BREEDING INVESTIGATIONS ON UTILITY OF MAIZE STREAK VIRUS RESISTANT GERMPLASM FOR HYBRID DEVELOPMENT IN THE TROPICS

by

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THESIS ABSTRACT

Maize (*Zea mays* L.) supports millions of livelihoods in sub-Saharan Africa (SSA) in terms of food and feed. Production of the crop is however limited by several factors, among these, maize streak virus (MSV) disease. Although extensively studied, MSV remains a serious problem in SSA due to several challenges in breeding MSV resistant maize varieties. These include integration of MSV resistant germplasm from different backgrounds, reliance on a few resistant sources, and genotype x environment interactions. This study was designed to assess the breeding potential of several MSV resistant lines in hybrid combinations. Understanding architecture of genetic divergence and background of these genotypes would greatly aid in breeding high yielding and stable MSV resistant hybrids. Experiments were conducted during 2010 to 2012 seasons in Kenya. Diallel crosses and SSR markers were used to characterize MSV resistant maize inbred lines from three programs of CIMMYT, KARI and IITA.

In general, this study revealed that MSV is still an important problem in Kenya with high incidence and severity levels in the farmers' fields. The levels of MSV resistance in locally grown hybrids needs to be improved. Farmers challenged breeders to develop new hybrids that combine early maturing, high yield potential and MSV resistance.

The study was successful in identifying the best eight inbred lines for use in breeding new maize hybrids with MSV resistance. The nature of gene effects was established for the first time, in particular the role of epistasis and G x E in conditioning MSV resistance in hybrids. Results indicate serious implications for previous models that ignored epistasis in studying MSV resistance in maize. The inbreds Z419, S558, CML509 and Osu23i, displayed high levels of epistasis for MSV resistance. Unless strong sources of MSV resistance, such as MUL114 and CML509, are used, breeding resistant hybrids will require parents that carry dominant resistance genes. The additive-dominance model was adequate to explain northern leaf blight (NLB) resistance in hybrids, indicating fewer complications in breeding NLB resistant hybrids.

The study also reveals that SSR genetic distance data can be used to predict hybrid performance, especially when the correct set of markers is used. Many previous studies have not found any significant relationship between genetic distance and heterosis, due to large G x E and use of a wrong set of markers. The diallel analysis and SSR data established the important heterotic groups, which will be exploited for efficient development of MSV resistant maize hybrids. These strategies will be recommended to programs that emphasize MSV resistance in maize hybrids.

DECLARATION

I, Lilian Njeri Gichuru, declare that:

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

1. This thesis has not been submitted for any degree or examination at any other university.
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……Date……..

GICHURU Lilian Njeri

As the candidate's supervisors we agree to the submission of this thesis

…….....….Date……..……..

Professor John Derera (Supervisor)

……Date…….

Professor Pangirayi Tongoona (Co-Supervisor)

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This Work is dedicated to My Father HUMPHREY GICHURU NJUGUNA

To My Mother RUTH WANJIRU GICHURU

To My Sisters SUSAN NYAMBURA and ELIZABETH WANJIRU GICHURU

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Introduction to Thesis

1. Background

Maize (*Zea mays*. L) is among the most important cereals globally along with wheat and rice, providing basic diet to millions of people in sub-Saharan Africa (SSA). Maize provides 35.2% of the total calories intake in Kenya, which is the highest in East Africa, followed closely by Tanzania (34.1%), with Uganda having the lowest (9.2%). Maize yields in Kenya are however low at 1.67 t ha^{-1} which is almost half of the yield realized in South Africa (3.17 t ha^{-1}), the latter has the highest maize grain yield in Africa (FAOSTAT, 2010). Therefore, maize yields in larger parts of SSA have remained too low to meet demand. Breeders among other partners have a great challenge to ensure increased and sustained yields for food security in the midst of the growing population and climate change in the region. To improve maize productivity, measures have to be taken to reduce the yield gap that causes frequent deficits and severe food shortages. Constraints contributing to significant yield and economic losses in SSA annually include drought, low soil fertility, pests and diseases (Cairns et al., 2012). Insect pests and diseases are, however, defined within geographic and ecological boundaries and therefore management should be prioritized for the most prevalent and most severe.

Several maize diseases are endemic in eastern and southern Africa. These include turcicum leaf blight caused by *Exserohilum turcicum*, common rust (*Puccinia sorghi*), MSV caused by maize streak Mastrevirus, grey leaf spot (*Cercospora Zea maydis*), ear rots (*Fusarium* and *Diplodia*), head smut (*Sporisorium reilianum*) and Phaeosphaeria leaf spot (PLS) caused by *Phaeosphaeria maydis* (Vivek et al., 2010). Recently there has been a new maize disease in the eastern African region, maize lethal necrotic virus (Wangai et al., 2012). The incidence and severity of most of these diseases can be reduced by chemical control methods ranging from seed dressing to foliar spraying; but host plant resistance provides the most economical management option. Climatic conditions across SSA are largely diverse and as such these diseases can occur and express inconsistently and sometimes simultaneously (Vivek et al., 2010) that epidemics can rarely be predicted. Further, as maize production intensifies even in unsuited areas, disease pressure is likely to increase, impacting on total yield losses. Resistant varieties are therefore required in the regions the diseases occur. Thus, there is need to identify and screen adapted and new maize germplasm for resistance to diseases and assess their usefulness in high yielding and adapted hybrid varieties. This study focuses on maize streak virus (MSV) which occurs mainly in the mid-altitude/subtropical and tropical lowlands

environments and northern leaf blight which is typically found in the highland and moist transitional areas of SSA.

Maize streak virus is becoming an increasing threat in Africa especially as maize area increases and agricultural production intensifies (necessitating successive cropping); practices that facilitate the over-wintering of both the virus and the vectors. Some agronomic practices have been recommended for reducing MSV incidence including, crop rotation, timely planting, seed treatment with systemic insecticides, and removal of infected plants (Mawere et al., 2006). However, some of these practices are not tenable for smallholder farmers. Sources are available in different regions but local response to MSV is required. Information on genetic variability and inheritance of resistance will guide effective selection procedures. Development of high yielding maize varieties that are resistant to MSV is a viable option to minimizing yield losses in maize.

Northern leaf blight (NLB) is increasing and becoming endemic in the eastern Africa region affecting not only grain yield due to high defoliation of maize plants but also fodder availability. Several sources of resistance to NLB have been identified in the region for the control of this disease but high susceptibility has been reported in several commercial hybrids in Kenya (Njoroge and Gichuru, 2013). Therefore, more sources of resistance have to be identified and inheritance of NLB resistance investigated.

The concept of heterotic groups and patterns has been widely used to simplify maize breeding and is critical for maximizing expression of heterosis in hybrids (Tracy and Chandler, 2006). Crossings between representative individuals of different heterotic pools maximize heterozygosity, heterosis and yield stability of the new maize cultivars. Maize inbred lines can be assigned to heterotic groups based on either pedigree, estimates of specific combining ability (SCA) (Fan et al., 2001) and genetic divergence based on molecular marker data (Pinto et al., 2003).

