DEVELOPMENT OF TOPCROSS HYBRID MAIZE (*Zea mays* L.) FOR YIELD AND RESISTANCE TO MAIZE STREAK VIRUS DISEASE

By

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHILOSOPHY DEGREE IN PLANT BREEDING

WEST AFRICA CENTRE FOR CROP IMPROVEMENT

SCHOOL OF AGRICULTURE

COLLEGE OF AGRICULTURE AND CONSUMER SCIENCES

UNIVERSITY OF GHANA, LEGON

DECEMBER 2013

DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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Dedication

This work is dedicated to my father Very Rev. William Ofori of blessed memory, my mother Grace, my siblings, my wife Linda, my little daughter, Marilyn and rest of the family.

General Abstract

Studies were conducted to explore the potential of utilizing Ghanaian maize landraces for improved grain yield and resistance to the maize streak virus disease. Farmer perceptions of the maize streak virus disease as well as constraints to the adoption of improved varieties and farmers' continuous reliance on local landraces or farmer varieties and production constraints were investigated. Genomic characterization of Maize streak virus (MSV) strains found in the forest and transitions zones of Ghana were also undertaken to identify virulent strains of the virus that can be used to screen novel genotypes with adequate resistance to the disease. Genetic relationships among selected Ghanaian maize landraces and "exotic" germplasm were analyzed using SSR markers. Studies on general combining ability (GCA) and specific combining ability (SCA) on yield and related traits as well as MSV disease incidence and severity were conducted on selected local landraces and some CIMMYT and IITA inbred line testers using the line by tester approach. Percentage heterosis and heterotic groups were assessed in the local germplasm. The yield stability of selected topcross hybrids generated together with their landrace parents were assessed using AMMI and yield stability index in six environments.

Sixty four (64%) of respondents cultivated improved OPVs released by the research institutes and about 36% and 3% depend on landraces and hybrids respectively. Majority of the farmers preferred cultivars with slender cobs and lot of grains. Constraints identified by the farmers included climate change, poor road infrastructure, lack of farming inputs and cheating by middlemen. Majority of farmers interviewed had knowledge of the maize streak virus disease. Maize streak virus disease was mentioned as a seasonal disease which affects about 20% of their yields. Variants of MSV A_1 identified from the disease specimen also aligned closely with those

that have been detected in West Africa. The UPGMA dendrogram showed one big cluster with two outliers. However, eight minor clusters and nine outliers were found, if a perpendicular line is drawn from 0.22 genetic similarity, whilst Bayesian analysis indicated four sub populations and a mixed group. Significantly high mean yields were obtained for some of the topcross hybrids compared with their parents and the local checks with as much as 111% heterosis being observed for some of topcross hybrids over the best parent yield. Heterotic groups were assigned to 11 of the landraces (farmer varieties) whilst the remaining 5 could not be assigned. Significantly positive general combining abilities (GCA) effects with respect to yield were observed for lines LA30, LA3 and LA80, while significantly positive specific combining ability effects were observed for crosses involving lines LA558, LA76, LA80, LA457 and LA3. Testers CML442, CML444 and TZEI17 GCA effects significantly contributed positively to yield increase but significantly negative to MSV disease incidence and severity. Yield stability index ranked most of the topcross hybrids as high yielding and stable across environments.

This work has demonstrated the potential of utilizing Ghanaian maize landraces for improved yield and resistance to the Maize streak virus disease. It is recommended that inbred lines are developed from some of the promising landraces identified which can be used to produce single or double cross hybrids with yields and Maize streak virus disease resistance better than what was obtained in this study for wider adoption.

Acknowledgement

My greatest acknowledgement goes to the Almighty God for His mercies this far. I humbly acknowledge my supervisors; Prof S.K. Offei, Prof. K. Ofori, Dr. Charles Thé (of blessed memory) and Dr. Hans Adu-Dapaah for their pieces of advice, encouragement and support which enabled successful completion of this study. Most special appreciation and thanks go to the Alliance for the Green Revolution in Africa (AGRA) for granting me a scholarship to undertake this study. Worthy of special mention are Prof. Eric Y-Danquah, Director of the West Africa centre for Crop Improvement (WACCI), University of Ghana, Legon and Dr. Rufaro Madakadze of AGRA for their unflinching support. I am grateful to the Director General of the CSIR and the entire management of the CSIR-Crops Research Institute, Kumasi for granting me study leave and providing a congenial set-up that enabled this study to be carried out. I am grateful to the Generation Challenge Programme (GCP) for providing genotyping support service through BeCA, Nairobi, Kenya as well as some equipment and training through the 'Capacity building a la carté programme. I dare not forget to acknowledge the immense support I have received from Dr. Marilyn L. Warburton of USDA-ARS CHPRRU, Mississippi State, MS, USA, for supporting my application to WACCI and reading through some portions of this work. Similarly, I am grateful to Dr. F.O Anno-Nyako of CSIR and Dr. F. A. Ofori formerly, of the KNUST Crops Science Department who in several ways have supported me this far. I recognize the immense support of Dr. Arvind Varsani of the University of Canterbury, New Zealand and Dr. Martin P. Darren of the University of Cape Town, South Africa for their support for the genomic characterization of Ghanaian Maize streak virus isolates. Special thanks go to Dr. Vernon Gracen of WACCI and Cornell University, New York, USA, for his support and advice. I am grateful to Dr. Cosmos Magorokosho of CIMMYT Zimbabwe and Dr. Badu-Apraku of

IITA for freely supplying me inbred lines. Worthy of mention are the staff of the Pathology and Entomology sections, PB maize and Socio-economic divisions of the CSIR-CRI for their total support and commitment. Of particular mention, are Dr. M. Owusu-Akyaw, Dr. B.M. Mochia, Dr. J.N.L. Lamptey, Dr. E. Moses, Dr. K. Obeng-Antwi, Mr. Ibrahim Adama and Mr. Manfred B. Ewool. Others are Mr. Lord Ohemeng Appiah, Bright Asante, Benedicta Nsia-Frimpong, Mr. and Mrs Appiah Kubi, Esther Ntim Oheneba, Isaac Ativor, Kwasi Paul, Francis Dadzie, Francis Mensah, Mr. Joseph Kyeremeh, Stephen Oware Kunkuma, Kwadwo Alale, Kwame Aponye, Nana Ebo, Ebenezer (Entomology), Rose Osei Kofi, Kwabena Ali Balenge, Bawuah, Samuel Wiafe, Rose Baafi and Comfort Nkrumah Gyamera. Special thanks go Alhaji Yakubu and Mr. Emmanuel Donkor of the Forestry Commission, Kumasi, the entire staff of WACCI, Dr. Maxwell D. Asante, and the numerous friends who in no small measure have contributed to this work. I say thank you to all the International Scientists who delivered modules on plant breeding at WACCI and reviewed this work at the conception and final stages. Last but not least; I am indebted to my family, wife Linda, my course mates (Cohort two members) and the numerous well wishers for their support, prayers and encouragement.

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Acronyms and abbreviations

AGRA	Alliance for Green Revolution in Africa
AMMI	Additive main effects and multiplicative interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
1000Gwt	1000 grain weight
A/R	Ashanti region
ASI	Anthesis silking interval
ASV	AMMI stability value
B/A	Brong Ahafo
CIMMYT	International Maize and Wheat Improvement Center
CM	Centimeter
CML	CIMMYT maize line
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
CW	Cob width
DNA	Deoxyribonucleic acid
E/R	Eastern region
Ed,	Ear diameter
FGD	Focus group discussion
GCA	General combining ability
GLM	General linear model
GoG	Government of Ghana
GPS	global positioning systems
GSS	Ghana Statistical Service
Ha	Hectare
HPV	High parent value
IITA	International Institute of Tropical Agriculture
Kg	Kilogramme
LA	Landrace
LC	Leaf count
Masl	Meters above sea level
MC	Moisture content
MoFA	Ministry of Food and Agriculture
MSV	Maize streak virus
MSVD	Maize streak virus disease

N/R	Northern Region
NGO	Non-governmental organization
OPV	Open-pollinated variety
PCR	Polymerase chain reaction
PH	Plant height
PIC	Polymorphic information content
SARI	Savannah Agricultural Research Institute
SCA	Specific combining ability
SRID	Statistics Research Information Division
SSA	Sub-Saharan Africa
TZEI	Tropical *zea* early inbreds
Vi	Virus incidence
VR	Volta region
Vs,	Virus severity
W/R	Western region
Yld/ha	Yield per hectare
YSI	Yield stability index

Chapter One

1.0 General Introduction

Maize (*Zea mays* L.) has become a major cereal crop and an important component of human and animal diets as well as raw material for industry (http://www.iita.org/maize, USAID/EAT, 2012). It is a widely grown cereal in the tropics (Damsteegt and Igwegbe, 2005) and plays a major role as a food security crop in both rural and urban communities. It is the second most important crop after wheat in terms of area cropped and number one in terms of production (FAOSTAT, 2009). Maize is so important that in most of sub-Saharan Africa, maize yields are usually linked to food shortages and famine (ISAAA, 2006).

In Ghana, maize production is estimated at around 1.79 MT per annum (USAID/EAT, 2012) from an area of approximately 992,000 ha (MOFA, 2011) with yield estimates of about 1.7 ton/ha in farmers' fields. This is generally low compared to global average of 4-5 ton/ha and over 8 ton/ha in the United States of America (USA) (FAOSTAT, 2008). This low production may be partly attributed to frequent biotic and abiotic stresses including disease outbreaks, drought and poor farming practices (Morris *et al.*, 1999). Other factors include continuous use of unimproved landraces, poor soils and erratic rainfall patterns (Akande and Lamidi, 2006, Bua and Chelimo, 2010, USAID/EAT, 2012).

The demand for maize in Ghana is increasing and between 2010- 2015, it is projected to grow at a compound rate of 2.5 percent per annum (MIDA, 2010). Despite increases in production over the years, Ghana is still not self sufficient as the country experiences average shortfalls in

domestic supplies of about 12 percent which are off-set by imports (MOFA, 2010), which impacts negatively on foreign exchange reserves.

Maize streak virus disease (MSVD) is one of the most important biotic constraints affecting maize production in Africa including Ghana (ISAAA, 2006). MSVD is indigenous to Africa and adjacent islands (De Vries and Toenniessen, 2001. , Okori *et al.*, 1999). Serious MSVD epidemics have been reported in at least 20 African countries, including Ghana, Nigeria, Ethiopia, Sudan and Zimbabwe (Barrow, 1992, ISAAA, 2006). The disease causes serious yield losses, but its occurrence is sporadic and unpredictable, which makes breeding for resistance difficult (Mesfin and Bosque-Pe´rez, 1998). In addition to complicating breeding efforts, yield losses due to the disease are not easy to quantify and may range from 0-100% depending on the year and stage of growth of maize plant when it is attacked (Ampong-Nyarko *et al.*, 1998, Diallo, 1999). In Africa, economic losses of between US$120M and US$480M are recorded per year based on average annual yield loss of only 6%–10% (Martin and Shepherd, 2009). Despite considerable advances in control measures, which could reduce these monetary losses, farmers continue to suffer serious crop losses to MSVD (Martin and Shepherd, 2009) which requires continuous research efforts to manage or control in any maize breeding program especially, in Africa.

Attempts made so far include agronomic practices such as timely planting and treatment of seed with systemic insecticides among others. Research efforts have been intensified by Maize breeders in Ghana to develop varieties with adequate resistance or tolerance to the numerous constraints to maize production, especially drought, improved nutritional quality and the Maize streak virus disease (Ragasa *et al.*, 2013, Wiredu *et al.*, 2010). Even though considerable

research attention has been placed on controlling the disease in Ghana (Souza *et al.*, 1996), there is still room for serious research efforts to develop varieties that combine good yield and MSVD resistance or tolerance. For instance over the years that maize breeding with resistance to the maize streak virus disease started in Ghana, the strains of the virus that causes severe symptoms of the disease have not been characterized at the genomic level which in a way impedes efforts to breed for novel resistant cultivars. Similarly, farmers consider incidence of the disease in their farms as a constraint which requires research efforts if productivity can improve.

Incorporation of both exotic and local landraces or farmer materials into breeding programmes have been suggested by several researchers (Carena, 2005, Hallauer and Miranda, 1981, Soengas *et al.*, 2003). For instance Bertoia *et al.* (2006); Balestre *et al.* (2009) have all reported that landraces with no history of breeding for grain production generated crosses with good potential which can be exploited in breeding programmes. The phenotypic diversity of maize is quite high especially, with respect to kernel colour, starch content, flint, dent consistencies, height, maturity date and tolerance to diseases. Landraces and wild relatives represent an extraordinary genetic resource of the crop with significant allelic diversity, much of which has not been incorporated into improved varieties (Sharma *et al.*, 2010, Warburton *et al.*, 2008). These constitute a possible source of diversity that can be exploited to widen the gene pool from which breeders can harness useful genes and alleles for breeding to meet biotic and abiotic challenges. To achieve this in Ghana requires that the local landraces are characterized at the DNA level to establish relationships among them and other varieties to ascertain their true identity.

The exploitation of heterosis and heterotic groups for hybrid maize production for improved grain yield has been the cornerstone of maize yield increases in countries such as USA, China

and Zimbabwe (Gerdes and Tracy, 1993, Mickelson *et al.*, 2001, Reif *et al.*, 2009, Troyer, 2004). One way to do this is through studies on combining ability, a method that has successfully been used to assign inbred lines and populations into heterotic groups for the initiation of hybrid production (Vasal *et al.*, 1992, Pswarayi and Vivek 2008, Legesse *et al*., 2008) and such hybrids must also be stable (Balestre *et al.*, 2009, Scapim *et al.*, 2000).

In Ghana, however, hybrid maize cultivation has not received much patronage by Ghanaian farmers (Ragasa *et al.*, 2013, USAID/EAT, 2012). Most of the cultivated maize is dominated by open pollinated varieties (OPVs) and landraces with few hybrids (Ragasa *et al.*, 2013, USAID/EAT, 2012), compared to the situation in developed countries (Troyer, 2004). This may be due to lack of knowledge of availability and potential of hybrid varieties or the non availability of preferred hybrids which farmers can readily adopt. For instance, Mamaba, a hybrid released by the CSIR-Crops Research Institute (CRI) in the 1990s has not received the expected patronage and some of the newly hybrids varieties are not well adopted either (Ragasa *et al.*, 2013).

It has become imperative that maize productivity should be raised to meet the high demand of ever increasing consumers. The production of good hybrids hold the key in this direction and these hybrids if they are to succeed could be developed from or around local germplasm for wider adoption as has been achieved in Costa Rica and Honduras, where Almekinders *et al.* (1994) found that hybridization between local and improved maize was highly valued by farmers. It is anticipated that hybrids developed from or with contribution from local germplasm which are also resistant and/or tolerant to the maize streak virus disease will facilitate easy adoption by farmers.

Thus the main objective of this work is to exploit local landraces (farmer varieties) and exotic germplasm to identify suitable parents that can be used to produce new high yielding, MSV disease resistant and farmer-preferred hybrids.

The specific objectives were to:

1. Ascertain the strains of MSV, farmer awareness of MSV disease, production constraints and lack of adoption of newly released varieties in major maize growing areas of Ghana.
2. Determine genetic relatedness of Ghanaian maize landraces using microsatellite (SSR) markers.
3. Estimate heterosis and assign heterotic groups to a set of landraces with respect to grain yield.
4. Estimate the general and specific combining abilities among selected local landraces and inbred lines used as testers.
5. Determine the yield stability of potential topcross hybrids.

CHAPTER TWO

2.0 Literature review

This literature review has been compiled as a broad overview of advances in maize improvement research, particularly in the areas of breeding for MSV resistance and yield. The review covers botany and the origin as well as the importance and utilization of maize around the world; MSV as a pathogen, epidemiology, disease symptoms and control using both conventional and marker assisted selection (MAS) as well as genetic characterization using DNA markers. It also covers maize production and its constraints and farmer varietal preferences. It again covers hybrid maize breeding vis-a-vis, heterosis, heterotic groups, combining abilities, usefulness of landraces or farmer varieties in plant breeding and yield stability across environments.

2.1 Origin, Classification and Botany of maize

Maize belongs to the tribe Maydeae of the grass family *Poaceae*. The genus *Zea* consists of four species of which *Zea mays* L. is economically important. The other *Zea* sp., referred to as teosintes, are largely wild grasses native to Mexico and Central America (Doebley *et al.*, 1984, Dubreuil *et al.*, 2006, Rebourg *et al.*, 2003). The number of chromosomes in *Zea mays* is 2 n = 20. Maize is a monoecious plant, that is, the sexes are partitioned into separate pistillate, the female flower and staminate, the male flower. It has determinate growth habit and the shoot terminates into the inflorescences bearing staminate flowers. Maize is generally protandrous, that is, the male flower matures earlier than the female flower.

The center of origin of *Zea mays* has been established as the Mesoamerican region, now Mexico and Central America (Matsuoka *et al.*, 2002, Piperno and Flannery, 2001). It is believed that teosinte (*Z. mexicana*) is an ancestor of maize (Warburton *et al.*, 2011), although opinions vary as to whether maize is a domesticated version of teosinte. Pollen is produced entirely in the staminate inflorescence. Maize is wind pollinated and both self and cross pollination is usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe *et al.*, 1988). The ear is enclosed in numerous large foliaceous bracts and a mass of long styles (silks) protrude from the tip as a mass of silky threads (Hitchcock and Chase, 1971).

2.2 Importance of maize

Maize is a widely grown cereal in the tropics (Damsteegt and Igwegbe, 2005). In many countries including Ghana, maize has become a major cereal staple and an important component of animal and human diets. It was considered to be the third most important cereal crop in the world after wheat and rice up to the end of the 1980s (Sleper and Poehlman, 2006). Currently, maize ranks second in production among the major grain cereals worldwide but due to a shift in cereal demand, maize is expected to be the leading cereal surpassing both wheat and rice (Pingali, 2001).

In the developed countries, maize is used primarily as animal feed and secondarily for production of food and industrial products including starch, sweeteners, and alcohol (Prasanna *et al.*, 2001, Rosegrant, 2008). In developing countries, maize is often grown as a food crop for human consumption, as well as for the market, but it is increasingly being used as animal feed

(Morris *et al.*, 1999, WABS., 2008). In some developing countries elsewhere in the world, maize is a food crop of second choice after wheat or rice, but in Africa and Latin America, maize is usually the staple crop of first choice.

The industrialized world still produces and uses more maize than the developing world, but the trend indicates that by 2020, developing countries will demand more maize than the industrialized world as a result of both population growth and increasing urbanization (Pingali, 2001). Between now and 2050, the demand for maize in the developing world will double, and by 2025, maize production is expected to be highest globally, especially in the developing countries (Rosegrant *et al.*, 2009).

It is estimated that 140 million hectares of maize is grown globally and approximately 96 million of that total production area is in developing countries (Pingali and Pandey, 2001). Despite that, only 46% of the world maize is produced in developing countries. Low average yield in the developing world is considered one of the causes of the wide gap between the global share of area and share of production. The Food and Agricultural Organization reported worldwide average maize productivity at 4 t/ha, but yield in Africa averages only 1.7 t/ha (FAOSTAT, 2012). In Ghana, average yield in farmers fields is around 1.6 ton/ha (MOFA, 2011). The cropping system used in the USA leads to yields that are 65% above the global average, (FAOSTAT, 2012). Wide disparities in climatic conditions (tropical versus temperate) and farming technologies account for the yield differential between the developed and the developing world (Pingali, 2001). Latin America and sub-Saharan Africa produce the most tropical maize while temperate environment production mainly include the USA, China, and Argentina.

2.3 Maize streak virus

Maize streak virus (MSV) belongs to the genus *Mastrevirus* of the Geminiviridae family. It is an indigenous African virus transmitted by a range of leafhoppers in the genus *Cicadulina* (Storey, 1925) and causes maize streak disease (MSD), the most damaging viral disease of the crop in Africa (Thottappilly, 1992). Maize streak is caused by a single stranded DNA geminivirus (Bock *et al.*, 1974, Harrison *et al.*, 1977). Bock *et al.* (1974) discovered that MSV virions have a novel, twinned, quasi-icosahedral (geminate) shape, from which the name 'geminivirus' was given. This was followed by the unexpected discovery in 1977 that geminivirus particles contained circular single-stranded DNA (ssDNA), a genome type never before observed in plant viruses (Goodman, 1977, Harrison *et al.*, 1977). The virus is known to exist in many forms and vary according to agro-ecology, season and vector dynamics (Rybicki, 1994). Research has shown that there are many isolates or strains of MSV (Varsani *et al.*, 1998), with some being more virulent than others (Bock *et al.*, 1974, Clarke *et al.*, 1989, Rybicki *et al.*, 1988). MSV is a monopartite geminivirus containing a single 2.7-2.9 kb circular, single stranded DNA molecule, making it the smallest known plant virus (Lazarowitz, 1987, Mullineaux *et al.*, 1984). The single genomic components resemble the A component of bipartite geminiviruses (Lazarowitz, 1987). The genome organization of MSV is similar to that of other geminviruses (Lazarowitz, 1987, Stanley and Davies, 1985). MSV isolated from many different hosts have been considered as forms, isolates, or strains of the virus (Dekker *et al.*, 1988, McClean, 1947, Mesfin *et al.*, 1992, Pinner *et al.*, 1988, Plasvic-Blanjac and Maramorosch, 1972, Storey and McClean, 1930). Eleven strains of MSV are currently known, but only the MSV-A strain is known to cause economically significant streak disease in maize (Shephered *et al.*, 2010).

Although there are fairly obvious differences in the genetic composition of MSV-A populations in Eastern, Western and Southern Africa (Briddon *et al.*, 1994, Martin *et al.*, 2001, Willment *et al.*, 2001), full understanding of how these translate into differences in disease epidemiology is not known. Of the four main lineages of MSV-A currently circulating in Africa (MSV-A_1, MSV-A_2, MSV-A_3 and MSV-A_4), MSV-A_1 and MSV-A_4 are apparently responsible for more than 95% of all MSVD cases that have been analyzed over the past 20 years. It is therefore safe to assume that these are the main lineages driving MSVD epidemics throughout southern and East Africa where the bulk of virus sampling has been carried out (Martin *et al.*, 2001, Owor *et al.*, 2007, Varsani *et al.*, 2008, Willment *et al.*, 2001). Although MSV-A_4 appears to be confined to southern Africa, MSV-A_1 has a geographical range that spans the whole of sub-Saharan Africa (Martin *et al.*, 2001, Monjane *et al.*, 2011, Owor *et al.*, 2007, Varsani *et al.*, 2008, Willment *et al.*, 2001). A characteristic of MSV-A_4, which may have some bearing on its more restricted geographical range, is that it is apparently less severe in maize than MSV-A_1 (Martin *et al.*, 1999). The widespread distribution of MSV-A_1 is, however, somewhat unusual in that no other group of similar MSV variants (i.e. a MSV lineage displaying similar patterns of genetic diversity to each other but distinct from MSV-A_1) has ever been found to be spread between major regions of the continent, such as between East and West Africa or East and Southern Africa. Although MSV-A is the only strain known to cause severe MSVD (Martin *et al.*, 2001, McClean, 1947, Storey and McClean, 1930), 10 non-maize-adapted MSV strains (MSV-B to MSV-K) have also been identified (Martin *et al.*, 2001, Schnippenkoetter *et al.*, 2001., Varsani *et al.*, 2008, Willment *et al.*, 2002). Although they are normally found infecting wild grasses, some of these (including MSV-B, MSV-C, MSV-D and MSV-E) are also known to produce mild infections in MSV-susceptible maize genotypes (Martin *et al.*, 1999; 2001). Although they have

no known direct impact on African agriculture, these MSV strains have probably had a large indirect influence on the evolution of the economically significant MSV-A strain. For example, the main genetic feature differentiating MSV-A4 from other MSV-A lineages is that it is the product of a recombination event between MSV-A and MSV-B viruses found in southern Africa (Martin *et al.*, 2001).

2.3.1 Maize streak virus disease

Maize streak virus disease (MSVD) was first reported in South Africa by Claude (Fuller, 1901). Fuller also quoted personal sources who noticed the disease of 'mealie variegation', as it was then described, as early as the 1870s. The disease was therefore not new at that time, and had probably been around as long as maize had been grown in the region. Fuller's investigation of MSVD was motivated by an increase in incidence of the disease, marked by a serious outbreak in 1896. Although Fuller was ignorant as to its cause, he thought it was caused either by a soil nutrient deficiency or a 'chemical enzyme' acquired from the soil, he accurately described many features of the disease as it manifests today. Symptoms of the disease in susceptible plants begin as inter-veinal, circular to oval, nearly white spots 0.5-2.0 mm in diameter, near the base of the newest expanding leaf. The spots may be scattered over the entire leaf or confined to a few adjacent inter-veinal areas. As the leaves expand, spots become numerous and elongate until long streaks running parallel to leaf veins become apparent (Bock *et al.*, 1974, Damsteegt, 1981, Efron *et al.*, 1989, Guthrie, 1976). Highly susceptible genotypes may exhibit widespread, almost complete chlorotic streaking of all new leaves following infection, often associated with morphological teratology, including leaf margin splitting, leaf tip twisting, necrosis of emerging leaf and reduced leaf size, tassel sterility and shoot stunting (Bock *et al.*, 1974, Damsteegt, 1981,

Fajemisin, 1984). Streaks are broad pale cream-coloured to light green or yellow against a darker green background with a glassine appearance in the first affected leaves (Storey, 1925). Tolerant genotypes exhibit more scattered, discontinuous streaks with less plant stunting and discoloration.

MSVD severity depends on the age at which the plant is infected, on the genotype, and the virus strain (McClean, 1947, Storey and McClean, 1930). Chlorosis is caused by failure of chloroplasts to develop in the tissue surrounding the vascular bundles, which results in reduced photosynthesis and increased respiration (McClean, 1947). Severe chlorosis occurs in very susceptible maize cultivars, leading to stunted growth and premature death, poor ear formation, reduced seed setting, and heavy yield losses (Monjane *et al.*, 2011).

2.3.2 MSV Disease epidemiology

MSVD is endemic to Africa and its adjacent Indian Ocean Islands. The disease occurs in the forest and the savanna zones from sea level to 1800m (meters above sea level MASL) (Bjarnason, 1986). Disease incidence and destructiveness varies from year to year and from season to season (Rossel and Thottappilly, 1985). The disease is transmitted by several leafhopper species of the *Cicadulina* genus (Rose, 1973). The most prevalent and efficient in transmission is *Cicadulina mbila* (Naude) which exists in populations of both active and inactive vectors. The exact make-up of MSV-A populations in different parts of Africa probably also influences MSVD epidemiology. Rapid increases in virus populations and epidemic spread between crops are usually attributable to the convergence of factors, such as: (i) staggered growing seasons in which MSV-A populations can bulk up in early planted maize and devastate

seedlings that germinate in successive plantings (Dabrowski *et al.*, 1991, Fajemisin and Shoyinka, 1976); the population density of wild grasses that are reservoirs of both MSV-A and leafhopper vectors (Autrey and Ricaud, 1983); (iii) the presence within leafhopper populations of a high percentage of MSV transmitters; and (iv) environmental factors that drive population sizes and long distance movement of leafhoppers (Rose, 1978). MSVD outbreaks are often associated with drought conditions, followed by irregular rains at the beginning of the growing season (Efron *et al.*, 1989), as in the savanna regions of West Africa in 1983 and 1984 (Rossel and Thottappilly, 1985), or in Kenya in 1988–89 (Njuguna *et al.*, 1990). The relative abundance of various *Cicadulina* species with differing abilities to transmit the virus in different parts of Africa is influenced by altitude, temperature and rainfall (Dabrowski *et al.*, 1987). The interplay of all these factors makes MSVD epidemiology rather erratic, with the disease being devastating in some years and insignificant in others (Efron *et al.*, 1989). In forest areas of West Africa with bimodal rainfall distribution and two cropping seasons, the second crop is more severely affected, and in savannah areas with unimodal rainfall distribution, the incidence is higher for later plantings (Rose, 1973). Irrigated plots usually suffer more damage (Fajemisin *et al.*, 1984, Rose, 1973). Population cycles follow a distinct pattern with young annual grasses and cereals being colonized predominantly by female leafhopper populations (Rose, 1973, Rose 1978). Patterns of streak infection in maize fields conform closely to seasonal distributions of different *Cicadulina* vectors (Rose, 1973, 1974), females tend to accumulate more on the edge/outer rows while males are found uniformly throughout the fields. Females are better transmitters of the disease than males (Heathcote, 1975). *Cicadulina* species survive and reproduce readily at temperatures ranging from 20^{o}C-28^{o}C (Asanzi *et al.*, 1995b).

Several reports have indicated differences among maize streak symptom severity caused by different MSV strains or isolates. Pinner et al. (1988) transmitted MSV isolates found on different hosts from Nigeria, Kenya, South Africa, Mauritius, Ethiopia, and Reunion to maize cv. Golden Batam, and reported that MSV symptoms varied from severe to mild streaks or lesions, sometimes consisting of only a few sparse or small dots on the leaves. Boulton *et al.* (1991) also concluded that "Nigeria mild" (MSV-Nm) and "Nigeria severe" (MSV-Ns) MSV strains could be differentiated based on five distinguishable characteristics that included severity of chlorosis, streak width, streak length. Njuguna (1996), (Rodier *et al.*, 1995) reported variations in maize streak development, and in some cases, resistant germplasm became susceptible when exposed to different strains of MSV. Perhaps the most elegant study was that of Martin *et al.* (1999), who used *Agrobacterium tumefaciens*–mediated inoculation with constructs for 11 MSV isolates collected in Kenya, Nigeria, Reunion, South Africa, and Zimbabwe, to quantify differences for relative susceptibility of maize genotypes to different MSV isolates. MSV-resistant maize lines developed at International wheat and maize improvement centre (CIMMYT) may experience small changes in MSV resistance, but resistant lines have not been reported to become susceptible when grown across a wide range of sites in eastern and southern Africa (Danson *et al.*, 2006).

2.3.3 Economic importance of Maize streak virus disease

Maize streak disease has played a major role in maize production in Africa although the importance of MSV on maize varies from place to place or location to location (Damsteegt and Igwegbe, 2005). Over the past three decades, the increasing popularity of maize has transformed it from a garden or backyard crop to a major field crop in most of West Africa (Efron *et al.*, 1989). This has led to greater ecological opportunities for the virus and its leafhopper vector. Increased prevalence of maize production is directly correlated to the increases in MSV disease (Rose, 1978). MSVD currently remains the most significant viral disease of maize in Africa (Bosque-Pérez, 2000) costing between US$120M and US$480M per year according to a conservative estimate based on average annual yield losses of only 6%–10% (Martin and Shepherd, 2009). Poorer farmers continue to suffer serious yield losses despite considerable advances in control measures, which could reduce these monetary losses (Martin and Shepherd, 2009) but are unavailable to farmers without the resources to obtain them. The magnitude of yield loss due to MSV is dependent on weather, vector population densities, percent carry over inoculums and growth stage of the crop when infection occurred (Bjarnason, 1986). Severe outbreaks are often associated with late plantings or second season cropping (Bjarnason 1986, Efron et al., 1989). In situations of severe disease incidence, the disease can result in up to 100% yield loss especially when susceptible genotypes are infected (Danson *et al.*, 2006), which is a major contributor to shortages of this staple in endemic regions. The pathogen causes serious yield losses, but its occurrence is sporadic and unpredictable (Diallo, 1999, Mesfin and Bosque-Pe´rez, 1998). In addition, yield losses due to the disease are not easy to quantify and may range from 0-100%, depending on the year and stage of growth of the maize plant when it is attacked

(Ampong-Nyarko *et al.*, 1998, Diallo, 1999). Plants attacked at early stages of growth (up to seven-leaf stage) sustain losses of 80% or more, while those attacked shortly thereafter (at the nine-leaf stage), incur only 20% yield loss (Ampong-Nyarko *et al.*, 1998). Van Rensburg and Kuhn (1977) reported losses of 50% from plants infected at 1 and 3 weeks after planting, and negligible loss in plants infected 8 weeks after planting. Fajemisin *et al.* (1984) reported that early losses occurred from lack of plant survival and later yield losses resulted from fewer harvestable ears, poorly formed ears, shorter plants, narrower stem diameter (weak stems), smaller leaf area, and smaller tassels.

2.4 Management of Maize Streak Virus Disease (MSVD)

Management strategies for MSV are interdependent on cultural practices, source of resistance in germplasm, source of resistance to the *Cicadulina* spp. and populations of different *Cicadulina* spp. (Efron et al., 1989). Management of MSVD is difficult partly due to the variability of the virus, and partly due to the susceptibility of locally adapted maize lines and unpredictable vector migratory and survival patterns. The development and deployment of resistant genotypes is the most appropriate and cost-effective approach to controlling MSVD (Danson *et al.*, 2006).

Agronomic or cultural practices such as field sanitation, crop rotation, both in time and in space, timely planting, seed treatment with systemic insecticides, and removal (rogueing) of infected plants have been recommended for reducing MSV incidence (Efron *et al.*, 1989, Gorter, 1959, Rose, 1978). Some of these practices conflict, however, with resource poor farmers' efforts to cope with the risks of uncertain rainfall. In Southern Africa, for example, many smallholder farmers use multiple planting dates over extended periods, a practice that results in greater

exposure to maize streak disease, to ensure that at least part of their crop successfully avoids periods of severe drought stress (Rohrbach, 1998). Furthermore, prices of imported chemicals and equipment for spraying often limit smallholder farmers' access to these MSV control options. Other cultural practices that have been suggested include planting downwind of earlier gramineous crops (Gorter, 1951), leaving barriers of 10 meters of bare ground around the crop (Gorter, 1951) and planting all susceptible crops concurrently (Mzira, 1984).

Disease avoidance can be practiced by only planting maize during the early season when viral inoculum loads are lowest. Leafhopper vectors can also be controlled with insecticides such as carbofuran applied at a rate of 10% granular formulation in the planting furrow together with the seed (Van Rensburg and Giliomee, 1990). However, the development and use of streak-resistant cultivars is probably the most effective and economically viable means of preventing streak epidemics. Naturally occurring tolerance to MSV (that is although plants become systemically infected, they do not suffer serious yield losses) has been found, which has primarily been attributed to a single gene, *msv-1* (Kim et al., 1989). However, other MSV resistance genes also exist and improved resistance has been achieved by pyramiding these genes within individual maize genotypes (Shephered *et al.*, 2010). Whereas true MSV immunity (meaning that plants cannot be symptomatically infected by the virus) has been achieved in lines that include multiple small-effect resistance genes together with *msv-1*, it has proven difficult to transfer this immunity into commercial maize genotypes (Shepherd *et al.*, 2007).

2.4.1 Genetic basis of MSV resistance

Tolerant or resistant sources of maize germplasm have been known for many years. Gorter (1959) reported on several resistant varieties from Zimbabwe and (Storey and Holland, 1967) described the incompletely dominant, single gene resistance mechanism. MSV resistance in maize is controlled by a major gene, with few modifying genes (Efron *et al.*, 1989, Kim *et al.*, 1989, Pernet *et al.*, 1999a, Rose, 1978). Kyetere *et al.* (1995) were first to map this major gene to chromosome 1, and several authors have subsequently confirmed the importance of this chromosomal segment, which has a major effect (typically explaining 50 to 60% of the phenotypic variation) on resistance to MSV (Kyetere *et al.*, 1999, Lu *et al.*, 1999, Pernet *et al.*, 1999, Welz *et al.*, 1998).

Breeding research using diverse germplasm under natural infection conditions led to the conclusion that resistance to MSV is under polygenic control in maize (Kim *et al.*, 1989). Generation mean analysis based on selected parents under controlled infections, however, led to the suggestion that a single major gene confers resistance to MSV (Storey and Holland, 1967). Sources of resistance have been identified in the varieties TZ-Y (Tropical Zea-Yellow) derived from a cross between Tuxpeno Planta Baja from CIMMYT and sources in East Africa. After six generations, a highly resistant line designated IB32 was selected for studying the mode of inheritance. Crosses with four susceptible inbred lines (B14, B68, B73 and Mo17) produced segregating lines without distinct reaction classes, indicating quantitative inheritance with three major genes (Kim *et al.*, 1989) and additional modifier genes affecting growth characteristics (Bjarnason, 1986). In another resistant source, "La Revolution", resistance is controlled by a single dominant gene (Kim *et al.*, 1989).

With further use of Recombinant Inbred Lines (RILs) developed in Hawaii, major controlling Quantitative Trait Loci (QTLs) (genomic regions influencing the phenotype identified by molecular makers) have been identified for MSV (Kyetere *et al.*, 1995, Kyetere *et al.*, 1999). The RILs were planted repeatedly under natural infection conditions to unequivocally identify phenotypes, laying the foundation for precise mapping with molecular markers. It may be debated whether these results reflect action of single loci within each QTL and to what extent other genes modify expression. It is clear, however, that the identification and mapping of single major genes or QTL regions can be a significant tool in breeding for general resistance to this disease. Tropical maize breeding focuses heavily on resistance to diseases and pests that is durable and not easily broken down by evolution of the pathogen or pest. Durable resistance is viewed as 'general' (or horizontal), and often assumed to be under polygenic control. Genetic resistance to many of these diseases and insect pests of maize have been found more readily among tropical germplasm than in the relatively narrow gene base of temperate hybrids (Kim *et al.*, 1988).

2.4.2 Breeding for MSV resistance using conventional methods

Use of resistant cultivars is probably the most appropriate, cost-effective, and long term approach to reducing yield losses due to maize streak epidemics (Efron *et al.*, 1989) .

