.56	4.12	4.05	4.69	0.363	0.156	-0.377
G15	TL2012-42/TL2012-17	6.07	4	8.48	8.44	6.92	4.42	3.48	4.70	0.278	0.039	-0.278
G16	TL2012-68/TL2012-17	5.01	27	9.26	7.17	4.71	0.62	3.72	4.57	0.742	0.515	0.293
G17	TL2012-7/TL2012-17	4.58	41	8.55	7.55	4.78	1.08	1.56	3.93	-1.779	0.665	-0.093
G18	TL2012-1/TL2012-41	5.14	22	9.58	6.96	4.38	2.78	3.36	3.78	0.065	0.327	-0.331
G19	TL2012-26/TL2012-41	5.20	20	7.77	7.09	5.63	2.94	3.51	4.26	0.064	-0.038	-0.056
G20	TL2012-25/TL2012-41	4.91	30	9.20	7.47	2.23	3.33	3.45	3.79	0.805	0.228	-0.814
G21	TL2012-38/TL2012-41	5.21	18	8.75	7.39	5.17	1.37	3.16	5.44	0.598	0.379	0.166
G22	TL2012-42/TL2012-41	5.84	7	7.94	7.35	7.16	2.33	6.18	4.06	0.639	-0.205	0.649
G23	TL2012-68/TL2012-41	4.88	36	8.63	8.17	4.66	0.22	4.35	3.23	0.784	0.562	0.377
G24	TL2012-7/TL2012-41	4.91	31	8.24	7.45	6.18	0.90	2.32	4.34	0.732	0.515	0.311
G25	TL2012-26/TL2012-1	5.96	6	9.45	6.96	5.24	5.65	3.84	4.62	0.754	-0.185	-0.748
G26	TL2012-25/TL2012-1	4.94	29	8.29	8.88	3.86	1.63	2.12	4.85	-0.722	0.595	-0.333
G27	TL2012-38/TL2012-1	4.90	33	7.75	8.27	5.33	3.80	0.98	3.26	0.693	0.339	-0.731

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GID		Mean	Rank							IPCAe[1]	IPCAe[2]	
G28	TL2012-42/TL2012-1	6.23	3	9.19	7.50	8.62	0.76	6.84	4.44	1.287	0.159	1.286
G29	TL2012-68/TL2012-1	5.21	19	7.84	7.86	6.77	1.31	2.84	4.61	0.522	0.364	0.384
G30	TL2012-7/TL2012-1	4.90	34	8.25	7.09	5.51	1.11	4.06	3.35	0.431	0.272	0.360
G31	TL2012-25/TL2012-26	1.95	49	2.05	2.69	1.27	0.09	2.35	3.26	-2.412	-1.033	0.188
G32	TL2012-38/TL2012-26	5.28	14	8.52	7.91	5.53	0.60	4.64	4.50	0.618	0.370	0.536
G33	TL2012-42/TL2012-26	5.05	23	8.50	7.17	5.49	0.86	3.73	4.56	0.515	0.348	0.400
G34	TL2012-68/TL2012-26	1.94	50	3.87	2.88	1.44	1.05	1.13	1.25	0.842	-0.579	-0.319
G35	TL2012-7/TL2012-26	4.38	45	7.13	6.91	3.41	0.99	4.26	3.56	0.132	0.027	0.131
G36	TL2012-38/TL2012-25	4.52	43	7.61	5.15	5.59	1.09	3.84	3.82	0.506	-0.072	0.507
G37	TL2012-42/TL2012-25	5.24	17	8.92	8.25	5.21	1.59	3.47	3.98	1.607	0.512	0.052
G38	TL2012-68/TL2012-25	5.01	26	8.92	7.81	5.41	0.44	3.08	4.40	0.954	0.646	0.339
G39	TL2012-7/TL2012-25	4.34	47	8.32	4.58	5.36	0.42	2.48	4.89	0.505	0.195	0.490
G40	TL2012-42/TL2012-38	4.88	35	8.93	6.68	2.43	2.29	3.77	5.19	0.388	0.126	-0.394
G41	TL2012-68/TL2012-38	4.36	46	8.41	4.68	4.60	1.72	3.39	3.38	0.120	-0.002	0.120
G42	TL2012-7/TL2012-38	5.69	9	9.08	6.71	4.56	4.54	4.46	4.76	0.512	-0.213	-0.492
G43	TL2012-68/TL2012-42	6.70	2	7.87	6.18	7.65	2.80	8.04	7.65	0.788	-0.811	1.061
G44	TL2012-7/TL2012-42	5.51	12	8.97	6.79	5.12	3.54	4.23	4.39	0.214	-0.039	-0.213
G45	TL2012-7/TL2012-68	4.55	42	3.37	6.44	4.89	4.11	3.52	4.94	2.587	-1.096	-0.198
G46	UH615	5.28	15	6.73	7.97	2.96	2.42	5.38	6.22	0.804	-0.366	-0.077
G47	SARI H208	4.95	28	6.00	4.49	4.71	3.98	6.39	4.11	-3.310	-1.165	0.144
G48	SARI H308	5.05	24	6.51	6.25	4.74	4.73	3.98	4.07	0.921	-0.664	-0.457
G49	SC627	4.50	44	7.28	6.34	4.36	2.33	2.89	3.80	0.159	-0.041	-0.158
G50	PANNAR 4M-19	5.62	10	7.11	6.04	4.71	4.02	6.48	5.38	-3.348	-0.849	0.055
Environmental mean [EM]		5.00		7.90	6.82	4.83	2.32	3.76	4.35			
IPCAe[1]		2.151		1.488	0.035	-1.662	-1.479	-0.532				
IPCAe[2]		0.409		0.571	-1.613	2.231	-1.25	-0.349				

GID= Genotype identifier

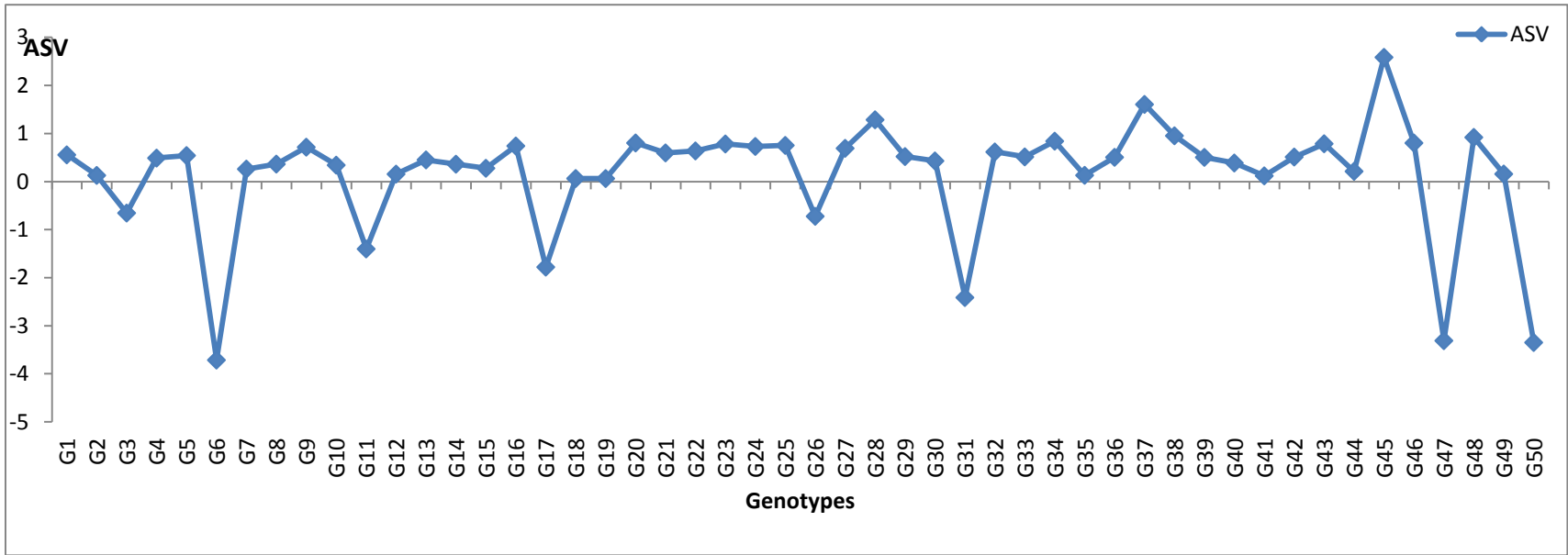| | 1st rank | | 2nd rank | | 3rd rank | | least |