Efficiency in breeding programs is heavily dependent on sustainable exploitation of genetically diverse germplasm especially if this germplasm presents high values in traits of interest (Benchimol et al., 2000, Reif et al., 2003b). Genetic diversity using SSR markers would provide a useful guide for selecting specific germplasm with distinct genetic background for diversifying MSV resistance breeding. A carefully chosen set of SSR markers providing genome-wide coverage will facilitate an unbiased assay of genetic diversity, thus giving a robust unambiguous description of maize inbred lines. Some studies have shown that molecular markers can be

efficient in assigning lines into heterotic groups but the prediction of yield performance of single crosses is still doubtful (Benchimol et al., 2000, Lanza et al., 1997). Thus genetic divergence based on molecular markers has been viewed as inadequate for designing best performing hybrids (Oliboni et al., 2012). However, reliable prediction of the magnitude of heterosis based on molecular marker genetic distance will have significant impact on efficiency of a maize breeding programme.

Per se performance of parents does not always reflect good or poor combiners in maize breeding. Improvement of F_1 hybrids generally relies on identification of good combining abilities of parents that belong to unrelated heterotic backgrounds. General and specific combining ability (GCA and SCA, respectively) estimates are dependent on the particular set of materials (inbreds, populations or varieties) included in the test, therefore any new germplasm introduced in a breeding programme have to be tested for GCA and SCA effects (Hallauer and Miranda, 1988). Extraction of useful alleles from diversely sourced germplasm can present difficulties due to poor adaptation and agronomic deficiency. Potential utility of diversely sourced MSV germplasm can be assessed with some certainty based on combining ability. Different kinds of gene action and their interactions control the inheritance of various plant attributes in maize. It is necessary to investigate genetic control of MSV, NLB, grain yield and other important agronomic traits to fully exploit these sources of resistance in breeding hybrids.

The outstanding feature of maize production areas in SSA is the variation experienced between seasons and locations even within short distances. This would contribute to complicated genotype x environment (G x E) interactions. Studies on the magnitude and patterns of G x E interaction effects of specific sets of germplasm should be an integral part of varietal development process to identify genotypes with broad or specific adaptation (Epinat-Le Signor et al., 2001). Assessment of stability for suitable varieties is becoming a continuous process as environmental conditions change and maize production expands to new ecologies (Tefera et al., 2013). Further, G x E analysis would develop essential information on the pattern of adaptation in breeding lines. Successful maize cultivars should produce good yields under common production constraints (Vivek et al., 2010). Moreover, evaluation of disease resistance in varying environmental conditions may provide indications of stable resistance.

2. Rationale for research focus

Improving grain yield and foliar disease resistance are major objectives in maize improvement programs in SSA where livelihoods of resource poor farmers depend on successful maize production. In spite of the efforts of breeding of varieties against MSV disease, sporadic outbreak of MSV have continued to occur in much of Africa, with significant yield losses. The disease is ecologically versatile occurring sporadically following climatic instabilities. Selection based on disease resistance only may not always be adequate for new maize hybrids. The choice of breeding strategy for incorporating resistance to MSV into agronomically acceptable cultivars depends on the type and magnitude of gene effects.

The impact of climate change on environmental conditions such as rainfall, temperature and relative humidity are likely to have an impact on the prevalence of plant diseases. In Kenya, maize streak virus is known to be restricted to the central highlands, coastal lowlands and the lake region (Mwangi, 1998). However, climate change induced geographical distribution of insect vector may result in the disease being reported in new regions causing problems to maize production. The long-term success of breeding for disease resistance including MSV and NLB, will depend on a more in-depth and clear understanding of availability, diversity and type of genetic resistance involved. While the genetics of resistance for MSV and NLB have been explored widely, maize breeding programmes in Africa need resistance combination patterns supporting their usefulness in high yield breeding. Further, the role of epistasis in the control of resistance to these diseases is unclear. The successful introgression of different resistance factors into locally adapted germplasm, combined with already available resistance factors, will be instrumental in forming combinations that are more durable of resistance to diseases.

The genetic variability in the base populations for developing new varieties needs to be broadened for sustaining genetic improvement and reducing genetic vulnerability to abiotic and biotic stresses. Exploitation of heterosis in maize breeding for hybrid varieties is a well-established approach for yield increment. Genetically divergent germplasm plays a great role for the phenomenon of heterosis to occur, and contributes to yield performance and stability of elite germplasm. Reliable estimates of genetic distances among lines allows genotypic classifications which aids in assigning of lines into heterotic groups (Lu et al., 2009). The future of maize breeding in Kenya will therefore depend on the discovery or introduction of new gene combinations and heterotic patterns that are more efficient in adapted germplasm. A wide diversity within the maize germplasm of the mid-altitude breeding programmes remains

unexploited resulting in the use of very few MSV resistant and heterotic sources to develop new commercial hybrids to increase the yields further.

The results from the current study will feed into the strategy for conserving genetic resources, which is important for future gains in the maize breeding programmes in Kenya, amidst climate change. Availing genetic resistance to maize streak virus and northern leaf blight in commercial varieties would further permit the production of maize in areas that would otherwise be non-profitable because of these diseases. Heterosis coupled with improved agronomic management and stress tolerance forms the basis of maize breeding. In this case, yield will be enhanced if foliar disease resistance is bred into materials that are heterotic.

3. Research objectives

The specific objectives of this study were:

1. To assess maize streak and other maize production constraints with farmer perceptions in central Kenya.
2. To phenotypically characterize diverse genotypes for MSV disease response and assess disease progression.
3. To investigate genetic diversity of 40 diverse lines with simple sequence repeat (SSR) markers.
4. To investigate relationship of gene action for grain yield and other agronomic traits and relationship of genetic distance by SSR markers with F_1 performance for grain yield.
5. To assess gene action and the role of epistasis in inheritance of MSV and NLB resistance.
6. To assess grain yield stability of F_1 maize hybrids developed from a 12 x 12 maize half-diallel cross.

4. Research hypotheses

To achieve high genetic gains with the available germplasm, and to improve resistance to field protection levels, the following assumptions were made.

1. Farmers are aware of the most serious production constraints in their production zones and can provide succinct focus for agricultural development and the breeding research agenda.

2. Selected inbreds have genes for resistance to MSV which can be transmitted effectively to progeny and that disease progress is uniform among genotypes
3. The inbreds have substantial genetic divergence that can be exploited for breeding for MSV disease resistance and grain yield performance.
4. The diverse maize accessions have potential of improving grain yield and its associated traits and that heterotic patterns can be delimited through diallel mating system and SSR marker based genetic distances
5. Inheritance of MSV resistance is controlled by simple mechanisms that can be introgressed into susceptible backgrounds and/or stacked into an elite line for hybrid formation
6. Hybrids with high MSV resistance are high yielding and have stable yield performance and wide adaptation. The selected inbred lines have stable performance across the target regions, which can be transmitted to stable and highly adapted F_1 hybrids.