CIMMYT, International Institute of Tropical Agriculture (http://www.iita.org/maize), and others have produced numerous MSV-resistant maize cultivars. East Africa's contribution to the pool of resistant germplasm started in the 1970s with the ultimate discovery and development of a source of resistance called "La Revolution". This was found in the Reunion Island (Engelbrecht, 1973)

and has remained very effective (Damsteegt, 1983). The National plant breeding programme in South Africa has produced several tolerant or resistant lines (Fourie and Pienaar, 1983). Two released Vaalhart lines are susceptible as young seedlings, but were somewhat resistant by the six to eight weeks stage (Damsteegt, 1983). A Vaalharts composite line, VHCY, developed by Engelbrecht (1973) was used as a non-recurrent parent in crosses with US inbreds Mo17 and B73 in 1989 to develop streak-resistant inbred lines with improved combining ability for yield (Kuhn and Van Rensburg, 1995). From 68 S_5 lines characterized in 1995, 15 superior inbred lines were released in 1996 (Kuhn and Van Rensburg, 1995). Breeding efforts have depended on availability of vectors together with alternate plant hosts of MSV, or use of artificial inoculation techniques involving mass rearing of *Cicadulina mbila* (leafhoppers) together with maintenance of suitable MSV inoculum (Bosque-P´erez and Alam, 1992). One OSU 23i line from CIMMYT was found to contain good resistance and was used to convert one locally adapted line (EM12-210) in Kenya (Danson *et al.*, 2006). An S_6 line derived from the backcross of the two lines was released. Since conventional methods generally take approximately 8 years to release a variety with good levels of MSV resistance, there is a need to develop, apply and adopt DNA markers in selection as an additional plant improvement tool (Danson *et al.*, 2006). In Ghana since 1979 all breeding efforts have placed emphasis on developing MSV resistant varieties which has led to the release of cultivars such as Obatanpa, Okomasa and Mamaba, (Souza *et al.*, 1996).

2.5 Combining abilities, heterosis and heterotic groups

Information on combining abilities, heterosis and heterotic grouping are important components for the successful development or breeding of new high yielding hybrids in any breeding programme (Legesse *et al.*, 2009, Romanus *et al.*, 2007). Such information can show the type of gene action involved in controlling quantitative characters, thereby assisting breeders in selecting suitable parents (Hallauer and Miranda, 1988). Significant values of general combining ability (GCA) and specific combining ability may be interpreted as indicating the performance of additive and non-additive gene action, respectively (Sprague and Tatum, 1942). GCA helps breeders to exploit existing variability in breeding materials to identity individual genotype(s) conferring desirable attributes and to distinguish relatedness among genotypes (Melania and Carena, 2005, Vacaro *et al.*, 2002). SCA helps breeders to determine heterotic patterns among populations or inbred lines, to identify promising single cross hybrids and to assign them into heterotic groups (Hede *et al.*, 1999, Parentoni *et al.*, 2001, Revilla *et al.*, 2002, Vasal *et al.*, 1992). The estimation of additive and non-additive gene action through this technique is useful in determining the possibility of commercial exploitation of heterosis and isolation of pure lines among the progenies of the good hybrids (Stuber 1994).

The phenomenon of heterosis was defined by Shull (1952) as "the interpretation of increased vigor, size, fruitfulness, speed of development, resistance to disease and to insect pests, or to climatic rigours of any kind manifested by crossbred organisms as compared with corresponding inbreds, as the specific results of unlikeness in the constitution of the uniting parental gametes". However, Schnell (1961) defined heterosis as the difference between the hybrid and the mean of its two parents. Heterosis has been extensively studied in maize because of: i. its expression for

grain yield increase (100-200%), ii. its intensive exploitation in hybrid breeding of maize, and iii. the favorable biological prerequisites such as large multiplication coefficient and ease of both self- and controlled cross-fertilization (Reif *et al.*, 2005b) Although many hypotheses have been suggested to explain heterosis, its genetical, physiological, and biochemical bases still remains largely unexplained (Reif *et al.*, 2005b). Heterosis is a major yield factor in all breeding categories except line breeding (Schnell, 1982). To systematically exploit heterosis in hybrid breeding, the concept of heterotic groups and patterns was suggested. Melchinger and Gumber (1998) defined a heterotic group "as a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups. By comparison, the term heterotic pattern refers to a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross." The concept of heterotic patterns includes the subdivision of the germplasm available in a hybrid breeding program in at least two divergent populations, which are improved upon with inter-population selection methods. Heterotic patterns have a strong impact in crop improvement because they pre-determine to a large extent the type of germplasm used in a hybrid breeding programme over a long period of time (Melchinger and Gumber, 1998).

According to Reif *et al.* (2005b) several genetic hypotheses have been proposed to explain concept of heterosis; amongst the oldest but still most prevailing explanations are: (i) the dominance hypothesis which explains heterosis by the joint action of multiple loci with the favorable allele being either partially or completely dominant (Bruce, 1910, Collins, 1921, Jones, 1917, Keeble and Pellew, 1910), the overdominance hypothesis assumes overdominant gene

action at many loci (Crow, 1948, East, 1936, Hull, 1945), (iii) the epistasis hypothesis attributes heterosis to epistatic interactions between non-allelic genes (Richley, 1942, Schnell and Cockerham, 1992). An extension of the dominance hypothesis was recently suggested on the basis of DNA sequencing data (Fu and Dooner, 2002). Accordingly, functional genes are often absent in maize lines, and lines lacking different genes would complement one another in the F1 hybrid, resulting in heterosis.

Heterosis and increased uniformity are the basis of the modern hybrid maize seed industry (Gerdes and Tracy, 1993). Early hybrid maize breeders observed that heterosis was greater in crosses between genetically diverse inbreds than in crosses between related inbreds. By relating levels of heterosis with pedigrees, the concept of heterotic groups was established (Anderson, 1944, Hallauer and Miranda, 1981). Maize breeders have relied on maintenance and exploitation of two or more heterotic breeding groups in development of inbreds and subsequent hybrids. The recognition and use of heterotic groups has contributed to the efficiency and success of hybrid maize breeding programme. United States of America maize inbreds are commonly classified into heterotic groups based on pedigree and/or combining ability and this information is used in making decisions on how germplasm is used in a breeding program (Hallauer and Miranda, 1981). The Reid Yellow Dent by Lancaster Surecrop heterotic pattern was identified shortly after hybrid maize was introduced and continues to be the most widely used combination (Darrah and Zuber, 1986; Hallauer, 1990).

Han *et al.* (1991) studied combining ability effects of inbred lines derived from CIMMYT populations and germplasm pools. They observed significantly positive SCA effects for crosses of inter-population inbred-line and concluded that inbred lines derived from different populations

are more likely to show superior yield performances. Zehui *et al.* (2000) studied inbred lines derived from different populations and found significant GCA effects for a number of yield related and morphological traits. Kadlubeic *et al.* (2001) using flint and dent types of maize inbred lines reported a higher proportion of GCA effects than SCA effects for yield and various other agronomic traits. Studies conducted on the inheritance of Gray leaf spot (GLS) in maize revealed the importance of both additive and non-additive type of gene action (Gevers *et al.*, 1994, Menkir and Ayodele, 2005) while others found GLS under control of additive gene action (Donahue *et al.*, 1991).

Beck *et al.* (1991) studied combining abilities and heterotic patterns among CIMMYT's maize gene pools population for hybrid development and reported highly significant, positive general combining ability (GCA) and specific combining ability effects for yield for some populations. In a study to classify early maturing CIMMYT, Zimbabwe maize genotypes into different heterotic groups and identification of suitable testers Pswarayi and Vivek (2008) identified good GCA effects for yield for some of the lines tested whilst others contributed to low plant height and reduced anthesis silking interval. Uhr and Goodman (1995) compared the performance of testcrosses between tropical-derived lines and an elite U.S. tester with the performance of adapted commercial hybrids. They concluded that since tropical-derived lines appear to combine well with B73/Mo17 they could be used as a source of disease resistance for either heterotic group in addition to having potential to enhance the combining ability of inbreds in the two heterotic groups. Tallury and Goodman (1999) evaluated the potential of tropical germplasm for temperate maize improvement and concluded that hybrids containing 10–60% tropical germplasm yielded within the range of the commercial hybrid checks.

Vasal *et al.* (1992) conducted a study to determine the heterosis and combining ability among CIMMYT's subtropical and temperate early-maturity maize germplasm. They found general combining ability (GCA) effects for yield were significant in some environments, while specific combining ability effects were significant only in temperate environments. Highly significant positive GCA effects for yield were also observed with some populations and pools under temperate conditions whilst other pools were found to be poor general combiners. Bertoia *et al.* (2006) evaluated 28 F1 hybrids, eight parental populations, and four commercial hybrids in four environments in Argentina for combining abilities and to determine heterotic patterns among germplasm sources. They reported significant midparent heterosis (MPH) for ear yield, stover yield, and whole plant yield with general combining ability (GCA) effects being significant for some of the traits studied and concluded that some of the landrace crosses showed potential for breeding. Darrah *et al.* (1987) studied twenty-one F_1 crosses of seven inbred lines of maize for afltoxin resistance in the USA and reported that the general combining ability mean square for aflatoxin B_1 was significant. Gissa *et al.*, (2007) reported significant differences between the genotypes for grain yield components and other agronomic traits. Their study also identified inbred lines and hybrid combinations that had desirable expression of important traits useful for the development of high yielding hybrids.

2.5.1 Hybrid maize production

Hybrid maize breeding has been effectively used in the last 100 years (Hallauer *et al.*, 2010). The hybrid maize concept (Shull, 1909), was developed in the public sector and is still considered one of the greatest achievements in crop breeding. It is considered the most successful plant breeding breakthroughs as a consequence of private-public cooperation and business vision.

Although the essential features of modern hybrid maize breeding were outlined before 1920, several distinct phases have been recorded (Hallauer and Miranda, 1981).

All of the maize acreage grown in the USA and Canada today is planted to hybrid maize, with an increasing percentage of the acreage worldwide (*about* 65%) moving from open-pollinated populations, improved synthetics, and variety crosses to hybrids (Duvick, 1999). Production of hybrid maize seed is an intricate process as seed quality, seed purity, and cost of production are all critically important factors (Lee *et al.*, 2006, Wych, 1988). One aspect that influences cost of production is grain yield of the female seed parent. Generally, grain yield of inbred lines is in the range of 3.8 to 5.4 Mg /ha, which is two to three times lower than hybrid grain yields (Duvick, 1999). Historically poor inbred seed yield was the impetus behind using double-cross and three-way hybrids (Hallauer and Miranda, 1988). With the improvement in performance of inbred lines, single cross hybrids began to replace double-cross and three-way hybrids in the market (Duvick, 1999, Hallauer and Miranda, 1988, Wych, 1988). In the late 1980s, it was estimated that single cross (SC) hybrids represented about 90% of the hybrid seed sold in the USA and Canada (Wych, 1988). In the 1960s and early 1970s, modified single cross (MSC) hybrids and three-way hybrids were grown extensively in the USA and Canada and in the late 1980s, it was estimated that they still occupied about 10% of the hybrid market (Wych, 1988). Double-cross, three-way, and MSC hybrids represent heterogeneous mixtures, with double-cross hybrids potentially being the most heterogeneous (four different parental genotypes). Heterogeneous mixtures of genotypes may result in temporal variability in the field. The results of hybrid type comparison studies concluded that single cross hybrids are higher yielding and more uniform in appearance (Wych, 1988).

Aguiar *et al.* (2008) determined heterotic groups of tropical maize by test crosses and by simple sequence repeat (SSR) markers to compare five grouping methods of heterogeneous maize populations. Sixteen lines of nine populations in the S_5 generation were evaluated in test crosses with three testers. The results of four experimental trials over two years were used to group the lines by five methods: evaluation based on the hybrid mean in topcross tests, hybrid index, genetic diversity by the Mahalanobis distance, genetic diversity by the Euclidean distance, and genetic diversity by SSR markers. The concordance of grouping by the Mahalanobis and Euclidean distance amounted to 87.50%, and the concordance of these methods and grouping by SSR markers was 56.25%. Grouping by SSR markers was consistent with the genealogy of the lines and is a useful procedure for the formation of heterotic groups of tropical maize lines. In Africa, using the resistance from IB32 and La Revolution, IITA and CIMMYT scientists, in collaboration with national programmes, have developed over 115 different inbreds with stable resistance to MSV both in the inbred *per se* and in hybrids developed from these inbreds. These are adapted to different ecological habitats and consumer preferences in Africa (Kim *et al.*, 1989). National programmes in South Africa, Zimbabwe, Kenya, DR Congo, Nigeria and others (including Ghana) in collaboration with IITA continue to screen maize germplasm for sources of usable resistance. Unfortunately, yields of resistant hybrids in the absence of MSV resistance have continued to be a problem in widespread acceptance of new hybrids. It is obvious that any new resistant hybrid varieties need to compete with the present day commercial hybrids (Damsteegt and Igwegbe, 2005). New resistant hybrids and inbreds are being released from national programmes and in Ghana, Mamaba known be resistant/tolerant to MSV, has been released (Souza *et al.*, 1996).

In 1992, MSIRI 3B, developed by the Mauritius sugar cane Industry Research Institute, was released in Mauritius as a new source of resistance different from that used by IITA. In the absence of MSV, this variety out-yielded the composite variety from which it was selected by 15-30%. Currently, extensive testing of several MSV resistant hybrids is being undertaken in several African countries. The ultimate goal is to identify and encourage wide scale cultivation of MSV resistant maize hybrids by farmers to remove MSV as a major limiting factor in maize production in Africa (Damsteegt and Igwegbe, 2005).

2.6 Marker assisted selection

Marker assisted selection, when based on sound phenotypic associations, has changed maize breeding considerably (Sleper and Poehlman, 2006). Marker assisted selection has been used in three areas namely; marker assisted backcrossing, marker assisted selection for quantitative traits, and prediction of heterotic crosses based on DNA markers. Markers often reduce the time needed to release new hybrids. DNA markers detect the recovery of the recurrent genome in backcross breeding, and are more efficient in selection of genomes that have recombinant events close to the target gene (Danson *et al.*, 2006). Markers may enable breeders to pyramid genes against different strains of a pathogen (Danson *et al.*, 2006). Various aspects of the use of molecular markers (for controlling the target genes, accelerating the recovery of recurrent genome or reducing linkage drag) to improve the efficiency of introgression in backcross breeding programmes have been investigated from a theoretical standpoint in recent years. Selection against genetic drag can save tens of generations but not necessarily high costs (Hospital, 2001). This is true even if the target gene is a QTL located with a given confidence interval on the genetic map (Hospital and Charcosset, 1997, Visscher *et al.*, 1996).

It must be noted that considerable research has been undertaken to investigate suitable molecular markers linked to resistant loci for MSV (Weltz *et al*., 1998; Kyetere *et al*., 1999; Pernet *et al*., 1999a, b). The loci for MSV have been mapped consistently to the same chromosome location in multiple field tests in Zimbabwe (Kyetere *et al*., 1999; Pernet *et al*., 1999a and b), Reunion (Weltz *et al*., 1998; Pernet *et al*., 1999a and b) and Uganda (Kyetere *et al*., 1999). Following the mapping of MSV QTL, molecular markers associated with MSV resistance QTL have been identified by CIMMYT and are accelerating the development of resistant cultivars by many breeding programmes. Application of marker assisted selection in the breeding process has been carried out mostly based on available information on map position of traits with agronomic importance and on the linked molecular markers. Simple sequence repeat (SSR) markers, known to amplify bordering the location where a major MSV QTL was detected (chromosome 1, bin 1.04) have been used (Kyetere *et al.*, 1999, Pernet *et al.*, 1999, Welz *et al.*, 1998). Simple sequence repeats are PCR based, multi-allelic and markers which have been used extensively for linkage map development in plants (Chin *et al.*, 1996, Taramino and Tingey, 1996) and for MAS.

Other types of molecular markers are available for MAS procedure, but not all can be used efficiently. Mohan *et al.* (1997) suggested two criteria for selection of markers for MAS: 1) the markers should be efficient in screening large populations, and 2) markers should show a high degree of reproducibility across laboratories. In addition, molecular markers should all be cost-effective (Thomas, 2003). Restriction fragment length polymorphisms (RFLPs) are reliable and yield co-dominant data, but are also time-consuming and expensive, requiring relatively large amount of highly purified DNA and they do not lend themselves to automation. SSR markers

combine reliability and genomic abundance with high levels of polymorphism and co-dominance (Chin *et al*., 1996; Taramino and Tingey, 1996). They do not require sophisticated DNA extraction methods, making them suitable for Ghana where simple DNA extraction techniques (e.g. manual leaf grinding in liquid nitrogen) are available but proper bio-safety measures and equipments to handle radioactive compounds are not. The main drawback of SSRs is the initial identification of primer sites to amplify SSR loci, a procedure which is time and resource demanding however, currently, a large number of SSR markers are already available. Thus, MAS using SSR markers is a valuable and ready to use tool for breeding.

Pernet *et al.* (1999), investigated QTLs responsible for resistance to MSV and their study confirmed that MSV was quantitatively inherited. They detected at least five significant QTLs on chromosomes 1, 2, 3, and 10 in the genotype D211. These QTLs explained between 48% and 62% of the total phenotypic variation observed. In a different study with genotype CIRAD390 as a source of resistance, they identified eight QTLs (Pernet *et al*., 1999b). Still, much of the resistance was reported to be controlled by a few genes; with two QTLs on chromosome 1 and 10 (bin 1.05 and 10.05) stable across dates and environments in the two populations. Their locations were consistently mapped in different populations as well (Welz *et al*., 1998; Kyetere *et al*., 1999; Pernet *et al*., 1999a; Pernet *et al*., 1999b). According to Pernet *et al*. (1999a), two systems exist: one major gene conferring stronger resistance and the other, polygenic, conferring partial resistance. In terms of gene action, the major QTL in bin 1.05 appeared to be partially dominant at the early stage of disease and additive for all the other scoring dates.

Reyna and Sneller (2001) concluded that it may be difficult to realize the value assigned to QTL alleles derived from diverse parents with variable relative genetic values when the alleles are

introgressed into populations with different genetic backgrounds, or when tested in different environments. This, however, is dependent on the trait, as Yousef and Juvik (2002) have shown that QTL identified in one mapping population has a positive effect across different genetic backgrounds and across different environments.

In populations segregating for an entire genome, the presence of epistasis between measured QTL has been found at a frequency close to that expected by chance alone (Xiao *et al.*, 1995) However, when RILs, double haploids (DH)s and isogenic lines are used, epistasis is detected more frequently (Eshed and Zamir, 1996). The presence of epistasis would imply that the QTL detected are not completely independent of QTLs located elsewhere in the genome. As such, MAS selection would not be effective if the epistatic QTLs are not both selected for in the segregating population. This would result in differences in genetic and phenotypic variation of the traits of interest making MSV a difficult trait to select for (Pernet *et al.*, 1999). Nevertheless, the magnitude of the epistasis in the overall phenotypic effect is generally quite small.

Marker assisted selection is a breeding strategy applying indirect selection. Instead of selecting for the gene itself, the molecular markers closely linked to the genes are used to monitor the incorporation of the desirable alleles from the donor source (Dudley, 1993). The strategy also finds application for quantitative traits. By the use of markers linked to specific QTLs it is possible to introgress specific regions of the genome that confer desirable quantitative characteristics to an elite variety (Hospital, 2001). Marker screening within the early generations of a breeding program means that MAS can help to accelerate the backcrossing and develop improved lines or populations. It may especially have advantages in some cases where phenotypic selection is difficult (Chen *et al.*, 2000).

Marker assisted selection with backcrossing has been suggested as a breeding strategy for the introgression of a limited number of QTLs into elite germplasm (Danson *et al.*, 2006, Dudley, 1993). Barata and Carena, (2006) applied 49 SSR markers to classify elite North Dakota (ND) maize inbred lines into heterotic groups to evaluate the consistency between simple sequence repeat (SSR) grouping and testcross data for hybrid maize development. However, their result showed that genetically similar germplasm could not be identified accurately and reliably with molecular markers even when the available germplasm was diverse contrary to what has been suggested. The development of molecular marker techniques has provided new tools for heterosis prediction and DNA markers have been used extensively in investigating correlations between parental genetic distance and F_1 performance or mid-parent heterosis (MPH). If well-established heterotic groups are not available, marker-based genetic distance estimates can be used to avoid producing and testing of crosses between related lines. Furthermore, crosses with inferior MPH could be discarded prior to field testing based on prediction. Genetic distance could also be used in the choice of an appropriate tester for evaluating the combining ability of lines in testcrosses (Melchinger, 1999).

2.6.1 Genetic characterization for Diversity studies

Genetic distance based on molecular markers has been suggested as a tool for grouping of similar germplasm as a first step in identifying promising heterotic patterns (Melchinger, 1999).

Melchinger *et al.* (1990a) evaluated diversity for restriction fragment length polymorphisms and heterosis in two sets of maize inbreds. They reported positive correlations between Roger's distance (RD) and F_1 performance for grain yield, specific combining ability effects heterosis and

showed the predictive value of RDs of the parental lines the yield of single crosses. Melchinger *et al.* (1992) reported positive correlations of genetic distance with F_1 performance, MPH, and SCA effects among flint and dent maize inbred lines.

Senior *et al.* (1998), in a study to assess genetic similarities among 94 maize inbred lines, used 70 simple sequence repeat (SSR) marker loci and reported average polymorphic information content (PIC) of 0.59 with a range of 0.17 to 0.92. Melchinger *et al.* (1991) assessed genetic diversity among thirty-two U.S. maize inbred lines belonging to the Iowa Stiff Stalk Synthetic (BSSS), Reid Yellow Dent (RYD), and Lancaster Sure Crop (LSC) groups using restriction fragment length polymorphism (RFLP). Genetic distance (Roger's Distance, RD) averaged 0.54, 0.57, 0.60, 0.58, and 0.60 for line combinations BSSS x BSSS, LSC x LSC, BSSS x LSC, RYD x BSSS, and RYD x LSC, respectively. Reif *et al.* (2003b) using 85 SSR markers studied the relationship between genetic distance and heterosis in seven tropical maize populations. Genetic distance (modified Roger's distance, MRD) between pairs of populations averaged 0.26 with a range of 0.20 to 0.32. Their results showed that in the analysis of molecular variance (AMOVA), 89.8% of the molecular genetic variance was found within populations and 10.2% between populations. Principal coordinate analysis based on modified Roger's distance revealed that the first three principal coordinates explained 65.2% of the total variation. Squared modified Roger's distance was significantly correlated with panmictic mid-parent heterosis (PMPH) for grain yield (r = 0.63) and negatively correlated for days to silking (r = -0.44) and plant height (r = -0.13). Reif et al. (2003b) concluded that the low correlations between squared modified Roger's distance (MRD^2) and PMPH for plant height and days to silking were mostly due to small PMPH

estimates for the two traits. A similar result was reported by Reif et al. (2004) using SSRs and Parentoni et al. (2001) using RAPDs among tropical maize populations.

In another study involving 20 pools and populations in three separate experiments, MRD between pairs of populations based on SSR data ranged from 0.21 to 0.30, 0.21 to 0.31, and 0.27 to 0.33 for experiment 1, 2, and 3, respectively (Reif *et al.*, 2003a). Polymorphism information content ranged from 0.10 to 0.85 for the SSR loci and analysis of molecular variance revealed that about 11% of the molecular variance was among and the rest within populations. Specific combining ability was found to be highly correlated to the specific MRD^2 in tropical and sub-tropical environments while PMPH was highly correlated to MRD^2 (Reif *et al.*, 2003a).

Reif et al. (2004) reported that principal coordinate analysis based on MRD estimates of tropical, subtropical, and temperate maize populations revealed a total of 34.2% of the molecular variance to be explained by the first two principal coordinates (PC), with PC1 separating the tropical populations from the others. They also reported that most of the variation was within the populations and very little between populations. Xia *et al.* (2004) studied genetic diversity among 86 and 69 yellow lowland tropical maize inbred lines using SSR markers. Polymorphism information content of the SSR markers ranged from 0.13 to 0.87. Genetic distance for yellow x yellow and white x white line combinations ranged from 0.44 to 0.88 and 0.37 to 0.89, respectively, with an average of 0.76. The average genetic distance for white x yellow line combinations was 0.77. Cluster analysis showed that among the white inbreds, lines derived from the Tuxpeño synthetic Pop43 formed one group while lines derived from quality protein maize (QPM) populations also clustered together. Xia et al. (2004) reported that few clear groups could be identified through cluster analysis of the yellow tropical maize inbred lines. In a study

to characterize maize inbred lines and open pollinated populations using SSR markers, the open pollinated populations clustered as predicted based on pedigree and known heterotic groups (Warburton, *et al.,* 2002) whilst dendrogram generated did not show good association based on heterotic grouping as assigned by field evaluations and testers. Melchinger et al. (1990a) and Smith *et al.* (1997) reported that cluster analysis using data from RFLP and SSR revealed associations of inbreds similar to that expected based on pedigree data.

Benchimol *et al.* (2000) calculated genetic distance among eighteen tropical maize inbred lines derived from a synthetic population and a composite population using RFLP markers. Modified Roger's Distance ranged from 0.39 to 0.83 with a mean of 0.74, with the 15 Brazilian composite population showing a greater range (0.39 to 0.80) compared to the Thai synthetic population (0.57 to 0.76). Cluster analysis led to grouping of the populations into two according to their heterotic patterns. Benchimol *et al.* (2000) again reported that simple correlations of genetic distance and with F1 performance and heterosis were highly significant (0.60 and 0.57, respectively). Barbosa *et al.* (2003) also reported highly significant correlation between genetic distance and F_1 performance (0.71) and genetic distance and heterosis (0.67) in a study using AFLP markers on inbred lines derived from the same populations used by Benchimol *et al.,* (2000).

Parentoni *et al.*, (2001) in a study involving twenty eight open pollinated varieties reported a low but significant correlation (r = 0.16) between marker genetic distance and specific combining ability. Lubberstedt *et al.*, (2000) evaluated genetic diversity among 51 early European maize inbreds and reported that genetic similarity estimates for unrelated line combinations of flint x flint ranged from 0.47 to 0.77 while those of dent x dent ranged from 0.45 to 0.69 with a mean of

0.57 and 0.55, respectively. Principal coordinate analysis calculated from AFLP genetic similarity estimates clearly separated the dent from the flint lines. Lübberstedt *et al.* (2000) noted that correlation between genetic similarity estimates based on AFLP, RAPD, and RFLPs were highly significant and ranged from 0.43 to 0.67 for flint and dent lines, with the highest correlation being between genetic similarity estimate based on AFLP and RFLP data. Betrán *et al.* (2003) evaluated tropical maize inbreds under stress and nonstress conditions and estimated genetic diversity for RFLPs, genetic distance, and heterosis. Polymorphism information content ranged from 0.28 to 0.82 for the RFLP probes. Average genetic distance among the inbred lines ranged from 0.20 to 0.84 with an average of 0.72, with sister lines having a low GD (<0.25). Principal component analysis using the calculated GD classified the inbred lines according to their origin and pedigree. Genetic distance was positively correlated with F_1 performance, MPH and high-parent heterosis (HPH) in all environments. Betrán *et al.*, (2003) indicated that correlations of GD with MPH and HPH increased when the drought-stress levels decreased.

2.7 Farmer participation in breeding and varietal selection

Atlin *et al.* (2002) reported that the success of new crop varieties depends upon quick adoption. Many times the improved varieties from breeders fail due to the reason that farmers' perceptions change over time. Lines selected by consensus based on networked Participatory Varietal Selection (PVS) trials are broadly adapted, stress tolerant, and acceptable to farmers (Atlin *et al.*, 2002).

Farmers have preferences that are key factors when selecting varieties for production. Farmers in Africa have special preferences for maize varieties such as taste, cooking qualities and high

biomass for stock feed. Generally, farmers are heterogeneous in their needs, priorities, and preferences. Failure to consider these could result in rejection of otherwise promising new varieties. Engaging farmers would help breeders understand their needs and preferences which in turn would help in selecting appropriate genetic materials (Witcombe *et al*., 1996). Unfortunately, formal research systems in developing countries are highly centralized and do not target the problems of resource-poor farmers. While enormous resources have been directed towards breeding maize in most of Africa over the past three decades, only an estimated 37% of the farmers regularly plant improved varieties (Morris, 1998, cited by DeVries and Toenniessen, 2001). It has been reported that, in most cases, breeders do not have a clear understanding of the farmers' requirements; hence breeding programmes might not have sufficiently considered the needs and preferences of farmers (Banziger and Cooper, 2001; Banziger and de Meyer, 2002; Toomey, 1999). This impedes the adoption of new cultivars, and in fact, in developing countries most cultivars grown by farmers were old and only a few of the released cultivars are grown (Witcombe *et al*., 1996). Studies in India have shown that the average age of cultivars grown by farmers was more than 12 years for rice, 15 years for groundnuts, 16 for sorghum and 17 for maize, and in Africa the varieties grown could be even older. It thus becomes imperative to ascertain farmers' preferences before any attempt is made to develop varieties for widespread adoption by farmers and consumers.

Mahendran *et al.* (2004) used PRA to characterize the farmers' crop management practices, gender roles, and farmers' preferences for traits in improved varieties to suit the local needs. Farmers were selected based on their primary occupation and degree of literacy. Five rice varieties were selected and raised in an area of 50% of the farmers' fields and managed by the

farmers under the supervision of scientists again in participatory varietal selection process at the research farm. The results revealed that the awareness about high yielding varieties was as high as 93%. However, the farmers preferred low yielding landraces to high yielding varieties for their tolerance to drought, low input costs, and readily available seed materials. The on-farm participatory breeding programme (PBP) revealed that farmers' preference rankings were not always correlated with scientists' ranking. However, the lines selected by farmers almost tallied at 75% with scientist selection, indicating that farmers are capable of identifying varieties by considering important plant growth characteristics of their choice. Mwala *et al.* (2004) reported that in a study by the national maize programmes of SADC and CIMMYT in the sub-region, development and transfer of improved maize varieties has often been found to take too much time, resulting in little impact on the farmers. Adoption of a participatory variety evaluation method, the Mother/Baby scheme, through which evaluation of the varieties was done under conditions representing those of the farmers and resulted in selection of varieties that possessed important characteristics that conformed to farmers' production and utilization expectations which resulted in faster adoption of varieties. The scheme led to rapid release of ZM 421 and ZM 521 in RSA, Malawi, Tanzania, and Zimbabwe, and ZM 621 in Malawi.

2.8 Importance of Landraces in modern maize breeding

Landraces and wild relatives represent an extraordinary genetic resource of maize with significant allelic diversity, much of which has not been incorporated into improved varieties (Sharma *et al.*, 2010, Warburton *et al.*, 2008). Landraces are genetically diverse, heterogeneous populations that are typically selected by farmers for their adaptation to specific local environments and needs (Prasanna and Sharma, 2005). High levels of genetic diversity in maize

are caused by active transposable elements, meiotic recombination following outcrossing, new introgressions from exotic germplasm, genetic drift following new introductions, and natural and artificial selection by farmers as the crop adapts to new environments (Doebley, 2004). In maize these factors have produced numerous open-pollinated landraces, which constitute a possible source of diversity that can be exploited to widen the improved gene pool from which breeders can harness useful genes and alleles to meet the challenges of climate change and other objectives of modern breeding programmes.

In Argentina, Bertoia *et al.* (2006) explored the possibility of using landraces as germplasm sources to enhance forage yield and quality in warm temperate areas. They reported that some landrace crosses showed superior yield than commercial checks, indicating the breeding potential of the evaluated germplasm.

Using Suwan-1, a popular OPV from Thailand, a composite 'Parbhat' has been developed at Punjab Agricultural University, Ludhiana (India), which shows resistance to multiple diseases, high yield and stability in performance (Dhillon *et al.*, 2002). Improved germplasm that is well adapted to the hill areas have been derived at Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, Uttarakhand (India), using landraces from the states of Jammu & Kashmir and Uttarakhand in India. The popular hybrids derived through this strategy include Him-129 (yellow, flint, 85–90 days maturity, highly tolerant to leaf blight), Him-128 and several 'Vivek' hybrids (Prasanna *et al.*, 2010). The utility of wild relatives of maize (teosintes and Tripsacum dactyloides) for developing genetically improved maize was well illustrated by Rich and Ejeta (2008) in terms of resistance to the 'witch weeds' (Striga species), which are particularly prevalent in Africa. While there appears to be paucity of Striga resistance genes

among maize landraces in Africa, although some resistance sources have been identified (Kim *et al.*, 1999); both perennial teosintes (*Zea diploperennis*) and Tripsacum dactyloides showed relatively higher levels of resistance (Gurney *et al.*, 2003, Lane *et al.*, 1997). Through a long-term breeding effort, researchers from the IITA (http://www.iita.org/maize) developed a Striga hermonthica–resistant inbred, ZD05; this inbred has in its pedigree a *Zea diploperennis* accession as well as tropical maize germplasm (Amusan *et al.*, 2008, Menkir *et al.*, 2006). Maize farmers often make intensive efforts to maintain the genetic identity of their favourite local varieties or landraces for several reasons. For example, farmers in Honduras grow hybrids in valleys and local varieties on hillsides – the purpose of growing the varieties on the hillsides is to maintain the genetic purity (Almekinders *et al.*, 1994). Gene exchanges among the maize landraces is often encouraged (a process called 'creolization') in many traditional farming systems (especially in Mexico). Similarly, in Costa Rica and Honduras, Almekinders *et al.* (1994) found that hybridization between local and improved maize is highly valued by farmers. Varieties derived through creolization (popularly referred to as Criollo varieties) provide an opportunity to smallholder farmers to gain access to improved farming technology and adapt new varieties to their local conditions without the cost of buying seed every year (Bellon and Risopoulos, 2001).

In both the USA and China which happens to be the two leading producers of the crop in the world the genetic base of the varieties used are narrow (Prasanna, 2012). This is because in most breeding programmes, there is an unwillingness to 'dilute' the present-day elite stocks with unimproved germplasm, as development of elite inbreds takes several generations of intensive breeding to bring to their present level of agronomic performance (Kannenberg and Falk, 1995);

and secondly, most of the public sector maize breeding programmes, especially in the developing countries, do not have adequate resources or strategies to devote for systematic characterization and utilization of landraces or exotic maize germplasm. Several programmes are being initiated to explore the potential of landraces to broaden the genetic base of maize: LAMP was the first internationally coordinated project (1987–1996) for evaluation of maize germplasm. This project generated information on maize germplasm in 11 South American countries (Argentina, Bolivia, Brazil, Colombia, Chile, Guatemala, Mexico, Paraguay, Peru, Uruguay and Venezuela) and the USA, and facilitated breeders to access this information and for the creation of superior varieties and hybrids. Besides yielding ability, the important agronomic traits evaluated in LAMP were standability (root-lodging and broken stalks), earliness, and plant and ear height. Another project is the USA-GEM Project which was a collaborative research effort of the USDA-ARS, land grant universities, private industry, and international and non-governmental (NGO) organizations to broaden the germplasm base of maize (Prasanna, 2012). The primary purpose of the project was to introgress useful genetic diversity from Latin American maize races and other tropical maize donor sources (lines and hybrids) into US maize germplasm to broaden the genetic base of the corn belt hybrids (Balint-Kurti *et al.*, 2006, Goodman, 2005). The project used the Latin American landrace accessions selected by LAMP and crossed them with elite temperate maize lines provided by private companies in North America (Salhuana and Pollak, 2006).

Another project 'Seeds of Discovery' a CIMMYT initiative, aims to discover the extent of allelic variation in the maize germplasm available or conserved in the Gene Bank, formulate core sets

based on genotyping and phenotyping, for utilization of rare useful alleles in breeding programmes (Prasanna, 2012).

2.9 Yield Stability

An important element affecting selection apart from heritability is genotype and environment interaction (G×E). Interpretation of G×E influences plant breeders' approaches to developing and improving crop varieties and their choices of how many and which varieties will be released across agricultural environments (Cooper and Hammer, 1996). Tollenaar and Lee, (2002) studied the relationship between yield and yield stability using a number of high-yielding maize hybrids, including three hybrids that have been involved in some of the highest maize yields recorded in producers' fields. Results of their stability analyses showed that high-yielding maize hybrids can differ in yield stability, but results do not support the contention that yield stability and high grain yield are mutually exclusive.

Scapim *et al.*, (2000) studied the importance of stability and adaptability of a genotype to different environments for recommending cultivars for known conditions of cultivation and requirements in breeding programmes. Twenty maize cultivars were evaluated at eight locations in Brazil for two years. They reported cultivar 'G-96C' showed medium adaptation to all environments (ideal cultivar) and had good stability. Cultivars 'C 505' and 'C435' were alternatives for 'G-96C'. 'DINA 70' showed good adaptability but had low stability.

Fan *et al.* (2007) studied the performance of maize hybrids in multi-environment trials (MET) to ascertain grain yield stability of 13 Chinese hybrids tested across 10 locations in 2002 and 2003 via GGE biplot analysis and Kang's yield-stability statistic (YS_i) and to identify non

representative and/or non discriminating locations. Within years, cultivars and cultivar-by-location (C × L) interactions were significant. Heterogeneity caused by environmental index did not contribute appreciably to C × L interactions. The YS_i identified, among the top five hybrids, LD10, Hai He, and YR1 as common between years. The GGE biplot analysis ranked hybrids with above-average yield across years as Hai He > LD10 > YR1 > Tun004 and for stability of performance as LD10, Hai He, Tun004, and YR1. Three different locations were identified in 2002 and 2003 as the least discriminating. The YS_i indicated ZZY6 and SB21-3 to be the most unstable hybrids between years. The only hybrid showing stable performance across locations was Tun004 in 2002.

Crossa *et al.* (1987) evaluated yield stability of a group of CIMMYT tropical maize populations across international environments, measured by the performance of varieties derived from them, showed that the populations Mezcla Tropical Blanca, La Posta and Amarillo Dentado produced the most stable varieties across environments. Mezcla Tropical Blanco and Amarillo Dentado produced selections with good stability in both low and high yielding environments while La Posta produced varieties with better performance in favourable environments. 'Across-site' varieties were not consistently more effective than 'site-specific' varieties in improving yield stability. Varieties selected for some specific population-environment combinations have been very stable in other areas of the world. Again Crossa *et al.* (1988) evaluated the yield stability of some CIMMYT tropical maize populations of early and intermediate maturity, measured by the performance of varieties derived from them. Results of the stability analyses, conducted over international environments from 1980 to 1983, indicated that selections from Mezcla Amarilla exhibited good stability in high yielding sites. Varieties derived from Antigua-Republica

Dominicana tended to be more stable in unfavourable environments, whereas selections from Blanco Cristalino-1 and Blanco Dentado-2 were stable in both low and high production sites. The combination of environmental factors in the specific test allowed selection of varieties that are very stable in other regions of the world. The varieties formed on the basis of multi locational data did not seem to be more stable than those formed using data from a single location.