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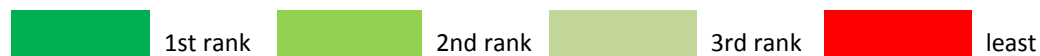
Figure 6.2: Variation of AMMI stability values (ASV) of 50 genotypes evaluated across six environments for grain yield. See code descriptions of genotypes in Table 6.5

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Genotypes ID selections per environment

The AMMI analysis identified four best hybrids in terms of grain yield performance across six environments. Hybrids G10 was selected as number one in environments E1 and E2. It was also selected as second in E6 and third in E3 (Table 6.6). G43 was selected number one in E3, E5 and E6. Other genotypes with multiple selections were G3, G25, G14, G28 and G22. All these hybrids with multiple selections of choices can be recommended for production in a wide range of environments as opposed to hybrids G26, G15, G1, G37, G42 and G50 which had only one choice, limited to a single location or environment therefore could be recommended for cultivation and production in that specific environment (Table 6.6). Related findings were documented by Mengesha (2013) when studying genotype by environment interaction the mid altitude sub-humid agro-ecologies of Ethiopia

Table 6.6 First four AMMI genotype ID selections per environment

Number	Environment	Mean	Score	1st	2nd	3rd	4th
1	E1	7.90	2.151	G10	G14	G26	G37
2	E2	6.82	1.488	G10	G14	G15	G25
3	E3	4.83	0.035	G43	G28	G10	G22
4	E4	2.32	-1.662	G25	G48	G1	G42
5	E5	3.76	-1.479	G43	G28	G22	G50
6	E6	4.35	-0.532	G43	G10	G28	G22

6.3.4. GXE interaction and identification of stable genotypes using AMMI biplot analysis

The biplot of the AMMI model presented in Figure 6.3 depicts the means of genotypes and environments against their respective interaction principal component (IPCA) scores. G11 and G10 appeared to have similar interaction with environment but differ significantly in yield. Yield performance of G10 was higher than that of G11 by 47.05% and was above average by 34.4% while G11 was below average by 51.6%. More than 40% of all tested hybrids performed above average such as G28, G43, and G10, G14, G15, G42 and G25. In the AMMI biplot, these hybrids were adapted to high yielding environment while about 22% of all the genotypes tested were below average including G11, G36, G8, G17, G25, G40, G35 and G45 and located or adapted to lower yielding environments (Figure 6.3). Such types of relationships among genotypes were reported by Abuali et al. (2014) and Kandus et al. (2010). Among the test

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environments, E1 and E2 were considered as high yielding environments as were located in the right hand side of the AMMI biplot while the rest four environments (E3, E4, E5, and E6) were lower performers (Figure 6.3). The first two environments performed above average with highest mean environmental mean yield of 7.90 t/ha and 6.23 t/ha, respectively (Table 6.5).
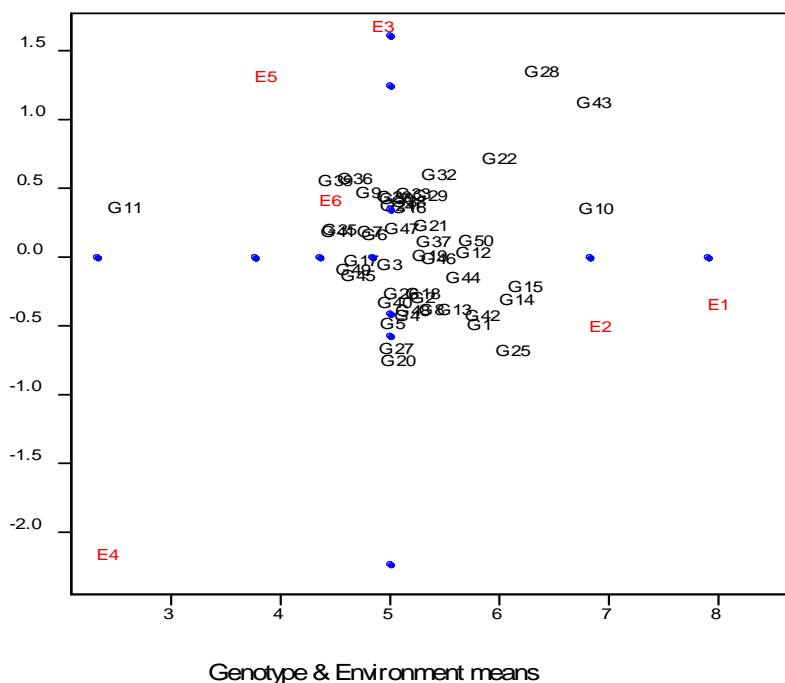

Figure 6.3: AMMI biplot with IPCA1 scores against means of genotypes and environments showing patterns of distribution of 45 F1 hybrids (G1-G45) across six environments (E1-E6). See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively.

6.3.5 GGE biplot analysis

Relationships among test environments and their discriminating ability

The GGE biplot (Figure 6.4) accounted 67.695 of the total phenotypic variation. The first principal component explained 40.97% while the second explained 26.72%. Tonk et al. (2011) found that 61.2% of total variation resulting from the two principal components in their GGE studies. The length of vectors of each environment and their cosine angles among them were analyzed. Environments, E1 and E2 had relatively long vectors and their cosine angle between them was significantly small indicating that they are positively strongly correlated and had high discriminating ability about the genotypes (Figure 6.4). These two environments can be used in

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evaluation studies because they have ability to discriminate the genotype and they give more information about them. Similar finding were reported by (Dagnachew et al., 2014). But the same environments showed had negative relationships with the remaining environments (E3, E5, D6 and E4) as reflected by the obtuse cosine angle between them. Yan and Tinker (2006) studied that strong negative correlation of this type causes significant crossover in performance of genotypes and therefore affect areas of recommendation for cultivation and production of the developed genotypes. E3 and E5 had moderately long vectors 450 angle between them. This implies that their correlation was 0.707 because cosine of 45^0 is approximately to 0.707. E6 was considered to have low discriminating ability because of its shortest vectors (Figure 6.4). This environment   gives little information about the performance of the genotypes under study thus should not be used in evaluation studies. A similar pattern of environments was reported in Ethiopia when Rezene et al.(2014) studying GGE biplot anlaysis on grain yield of pea.
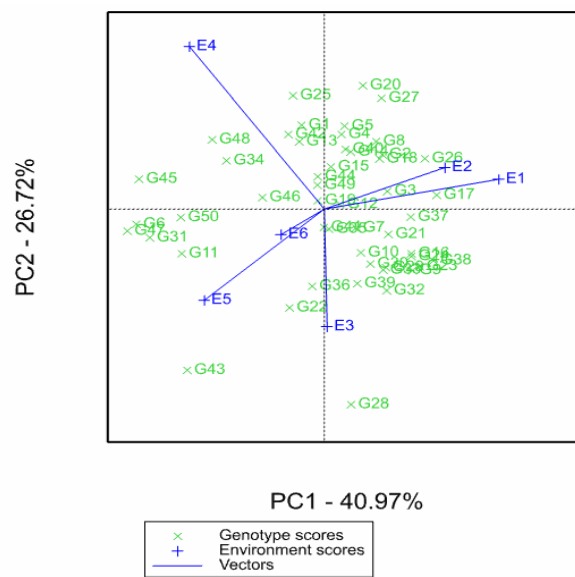
Figure 6.4: GGE Biplot showing relationships of the test environments and their discriminating ability. See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively.