5. Outline of thesis

The specific objectives mentioned in the foregoing were achieved and addressed in the various chapters that constitute this thesis. Each chapter is an independent, potential manuscript for journal publication and as such overlapping of content and reference may be inevitable. The referencing system used in the chapters is based on the journals of the American Society for Agronomy, the Soil Science Society of America, and the Crop Science Society of America. The chapters are divided as follows:

1. Introduction to thesis.
2. Chapter 1: Literature review.
3. Chapter 2: Maize streak and other maize production constraints with farmer perceptions in central Kenya.
4. Chapter 3: Phenotypic characterization of maize inbreds for maize streak virus and disease progression in Kenya.
5. Chapter 4: Molecular characterization of maize streak virus resistant germplasm using SSR markers.
6. Chapter 5: Gene action for grain yield and associated traits and prediction of maize single cross performance using SSR markers.

7. Chapter 6: Genetic analysis and role of epistasis in resistance to maize streak virus and northern leaf blight in tropical maize lines.
8. Chapter 7: Genotype x environment interaction and stability analysis of half-diallel crosses in maize.
9. Chapter 8: General Overview.

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CHAPTER 1

Literature review

1.1 Introduction

This review covers aspects relevant to research focus with emphasis on disease resistance breeding in tropical maize germplasm to create a frame of reference for the research study. The importance of maize streak virus and northern leaf blight diseases in the region are discussed including genetics and breeding strategies for these diseases. Tools used for breeding decisions including combining ability analyses, molecular marker analyses and stability analyses are also reviewed.

1.2 Importance of maize

Maize (*Zea mays* L.) belongs to the grass family Poaceae and tribe Maydeae. The maize plant has characteristics of wide adaptability in the different ranges of conditions. Thus, it is one of the world's three most important cereals along with wheat and rice. Maize is the major staple food crop in sub-Saharan Africa (SSA) comprising a significant part of the diet to millions of people in the region. In the region approximately 15 million ha are planted annually across all major agro-ecological zones up to 2400 m above sea level (asl) (Twumasi-Afriyie et al., 2001)

Maize in Kenya is currently grown on 2.26 million hectares with a production of 3.6 metric tons (MoA, 2013). In Kenya, maize ranks first in total area coverage (2.26 M Ha) among the cereals compared to wheat (0.15 M Ha) and rice (0.03 M Ha), however, in terms of production per unit area nationwide, maize (1.53 t ha^{-1}) comes third after rice (5.3 t ha^{-1}) and wheat (2.9 t ha^{-1}) (MoA, 2013). Therefore, maize is plagued by many production constraints and production is not intensified as in the other major cereals. The highest grain yield, 6 t ha^{-1}, is achieved in the high potential areas which are in the highlands but current production elsewhere stands at 1.6 t ha^{-1} (FAOSTAT, 2010). Research efforts in Kenya should focus on ensuring maize productivity growth of 1.5 to 2% per year by intensifying production in existing areas (De Groote et al., 2005), since the area planted with maize in Kenya seems to have reached a stagnation point (Mbithi and Van Huylenbroeck, 2000). The ministry of Agriculture in Kenya in 2012 listed the key constraints affecting maize production as the following: maize lethal necrosis, maize streak virus, head smut, maize stem borers and Striga weed. Other constraints included use of uncertified seeds, prolonged rainfall during harvest contributing to high post-harvest losses and

lack of ready markets (MoA, 2013). The present study focuses on maize streak and northern leaf blight diseases.

1.3 Maize streak virus (MSV) disease

Maize streak virus (MSV) disease ranks third in economically damaging maize foliar diseases world-wide, after northern leaf blight and grey leaf spot (Martin and Shepherd, 2009). In Africa, however, MSV is economically the most important foliar disease of maize (Pingali and Pandey, 2001). MSV is the most significant type member of the Mastrevirus genus of the taxonomic family *Geminiviridae* (Willment et al., 2001) although recently, a new species from the genus Mastrevirus has been isolated, called maize streak Réunion virus (Pande et al., 2012). Geminiviruses are plant viruses with a circular single stranded DNA genome of approximately 2.7 kb, with characteristic geminate virions (Pratt and Gordon, 2006).

1.3.1 The distribution of maize streak virus disease

Maize streak is reported as widely distributed in SSA from Sudan to South Africa and Kenya to Senegal and in adjacent islands, including Mauritius, Réunion, Madagascar, Sao Tomé and Principe (Figure 1.1) (Thottapilly et al., 1993).

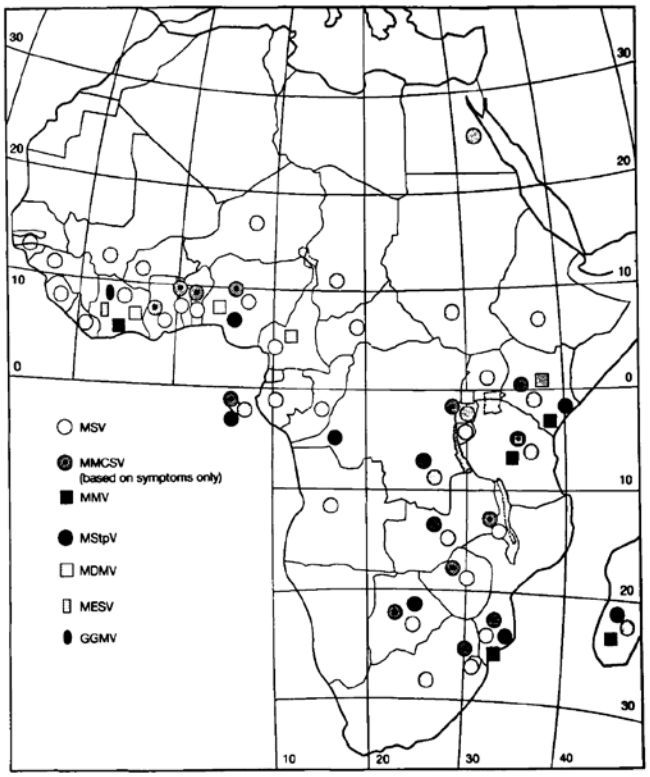

Figure 1.1. Distribution of MSV disease and other viral diseases of maize in Africa
Source: Thottapilly et al. (1993).

1.3.2 Economic importance of maize streak virus

There is a close relationship between plant age, time of infection, and the yield losses that accrue due to maize streak disease (Bosque-Perez et al., 1998). Doyle and Autrey (1992) reported a 91% reduction in grain production when plants were infected at 3-5 leaf and a 65% reduction when infection occurred at 7-10 leaf stage. Yield reduction in susceptible varieties has been noted to exceed 70% (Bosque-Perez et al., 1998). Recently, Magenya (2009) reported yield losses estimates of between 30-40% in geographically separated maize producing regions of Kenya. During the 1980s, several epidemics were reported in African countries (Kim et al., 1989, Malithano et al., 1997, Rossel and Thottapilly, 1985), including Kenya. However, these outbreaks have been erratic and very difficult to predict. Whilst MSV eradication is infeasible, development of immune genotypes could result in 5-20% annual yield increases in a country such as Kenya translating to between US$ 4 M and US$ 15 M (Martin and Shepherd, 2009).