Pixley *et al.* (2002) assessed stability of grain yield, protein content, protein quality, and endosperm modification of QPM cultivars. They evaluated 18 single-cross, 18 three-way, and 18 double-cross hybrids, and eight open-pollinated cultivars (OPVs) in 13 tropical locations on four continents. They concluded that protein quality and endosperm modification score were always within expected values for QPM and tryptophan concentration in protein was the most stable trait, followed by protein concentration in grain, then endosperm modification score and finally grain yield. Balestre (2009) evaluated the stability and adaptability of the grain yield of commercial intervarietal maize hybrids by the GGE (Genotype and Genotype by Environment Interaction) biplot and AMMI (Additive Main Effects and Multiplicative Interaction) analyses. Two intervarietal hybrids (BIO 2 and BIO4) were evaluated together with single, double and three-way cross hybrids. They reported that the performance of the intervarietal hybrid was superior to all double and three-way cross hybrids and outmatched the single-cross hybrids by 43%. In terms of stability, BIO2 was more stable than BIO4, which is desirable.

Gama and Hallauer (1980) compared relative stability of grain yield among hybrids produced from selected and unselected lines. Mean grain yield and stability analysis of variance, which included linear regression coefficient and deviations from the regression, were used to determine relative stability of the single-cross hybrids. Simple correlation coefficients among mean grain

yields also were determined. They reported that hybrids of selected lines produced significantly greater grain yield than hybrids of unselected lines and significant hybrid-environment interactions with similar contributions to total hybrid-environment variances. They suggested that selection of hybrids for mean yield across environments should be emphasized first before the relative stability of the elite hybrids acrossr environments is determined. Byrne *et al.* (1995) evaluated progress from selection of two related tropical populations across a broad range of environmental conditions and reported that the difference between the populations was not significant at ($P < 0.10$). Stability analysis indicated that the check variety 'La Posta Sequia Best' was the most stable and high yielding genotype. They concluded that selection under managed levels of drought stress at one location together with multi-location testing may be desirable components of maize breeding for drought-prone tropical areas.

CHAPTER THREE

3.0 Farmers' perceptions on maize streak virus disease, production constraints, and preferred varieties in the forest-transition zone of Ghana

3.1 Introduction

The Maize Streak Virus Disease (MSVD) has been noted as one of the most serious biotic constraints to maize production in Sub Saharan Africa and hence a threat to food security(Asiedu *et al.*, 2001, Bello *et al.*, 2012, Monjane *et al.*, 2011). However, farmers' awareness, perceptions and general knowledge of this important disease has not been adequately assessed in the forest transitions ecologies of Ghana (M.B. Ewool, personal communication). It is imperative to ascertain farmers' preferences before any attempt is made to develop varieties for widespread adoption. This is because farmers have different needs, priorities, and preferences. Failure to consider these could result in rejection of otherwise promising new varieties. Engaging farmers can help breeders to understand their needs and preferences which in turn would help in selecting preferred genetic materials (Witcombe *et al.*, 1996).

In Ghana most farmers still rely on the cultivation of maize landraces. Formal research systems in developing countries are highly centralized and do not target the problems of resource-poor farmers. While enormous resources have been directed towards breeding maize in most of Africa over the past decades, in 1998 it was reported only an estimated 37% of the farmers regularly plant improved varieties (Doss and Morris, 1998). Recent estimates puts at over 60% with a sizable percentage still depending on landraces (USAID/EAT, 2012). It has been reported that, in

most cases, breeders do not have a clear understanding of farmers' requirements, hence breeding programmes might not have sufficiently considered their needs and preferences ((Banziger and Cooper, 2001, Banziger and Meyer., 2002, Toomey, 1999). This situation impedes the adoption of new cultivars (Witcombe *et al.*, 1996). Engaging farmers in consultation and collaboration will help to exploit their knowledge and preferences in developing new varieties which will gain wider acceptability.

The knowledge gained in this study will give a clear picture of the extent of damage caused by the maize streak virus disease, production constraints and also provide a reliable guide for breeders in developing farmer acceptable maize varieties with adequate resistance to the MSVD.

The objectives of the study were to:

a) assess farmers' awareness of the Maize streak virus (MSV) disease incidence and its effects on production.

b) ascertain characteristics of farmer preferred maize varieties and

c) ascertain production constraints in relation to maize streak virus disease.

3.2 Materials and Methods

3.2.1 Focus group discussions

Prior to the formal survey, an informal survey was conducted. It involved Farmer Focus Group Discussions (FFGD) on key issues to ascertain the existence of the disease in the study areas, constraints in maize production and the production systems employed in the areas especially in the presence of the MSV disease.

Two focus group discussions were organized in two important maize growing districts in the Ashanti and Brong Ahafo regions, namely Ejura and Wenchi respectively. These districts which fall within the forest transition zones were selected because of their output to total national maize production (Morris *et al*., 1999). They fall within the zones where maize streak disease is prevalent. Majority of farmers in these districts grow maize which is sold throughout Ghana. Farmers were assembled through the efforts of the district and local Agricultural Extension Officers.

At Tromeso in the Wenchi district which is a major food crop production community, 40 male and female maize farmers of varied age groups, levels of education and experience in maize production were randomly selected and then sub-divided into four groups of ten to enhance participation. A similar number with similar characteristics was also randomly selected at Hiawoanwo, a predominantly maize growing community in the Ejura district. The discussion followed a guideline that focused on farmers' knowledge of the maize streak virus disease, preferred varieties, farming practices and production constraints (Appendix 3.1). Farmer concerns, constraints and opinions were recorded on a flip chart after a consensus had been reached on the topics.

3.2.2 Questionnaire administration

Based on the information gathered from the FGD, a detailed questionnaire was designed for a formal survey (Appendix 3.2). The questionnaire had semi-structured questions on knowledge of the maize streak virus disease and farmers' perceptions of the disease. Others were preferred varieties, production constraints and marketing. One hundred and ninety six (196) questionnaires

were administered to randomly selected farmers in the selected communities, namely Hiawoanwo, Boyon, Dromankoma, Nkrampa, Sekyidumasi in the Ejura-Sekyidumasi municipality. All these communities are pre-dominantly farming communities cultivating maize and other food crops such cassava cowpeas, etc. Others were Tromeso, Amoakrom, Nyamebekyere and Twum krom in the Wenchi Municipality which are also important food crop growing community.

3.2.3 Data analyses

The data were analyzed using Social Science package for statistical analyses (SPSS) version 16 to generate frequencies and percentages. Associations between farmer responses and demographic data were also determined. This was done by entering all data collected onto Windows Excel spreadsheet for the analysis using the appropriate commands.

3.3 Results

The age of respondents ranged from 20 to 80 years with an average of about 41 years (Table 3.1). Experience in maize production also ranged from 3 to 50 years with an average of 16 years. Household size averaged 7 and an active working group per household was 4. Taking the total members of the household, about 3 out of the 7 members were educated up to the basic level, with 2 males and 1 female being educated per household.

Farmers on the average had farm sizes of about 2.5 (hectares) and cropped this land two times in a year. The farmers reported average yield loss as a result of the disease infestation was about 19

percent. Each land is cultivated for about two years after which new plots are farmed and the old one allowed to fallow.

Table 3. 1 Summary statistics of quantitative variables used in the analysis

Variable	Minimum	Maximum	Standard deviation	Mean
Age	20	80	11.959	40.73
Experience in Maize production	1	50	10.479	15.82
Years of residence of non- natives	3	50	12.415	19.26
Household size (total)	1	16	2.927	6.68
Number of males in household	1	10	1.851	3.42
Number of females in household	0	10	1.904	3.26
Number of males educated in household	0	6	1.277	1.54
Number of females educated in household	0	9	1.426	1.46
Number educated in household (total)	0	10	2.351	2.83
Average farm size (acres)	1	40	5.699	6.49
Number of times land cropped	1	3	0.407	1.84
Percent yield loss from MSV disease infestation	1	70	15.026	18.99

In all the qualitative variables considered, with the exception of farmers' engagement in off-farm work, basic education and whether they practiced rotation, the males outnumbered the females in terms of percentage (Table 3.2).

Table 3. 2 Descriptive statistics of qualitative variables in the analysis

Variables	Frequency		Percent	
	Males	Females	Males	Females
Gender	142	54	72.45	27.55
Educational level				
None	47	16	33.1	29.6
Basic	87	37	61.3	68.5
Secondary	6	1	4.2	1.9
Tertiary	2	-	1.4	-
Head of household	127	21	89.4	38.9
Nativity				
Native	105	38	73.9	70.4
Settler	23	12	16.2	22.2
Migrant	14	4	9.9	7.4
Main Occupation				
Crop production	140	51	98.6	94.4
Engagement in Non- farm	97	41	68.3	75.9
Practice of rotation	63	27	44.4	50.0
Persons within 20-35 years	55	17	38.7	31.5
Persons 36-50 years (total)	63	24	44.4	44.4
Persons 51-65 years (total)	19	10	13.4	18.5
Persons above 65	5	3	3.5	5.6

Results of farmers awareness of the maize streak virus disease is presented in Table 3.3. About 81 percent of the farmers were aware or had heard of the disease. However, about 76 percent of the respondents affirmed they had experienced the disease on their farms. More than half of the respondents agreed that the disease is very important or needed attention.

Table 3. 3 Farmers' knowledge of Maize Streak Virus

Variables	Frequency	Percentage (%)
Awareness of maize streak	159	81.1
Plant parts affected		
Leaves	119	60.7
Importance of maize streak		
Very important	63	32.1
Important	59	30.1
Not so important	32	16.3
Not important	12	6.1
Experience streak on farm	148	75.5
Is variety tolerant		
Yes	103	52.6
No	42	21.4
Don't know	21	10.7
Method of control		
Fungicide	19	9.7
Nothing	71	36.2
Roguing	53	19.7
Symptoms of streak		
Leaf rot	5	2.6
Yellowing along leaf blade	110	56.1
Don't know	55	28.1
Perception on resistant varieties		
OPVs'	43	21.9
Landraces	37	18.9
Hybrid	6	3.1
Don't know	56	28.6
Perception on susceptible varieties		
OPVs'	38	19.4
Landraces	20	10.2
Don't know	44	22.4
Stage of growth disease occur		
Vegetative	90	45.9
Reproductive	36	18.4
Season streak is prevalent		
Major	35	17.9
Minor	126	64.3
Is disease transmissible		
Yes	97	49.5
No	38	19.4

Don't know	23	31.1
Causes of diseases		
Insects	39	19.9
Don't know	104	53.1
Month disease is prevalent		
June	26	13.3
August	25	12.8
September	39	19.9
October	37	18.9

Fifty three percent of the respondents said the varieties they grow were tolerant to the disease so during the time of disease attack they did nothing. The few who acted in the presence of the disease either applied fungicides or rouged affected plants (up-root the affected plants and burn or bury them).

Majority of the respondents could not be specific on the month of the year when the disease was prevalent even though they mentioned the minor season as the most worrying season (Table 3.3). However, most of the respondents mentioned June, August, September and October and these months fall in both the major and minor seasons.

Another challenge to farmers was knowledge of varieties which were susceptible or resistant to the disease. The majority did not know. However, farmers rated the OPVs and their local varieties as being resistant. Farmers also rated the OPVs as being susceptible to the disease caused by fungi, etc.

Farmers within the surveyed areas cultivated both improved and local varieties. The common varieties identified were Obatanpa, and Aburohoma, and others (Abelehi, Aburotia, Okomasa, and Mamaba) were less mentioned, (Figure 3.1). These varieties on the average had been cultivated for over 7 years. Farmers generally used their saved seeds from previous harvest and

this practice according to them had continued for many years. Farmers harvested higher bags of maize grains in the major season than in the minor season.

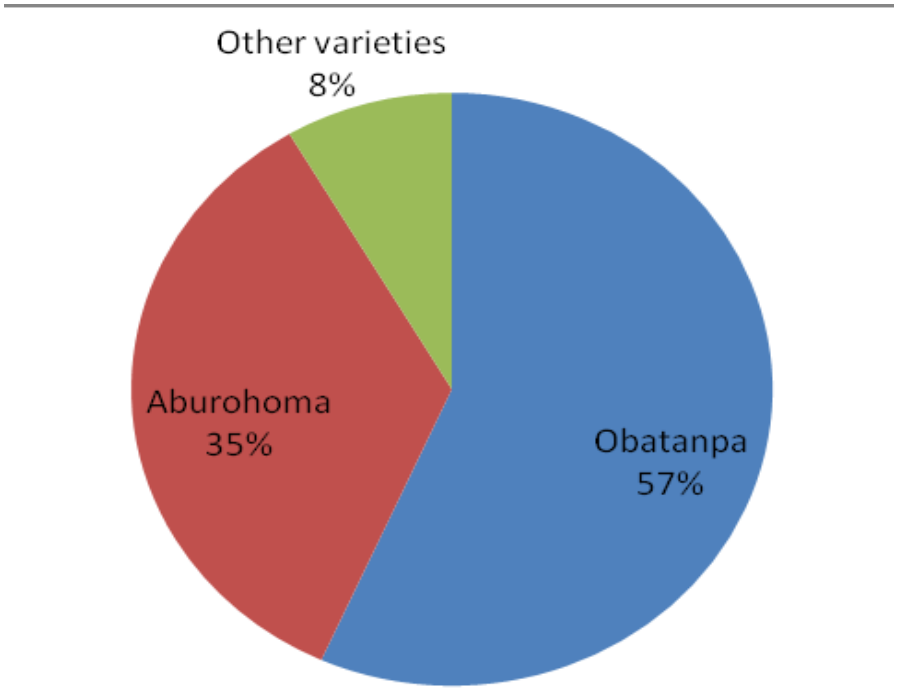


Figure 3. 1 Varieties cultivated by farmers

Varieties that farmers were familiar with were improved OPVs, and their local landrace (Table 3.4). The common among them were Obatanpa and Aburohoma, also known as Appiah in other localities. These varieties were both cultivated in both the major and minor seasons. However, farmers were not very sure of their tolerance to the maize streak virus. Most of the newly released varieties like Enibi, Omankwa, etc. were not mentioned by the farmers.

Table 3. 4 Farmers' preference for new variety and characteristics associated with it

Preference for new variety	Freq	%	Traits desired in order of preference	Variety colour preferred	Reason for colour prefernce	Variety type most preferred	Main information source about variety
Yes	119	60.7	Yield Early maturity Pest and disease resistant (including MSVD)	White	Highly demanded and better market price	OPVs	Extensionist from MoFA, Fellow farmers Colleague farmers
No	77	39	N/A	N/A	N/A	N/A	N/A

Farmers were willing to adopt a new variety as shown in table 3.4. This they indicated should be varieties that possess some traits of interest. They expressed preference for white OPV variety which is high yielding, early maturing and pest and diseases resistant including MSV disease. They desired such varieties because that was to them what the market preferred and could therefore provide them with higher incomes to improve their livelihoods.

The main sources of information about new varieties and farming technologies as indicated by the respondents were from Ministry of Agriculture (MoFA) extension agents and their colleagues, (Table 3.4).

Technologies currently used by farmers

During the PRA, farmers mentioned that they employed row planting and fertilizer application technologies on their fields especially during disease outbreaks. Others applied techniques like crop rotation; especially for the management of diseases including maize streak virus disease was also mentioned.

Constraints to maize production

Framers in the two districts ranked their production constraints in terms of its seriousness from highest to lowest. Even though there were similar issues of concern in the two districts, the weight placed on them differed. Whilst financial constraints, unavailability of seeds, erratic rainfall, low yields, poor roads and unavailability of tractor services followed in that order for farmers in Wenchi district, farmers in Ejura Sekyeredumase Municipality ranked erratic rainfall, unavailability of tractor services, unavailability of labour, high cost of fertilizer, low prices by middlemen, lack of drying facilities and pest and diseases (including maize streak disease) as their constraints in that order.

3.4 Discussion

Breeding programmes that have included the participation of the target communities have always come out with varieties with high adoption rate (Imanywoha *et al.*, 2004, Jeyaprakash *et al.*, 2004, Joshi and Witcombe, 1996, Witcombe *et al.*, 1996). The present study has revealed the low adoption of recently released maize varieties by the research institutions in the country. Of the over 40 maize varieties that have been released only Obatanpa enjoyed widespread adoption by

the farmers. Varieties like Abelehi, Mamaba and Aburotia, were mentioned by a few respondents. Others like Enibi, Etubi, Omankwa, etc which were released recently were not mentioned. The wider adoption of the Obatanpa variety could be attributed to the widespread publicity that accompanied its release (Morris *et. al*., 1999). Low adoption of the other varieties may in part be attributed to lack of interest or knowledge of these varieties by the farmers. Possibly, these varieties do not possess the qualities preferred or farmers may not have had the opportunity to try them as have been reported by (Harris *et al.*, 2001) in India and Zimbabwe where farmers refused to adopt simple technologies like seed priming for better establishment. Thus the need to involve farmers at the initial stages of varietal development to incorporate their input/preferences should not be underestimated (Imanywoha *et al.*, 2004).

Awareness of the maize streak virus disease was widespread among the farmers interviewed. One disturbing fact, however, was lack of knowledge on the causes and the spread of the disease as a result some farmers apply various chemicals, including fungicides for its control. Awareness of the disease also appeared to affect the choice of varieties cultivated. Varieties that farmers were not sure of its disease resistance were not readily adopted as has been reported in some countries (Ramasamy *et al.*, 2004). This is also supported by cost of production which the farmers complained keep rising. For instance, if the farmer grows varieties that succumb to the disease they will lose out and as much as possible they try to avoid this by limiting themselves to varieties they are sure of getting something from (Singh *et al.*, 2004). This is expressed in the continuous reliance on landraces by many rural farmers (Prasad *et al.*, 2004), which is confirmed by the results of this study.

The continuous reliance on maize landraces shows that they possess some characteristics of interest to the rural communities which should attract the attention of scientists if efforts to improve rural agricultural productivity were to succeed. The survey revealed that about 37 percent of respondents still depended on landraces. The farmers rated the performance of these landraces as comparable to those of the improved OPVs which will have to be investigated. Elsewhere in the world the exploitation of local landraces for varietal development is gaining momentum(Carena, 2005, Michelini and Hallauer, 1993, Soengas *et al.*, 2006) and it is about time Ghana also looks inwards and exploit the potential of its maize landraces to ensure easy varietal adoption for increased productivity.

Important information that came out was the role the market/consumers play in the adoption of a variety. Invariably, farmers respond to what the market demands which dictates the adoption or cultivation of a variety (Imanywoha *et al.*, 2004). This piece of information is very useful. It perhaps corroborates the wider adoption of the Obatanpa variety (57% of respondents grow it) because as has been explained earlier, this variety was highly popularized through media campaigns by extolling its nutritional attributes (quality protein maize) which generated consumer or market interest thereby compelling farmers to adopt. Farmers continue to grow landraces because consumers, and for that matter, the market rewards them by patronizing. This shows the potential of landraces. There is a market readily available. Farmers will not struggle for markets if its productivity can be improved upon through yield increases.

One major constraint that was mentioned was irregular rainfall which has also been of immense concern in many countries worldwide in recent times (http://hdl.handle.net/10568/24696, Prasanna, 2012, Tachie-Obeng *et al.*, 2013). Irregular rainfall is linked to larger phenomenon of

climate change. The incidence of maize streak virus disease has an element of weather or time dependence (Bosque-P´erez *et al.*, 1998, Dabrowski *et al.*, 1987). The disease is transmitted by insect vectors; leafhoppers (*C. mbila*) and is mostly prevalent during the minor season. This requires that new varieties that are being developed should genetically include MSV resistant genes so that irrespective of the time planted they can resist or tolerate the disease and thereby improve productivity.

It again emerged from the study that most farmers preferred to grow landraces and improved open pollinated varieties (OPVs) compared to hybrids, although the preference for high yielding maize cultivar was preferred by the majority. The few who grew hybrid maize (supplied by companies from South Africa) were of the view that some of the varieties did not meet their preferences. They preferred slender cobs, light in weight and with lots of grain which can be transported easily from the farm gate to the processing centre without incurring much cost of transportation and at the same time obtaining large number of tons/ha. This was the main reason most of the farmers preferred the landrace (Aburohoma) which is noted for slender cobs with large amounts of grain. Thus any efforts to successfully develop high yielding hybrid maize for the market should be to develop varieties with these attributes and also appreciable percentage of yield higher than what they currently have.

Most of the farmers applied modern agronomic practices such as crop rotation or mixed cropping (Morris et. al., 1999). The practice of crop rotation is an important management practice that reduces disease incidence when done properly, crop rotation disrupts the life cycle or host of most vectors which is very important in the management of plant diseases outbreaks including MSVD (Zitter and Simons, 1980).

3.5 Conclusion

Farmers reported that they lost about 20% of their produce to MSVD. Majority of them cultivated improved open-pollinated varieties (OPVs), particularly Obatanpa variety developed by the CSIR Crops Research Institute and the local landrace called *Aburohoma* or *Appiah*. Only about 3% grew hybrid varieties. Farmers were not sure which of their varieties were resistant or susceptible to the maize streak disease because over 70% of them have experienced the disease on their fields and these involved improved OPVs and the local landraces. Control of the disease varied with some uprooting and burning infected plants, others applied fungicides whilst others did nothing. Majority of the farmers also indicated preferred varieties as those that are high yielding with slender cobs and lots of grains that are also resistant to the maize streak virus disease. Constraints to production in the districts ranked financing, lack of tractor services, poor transportation, and lack of labour, climate change, high cost of fertilizer, low prices by middlemen, lack of drying facilities and pest and diseases. Majority employ modern agronomic practices such as planting in rows, application of fertilizer (NPK and ammonia/urea). Adoption of recently released varieties was found to be low or non-existent.

CHAPTER FOUR

4.0 Genomic characterization of Maize streak virus (MSV) strains found in the forest and transition zones of Ghana

4.1 Introduction

Maize streak virus (MSV) belongs to the genus *Mastrevirus* of the family Geminiviridae. The virus has been found to infect maize in the forest and the savanna zones from sea level to 1800 m (Bjarnason, 1986) in Africa. MSV is endemic in Africa and the adjacent Indian Ocean islands (Bock *et al.*, 1974, Rose, 1978, Soto *et al.*, 1982). It is transmitted by leafhoppers of the genus *Cicadulina* (Storey, 1925) and causes maize streak disease (MSVD), the most damaging viral disease of the crop in Africa (Martin and Shepherd, 2009, Thottappilly, 1992). Maize streak virus is a single stranded DNA virus of ~2.7kb in size encapsidated into geminate particles (Bock *et al.*, 1974, Harrison *et al.*, 1977).

The virus is known to exist in many forms and vary according to agro-ecology, season and vector dynamics (Mawere *et al.*, 2006, Rybicki, 1994). Research has shown that there are many isolates or strains of MSV (Martin *et al.*, 2001, Varsani *et al.*, 2008, Willment *et al.*, 2001) with some being more virulent than others (Bock *et al.*, 1974, Clarke *et al.*, 1989, Rybicki *et al.*, 1988). Eleven strains of MSV are currently known, but only the MSV-A strain is known to cause economically significant streak disease in maize (Shephered *et al.*, 2010). Severe chlorosis occurs in very susceptible maize cultivars, leading to stunted growth, poor ear formation,

reduced seed setting, and heavy yield losses or premature death (Danson *et al.*, 2006, Mawere *et al.*, 2006).

The exact make-up of MSV-A populations in different parts of Africa including Ghana could influence MSVD epidemiology. Rapid increases in virus concentration and epidemic spreads of the disease are usually attributable to the convergence of factors, including build up of MSV-A in early planted maize which affect successive plantings (Dabrowski *et al.*, 1991, Fajemisin and Shoyinka, 1976), population density of wild grasses which serve as reservoirs of both MSV-A and leafhopper vectors (Autrey and Ricaud, 1983) and environmental factors that facilitate vector population increases and long distance movement of leafhoppers (Rose, 1978).

Several reports indicate that differences exist among maize streak symptom severity caused by different MSV strains or isolates (Pinner *et al.*, 1988). Boulton *et al.* (1991) concluded that "Nigeria mild" (MSV-Nm) and "Nigeria severe" (MSV-Ns) MSV strains could be differentiated based on five distinguishable characteristics that included severity of chlorosis, streak width, and streak length. Njuguna (1996), and Rodier (1995) reported variations in maize streak development, and in some cases, resistant germplasm became susceptible when exposed to different strains of MSV.

In Ghana the disease is known to spread across the maize growing zones and since 1979 all maize breeding efforts have placed emphasis on developing maize streak resistant cultivars (Souza *et al.*, 1996). However, little information is available on different strains of MSV that cause the maize streak disease in the important maize growing areas.

With improved virus detection and sequencing techniques developed over the last decade, new efforts to develop MSV resistant maize genotypes should include the detailed characterization of MSV strains against which these genotypes will be deployed. Identification of the key Maize streak disease causing MSV strains will enable successful screening of novel maize genotypes with the most relevant range of MSV strains.

The objectives of this study were to:

1. identify strains of MSV found in the important maize growing areas in the forest and transition zones of Ghana, and
2. identify virulent MSV inoculum sources for the screening of novel maize genotypes against the maize streak virus disease.

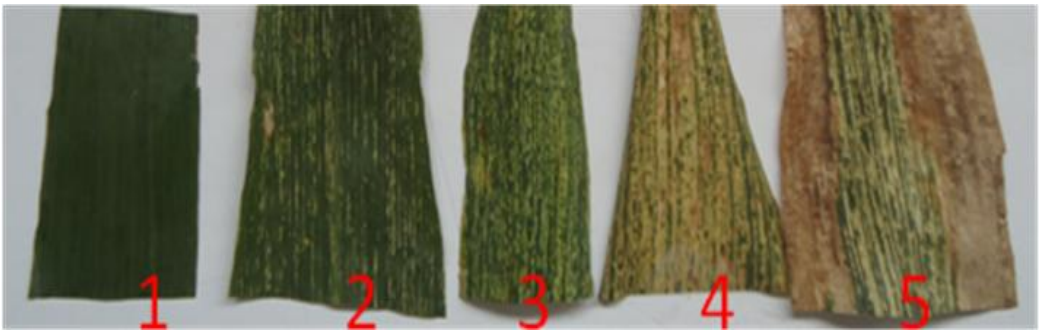
4.2 Materials and Methods

4.2.1 Sample collection

A survey was conducted in the main maize-growing areas of Ghana, namely the forest and transition zones, that is Brong Ahafo, Ashanti, Eastern and parts of the Central regions of Ghana (Figure 1) in the November/December (minor season) of 2010 to collect MSV infected maize leaf samples. Over 79 farms were randomly visited and the specific locations which were captured with global positioning system (GPS) are shown in Table 1 and Figures 4.2, 4.2 & 4.3. The distance between two nearest farms was more than five kilometers. Transects walks were made in each farm and samples were collected from plants showing disease symptoms. Each sample collected was scored for disease severity on the scale of 1-5, where 1 represents no

infection, 2 mild infection, 3 moderate infection, 4 severe infection and 5 very severe infection, (Kyetere *et al.*, 1999). In each farm not more than five samples were collected. Samples were labeled and then stored by pressing on paper and then shipped to the University Of Cape Town, South Africa for further processing.

Plate 4. 1 Maize streak virus disease scoring scale



NB: scale adapted from (Kyetere et al., 1999). 1= no infection, 2= mild; infection, 3= moderate infection, 4= severe infection, 5=very severe infection

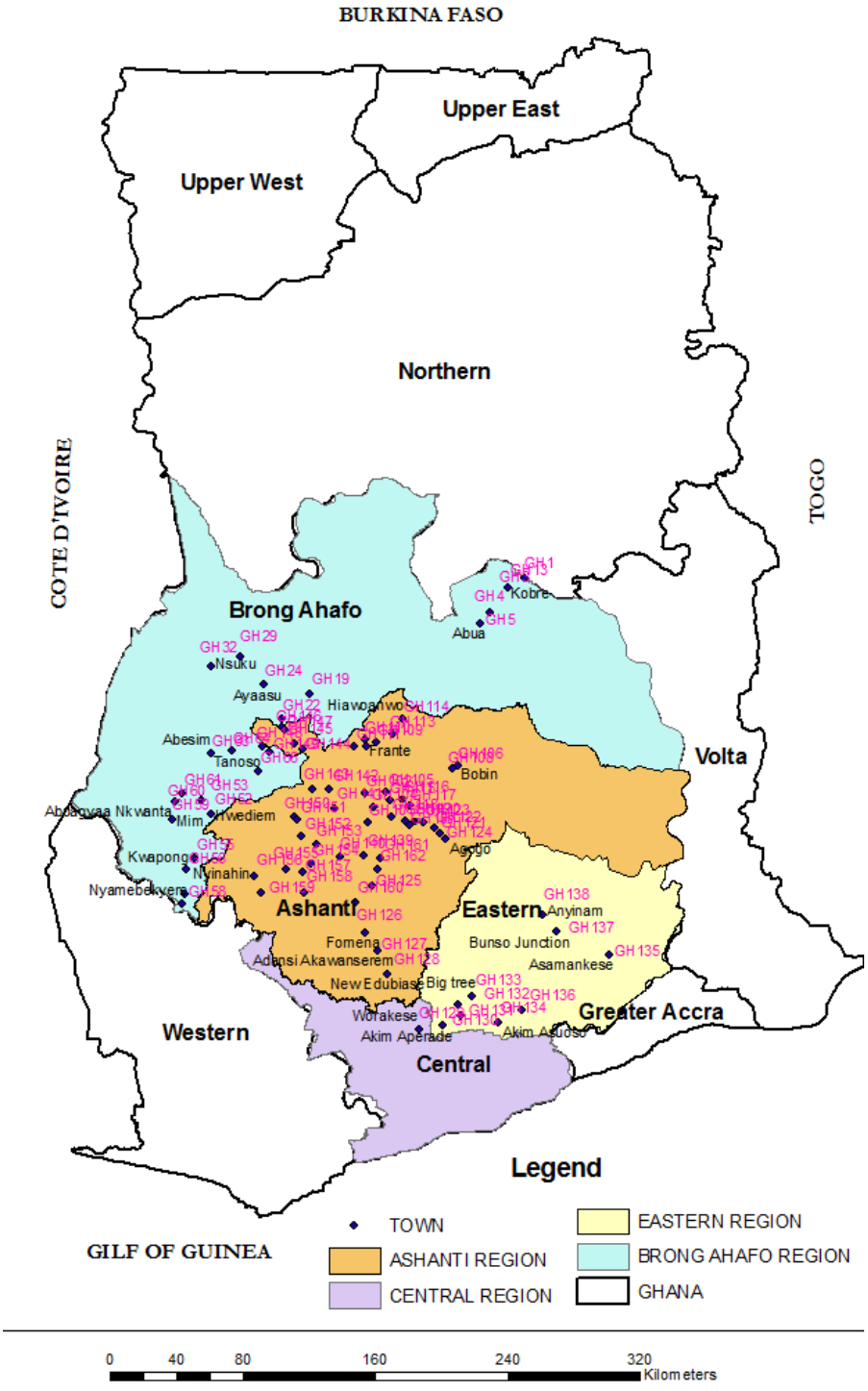


Figure 4. 1 Map of Ghana showing areas where maize leaves infected with maize streak virus disease were sampled.

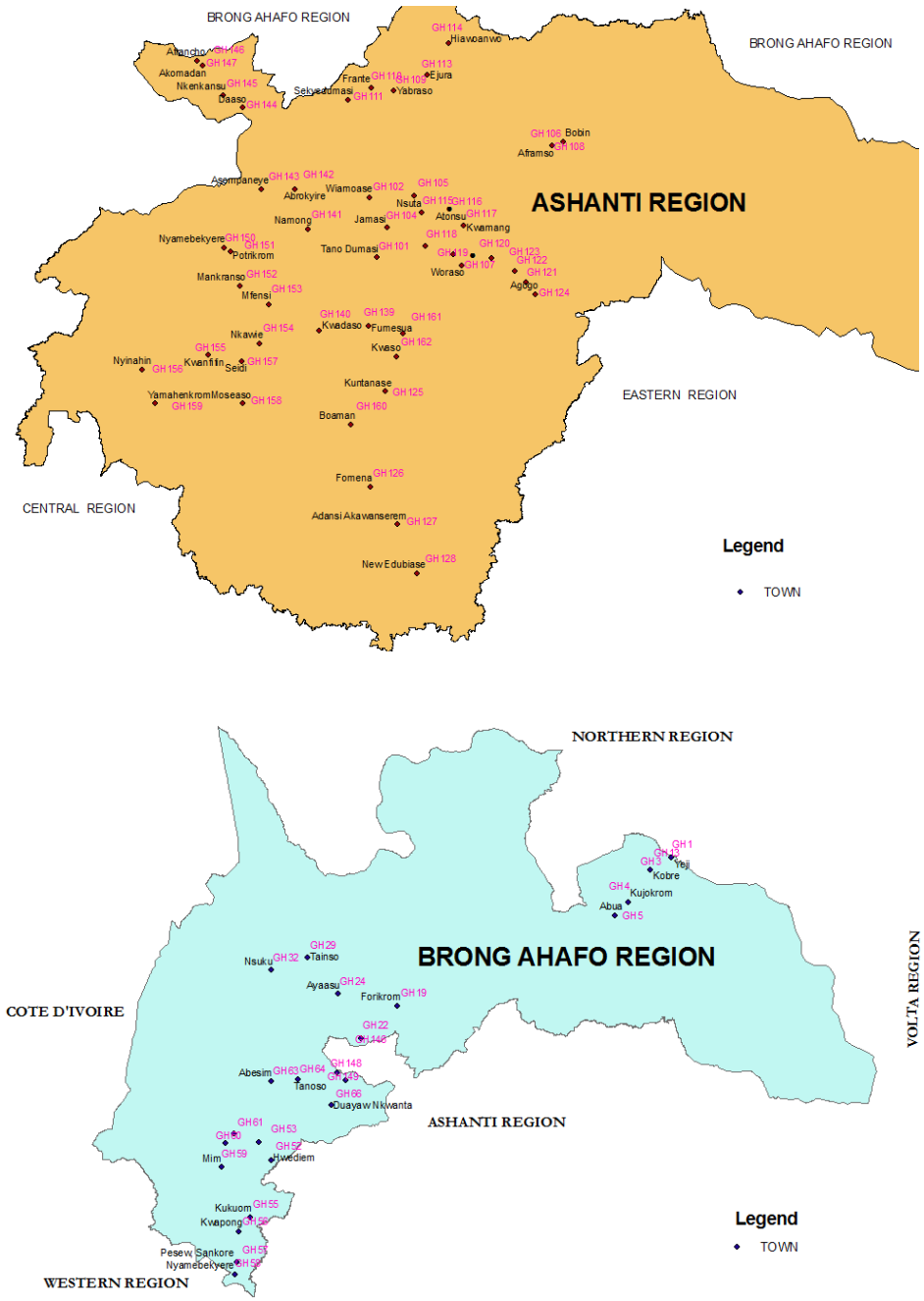


Figure 4. 2 Map showing locations where MSV specimens were collected from the Ashanti and Brong Ahafo regions respectively.

Figure 4. 3 Map showing Eastern and Central regions where MSV specimens were collected respectively

4.2.2 Maize streak virus DNA extraction, cloning and nucleotide sequencing

Fifty four random leaf samples were taken from the sampled lot and total DNA was extracted from the dried plant material using Epoch nucleic acid purification kit (Epoch Life Science, Inc, USA). The full MSV viral genomes were enriched for circular DNA using phi29 DNA polymerase (TempliPhi^{TM}, GE Healthcare, USA), as described by Owor *et al.* (2007) and Shepherd *et al.*, (2008). The concatenated viral genomes were then digested using restriction enzymes *Bam*HII to yield linearized genomes (~2.7 kb). The fragments were resolved on a 0.7% agarose gels and fragments of 2.7 kb were gel purified using the Intron Gel Purification Kit (Intron, Korea) and cloned into the *Bam*HI site of the pGEM3Zf+ vector (Promega Biotech, USA). The resulting clones were sequenced by Macrogen Inc. (Korea) by primer walking. The viral genomes were assembled using DNAMAN (version 7; Lynnon Biosoft).

4.2.3 Maize streak virus sequence analyses

Publicly available genome sequences of MSV from West African countries, namely; Burkina Faso [BF], Benin [BJ], Nigeria [NG], Cameroun [CM], and others from Uganda [UG], Kenya [KE], South Africa [ZA], Zambia [ZM], Zimbabwe [ZW], Chad [TD], Central African Republic [CF] and Mozambique [MZ] were downloaded from NCBI GenBank (www.ncbi.com//maizestreakvirus).

Viral nucleotide sequences obtained from 4.3.2 and those downloaded from Genbank were used to classify these isolates into their respective classification/groups. The nucleotides were aligned using muscle implemented in MEGA and then saved in fasta format. Phylogenetic and molecular evolutionary analyses were conducted using MEGA (version 5) (Tamura *et al.*, 2011) using

neighbor joining tree with 1000 bootstrap support. Degree of similarity among strains was calculated using SDT v 1.0 (Muhire *et al.*, 2013). This was done to show the relationships between the strains found in Ghana and those of the other countries.

Table 4. 1 List of Locations where maize streak virus disease infected leaf samples were collected. Samples from locations with asterisk(*) virus DNA were fully sequenced and characterized.