Stability and representativeness of environment s and genotypes

The concentric circles drawn on the biplot assisted breeders to visualize the stability of environments and genotypes in yield performance (Asnake et al., 2013, Dagnachew et al., 2014). Environments or genotypes that fall onto the centre of the innermost concentric smallest circle are considered ideal while those located closer to it (innermost circle) are considered

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desirable and discriminating (Naroui et al., 2013). In the present study, E1 was most stable and representative because it was found in the innermost concentric small circle (Figure 6.5). E2 located on the second circle next to this smallest circle suggests that it was relatively most desirable. In contrast, E3, E4, E5 and E6 located far away from the concentric innermost circle hence were considered undesirable with E4 and E5 being the most undesirable. These environments were neither representative nor discriminating (Figure 6.5). On the other hand, hybrids G44, G12, G19, G12, G1, G13 and G42 were found within the innermost concentric smallest circle and had performance above average suggesting that they are most ideal hybrids (Figure 6.6). Such genotypes are considered to be stable and can be used as reference genotypes or hybrids for evaluating (Karimizadeh et al., 2013; Mohamed et al., 2013). G14, G4, G46 and G25 were located in the concentric circle next to the inner most concentric smallest circle therefore were considered more desirable. Other genotypes such as G31 were undesirable with G34 and G11 being the most undesirable (Figure 6.6). Several studies have reported similar phenomenon (Jandong et al., 2011; Asnake et al., 2013).

PC1 - 44.69%

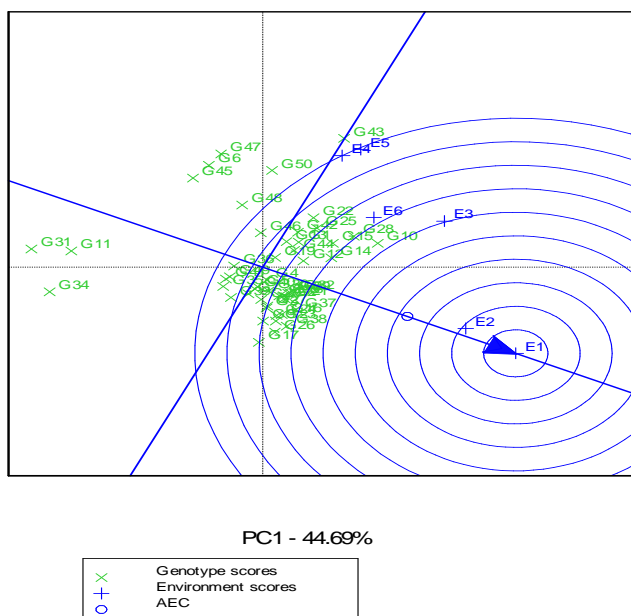|	Genotype scores
×	
+	Environment scores
○	AEC

Figure 6.5: GGE Biplot showing ranking of environments based on ideal test environments or representativeness. See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively

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PC1 - 44.69%

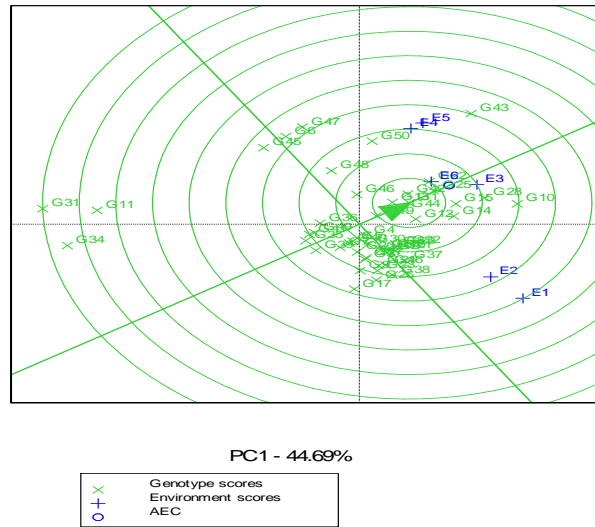|	
×	Genotype scores
+	Environment scores
○	AEC

Figure 6.6: GGE biplot showing genotypes based on ideal genotype. See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively.

Identification of superior genotypes in each mega environments

Figure 6.7 presents the which won where pattern view of the GGE biplot. This biplot accounted 65.72% of the total variation of the data with each component 44.69% for the first and 21.03% for the second component. This biplot is important it is used to indicate the most performing genotypes (superior) in each of the possible mega environments identified. The vertices of the irregular polygon drawn on the GGE biplot represent the yield potential of the wining genotypes (Yan et al., 2007). Hybrids G43 and G10 were considered superior because they were located at the vertices of the polygon. These hybrids were also very close to E5, E3 and E6 suggesting that they adapted well to these environments (Figure 6.7). G31 and G34 were also among the superior hybrids but in lower yielding environments.
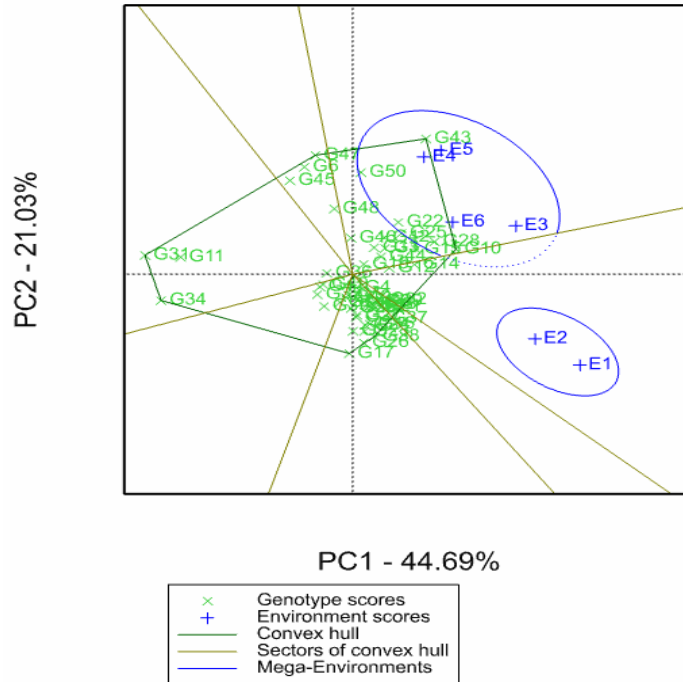
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Figure 6.7: The polygon view of the GGE biplot analysis showing the which won where pattern for selecting superior genotypes. See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively.

Ranking of genotypes and environments based on yields and stability

Figure 6.8 presents the average environment coordination (AEC) or average environment axis (AEA) view of the GGE biplot showing stability and mean performance ranking of genotypes. The biplot view consisted of two principal lines. The single arrowed line is the AEC or AEA abscissa points to higher mean yield across environments and the crossing line that points to greater variability (poor stability) in either direction. This biplot explained 64.19% of the total variation with the first and second PCs contributing to 43.43 and 20.77%, respectively. G10 had highest mean yield, followed by G28 and G43 but were unstable. G31 appeared to have the same variability levels with G10 but differed considerably in yield performance (Figure 6.8). These two hybrids had grain yields of 1.95 and 6.70 t/ha, respectively (Table 6.5). The hybrid G43 demonstrated highest level of variability in yield performance because it had lower (2.80 t/ha) than expected yield in E3 but produced highest yield in E5 and E6 (Table 6.5). Tonk et al. (2011) and Nzuve et al. (2013) reported similar finding when analyzing multi-environment trial data using GGE biplot.