1.3.3 Disease symptoms

Maize streak disease first presents as round yellow spots scattered on the youngest leaves and subsequently streaks develop at an increasing density (Thottapilly et al., 1993). Symptoms vary from severe narrow, broad to chlorotic streaks centred on secondary and tertiary leaf veins and uniformly distributed on the leaf surface. Infection of seedlings of susceptible plants causes chlorosis of entire leaves, shortening of stalk internodes, poor cob formation and sometimes complete necrosis of the plant. Chlorosis results from failure of chloroplasts to develop in the tissue surrounding the vascular bundles (Fajemisin et al., 1976). Very substantial damage including total yield loss has been observed in maize crops.

Standard terms, ‘complete resistance’ have been used to refer to symptom-free plants in which virus multiplication is totally prevented and ‘partial resistance’ referring to plants that exhibit symptoms, but to a lesser extent than the susceptible control, and in which virus multiplication is partially suppressed (Rodier et al., 1995). Detection of the virus is achieved using enzyme-linked immunosorbent assay (ELISA) to determine virus concentration in the leaf. Salaudeen et al. (2010) reported positive correlation between symptoms and virus titre in plants. Symptom rating is usually done on a scale of 1 (symptomless) to 5 or 9 (severe streaking on 75% or more of the leaf area, plants severely stunted, dying or dead) (Tefera et al., 2013). Besides visual score rating image analysis technique has also been used to estimate chlorotic lesions in symptomatic plants (Martin et al., 1999).

1.3.4 Disease transmission

Maize streak is obligately transmitted by leafhoppers of the *Cicadulina* species in a persistent manner (Rose, 1978). There are 22 recognized species of *Cicadulina*, 18 of which occur in Africa (Webb, 1987). Nine species have been shown to be important vectors: *C. mbila. C. similis, C. arachidis, C. storeyi* (= *triangula*), *C. bipunctata, C. latens, C. ghaurii, C. niger, and C. parazeae* (Dabrowski, 1987, Fajinmi et al., 2012, Okoth et al., 1987, Webb, 1987). The efficiency in virus transmission of *C. mbila* is reported in several African countries including, Kenya, Tanzania, Ethiopia, South Africa (Asanzi et al., 1995). In Nigeria *C. storeyi* was found to have higher transmission efficiency than *C. mbila* (Oluwafemi et al., 2007). Within the populations of *C. mbila,* there are genetically distinguishable active and inactive vector individuals with differences in ability to transmit the virus (Alegbejo et al., 2002). The percentage of active transmitters in populations vary between 60 and 100% (Markham et al., 1984). The time required for *C. mbila* to acquire the virus from an infected plant is about 15 to 30 seconds, referred to as acquisition access period (Okoth et al., 1987). Although successful inoculation of MSV into a healthy plant takes about five minutes, the inoculation access period (IAP) can be up to 1-3 hours since it can only occur when the vector salivates into the phloem tissue (Okoth et al., 1987, Peterschmitt et al., 1991). This information implies that successful viral transmission depends on availability of the virus in the plant and adequate populations of transmitting vectors.

1.3.5 MSV strains and isolates

A strain has been defined as a group of isolates sharing greater than 94% nucleotide sequence identity. Of the 11 known MSV strains designated “A” through “K”, only one, MSV-A, is usually found infecting maize plants (Vasani et al., 2008, Willment et al., 2001). Martin et al. (2001) designated six subtypes within strain “A” as MSV-A_1, -A_2 -A_3, -A_4, -A_5 and -A_6. Differences in pathogenicity exist and have been noted among the subtypes (Martin et al., 2001). Subtypes A_1, A_2, and A_5, produces more severe symptoms, A_3 andA_6 intermediate symptoms while A_4 produces mild symptoms (Martin et al., 2001). Severe isolates cause earlier symptoms with wider and more chlorotic streaks than mild isolates (Bosque-Perez, 2000). Distinction between mild and severe strains have been made through five characteristics namely, severity of chlorosis, streak width, streak length, latency and host range (Bolton et al., 1991). Geographically, subtype A_1 isolates occurs throughout Africa from Nigeria to South Africa, subtypes A_2, A_3, and A_4 were found only in western, eastern, and southern Africa, respectively

(Martin et al., 2001), while subtype A_6 isolates only in the Indian Ocean islands of La Réunion and Mauritius (Briddon et al., 1994).

The presence of various isolates complicates the process of finding broad resistance. If MSV isolates overcome resistance in commonly used highly resistant germplasm, this could render germplasm for breeding programmes vulnerable to possible future MSV epidemics. Fortunately, various genotypes have not been found to vary in resistance to different MSV isolates (Martin et al., 1999; Mawere et al., 2006) indicating limited specialization of MSV isolates to resistance genes. It is however recommended that MSV isolates be specifically chosen for particular resistance breeding programmes (Pernet et al., 1999a, Rodier et al., 1995). Mixed isolates of MSV could, however, be used to facilitate screening, an approach that has been successful for the improvement of resistance in breeding lines (Pratt and Gordon, 2006).

1.3.6 The control of maize streak virus

MSV disease has a complex epidemiology therefore a combination of many control measures should be integrated for effective control of the disease. The incidence of the disease could be reduced through several control options broadly classified into three main categories: (1) cultural or avoidance practices; (2) chemical use (insecticide use for control of the leafhopper and herbicide use for control of weeds); and (3) use of MSV-resistant genotypes (Martin and Shepherd, 2009).

Cultural practices are aimed at reducing leafhopper movement disrupting leafhopper mating behaviour and the subsequent spread of MSV disease between farms. They include incorporating barriers of bare ground between early- and late-planted maize fields (Bosque-Perez, 2000); avoiding the planting of maize downwind from older cereal crops; planting early during the second season to prevent early infection (Fajemisin et al., 1976); and the use of crop rotations and intercropping (Page et al., 1999). A further possible strategy is the better use of basic agricultural tools such as fertilizers and irrigation, which could have a substantial impact on MSV disease and other maize diseases, simply because stronger, healthier plants are better able to resist pathogens (Magenya et al., 2008).

Carbamate insecticides such as systemic carbofuran, applied either to the planting furrow or as a seed coating, have been shown to reduce the incidence of MSV in the field by killing leafhoppers (van Rensburg and Giliomee, 1991). Besides controlling leafhoppers, it may be possible to control MSV disease through chemical control of wild grasses within and around maize fields that serve as reservoirs of MSV (Martin and Shepherd, 2009). There is, however,

currently no information available on the impact of controlling the alternative hosts on MSV disease incidence. Chemicals are potentially hazardous to users and to the environment, in addition to being beyond the economic means of most small-scale farmers. In addition, absolute protection against MSV disease is not achievable with insecticides, as repeated insecticide applications are often necessary to control new influxes of migrant leafhoppers (Magenya et al., 2008).