Code	Location		Symptom severity score	Code	Location		Symptom Severity Score
	Lat (deg. dec. N)	Long (deg. dec. W)			Lat. (deg. dec. N)	Long (deg. dec. W)	
GH101	6.892	1.503	2.5	GH145	7.318	1.905*	3.0
GH102	7.05	1.522	3.0	GH149	7.274	2.039	2.0
GH103	7.05	1.623*	3.0	GH147	7.396	1.959	2.5
GH105	7.054	1.405*	2.5	GH150	6.917	1.903	2.5
GH106	7.196	1.013*	3.5	GH151	6.907	1.886*	2.5
GH107	6.871	1.279	3.0	GH152	6.817	1.862	2.0
GH108	7.185	1.043	2.5	GH156	6.598	2.119	2.5
GH109	7.33	1.459	2.5	GH158	6.508	1.853*	2.5
GH110	7.338	1.517*	2.5	GH160	6.453	1.57	3.0
GH112	7.302	1.512*	3.0	GH13	8.222	0.655*	3.5
GH114	7.456	1.314*	3.0	GH1	8.125	0.385*	3.0
GH115	7.01	1.384	3.0	GH29	7.795	2.20*	2.5
GH116	7.019	1.312	3.0	GH32	7.743	2.353*	3.5
GH117	6.977	1.274	3.0	GH104	6.97	1.475*	3.5
GH120	6.898	1.25*	3.0	GH111	7.306	1.577*	3.0
GH121	6.827	1.111*	2.5	GH113	7.372	1.369*	3.0
GH122	6.856	1.141	3.0	GH118	6.921	1.375*	3.5
GH123	6.891	1.20*	3.5	GH119	6.899	1.301*	3.0
GH124	6.796	1.08	3.0	GH129	5.76	1.225*	2.0
GH125	6.54	1.479	3.0	GH130	5.779	1.098*	2.0
GH126	6.209	1.518	3.5	GH135	6.168	0.189*	3.0
GH127	6.19	1.449*	2.5	GH146	7.409	1.973*	3.0
GH128	6.062	1.397*	2.5	GH148	7.306	2.074*	3.5
GH131	5.836	0.999*	2.0	GH153	6.768	1.786*	3.0
GH132	5.894	1.014*	3.0	GH154	6.635	1.81*	3.5
GH133	5.98	0.94*	3.0	GH155	6.635	1.945*	3.5
GH134	5.795	0.792	3.0	GH157	6.619	1.856*	2.0
GH136	5.865	0.67 *	2.5	GH159	6.508	2.083*	3.0
GH137	6.291	0.476	2.0	GH161	6.692	1.434	2.5
GH138	6.383	0.552	3.0	GH162	6.631	1.451*	3.5
GH139	6.712	1.523*	4.0	GH19	7.596	1.818*	3.5
GH140	6.699	1.653*	4.0	GH22	7.454	1.973	2.5
GH141	6.967	1.683*	3.5	GH24	7.642	2.072*	3.5
GH142	7.072	1.717*	3.0	GH3	8.17	0.746*	3.0
GH143	7.231	1.806*	3.5	GH4	8.034	0.838*	3.5
GH144	7.286	1.855	3.5	GH52	6.932	2.357*	3.5
GH53	7.008	2.409*	3.5	GH58	6.446	2.51*	3.0
GH55	6.69	2.444*	3.0	GH59	6.903	2.567	3.0
GH56	6.63	2.494	2.0	GH5	7.976	0.894*	3.5
GH57	6.499	2.502*	4.0				

4.3 Results

All the Ghanaian samples sequenced and analyzed belonged to the virulent MSV-A group, precisely the MSV-A_1 strain. None belonged to the other MSV-A strains (A_2-A_6; Figs 4.4 and 4.5). All isolates showed close relationship with those found in neighbouring West African countries; namely, Burkina Faso (BF), Benin (BJ), Nigeria (NG) and Comeroun (CM). Similarly, some of the Ghanaian strains showed close relationship with some of the isolates from Uganda [UG], Kenya [KE], Zimbabwe [ZW], Zambia [ZM], South Africa [ZA], Central African Republic [CF] and Mozambique [MZ]. The MSV-A_2 isolate from Chad [TD] did not group with any of the Ghanaian isolates.

The relationship between isolates from Ghana did not show any particular pattern. There were no distinctions between isolates obtained from the transition zones and those from the forest zone. In some cases specimens obtained from the transition zone showed over 98% similarity with those obtained from the forest zone. For instance, isolate number GH114 which was identified in the transition zone showed 98.5% similarity to isolate number GH151 which was isolated from a sample in the forest zone. On the other hand, isolates from the same ecology differed in their position on the phylogenetic tree as shown by isolate GH122 and GH123 which were collected within a distance of 10 km apart but grouped differently as shown in Figure 4.4. Similarly, disease severity scores on sampled leaves did not show any particular pattern or correlation. For instance, specimen GH29 and GH162 were scored 2.5 and 3.5 respectively both shared over 80% virus similarity. On the other hand specimens GH123 and GH144 were both scored 3.5 each and both shared over 98% similarity (Fig. 4.4).

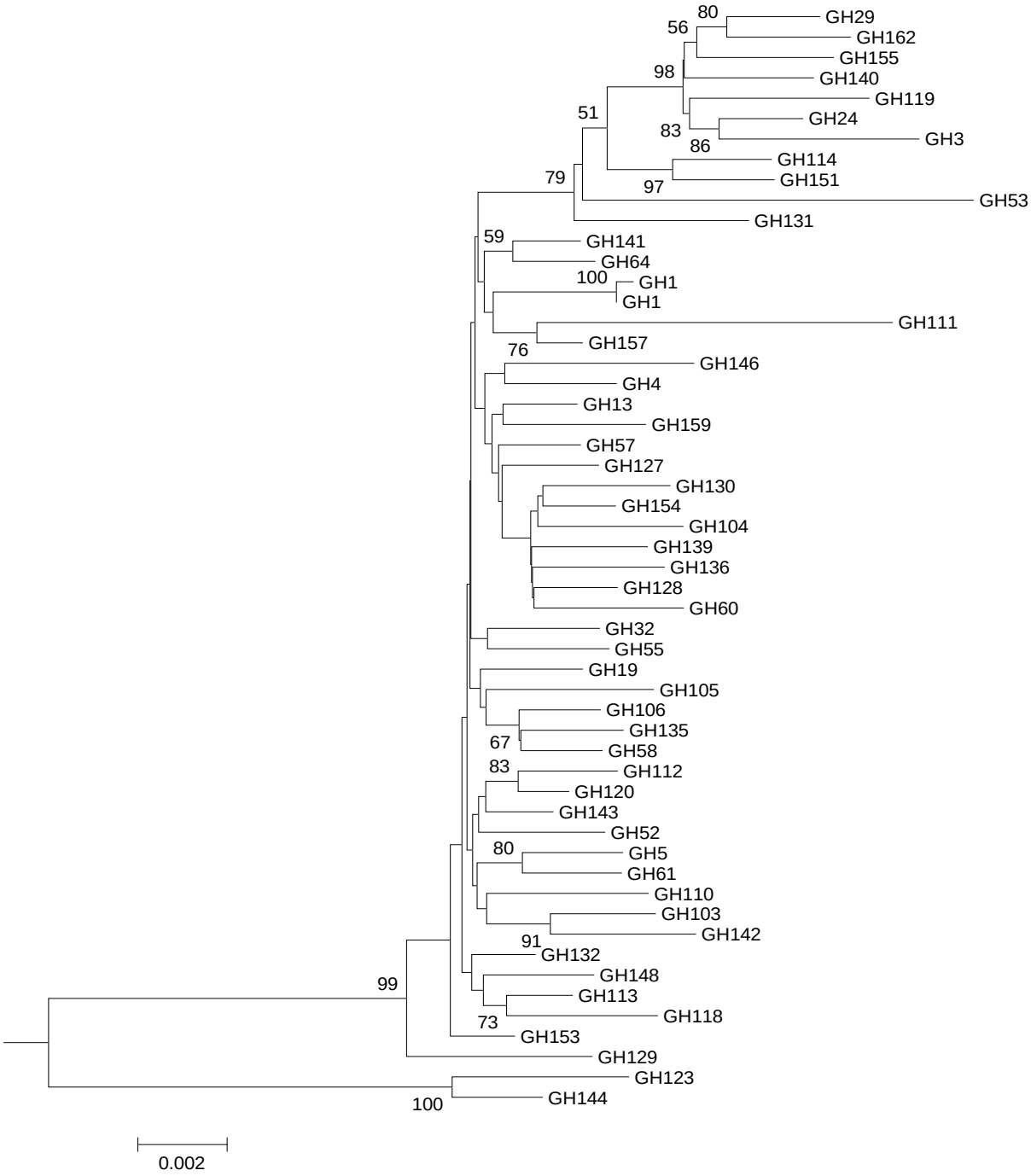


Figure 4. 4 Phylogenetic tree showing the relationship between isolates of maize streak virus isolates collected from the forest and transition zones of Ghana. Numbers associated with nodes represent the percentage of 1000 bootstrap iterations supporting the nodes. Nodes with less than 50% bootstrap support were collapsed.

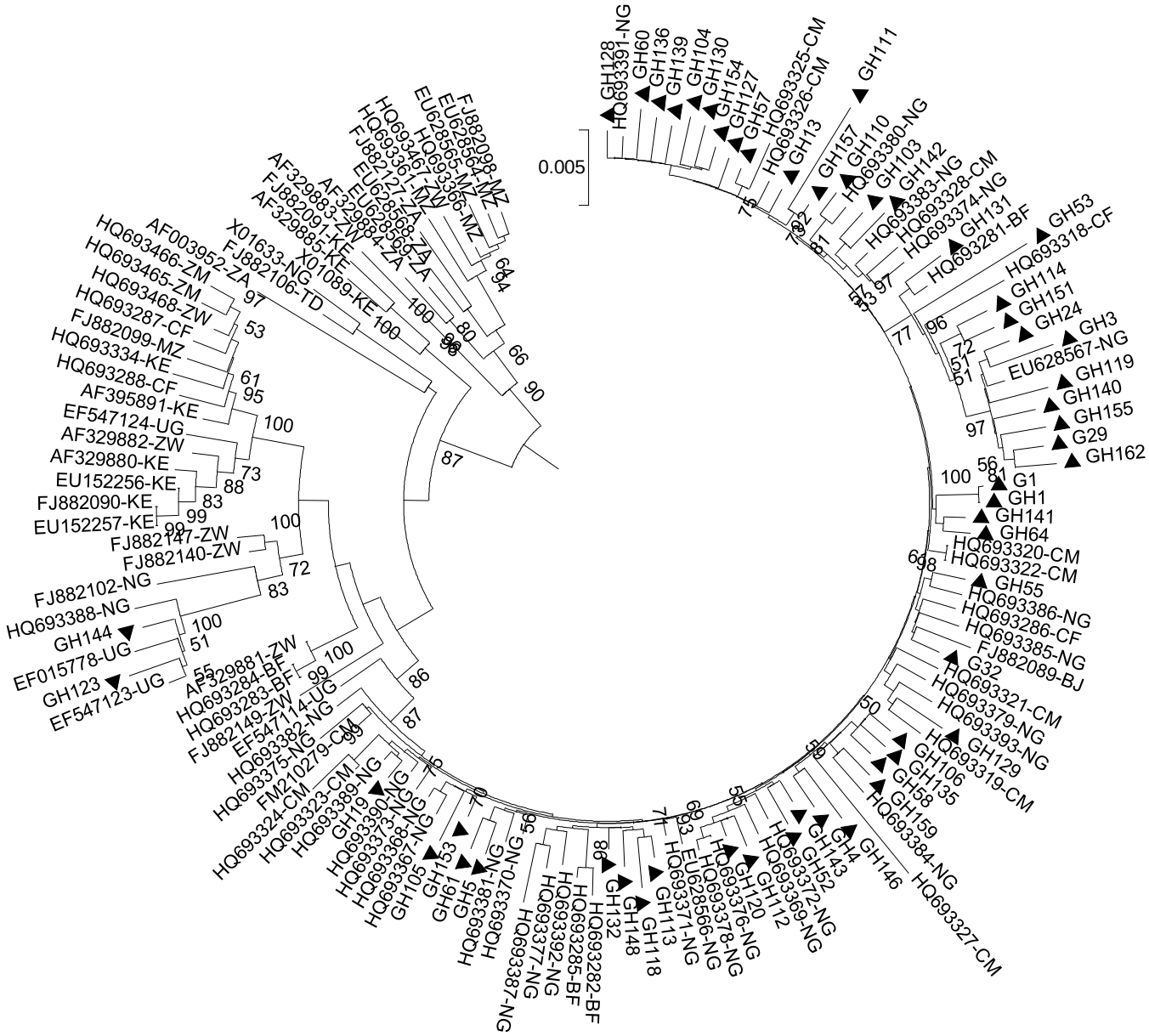


Figure 4.5. Phylogenetic tree showing the relationship between isolates of maize streak virus collected from Ghana (indicated by dark triangles) and other African countries (indicated their initials). Numbers associated with nodes represent the percentage of 1000 bootstrap iterations supporting the nodes. Nodes with less than 50% bootstrap support were collapsed.

4.4 Discussion

Maize streak virus is known to exist in many forms and vary according to agro-ecology, season and vector dynamics (Rybicki, 1994). Research has shown that there are many isolates or strains of MSV (Varsani *et al.*, 2008), with some being more virulent than others (Bock *et al.*, 1974, Clarke *et al.*, 1989, Rybicki *et al.*, 1988). MSV is a monopartite geminivirus containing a single 2.7-2.9 kb circular, single stranded DNA molecule (Lazarowitz, 1987, Mullineaux *et al.*, 1984). This result showed that the isolates sequenced contained 2.6-2.9 kb indicating that they belong to the geminivirus group.

MSV samples isolated from many different hosts have been considered as isolates, or strains of the virus (Dekker *et al.*, 1988, McClean, 1947, Mesfin *et al.*, 1992, Pinner *et al.*, 1988, Plasvic-Blanjac and Maramorosch, 1972, Storey and McClean, 1930). Eleven strains of MSV are currently known, but only the MSV-A strain is known to cause economically significant maize streak disease (Martin *et al.*, 2001, Shephered *et al.*, 2010). Of the 54 samples sequenced none of the isolates belonged to any other group than MSV-A group. This again confirms the widespread distribution of the MSV-A isolates which have been identified in most parts of Africa where the disease is prevalent (Martin *et al.*, 2001).

There are differences in the genetic composition of MSV-A populations in eastern, western and southern Africa (Briddon *et al.*, 1994, Martin *et al.*, 2001, Willment *et al.*, 2001). In West Africa, two MSV-A lineages have been identified, namely MSV-$A_{1,}$ which is the most dominant and then MSV-A_2 (Martin *et al.*, 2001). These isolates have been identified from Nigeria, Benin, Burkina Faso and Cameroun (Martin *et al.*, 2001, www.ncbi.com//maizestreakvirus). In this

study only MSV-A_1 isolate was identified in all areas sampled which also confirms its prevalence in West Africa.

According to Martin *et al.* (2001) currently there are six main lineages of MSV-A circulating in Africa; MSV-A_1, MSV-A_2, MSV-A_3, MSV-A_4 and MSV-A_6 with varying degrees of virulence. A recent study conducted by Monjane *et al.* (2011) suggests various haplotypes or recombinant lineages within the various subtypes/strains of MSV-A across Africa with MSV A_1 alone having 14 haplotypes or recombinant lineages. The phylogenetic tree of the Ghanaians isolates (Fig. 4.4) could be subjected to this test to ascertain if any of these haplotypes can be found. However, MSV-A_1 has a wider geographical range that spans the whole of sub-Saharan Africa (Martin *et al.*, 2001, Monjane *et al.*, 2011, Owor *et al.*, 2007, Varsani *et al.*, 2008, Willment *et al.*, 2001). MSV-A_1 has been reported to be the most virulent, particularly in Sub-saharan Africa. The present study again appears to confirm the virulence of the MSV-A_1 isolate. All the samples sequenced showed disease symptoms with a disease severity score of between 2 and 4. It is worthy to note that some of these samples, including those sequenced, were collected from fields planted with "certified" MSV resistant maize cultivars. The presence of the maize streak virus disease in these genotypes suggest the virulence of the MSV-A_1 which also indicates that the different variants within the MSV-A_1 could influence infection thereby making breeding for MSVD resistance rather difficult.

Although MSV-A is the only strain known to cause severe MSVD (Martin *et al.*, 2001), ten non-maize-adapted MSV strains (MSV-B to MSV-K) have also been identified (Martin *et al.*, 2001, Schnippenkoetter *et al.*, 2001., Varsani *et al.*, 2008, Willment *et al.*, 2002). These are normally found infecting wild grasses, some of these (including MSV-B, MSV-C, MSV-D and MSV-E)

are also known to produce mild infections on MSV-susceptible maize genotypes (Martin *et al.*, 2001, Martin *et al.*, 1999) however, none of these strains were identified in the samples studied. It could be possible that these strains of the virus could be present in Ghana if the study had also focused on some of the alternate host plants the virus is known to infect.

4.5 Conclusion

Over 100 MSVD infected specimens were collected from over 79 locations. All samples collected were scored for disease severity. Fifty four of the samples were randomly selected and total DNA was extracted from the dried plant material using Epoch nucleic acid purification kit. MSV genome sequences from various regions in Africa were downloaded from GenBank for comparative phylogenetic analysis. All 54 Ghanaian specimens analyzed belonged to the virulent MSV-A strain, and subtype MSV A_1, which also aligned closely to those found in West Africa. Sources of the virulent strains of the virus were identified that can be used to screen novel genotypes against the disease in Ghana. This report is the first characterizing the virus at the genomic level in Ghana.

Chapter Five

5.0 Determination of genetic relationships among Ghanaian maize landraces and "exotic" germplasm using SSR markers

5.1 Introduction

Information on relationships between breeding materials is an important requirement for the selection of parents in plant breeding programmes. This is especially important in hybrid maize breeding where the recognition and exploitation of heterotic patterns are vital for maximizing heterosis (Mesmer *et al.*, 1992, Reif *et al.*, 2003b, Xia *et al.*, 2005). Crosses between genetically distant/divergent lines generally produce better hybrids than crosses between closely related parents (Lu and Bernardo, 2001, Melchinger, 1999, Xia *et al.*, 2005). Studies have shown that when there are no established relationships among a collection of germplasm, genetically similar lines can be first identified based on DNA marker estimates of genetic distances among the populations (Melchinger *et al.*, 1999). Exploitation of heterosis can then be carried out by evaluating crosses among representative genotypes in each group (Melchinger *et al.*, 1999).

Molecular markers are used to characterize genotypes at the DNA level (Warburton *et al.*, 2011). With a sufficient number of markers, groups can be identified in a systematic way, and genotypes of unknown genetic origin can predictably be assigned to established or new heterotic groups (Bernardo, 1994, Schrag *et al.*, 2010). Several studies have successfully used microsatellite or simple sequence repeats (SSRs) markers to study maize genetic diversity and

define heterotic groups in temperate maize germplasm (George *et al.*, 2004, Xia *et al.*, 2005, Yuan *et al.*, 2001).

However, studies conducted on tropical inbred lines and populations from which these lines were developed revealed a large amount of diversity that made it difficult to find a clear-cut structure of the inbred lines (Warburton *et al.*, 2002). A similar observation was made in a regional diversity study of representative inbred lines from several Asian countries (George *et al.*, 2004).

SSR markers were the choice for DNA fingerprinting until recently; now SNPs are increasingly being used for diversity analysis, although they may not be as informative as SSRs (Hamblin *et al.*, 2007, Prasanna, 2012). Simple Sequence Repeat (SSR) markers have been used to characterize CIMMYT tropical, sub-tropical and temperate maize breeding materials (Reif *et al.*, 2004) and CIMMYT highland and mid-altitude lines bred in Africa (Legesse *et al.*, 2007), and to compare CIMMYT breeding populations with inbred lines with maize landraces from Mexico (Warburton *et al.*, 2008). Other recent studies with SSR markers include characterization of indigenous landraces of Argentina, China, Ethiopia, Portugal, Switzerland, India, and others (Beyene *et al.*, 2006, Bracco *et al.*, 2009, Eschholz *et al.*, 2006, Patto *et al.*, 2004, Prasanna *et al.*, 2010, Qi-Lun *et al.*, 2008, Sharma *et al.*, 2010). Molecular characterization of maize landraces of India, the Americas and Europe led to significant insights into the genetic diversity and population structure (Prasanna *et al.*, 2010, Sharma *et al.*, 2010, Warburton *et al.*, 2011).

In Ghana, many of the local maize landraces currently grown by farmers have not been adequately characterized at the genetic level, nor assigned to any heterotic group(s). The successful utilization of these landraces in a breeding programme would require the

understanding of the relationships among them in order to select useful lines/populations for breeding. This can be efficiently done at the molecular level, and it is anticipated that characterization could point to sources of new alleles that can be exploited to develop disease and other stress resistant, high yielding hybrid varieties that would be acceptable to farmers.

The objective of this study was to characterize a set of Ghanaian maize landraces and inbred lines obtained from CIMMYT and IITA, to determine their genetic relatedness.

5.2 Materials and Methods

5.2.1 Germplasm

The germplasm used for this study was made of 4 CIMMYT inbred lines, 8 IITA inbred lines, 4 CSIR-CRI released varieties (checks), 10 F_1 topcross and single cross hybrids (checks). The landraces are varieties collected from farmers which according to them are their own varieties they have been growing for a long while (over 10 years). They were collected from 6 different regions namely; Ashanti, Brong Ahafo, Northern, Western Eastern and Volta regions of Ghana (Table 5.1).

5.2.2 DNA Extraction

Genomic DNA was extracted using a modified Cetyl tri-methylammonium bromide (CTAB) (Warburton *et al.*, 2002) method. Leaves of three week old maize seedlings were harvested and freeze-dried in liquid nitrogen. At least 0.01 g lyophilised leaf tissues were ground using steel balls into fine powder by shaking for 4 minutes with the GenoGrinder-2000 at a speed of 500 strokes per minute. Tubes were spun down for about 2-3 minutes to bring the ground tissue to the bottom of the

tube. Six hundred microliters (600 ul) of freshly prepared modified CTAB extraction buffer was added to each sample followed by additional 2 minutes of grinding. The samples were incubated for 30 minutes at 65^{o}C with continuous gentle rocking with tubes inverted every 10 minutes to ensure proper homogenization with buffer. The tubes were removed from the waterbath and allowed to cool for about 10 minutes in the fumehood before they were centrifuged at 3500 revolutions per minute (rpm) at 15^{o}C for 10 minutes after which 500 ul of the supernatant was transferred into freshly labeled tubes. Four hundred micro litres (400 ul) chloroform: isoamylalcohol (24:1) was added into the new tubes by pipetting along the side of the tubes. The contents were mixed gently with continuous rocking for up to 30 minutes at room temperature after which it was centrifuged at 3500 rpm for 10 minutes. A repeat of the chloroform: isoamylalcohol extraction was done in freshly labeled tubes to obtain a pure DNA. About 400 ul of the aqueous layer was transferred into freshly labeled tubes. Three hundred microliters (300 ul) of 100% ice cold Isopropanol was then added to each tube with gentle mixing for 5 minutes to precipitate the nucleic acid. The tubes were kept at -20˚C overnight after which they were placed on the bench for 5-10 min with gentle inverts (about 50 times). Tubes were then centrifuged at 3500 rpm for 30 minutes to pellets the DNA at the bottom of the tubes. The DNA was then washed by dissolving in 400 ul 70% ethanol for 15 minutes after which the ethanol was discarded and the pellet allowed to dry for about one hour and then resuspended in 150 ul of 10mM Tris-HCl pH 8.3 and incubated for about 45 min at 45˚C in a waterbath with gentle tapping every 10 min. Three microliters (3 ul) of RNAse was added to the DNA and finally centrifuged at 3500 rpm for 1-2 minutes and the DNA stored at 4^{o}C for PCR. Quality (purity) and quantity of extracted genomic DNA were assessed on 0.8% agarose using gel electrophoresis at 120 V. Further DNA quality or purity and quantity test was determined by using the NanoDrop spectrophotometer (ND8000, Thermo Scientific, Wilmington DE) according to manufacturer's

instructions. This was done to ensure that the optical density values were within the accepted range of 1.8 – 2.0 (which indicated pure DNA) and to determine the concentration of DNA.

Table 5. 1 Genotypes used in the study

18	Genotype	PopID	Ind. No.	Genotype	PopID
1	CML444 x LA296	1	23	LA409	8
2	CML444 x LA30	1	24	LA435	8
3	CML-202 x LA 30	1	25	LA540	8
4	TZE1-17 x LA30	2	26	LA571	8
5	TZE1-17 x LA296	2	27	LA276	8
6	TZE1-23 X LA30	3	28	LA080	8
7	TZE1-23 X LA296	3	29	LA467	8
8	TZE1-17 X TZE1-23	4	30	LA400	8
9	TZE1-17 X CML-202	5	31	LA493	8
10	TZE1-17 X CML-444	5	32	LA580	8
11	CML-202	6	33	LA492	8
12	CML-444	6	34	LA462	8
13	CML442	6	35	LA504	8
14	CML-312	6	36	LA424	8
15	TZE1-17	7	37	LA097	8
16	TZE1-23	7	38	LA518	8
17	TZE1-122	7	39	LA076	8
18	TZE1-121	7	40	LA537	8
19	VLO-511298	7	41	LA-30	8
20	TZE1-124	7	42	LA463	8
21	TZE1-151	7	43	LA558	8
22	TZE1-16	7	44	LA246	8
49	ETUBI	9	45	LA099	8
50	OBATANPA	9	46	LA457	8
51	MAMABA	9	47	LA296	8
52	ENIBI	9	48	LA030	8

NB: *Genotypes with LA initial are local landraces collected from various locations in Ghana. Those with CML initials are inbred lines obtained from CIMMYT. Genotypes with initials TZEI and VLO are inbred lines obtained from IITA whilst Etubi, Einbi, Obatanpa and Mamaba are CSIR-CRI released genotype which were used as checks or control. In addition, are 10 F1 topcross hybrids and single cross hybrids consisting of CIMMYT, IITA and local landraces also used as checks.*

Table 5. 2 List of SSR primers

Marker	Chromosome	Motif	Forward_Primer	Reverse_Primer
nc130	5	AGC	GCACATGAAGATCCTGCTGA	TGTGGATGACGGTGATGC
nc133	2	GTGTC	AATCAAACACACACCTTGCG	GCAAGGGAATAAGGTGACGA
phi029	3	AGCG	TTGTCTTTCTTCCTCCACAAGCAGCGA	ATTTCCAGTTGCCACCGACGAAGAACTT
phi031	6	GTAC	GCAACAGGTTACATGAGCTGACGA	CCAGCGTGCTGTTCCAGTAGTT
phi041	10	AGCC	TTGGCTCCCAGCGCCGCAAA	GATCCAGAGCGATTTGACGGCA
phi046	3	ACGC	ATCTCGCGAACGTGTGCAGATTCT	TCGATCTTTCCCGGAACTCTGAC
phi056	1	CCG	ACTTGCTTGCCTGCCGTTAC	CGCACACCACTTCCCAGAA
phi062	10	ACG	CCAACCCGCTAGGCTACTTCAA	ATGCCATGCGTTCGCTCTGTATC
phi065	9	CACTT	AGGGACAAATACGTGGAGACACAG	CGATCTGCACAAAGTGGAGTAGTC
phi072	4	AAAC	ACCGTGCATGATTAATTTCTCCAGCCT	GACAGCGCGCAAATGGATTGAACT
phi075	6	CT	GGAGGAGctCACCGGCGCATAA	AAAGGTTACTGGACAAATATGC
phi076	4	GAGCGG	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC
phi079	4	CATCT	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA
phi084	10	GAA	AGAAGGAATCCGATCCATCCAAGC	CACCCGTACTTGAGGAAAACCC
phi102228	3	AAGC	ATTCCGACGCAATCAACA	TTCATCTCCTCCAGGAGCCTT
phi112	7	AG	TGCCCTGCAGGTTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC
phi114	7	GCCT	CCGAGACCGTCAAGACCATCAA	AGCTCCAAACGATTCTGAACTCGC
phi123	6	AAAG	GGAGACGAGGTGCTACTTCTTCAA	TGTGGCTGAGGCTAGGAATCTC
phi227562	1	ACC	TGATAAAGCTCAGCCACAAGG	ATCTCGGCTACGGCCAGA
phi299852	6	AGC	GATGTGGGTGCTACGAGCC	AGATCTCGGAGCTCGGCTA
phi308707	6	AGC	GCAACAAGATCCAGCCGAT	GTCGCCCTCATATGACCTTC
phi331888	5	AAG	TTGCGCAAGTTTGTAGCTG	ACTGAACCGCATGCCAAC
phi374118	3	ACC	TACCCGGACATGGTTGAGC	TGAAGGGTGTCCTTCCGAT
phi96100	2	ACCT	AGGAGGACCCCAACTCCTG	TTGCACGAGCCATCGTAT
umc1161	8	GCTGGG	GGTACCGCTACTGCTTGTTACTGC	GCTCGCTGTTGGTAGCAAGTTTTA
umc1304	8	TCGA	CATGCAGCTCTCCAAATTAAATCC	GCCAACTAGAACTACTGCTGCTCC
umc1367	10	CGA	TGGACGATCTGCTTCTTCAGG	GAAGGCTTCTTCCTCGAGTAGGTC
umc1545	7	AAGA	GAAAACTGCATCAACAACAAGCTG	ATTGGTTGGTTCTTGCTTCCATTA
umc1917	1	CTG	ACTTCCACTTCACCAGCCTTTTC	GGAAAGAAGAGCCGCTTGGT
umc2250	2	ACG	ACAGGTCACAGATGTTCATCCAGG	CTCGACTGGATCGCCTCCTC

5.2.3 PCR and Genotyping

Thirty SSR primers randomly distributed within the 10 chromosomes of the maize genome (Table 5.2) were used based on repeat unit and bin location to provide a uniform coverage. These include those known to be linked to MSV resistance. These selected primers have been optimized for diversity studies (Warburton *et al.*, 2011, www.maizegdb.org). Detailed procedure for PCR can be found in appendix 5.1.

Capillary electrophoresis was carried out following PCR amplification with fluorescently labelled (Ned (Y), Pet (R), 6-FAM (B) and Vic (G)) primers, using an automatic sequencer ABI-3730 (Applied Biosystems, Foster City, CA) and LIZ500 as internal size standard to separate and size the fragments generated via PCR according to standard protocols (http://www.cimmyt.org/english/docs/manual/protocols/abc_amgl.pdf, page 48).

The data were captured using the Genescan^{R} collection software (Applied biosystems, Forster City, CA) and the fragments analyzed using the Genemapper software ver4.1 (Applied Biosystems). A total of 1566 datapoints were collected out of the expected 1590 data points giving an overall success rate of 98.5%. The data were compiled into a spreadsheet as a standard Genemapper output file. The sizes for each detected allele were indicated in base pairs. Parameters considered for data quality were indicated in the peak height with the lower peaks being verified manually and discarded as appropriate.

5.2.4 Data analyses

The genetic data were analyzed using the PowerMarker software package (Liu and Muse, 2005) for the statistical analyses and the phylogenetic tree (UPGMA dendrogram) was viewed using *MEGA*, version 5.05 (Tamura *et al.*, 2011). Basic summary statistics including Polymorphic information content (PIC) (Botstein *et al* 1980), heterozygosity (He), number of alleles, gene diversity, allelic and genetic frequencies were calculated. F statistics were calculated according to Berg and Hamrick (1997). A distance based phylogenetic tree using shared allele distance (Chakraborty and Jin, 1993) and 1000 bootstrap support was drawn to show the relationship between the different genotypes. Analysis of molecular variance (AMOVA) (Michalakis and Excoffier, 1996) was also calculated. Heterozygosity is defined as the proportion of heterozygous individuals in the population. Gene diversity often referred to as expected heterozygosity, is defined as the probability that two randomly chosen alleles from the population are different. Polymorphism information content (PIC) is a diversity measure showing how genotypes are closely related (Botstein *et al.*, 1980)(Botstein *et al.* 1980).

Structure, a model-based (Bayesian) clustering method for using multilocus genotype data to infer population structure and assign individuals to populations (Pritchard *et al.*, 2000), was used to analyze population structure which was grouped into 9 populations based on their origin (Table 5.1). This was done to verify if all genotypes fall into the predetermined groups defined by the origin of the 9 populations namely: CIMMYT lines only; IITA lines only; Landraces only; topcross hybrids of landraces and CIMMYT inbred lines, F_1 hybrid of IITA inbred lines, topcross hybrids of IITA and landraces, F_1 hybrids of CIMMYT and IITA inbred lines; and 4

local checks (namely; Etubi, Enibi, Obatanpa and Mamaba which are CSIR-CRI released materials).

The analysis was run with the admixture model to assign each individual to a population with similar allele frequencies at each locus. The method of Evanno *et al.* (2005) was used to calculate the most suitable number of clusters or subpopulations (K). Probabilities for *K* were calculated from 1 to 10, using 100,000 replications after a burn-in period of 50,000 iterations, and the procedure was repeated 5 times for each *K* value. Value of ΔK was calculated and chosen based on the rate of change in the logarithm of the probability of data between successive values of *K;* higher values of ΔK represent the appropriate number of groups defining the level of structure. K = 4 was chosen as the most likely, and populations were assigned to each one of the 4 groups for which they had an ancestry proportion greater than 51.0% (Reif *et al.*, 2006); if an individual did not show an ancestry proportion higher than this value, it was assigned to the mixed group.

5.3 Results

Results from the dendrogram, which was drawn based on UPGMA using shared allele distance, revealed no clear clustering. The germplasm fell into 1 main group with two outliers (fig. 5.1). However, if a vertical line is placed on the genetic similarity level 0.22, it can be seen that the main cluster also consist of 8 smaller groups with genotypes in each group more related and 9 individuals, including the two outliers and then other 7 outliers more closely related to one of the eight groups (fig. 5.1). There are a few others when crosses clustered with parents (cluster 1, 2, 4 and 5). Cluster 3 consisted of only landraces (fig. 5.1).

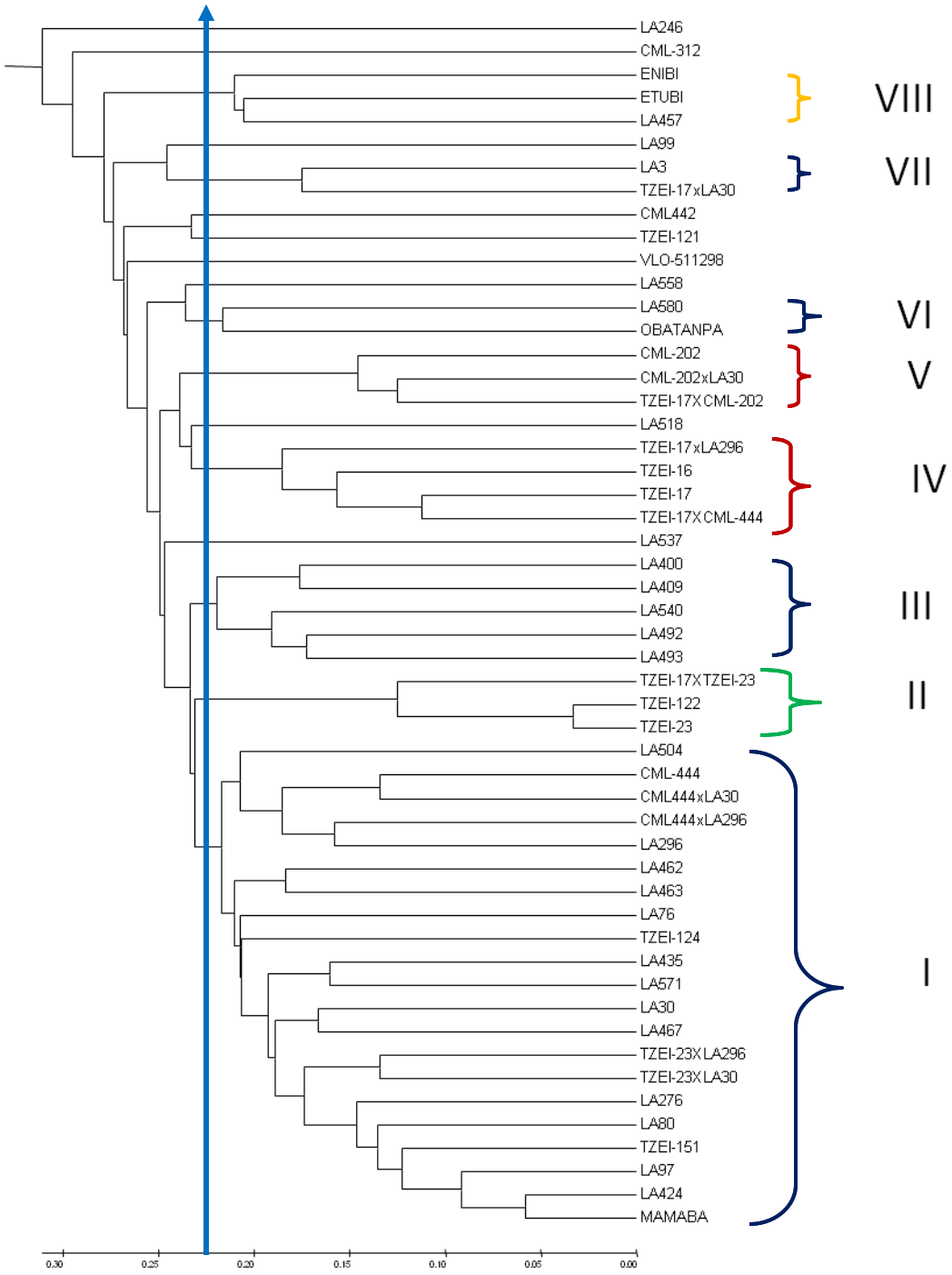


Figure 5. 1 Dendrogram showing relationships between 52 individual maize genotypes including four controls as created using the shared allele distances between all pairs of individuals in Powermarker

Summary statistics can be found in Table 5.2. Mean PIC was found to be 0.492 and it ranged from a 0.158 for marker phi112 to 0.795 for marker phi225562. Mean Heterozygozity (He) was found to be 0.350 and it ranged from 0.039 for marker phi331888 to 0.843 for marker phi031. Gene diversity ranged from 0.058 for marker umc1367 to 0.819 for marker phi2275562 with a mean of 0.533. Total number of alleles was found to be 160 with a mean of 5.333 per allele. Markers phi23 and phi062 produced the least number of alleles (2 each) whilst marker phi056 produced the highest (9 alleles). The highest mean allele frequency was produced by marker umc1367 (0.971) and the least by marker phi96100 (0.260) with a mean of 0.597.