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PC1 - 43.42%

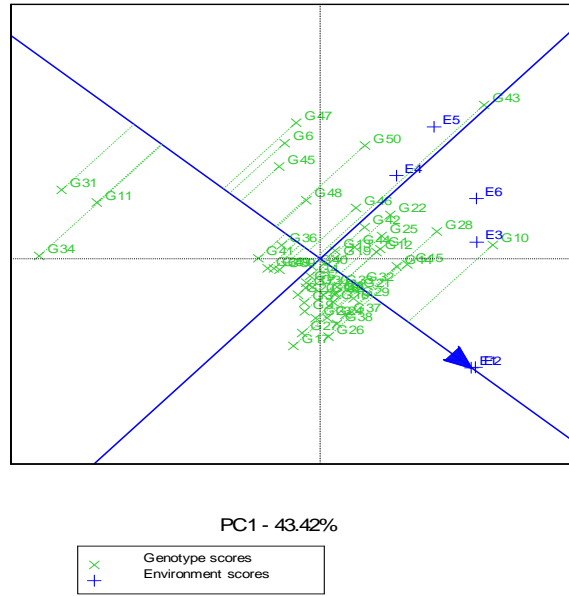|	
×	Genotype scores
+	Environment scores

Figure 6.8: The average environment coordination (AEC) view showing mean performance and stability of 45 F1 hybrids tested across six environments (E1-E6). See code descriptions of environments and genotypes in Tables 6.1 and 6.5, respectively.

6.3.6 Means of MSV disease reaction of F1 maize hybrids and standard checks evaluated across six environments

The mean MSV, ASV and IPCAs scores of newly developed 45 F1 maize hybrids and five standard checks evaluated across six different environments are presented in Table 6.7. Among the checks, hybrids UH615 and SC627 were the only checks that were used to compare disease reaction with the newly developed test materials (Table 6.2) the rest three were checks were for grain yield comparison. About 33.3% of the test hybrids had substantially low reaction to MSV disease, some of which include G31 (21.96%), followed by G23 (23.49%), G18 (25.07%) and G22 (26.06%). Others were G33 (28.89%), G34 (29.70%), G19 (30.07%), G26 (31.55%) and G43 (31.88%). Mukherjee et al. (2013) reported similar mean severity of blast in rice. In general, most of these hybrids had ASV value ranging between below one and a value greater than -1 (Figure 6.9) indicating that their performance does not differ significantly among the testing locations.

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Table 6.7 Mean MSV disease severity (%) of 45 F1 maize hybrids (G1-G45) and five standard checks (G46-G50) evaluated across six environments (E1-E5) with ranks, ASV and IPCA scores

GID	Genotype	E1	E2	E3	E4	E5	E6	Mean	Rank	ASV	IPCAg[1]	IPCAg[2]
G1	TL2012-17/TL2012-55	75.00	66.20	77.65	78.30	76.73	71.9	74.3	6	1.968	-0.662	-0.075
G2	TL2012-41/TL2012-55	61.13	77.69	35.17	27.33	44.14	59.72	50.86	31	-5.579	2.945	-0.804
G3	TL2012-1/TL2012-55	31.79	22.01	71.94	43.89	52.47	43.18	44.21	34	-6.395	-2.563	0.410
G4	TL2012-26/TL2012-55	62.97	49.66	71.47	64.50	65.73	63.60	62.99	22	2.602	-1.080	-0.187
G5	TL2012-25/TL2012-55	62.18	64.81	60.13	63.21	48.72	71.65	61.78	23	-1.515	0.935	-0.339
G6	TL2012-38/TL2012-55	77.37	37.30	21.79	83.20	67.97	67.88	59.25	24	3.077	-0.256	-3.076
G7	TL2012-42/TL2012-55	42.26	41.09	44.78	35.70	44.20	38.84	41.15	37	-0.243	-0.104	0.019
G8	TL2012-68/TL2012-55	65.56	55.69	58.41	39.35	22.39	37.18	46.43	33	2.223	1.253	1.988
G9	TL2012-7/TL2012-55	83.68	91.33	80.54	92.13	87.73	23.79	76.53	5	3.577	-0.504	3.582
G10	TL2012-41/TL2012-17	84.41	97.13	47.68	58.49	24.39	76.13	64.71	19	15.061	4.470	0.394
G11	TL2012-1/TL2012-17	61.69	56.51	13.71	46.73	68.82	56.23	50.62	32	2.635	1.228	-2.759
G12	TL2012-26/TL2012-17	51.93	63.91	36.78	77.68	27.34	78.65	56.05	27	-2.954	2.275	-1.167
G13	TL2012-25/TL2012-17	55.54	33.89	86.66	81.55	100.74	78.43	72.80	9	6.118	-3.861	-1.660
G14	TL2012-38/TL2012-17	98.23	95.70	81.38	82.42	83.04	101.37	90.36	1	-1.148	1.308	-0.981
G15	TL2012-42/TL2012-17	41.99	81.78	66.19	93.40	53.58	92.18	71.52	12	-1.588	1.274	-0.690
G16	TL2012-68/TL2012-17	39.38	93.57	78.65	81.02	26.55	68.46	64.61	20	3.308	2.320	2.392
G17	TL2012-7/TL2012-17	64.55	97.15	74.30	98.48	99.33	83.94	86.29	2	0.613	0.242	-0.631
G18	TL2012-1/TL2012-41	13.18	21.09	32.23	34.87	18.69	30.36	25.07	48	-0.713	-0.304	0.055
G19	TL2012-26/TL2012-41	35.80	30.69	23.80	25.00	19.98	48.70	30.66	44	0.875	1.102	-1.331
G20	TL2012-25/TL2012-41	51.83	21.63	38.59	28.13	44.71	39.57	37.41	39	1.240	-0.850	-0.941
G21	TL2012-38/TL2012-41	73.45	80.66	71.78	58.07	47.76	79.86	68.60	17	5.860	1.800	0.170
G22	TL2012-42/TL2012-41	22.18	20.55	40.50	23.28	28.55	21.27	26.06	47	-0.875	-0.878	0.601
G23	TL2012-68/TL2012-41	24.39	30.48	24.90	8.310	27.74	25.12	23.49	49	-0.600	0.514	-0.301
G24	TL2012-7/TL2012-41	80.03	29.90	90.71	82.81	74.07	55.05	68.76	16	-6.897	-3.367	0.792
G25	TL2012-26/TL2012-1	27.10	10.57	46.03	35.12	63.00	38.13	36.66	41	4.199	-2.765	-1.333