Resistance to MSV would be the far most attractive disease control option, if achieved in agronomically and commercially appealing maize varieties. Despite significant progress having been made in the breeding of MSV-resistant maize, in practice, commercially available maize hybrids are reported to be moderately tolerant to MSV (Martin and Shepherd, 2009). The agronomic and epidemiological benefits of complete and partial resistance include reduced yield losses and fewer inoculum sources, particularly host resistance to virus multiplication (Rodier et al., 1995).

More recently, the control of MSV has also been achieved using biotechnology to develop the first transgenic MSV tolerant maize, in South Africa (Shepherd et al., 2007). The transgenic maize was found to delay symptom development, reduce symptom severity and increase survival rates after MSV infection. Genetic engineering however has several significant drawbacks including transformation and regeneration difficulties, potential disruption of important regulatory or coding sequences, and costly risk assessment and awareness measures. Thus, currently the use of conventionally bred resistant varieties combined with sound crop management practices provide the best means of reducing the impact of MSV on maize yields (Shepherd et al., 2010).

1.3.7 Mechanisms of resistance

Studies on mechanisms of resistance to MSV would be useful in determining the selection scheme (Pernet et al., 1999a). Rodier et al. (1995) suggested that more than one mechanism is involved in a field resistance where plants are able to outgrow symptoms. The resistant type was referred to as recovery type where plants showed severe symptoms at early stages and reduction in symptoms in subsequently emerged leaves (Mawere et al., 2006, Salaudeen et al., 2010).

Kairo et al. (1995) reported some evidence for resistance against *Cicadulina mbila* Naude, the main vector, whose effectiveness is to reduce prolonged probing into the phloem. They

indicated that these resistance mechanisms are manifested in varying degrees in different resistant germplasm. Other authors have found the mechanism of resistance to be antiviral in nature (Martin et al., 1999) with an antixenosis component (van Rensburg, 2001). Antiviral resistance is where host plants poses small regulating RNAs (miRNAs) for viral gene silencing and these can be engineered into plants to down regulate the expression of viral defence mechanism (Ramesh et al., 2014). On the other hand antixenosis (non-preference) describes resistance in which insects is either repelled from host plants. These two resistance mechanisms can operate in the same resistance source and sometimes cannot be distinguished. Combining resistance to the insect vector and resistance to the virus could further contribute to the durability of the resistance.

1.3.8 Source germplasm and origin of resistance

Several sources of resistance have been identified in breeding programmes in Africa and have been utilized in conversions of adapted streak susceptible genotypes. The earliest sources were identified in 1931 in variety 'Peruvian Yellow and later in 'Arkels Hickory' in South Africa (Efron et al., 1989). In West Africa, sources were identified in 1976 in TZY population from which inbred line Ibadan 32 (IB32) was fixed and has been widely used as a donor of MSV resistance at IITA (Efron et al., 1989). Despite these early efforts in search of resistance, other sources of resistance that are in current use are mostly derivatives of the initial sources of resistance. MSV resistance was also identified in the variety 'La Revolution' from Reunion Island and in variety Tuxpeno x Ilonga composite from Tanzania (Bjarnason, 1986). The Réunion island maize germplasm, a composite, Viroses Résistant 3- cycle 3 (CVR3-C3) was found resistant to three viruses and became a source of highly resistance lines namely, CIRAD390 and D211 (Pernet et al., 1999b, Pernet et al., 1999a, Rodier et al., 1995). Other sources that have been utilized in East Africa breeding programmes are lines extracted from the tropical *Zea* mid-altitude streak resistant population from IITA, CIMMYT maize Pool-9A and Osu23i (Gibson et al., 2005). All CIMMYT mid-altitude inbreds ranging from CML195 to CML215 have average or above-average resistance to MSV (CIMMYT, 2004) including some recently released lines including CML505, CML509, CML544, CML545, CML548 (http://www.cimmyt.org, verified 28 Nov 2013).

1.3.9 Genetics of MSV resistance

The mode of gene action for MSV resistance was first studied by Storey and Howland (1967) who reported a major gene, but with some deviations attributed to minor genes acting as modifiers. This was later ascertained by Rodier et al. (1995) reporting two genetic systems, a

major gene, with dominance for resistance controlling high to complete resistance in composite CVR3-C3, while minor genes were associated with partial resistance. Kyetere et al. (1999) were first to map this major gene on chromosome 1 concluding that resistance simply inherited and easily transferred to susceptible types. Pernet et al. (1999a) found a gene action of partial dominance to over-dominance with major gene on chromosome 1 in CIRAD 390. Despite the presence of a major gene for resistance in line CIRAD390, resistance to MSV was quantitatively inherited. Therefore, Pernet et al. (1999a) concluded two genetic systems, one arising from a major gene on the short arm of chromosome 1 with dominance effects (msv1), and with other minor genes on chromosome 2, 3, and 10, that confer quantitative resistance. Genetic control of resistance components, i.e., has also been found to be controlled by additive gene action (Pernet et al., 1999a). Quantitative inheritance of MSV is also supported by the findings of Kim et al. (1989) in germplasm IB32, although, a relatively small number of genes were involved. The effect of maternal influences on MSV resistance has been reported (Bombom et al., 2012) as reciprocal differences in disease reaction in F_1 generation.

1.3.10 Breeding for MSV disease resistance

Breeding for MSV resistance has been a long-term effort in most of Africa. Early breeding strategies used selective inbreeding and backcross technique to transfer resistance into East African germplasm (Storey and Howland, 1967). Several breeding techniques have been applied in breeding maize for MSV resistance. Breeding for resistance to MSV has also been achieved with inbreeding in full sib selection without a compromise on yield (Pixley et al., 2006). High levels of resistance have been achieved in a full-sib family selection after five cycles of selection (van Rensburg, 2005). Rodier et al. (1995) also developed lines expressing complete resistance to MSV within five cycles of inbreeding and selection. While comparing conventional selection to marker-assisted selection, Abalo et al. (2009) indicated that higher selection gains selection are achieved in lines from a segregating F_2 population than from a backcross procedure. Moreover, the lines they developed using marker-assisted selection had higher levels of resistance that those obtained with conventional recurrent selection.

QTLs for resistance to MSV have been identified and mapped in maize. Welz et al. (1998) using CIMMYT line 202 detected a major QTL on chromosome 1 and minor QTL on chromosome 2, 3 and 4. Kyetere et al. (1999) also detected a major QTL on short arm of chromosome 1 with resistant parent Tzi4. The presence of a major QTL on the short arm of chromosome 1 has been replicated by other authors (Lagat et al., 2008, Pernet et al., 1999b, Pernet et al., 1999a)

explaining 50 to 60% of phenotypic variation although other minor QTL have also been implicated. Alleles at this locus were additive or partially dominant depending on the resistance source (Redinbaugh et al., 2004). A major QTL has also been detected on chromosome 3 accounting for more than 60% of phenotypic variance in disease severity (IITA, 2012).