Table 5. 3 Summary statistics of the markers used in the study including the frequency of the most common allele, number of genotypes, gene diversity, number of alleles per marker, heterozygosity, Polymorphic Information Content (PIC) and Fstat

Marker	Major.Allele.Frquency	No. of Genotype	AlleleNo	GeneDiversity	Heterozygosity	PIC	Theta= F_{IS}	F= F_{IT}	f= F_{ST}
nc133	0.578	6.000	4.000	0.592	0.333	0.540	0.037	0.450	0.429
nc130	0.692	5.000	5.000	0.467	0.577	0.415	0.101	-0.191	-0.325
phi029	0.548	10.000	6.000	0.598	0.500	0.536	-0.070	0.157	0.212
phi031	0.294	15.000	8.000	0.781	0.843	0.747	0.035	-0.060	-0.098
phi041	0.774	7.000	6.000	0.384	0.095	0.363	0.177	0.769	0.719
phi046	0.615	8.000	8.000	0.531	0.327	0.462	0.016	0.418	0.409
phi056	0.480	13.000	9.000	0.709	0.686	0.680	0.064	0.059	-0.005
phi062	0.824	3.000	2.000	0.291	0.196	0.248	0.081	0.350	0.293
phi072	0.394	13.000	6.000	0.683	0.538	0.628	0.042	0.229	0.196
phi075	0.441	11.000	7.000	0.710	0.431	0.669	0.157	0.427	0.320
phi076	0.500	11.000	5.000	0.670	0.423	0.626	-0.090	0.361	0.414
phi079	0.330	13.000	6.000	0.759	0.360	0.721	-0.038	0.528	0.546
phi084	0.769	6.000	4.000	0.386	0.365	0.359	0.027	0.070	0.044
phi102228	0.692	7.000	5.000	0.478	0.288	0.437	0.023	0.408	0.395
phi112	0.913	6.000	4.000	0.163	0.058	0.158	-0.064	0.645	0.667
phi114	0.450	9.000	4.000	0.669	0.380	0.611	-0.015	0.438	0.446
phi123	0.725	3.000	2.000	0.398	0.275	0.319	-0.017	0.317	0.328
phi2275562	0.275	19.000	8.000	0.819	0.529	0.795	0.037	0.368	0.344
phi308707	0.375	10.000	5.000	0.691	0.462	0.631	-0.009	0.339	0.345
phi299852	0.442	17.000	8.000	0.717	0.365	0.680	0.024	0.501	0.489
phi331888	0.731	5.000	4.000	0.426	0.038	0.383	0.010	0.912	0.911
phi374118	0.510	11.000	6.000	0.626	0.442	0.565	0.063	0.315	0.269
phi96100	0.260	20.000	7.000	0.806	0.519	0.778	0.016	0.367	0.357
umc 2047	0.804	4.000	3.000	0.322	0.275	0.281	-0.048	0.146	0.185
umc1161	0.608	12.000	8.000	0.583	0.118	0.547	-0.061	0.798	0.810
umc 1304	0.755	5.000	3.000	0.383	0.235	0.329	0.196	0.426	0.286
umc 1367	0.971	3.000	3.000	0.056	0.058	0.056	-0.100	-0.042	0.053
umc1545	0.598	10.000	6.000	0.585	0.294	0.542	0.126	0.523	0.454
umc 1917	0.650	8.000	4.000	0.534	0.360	0.496	0.006	0.336	0.332
umc 2250	0.902	4.000	4.000	0.180	0.137	0.170	-0.081	0.230	0.287
Mean	0.597	9.133	5.333	0.533	0.350	0.492	0.027	0.355	0.337

NB: F_{IS} = amount of inbreeding-like effects or correlation of alleles within individuals within subpopulations (level 2)
F_{IT} = amount of correlation of alleles (identity by descent, or inbreeding-like effects)
within the entire population (all individuals from all populations combined)
F_{ST} = amount of correlation of alleles (identity by descent, or inbreeding-like effects) among (between) subpopulations (level 2)

When a breakdown of alleles contributed by each individual based on the 30 markers was done it was realized that on the whole, landraces contained more alleles than other populations (Table 5.4).

Table 5. 4 Contribution of number of alleles and gene diversity by each individual indicated by the 30 primers.

Genotype	Mean Allelic richness/genotype	Mean Gene diversity/genotype	Genotype	Mean Allelic richness/genotype	Mean Gene diversity/genotype
CML444 x LA296	1.4333	0.2167	LA571	1.2333	0.2167
CML444 x LA30	1.3333	0.2667	LA080	1.2667	0.1333
CML-202 x LA30	1.5667	0.2833	LA467	1.5000	0.2500
TZE1-17 x LA30	1.5333	0.2667	LA400	1.3667	0.2833
TZE1-17 x LA296	1.4667	0.2333	LA493	1.3000	0.2000
TZE1-23 X LA30	1.4667	0.2333	LA580	1.3667	0.1833
TZE1-23 X LA296	1.3333	0.2667	LA492	1.5000	0.2500
TZE1-17 X TZE1-	1.1000	0.0500	LA462	1.2333	0.1167
TZE1-17 X CML-	1.4333	0.2167	LA504	1.2667	0.1333
TZE1-17 X CML-	1.4000	0.2000	LA424	1.4000	0.2000
CML-202	1.1333	0.0667	LA097	1.5000	0.2500
CML-444	1.1000	0.0500	LA518	1.0667	0.0833
CML442	1.2000	0.1500	LA076	1.2667	0.2333
CML-312	1.0333	0.1667	LA537	1.2667	0.1333
TZE1-17	1.1000	0.1000	LA003	1.2667	0.1333
TZE1-23	1.1000	0.0500	LA463	1.3000	0.1500
TZE1-122	1.1000	0.0500	LA558	1.1000	0.2500
TZE1-121	1.3333	0.1667	LA246	1.3667	0.2333
VLO-511298	1.0333	0.0667	LA099	1.3667	0.2333
TZE1-124	1.4333	0.2167	LA457	1.3667	0.2333
TZE1-151	1.5667	0.2833	LA296	1.5000	0.2500
TZE1-16	1.2333	0.1167	LA030	1.3000	0.1500
LA409	1.3333	0.2167	ETUBI	1.3333	0.2667
LA435	1.2333	0.1167	OBATANPA	1.5333	0.2667
LA540	1.2667	0.1333	MAMABA	1.4667	0.2333
LA276	1.4667	0.2333	ENIBI	1.5000	0.2500

Analysis of molecular variance (AMOVA) showed more variation within the "populations", 87.63% than among them; 12.37% (table 5.5).

Table 5. 5 Analysis of molecular variance of the 9 maize populations analyzed with 30 SSR markers

Sources of Variation	Degree of freedom	Sum of squares	Percentage (%) Variation
Among Populations	8	202.1060	12.37
Within Population	95	1432.2378	87.63
Total	103	1634.3439	100.00

Mean F_{IS} was found to be 0.027 and F_{IT} was 0.355 and F_{ST} was 0.337. Highest population pairwise Fstat of 0.5452 was between population 8 and 5 (Table 5.5)

Table 5. 6 Population pairwise Fstat as revealed by the 30 SSR marker for the 9 populations used in the study

	1	2	3	4	5	6	7	8	9
1									
2	0.0756								
3	NaN	NaN							
4	NaN	NaN	NaN						
5	0.0516	-0.0404	NaN	NaN					
6	-0.1024	0.1178	NaN	NaN	0.0669				
7	0.1056	0.1552	NaN	NaN	0.2065	0.1072			
8	0.2538	0.4594	NaN	NaN	0.5452	0.2419	0.0962		
9	0.0689	0.1359	NaN	NaN	0.1939	0.1039	0.1133	0.1905	

NaN= Not available

Analyses done with Structure clustered the germplasm into 4 main groups or sub-populations, and the mixed individuals that did not have percentage lineage greater than 51% from any single cluster or sub-population (Fig.5.2). The results from Structure grouped some of the landraces, CSIR-CRI released materials, CIMMYT and the IITA inbred lines into the same sub-

population/cluster (Table 5.7). Most of the genotypes were in the blue cluster, consisting of 20 genotypes sharing similar ancestry the majority of which correspond to sub group 1 of the UPGMA dendrogram (Fig, 5.1) with the exception of LA30, LA435, LA504 and TZEI124. This group is made of landraces, IITA and CIMMYT inbred lines as well as crosses involving CIMMYT lines and landraces and 1 check (Mamaba). The red group consisting of 11 genotypes contributed from the various populations and the majority of the individuals belong to groups IV and V of the dendrogram and few individuals from the other clusters. They are mainly inbred lines and crosses involving CML202 and a few of the landraces. The green group consists of 3 genotypes namely TZEI23, TZEI122 and their F1 hybrid; TZEI23 X TZEI17 which corresponds exactly to group II in the UPGMA dendrogram. The yellow group consists of 14 genotypes comprising individuals from all populations including one CIMMYT inbred line; CML312. Again most genotypes in this sub population belong to group VIII and all the outliers of the small groups of the UPGMA dendrograme with exception of CML442 and TZEI121. Four genotypes fell into the mixed group, namely TZEI121, LA540, LA580 and LA504. None of the known testers belonging to the different heterotic groups clustered together. CML442 which belongs heterotic group A (CIMMYT classification) grouped differently from CML202 which has been assigned to the group B. Similarly, TZEI17 which belongs to heterotic group A did not group together with TZEI23 which belongs heterotic group B.

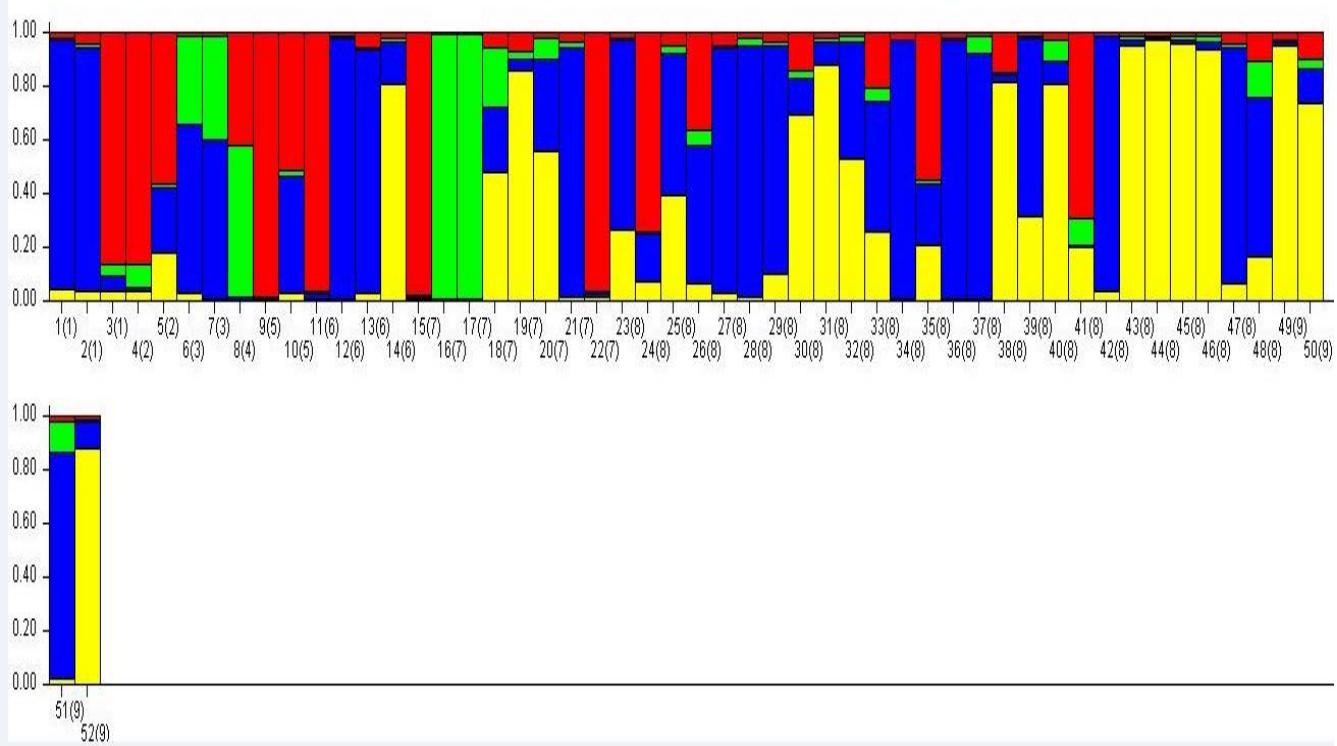


Figure 5. 2 Plot of multiple lines of individuals within each sub-population from the 4 sub-populations based on results obtained from analysis done with Structure (k=4). Each individual was considered mixed if it did not have percentage lineage greater than 51% to fall into a unique cluster/group

Table 5. 7 Number of individuals within each sub-population from the 4 colour groups based on results obtained from analysis done with Structure (k=4).

Sub-populations	Individuals belonging to cluster	Total number of individuals in a group/cluster
Sub-population 1	CML444 x LA296, CML444 x LA30, CML444, CML442, TZE1-151, LA409, LA276, LA080, LA467, LA462, LA424, LA3, LA097, LA76, LA463, LA296, LA571, Mamaba, TZEI23XLA30, LA23X296,	20
Sub-population 2	CML202 x LA30, TZE1-17 x LA30, TZE1-17 x LA296, TZE1-17 X CML202, CML202, TZEI17, TZEI16, LA435, LA30, LA504, TZEI17XCML444	11
Sub-Population 3	TZEI23, TZEI122, TZEI17XTZEI23	3
Sub-population 4	CML312, VL0511298, LA400, LA493, LA518, LA537, LA558, LA246, LA99, LA457, Etubi, Obatanpa, Enibi, TZEI124,	14
Undefined population	TZEI121, LA540, LA580, LA492, ,	4

NB: Individuals were assigned to a sub population (colour) based on ancestry proportion greater than 51 percent. Individuals were considered mixed if ancestry proportion of any colour was less than 50 Percent

5.4 Discussion

The 1.5% missing data obtained for this study compares favourably with those obtained by Reif *et al.* (2006) when a similar study was conducted. The number of alleles obtained also compares well with previously reported studies of maize using SSRs (Dubreuil *et al.*, 2006, Qi-Lun *et al.*, 2008). Reif *et al.* (2006) reported a mean gene diversity of 0.61 when they examined 25 accessions of 24 races of maize from Mexico, and 0.53, when they analyzed 150 European maize cultivars (Reif *et al.,* 2005a) similar to the 0.533 obtained in this study. The AMOVA showed a higher percentage variance within populations than among populations which is in harmony with the broad genetic base of the materials used, and compares with that obtained by Reif *et al.* (2003b), Warburton *et al.* (2010). It was not surprising that a smaller between populations variation was observed. A more detailed analysis of the population subdivision with test statistics of the AMOVA was not possible, because it would require knowledge of the gametic phase linkage for linked loci (Michalakis and Excoffier, 1996), which cannot be determined from SSR analyses of heterozygous individuals (Reif *et al.*, 2003b).

The F statistic values indicated that each population was not well differentiated. In fact, overall F_{ST} (correlation of alleles among populations) is only slightly lower than F_{IT} (correlation of alleles within the entire study, all individuals from all populations combined), and thus each population probably contains a great deal of mixed germplasm. However, the partitioning within did not agree with the original populations as proposed as can be seen in the Structure analysis. This is not uncommon of maize populations, which are outcrossing by nature, and farmers, seed dealers and breeders often exchange seeds of their best populations. Mixing thus occurs via pollen and gene flow. It is however, possible that some of the inbred lines from CIMMYT and

IITA might have influenced the variation within the subpopulations resulting in lower value for mean F_{ST} compared with mean F_{IT} (Table 5.3 & 5.5).

Cluster analysis using shared allele distances and the UPGMA algorithm did not reveal any clear clustering according to the source of the populations, although eight clusters and nine individuals can be seen when a perpendicular line is drawn from 0.22 genetic similarity (Fig. 5.1). This can be explained by the sources of germplasm used in the study. The landraces were collected from across Ghana (Table 6.2). The controls used, that is Obatanpa, Mamaba, Etubi and Enibi were developed from CIMMYT lines or populations. Similarly, breeding in Ghana has also always used IITA germplasm that have also led to the release of some varieties. Maize being an outcrossing crop, there is the possibility that gene flow via pollen might have occurred resulting in the not clearly defined clusters depicted by the UPGMA dendrogram. It is also possible that some of the ancestry of the released materials traced their origins from these CIMMYT lines. Similar reports or findings have been made by several authors (Warburton *et al*., 2011, Xia *et al*., 2004). In all, the landraces contained larger proportion of alleles compared with the other populations. This is not surprising because again maize being an outcrossing crop it is possible that pollen flow might have occurred resulting in new gene combinations (Doebley, 2004).

The Bayesian analysis done with Structure, which is a powerful statistical tool, was able to define the populations into 4 subpopulations and was able to show that many of the germplasm were of mixed ancestries. The use of Structure analysis broke the large UPGMA clusters into subgroups/populations, which is additional information. It was interesting to see that the known testers belonging to different heterotic groups (CML442 and CML202 and TZEI17 and TZEI23) did not cluster together in the same sub-populations (Table 5.7). The landraces were distributed

fairly into all sub-populations with the exception of the sub-population three. This suggests that the landraces used in the study can be grouped into 3 sub-populations. This again confirms the diversity of maize germplasm found in Ghana. Although this is a small sample of only twenty six originating from 6 regions namely, Northern, Brong Ahafo, Ashanti, Eastern, Volta and Western regions, we still see a diverse range of diversity and non-uniformity of maize populations grown in Ghana. It however remains to be seen if the clustering can be used to guide the testcrossing necessary to define heterotic patterns as has been mentioned by other authors (Melchinger and Gumber, 1998, Reif *et al.*, 2005b).

Landraces from Ashanti belonged to all three clusters which demonstrate the peculiar location of the region and probably its importance in maize cultivation. Ashanti is centrally located in Ghana and it is one of the main centres of the maize trade. Farmers from all over the country converge there to trade. Similarly, the vegetation in Ashanti is both forests with some portions being forest/savannah transition agroecology, which is also similar to those obtained in the other regions, thus supporting the cultivation of the different landraces. Another factor may be due to demographics. Ashanti is inhabited by people from all other regions of Ghana and it might be that people cultivate landrace materials they are used to in locations they might find themselves. In maize these factors have produced numerous open-pollinated landraces (Louette *et al.*, 1997 , Louette and Smale, 2000) which constitute a possible source of diversity that can be exploited to widen the gene pool from which breeders can harness useful genes and alleles to meet the challenges of climate change and other objectives of modern breeding programmes (Sharma *et al.*, 2010, Warburton *et al.*, 2008).

On the other hand, another factor working against geographic partitioning may be attributed to trade within Ghana by women traders and distributors who dominate the maize seed supply chain. These small scale seed distributors buy seed from all sections of the country and sell them wherever they can, and the speculative nature of their trade brings about movement of germplasm into areas where seeds are not necessarily adapted. This is probably one of the causes of most of the population mixing seen in this study, and possibly much of the low yield in Ghanaian maize production, as some farmers are probably growing maize that is not well adapted to their local regions.

Efforts to breed new maize genotypes should improve targeting specific climatic conditions for higher productivity. Unfortunately, this study has demonstrated that new germplasm could be found in places where they are not intended for optimum yield and productivity.

5.5 Conclusion

This study has been able to establish the relationships existing among the various germplasm used in the study. The UPGMA cluster showed one big cluster with two individuals as outliers. However, if a perpendicular line is drawn along the 0.22 genetic similarity eight other clusters were identified with nine individuals closely related to these clusters. The AMOVA showed a high within population variation. The F statistics showed that the populations were not well differentiated as they were defined. A similar observation was made with Bayesian analyses done with Structure. However, the Bayesian analysis grouped the genotypes into 4 main clusters/sub-populations and none of the landraces clustered with the sub-population three. Further the Bayesian analysis showed that four of the genotypes origins were mixed and

therefore, could not be assigned to any of the 4 sub-populations. The diversity of maize germplasm found in the landraces was also established which shows their potential as a breeding material for any future maize improvement efforts. However, targeting of specific ecologies for specific varieties that provide high yield and better resistance to disease should be made known to other stakeholders including seed dealers.

Chapter Six

6.0 Studies on Combining ability, heterotic patterns and yield stability of crosses involving selected Ghanaian maize landraces and inbred line testers

6.1 Introduction

Information on combining abilities, heterosis and heterotic grouping are important for the successful development of new high yielding hybrids in any breeding programme (Legesse *et al.*, 2009, Mohammed, 2009, Romanus *et al.*, 2007). Such information can show the type of gene action involved in controlling quantitative characters or traits, thereby assisting breeders in selecting suitable parent materials (Hallauer and Miranda, 1988). Significant values of general combining ability (GCA) and specific combining ability (Scapim *et al.*, 2000) may be interpreted as indicating the performance of additive and non-additive gene action, respectively (Sprague and Tatum, 1942). GCA helps breeders to exploit existing variability in breeding materials to identity genotypes conferring desirable attributes and to distinguish relatedness among genotypes (Melania and Carena, 2005, Vacaro *et al.*, 2002). SCA helps breeders to determine heterotic patterns among populations or inbred lines to identify promising single crosses and assign them into heterotic groups (Hede *et al.*, 1999, Parentoni *et al.*, 2001, Vasal *et al.*, 1992). The estimation of additive and non-additive gene action through this technique is useful in determining the possibility of commercial exploitation of heterosis for hybrid development (Stuber, 1994).

Heterosis and increased uniformity are the basis of the modern hybrid maize seed industry (Gerdes and Tracy, 1993). Early hybrid maize breeders observed that heterosis was greater in crosses between genetically diverse inbreds than in crosses between related inbreds. By relating levels of heterosis with pedigrees, the concept of heterotic groups was established (Anderson, 1944, Hallauer and Miranda, 1981). Maize breeders have relied on maintenance and exploitation of two or more heterotic breeding groups in the development of inbreds and subsequent hybrids. The recognition and use of heterotic groups has contributed to the efficiency and success of hybrid maize breeding programs (Barata and Carena, 2006). Grain yield of the first maize hybrids in the 1930s was about 15% greater than that of the best open pollinated varieties and approximately 60% of the yield of the 1930s single crosses was attributable to heterosis (Duvick, 1999).

In the United States of America for instance, maize inbreds are commonly classified into heterotic groups based on pedigree and/or combining ability and this information is used in making decisions on how germplasm is used in a breeding program (Hallauer and Miranda, 1981). For instance, the Reid Yellow Dent by Lancaster Surecrop heterotic pattern was identified shortly after hybrid maize was introduced, and continues to be a widely used combination (Darrah and Zuber, 1986, Hallauer, 1990, Reif *et al.*, 2005b).

An important element affecting selection apart from heritability is genotype and environment interaction (G×E). Interpretation of G×E influences breeders' approach to developing and improving varieties which influences the number and the types of varieties to be released across environments (Cooper and Hammer, 1996). According to Tollenaar and Lee (2006) yield stability and general stress tolerance are highly associated and yield stability does not appear to

decline with increasing yield potential. Yield stability is generally a highly desirable trait and maize breeding programs select for yield stability (Scapim *et al.*, 2000). In order to identify widely adapted maize hybrids, hybrid evaluation in commercial maize breeding programs is carried out across a large number of locations or environments where there is the possibility of commercial production by farmers, including stressful environments (Bradley *et al.*, 1988). The success of a hybrid is determined by both yield and yield stability in farmer's field (Balestre *et al.*, 2009).

In Ghana hybrid maize production is at its infant stages with only about 3% of farmers cultivating hybrids which are mainly imported (Ragasa et al, 2013). It is surprising to know for instance, that there are no known developed local inbredlines (M.B. Ewool Personal communication) that can be used to initiate hybrid maize production as Ghana depends on CIMMYT and IITA for supply of inbred lines. As Ghana aspires to increase maize productivity it is time that studies are conducted to identify potential landraces that can be used for hybrid maize production.

Thus the objectives of this work were to:

a. determine general and specific combining ability of crosses involving selected Ghanaian maize landraces and inbred line testers;
b. determine heterosis and assign heterotic groups with respect to yield ability of crosses
c. determine yield stability of potential topcross hybrids to identify potential hybrid combinations with resistance and/or tolerance to MSV disease for higher productivity.

6.2 Materials and Methods

6.2.1 Location of Experiments

Rearing of leafhoppers *Cicadulina mbila* (Naude) vector colonies for maize streak virus disease screening took place at the Entomology section of the CSIR-Crops Research Institute, Kwadaso station. Crossing blocks were established at the experimental fields of the CSIR-CRI research fields at Fumesua. Evaluation trials were conducted in three locations namely; Fumesua (6.712N; 1.523W) and Akomadan (7.396 N; 1.973W) in the Ashanti region and Wenchi (7.7333N; 2.1W) in the Brong Ahafo region. Wenchi lies in the heart of the transition zone of Ghana. Fumesua and Akomadan both lie in the semi-deciduous rain forest of Ghana. All the locations have two seasons of rainfall with the major season starting from March and ending in July. The minor season starts from September and ends in November or December. Wenchi has been identified a good MSV hotspot for disease evaluation under natural conditions (personal communications, M.B. Ewool).

6.2.2 Source of Germplasm

Inbred lines with resistance to the maize streak virus disease were supplied by CIMMYT Zimbabwe and IITA, Ibadan Nigeria. Local maize landraces or farmer varieties were collected from farmers in various locations across Ghana by the CSIR-CRI. The collections were made in December 2007 and have undergone two cycles of selection in the main seasons of 2008 and 2009. In addition, these materials had previously been screened in the screen house for their response to the maize steak virus disease. Four CSIR-CRI released varieties namely; Obatanpa,

Mamaba, Etubi and Enibi in addition to F_1 hybrids of the crosses TZEI17 x TZEI23 and CML202 x CML442 were used as checks for MSV resistance and yield in this study (Table 6.1).

Table 6. 1 Characteristics of genotypes selected for genetic studies

Genotype	Pedigree	Colour	Maturity	Source	Heterotic group
CML202	ZSR923-S4BULK-5-1-BBB	White	Intermediate/late	CIMMYT	B
CML444	P43C9-1-1-1-1-1-BBBBB	White	Intermediate/late	CIMMYT	B
CML442	CIMMYT M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BBBB	White	Intermediate/late	CIMMYT	A
TZEI17	TZE Comp5-YC6 inbred 35	Yellow	Early	IITA	A
TZEI23	TZE-Y Pop STR Co S6 inbred 62-2-3	Yellow	Early	IITA	B
LA03	N/A	White	Intermediate	Wenchi B/A	-
LA30	N/A	White	Intermediate	Bekwai, A/R	-
LA76	N/A	White	Intermediate	Juapong E/R	-
LA80	N/A	White	Intermediate	Ejura, A/R	-
LA97	N/A	White	Intermediate	Golokwati VR	-
LA99	N/A	Yellow	Intermediate	Anum E/R	-
LA246	N/A	Yellow	Intermediate	Akrofu-Agove,V/R	-
LA276	N/A	White	Intermediate	Kpeve, V/R	-
LA400	N/A	White	Intermediate	Ejura, A/R	-
LA424	N/A	Yellow	Intermediate	Ejura, A/R	-
LA457	N/A	White	Intermediate	Golokwati, V/R	-
LA463	N/A	White	Intermediate	Kpong, E/R	-
LA467	N/A	White	Intermediate	Golokwati, V/R	-
LA518	N/A	Yellow	Intermediate	Kpana, N/R	-
LA537	N/A	Mixed	Intermediate	Elubu, W/R	-
LA558	N/A	White	Intermediate	Nabogu, N/R	-
LA580	N/A	White	Intermediate	Wenchi, B/A	-

N/A= Not available

6.2.3 Establishment of Crossing blocks

Two crossing blocks were established at Fumesua; one in the major season of 2012 (April-July) and another in the minor season of 2012 (August-December). In the major season crossing block, the 17 local landraces were crossed with 4 inbred line testers (CML202, CML444, TZEI17 and TZEI23). The North Carolina Design II (line by tester) was used with the four inbred lines used as males. In the crossing block each line was planted in two rows at spacing of 75 cm by 40 cm. Because of the varying maturity dates of the different germplasm, planting dates of the landrace parents and the inbred lines were staggered to ensure synchronization of flowering. All cultural practices, including fertilizer application and weeding were done to ensure good growth and yield.

Artificial pollination was done by collecting pollen from male parents and then crossed with the female parents. The male pollen was collected by covering tassels of male plants very early in the morning (before 6:00 am) until mid-day to allow enough pollen to be collected for the day and then bulked for each line. Sufficient pollen was poured to completely cover the emerged silks of each female plant that had previously been shoot-bagged with plastic polythene bagged. To generate enough seeds for multi-location trial, between 5 to 7 female plants of each landrace were pollinated. Sixty eight topcross and one single cross hybrids were generated.

Similarly, in the minor season of 2012 (August-December) another crossing block was established again at Fumesua. This time five inbred lines (CML202, CML444, CML442, TZEI17 and TZEI23) were crossed with the 17 local landraces. Pollen from the five inbred lines was used

to cross with each of the local landrace to generate 85 F_1 topcross hybrids and two extra controls made up of crosses between CML202 x CML442 and TZEI17 x TZEI23.

6.2.4 Evaluation of F1 topcross hybrids and parents

Two evaluation trials were established; one in the minor season of 2012 (September-January) and the other in the major season of 2013 (March-July) in three locations namely; Fumesua and Akomadan in Ashanti and Wenchi in the Brong Ahafo region. The different locations were selected to provide diversified environments to assess the performance of the F1 hybrids generated above. Of the three locations Wenchi is a hotspot for MSV disease especially, in the minor season.

Seeds were planted in two rows per plot in two replications at planting density of approximately 66,667 plants per hectare. A spacing of 75 cm between rows and 25 cm between plants was used with one plant per hill. Each row was 5 m long. In the minor season of 2012, the trial was set up using a 9 by 10 alpha-lattice design with two replications. The evaluation trial involved 68 F_1 topcross hybrids, 17 landrace parents, 1 F_1 hybrid (TZEI17/TZEI23) and 4 CSIR-CRI released varieties (Obatanpa, Mamaba, Etubi and Enibi).

The 2013 major season trials were also conducted in Fumesua, Akomadan and Wenchi. Planting was done as in the previous year using the same planting distance and population density per hectare (66,667 per hectare). A 9 by 12 rectangular lattice with two replications was used. In this trial there were 6 controls including 2 F_1 hybrids; TZEI17 x TZEI23 and CML202 x CML442 and the 4 CSIR-CRI released varieties (Obatanpa, Mamaba, Etubi and Enibi).

In both trials all agronomic practices followed prescribed recommendations by maize breeders at the CSIR-CRI. A basal NPK application at a rate of 5 bags/ha and a top dressing of 2.5 bags sulphate of ammonia were applied. Guard rows were planted around all trials to avoid biases.

6.2.5 Data Collection

Data were collected on the following:

1. MSV disease incidence (Vi): was measured by counting number of infected plants per plot and then expressed as a percentage.
2. Virus severity (Vs) was scored for each plot based on a scale of 1 to 5, where, 1=no disease, 2=mild infection, 3=moderate infection, 4=severe infection and 5=very severe infection (Plate 4.1).
3. Plant height (PH in centimeters, cm) was measured from base of the plant to tassel leaf,
4. Total leaf count (LC) mean of the number of leaves per plot at 65 days, after planting
5. Anthesis silking interval (ASI), number of day between 50% anthesis date and 50% silking date
6. Ear diameter (Ed, in cm), was measured as the diameter of the cob with grains per plot
7. Thousand grain weight (1000Gwt in grammes) weight of 1000 grains per plot.
8. Cob width (CW in cm) was measured as the diameter of cob after shelling per plot
9. Yield per hectare (Yld/ha in kg). Yield per plant was calculated by dividing the shelled grain weight at 15% moisture content (MC) by the number of plants per plot. Yield per hectare was estimated by multiplying the yield per plant per plot by plant density per hectare as described by Tollenaar and Lee (2006).

6.2.6. Yield Stability

Yield stability of crosses and the landrace parents were assessed by analyzing the 2012 and 2013 evaluation trials in all locations to ascertain the most stable genotype(s) across the environments with respect to grain yield per hectare. However, crosses involving tester CML442 and those that did not produce enough seed for all locations were not included in this analysis to avoid bias.

6.2.7 Statistical analyses

All analyses were carried out with PROC GLM in SAS (SAS Institute, 2009) using the mixed model with test option and environments and year considered as random except yield stability index which was analysed with Genstat version 12. Analysis of variance (ANOVA) was computed for the genotypes for separate locations (data not shown) and then across the locations or environments for 2012, 2013 and combined 2012 and 2013 seasons also across locations to generate entry means adjusted for block effects according to the lattice design (Cochran and Cox, 1960, Menkir *et al.*, 2003, Vasal *et al.*, 1992). The pooled error mean square was calculated for each trait. Format of ANOVA far individual location and across locations are presented in table 6.2 & 6.3 respectively.

Table 6. 2 Format of ANOVA for individual location

Source of variation	Df	MS	EMS	F – test
Replication	$r-1$	MS_r		
Genotypes	$g-1$	MS_g	$\sigma_e^2 + r\sigma_g^2$	MS_g / MS_e
Error	$(g-1)(r-1)$	MS_e	σ_e^2	
Total	$gr-1$			

Table 6. 3 Format of ANOVA for the combined locations

Source of variation	Df	MS	EMS	F – test
Replication	$r-1$	MS_b		
Replication (Location)	$r(l-1)$			
Location	$l-1$	MS_l	$\sigma_e^2 + r\sigma_{gl}^2 + rg\sigma_l^2$	MS_l / MS_{ge}
Genotype	$g-1$	MS_g	$\sigma_e^2 + r\sigma_{gl}^2 + rl\sigma_g^2$	MS_g / MS_e
Genotype x Location	$(g-1)(l-1)$	MS_{ge}	$\sigma_e^2 + r\sigma_{gl}^2$	MS_{ge} / MS_e
Error	$(gl-1)(r-1)$	MS_e	σ_e^2	
Total	$glr-1$			

Where:

$$\sigma_l^2 = \frac{MS_l - MS_{gl}}{rg}$$

$$\sigma_g^2 = \frac{MS_g - MS_{gl}}{rl}$$

$$\sigma_{gl}^2 = \frac{MS_{gl} - MS_e}{r}$$

$$\sigma_e^2 = MS_e$$

MS_l = Means square due Location

MS_g = Mean square due genotype

MS_{gl} = Mean square due Genotype x Location

MS_e = Error mean square

σ_l^2 = Location variance

σ_g^2 = Genotypic variance

σ_{gl}^2 = Genotype x Location variance

σ_e^2 = Error variance

r, l, g are number of replication, location, genotype respectively and e experimental error

Components of variance

$$\sigma_g^2 = \sigma_a^2 + \sigma_e^2$$

$$\sigma_e^2 = MS_a - MS_e$$

Where:

MS_g = Genotypic means square

MS_e = Error mean square

σ_g^2 = Genotypic variance

σ_e^2 = Error variance

Combining ability

Line by tester analysis was done to partition the genotype source of variation into that due to parental line and tester general combining ability (GCA) effects as well as due to specific combining ability effects of crosses from the adjusted means using the method of Kempthorne (1957). In these analyses the checks and the parents were not included. The mean squares for GCA effects for the line and testers were tested for significance using the interactions with site x line and site x tester as error term respectively. The SCA for line x tester was tested with the interaction of mean squares due to site*Line*Tester as an error term. Interaction of site x GCA and site x SCA effects were tested with error mean square of the error term. In the combined

2012 and 2013 GCA and SCA analysis year was considered as environment for the traits to obtain 6 environments. The model below by Fan *et al.* (2009) was used for data analysis:

$Yijkl = \mu + al + bkl + vij + (av)ijl + eijkl$

$vij = gi + gj + sij$

where Y*ijk*l = observed value from each experimental unit;

μ =population mean;

al = location effect;

*b*kl = block or replication effect within each location;

*v*ij = F1 hybrid effect = *g*i + *g*j+ sij

[where *gi* = general combining ability (GCA) for the *i*th

parental line;

gj = GCA effect of *j*th tester;

and s*ij* = specific combining ability for the *ij*th F1 hybrid];

(*av*)*ij*l = interaction effect between *i*th F1 hybrid and *l*th location;

and

*e*ijkl = residual effect.

Analysis of variance for combining ability for line x tester across environments and one location are presented in table 6.4 and 6.5 below.

Table 6. 4 Analysis of variance of combining ability for line x tester across environments

Source of variation	Df	MS	EMS
Environment	$e-1$		
GCA_{Tester}	$t-1$	M_5	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2 + re\hat{\sigma}_{SCA}^2 + rel\hat{\sigma}_{GCA}^2$
GCA_{Line}	$l-1$	M_4	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2 + re\hat{\sigma}_{SCA}^2 + ret\hat{\sigma}_{GCA}^2$
SCA	$(t-1)(l-1)$	M_3	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2 + re\hat{\sigma}_{SCA}^2$
GCA_{Tester} x E	$(t-1)(e-1)$	M_{22}	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2 + rl\hat{\sigma}_{GCA*E}^2$
GCA_{Line} x E	$(l-1)(e-1)$	M_{21}	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2 + rt\hat{\sigma}_{GCA*E}^2$
SCA x E	$(t-1)(l-1)(e-1)$	M_2	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA*E}^2$
Error	$e-1(lte-1)$	M_1	$\hat{\sigma}_e^2$

Table 6. 5 Analysis of variance of Line x Tester for one environment or location

Source of variation	Df	MS	E(MS)
Replication	$r-1$		
GCA_{Tester}	$t-1$	M_5	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA}^2 + rl\hat{\sigma}_{GCA}^2$
GCA_{Line}	$l-1$	M_4	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA}^2 + rt\hat{\sigma}_{GCA}^2$
SCA	$(t-1)(l-1)$	M_3	$\hat{\sigma}_e^2 + r\hat{\sigma}_{SCA}^2$
Error	$r-1(lt-1)$	M_1	$\hat{\sigma}_e^2$

Heterosis

Percentage heterosis was calculated for the combined 2012 and 2013 yield data based on the formula of Flint-Garcia *et al.* (2009) as follows:

High Parent Heterosis = (F_1-HPV)/HPV x 100

Where HPV is the High parent value and

F_1 is the mean of the F_1 hybrid

Heterotic groups

Lines were assigned to heterotic groups by using SCA effects for yield where positive SCA between lines and tester generally indicates that lines are in opposite heterotic groups while lines in the same heterotic group with tester exhibit negative SCA effects as described by Vasal *et al.* (1992). Based on the combined 2012 and 2013 GCA and SCA results, testers CML442 and CML202 were used to assign opposite heterotic groups to the lines.

Yield Stability

Yield stability index was calculated based on yield per hectare using the Additive main effects and multiplicative interaction (AMMI) which combines the analyses of variance for the genotypes and environment main effect with principal component analysis and G x E interaction (Gauch and Zobel, 1996, Zobel *et al.*, 1988). The AMMI model was based on equation according to Gauch and Zobel (1996) as follows:

$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$

Where: Y_{ger} = yield for genotype (g) in environment (e) for replicate (r)

μ = the grand mean

α_g = genotype (g) deviation

β_e = the environment (e) deviation

λ_n = singular value for IPCA axis n

γ_{gn} = eigenvector value for genotype (g) of IPCA axis n

δ_{en} = eigenvector value for environment (e) of IPCA axis n

ρ_{ge} = the residual

ε_{ger} = error

AMMI stability value (Purchase *et al.*, 2000) was calculated using the IPCA1 (interaction principal component axis) and IPCA2 using the formula

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}\ x\ (IPCA1score)\right]^2 + [IPCA2score]^2}$$

Where SSIPCA1 is the sum of squares of the IPCA1 and SSIPCA2 is the sum of squares of IPCA2.