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G26	TL2012-25/TL2012-1	24.84	27.13	42.95	38.63	23.05	32.69	31.55	43	0.472	-0.370	0.560
G27	TL2012-38/TL2012-1	50.60	28.90	101.48	85.81	78.80	61.26	67.81	18	-10.075	-4.190	0.721
G28	TL2012-42/TL2012-1	43.76	46.02	45.56	49.06	19.35	44.36	41.35	36	1.382	1.005	0.781
G29	TL2012-68/TL2012-1	67.13	63.23	24.45	50.06	15.91	10.68	38.58	38	3.579	2.552	2.460
G30	TL2012-7/TL2012-1	69.34	41.63	74.74	48.17	64.06	31.66	54.93	28	-1.032	-1.803	1.607
G31	TL2012-25/TL2012-26	18.96	23.66	19.57	36.36	10.98	22.22	21.96	50	0.678	0.469	0.265
G32	TL2012-38/TL2012-26	66.42	79.37	98.81	93.33	74.76	29.36	73.68	7	4.106	-1.429	4.190
G33	TL2012-42/TL2012-26	20.87	21.47	31.96	41.95	21.73	35.36	28.89	46	0.448	-0.325	-0.241
G34	TL2012-68/TL2012-26	20.42	34.63	26.79	39.63	25.64	31.09	29.70	45	-1.628	0.430	-0.03
G35	TL2012-7/TL2012-26	74.21	48.47	93.02	92.31	54.31	58.15	70.08	15	0.924	-1.763	1.924
G36	TL2012-38/TL2012-25	71.70	75.73	87.47	76.26	67.20	63.18	73.59	8	1.471	-0.219	1.473
G37	TL2012-42/TL2012-25	35.83	36.51	18.68	20.23	50.97	58.70	36.82	40	3.043	0.674	-3.059
G38	TL2012-68/TL2012-25	48.38	74.02	36.38	12.69	27.73	63.20	43.73	35	-7.120	3.407	-0.771
G39	TL2012-7/TL2012-25	90.58	83.89	81.19	86.13	62.72	70.96	79.25	3	1.382	0.807	1.215
G40	TL2012-42/TL2012-38	69.41	66.65	24.26	63.36	64.65	38.39	54.45	29	-1.853	1.224	-0.498
G41	TL2012-68/TL2012-38	89.34	36.29	97.02	66.16	74.60	69.03	72.07	11	-9.377	-2.721	0.229
G42	TL2012-7/TL2012-38	74.63	86.18	78.24	84.79	56.56	80.24	76.77	4	1.856	1.265	0.678
G43	TL2012-68/TL2012-42	36.43	32.94	39.12	36.37	22.10	24.32	31.88	42	1.082	0.147	1.081
G44	TL2012-7/TL2012-42	72.68	75.55	89.75	67.23	52.68	76.85	72.46	10	1.149	0.556	1.077
G45	TL2012-7/TL2012-68	74.97	21.93	37.13	49.90	68.25	56.83	51.50	30	2.697	-1.444	-2.459
G46	UH615	83.46	70.10	85.07	62.99	64.49	61.34	71.24	14	1.273	-0.114	1.273
G47	SARI H208	66.09	42.80	57.63	87.97	77.98	96.04	71.42	13	3.250	-1.251	-3.153
G48	SARI H308	55.23	48.36	75.22	67.25	63.29	75.49	64.14	21	1.593	-1.123	-0.684
G49	SC627	54.46	41.68	58.30	66.52	50.30	80.03	58.55	25	1.619	-0.399	-1.607
G50	PANNAR 4M-19	39.22	58.72	53.76	62.28	59.98	70.13	57.35	26	1.158	0.144	-1.159
Environmental means [EM]		56.13	52.74	57.09	58.57	51.41	55.86	55.3				

<!-- legend -->
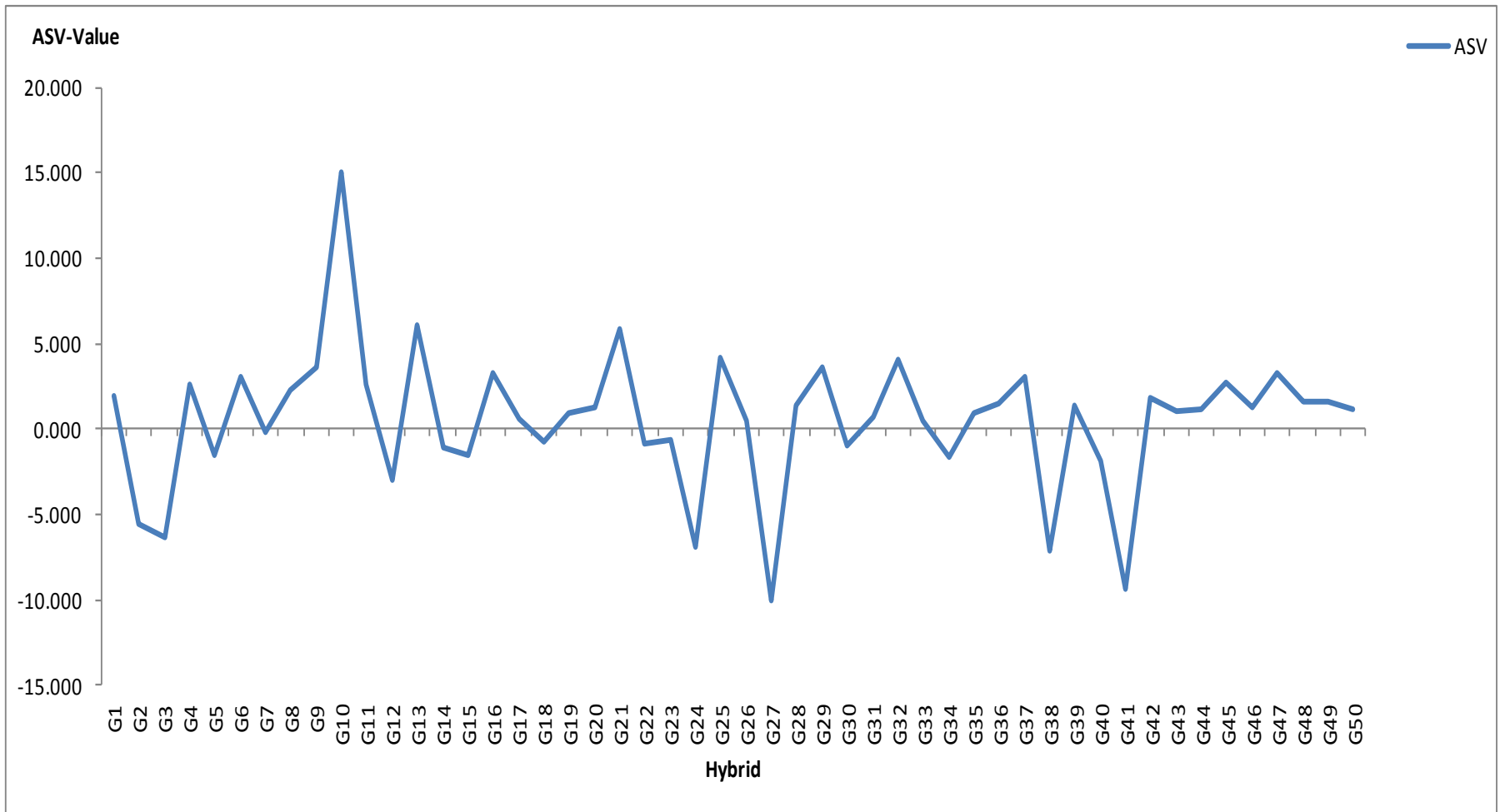██ 1st rank   ██ 2nd rank   ██ 3rd rank   ██ Least

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Figure 6.9: Variation of AMMI stability value (ASV) among 50 F1 maize hybrids evaluated across six environments for resistance to MSV disease. See description of codes for genotypes in Table 6.5

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6.3.7 GGE biplot analysis of genotypes based on MSV severity (%)