1.4 Northern leaf blight disease

Northern leaf blight (NLB) is a serious foliar disease of maize that occurs virtually everywhere maize is grown (Smith and White, 1988). The disease is caused by the ascomycete fungus *Setosphaeria turcica* Leonard and Suggs with its conidial state *Exserohilum turcicum* Leonard and Suggs. The characteristic symptom of NLB is a grey-green, elliptical or cigar-shaped lesion typically 3-15 cm in length. These symptoms can range from the small cigar shaped lesion to complete destruction of the foliage. Lesions first appear on the lower leaves and progress upwards on susceptible maize types until nearly all of the leaves are infected (Anon., 1997).

The advent of some practices such as reduced tillage and continuous sowing of maize year round have greatly increased importance of NLB disease across SSA. *E. turcicum* overwinters in crop debris from season to season as mycelia, sclerotia or chlamydospores or in the soil (Levy, 1984). Seed transmission has also been reported (Nobel and Richardson, 1968). Lesion formation is favoured by temperature of about 25^0C coupled with minimum dew period of 14 hours and increasing inoculum concentration (Levy and Pataky, 1992). Levy (1989) however emphasized that in addition to weather conditions, the components of fitness of the pathogen have to be considered in determining the severity of NLB. Muiru et al. (2008) studied the invasion patterns of the NLB pathogen suggesting that resistance seems to be inferred by restricted mycelia growth on leaves that results into reduced lesions, delayed wilting and tissue necrosis.

1.4.1 Reduction of yield associated with northern leaf blight

The leaf blight lesions can result in extensive defoliation during the grain-filling period with grain yield losses of 50% or more (Perkins and Pedersen, 1987) in the highland and moist transitional regions of SSA. Northern leaf blight reduces the productivity of the maize crop not only in terms of grain loss, but also in forage value and predisposing plants to cob rot (Anon., 1997). High yield reduction seem to occur with early infections, however, if disease onset is delayed until six weeks after silking, losses are minimal (Perkins and Pedersen, 1987).

Vulnerability of commercial varieties to the disease has been variably reported. Muriithi and Mutinda (2001) rated three varieties in Kenya, H513, H622 and H614 as susceptible suggesting genetic resistance in commonly grown varieties was either very low or non-existent. Shivankar and Shivankar (2000) reported grain yield losses due NLB under artificial inoculation in some commercial varieties in India in the range of 10-18%. When NLB severity is high >75%, maize yield decrease of up to 0.6 t ha^{-1} have been estimated (Sibiya et al., 2013).

1.4.2 The control of northern leaf blight

Maize fungal diseases including NLB can be effectively managed with the adoption of a proper routine scouting program combined with knowledge of the disease and its life cycle. Disease control is best achieved using an integrated disease control program.

Cultural disease control measures such as crop residue burial and crop rotation could reduce NCLB levels. Although the spores are easily disseminated by winds, rotation of maize with other non-host crops could lessen inoculum and thus disease levels (Anon., 1997). Improved soil nutrition, particularly nitrogen and pest control has been associated with low leaf blight infections.

Berger (1972) emphasized that in the use of aerial fungicidal sprays (such as maneb) to control leaf blight, proper timing of the first spray just before spore deposition is essential to prevent early infection. Foliar fungicides are particularly essential in seed production fields of susceptible inbred parents (Anon., 1997).

Host plant resistance is however the most feasible and effective control of *Exserohilum turcicum* (Welz and Geiger, 2000). Northern leaf blight is thus mainly controlled by developing resistant hybrids with high level of polygenic resistance or incorporation of single gene resistance in hybrids with low polygenic resistance (Perkins and Pedersen, 1987).

1.4.3 Sources of resistance

Several authors have tested and identified inbred lines and hybrids with resistance to NLB. Some inbreds exhibiting quantitative resistance to NLB include CML197, CML202, CML204 and inbred A (parent of H632) (Schechert et al., 1997, Welz et al., 1998). Ngwira and Khonje (2005) identified resistance in 37 inbred lines and hybrids developed by CIMMYT in Malawi. Singh et al. (2004) identified CIMMYT lines 145 and CML104 as showing high degree of resistance to *Exserohilum turcicum*. In Kenya, several sources of resistance to NLB were identified in diverse germplasm including lines derived from Ecuador 573 population such as EC573-93 and EC573-

14 (Ininda et al., 2007). Maize lineL30R with HtP gene was found a good source of resistance conferring wide resistance to E. turcicum races such as 123x and 23rx that combine multiple virulence factors (Ogliari et al., 2005).

1.4.4 Genetics of NLB resistance

Components of NLB resistance in maize include lesion types, number of lesions, percent leaf area affected (severity), incubation period, and area under the disease progress curve (AUDPC). Most of these components are favourably correlated and highly heritable (Welz and Geiger, 2000). Resistance to NLB in maize is reported as either as monogenic with several dominant genes (*Ht*, *Ht2*, *Ht3*, *HtM*, and *HtN)* (Robbins and Warren, 1993, Simcox and Bennetzen, 1993) and one recessive gene (Carson, 1995). Polygenic resistance has a few genes (Hudges and Hooker, 1971) or many genes having major effect (Pratt et al., 1997). Monogenic resistance is observed by suppressed sporulation, delayed necrosis, delayed lesion formation, small necrotic streaks and chlorotic flecks and halos (Pratt et al., 2003, Welz and Geiger, 2000). Polygenic resistance on the other hand, or partial resistance is characterized by a prolonged incubation period, a reduction in lesion size and number, and infection efficiency (Carson, 2006, Durga and Reddy, 2001). The *Ht* genes can be unstable as they suffer from climatically sensitive expression, therefore, quantitative resistance alone or in combination with *Ht* genes, is necessary to manage NLB (Lipps et al., 1997, Pratt and Gordon, 2006). Additional factors such as genotype-environment interaction cannot be ignored as an additional source of bias contributing to observed discrepancies in different germplasm (Beshir et al., 2012, Hudges and Hooker, 1971).

Putative QTL for NLB have been reported to be unlinked and dispersed across the genome (Wisser et al., 2008). QTL for AUDPC have been located on chromosomes 1 through to 9 (Dingerdissen et al., 1996, Welz and Geiger, 2000). Pratt and Gordon (2006) reported three QTL from chromosome 3, 5 and 8 being consistent across studies which may be considered consensus QTL for NLB and thus candidates for marker assisted selection. Asea et al. (2009) validated the NCLB rQTL in bin 5.04 as an excellent target rQTL for breeding purposes.

Gene action controlling resistance is reported as additive (Vieira et al., 2009) to partially dominant (Welz and Geiger, 2000). In sorghum, additive, dominance, and epistatic effects have been reported (Beshir et al., 2012). Sigulas et al. (1988) reported additive and unequal genetic effects for lesion expansion in maize. Extended latent period length, an important component of partial resistance, was also found to be controlled by additive effects (Carson, 1995). To exploit

additive gene effects, recurrent mass selection has been used to improve general resistance and disease severity to *Exserohilum turcicum* race 1 (Campaña and Patacky, 2005).