Yield stability index (YSI) was calculated using Microsoft Excel 2007 by ranking the yield per hectare from highest to lowest and ASV from lowest to highest and the sum of the two rankings with least value was declared the most high yielding and stable genotype across the environments in that order (Farshadfar *et al.*, 2011).

6.3 Results

There were significant ($p < 0.05$) differences between environments and years for all traits for the combined 2012 and 2013 trials (Table 6.6 & 6.7).

Table 6.6 Means squares of general ANOVA for MSV severity, incidence, leaf count and plant height for the combined 2012 and 2013 seasons

Source	DF	Vs	Vi	LC	PH
Environment (Env)	2	4.26**	10901.69***	210.15***	31778.62***
Year	1	160.65***	85880.84***	362.23***	106921.42***
Block(Replication*Env*Year)	96	0.68	82.44***	0.91***	506.37***
Rep(Env*Year)	6	0.27	425.87**	6.33***	830.97
Genotypes	105	1.31	486.70	4.63***	1785.99***
Genotypes*Year	81	1.20***	565.23***	1.27***	436.10**
Genotypes*Env	210	0.52	105.12	0.57	307.84
Genotypes*Env*Year	1622	0.68*	130.57***	0.70	281.80
Error	450	0.55	41.32	0.57	264.22

DF= degrees of freedom, Vs= virus disease severity, Vi= virus disease incidence, LC= leaf count, PH= Plant height

Table 6. 7 Means squares of general ANOVA for grain yield per hectare, 1000 grain weight, ear diameter anthesis interval and cob with for the combined 2012 and 2013 seasons

Source	DF	Yld/Ha	1000Gwt	Edcm	ASI	CW
Env	2	10984056	207745.73***	0.78***	732.786***	0.401**
Year	1	261189088.5***	777462.49***	8.16***	63.455**	1.375***
Block(Rep*Env*Year)	96	2218052.7*	792.35	0.05**	1.770**	0.019*
Rep(Env*Year)	6	5930503	1266.06	0.04	5.265145	0.031685
Genotypes	105	5569524.3***	2850.26***	0.35***	2.693945	0.171***
Genotypes*Year	81	1972584	809.61*	0.10***	1.966527	0.045***
Genotypes*Env	210	1771387	720.99	0.05	1.609702	0.01837
Genotypes*Env*Year	1622	1789692	533.3	0.04*	1.868***	0.016182
Error	450	1563387	429.8	0.03	1.127997	0.01363

Yld/Ha=yield/ha, 1000Gwt= 1000 grain weight, EDcm =ear diameter, ASI= anthesis silking interval, CW=cob weight

Yield:

Highest yield across locations was 8902.34 kg/ha for the cross CML442/LA80 followed by CML444/LA3 with a yield per hectare of 7818.14. In general, crosses involving CML444 gave higher yield per hectare whilst those involving TZEI23 gave the lowest yields. The highest yield for the controls was CML202/CML442 (single cross hybrid) with a yield per hectare of 7059.26kg/ha. For the local checks, Obatanpa performed best with a mean yield of 5612.43kg/ha. The cross TZEI17/TZEI23 gave the lowest yield for the controls (4988.99kg/ha). The highest yielding parent was LA276 with a yield of 5986.54kg/ha with LA537 with a yield of 3491.44kg/ha being the lowest (Table 6.8).

Table 6. 8 Mean grain yield (t ha-1) across locations for combined 2012 and 2013 seasons

Genotypes	Yld/ha (Kg	Genotypes	Yld/ha	Genotypes	Yld/ha
CML202/LA246	6693.57	TZEI17/LA246	6110.81	CML444/LA276	6508.14
CML202/LA276	6937.61	TZEI17/LA276	5842.86	CML444/LA3	6099.49
CML202/LA3	6771.49	TZEI17/LA3	6402.47	CML444/LA30	7818.14
CML202/LA30	7240.65	TZEI17/LA30	6468.95	CML444/LA400	6273.89
CML202/LA400	6164.56	TZEI17/LA400	6763.95	CML444/LA424	7037.25
CML202/LA424	6798.72	TZEI17/LA424	6031.69	CML444/LA457	6518.02
CML202/LA457	5571.08	TZEI17/LA457	7026.06	CML444/LA463	7000.49
CML202/LA463	5975.89	TZEI17/LA463	6135.59	CML444/LA467	6609.84
CML202/LA467	6439.93	TZEI17/LA467	6228.35	CML444/LA518	6819.28
CML202/LA518	5208.08	TZEI17/LA518	5807.63	CML444/LA537	6284.39
CML202/LA537	5982.38	TZEI17/LA537	4885.48	CML444/LA558	6110.08
CML202/LA558	5861.60	TZEI17/LA558	5780.70	CML444/LA580	7586.02
CML202/LA580	6591.16	TZEI17/LA580	6211.45	CML444/LA76	7554.09
CML202/LA76	5773.65	TZEI17/LA76	6289.50	CML444/LA80	6128.60
CML202/LA80	6229.48	TZEI17/LA80	7247.36	CML444/LA97	6235.38
CML442/CML202	7059.26	TZEI17/LA97	6452.79	CML444/LA99	6424.99
CML442/LA246	6764.94	TZEI17/LA99	5609.06	LA246	4562.17
CML442/LA276	6680.26	TZEI17/TZEI23	4988.99	LA276	5986.54
CML442/LA3	7455.18	TZEI23/LA246	5752.15	LA3	5557.40
CML442/LA30	6641.65	TZEI23/LA276	6058.04	LA30	5260.38
CML442/LA400	7327.73	TZEI23/LA3	6876.83	LA400	5444.70
CML442/LA424	6243.79	TZEI23/LA30	5912.89	LA424	5893.95
CML442/LA457	6669.36	TZEI23/LA400	5761.88	LA457	4205.10
CML442/LA463	6657.62	TZEI23/LA424	5863.35	LA463	5291.54
CML442/LA467	6546.86	TZEI23/LA457	5760.18	LA467	5130.21
CML442/LA518	6028.99	TZEI23/LA463	5577.71	LA518	4412.72
CML442/LA537	6028.14	TZEI23/LA467	5008.19	LA537	3491.44
CML442/LA558	7307.97	TZEI23/LA518	5692.63	LA558	5015.81
CML442/LA580	6609.40	TZEI23/LA537	5482.05	LA580	4222.05
CML442/LA76	6320.50	TZEI23/LA558	5664.58	LA76	4588.91
CML442/LA80	8902.34	TZEI23/LA580	6031.46	LA80	4209.55
CML442/LA97	6645.25	TZEI23/LA76	5762.42	LA97	4095.94
CML442/LA99	6050.68	TZEI23/LA80	6021.98	LA99	4728.72
CML444/LA246	6045.28	TZEI23/LA97	5323.01	Enibi	5422.92
Obatanpa	5612.28	TZEI23/LA99	5983.54	Etubi	5046.21
SE	930.92	Mamaba	5173.18		
Lsd	**2585.43**				

Virus disease incidence and severity

Virus disease incidence was highest for the landrace parents compared with the crosses. Across years the line with the highest mean virus incidence was LA537 (35.85%) followed by LA580 (32.49%), LA99 (27.85%). Generally, virus disease incidence and severity were low for the 2013 trial because there was low disease pressure. High scores were observed in 2012. The parents scored higher disease incidence and severity than the crosses, eg. LA537 scored highest disease incidence of 70% with a mean severity of 3.5 followed by LA97 (53.69%), LA99 (53.83%). However, crosses involving the landraces had a reduced disease severity and incidence, eg. CML444/LA99 had mean incidence of 2.8% and CML444/LA97 was 16.73% (Table 6.9 and 6.10)

Table 6. 9 Mean of virus incidence (%) and severity scores for 2012 seasons

Gentypes	Vs	Vi	Gentypes	Vs	Vi	Gentypes	Vs	Vi
CML202/LA246	1.92	16.14	LA03	2.42	39.15	TZEI17/LA558	1.50	3.15
CML202/LA276	1.80	26.63	LA246	2.08	29.69	TZEI17/LA580	1.33	6.15
CML202/LA400	1.83	10.46	LA276	1.75	10.25	TZEI17/LA76	1.33	2.23
CML202/LA424	1.67	11.11	LA30	1.92	10.92	TZEI17/LA80	1.67	8.85
CML202/LA457	2.20	33.60	LA400	2.75	43.95	TZEI17/LA97	1.75	17.50
CML202/LA463	1.50	9.33	LA424	2.75	48.85	TZEI17/LA99	1.75	14.27
CML202/LA467	1.58	9.32	LA457	2.50	16.19	TZEI23/LA03	2.00	10.79
CML202/LA518	1.75	24.23	LA463	2.17	24.32	TZEI23/LA246	1.83	12.27
CML202/LA537	1.42	5.31	LA467	2.50	36.18	TZEI23/LA276	1.42	4.12
CML202/LA558	1.38	2.51	LA518	2.83	48.73	TZEI23/LA30	1.75	8.68
CML202/LA580	2.00	19.14	LA537	3.25	70.30	TZEI23/LA400	2.08	29.61
CML202/LA76	1.50	6.96	LA558	2.17	22.23	TZEI23/LA424	2.17	24.76
CML202/LA80	2.00	28.52	LA580	2.75	59.57	TZEI23/LA457	2.08	16.35
CML444/LA03	1.58	7.33	LA76	6.42	39.56	TZEI23/LA463	1.67	11.94
CML444/LA246	1.50	3.87	LA80	3.00	48.04	TZEI23/LA467	2.33	18.31
CML444/LA276	1.63	29.87	LA97	2.92	53.69	TZEI23/LA518	2.00	19.62
CML444/LA30	1.75	20.34	LA99	2.83	53.83	TZEI23/LA537	2.33	34.60
CML444/LA400	1.75	12.74	TZEI17/LA03	1.58	8.20	TZEI23/LA558	1.75	16.30
CML444/LA424	2.00	13.95	TZEI17/LA24	1.42	2.73	TZEI23/LA580	2.20	18.51
CML444/LA457	1.50	4.32	TZEI17/LA27	1.17	1.35	TZEI23/LA76	2.17	24.04
CML444/LA463	1.67	5.95	TZEI17/LA30	1.08	1.47	TZEI23/LA80	2.33	32.36
CML444/LA467	1.67	10.20	TZEI17/LA40	1.58	9.30	TZEI23/LA97	2.42	33.64
CML444/LA518	1.75	16.32	TZEI17/LA42	1.58	6.93	TZEI23/LA99	1.92	14.01
CML444/LA537	1.50	7.15	TZEI17/LA45	1.17	2.78	MAMABA	2.25	33.19
CML444/LA558	1.67	12.88	TZEI17/LA46	1.58	12.86	OBATANPA	2.00	25.22
CML444/LA80	2.08	24.31	TZEI17/LA46	1.92	15.12	ENIBI	2.58	33.37
CML444/LA97	1.92	16.73	TZEI17/LA51	1.75	21.85	ETUBI	1.92	9.78
CML444/LA99	1.33	2.84	TZEI17/LA53	1.92	12.61	TZEI17/TZEI23	1.08	0.64
LSd	2.11	18.65						
SE	0.76	6.69						

Vs= virus disease severity score, Vi= virus disease incidence

Table 6. 10 Mean virus incidence (%) and severity scores for the combined 2012 and 2013 seasons

Genotypes	Vs	Vi	Genotypes	Vs	Vi	Genotypes	Vs	Vi
CML202/LA246	1.54	8.43	TZEI17/LA246	1.21	1.36	CML444/LA400	1.38	6.37
CML202/LA276	1.45	13.11	TZEI17/LA276	1.17	0.90	CML444/LA424	1.54	7.17
CML202/LA3	1.00	0	TZEI17/LA3	1.33	4.31	CML444/LA457	1.25	2.16
CML202/LA30	1.00	0	TZEI17/LA30	1.04	0.74	CML444/LA463	1.33	2.98
CML202/LA400	1.42	5.23	TZEI17/LA400	1.38	4.82	CML444/LA467	1.38	5.30
CML202/LA424	1.33	5.56	TZEI17/LA424	1.29	3.46	CML444/LA518	1.38	8.16
CML202/LA457	1.65	12.93	TZEI17/LA457	1.17	1.60	CML444/LA537	1.25	3.58
CML202/LA463	1.50	9.33	TZEI17/LA463	1.29	6.43	CML444/LA558	1.33	6.44
CML202/LA467	1.42	4.91	TZEI17/LA467	1.54	7.73	CML444/LA580	1.00	0
CML202/LA518	1.50	12.48	TZEI17/LA518	1.38	10.92	CML444/LA76	1.00	0
CML202/LA537	1.35	6.28	TZEI17/LA537	1.54	6.59	CML444/LA80	1.54	12.16
CML202/LA558	1.25	1.23	TZEI17/LA558	1.25	1.58	CML444/LA97	1.92	16.73
CML202/LA580	1.45	8.70	TZEI17/LA580	1.17	3.07	CML444/LA99	1.38	1.91
CML202/LA76	1.29	3.69	TZEI17/LA76	1.17	1.11	LA246	1.54	14.84
CML202/LA80	1.50	14.26	TZEI17/LA80	1.33	4.42	LA276	1.38	5.12
CML442/CML202	1.00	0	TZEI17/LA97	1.54	9.15	LA3	1.71	19.57
CML442/LA246	1.08	0.52	TZEI17/LA99	1.38	7.88	LA30	1.54	5.72
CML442/LA276	1.00	0	TZEI17/TZEI23	1.04	0.32	LA400	2.21	22.96
CML442/LA3	1.25	0.77	TZEI23/LA246	1.58	6.59	LA424	1.96	26.51
CML442/LA30	1.00	0	TZEI23/LA276	1.21	2.06	LA457	1.75	8.10
CML442/LA400	1.00	0	TZEI23/LA3	1.50	5.39	LA463	1.63	12.52
CML442/LA424	1.33	0.40	TZEI23/LA30	1.54	4.52	LA467	1.88	18.70
CML442/LA457	1.00	0	TZEI23/LA400	1.63	15.01	LA518	2.25	25.03
CML442/LA463	1.00	0	TZEI23/LA424	1.58	12.38	LA537	2.33	35.82
CML442/LA467	1.00	0	TZEI23/LA457	1.54	8.35	LA558	1.71	11.30
CML442/LA518	1.42	0.95	TZEI23/LA463	1.33	5.97	LA580	1.95	32.49
CML442/LA537	1.00	0	TZEI23/LA467	1.75	9.32	LA76	3.71	19.78
CML442/LA558	1.00	0	TZEI23/LA518	1.55	9.17	LA80	2.14	22.11

CML442/LA580	1.17	0.50	TZEI23/LA537	1.83	17.76	LA97	2.33	28.10
CML442/LA76	1.00	0	TZEI23/LA558	1.46	8.40	LA99	2.13	27.85
CML442/LA80	1.00	0	TZEI23/LA580	1.77	9.29	Mamaba	1.88	17.19
CML442/LA97	1.08	0.52	TZEI23/LA76	1.67	12.24	Obatanpa	1.77	13.30
CML442/LA99	1.00	0	TZEI23/LA80	1.79	16.66	Enibi	2.08	17.23
CML444/LA246	1.33	2.19	TZEI23/LA97	1.83	17.19	Etubi	1.54	5.19
CML444/LA276	1.25	11.95	TZEI23/LA99	1.46	7.01			
CML444/LA3	1.29	3.66	CML444/LA30	1.38	10.17			
SE	0.53	5.1						
Lsd	1.48	14.17						

Vs= virus disease severity score, Vi= virus disease incidence

Plant Height

Plant height ranged from 212 cm for line LA424 to 146.1cm for the cross TZEI17/TZEI23 (Table 6.11).

Table 6. 11 Mean plant height (cm) across locations for combined 2012 and 2013 seasons

Genotypes	PH	Genotypes	PH	Genotypes	PH	Genotypes	PH
CML202/LA246	185.75	CML444/LA246	175.92	LA246	178.00	TZEI17/LA467	168.33
CML202/LA276	180.82	CML444/LA276	169.30	LA276	168.75	TZEI17/LA518	166.00
CML202/LA3	182.00	CML444/LA3	192.08	LA3	194.17	TZEI17/LA537	164.08
CML202/LA30	181.67	CML444/LA30	188.25	LA30	197.50	TZEI17/LA558	168.42
CML202/LA400	189.58	CML444/LA400	187.58	LA400	201.00	TZEI17/LA580	170.75
CML202/LA424	190.25	CML444/LA424	203.00	LA424	212.67	TZEI17/LA76	173.33
CML202/LA457	179.20	CML444/LA457	186.33	LA457	180.92	TZEI17/LA80	182.50
CML202/LA463	192.50	CML444/LA463	191.75	LA463	177.08	TZEI17/LA97	173.17
CML202/LA467	191.58	CML444/LA467	197.67	LA467	185.25	TZEI17/LA99	169.92
CML202/LA518	172.42	CML444/LA518	171.58	LA518	155.25	TZEI17/TZEI23	146.17
CML202/LA537	176.31	CML444/LA537	184.83	LA537	153.08	TZEI23/LA246	166.92
CML202/LA558	163.90	CML444/LA558	169.75	LA558	178.92	TZEI23/LA276	162.00
CML202/LA580	189.73	CML444/LA580	162.33	LA580	164.27	TZEI23/LA3	175.92
CML202/LA76	184.75	CML444/LA76	186.33	LA76	176.92	TZEI23/LA30	166.42
CML202/LA80	179.33	CML444/LA80	195.58	LA80	177.55	TZEI23/LA400	160.25
CML442/CML202	176.67	CML444/LA97	176.17	LA97	163.17	TZEI23/LA424	165.67
CML442/LA246	187.83	CML444/LA99	197.08	LA99	186.42	TZEI23/LA457	166.50
CML442/LA276	175.83	CML442/LA518	173.33	TZEI17/LA246	174.00	TZEI23/LA463	162.58
CML442/LA3	193.33	CML442/LA537	167.83	TZEI17/LA276	163.67	TZEI23/LA467	158.08
CML442/LA30	185.67	CML442/LA558	156.83	TZEI17/LA3	179.33	TZEI23/LA518	151.00
CML442/LA400	182.17	CML442/LA580	169.33	TZEI17/LA30	167.67	TZEI23/LA537	153.17
CML442/LA424	188.83	CML442/LA76	180.67	TZEI17/LA400	180.75	TZEI23/LA558	170.33
CML442/LA457	177.67	CML442/LA80	193.67	TZEI17/LA424	173.42	TZEI23/LA580	161.64
CML442/LA463	172.83	CML442/LA97	170.50	TZEI17/LA457	175.75	TZEI23/LA76	158.25
CML442/LA467	170.17	CML442/LA99	176.50	TZEI17/LA463	175.58	TZEI23/LA80	165.50
SE	12.53	Enibi	160.00	Mamaba	156.50	TZEI23/LA97	163.83
Lsd	34.81	Etubi	161.75	Obatanpa	190.33	TZEI23/LA99	169.50

PH= Plant height

Heterosis

Heterosis for yield ranged from -2.40% for TZEI17/LA276 to 111.48% for CML442/LA80 (Table 6.12). The highest heterosis was found with crosses involving tester CML444 and CML442 whilst TZEI23 gave the lowest heterosis.

Table 6. 12 Percentage heterosis for grain yield measured across locations for the combined 2012 and 2013 seasons

LINE	CML202	CML442	CML444	TZEI17	TZEI23
LA246	46.72	48.28	32.51	33.95	26.08
LA276	15.89	11.59	8.71	-2.40	1.19
LA3	21.85	34.15	9.75	15.21	23.74
LA30	37.64	26.26	48.62	22.97	12.40
LA400	13.22	34.58	15.23	24.23	5.83
LA424	15.35	5.94	19.40	2.34	-0.52
LA457	32.48	58.60	55.00	67.08	36.98
LA463	12.93	25.82	32.30	15.95	5.41
LA467	25.53	27.61	28.84	21.41	-2.38
LA518	18.02	36.63	54.54	31.61	29.01
LA537	71.34	72.65	79.99	39.93	57.01
LA558	16.86	45.70	21.82	15.25	12.93
LA580	56.11	56.54	79.68	47.12	42.86
LA76	25.82	37.73	64.62	37.06	25.57
LA80	47.98	111.48	45.59	72.16	43.06
LA97	N/A	62.24	52.23	57.54	29.96
LA99	N/A	27.96	35.87	18.62	26.54

NB: N/A shows that data could not taken for the respective cross

Combining ability analysis

General combining ability effects of 2012 and 2013 of combined trials across locations found that all the traits studied were not significant for replication except virus incidence which was significant at ($p<0.01$) (Table 6.13 and 6.14). All traits were significant for environment except Yld/ha and 1000 grain weight (1000Gwt). Virus disease incidence (Vi) and anthesis-silking interval (ASI) were significant at ($p<0.05$). Plant height (PH) was significant at ($p<0.01$) while cob width (CW) was significant at ($p<0.001$). Line GCA effects were significant at $p<0.05$ for ASI, 1000Gwt, Vi and Yld/ha. GCA effects for Plant height were significant at $p<0.01$ whilst those for virus severity (Vs) and cob width were highly significant at ($p<0.001$). Ear diameter (Ed) GCA effects were not significant. Tester GCA effects were significant at ($p<0.05$) for ASI; plant height and virus severity were significant at ($p<0.01$). Tester GCA effect for ear diameter and cob width were significant at $p<0.001$ but yield per hectare and virus severity were not significant. Line by environment GCA effects were highly significant for ear diameter, cob width and virus incidence at ($p<0.001$). ASI GCA effects for line by environment were significant at $p<0.05$ whilst 1000Gwt GCA effects were significant at $p<0.01$. Line by environment GCA effects plant height, leaf count and virus disease score were not significant at $p<0.05$. Tester by environment GCA effects were highly significant at $p<0.001$ for ASI, virus severity and cob width and significant at $p<0.01$ for ear diameter, yield per hectare and virus incidence. GCA effects for Leaf count and 1000Gwt were significant at ($p<0.05$) but plant height GCA effect were not significant. Line by tester GCA effects were significant for ear diameter and virus incidence at ($p<0.01$) whilst virus severity GCA effect were significant at $p<0.05$ and with cob width being significant at $p<0.001$. The remaining traits GCA effects for line by tester were not

significant. SCA by site interaction effects were significant at p<0.01 for ASI, 1000 grain weight and ear diameter. Virus incidence and cob width SCA effects were highly significant at (p<0.001) whilst the other traits SCA effects were all not significant.

Table 6. 13 Mean squares of combined ANOVA for anthesis silking interval, ear diameter, 1000 grain weight and plant height for the combined 2012 and 2013 seasons

Source	DF	ASI	Edcm	1000Gwt	PH
Replication	1	3.494	0.124	11.478	1025.61
Environment (Env)	5	352.266***	6.859***	210227.652*	92665.309**
GCA_{LINE}	16	3.933*	0.13	2857.918***	1617.668***
GCA_{TESTER}	4	14.832*	3.081***	26671.858**	17845.403**
$GCA_{env*LINE}$	80	1.841*	0.074***	866.694***	338.932
$GCA_{env*TESTER}$	17	4.746***	0.2067***	4064.155***	465.071
$SCA_{LINE*TESTER}$	63	1.853	0.0886***	770.1913	406.928
SCA*Env	244	1.73**	0.0467**	604.24**	338.308
Error	417	1.287	0.033	451.047	299.724

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively; ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height

Table 6. 14 Mean squares of combined ANOVA for leaf count, MSV incidence and severity score, yield per hectare and 1000 grain weight for the combined 2012 and 2013 seasons

Source	DF	LC	Vi	Vs	Yld/ha	CW
Replication	1	0.528	373.211**	0.005	2128025	0.017
Environment	5	143.985***	9631.047***	15.587***	300300978***	0.574***
GCA_{LINE}	16	5.165***	244.358*	0.355***	3432008*	0.118***
GCA_{TESTER}	4	63.089***	869.428	2.424**	1.5E+07	1.674***
$GCA_{env*LINE}$	80	0.715	111.98***	0.123	1746484	0.036***
$GCA_{env*TESTER}$	17	1.302*	528.295***	0.418***	5181562***	0.068***
$SCA_{LINE*TESTER}$	63	0.808	140.957**	0.176*	2213277	0.054***
SCA*Env	244	0.729	81.292***	0.116	1938623	0.019***
Error	417	0.676	37.857	0.113	1871528	0.014

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively
LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

GCA effects for Lines and testers

GCA effects for ASI for the combined 2012 and 2013 analysis for lines ranged from -0.73 (LA518) to 0.49 (LA424). ASIs GCA effects were negative for LA246, LA276, LA30, LA457, LA558, LA97, LA467 and LA518 which was significant at $p<0.01$. The rest had positive GCA effects with LA424 being significant at $p<0.01$. For the testers CML444 and TZEI17 negative GCA effects were detected whilst the rest were positive with CML442 being significant at $p<0.05$. GCA effects for Cob width ranged from -0.01 to 0.09 for lines and -.09 – 0.17 for testers. Lines LA3, LA400, LA457, LA518, LA580, LA80 and LA97 had negative GCA effects for cob width whilst the rest were positive with line LA3 and LA99 being significant at $p<0.05$. All the tester GCA effects were negative for this trait except that for CML444 which was positive and significant at $p<0.01$. Ear diameter GCA effects for lines ranged from -0.004 to 0.13. Ear diameter GCA effects were negative for lines LA246, LA457, LA467, LA518, LA537, LA558, LA80 and LA97 with line LA518, LA80 and LA97 being significant at ($p<0.01$) and ($p<0.05$) respectively. Tester GCA effects were negative and significant at $p<0.01$ for CML202 and TZEI17 whilst TZEI23 was not significant. The rest were positive and significant at $p<0.01$. 1000Gwt GCA effects ranged from -14.07 to 11.54. Lines LA400, LA467, LA537, LA580, LA76, LA97 and LA518 had negative GCA effects with lines LA400, LA467, LA76 being significant at $p<0.01$ and LA537 significant at $p<0.05$. The rest had positive GCA effects with LA424, LA99 and LA457 being significant at $p<0.05$ and $p<0.01$ respectively. Testers CML202, TZEI17 and TZEI23 had negative and significant GCA effects whilst the rest were positive and significant for CML442 at $p<0.01$ and $p<0.05$ for CML444.

GCA effects for Leaf count of the lines ranged from -0.68 to 0.64. Leaf count GCA effects were negative for LA276, LA457, LA563, LA537, LA558, LA580, LA76, and LA97 with LA558, LA580, LA76, and LA97 being significant at $p<0.01$. Lines LA3, LA30 and LA400 had positive significant GCA effects at $p<0.01$ whilst LA424 was positive for LA424 at $p<0.05$. The rest of lines GCA effects were positive but not significant for the trait. For the testers CML442, TZEI23 and TZEI17 GCA effects were negative and significant at $p<0.01$ and $p<0.05$ respectively whilst the rest had positive significant GCA at $p<0.01$. Virus severity GCA effects ranged from -0.14 to 0.25. GCA effects for virus severity were negative for lines LA276, LA3, LA30, LA457, LA463, LA558, LA580, LA76 and LA99 but those for LA276 were significant at $p<0.01$, the rest were not significant. For virus incidence GCA estimates ranged from -2.67-5.55 for lines. Line LA80 had positive significant GCA effects at $p<0.01$ whilst the rest were positive but not significant whilst LA276 had negative and significant GCA effects. For the testers, GCA effects for CML442 were negative and significant at $p<0.01$, those for CML444 and TZEI17 were negative but not significant. The other testers GCA effects were positive with TZEI23 being significant at $p<0.01$. GCA estimates for Plant height ranged from -9.16 to 8.15. GCA effects for Lines LA276, LA518, LA537, LA558 were all negative at $p<0.01$ for plant height whilst those for LA97 were significant at $p<0.05$. GCA effects for LA580 and LA76 were negative but not significant. Those for Lines LA3, LA30, LA424 and LA80 were positive and significant at $p<0.01$. The rest were positive but not significant. Testers TZEI17 and TZEI23 had negative and significant GCA effects at $p<0.05$ and $p<0.01$ respectively. Yield per hectare GCA effects ranged from -559.48 to 521.01. Lines LA246, LA463, LA467, LA558, LA76, LA97, LA99 had negative GCA effects whilst LA518 and LA537 had negative significant GCA effects at ($p<0.05$) and ($p<0.01$) respectively. Lines LA3, LA30 had significant GCA effects at $p<0.01$ whilst LA80

had significant GCA effects at $p<0.05$ with rest of lines having positive but not significant GCA effects. Tester CML202, TZEI17 had negative none significant GCA effects whilst TZEI23 had significant negative GCA effects. Tester CML442 had positive significant GCA effects at $p<0.01$ whilst those of CML444 were positive and significant at $p<0.05$.

Table 6. 15 General combining ability effects of various traits of lines and testers evaluated across environments for the combined 2012 and 2013 seasons

LINE	ASI	Edcm	1000Gwt	LC	Vi	Vs	PH	Yld/ha	CW
LA246	-0.27	-0.01	3.11	0.04	-1.92	0.01	1.40	-45.53	0.09**
LA276	-0.023	0.10	4.65	-0.04	-0.24	-0.14*	-6.09**	94.32	0.05
LA3	0.11	0.09*	7.18	0.64**	-2.67*	-0.06	8.15**	358.77*	-0.04
LA30	-0.01	0.07	6.62	0.35**	-2.25	-0.13*	0.90**	521.02**	0.02
LA400	0.21	0.02	-13.82**	0.63**	0.88	0.03	4.23	97.55	-0.01
LA424	0.49**	0.02	8.94*	0.23*	0.29	0.05	8.12**	147.50	0.04
LA457	-0.13	-0.05	11.54**	-0.24*	-0.83	-0.03	1.34	31.47	-0.09**
LA463	0.26	0.004	6.90	-0.02	-1.10	-0.07	2.55	-6.63	0.02
LA467	-0.20	-0.003	-11.07**	0.11	-0.05	0.09	2.34	-139.87	0.02
LA518	-0.73**	-0.11**	-4.71	0.09	3.05*	0.07	-9.16**	-362.13*	-0.08**
LA537	0.10	-0.07	-9.86*	-0.17	1.47	0.06	-6.07**	-559.48**	0.01
LA558	-0.10	-0.01	2.36	-0.68**	-2.08	-0.08	-8.64**	-267.79	0.03
LA580	0.20	0.13**	-3.54	-0.50**	-0.94	-0.01	-3.77	226.15	-0.03
LA76	0.19	0.01	-14.07**	-0.30**	-1.85	-0.09	-0.64	-73.54	0.02
LA80	0.01	-0.10*	4.46	0.15	4.45**	0.11*	6.57**	419.87*	-0.08**
LA97	-0.17	-0.10*	-7.69	-0.53**	5.55**	0.25	-5.49*	-192.22	-0.03
LA99	0.10	0.03	9.71*	0.08	-1.31	-0.03	2.90	-252.00	0.06*
SE	0.17	0.03	4.04	0.11	1.33	0.04	2.31	165.52	0.02
Lsd 0.05	0.33	0.07	7.94	0.21	2.61	0.09	4.53	325.35	0.05
Lsd 0.01	0.44	0.09	10.45	0.27	3.43	0.11	5.97		0.06
TESTER									
CML202	0.16	-0.10**	-11.145*	0.38**	1.37	0.03	6.95**	-18.79	-0.05**
CML442	0.33*	0.11**	28.67**	-0.20**	-5.89**	-0.29**	2.22	487.98**	-0.09**
CML444	-0.37	0.22**	19.13*	0.78**	-0.20	-0.02	9.94**	341.02*	0.17**
TZEI17	-0.07	-0.12**	-10.36*	-0.15*	-1.63	-0.07	-3.41*	-70.45	-0.06**
TZEI23	0.11	-0.05	-15.34**	-0.76**	3.74**	0.22**	-12.15**	-468.86**	-0.01
SE	0.14	0.029	4.37326	0.07	1.44	0.04	1.35	142.55	0.02

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Specific combining ability

SCA effects (line by tester) for all the traits studied were analyzed but that of yield per hectare, virus incidence and severity for the combined 2012 and 2013 trials are presented in Table 6.16 and 6.17 respectively. Crosses involving lines LA400, LA457, LA463, LA518, LA558, LA76, LA80 with the tester CML202 had negative SCA effects for yield per hectare whilst crosses with LA457 had significant negative SCA at ($p<0.05$). The rest of the crosses with this tester had positive SCA effects. Crosses of tester CML442 and lines LA276, LA30, LA424, LA457, LA463, LA518, LA518, LA537, LA580, LA76 and LA99 had negative SCA effects. Crosses the tester with line LA80 and LA558 had positive significant SCA effects at $p<0.01$ and $p<0.05$ respectively whilst the rest had positive SCA effects with the same tester. Crosses of tester CML444 and lines LA246, LA276, LA400, LA457, LA558 had significantly negative SCA effects whilst crosses of the tester with LA3 and LA80 had significantly negative SCA effects at $p<0.05$ with the rest of the crosses SCA effects being negative with the tester. The rest of the crosses with the tester had positive SCA with LA76 and LA580 being significant at $p<0.01$ and $p<0.05$ respectively. Crosses involving Tester TZEI17 and lines LA400, LA467, LA76, LA80, LA97 had positive SCA effects with the cross involving LA457 having significant SCA effects at $p<0.05$. The rest of the crosses with the same tester had negative SCA effect whilst a cross with LA537 had significantly negative SCA effects. For the tester TZEI23 the following crosses had negative SCA effects with tester; LA30, LA400, LA434, LA457, LA463, LA467, LA80 and LA97. The rest of the crosses had positive SCA effects with the cross involving LA3 having a significantly positive SCA effect with the tester at $p<0.05$ (Table 6.16).

For virus severity, the following crosses with tester CML202 and lines LA424, LA467, LA537, LA76, LA80 had negative SCA effect whilst crosses of the same tester with LA3 and LA30 were negative and significant (Table 6.17). The rest of the crosses had positive SCA effects with crosses involving lines LA457 and LA276 being significant at $p<0.01$ and $p<0.05$ respectively. For tester CML442, 7 crosses had negative SCA effect with this tester, namely; LA400, LA457, LA463, LA467, LA537, LA80 and LA97. LA80 and LA97 SCA effects were significant at $p<0.05$ and $p<0.01$ respectively. The rest of the crosses with this tester had positive SCA effects except LA518 and LA424 which were significant at $p<0.01$ and $p<0.05$ respectively. Seven crosses showed negative SCA effects with tester CML444 that is, Lines LA246, LA457, LA467, LA518, LA537 whilst LA580 and LA76 were significant at $p<0.01$. The rest of the crosses had positive SCA effects with the tester. Tester TZEI17 had negative SCA effects with crosses involving lines LA246, LA30, LA424, LA457, LA580, LA76, LA80 and LA97. The rest of the crosses had positive SCA for the tester. For Tester TZEI23, crosses involving lines LA246, LA276, LA3, LA424, LA457, LA463, LA518, LA558, LA97 and LA99 had negative SCA effects with this tester whilst the rest were positive with LA537 and LA580 being significant at $p<0.05$ (Table 6.17).

SCA effects for virus incidence were negative for crosses involving the tester CML202 and lines LA3, LA30, LA400, LA424, LA467, LA537, LA558 and LA76 whilst the rest were positive with LA246 and LA457 being significant at $p<0.01$ (Table 6.17). Crosses involving lines LA400, LA424, LA467, LA518, LA537, LA80 and LA97 had negative SCA effects whilst LA80 and LA97 were significant with tester CML442 at $p<0.05$. The rest of the crosses were positive but not significant with this tester. Crosses involving lines LA276 and LA30 and teste CML44 had

positive and significant SCA effects at $p<0.01$ whilst SCA effects involving that of LA97 was positive and significant at $p<0.05$. LA424, LA558 and LA80 had positive SCA effects with tester the CML444, whilst the rest had negative SCA effects except LA580 which was significant at $p<0.05$. Crosses of lines LA3, LA463, LA467, LA518, LA537 had positive SCA effects with tester TZEI17 except LA99 which had significantly positive SCA effects at $p<0.05$. The rest of the crosses had negative SCA effects with this tester except LA80 which was significant at $p<0.05$. Crosses of lines LA246, LA3, LA30, LA457, LA463, LA518, LA99 had negative SCA effects with tester TZEI23 except LA276 which was significant at $p<0.01$. The rest of the crosses had positive SCA with this tester except LA537 which was significant at $p<0.01$.

Heterotic groups:

The tester CML202 and CML442 were used to group the lines into opposite heterotic groups as can be found in Table 6.16. Lines LA276, LA30, LA424, LA467, LA537, LA580 and LA99 classified as belonging to the tester CML442 (group A). Lines LA400, LA558 and LA80 were grouped as belonging to the same group as tester CML202 (group B). Lines LA246 and LA3 were assigned to both groups. The rest of the lines could not be assigned to any of the two testers (Table 6.16).