The GGE biplot (Figure 6.10) represent the distributions of genotypes based on their relative mean severity (%) performance and IPCA scores. G7 was located on horizontal line having IPCA score value close to zero together with G5 and G48 indicating that all had low variability but G7 had lower disease severity performance than G5 and G6. Similarly, G28 exhibited the same IPCA scores with G27 and G24 but had different disease severity levels (Figure 6.10). On the other hand, all G28, G7 and G38 were located on the same perpendicular line of the biplot suggesting that they have equal disease severity performance. Similarly, G16 and G10 had the same performance in disease severity because were located on the same perpendicular line of the biplot. Furthermore G28, G3, G7, and G38 resided in lower disease reaction side of the biplot suggesting that they were resistant whereas G27 and G28 were most susceptible followed by G16, G10, G4, G48 and G5 since were located on the right hand side of the biplot. These results did not differ from those that reported by Mukherjee et al. (2013) in rice blast. Interestingly, E3, E1 and E6 were located on the same perpendicular line of the biplot indicating that they had similar performance in disease severity but differed significantly in terms of stability because E1 was more stable than E3 and E6, had IPCA score value close to zero (Figure 6.10). To depict clearly the most resistant and susceptible genotypes the which won where pattern view of GGE biplot was generated (Figure 6.11). This biplot accounted 75.52% of the total variation attributable to GGE biplot model on the data with its first and second PC scores contributing to 60.60% and 14.92%, respectively. This indicates that the model described adequately the variation present among the tested genotypes. From this graph G31, G23, G22, G25 and G38 were superior genotypes but adapted to lower disease increasing environment while G14, G27 and G10 were also superior candidates but adapted to high disease increasing environment (Figures 6.10 & 6.11). These types of results were reported by Mukherjee et al. (2013) in rice blast pathosystem and Farshadfar et al. (2013) in chickpea.
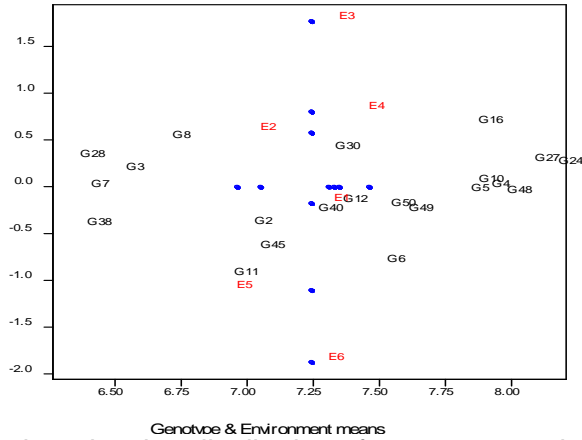
Figure 6.10: The GGE biplot view showing distribution of genotypes and environments based on their respective mean severity (%) performance for MSV disease and IPCA scores. See Tables 6.1 and 6.5 for descriptions of environments and genotypes codes, respectively

PC1 - 60.60%

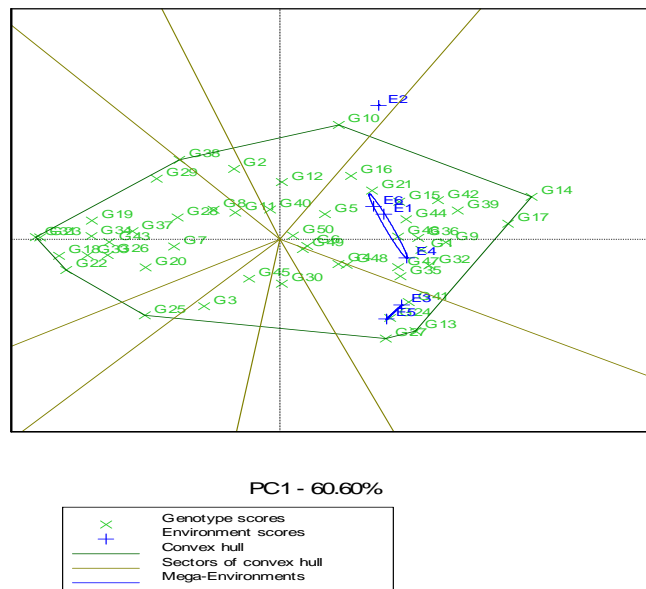|	Genotype scores
×	Environment scores
+	Convex hull
	Sectors of convex hull
	Mega-Environments

Figure 6.11: The which won where pattern view of GGE biplot showing most resistant and susceptible genotypes to MSV disease. See Tables 6.1 and 6.5 for descriptions of environments and genotypes codes, respectively.

Ranking of hybrids based on mean MSV severity and stability

The GGE biplot (6.12) shows the MSV severity % of 50 genotypes and their relative GXE interaction. It accounted 74.72% of the total variation of the data with its two IPCs contributing 60.63% and 14.09%, respectively (Figure 6.12). The average environment coordination (AEC) line with an arrow passes through the biplot origin line gives estimates performance of each genotype while other line crossing AEC estimates variation due to GXE interaction. From this

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biplot G21, G15, G42, and G5 although showed low variability, yet performed substantially high above average indicating that they had high level of susceptibility while G20, G7, G3, G22 and G25 recorded low MSV severity and had low variability suggesting that these hybrids are resistant and could be selected for production across the target environments, especially in areas of Tanzania where MSV incidence is frequently high. Consistently low disease expressions were reported by Hamidou et al. (2014) in a study of aflatoxin contamination. Additionally, G14 was the most susceptible genotype and relative unstable followed by G14, G10 and G17 which showed high levels of susceptibility and were most unstable (Figure 6.12).

PC1 - 60.63%
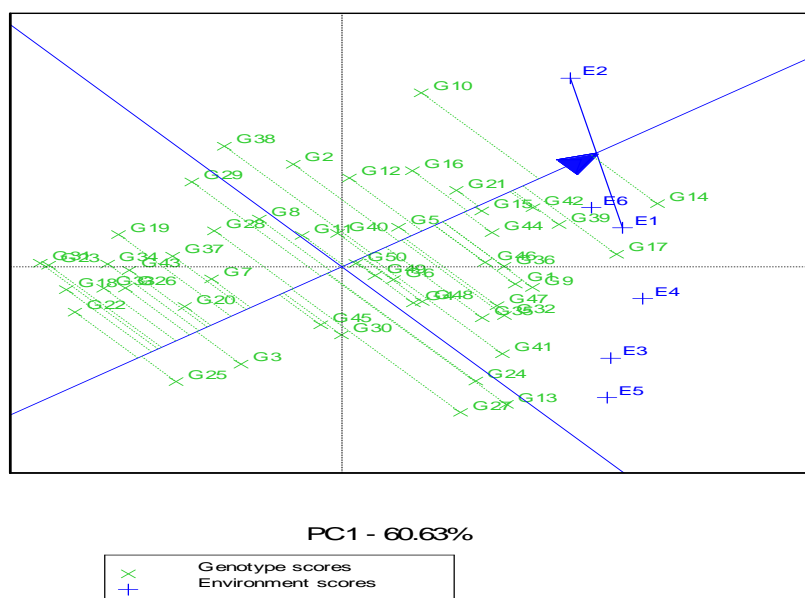
Genotype scores
Environment scores

Figure 6.12: GGE biplot view showing ranking of genotypes based on their reaction to MSV disease and stability across environments. See Tables 6.1 and 6.5 for descriptions of environments and genotypes codes, respectively

Ranking of genotypes and environment based on ideal performance of genotypes and test environments on MSV reaction

The GGE biplot view of concentric circles showing ranking of genotypes and environments based on ideal genotypes and test environments in performance to MSV reaction is presented in Figure 6.13. This biplot captured 75.52% of the total variation of the experimental materials. The first and second IPC scores of this biplot explained 60.60% and 14.92%, respectively. The biplot assisted to assess visually the stability and variability levels of genotypes. G32, G1, G47,