1.5 Gene action

1.5.1 Combining ability

Information on combining ability of suitable parents is necessary for variety development for diverse production areas. The analysis of combining abilities of parental germplasm construes gene actions at play and assists in the choice of parents with high general combining ability and hybrids with high specific combining ability. General combining ability (GCA) is the average performance of a genotype in its hybrid combination while specific combining ability (SCA) refers to the superiority of a certain hybrid compared to other hybrids derived from crossing different genotypes (Sprague and Tatum, 1942). The variance for GCA is associated with additive genetic variance while SCA variance relates to non-additive genetic effects, primarily dominance and epistatic deviations (Hallauer and Miranda, 1988). The significance of GCA variance and hence additive variation shows that selection for the trait could be successful. General combining ability deviations can be positive or negative (Kearsey and Pooni, 1996). A positive deviation can be favourable or unfavourable, depending on the trait under consideration. For example, positive deviation for yield is desirable as this indicates high yielding potential. On the contrary, positive high values on foliar disease ratings would not be desirable. Negative GCA values on the anthesis date are more desirable for selection of early maturing combinations. In addition, the stability of GCA and SCA effects is key in identifying parents and hybrids with improved homeostasis to cater for environmental variations (Dehghanpour and Ehdaie, 2013).

The effect of both additive and non-additive effects have been reported for different agronomic traits in maize including grain yield and hence the gene action conditioning most complex traits cannot be generalized. Preponderance of additive effects has been noted in determining resistance to several maize diseases including ear rots, NLB, GLS, PLS, common rust, head smut and MSV (Vivek et al., 2010). However, the importance of non-additive effects cannot be overlooked since in some cases two parents with average resistance could give above average resistance when crossed with each other (Vivek et al., 2010).

1.5.2 Diallel mating designs

Diallel crosses have been used extensively for studies of quantitative characters with the methods developed mainly by Griffing (1956), Hayman (1954), Jinks (1954), and Kempthorne (1957) and Gardner and Eberhart (1966). A diallel cross is a set of parents and their crosses in all possible combinations. The theory of homozygous diallel crosses is based on the following assumptions: (i) homozygous parents, (ii) diploid segregation, (iii) no difference between reciprocal crosses, (iv) independent gene action of non-allelic genes, (v) no multiple allelism, (v) no linkage and (v) random selection of parents.

Griffing (1956) presented diallel crossing schemes depending on the inclusion of reciprocal crosses and/or parental lines. Method 1 includes the parents, one set of F_1's, and the reciprocal F_1's (all p^2 combinations); method 2 has parents and one set of F_1's without reciprocals ($\frac{1}{2}p(p+1)$ combinations); method 3 has one set of F_1's and reciprocals but not the parents ($p(p-1)$ combinations); while method 4 has one set of F_1's but neither the parents nor reciprocal F_1's are included ($\frac{1}{2}p(p-1)$ combinations. Since methods 3 and 4 do not involve selfing; only these methods can be used to estimate variance components (Wricke and Weber, 1986). Further, the fixed model of method 3 or 4 is the most appropriate for obtaining unbiased estimates of combining abilities and gene action (Shattuck et al., 1993). Further, reciprocal or maternal effects can be determined with Griffing method 1 and 3 (Wricke and Weber, 1986).

If the assumptions of the diallel are met, in the analysis of Hayman (1954) for an $p \times p$ diallel, the variance of all the offspring of the ith parent, V_i, is simply related to the covariance between these offspring and their non-recurrent parents, W_i. The positions of the pairs of points V_i, W_i relative to the regression line through the mean $\bar{V}_i$, $\bar{W}_i$ indicates the nature of dominance. The line through the origin indicates complete dominance: the greater the intercept with the abscissa, the more tendency to overdominance (Mayo, 1987). In the absence of non-allelic interactions, the regression of W_i on V_i is expected to have a slope of unity (Hill et al., 1998). The Wr - Vr analysis provides a test for epistasis and a further test is available if the F2 generation is also grown (Jinks and Hayman, 1953).

The diallel thus provides an assessment of the relative merits of parents and crosses and information on the relative contribution of additive and non-additive gene action to guide selection and testing methodologies. Traits controlled by additive effects respond efficiently to selection while those controlled by non-additive effects, mainly dominance and epistasis, manifest in hybrid combinations.

1.6 Heterosis

Heterosis describes the superior phenotypes observed in hybrids relative to their inbred parents. Heterosis is of outstanding agronomic importance and its extensive exploitation in maize breeding is based on the findings of East and Shull in 1908. Heterosis is manifested in various traits such as increased biomass, size, agronomic yield, pest resistance, tolerance to abiotic stress, and other parameters in the F_1 generation in crosses between inbred lines (Falconer and Mackay, 1996). The exploitation of heterosis is largely responsible for the tremendous increase in maize yield in many developed nations (Duvick, 2001). Heterosis among maize lines can be assessed by observing per se performance, determining combining ability, and genetic diversity as determined through genetic origin, morphological and agronomic traits as well as molecular markers (Konstanovic and Drinić Mladenović, 2007).

Falconer and Mackay (1996) derived an expression for mid-parent heterosis (H) that considers the joint effects of all loci that are different in the cross of two parental lines as H = $\sum dy^2$; d includes the effect of dominance and therefore heterosis depends on the occurrence of dominance and y^2 is the square of the difference in allele frequency between the lines and determines the amount of heterosis expressed in the cross. The ability to predict heterosis levels using inbred phenotype or genetic distance between parents varies for different traits. For some traits, it is possible to explain a significant proportion of heterosis variation using linear modelling while for other traits is it more difficult to predict (Flint-Garcia et al., 2009).

The underlying genetic basis of heterosis has not been satisfactorily explained. Several probable explanations have been given, for instance, heterosis is due complementary effects of dominant alleles at two loci (Stuber, 1994). Over-dominance has also been advanced as another explanation to heterosis, where single locus at which two alleles have the property that the heterozygote is truly superior to either homozygote (Stuber, 1994) . However, this is very limited in plants and cannot be distinguished from pseudo-overdominance. There is some evidence of epistasis (non-alleles) in maize single crosses where an allele is masked in a hybrid by a favourable dominant allele from another parental line (Acquaah, 2009). A special type of epistasis is also given where there are multiplicative effects between genes for complex characters, which are a product of two or more characters (Schnell and Cockerham, 1992).

Hybrid testing programmes are expensive and limited in the number of hybrids that can be generated and tested each year. Thus, the ability to predict hybrid performance without producing hybrid progeny or conducting field trials would be valuable, and heterotic groups have

been established to facilitate breeding efforts (Troyer, 2006). The prediction of the degree of heterosis using heterotic groups or genetic distance between parents based on only yield has had limited success (Flint-Garcia et al., 2009). It is clear that heterosis is also expressed for phenotypic traits other than yield (Hoecker et al., 2006, Tollenaar et al., 2004). Flint-Garcia et al. (2009) found that the prediction of hybrid phenotypes based upon the parental phenotype decreases as the amount of heterosis for a trait increases. Divergent parents do not always produce heterotic crosses (Oliboni et al., 2012) since heterosis depends not only on differences in allele frequencies but also on dominance and epistatic interactions. Predicting hybrid performance has remained a significant challenge for several reasons according to Lee (1995) (i) progeny derived from source populations must be tested in intergroup combinations; (ii) a heterotic group may have unperceived genetic substructure, and (iii) uncharacterized inbreds or those from mixed origin may not fit into established groups. The future of maize breeding will however depend on the discovery of new gene combinations more efficient than the currently available, and on their successful concentration in different sources of heterosis.