Table 6. 16 Specific combining ability effects for grain yield across locations for combined 2012 and 2013 seasons and heterotic groups of lines,

	CML202	CML442	CML444	TZEI17	TZEI23	
LINE	**SCA Yld/ha(kg)**	**SCA Yld/ha(kg)**	**SCA Yld/ha(kg)**	**SCA Yld/ha(kg)**	**SCA Yld/ha(kg)**	**Heterotic Group**
LA246	493.63	58.23	-514.47	-37.46	2.28	A,B
LA276	597.82	-166.3	-191.46	-445.26	168.32	A
LA3	167.26	344.17	-864.56*	-150.11	722.66*	A,B
LA30	474.16	-631.61	691.84*	-245.88	-403.53	A
LA400	-178.46	477.94	-428.94	472.59	-131.07	B
LA424	405.76	-655.94	284.47	-309.61	-79.55	A
LA457	-705.86*	-114.35	-118.73	800.78*	-66.69	-
LA463	-262.96	-87.99	401.84	-51.59	-211.07	-
LA467	334.34	-65.5	144.44	174.42	-647.34	A
LA518	-675.26	-361.12	576.13	-24.04	259.37	-
LA537	296.39	-164.62	238.59	-748.84*	246.13	A
LA558	-116.08	823.52*	-227.42	-145.32	136.97	B
LA580	119.55	-368.98	754.59*	-208.5	9.91	A
LA76	-398.27	-358.19	1022.36**	169.24	40.56	-
LA80	-435.86	1730.23***	-896.55*	633.68	-193.29	B
LA97	N/A.	85.23	-177.68	451.21	-280.17	-
LA99	N/A.	-449.56	71.71	-332.75	440.13	-
SE	348.768					
Lsd.05	685.5625					
Lsd .01	902.4967					

NB: N/A shows that data could not taken for the respective cross: SCA= specific combining ability, Yld/ha= grain yield per hectare, CML= CIMMYT maize lines, TZEI= Tropical zea early inbredline

Table 6. 17 SCA effects of Virus severity and incidence across locations for the combined 2012and 20/13 trials

SCA for Virus severity						SCA for virus incidence					
LINE	CML202	CML442	CML444	TZEI17	TZEI23	LINE	CML202	CML442	CML444	TZEI17	TZEI23
LA246	0.14	0	-0.03	-0.1	-0.01	LA246	2.87	2.22	-1.79	-1.19	-1.33
LA276	0.19*	0.06	0.03	0	-0.24	LA276	5.88**	0.02	6.28**	-3.33	-7.55**
LA3	-0.34**	0.23	0	0.09	-0.03	LA3	-4.81	3.22	0.43	2.5	-1.78
LA30	-0.26**	0.05	0.15	-0.13	0.09	LA30	-5.23	2.03	6.51**	-1.49	-3.07
LA400	-0.01	-0.1	0	0.05	0.01	LA400	-3.13	-1.09	-0.41	-0.54	4.29
LA424	-0.12	0.20*	0.14	-0.07	-0.06	LA424	-2.21	-0.1	0.98	-1.3	2.24
LA457	0.28**	-0.05	-0.08	-0.11	-0.02	LA457	6.28**	0.61	-2.92	-2.05	-0.67
LA463	0.17	-0.01	0.05	0.06	-0.19	LA463	2.95	0.88	-1.83	3.05	-2.78
LA467	-0.07	-0.17	-0.07	0.15	0.07	LA467	-2.52	-0.17	-0.56	3.3	-0.48
LA518	0.03	0.27**	-0.05	0	-0.11	LA518	1.96	-2.32	-0.8	3.4	-3.73
LA537	-0.12	-0.14	-0.17	0.17	0.18*	LA537	-2.67	-1.68	-3.8	0.65	6.44**
LA558	-0.06	0.01	0.06	0.03	-0.05	LA558	-4.16	1.87	2.62	-0.82	0.63
LA580	0.07	0.1	-0.34**	-0.12	0.20*	LA580	2.16	1.22	-4.97*	-0.47	0.38
LA76	-0.01	0.01	-0.26**	-0.05	0.17	LA76	-1.94	1.63	-4.06	-1.51	4.24
LA80	-0.01	-0.19*	0.08	-0.08	0.09	LA80	2.34	-4.66*	1.8	-4.50*	2.36
LA97	N/A	-0.25**	0.31	-0.01	-0.01	LA97	N/A	-5.24*	5.27*	-0.87	1.79
LA99	N/A	-0.05	0.05	0.1	-0.1	LA99	N.A	1.09	-2.69	4.71*	-1.53
SE	0.09					SE	2.26				
LSd 0.05	0.17					Lsd 0.05	4.44				
Lsd 0.01	0.22					Lsd 0.01	5.84				

NB: N/A shows that data could not taken for the respective cross SCA= specific combining ability, CML= CIMMYT maize line

Stability Analyses

The most stable yielding genotype across the environments was found to be CML444/LA457 followed by TZEI17/LA97, CML202/LA246 in that order. The top ten most stable yielding genotypes included 4 crosses involving TZEI17, 3 crosses involving CML444, 2 involving TZEI23 and 1 involving CML202. The most stable control genotype for yield was Etubi with a score of 63 compared to 17 by the most stable genotype. The bottom 10 comprised mainly of landrace parents (http://hdl.handle.net/10568/24696) with least score being 130 (Table 6.18). AMMI analysis of variance showed highly significant ($p<0.001$) differences for genotypes, environment and ($p<0.05$) for blocks and interactions (Table 6.19).

Table 6. 18 Yield stability index measured for selected topcross hybrids and parents

Genotype	NG	Gm	RY	ASV-Yieldha	RASV	YSI	Genotype conti.	NG	Gm	RY	ASV-Yld/ha	RASV	YSI
CML444/LA4	14	6518	12	4.27	5	**17**	**CML444/LA80**	20	6129	25	25.45	47	**72**
TZEI17/LA97	53	6453	14	4.99	8	**22**	**CML444/LA518**	17	6819	7	49.25	65	**72**
CML202/LA2	1	6694	10	10.32	18	**28**	**TZEI23/LA97**	69	5323	57	10.28	17	**74**
CML444/LA4	15	7000	5	12.90	23	**28**	**OBATANPA**	38	5612	48	13.51	26	**74**
CML444/LA4	13	7037	3	14.32	28	**31**	**TZEI17/LA457**	44	7026	4	121.52	70	**74**
TZEI17/LA76	51	6290	17	8.77	15	**32**	**TZEI23/LA467**	64	5008	64	8.15	11	**75**
TZEI17/LA42	43	6032	31	4.044	3	**34**	**CML444/LA99**	21	6425	16	35.54	59	**75**
TZEI17/LA46	46	6228	21	8.38	13	**34**	**TZEI23/LA80**	68	6022	32	24.96	44	**76**
TZEI23/LA99	70	5984	34	4.23	4	**38**	**TZEI17/LA400**	42	6764	9	64.37	67	**76**
TZEI23/LA3	58	6877	6	15.83	32	**38**	**LA463**	31	5364	56	12.25	21	**77**
CML444/LA4	12	6274	19	12.34	22	**41**	**LA400**	28	5445	53	13.81	27	**80**
TZEI17/LA55	49	5781	41	2.852	1	**42**	**TZEI17/LA518**	47	5841	40	21.42	40	**80**
TZEI23/LA27	57	6058	29	8.40	14	**43**	**TZEI23/LA457**	62	5760	45	18.78	37	**82**
CML202/LA4	3	6799	8	18.06	35	**43**	**MAMABA**	37	5173	60	13.38	24	**84**
TZEI23/LA42	61	5863	38	5.83	10	**48**	**TZEI23/LA76**	67	5762	43	22.33	42	**85**
CML202/LA7	7	5774	42	5.27	9	**51**	**LA276**	25	5987	33	31.37	52	**85**
CML202/LA8	8	6229	20	16.20	33	**53**	**CML444/LA3**	10	6099	28	34.56	57	**85**
TZEI23/LA30	59	5913	36	11.94	20	**56**	**LA537**	34	3491	70	8.84	16	**86**
CML444/LA5	18	6284	18	19.32	38	**56**	**CML444/LA246**	9	6045	30	33.86	56	**86**
TZEI17/LA30	41	6469	13	22.92	43	**56**	**TZEI23/LA400**	60	5762	44	25.20	45	**89**
CML444/LA5	19	6110	27	15.59	30	**57**	**CML202/LA400**	2	6165	23	60.71	66	**89**
TZEI17/LA24	39	6111	26	15.62	31	**57**	**TZEI17/TZEI23**	55	4989	65	15.16	29	**94**
LA3	26	5571	51	4.48	7	**58**	**LA30**	27	5260	58	18.64	36	**94**
TZEI23/LA55	66	5665	47	8.23	12	**59**	**TZEI23/LA463**	63	5578	50	25.25	46	**96**
TZEI17/LA58	50	6211	22	21.37	39	**61**	**ENIBI**	22	5423	55	28.87	50	**105**
CML444/LA4	16	6610	11	30.6683	51	**62**	**LA424**	29	5894	37	66.120	68	**105**

TZEI17/LA27	40	5843	39	13.4609	25	**64**	**TZEI17/LA537**	48	5443	54	32.180	53	**107**
TZEI17/LA46	45	6136	24	21.9676	41	**65**	**CML202/LA518**	5	5208	59	27.617	49	**108**
CML444/LA3	11	7818	1	46.3515	64	**65**	**TZEI23/LA246**	56	5752	46	43.845	62	**108**
ETUBI	23	5046	62	4.30672	6	**68**	**TZEI17/LA99**	54	5609	49	44.91	63	**112**
LA246	24	4562	67	3.49934	2	**69**	**LA518**	33	4413	68	26.806	48	**116**
CML202/LA5	6	5967	35	17.4108	34	**69**	**LA76**	36	4589	66	32.484	54	**120**
CML202/LA4	4	6440	15	33.3651	55	**70**	**LA558**	35	5016	63	34.630	58	**121**
TZEI23/LA53	65	5482	52	11.5001	19	**71**	**LA467**	32	5130	61	35.712	60	**121**
TZEI17/LA80	52	7247	2	78.8362	69	**71**	**LA457**	30	4205	69	37.648	61	**130**

NG= number of genotype, Gm= mean grain weight, RY= Rank of yield, ASV= AMMI stability value, RASV= rank of AMMI stability value, YSI= yield stability index

Table 6. 19 AMMI analysis for grain yield of 70 topcross hybrids and landrace parents evaluated in six environments (three locations and two seasons)

Source	df	SS	MS	F	F_prob
Total	839	340803633	4062022		
Treatments	419	266250177	6354420	3.66	0.0000
Genotypes	69	456025776	6609069	3.81	0.0000
Environments	5	148614343	2.97E+08	64.49	0.0000
Block	6	27651602	4608600	2.66	0.01532
Interactions	345	720332565	2087920	1.2	0.03536
IPCA	73	320808933	4394643	2.53	0.0000
IPCA	71	149381998	2103972	1.21	0.1292
IPCA	69	126933641	1839618	1.06	0.35678
Residuals	132	123207993	933394	0.54	0.99998
Error	414	717882960	1734017		

6.4 Discussion

The highest yield obtained across locations and years was 8029 kg/ha for the topcross hybrid CML442/LA80. This was significantly higher than any of the landrace parents and better than F_1 hybrid (CML442/CML202) as well as the other controls. The high yields obtained from some of the topcross hybrids give indication that there is the potential to raise substantially the yield of some of Ghanaian maize landraces. Similar findings have been reported in other parts of the world such as India, Honduras, and Thailand where yield and disease resistance of landraces have been improved in a similar manner (Almekinders *et al.*, 1994, Dhillon *et al.*, 2002, Prasanna, 2012, Vasal *et al.*, 1987).

The results also demonstrate that the Maize streak virus disease severity and incidence can be managed if farmers adopt hybrid varieties that have at least one resistant parent. The incidence and disease severity observed in the landrace parents and that of the corresponding topcross hybrids especially, in the 2012 trial where disease pressure was high with significant correlation ($r=-0.2$, $p<0.05$) buttresses this point. Parents were more susceptible to severe maize streak disease compared with the hybrids which also reflected in the grain yield per hectare. Most of the topcross hybrids performed better than the parents in disease severity scores and incidence indicating dominance gene action. This observation supports report that MSV disease is controlled mostly by a dominant gene (Storey and Howland, 1967; Efron *et al.*, 1989, Kim *et al.*, 1989, Pernet *et al.*, 1999a, Rose, 1978; Kyetere *et al.*, 1995). This conclusion was arrived at based on the fact that all the testers used have been certified as resistant/tolerant to the disease by the suppliers and which was also confirmed during the preliminary screening. However, it appears that the resistance becomes stronger with certain tester/parent crosses suggesting

different levels of resistance among the genotypes which also agrees with similar observations by Kim *et al.*, (1989) when they studied the resistance of Maize streak virus disease. On the other hand, it could also be argued that those topcross hybrids that suffered mild disease symptoms or disease effects might have escaped or suffered late infection. This then requires that the most resistant/tolerant genotypes identified should be subjected to further study.

The results provide information on the heterotic patterns for selected local landraces with respect to two of the five testers used in the study namely; CML442 and CML202. The two IITA testers (TZEI17 and TZEI23) could not be used to assign heterotic groups to the landraces because their GCA were both negative with respect to grain yield which is considered the most important trait for heterotic grouping (Legesse *et al.*, 2009, Soengas *et al.*, 2003, Vasal *et al.*, 1992). Some of the lines could not be assigned to any of the two groups because their SCAs with the two testers were negative suggesting they did not belong to any of the testers' group (Table 6.16). Two other lines LA97 and LA99 could not be assigned because data could not be taken on their crosses with the tester CML202. Two of the lines were assigned to both groups because their SCAs were positive with both testers. Similar groupings have been made by Legesse *et al.* (2009) ; Parentoni *et al.* (2001); Vasal *et al.* (1992). The results suggest the possibility of developing some of these landraces into inbred lines which can be utilized for hybrid maize production as has been reported by several authors (Beck *et al.*, 1990, Fan *et al.*, 2003, Hallauer and Miranda, 1988, Vasal *et al.*, 1987, Vasal *et al.*, 1992, Vasal *et al.*, 1992b).

Estimates of heterosis were found to be very high among some of the crosses. Particularly, crosses with Testers CML442 and CML444 and a few of the crosses involving TZEI17 gave high heterosis. Expressions of high heterosis suggests the possibility of using these genotypes for

hybrid maize production as have been reported by Alvarez *et al.* (1993); Gissa *et al.* (2007) which is also a reflection of the existence of dominance and epistasis gene action with respect to grain yield (Crow, 1948; Schnell and Cockerham, 1992). Reports of estimates of grain yield heterosis have been reported in other parts of the world (Flint-Garcia *et al.*, 2009, Gissa *et al.*, 2007, Kara, 2001, Reif *et al.*, 2009).

The various environments used for the study showed significant differences for most of the traits studied. Although line x tester effects were not significant for yield, significant differences were found for some of the yield related traits like cob width, and ear diameter as well as virus incidence and severity (Table 6.14). Similar findings have been reported by other researchers (Fato *et al.*, 2012, Legesse *et al.*, 2009, Soengas *et al.*, 2003, Vasal *et al.*, 1992).

Results from the GCA and SCA studies also demonstrated the potential of using some of the landraces for hybrid maize production. Some of the lines, such as LA30, LA3 and LA80 had positive and significant GCA effects with respect to yield. Similarly, positive and significant SCA effects were observed for crosses involving LA558 LA76, LA80 LA457 and LA3 (Table 6.15 and 6.16). Significant GCA effects for yield has been mentioned by several authors as a requirement for the development of inbred lines for hybrid production (Pswarayi and Vivek, 2008; Vasal *et al*., 1992b). Significant and positive GCA effects imply additive gene action whilst significant SCA effects imply dominance and epistasis (Sprague and Tatum 1942; Legesse *et al.*, 2009). For hybrid development positive GCA for yield is required especially, if materials involved have not been previously selected (Sprague and Tatum 1942; Fan *et al.*, 2003 Legesse *et al.*, 2009) in addition to significant and positive SCA (Hallauer and Miranda 1988 Vasal *et al.*, 1992 Hede *et al.*, 1999; Revilla *et al.*, 2002). This again confirms the potential of the Ghanaian

landraces for improved productivity as has been achieved in other parts of the world (Soengas *et al.,* 2003; Almekinders *et al.,* 1994; Dhillon *et al.,* 2002). For resistance/tolerance to MSV disease, genotypes that contribute least or negative GCA and SCA effects are required (Fato *et al.,* 2012; Legesse *et al.,* 2009). Three of the testers CML442, CML444 and TZEI17 had negative GCA effects for MSV incidence and severity whilst CML442, CML444 and TZEI17 had negative SCA effects with a number crosses with some of the landraces parents. This implies that the testers contributed in reducing the incidence and severity of the disease and therefore can be relied on to improve productivity. A similar observation was made with some of the lines (Table 6.17). Several negative and significant SCA effects were also recorded between some of the testers and some lines. This again confirms the assertion made earlier that using at least a resistant parent for hybrid production can effectively manage the incidence and severity of the maize streak virus disease.

GCA and SCA effects for the yield related traits studied showed similar trends. For plant height low GCA and SCA effect are preferred because farmers prefer shorter plants for better crop husbandry (Pswarayi and Vivek, 2008). Significant differences also observed in some of the traits show that there is the possibility of selecting for these traits in the landraces as have been reported by other researcher (Pswarayi and Vivek, 2008, Vacaro *et al.*, 2002, Zehui *et al.*, 2000).

Stability is not the only parameter for selection because most stable genotypes necessarily do not give the best yield performance (Mohammadi *et al.*, 2007, Mohammadi and Amri, 2008). This has resulted in the development of approaches that incorporate both high mean yield and stability in a single index, known as yield stability index (YSI) (Babarmanzoor *et al.*, 2009, Eskridge, 1990, Farshadfar, 2008, Rao and Prabhakaran, 2005). In this regard, the AMMI stability value

takes into account both IPCA1 and IPCA2 that justify most of the variation in the GxE interaction and then rank the AMMI stability value (Plasvic-Blanjac and Maramorosch, 1972) and mean yield in such a way that the lowest ASV takes the number one rank, while the highest mean yield takes the rank one and then both ranks are summed in a single simultaneous selection index of high mean yield and yield stability which is referred to as yield stability index (YSI) (Farshadfar *et al.,* 2011). Stable and high yielding genotypes across environments were identified with the AMMI stability analysis as revealed by YSI (Table 6.18). The top ten most stable and high yielding genotypes involved crosses with all four testers. The best performing control/check genotype did not rank among the top 10. This information is very useful because it demonstrates that farmers who continue to depend on landraces can significantly improve their yields across environments if these genotypes are developed into topcross hybrid cultivars and then released to them for adoption and cultivation. Similar recommendations have been made by Annicchiarico (1997); Yaghotipoor and Farshadfar (2007); Gauch (1988).

Subjecting genotypes to stability analyses at the initial stages of cultivar development as has been done in this study has several advantages because it improves the accuracy of yield estimates (Farshadfar *et al.,* 2011). It has been reported by Zobel *et al.,* (1988); Crossa, (1990) that gains have been obtained in the accuracy of yield estimates that are equivalent to increasing the number of replications by a factor of two to five which effectively reduces cost of cultivar development.

6.5 Conclusion

The study has brought to the fore the potential of the local landraces for improved productivity through topcross hybrid production and resistance to the maize streak virus disease. This study was able to identify high heterosis among some of the topcross hybrids and assigned some of the landraces into heterotic groups using two inbred line testers namely; CML202 and CML442. Highly significant GCA effects were identified which implies maize streak virus incidence and resistance as well as other yield related traits can be selected for. CML442, CML444 and TZEI17 contributed positively to yield increases as well as reduction or tolerance/resistance to MSV. Landraces like LA3, LA80, LA76 and LA457 produced high significant SCA effects for yield. Highly significant SCA effects were observed in some crosses which suggest dominance and epistastic gene action. High yielding and stable topcross hybrids were identified which shows that hybrids developed from these landraces can significantly raise farmers' yields across different environments. This work is the first report in Ghana and it has shown the potential of utilizing Ghanaian maize landraces (farmer varieties) for improved productivity and resistance to the maize streak virus disease. It has contributed to the research on utilization of landraces for improved productivity and resistance to the maize streak virus disease.

CHAPTER SEVEN

7.0 General Discussion and Conclusion

This work was set up with the objective to identify suitable parents from Ghanaian maize landraces that can be used to improve the yield and resistance to the maize streak virus disease through the development of topcross hybrids. It also aimed at identifying farmer constraints that impede their production, their perceptions of the maize streak virus disease, preferred varieties in the context of the adoption of improved varieties and landraces. To help breeding for novel genotypes with adequate resistance to the maize streak virus disease, genomic characterization of the MSV from diseased samples collected from the forest and transition zones of Ghana was undertaken to identify virulent strains of the virus for screening purposes. Molecular characterization of Ghanaian maize landraces and exotic genotypes was also carried out using SSR markers to ascertain their genetic relationships. Finally studies on combining abilities, heterosis, assigning heterotic groups to the landraces and yield stability of some of the genotypes were verified across environments.

Reasons for farmer preferred varieties, production constraints and their perceptions/knowledge of the MSV disease were ascertained. A majority of the respondents (64%) cultivated improved OPVs whilst 36% of them continued to depend on landraces. Just about 3% of the respondents cultivated hybrids. The reason for cultivation or low adoption of hybrids was that the existing hybrid varieties (mostly imported) do not meet their preference. They preferred varieties with slender cob size with a lot of grain and would wish to have one with local input. MSV was indicated as a seasonal problem which affects about 20% of their yields which they wish a cure

could be found. Apart from financial constraints, climate change was mentioned in addition to lack of tractor service, poor road, cheating by middlemen, pest and diseases as the main constraints.

Several strains of the MSV have been identified with some being more virulent than others in Africa and the neighboring islands (Shephered *et al.*, 2010). Genomic characterization of the virus infected samples collected identified the most virulent strain of the virus that is the MSV-A_1 strain in sampled areas. The characterization of the virus in Ghana is a very useful contribution to the development of the maize streak resistant varieties in breeding programmes. It will be interesting to know if new strains can be identified within the MSV-A_1 identified in the study which may explain why certain resistant cultivars responded differently in certain environments and conditions.

The genomic characterization of the maize genotypes with SSR markers revealed some interesting findings. The UPGMA dendrogram showed one big cluster and two outliers. However, if a perpendicular line is drawn from 0.22 genetic similarity eight minor clusters within and nine outliers can be seen (Fig. 5.1). The Bayesian clustering grouped the germplasm into 4 sub-populations with one mixed sub-population/group. No clear/distinct clustering was identified between the Ghanaian landraces and CSIR released varieties that were used as checks. A similar observation was made with the IITA and CIMMYT lines (Fig. 5.1). This suggests that the landraces were not entirely different from the released varieties. Probably, the landraces might have been research materials from Research Institutions that might have found their way into farmers' hands and which they now consider as their own. Another reason possibly, may be that gene flow might have occurred between the local materials and the released varieties which

is not surprising considering the fact that maize is an open pollinated crop (Warburton *et al.*, 2010). It should also be mentioned that most of the released varieties were developed with the germplasm originating from either CIMMYT or IITA which could also be responsible the poor differentiation. It will be interesting to see as follow up to this finding the extent of genetic mixing between the released varieties, landraces as well the CIMMYT and IITA germplasm occurring in Ghana. However, the study contributed in reducing the number of landrace genotypes from 26 to 17 by removing closely related genotypes. These 17 genotypes were used for the studies on combining abilities.

The studies on combining abilities, heterosis, assigning heterotic group and identification of high and stable yields across environments showed that topcross hybrid varieties can be developed from the landraces. Some of the topcross hybrids gave high yields which were significantly higher than any of the landrace parents with as high as 111% heterosis for some of the hybrids. Opposite heterotic groups were identified among the landraces which means that in future inbred lines could be developed from them for hybrid maize production. Similarly, high and significant GCA and SCA effects were identified with both lines and testers which is basic information needed for any successful hybrid breeding programme. Most importantly, incidence and severity of the maize streak virus disease was significantly lower with the topcross hybrids than the landraces. Lower MSV disease severity and incidence were recorded with the topcross hybrids compared with the parents even though they had previously been screened with the virus. This was also confirmed by the negative and significant GCA and SCA effects for MSV disease incidence and severity of 3 testers; CML442, CML444 and TZEI17. All these testers had been certified as resistant/tolerant to the disease by their suppliers and their resistance/tolerance was

confirmed in the preliminary screening in the screen house. The implication of this finding is significant. It demonstrates the potential to increase farmers' productivity through the development of hybrid varieties that are also resistant or tolerant to MSV disease. Most significant is that it can be done with local germplasm. The stability of the topcross hybrids were also confirmed in the yield stability analysis across the environments. The top ten of the most stable and high yielding genotypes involved the topcross hybrids with majority of the landraces occupying the bottom 10 (Table 6.18).

The results demonstrate the potential for increased productivity from the local landraces which can also be used for controlling the effects of maize streak virus disease.

It is recommended that the landraces with significantly high GCA and SCA effects be developed into inbred lines for hybrid production which can further improve the yield figures obtained in this study.

In conclusion, this work has demonstrated the potential of using Ghanaian maize landraces for improved productivity as well as management of the maize streak virus disease albeit with the "exotic" testers used in the study. This work has also contributed to the research on utilization of landraces for improved productivity and demonstrated the potential of utilizing landraces in fighting hunger and poverty among rural farmers in Ghana and beyond as have been done elsewhere in the world (Almekinders *et al.*, 1994, Prasanna, 2012).

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Appendices

Appendix 3.1 Checklist for a PRA on the prevalence of maize streak virus disease in farmers fields in the forest and transition zones of Ghana

Region_____ District _____
Agro-ecological zone_____ Community _____

Number of participants _____
Male_____female_____

1. Importance of Mize in this community

Crop	Rank

2. List the Varieties of maize cultivated and their yield in this community

OPVs	Yield (Maxi bags)	Landraces	Yield (Maxi bags)	Hybrids	Yield (Maxi bags)

3. Have you heard about the maize streak disease?_____

4. Where did you hear it from?
_____

5. What are the symptoms?

_____

6. Which part of the crop is mostly affected by the streak disease?

_____

7. Which months in the year is the streak disease most prevalence

Statement	Month											
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Time of inception												
Most prevalence												
Very severe												
Time it goes												

8. Generally what is the percentage loss that can be obtained from incidence of streak

_____

9. Which varieties of maize are tolerant to the maize streak disease?

_____

10. Which of the verities are more susceptible to the maize streak disease?

_____

11. Is the disease transmissible on the field? _____

12. What are some of the courses of the maize streak disease?

_____

13. How do you control maize streak disease on your farm

_____

14. List and rank the major constraints to maize production in this community

Constraints	Rank

15. What is the importance of maize streak disease in this community?

_____

16. Preference for maize varieties (OPV, Hybrid)

Varieties preferred (OPV or Hybrid)	Reasons

Preference for a new maize variety

17. Characteristics you want to see in the new variety

Characteristics	Preference
Yield	
Maturity period	
Cob size	
Resistance to lodging	
Disease tolerance	
Taste	
Storability	
Colour	
Grain size	
Drought resistance	
Market price	

18. Recommended practices in maize production

Recommended practices	Response
Row planting	
Fertilizer application	
NPK	
Ammonia	
urea	
Rate of fertilizer application	

Maize Marketing

19. Where do you normally sell your maize?

_____

Appendix 3.2 A Survey on Maize production Systems in Forest Transition zones of Ghana

Ghana

Farmer Questionnaire

Questionnaire ID……
Name of enumerator……
Date of enumeration…….
Region…….... Region
Code……..
District…… District
Code……..
Community……. Community
Code……...

A. Background information

1 Household code: …….
2 Name of respondent……..
3 Sex of respondent 0=Female1=Male
4 Age of respondent……..
5 Highest level of formal education of respondent…… 0=None 1=Basic(Primary/JHS/Middle) 2=Secondary (Secondary/Vocational) 3=Tertiary (Training college/Polytechnic/University)
6 Marital status of respondent: …….. 0=Single 1=Married 2=Divorced/Separated 3=Widowed
7 Years of experience in Maize cultivation……
8 Is the respondent the head of the household? 1=Yes 0=No
9 Nativity of respondent: …… 1=Native 2=Settler 3=Migrant 4=Other (specify):……
10 If non-native, how long have you been a resident in the community? ……..
11 What is the number of persons in the household? Total: …….. Male: …… Female: …….
12 Number of persons who are ≤14 years. Total: …….. Male: …… Female: …….
13 Number of persons who are ≥15 and ≤60 years. Total: …….. Male: …… Female: …….
14 Number of persons who are >60 years. Total: …….. Male: …… Female: …….

15 Number of literate/educated persons. Total: …….. Male: ……
Female: …….
16 Number of persons attending school. Total: …….. Male: ……
Female: …….
17 What is your main occupation? …. 1=crop production 2=livestock rearing 3=petty trading 4=craftsmanship 5=labour 6=permanent employment
18 Are members of household engaged in off-farm income generating activities? 1=Yes 0=No
19 On the average, how many acres of land do you farm to maize every year?.....
20 How many times do you cultivate maize in a year? 1=once 2=twice 3=three times
21 Do you cultivate other crops? Yes No
22 If Yes, which other crops do you cultivate?
1= Maize 2= Cassava 3= Yam 4= Plantain 5= Cowpea 6= Vegetables 7=Cocoa 8= others
(specify)……

23 Do you rotate any crop with maize on the same piece of land? 1=Yes 2=No

B. Knowledge on streak

24. Do you know about the maize streak disease? 1= Yes 2=No

25. Which parts of the maize plant does streak affect? 1=leaves 2=stem (node)
3=tassels 4=don't know

26. How important is streak disease in your locality 1=very important 2=important 3=not so important 4=not important

27. Have you ever experienced streak on your farm? 1= Yes 2=No
28. How much yield loss did it cause? (In percentage)……
29. Is the variety you usually cultivate tolerant to streak? 1= Yes 2= No 3= don't know
Please mention variety……
30. How do you control streak outbreak on your field? 1=use fungicide 2=reduce Nitrogen fertilizer application 3=plant resistant/tolerant varieties 4=do nothing
5=other (specify)…….
31. What are some of the symptoms of streak that you know? 1=yellowing of leaves 2=leaf rot 3=streaks of yellowing along the blade 4=don't know
(Please provide some symptoms as answers)
32. Which varieties of maize are tolerant/resistant to the streak disease?
1=OPVs 2= Landraces 3=hybrid 4=don't know
33. Which varieties of maize are susceptible to the streak disease?

1=OPVs 2= Landraces 3=hybrid 4=don't know

34. At what stage of the growth of the crops does streak usually occur?

1= Vegetative stage 2= Reproductive phase

35. During what season is the streak disease more prevalent?

1=major 2=Minor 3=don't know

36. Is the disease transmissible on the field? 1=Yes 2=No

37. What do you think are the causes of the disease?

a. caused by insects b. Caused by the seed used c. Man d. implements

38. At what month of the year is the streak disease most prevalent?

January	February	March	April	May	June	July	August	September	October	November	December

C. Farmers' perception on Maize streak disease

39. How do you agree with the following statements about streak? (Please tick) (*1=strongly agree 2=agree 3=neutral 4=disagree 5=strongly disagree)*

Statement (Please put in some statements about streak that we intend to assess their perception on)	**1**	**2**	**3**	**4**	**5**
Maize streak is a major constraint to yield					
Not all varieties are susceptible to streak					
Local varieties are more susceptible to streak					
It is very easy to control during outbreak					
Improved varieties are more susceptible to streak					
Hybrid varieties have better resistance					
The disease occurs all seasons of planting					
The disease occurs only in the minor season					
Yield is not much affected by the disease					

E. Maize Production

41. How does the last maize season area compare with the previous ones and why?

1) Same	*2) Larger*	*3) Smaller*
Reason *1)Rainfall pattern unchanged* *2) Pests and diseases* *3) Weeds* *4) Yield* *5) Market price* *6) Seed quantity unchanged* *7) Seed price* *8) Labor force unchanged* *9) Cash for inputs unchanged* *10) Land size unchanged* *11) Not interested in expanding* *12) Other*	***Reason*** *1) Enough seed* *2) Enough labor* *3) Enough cash to buy inputs* *4) Enough land to expand* *5) Interested in expanding* *6) Better rainfall* *7) Other*	***Reason*** *1) Inadequate seed* *2) Reduced labor force* *3) Reduced cash for inputs* *4) Reduced land available* *5) Interest in intensive farming* *6) Poor rainfall* *7) Floods* *8) Pests and diseases* *9) Other*_____

42. Give the quantities of maize varieties[1] you purchased in previous year?

Name of maize variety	1 = Local 2 = Imp.	Seed quantity purchased (kg)	Month of purchase	Amount paid (GHC)	Transport charge for seed (per kg)	Name of seller
Major season						
Minor season						

Note: Varieties mean both OPVs and hybrids

43. What quantities of the following inputs did you purchase in the previous season?

Input	Quantity purchased	Month of purchase	Amount paid (LC)	Transport charge for input	Name of seller
Major season					
Basal (NPK) fertilizer (kg)					
Top dress (urea) fertilizer (kg)					
Herbicides (l)					
Insecticides (l/kg)					
Manure ( )					
Others ( )					
Minor season					
Basal (NPK) fertilizer (kg)					
Top dress (urea) fertilizer (kg)					
Herbicides (l)					
Insecticides (l/kg)					
Manure ( )					
Others ( )					

44. What quantities of the following inputs did you apply to the following varieties in crop season?

Crop	NPK (basal) (kg)	SA/Urea (top-dress) (kg)	Animal manure (carts)	Other (_____)
Major season				
Local Maize				
Improved maize				
Minor season				
Local Maize				

Improved maize				

45. Do you know the different types of maize known as improved OPV and hybrid? [1] Yes [2]

No

47. Which maize varieties have you planted over the years? [*List in order of importance in terms of planted area, and recall as good as possible the yield, especially if it failed*]

Crop season	Variety		Quantity of seed planted (kg)		Area planted (ha)		Production (kg)	
			Season 1	Season 2	Season 1	Season 2	Season 1	Season 2
2010/11	1							
	2							
	3							
	4							
	5							
	6							
2009/10	1							
	2							
	3							
	4							
	5							
	6							

See list of varieties below and indicate if you grow them or not

Varieties:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

7. _____
8. _____
9. _____
10. _____
11. _____
12. _____

Will you like to have a new (Ideal) maize variety? [1] Yes [2] No

49. List the three most important characteristics you desire in your ideal maize variety? ___ ___ ___

[1] Yield potential [2] Pest/disease resistance [3] Performance under poor soils
[4] Performance under poor rainfall [5] Superior storage pest resistance
[6] Cob size [7] Grain size [8] Cob filling
[9] Plant height [10] Yield stability [11] Resistance to lodging
[12] Early maturity [13] Drought tolerance [14] Number of cobs per plant
[15] Husk cover [16] Grain color [17] good market potential
[18] Taste [18] Other ( )

50. What grain colour will you prefer in the new variety? [1] White [2] yellow [3] mixed

What type of new (Ideal) variety would you like to have and why? [1] OPV [2] Hybrid [3] landrace

51. Have you **ever** planted any improved variety of maize during the last five years?
[1] Yes [2] No

52. If NO to question 51, why have you **never** planted any improved maize variety?
[1] S/A
[2] Not heard of any improved varieties
[3] Can't get the seeds to buy
[4] No money to buy the seeds
[5] Satisfied with the local varieties I plant
[6] Simply not interested in experimenting with new varieties
[7] Not seen any demonstration to show superiority of improved varieties
[8] Too risky
[9] Other: _____

55. What was the source of information about the improved variety?

[1] Fellow farmer [2] Local retail shop
[3] Ministry of Agric. Extension agent [4] Seed company staff
_____
[5] Staff of a Research Institute [6] NGO (specify) _____
[7] Radio [8] Television
[9] Newspaper [10] Other (specify) _____
[ ] farm group / cooperative [ ] women's group

56. What was your source of seed?

[1] Saved from last season's harvest [2] Free seed from a neighbor
[3] Free seed from government program [4] Free seed from an NGO program
[5] Purchased from a Seed company [6] Purchased from NGO
[7] Purchased from Ministry of Agriculture [8] Purchased from another farmer
[9] Purchased from market [10] Purchased at a seed fair
[11] Purchased from an agro-dealer [12] Other:
_____

57. What was the reason for your choice of seed source?

[1] N/A [2] Cheaper source [3] Available source
[4] Lack of cash [5] Near homestead [6] Free source
[7] Other: _____

F. Marketing of maize

71. Do you cultivate maize for home consumption or for selling?

1= selling 2= home consumption3=both

72. If both, what percentage of maize do you sell…….

73. Who buys your maize? 1=market women 2= kenkey sellers 3=consumers
4=government agency5=NGO 6= other…….

74. Is it difficult to sell the maize you produce 1= Yes 2=No

75. If Yes, what is the major reason for the lack of market for the rice you produce?.....

76. Do you make profits 1=Yes 2=No

Appendix 5.1 PCR PROCEDURE

Materials and Equipment

PCR machine

Taq polymerase

dNTP's

Buffer

Magnesium Chloride

DNA template

Primers

Latex gloves

Method

PCR reaction condition for Maize genotyping project Components	**Stock concentration**	**One reaction in 10ul**
Buffer	10X	1.0 ul
MgCl2	10mM	0.8 ul
dNTP's	2.5 mM	0.8 ul
Primer F&R	1.0pmoles/ul	0.2ul
TaqDNA polymerase	5.0 U/ul	0.075ul
H2O	4.725ul	
DNA	50ng/ul	1.0 ul
Final volume	**10 ul**	

Thermocycler programme

1. 94oC x 3 min
2. 94 oC x 30 sec
3. 52 oC- 60 oC x 1 min for 35 cycles.
4. 72 oC x 2.0 mins
5. 72 oC x 10 min
6. 4 oC Hold

DNA FRAGMENT ANALYSIS PROCEDURE

Materials and Equipment

PCR machine

PCR products for using various primers

LIZ-500 size standard

LIZ-1200 size standard

HIDI-formamide

PCR plates (96 well or 384 well)

Adhesive film and plates covers

Multichannel pipettes and single pipettes

Pipette tips

Latex gloves

Preparation of the cocktail mix

1. Pipette 1.0ml of HIDI into a 1.5ml eppendorf tube

2. Add 12.0ul of LIZ-500 (or 48ul of LIZ1200) size standard and mix by vortexing.

3. Aliquot 9.0ul of the mix into each well either 96well plates.

4. Add 1.2ul of PCR products.

5. Denature at 95oC for 3minutes and quickly chill in ice for 5minutes.

6. Run on the ABI-3730 after filling in the required sample sheet.

Fragment Analysis

The PCR products were run and detected on capillary system ABI-3730 using the LIZ500 as internal size standard.