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and G33 were considered ideal or representative because were located in the smaller innermost concentric circle while G44 and G35 were found within the second concentric implying that they were desirable. G23, G18, and G22 were among the most undesirable genotypes on MSV reaction (Figure 6.13). Similarly, environment E1 was plotted in the smallest and innermost circle of the concentric GGE biplot (Figure 6.14) suggesting that it was an ideal environment for further evaluation of foliar diseases like MSV. Results further indicated that E6 was located next to the ideal environment found within second concentric circle thus was considered most desirable environments while the remaining environments were undesirable; with E5 being the most undesirable one followed by E2 (Figure 6.14).
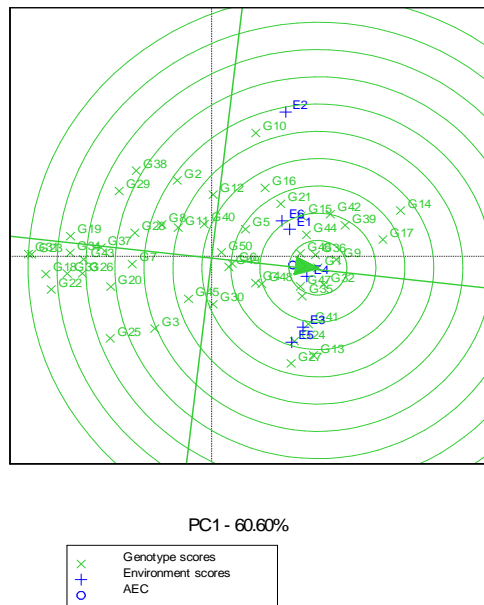

PC1 - 60.60%

×	Genotype scores
+	Environment scores
○	AEC

Figure 6.13: The GGE biplot view showing ranking of genotypes based on ideal or reference genotypes

PC1 - 60.60%

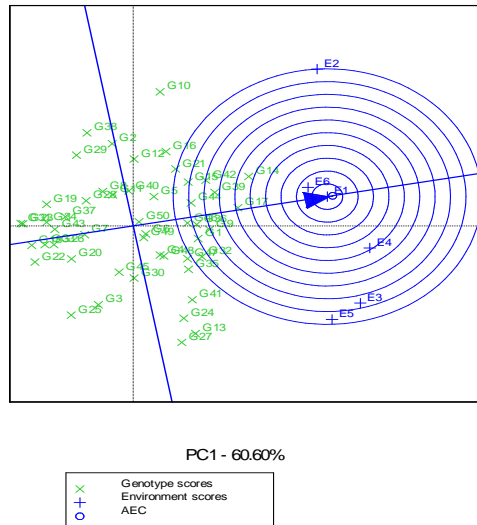|	
×	Genotype scores
+	Environment scores
○	AEC

Figure 6.14: GGE biplot view showing ranking of environments based on the ideal test environment for MSV reaction. See Tables 6.1 and 6.5 for descriptions of environments and genotypes codes, respectively.

6.4 Conclusions

Maize is widely grown in a wide range of diverse agro-ecologies in Tanzania. It accounts significantly for food security, income generation and rural livelihood of the majority poor smallholder farmers. However, productivity of maize is greatly affected by GXE interaction. GXE interaction is the most important factor that causes substantial yield variations under the smallholder farming systems and among maize growing agro-ecological zones in the country. GxE is also accelerated by the outbreaks of biotic stresses such as maize diseases like MSV, MLN and GLS and occurrence of random stresses and variability in soil fertility. Yield performance of genotypes is often confounded by GXE interaction and therefore reduces selection efficiency and response. Using the AMMI model the grain yield response of 45 novels F1 maize hybrids were evaluated across six environments. Results showed that environment accounted for 52.06% of the total variation in grain yield among genotypes. Therefore environments could be the major source of GXE interaction for grain yield observed among the test genotypes evaluated in this study although it was less important in the variations of MSV disease severity. The present study identified genotypes such as G10, G43, G14 and G28 showing respectively high mean grain yields of 6.72, 6.00, and 6.23 t/ha across environments showing minimal GXE interaction. These genotypes could be recommended for direct large scale production in northern Tanzania or similar environments. However some of these hybrids

were highly susceptible to the MSV disease. For example hybrids G14 and G10 had disease severities of 90.36 and 64.71%, respectively; hence they would not be recommended in MSV prone environments. Furthermore, hybrid G43 had good mean yield of 6.70 t/ha with consistently low reaction to MSV disease across locations except its variable yield expression across environments. This hybrid can be recommended in E5 and E6. In these environments, it yielded significantly high at 8.04 and 7.65 t/ha, respectively. Interestingly, most of MSV resistant hybrids identified in the current study were poor in yield performances. For example G31 had the least MSV infection but it yielded consistently low across environments. Other hybrids such as G23 with grain yield of 4.84 t/ha, G18 (5.14 t/ha), and G34 (1.94 t/ha) were not suitable for grain production but they can be exploited in MSV resistance breeding programs. In general, genotype by environment interaction is a big challenge for plant breeders. In this study, GGE biplot and AMMI models were particularly useful that revealed the magnitude of GXE interaction present in the study materials.

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General overview

7.1 Introduction and objectives

Maize (*Zea mays* L.) is the principal food security crop in Tanzania predominantly gown by smallholder farmers who account for more than 85% of the total maize production. Foliar diseases especially maize streak virus (MSV) remains the major challenge to maize productivity in Tanzania and east Africa. Yield losses due to MSV are substantially high reaching up to 100% on susceptible varieties. Different MSV management strategies have been identified and recommended including cultural method (early planting, crop rotation, intercropping with non-host species), phytosanitation, and chemicals to control its vectors, biological control and host resistance. Breeding farmers'-preferred, high yielding and MSV resistant maize varieties is probably the cheapest and the most practical means in Tanzania because chemicals are generally expensive, phytosanitation and cultural measures are difficult to apply, and biological control agents are not commercially available. This overview highlights the study objectives with subsequent summary of major findings of each objective. Finally, the implications of the findings are presented for maize breeding to MSV resistance and improved agronomic attributes according to the needs of the growers.

Objectives

The objectives of this study were to:

- determine farmers' preferred traits of maize and production constraints limiting maize production in the northern areas of Tanzania.
- determine agro-morphological diversity present among 80 local and introduced maize inbred lines under maize streak virus (MSV) prone environments of the northern zone of Tanzania.
- assess the genetic diversity and genetic relationship among 79 maize inbred lines collected from five different origins using 30 polymorphic simple sequence repeat (SSR) markers.
- determine combining ability and heterosis for grain yield and related traits and resistance to maize streak virus (MSV) among 10 elite maize inbred lines and their hybrid progenies, and
- investigate the GXE interaction for grain yield and MSV resistance among newly developed maize hybrids in Tanzania using AMMI and GGE biplot methods.

7.2 Main findings of the study

7.2.1 Key maize production constraints and identification of farmers' preferred traits in the mid-altitude maize agro-ecologies of northern Tanzania

A participatory rural appraisal (PRA) study was conducted in 2012 at Babati, Arumeru and Hai Districts in northern Tanzania. Data were collected involving 500 farmers using structured interviews and focused group discussions (FGD).

- Results showed that maize was the most important crop in the study areas and ranked first among other food crops. Grain yield potential, disease resistance and drought stress tolerance were farmers preferred traits with relative importance of 71.9, 70.0 and 69.9%, respectively.

- Through FGD farmers identified ear rot, MSV and common rust as most important diseases affecting maize production.

- High costs of production inputs and low price of maize were also among the challenges to maize production in the study area.

- Knowledge of the farmers' preferences and production constraints is required by breeders to enhance the productivity of maize in the northern areas of Tanzania

7.2.2. Agro-morphological characterization of maize inbred lines under maize streak virus prone environment

Eighty maize inbred lines were evaluated using ago-morphological traits. Field experiment was established during 2011/2012 at maize streak virus (MSV) prone environment of Ngaramtoni Research Farm of Selian Agricultural Research Institute in northern Tanzania using a 10 x 8 alpha lattice design with two replications.