Appendix 6.1 Table General ANOVA of 2013 trial across sites

Source	DF	Edcm	Vi	Vs	Yld/ha	LC	PH	ASI	CW
Environment (Env)	2	10.27119	54.40**	3.77140213	506975875*	26.2534	64734.5635	700.096397	0.151768
Block	48	0.05390749	1.60	0.121834	2760331	0.659437	566.2559**	2.135984**	0.0160048
Replication(Env)	3	0.060545	1.47	0.102039	4829669	4.0282453*	557.4189	6.889762	0.0546342
Gentypes	103	0.25450338	2.36	0.16480461	3978403***	2.6574567*	1223.608**	2.359261*	0.12403881
Gentypes*Env	206	0.041707	2.51	0.16607418	2360729	0.603898	206.6536	1.763818**	0.013076
Error	260	0.037328	2.22	0.10431	2148274	0.585837	183.4567	1.231207	0.0132673

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.2 Table of Means across site for 2012 and 2013 combined

Genotypes	Vs	Vi	LC	PH	Edcm	1000 Gwt	Yld/ha	ASI	CW
CML202/LA246	1.54	8.43	14.42	185.7	4.19	256.7	6693.	4.50	2.46
CML202/LA276	1.45	13.11	13.64	180.8	4.22	238.2	6937.	4.82	2.38
CML202/LA3	1.00	0	14.17	182	4.22	250.8	6771.	4.83	2.27
CML202/LA30	1.00	0	13.83	181.6	4.35	274.3	7240.	4.33	2.37
CML202/LA400	1.42	5.23	14.58	189.5	4.21	220.3	6164.	5.75	2.41
CML202/LA424	1.33	5.56	13.83	190.2	4.15	246.2	6798.	5.75	2.42
CML202/LA457	1.65	12.93	13.10	179.2	4.02	232.4	5571.	4.70	2.21
CML202/LA463	1.50	9.33	15.50	192.5	4.17	200.3	5975.	6.00	2.48
CML202/LA467	1.42	4.91	13.92	191.5	4.29	226.7	6439.	4.50	2.48
CML202/LA518	1.50	12.48	14.17	172.4	4.09	223.3	5208.	4.42	2.37
CML202/LA537	1.35	6.28	13.77	176.3	4.15	226.4	5982.	5.08	2.42
CML202/LA558	1.25	1.23	13.10	163.9	4.11	227.1	5861.	4.40	2.32
CML202/LA580	1.45	8.70	13.91	189.7	4.28	232.6	6591.	5.09	2.38
CML202/LA76	1.29	3.69	13.75	184.7	4.20	213.1	5773.	5.67	2.47
CML202/LA80	1.50	14.26	14.25	179.3	4.17	240.8	6229.	5.33	2.41
CML442/CML2	1.00	0	14.33	176.6	4.23	279.7	7059.	3.67	2.38
CML442/LA246	1.08	0.52	13.33	187.8	4.48	263.6	6764.	4.50	2.60
CML442/LA276	1.00	0	13.83	175.8	4.52	276.3	6680.	5.33	2.38
CML442/LA3	1.25	0.77	14.00	193.3	4.53	285.0	7455.	6.50	2.28
CML442/LA30	1.00	0	13.50	185.6	4.32	257.7	6641.	5.17	2.42
CML442/LA400	1.00	0	14.17	182.1	4.47	248.3	7327.	4.50	2.40
CML442/LA424	1.33	0.40	14.00	188.8	4.47	287.2	6243.	6.00	2.35
CML442/LA457	1.00	0	13.17	177.6	4.35	291.7	6669.	5.00	2.17
CML442/LA463	1.00	0	12.83	172.8	4.32	287.8	6657.	4.67	2.32
CML442/LA467	1.00	0	12.83	170.1	4.25	258.4	6546.	5.17	2.35
CML442/LA518	1.42	0.95	14.00	173.3	4.32	285.1	6028.	4.33	2.22
CML442/LA537	1.00	0	13.00	167.8	4.27	254.0	6028.	5.17	2.42
CML442/LA558	1.00	0	12.17	156.8	4.32	279.6	7307.	5.33	2.36
CML442/LA580	1.17	0.50	12.83	169.3	4.53	253.7	6609.	4.67	2.25
CML442/LA76	1.00	0	13.67	180.6	4.37	253.8	6320.	5.17	2.32
CML442/LA80	1.00	0	13.83	193.6	4.55	314.3	8902.	5.50	2.45
CML442/LA97	1.08	0.52	13.17	170.5	4.37	275.9	6645.	4.67	2.42
CML442/LA99	1.00	0	13.33	176.5	4.25	278.6	6050.	6.00	2.35
CML444/LA246	1.33	2.19	14.25	175.9	4.49	258.3	6045.	4.50	2.72
CML444/LA276	1.25	11.95	14.4	169.3	4.58	283.7	6508.	5.10	2.68
CML444/LA3	1.29	3.66	15.00	192.0	4.47	247.7	6099.	4.08	2.57
CML444/LA30	1.38	10.17	14.67	188.2	4.52	272.9	7818.	4.25	2.56
CML444/LA400	1.38	6.37	15.08	187.5	4.62	252.0	6273.	4.33	2.67
CML444/LA424	1.54	7.17	14.83	203.0	4.51	274.6	7037.	4.67	2.64

CML444/LA457	1.25	2.16	14.17	186.3	4.50	272.8	6518.	4.67	2.61
CML444/LA463	1.33	2.98	14.25	191.7	4.54	274.3	7000.	4.83	2.66
CML444/LA467	1.38	5.30	14.92	197.6	4.64	256.1	6609.	4.33	2.63
CML444/LA518	1.38	8.16	14.08	171.5	4.25	264.8	6819.	3.67	2.49
CML444/LA537	1.25	3.58	13.92	184.8	4.47	244.8	6284.	4.50	2.62
CML444/LA558	1.33	6.44	13.50	169.7	4.52	273.0	6110.	4.67	2.75
CML444/LA580	1.00	0	12.83	162.3	4.78	279.9	7586.	5.17	2.58
CML444/LA76	1.00	0	14.17	186.3	4.60	290.4	7554.	4.00	2.52
CML444/LA80	1.54	12.16	13.92	195.5	4.33	251.4	6128.	4.42	2.55
CML444/LA97	1.92	16.73	14.83	176.1	4.13	223.1	6235.	4.60	2.57
CML444/LA99	1.38	1.91	14.75	197.0	4.54	263.9	6424.	4.25	2.64
Enibi	2.08	17.23	13.58	160.0	4.35	228.5	5422.	4.92	2.63
Etubi	1.54	5.19	13.25	161.7	4.18	206.3	5046.	4.75	2.48
LA246	1.54	14.84	13.58	178.0	3.98	223.3	4562.	4.75	2.56
LA276	1.38	5.12	13.75	168.7	4.45	237.9	5986.	4.67	2.56
LA3	1.71	19.57	15.17	194.1	4.33	226.1	5557.	4.75	2.32
LA30	1.54	5.72	14.92	197.5	4.25	221.8	5260.	5.67	2.46
LA400	2.21	22.96	14.92	201.0	4.21	210.5	5444.	5.42	2.31
LA424	1.96	26.51	14.17	212.6	4.38	256.7	5893.	5.17	2.64
LA457	1.75	8.10	13.08	180.9	3.87	252.8	4205.	5.92	2.12
LA463	1.63	12.52	13.08	177.0	4.05	236.0	5291.	5.17	2.35
LA467	1.88	18.70	13.58	185.2	4.23	218.0	5130.	4.83	2.55
LA518	2.25	25.03	13.92	155.2	3.82	198.9	4412.	4.42	2.20
LA537	2.33	35.82	13.50	153.0	3.62	178.8	3491.	4.50	2.29
LA558	1.71	11.30	13.00	178.9	3.95	209.2	5015.	5.08	2.27
LA580	1.95	32.49	13.09	164.2	3.95	200.8	4222.	4.82	2.21
LA76	3.71	19.78	13.17	176.9	4.02	202.5	4588.	5.17	2.42
LA80	2.14	22.11	13.36	177.5	3.61	224.7	4209.	5.00	2.07
LA97	2.33	28.10	12.75	163.1	4.04	204.6	4095.	4.25	2.43
LA99	2.13	27.85	14.25	186.4	4.06	232.1	4728.	6.00	2.55
Mamaba	1.88	17.19	13.33	156.5	4.28	221.4	5173.	4.50	2.58
Obatanpa	1.77	13.30	13.58	190.3	4.62	263.9	5612.	5.00	2.85
TZEI17/LA246	1.21	1.36	13.33	174.0	4.14	235.9	6110.	4.67	2.44
TZEI17/LA276	1.17	0.90	13.58	163.6	4.28	230.6	5842.	4.58	2.44
TZEI17/LA3	1.33	4.31	14.00	179.3	4.24	242.2	6402.	5.17	2.34
TZEI17/LA30	1.04	0.74	14.00	167.6	4.22	240.1	6468.	4.58	2.41
TZEI17/LA400	1.38	4.82	13.83	180.7	4.13	224.7	6763.	5.08	2.31
TZEI17/LA424	1.29	3.46	13.67	173.4	4.22	238.4	6031.	5.17	2.50
TZEI17/LA457	1.17	1.60	13.50	175.7	4.07	251.8	7026.	4.17	2.27
TZEI17/LA463	1.29	6.43	13.33	175.5	4.11	245.8	6135.	5.17	2.34
TZEI17/LA467	1.54	7.73	13.58	168.3	4.10	228.7	6228.	4.50	2.39
TZEI17/LA518	1.38	10.92	13.50	166.0	4.09	229.0	5807.	4.17	2.31
TZEI17/LA537	1.54	6.59	13.42	164.0	4.00	225.5	4885.	5.17	2.37

TZEI17/LA558	1.25	1.58	13.17	168.4	4.13	228.8	5780.	4.75	2.38
TZEI17/LA580	1.17	3.07	13.25	170.7	4.33	233.1	6211.	4.58	2.42
TZEI17/LA76	1.17	1.11	12.92	173.3	4.18	213.6	6289.	4.67	2.43
TZEI17/LA80	1.33	4.42	13.50	182.5	4.08	242.0	7247.	4.50	2.31
TZEI17/LA97	1.54	9.15	12.67	173.1	4.28	234.6	6452.	4.83	2.47
TZEI17/LA99	1.38	7.88	13.25	169.9	4.18	241.5	5609.	5.17	2.50
TZEI17/TZEI23	1.04	0.32	12.50	146.1	4.07	224.8	4988.	5.25	2.44
TZEI23/LA246	1.58	6.59	12.67	166.9	4.18	232.8	5752.	4.58	2.51
TZEI23/LA276	1.21	2.06	12.58	162.0	4.41	235.2	6058.	4.50	2.58
TZEI23/LA3	1.50	5.39	13.83	175.9	4.40	247.2	6876.	4.83	2.44
TZEI23/LA30	1.54	4.52	13.42	166.4	4.33	224.0	5912.	5.67	2.52
TZEI23/LA400	1.63	15.01	13.20	160.2	4.20	216.8	5761.	5.27	2.37
TZEI23/LA424	1.58	12.38	12.83	165.6	4.24	235.9	5863.	5.33	2.47
TZEI23/LA457	1.54	8.35	12.67	166.5	4.25	245.0	5760.	5.08	2.41
TZEI23/LA463	1.33	5.97	12.5	162.5	4.26	234.0	5577.	5.00	2.46
TZEI23/LA467	1.75	9.32	12.75	158.0	4.11	209.0	5008.	4.92	2.42
TZEI23/LA518	1.55	9.17	12.73	151.0	4.21	213.9	5692.	4.00	2.35
TZEI23/LA537	1.83	17.76	12.75	153.1	4.20	232.9	5482.	4.83	2.44
TZEI23/LA558	1.46	8.40	12.25	170.3	4.27	241.8	5664.	4.75	2.50
TZEI23/LA580	1.77	9.29	12.36	161.6	4.34	225.2	6031.	5.55	2.47
TZEI23/LA76	1.67	12.24	12.58	158.2	4.32	216.0	5762.	5.17	2.53
TZEI23/LA80	1.79	16.66	13.25	165.5	3.97	224.2	6021.	4.75	2.14
TZEI23/LA97	1.83	17.19	12.50	163.8	4.03	222.1	5323.	4.50	2.29
TZEI23/LA99	1.46	7.01	13.17	169.5	4.27	243.8	5983.	5.25	2.45
SE	0.53	5.10	0.59	12.53	0.13	15.80	930.9	0.80	0.09
Lsd	1.48	14.17	1.63	34.81	0.37	43.92	2585.	2.23	0.24

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.3 Table of means of traits of 2013 trial across sites

Gentypes	Vs	Vi	PH	ASI	Edcm	LC	Yld/ha	CW
CML202/LA246	1.17	0.72	181.83	4.17	4.22	13.50	7206.01	2.38
CML202/LA276	1.17	1.85	165.50	3.83	4.55	13.00	6968.39	2.50
CML202/LA3	1	0	182.00	4.83	4.22	14.17	6771.49	2.27
CML202/LA30	1	0	181.67	4.33	4.35	13.83	7240.65	2.37
CML202/LA400	1	0	179.17	5.67	4.25	13.67	5948.39	2.30
CML202/LA424	1	0	193.33	5.00	4.27	13.50	7096.4	2.40
CML202/LA457	1.17	0.85	167.50	4.5	4.10	12.67	5748.06	2.18
CML202/LA467	1.25	0.50	172.00	4.33	4.37	13.17	6278.82	2.42
CML202/LA518	1.25	0.73	158.33	4.33	4.12	13.50	5790.27	2.28
CML202/LA537	1.25	1.00	160.67	4.67	4.17	12.50	6562.82	2.42
CML202/LA558	1.17	0.38	163.33	4.17	4.32	13.00	6762.74	2.35
CML202/LA580	1.00	0	173.50	4.67	4.42	12.67	7462.99	2.38
CML202/LA76	1.08	0.42	168.83	4.50	4.33	13.33	6009.26	2.40
CML202/LA80	1.00	0	174.67	4.83	4.23	13.50	6230.29	2.28
CML442/CML202	1.00	0	176.67	3.67	4.23	14.33	7059.26	2.38
CML442/LA246	1.08	0.52	187.83	4.50	4.48	13.33	6764.94	2.60
CML442/LA276	1.00	0	175.83	5.33	4.52	13.83	6680.26	2.38
CML442/LA3	1.25	0.77	193.33	6.50	4.53	14.00	7455.18	2.28
CML442/LA30	1.00	0	185.67	5.17	4.32	13.50	6641.65	2.42
CML442/LA400	1.00	0	182.17	4.50	4.47	14.17	7327.73	2.40
CML442/LA424	1.33	0.40	188.83	60.00	4.47	14.00	6243.79	2.35
CML442/LA457	1.00	0	177.67	5.00	4.35	13.17	6669.36	2.17
CML442/LA463	1.00	0	172.83	4.67	4.32	12.83	6657.62	2.32
CML442/LA467	1.00	0	170.17	5.17	4.25	12.83	6546.86	2.35
CML442/LA518	1.42	0.95	173.33	4.33	4.32	14.00	6028.99	2.22
CML442/LA537	1.00	0	167.83	5.17	4.27	13.00	6028.14	2.42
CML442/LA558	1.00	0	156.83	5.33	4.32	12.17	7307.97	2.36
CML442/LA580	1.17	0.50	169.33	4.67	4.53	12.83	6609.40	2.25
CML442/LA76	1.00	0	180.67	5.17	4.37	13.67	6320.50	2.32
CML442/LA80	1.00	0	193.67	5.50	4.55	13.83	8902.34	2.45
CML442/LA97	1.08	0.52	170.50	4.67	4.37	13.17	6645.25	2.42
CML442/LA99	1.00	0	176.50	6.00	4.25	13.33	6050.68	2.35
CML444/LA246	1.17	0.52	163.50	4.50	4.47	12.83	6045.00	2.62
CML444/LA276	1.00	0	165.33	4.33	4.87	13.67	6222.27	2.73
CML444/LA3	1.00	0	178.00	4.33	4.73	14.50	7318.19	2.63
CML444/LA30	1.00	0	191.67	4.33	4.95	14.83	8644.04	2.68
CML444/LA400	1.00	0	185.67	4.67	4.80	14.33	7568.80	2.58
CML444/LA424	1.08	0.40	194.83	4.83	4.54	14.33	7870.04	2.55
CML444/LA457	1.00	0	164.67	4.33	4.55	13.33	6914.63	2.50
CML444/LA463	1.00	0	180.33	5.17	4.57	13.00	7786.33	2.60

CML444/LA467	1.08	0.40	181.67	4.00	4.70	14.00	6235.53	2.70
CML444/LA518	1.00	0	147.17	3.83	4.27	12.67	7777.81	2.42
CML444/LA537	1.00	0	168.00	4.67	4.48	12.83	7126.78	2.55
CML444/LA558	1.00	0	166.33	4.83	4.52	12.50	6221.71	2.67
CML444/LA580	1.00	0	162.33	5.17	4.78	12.83	7586.02	2.58
CML444/LA76	1.00	0	186.33	4.00	4.60	14.17	7554.09	2.52
CML444/LA80	1.00	0	191.00	3.83	4.42	13.33	7180.66	2.48
CML444/LA99	1.42	0.98	182.00	4.83	4.57	13.83	6912.25	2.67
Enibi	1.58	1.08	150.50	4.67	4.47	13.00	6256.51	2.63
Etubi	1.17	0.60	150.67	4.33	4.33	13.17	6079.29	2.42
LA246	1.00	0	169.17	4.50	4.10	13.17	5335.52	2.53
LA276	1.00	0	152.17	4.50	4.45	13.00	6847.67	2.50
LA3	1.00	0	183.83	4.50	4.50	14.33	6383.58	2.32
LA30	1.17	0.52	192.33	5.17	4.57	14.67	6523.94	2.47
LA400	1.67	1.97	202.00	5.00	4.42	14.00	6101.53	2.28
LA424	1.17	4.17	196.83	5.17	4.50	13.83	7583.35	2.60
LA457	1.00	0	167.50	5.83	3.97	12.50	4481.56	2.08
LA463	1.08	0.72	157.33	5.67	4.10	12.50	5512.06	2.25
LA467	1.25	1.23	182.83	4.67	4.42	13.33	5625.60	2.52
LA518	1.67	1.32	146.50	4.17	3.92	13.17	4781.34	2.17
LA537	1.42	1.33	159.00	3.83	3.82	13.00	4385.79	2.33
LA558	1.25	0.37	163.67	3.83	4.02	12.33	5950.01	2.27
LA580	1.00	0	145.60	5.40	3.92	12.00	4829.65	2.16
LA76	1.00	0	167.17	4.50	4.17	12.50	5389.50	2.43
LA80	1.42	0.50	171.50	5.83	3.73	12.83	5020.69	1.98
LA97	1.75	2.50	169.83	4.17	4.23	12.67	5068.70	2.47
LA99	1.42	1.87	175.67	6.00	4.18	13.00	5644.77	2.53
Mamaba	1.50	1.20	141.50	4.33	4.28	12.33	5724.40	2.47
Obatanpa	1.50	1.38	183.33	4.50	4.78	13.17	6458.04	2.82
TZEI17/LA246	1.00	0	160.67	3.83	4.12	12.83	6452.82	2.32
TZEI17/LA276	1.17	0.45	147.5	4.33	4.30	12.33	6297.19	2.37
TZEI17/LA3	1.08	0.42	161.83	4.67	4.32	13.50	6707.79	2.27
TZEI17/LA30	1.00	0	158.17	4.00	4.28	13.50	6649.75	2.35
TZEI17/LA400	1.17	0.33	178.83	4.67	4.13	13.50	7400.75	2.20
TZEI17/LA424	1.00	0	163.00	4.33	4.18	13.17	6417.56	2.42
TZEI17/LA457	1.17	0.42	162.83	3.33	4.13	12.33	8571.00	2.22
TZEI17/LA463	1.00	0	157.67	5.50	4.12	12.17	6180.18	2.27
TZEI17/LA467	1.17	0.33	159.67	4.50	4.17	13.17	6755.61	2.37
TZEI17/LA518	1.00	0	156.00	3.67	4.00	13.00	5277.80	2.22
TZEI17/LA537	1.17	0.58	149.33	4.83	4.05	12.83	4811.56	2.30
TZEI17/LA558	1.00	0	158.50	4.33	4.13	12.83	5567.42	2.32
TZEI17/LA580	1.00	0	156.33	3.67	4.28	13.00	6288.37	2.25
TZEI17/LA76	1.00	0	166.67	4.33	4.27	12.83	6776.50	2.38

TZEI17/LA80	1.00	0	171.17	4.17	4.07	12.67	8589.15	2.18
TZEI17/LA97	1.33	0.80	164.83	4.33	4.32	12.33	7030.02	2.43
TZEI17/LA99	1.00	1.50	161.67	5.17	4.27	12.67	5903.71	2.50
TZEI17/TZEI23	1.00	0	131.67	5.83	4.13	11.83	5104.00	2.42
TZEI23/LA246	1.33	0.92	155.67	4.50	4.33	12.17	6814.32	2.53
TZEI23/LA276	1.00	0	151.17	3.83	4.50	12.67	6307.10	2.58
TZEI23/LA3	1.00	0	167.83	4.50	4.53	13.67	8092.00	2.38
TZEI23/LA30	1.33	0.37	159.00	6.00	4.43	13.17	6398.00	2.43
TZEI23/LA400	1.17	0.42	157.50	4.83	4.28	13.00	6391.25	2.33
TZEI23/LA424	1.00	0	148.00	5.00	4.30	12.17	6181.01	2.43
TZEI23/LA457	1.00	0.35	154.50	4.83	4.30	12.17	5813.63	2.37
TZEI23/LA463	1.00	0	153.50	4.50	4.35	11.83	5859.16	2.42
TZEI23/LA467	1.17	0.33	138.33	4.50	4.32	12.17	5775.81	2.47
TZEI23/LA518	1.17	0.45	142.00	3.00	4.18	12.17	5762.39	2.23
TZEI23/LA537	1.33	0.92	140.50	4.50	4.35	12.17	6215.82	2.37
TZEI23/LA558	1.17	0.50	144.00	4.33	4.27	11.5	6018.56	2.38
TZEI23/LA580	1.42	1.62	142.00	5.83	4.53	12.00	7115.56	2.45
TZEI23/LA76	1.17	0.43	146.50	5.33	4.32	11.83	6028.83	2.48
TZEI23/LA80	1.25	0.95	155.83	4.83	3.92	12.83	6537.84	2.02
TZEI23/LA97	1.25	0.73	156.00	3.83	4.32	12.00	5599.39	2.53
TZEI23/LA99	1.00	0	151.83	5.67	4.42	12.67	6423.61	2.48
SE	0.228	1.05	9.58	0.78	0.13	0.54	1036.41	0.08
Lsd	0.64	2.94	26.67	2.18	0.37	1.51	2886.20	0.23

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.4 Table of Means of 2012 trials across sites

Gentypes	Vs	Vi	Yld/ha	LC	EDcm	PH	ASI	CW
CML202/LA246	1.92	16.14	6181.10	15.33	4.15	189.67	4.83	2.53
CML202/LA276	1.80	26.63	6900.70	14.40	3.83	199.20	3.83	2.23
CML202/LA400	1.83	10.46	6380.70	15.50	4.16	200.00	5.83	2.53
CML202/LA424	1.67	11.11	6501.00	14.17	4.03	187.17	5.67	2.44
CML202/LA457	2.20	33.60	5479.10	14.00	4.04	187.80	4.33	2.35
CML202/LA463	1.50	9.33	5975.90	15.50	4.17	192.50	6.00	2.48
CML202/LA467	1.58	9.32	6601.00	14.67	4.20	211.17	4.67	2.54
CML202/LA518	1.75	24.23	4625.90	14.83	4.07	186.50	4.50	2.46
CML202/LA537	1.42	5.31	5370.20	14.83	4.06	196.00	5.33	2.39
CML202/LA558	1.38	2.51	4509.90	13.25	3.79	164.75	3.80	2.28
CML202/LA580	2.00	19.14	5545.00	15.40	4.12	209.20	4.67	2.38

CML202/LA76	1.50	6.96	5538.10	14.17	4.06	200.67	6.83	2.54
CML202/LA80	2.00	28.52	6228.70	15.00	4.11	184.00	5.83	2.53
CML444/LA03	1.58	7.33	4880.80	15.50	4.20	206.17	3.83	2.51
CML444/LA246	1.50	3.87	6045.60	15.67	4.51	188.33	4.50	2.82
CML444/LA276	1.63	29.87	6936.90	15.50	4.14	175.25	3.50	2.60
CML444/LA30	1.75	20.34	6992.20	14.50	4.09	184.83	4.17	2.43
CML444/LA400	1.75	12.74	4979.00	15.83	4.47	189.50	4.00	2.76
CML444/LA424	2.00	13.95	6204.50	15.33	4.49	211.17	4.50	2.73
CML444/LA457	1.50	4.32	6121.40	15.00	4.45	208.00	5.00	2.72
CML444/LA463	1.67	5.95	6214.70	15.50	4.52	203.17	4.50	2.72
CML444/LA467	1.67	10.20	6984.20	15.83	4.59	213.67	4.67	2.55
CML444/LA518	1.75	16.32	5860.80	15.50	4.23	196.00	3.50	2.56
CML444/LA537	1.50	7.15	5442.00	15.00	4.45	201.67	4.33	2.68
CML444/LA558	1.67	12.88	5998.40	14.50	4.53	173.17	4.50	2.84
CML444/LA80	2.08	24.31	5076.50	14.50	4.24	200.17	5.00	2.61
CML444/LA97	1.92	16.73	6235.40	14.83	4.13	176.17	3.83	2.57
CML444/LA99	1.33	2.84	5937.70	15.67	4.51	212.17	3.67	2.61
LA03	2.42	39.15	4731.20	16.00	4.16	204.50	5.00	2.33
LA246	2.08	29.69	3788.80	14.00	3.85	186.83	5.00	2.59
LA276	1.75	10.25	5125.40	14.50	4.45	185.33	4.83	2.61
LA30	1.92	10.92	3996.80	15.17	3.94	202.67	6.17	2.44
LA400	2.75	43.95	4787.90	15.83	4.01	200.00	5.83	2.33
LA424	2.75	48.85	4204.50	14.50	4.25	228.50	5.17	2.68
LA457	2.50	16.19	3928.60	13.67	3.78	194.33	6.00	2.15
LA463	2.17	24.32	5071.00	13.67	3.99	196.83	4.67	2.45
LA467	2.50	36.18	4634.80	13.83	4.04	187.67	5.00	2.59
LA518	2.83	48.73	4044.10	14.67	3.71	164.00	4.67	2.24
LA537	3.25	70.30	2597.10	14.00	3.43	147.17	5.17	2.24
LA558	2.17	22.23	4081.60	13.67	3.87	194.17	6.33	2.28
LA580	2.75	59.57	3715.70	14.00	3.97	179.83	4.33	2.25
LA76	6.42	39.56	3788.30	13.83	3.88	186.67	5.83	2.41
LA80	3.00	48.04	3236.20	14.00	3.46	184.80	3.33	2.16
LA97	2.92	53.69	3123.20	12.83	3.82	156.50	4.33	2.38
LA99	2.83	53.83	3812.70	15.50	3.94	197.17	6.00	2.56
TZEI17/LA03	1.58	8.20	6097.10	14.50	4.16	196.83	5.67	2.42
TZEI17/LA246	1.42	2.73	5768.80	13.83	4.16	187.33	5.50	2.55
TZEI17/LA276	1.17	1.35	5388.50	14.83	4.25	179.83	4.83	2.50
TZEI17/LA30	1.08	1.47	6288.10	14.50	4.15	177.17	5.17	2.46
TZEI17/LA400	1.58	9.30	6127.20	14.17	4.12	182.67	5.50	2.41
TZEI17/LA424	1.58	6.93	5645.80	14.17	4.25	183.83	6.00	2.58
TZEI17/LA457	1.17	2.78	5481.10	14.67	4.01	188.67	5.00	2.33
TZEI17/LA463	1.58	12.86	6091.00	14.50	4.09	193.50	4.83	2.40
TZEI17/LA467	1.92	15.12	5701.10	14.00	4.03	177.00	4.50	2.42

TZEI17/LA518	1.75	21.85	6337.50	14.00	4.17	178.00	4.67	2.41
TZEI17/LA537	1.92	12.61	4959.40	14.00	3.94	178.83	5.50	2.44
TZEI17/LA558	1.50	3.15	5994.00	13.50	4.13	178.33	5.17	2.43
TZEI17/LA580	1.33	6.15	6134.50	13.50	4.38	185.17	5.50	2.58
TZEI17/LA76	1.33	2.23	5802.50	13.00	4.09	180.00	5.00	2.47
TZEI17/LA80	1.67	8.85	5905.60	14.33	4.10	193.83	4.83	2.43
TZEI17/LA97	1.75	17.50	5875.60	13.00	4.24	181.50	5.33	2.51
TZEI17/LA99	1.75	14.27	5314.40	13.83	4.08	178.17	5.17	2.50
TZEI23/LA03	2.00	10.79	5661.70	14.00	4.26	184.00	5.17	2.50
TZEI23/LA246	1.83	12.27	4690.00	13.17	4.03	178.17	4.67	2.48
TZEI23/LA276	1.42	4.12	5809.00	12.50	4.33	172.83	5.17	2.57
TZEI23/LA30	1.75	8.68	5427.80	13.67	4.22	173.83	5.33	2.61
TZEI23/LA400	2.08	29.61	5132.50	13.40	4.11	163.00	4.83	2.41
TZEI23/LA424	2.17	24.76	5545.70	13.50	4.18	183.33	5.67	2.51
TZEI23/LA457	2.08	16.35	5706.70	13.17	4.19	178.50	5.33	2.46
TZEI23/LA463	1.67	11.94	5296.30	13.17	4.16	171.67	5.50	2.50
TZEI23/LA467	2.33	18.31	4240.60	13.33	3.89	177.83	5.33	2.37
TZEI23/LA518	2.00	19.62	5608.90	13.40	4.23	161.80	4.33	2.50
TZEI23/LA537	2.33	34.60	4748.30	13.33	4.04	165.83	5.17	2.50
TZEI23/LA558	1.75	16.30	5310.60	13.00	4.28	196.67	5.17	2.61
TZEI23/LA580	2.20	18.51	4730.50	12.80	4.11	185.20	5.20	2.50
TZEI23/LA76	2.17	24.04	5496.00	13.33	4.33	170.00	5.00	2.58
TZEI23/LA80	2.33	32.36	5506.10	13.67	4.03	175.17	4.67	2.26
TZEI23/LA97	2.42	33.64	5046.60	13.00	3.75	171.67	5.17	2.04
TZEI23/LA99	1.92	14.01	5543.50	13.67	4.12	187.17	4.83	2.41
MAMABA	**2.25**	**33.19**	**4621.90**	**14.33**	**4.28**	**171.50**	**4.67**	**2.70**
OBATANPA	**2.00**	**25.22**	**4766.50**	**14.00**	**4.46**	**197.33**	**5.50**	**2.89**
ENIBI	**2.58**	**33.37**	**4589.30**	**14.17**	**4.22**	**169.50**	**5.17**	**2.62**
ETUBI	**1.92**	**9.78**	**4013.10**	**13.33**	**4.03**	**172.83**	**5.17**	**2.54**
TZEI17/TZEI23	**1.08**	**0.64**	**4874.00**	**13.17**	**4.01**	**160.67**	**4.67**	**2.46**
LSd	**2.11**	**18.65**	**1727.60**	**1.47**	**0.33**	**38.27**	**2.35**	**0.24**
SE	**0.76**	**6.69**	**619.31**	**0.53**	**0.12**	**13.72**	**0.84**	**0.08**

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.5 General combining ability effects of various traits of lines and testers evaluated across environments for the 2013 seasons

Line	Vs	Vi	PH	ASI	Edcm	Yld/ha	1000Gwt	LC	CW
LA246	0.09	0.35*	1.01	-0.05	-0.09*	-170.27	-4.88	-0.11	0.05045*
LA276	-0.03	0.15	-6.29**	-0.32	0.16**	-197.36	7.38	-0.014	0.09711*
LA03	-0.03	-0.07	7.31**	0.38	0.11**	490.95	5.22	0.79**	-0.026
LA30	-0.03	-0.24	10.11**	0.05	0.12	614.53*	8.83	0.75**	0.044
LA400	-0.06	-0.25	8.78**	0.28	0.06	280.89	-13.49**	0.68**	-0.0329
LA424	0.02	-0.07	10.75**	0.38	-0.02	62.76	5.08	0.45	0.014
LA457	-0.03	0.01	-2.69	-0.25	-0.07	54.65	13.21**	-0.38	-0.10622*
LA463	-0.09	-0.31*	0.05	0.31	-0.02	-61.82	10.17*	-0.62*	-0.003
LA467	0.01	-0.06	-2.32	-0.15	-0.001	-358.75	-10.30*	0.02	0.047*
LA518	0.11*	0.18	-12.35**	-0.82*	-0.18**	-503.99	-5.10	-0.05	-0.10955*
LA537	0.06	0.19	-9.45**	0.05	-0.12**	-622.52*	-8.29	-0.41*	0.007
LA558	-0.03	-0.14	-9.89**	0.02	-0.04	-271.35	-0.22	-0.65**	0.004
LA580	0.02	0.11	-7.69**	0.08	0.13**	176.07	-8.22	-0.48*	-0.026
LA76	-0.04	-0.14	3.51	0.08	0.039	42.68	-12.61*	0.12	0.017
LA80	-0.08	-0.25	10.75**	-0.38	-0.09*	876.77**	12.24*	0.120	-0.08955*
LA97	0.13	0.37*	-3.54	-0.37	-0.03	-251.99	-9.53*	-0.58*	0.058*
LA99	0.01	0.31*	0.68	0.77**	0.01	-354.32	10.71*	0.05	0.097*
SE	0.05	0.18	2.38	0.27	0.04	273.91	4.88	0.13	0.025
Lsd 0.05	0.10			0.53	0.08	539.53	9.61		
Lsd 0.01	0.13			0.70	0.10	711.11	12.67		
Tester									
CML202	0.01	0.15	5.70**	-0.09	-0.08*	-99.99	-10.77**	0.21**	-0.05051*
CML442	-0.02	-0.10	10.50**	0.51**	0.03	75.35	9.42*	0.31**	-0.05041*
CML444	-0.05	-0.17	8.23**	-0.17	0.26**	508.37**	25.07**	0.48**	0.190*
TZEI17	-0.02	-0.03	-6.46**	-0.32	-0.18**	-107.64	-10.87**	-0.22**	-0.09014*
TZEI23	0.07	0.16	-16.49**	0.05	-0.03	-363.10*	-12.93**	-0.72**	0.003
SE	0.04	0.14	1.84	0.19	0.03	190.16	3.76	0.08	0.011

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.6 Mean squares of combined ANOVA for various traits of lines and testers evaluated across environments for the 2013 season

Source	Df	Vs	Vi	PH	ASI	LC	Edcm	1000Gwt	Yld/ha	CW
Rep	1	0.08	0.08	24.90	6.45	2.68	0.22*	264.33	556880.2	0.03
env	2	26.90**	26.90**	50233.26**	539.86**	19.31**	7.34**	95501.87**	395069074.3**	0.08**
Line	16	1.19	1.19	1700.61***	3.78	6.01***	0.26**	2238.03**	4518350	0.12**
Tester	4	1.91	1.91	12912.56**	10.11	21.33**	2.58**	25637.07**	9427260	1.22**
Env*Line	32	1.04	1.04	180.81	2.29	0.55	0.04	759.68**	2391447	0.02
Env*Tester	8	2.64	2.64	432.99	4.96	0.87	0.12**	1801.74***	4610606	0.02
Line*Tester	60	0.96	0.96	291.67	1.45	0.76	0.06	1036.07*	2871490	0.03**
Env*Line*Test	11	1.00	1.00	227.11	1.53	0.65	0.04	668.81***	2713851	0.02
Error	24	0.97	0.97	215.74	1.39	0.64	0.04	427.4491	2365234	0.02

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width

Appendix 6.7. General combining ability effects of various traits of lines and testers evaluated across environments for the 2012 season

Line	ASI	Edcm	LC	Vi	Vs	Yld/ha	PH	CW
LA03	-0.85323*	0.02487	0.40147*	-5.09645*	-0.02433	-166.424	8.9512*	-0.02498
LA246	0.3551	0.03264	0.23481	-5.11381*	-0.07989	-41.592	-0.8405	0.09308*
LA276	-0.18657	-0.02878	-0.07472	-0.27399	-0.27037*	450.667*	-5.144	-0.02752
LA30	-0.85323*	-0.02736	-0.04297	-3.70256	-0.21878*	523.097*	-8.1044*	-0.00164
LA400	0.52177	0.03431	0.51742*	1.65952	0.06594	-58.105	-2.9238	0.02308
LA424	0.93843*	0.05931	0.02647	0.32035	0.10761	261.294	4.6595	0.06141*
LA457	0.39677	-0.0033	-0.0478	-0.4459	-0.02917	-6.399	4.1541	-0.03406
LA463	0.68843*	0.05431	0.40147*	-3.84631	-0.14239*	181.49	3.4929	0.02225
LA467	0.27177	-0.00236	0.19314	-0.62715	0.12844*	168.758	8.2012*	-0.03192
LA518	-0.2699	-0.00851	0.21307	6.67976*	0.05779	-104.728	-5.17	-0.02362
LA537	0.56343	-0.05819	0.02647	1.04869	0.04511	-582.986*	-1.1321	-0.00025
LA558	0.17575	0.03658	-0.67428*	-4.59226*	-0.15565*	-173.969	-7.2609	0.06088*
LA580	-0.37704	0.03306	-0.39019*	0.20223	0.06594	-201.412	5.972	-0.01025
LA76	0.07101	-0.0218	-0.76519*	-2.79034	-0.07989	-100.769	-3.1599	0.02836
LA80	0.56343	-0.05986	0.10981	9.64494*	0.27428*	-33.734	1.5762	-0.04609
LA97	-0.93657*	-0.13958	-0.65408*	8.75744*	0.28122*	6.23	-10.271*	-0.12942*
LA99	-1.10323*	0.0582	0.1237	-3.4959	-0.07989	-114.422	5.7845	0.00613
SE	0.3213	0.037249	0.13337	2.43349	0.061975	197.69	3.91781	0.026535
Tester								
CML202	-0.62881*	-0.10918*	0.47453*	1.68383	-0.01368	151.138	6.7914*	-0.0581
CML444	-0.63215*	0.19442*	0.97344*	-1.67317	-0.05906	259.615	9.7163*	0.14588*
TZEI17	0.66637*	-0.04187	-0.24559	-5.19952	-0.21224*	105.409	-3.0917	-0.04099
TZEI23	0.56921*	-0.04869	-0.96216*	5.5467*	0.27844*	-446.31	-10.3855*	-0.04415
SE	0.22748	0.039017	0.056176	2.74729	0.042381	225.389	2.07588	0.027754

ASI= anthesis-silking interval, Edcm= Ear diameter in centimeter, 1000Gwt= 1000 grain weight, PH= Plant height, LC= leaf count, Vi= Virus disease incidence, Vs= Virus disease severity, Yld/ha= grain yield per hectare CW= cob width