- Lines TL2012-42 and TLl2012-41 were identified as superior lines with grain yields of 3.52 and 2.46 t/ha respectively. These genotypes showed low (< 30%) level of MSV reaction suggesting their suitability for hybrid breeding to achieve high grain yield and MSV resistance.

- Principal component analysis revealed 68.9% of the total variation explained by four principal components.

- The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the inbred lines into nine clusters consistent with their heterotic patterns

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- The study identified the following inbred lines: TL2012-53 and TL2012-61 from cluster II and TL2012-20, TL2012-70, and TL2012-78 from cluster IV for breeding.

7.2.3 Genetic diversity analysis of maize inbred lines collected from diverse origins using SSR markers

Genetic diversity and relationships of 79 maize inbred lines collected from five diverse sources were subjected to SSR analysis using 30 polymorphic markers.

- The mean numbers of observed and effective alleles were 4.70 and 2.40, respectively. The markers displayed high Shannon's information index of 0.96 and polymorphic information content (PIC) of 0.51.

- The mean values of observed and expected heterozygosity among lines were 0.136 and 0.508, respectively.

- A dendrogram constructed based on UPGMA clustered the inbred lines into three main genetic groups with varied sub-clusters.

- The principal coordinate analysis (PCA) explained 20.4% of the total genetic variation detected among inbred lines and separated them into two main clusters.

- Analysis of molecular variance (AMOVA) showed that 72% of the total variation was attributed to differences among inbred lines across locations, 26% of the total variation was due to inbred lines within sub-populations/locations and 2% was attributed to variation between the five geographic origins of inbred lines.

- The study identified inbred lines such as TL2012-20, TL2012-24 and TL2012-54 (from cluster I) and TL2012-25, TL2012-21 and TL2012-12 (from cluster III) showing genetic difference for hybrid breeding to exploit heterosis.

7.2.4 Combining ability and heterosis among maize genotypes for yield and yield components and resistance to maize streak virus disease

Ten selected inbred lines were crossed to generate 45 F_1 hybrids using a 10 x10 half diallel mating design. Parents, F_1 hybrids and five standard checks were evaluated using a 6 x 10 lattice design with two replications at Ngramtoni, Inyala and Igomelo during 2012/13 and 2013/14. General combining ability (GCA) of parents, specific combining ability (SCA) of

hybrids, heritability and heterosis of grain yield and related traits and MSV resistance were calculated.

- The SCA effect was important for all traits except for MSV, number of ears per plant and husk cover while the GCA effect was most important for resistance to MSV.

- Heritability estimates of traits were high associated with high GCA effects. Line TL2012-42 was a good general combiner for grain yield showing highly significant positive GCA effect of 0.695 t/ha while lines TL2012-41, TL2012-1 and TL2012-42 had significant negative GCA effects of -10.926, -10.792 and -10.748, respectively for MSV reaction. These inbred lines could be exploited in hybrid breeding to develop high yielding and MSV resistant varieties.

- Hybrids TL2012-38/TL2012-55 and TL2012-25/TL2012-26 had negative significant SCA effect of -10.892 and -19.451%, respectively for MSV reactions.

- Maximum mid-parent heterosis for grain yield was recorded for hybrid TL2012-7/TL2012-38 at 138% while TL2012-25/TL2012-26 had the lowest and negative heterosis of -38.2% for MSV reaction.

- Crosses TL2012-7/TL2012-42 and TL2012-7/TL2012-68 had significant positive SCA effects for grain yield which can be used for direct production as single cross hybrids or developed further as three way hybrids for large scale production.

7.2.5 Genotype by environment interaction of grain yield and MSV resistance among novel maize hybrids in the mid-altitude agro-ecologies of Tanzania

Genotype by environment interaction (GXE) of grain yield and MSV resistance was investigated among newly developed maize hybrids in Tanzania. Forty five novel single cross hybrids and five standard check three-way cross hybrids were evaluated using a 5x10 alpha lattice design with two replications across six environments. The Additive Main Effects and Multiplicative Interaction (AMMI) and genotype, and genotype by environment (GGE) biplot models were used to assess the magnitude of GXE interaction of grain yield and reaction to MSV disease among test genotypes.

- Results from the AMMI analysis of variance revealed high (52.06%) contribution of the environmental effect on grain compared to genotypes and GXE interaction which, respectively accounted for 12.4% and 17.76% of the total variation on this trait among hybrids tested.

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- Genotypes explained 45.52% of the total variation of hybrids for MSV resistance while the contribution of environments was minimal (2.77%).

- Hybrid G43 was identified with relatively high mean grain yield of 6.70 t/ha with low MSV severity of 31.88% across environments.

- Experimental hybrids such as G10, G14 and G28 had high yield performance of 6.72, 6.00, and 6.23 t/ha, in that order across environments but with highly susceptible reaction to MSV.

- Hybrid G31 expressed low MSV infection but yielded the lowest at each environment. Hybrids such as G23 with low grain yields of 4.84 t/ha, G18 (5.14 t/ha), and G34 (1.94 t/ha) showed relatively low MSV infection levels which are useful genetic resources for resistance breeding.

- Experimental hybrids with high grain yield and MSV resistance selected in this study are good candidates for direct production or for future three-way hybrid development in Tanzania.

7.3 Implications for breeding

The high level of genetic diversity present among 80 inbred lines examined in this study. This will aid sustainable selection and development of superior hybrids in the northern Tanzania or similar environments. Lines TL2012-42, TL2012-41 and TL2012-1 have good general combining ability which are ideal genotypes to develop maize varieties with resistance to MSV and good characteristics preferred by farmers. Hybrids TL2012-7/TL2012-42 and TL2012-7/TL2012-68 have positive and high SCA effects for grain yield which will be released for direct production as single cross hybrids or converted into three-way hybrid before release.

Appendix No. 1: Survey questionnaires

Questionnaires used during survey to identify farmers' key maize constraints and preferences for MSV resistant maize cultivars conducted in 2012 in northern Tanzania (specific districts were Arumeru, Babati and Hai)

By Lameck Nyaligwa

District: _____ Village: _____

Questionnaire Number: _____

Enumerator: _____

1. Household characteristics:

- Gender : Male ☐   Female ☐
- Age of respondent: What is your age? ≤ 30 years ☐   31-50 years ☐ above 50 years ☐
- Who is the head of the household: male ☐   female ☐
- How many people are present at your family?   one ☐   two ☐   three ☐ 4 and above ☐
- What level of education you have? primary ☐   secondary ☐   college ☐ no education ☐

2. Faming system:

- Where do you obtain maize seed? Farmers' own fields ☐   private seed ☐ company ☐   local market ☐   agro dealers ☐   public research institutes ☐
- What size of land do you grow maize (acres)? ≤ 1 ☐   1.5-3 ☐   3.5- 10 ☐ > 10 ☐
- By average how many bags of maize do harvest from your farm   ≤ 1 ☐ 1.1-3 ☐   3.1 - ☐   > 10 ☐

3 Maize traits of economic importance:

- What are the maize traits of economic importance? List...............................
- What are the uses of maize in your area? List them.....................................

- Which maize varieties do you grow in your area? List them...........................

4. Maize production constraints

- What are the major maize production constraints in your area? ....

- What are the maize diseases are you facing? ....

- What measures do you use to overcome the crop diseases problems?.............

5. Trait preferences

- Which MSV resistant cultivar have you planted in recent years? List ....

- Which MSV resistant maize cultivar you did not like to grow it again? Name and give reason for not rowing...

- What other traits of maize do you prefer? List...

6. Types of Fertilizer used

- What types of fertilizer do you use and why? ....

- Where do you get the fertilizer?..

- And how do you see its cost?...