1.6.1 Heterotic groups and patterns

The concept of heterotic groups and patterns has been widely used to simplify maize breeding (Tracy and Chandler, 2006) and is critical for maximizing expression of heterosis in hybrids. A heterotic group is a population of genotypes that, when crossed with individuals from another heterotic group or population, consistently outperform intra-population crosses (Hallauer and Miranda, 1988). A heterotic pattern, on the other hand, is defined as a specific pair of heterotic group, which may be populations or lines, that express in their crosses high heterosis and consistently high hybrid performance (Acquaah, 2009). Heterotic groups and patterns can be exploited by crossing elite lines within heterotic groups followed by inbreeding and selection and hybrids can be formed by crossing parents from opposite heterotic groups (Hallauer and Miranda, 1988).

Breeders have used different methods of selecting best parental and assigning lines to heterotic groups. These methods include phenotypic performance for specific traits (Menkir et al., 2004), pedigree relationships (Van Becelaere et al., 2005), adaptability and yield stability (Fan et al., 2009), diallel crosses (Fan et al., 2001) and genetic distances from morphological (Nagy et al., 2003) and molecular markers (Aguiar et al., 2008). The mixed genetic composition and the broad genetic base of the source populations for the tropical mid-altitude lines, makes it difficult to classify these lines into distinct heterotic groups based only on the results of combining ability studies. Therefore, the combined use of molecular markers, pedigree information and testcross

evaluation has been used to facilitate separation of lines into heterotic groups (Devi and Singh, 2011, Fuentes et al., 2005, Hartings et al., 2008).

In CIMMYT, tropical germplasm have been placed into two divergent heterotic groups (THGA and THGB) with “Pop 21” (Tuxpeno-1) and “Pop 25” (Blanco Cristalino) as respective testers using SCA estimates (Vasal et al., 1992a). Similarly sub-tropical CIMMYT maize lines have been assigned into two divergent subtropical heterotic group (STHGA and STHGB) using tester 2 (Pop 44) and tester 4 (Pop 34) (Vasal et al., 1992b). These were based on the hypothesis that positive SCA effects between inbred lines generally indicate that lines are in opposite heterotic groups and lines in the same heterotic group tended to exhibit negative SCA effects when crossed.

In Kenya, maize germplasm has been characterized according to the different maize agro-ecological zones (KARI, 1992). Generally, there are nine major heterotic maize groups in Kenya. The high altitude programme has three heterotic groups: Kitale Synthetic I and II, and Ecuador 573. The mid altitude programme has six heterotic groups; Embu 11, Embu 12, Muguga A, Muguga B, Kakamega pool A, and Kakamega pool B (KARI, 1992).

1.7 Genetic markers

Genetic markers are categorized as morphological, biochemical or molecular (DNA) markers. Morphological traits that exhibit continuous variation between individuals in a population often obscure the assessment of genetic diversity (Yong-Jin et al., 2009). Morphological markers have a shortcoming in their ability to detect genetic variation as they are usually masked by environmental factors and discernible morphological markers are often rare (Yong-Jin et al., 2009). Besides morphological markers, there are genes encoded proteins and isozymes markers, which can circumvent the effects of environment. However, they have the drawbacks in the number of detectable isozymes as well as tissue and development stage specificity (Brown, 1978).

1.7.1 Molecular markers

Markers based on DNA have several advantages over the traditional morphological and protein markers thus gaining popularity in genetic analysis. There are unlimited number of DNA markers that can be generated. Markers are unaffected by the environment and unlike isozyme markers, they are not constrained by tissue or developmental stage specificity (Yong-Jin et al., 2009). Molecular marker analyses are now an important part of breeding and are applied

routinely to test for polymorphism, checking the homogeneity of lines and purity of breeding stocks (Nagy et al., 2003) and removal of redundant genotypes (Yong-Jin et al., 2009). Moreover, in genetic mapping, markers offer great scope for improving the efficiency of conventional plant breeding by selection on molecular markers linked to a trait (Semagn et al., 2006).

The first generation of DNA marker systems employed Southern blot based markers which included restriction fragment length polymorphisms (RFLPs) and Minisatellite probes (Yong-Jin et al., 2009). The second-generation DNA markers were derived from polymerase chain reaction (PCR). These had several advantages over Southern blot based markers including requiring only small DNA amounts to allow analysis at very early stages and were relatively inexpensive and simple. The major systems in PCR-marker techniques include RAPD, AFLP and SSR with the other systems being modifications of these three. There has been a recent shift towards the use to single nucleotide polymorphisms (SNPs).

Amongst the procedures that facilitate the evaluation of molecular diversity, SSRs (simple sequence repeat) or microsatellite variation is the preferred system since it exposes a large number of DNA polymorphisms with relatively simple technical complexity (Yong-Jin et al., 2009), co-dominance and stability of results (Abdellatif and Khidr, 2010). SSR markers have been useful in identifying genetic relationships among diverse germplasm and have classified miscellaneous inbreds into known heterotic groups (Reif et al., 2003a, Wang et al., 2011, Zheng et al., 2008). Relationships between the populations obtained by SSR analyses have been found to be in excellent agreement with pedigree information (Reif et al., 2003b).

1.7.2 Use of molecular markers in genetic characterization of germplasm

Molecular markers can unveil unperceived substructure in a previously established heterotic pattern (Lanza et al., 1997). In comparative analysis of SSRs and SNP, SSRs provided more resolution in measuring genetic distance based on allele-sharing and thus large numbers of SNP loci will be required to replace highly polymorphic SSRs in studies of diversity (Hamblin et al., 2007). It was however noted that in highly diverged maize lines diversity is difficult to measure accurately regardless of the marker system (Hamblin et al., 2007). This happens to be the case in many tropical maize programs where germplasm is diversely sourced and recombined. Overall, information about the genetic diversity of germplasm would be crucial for early diagnosis of genetic narrowing of heterotic pools and in designing efficient strategies for broadening the populations.

1.7.3 Prediction of hybrid performance using markers

Several studies have been conducted on the prediction of maize single cross hybrids using molecular marker heterozygosity. Several studies have reported the limitation of using molecular marker data for hybrid prediction such that correlations of SSR marker distances with heterosis and SCA estimates are too low to be of any predictive value (Lanza et al., 1997, Wegary et al., 2013). It is hypothesized that for quantitative traits such as grain yield, bidirectional dominance can occur (Falconer and Mackay, 1996). Also, some characteristics used to estimate genetic divergence do not significantly influence grain yield (Oliboni et al., 2012). According to Bernardo (2002), effective prediction of hybrid performance using molecular heterozygosity depends on (i) strong dominance effects; (ii) strong correlations of allelic frequencies at individual loci in parental inbreds; (iii) high trait heritability; (iv) the narrow range of average parental allele frequencies; (v) 30-50% of QTLs (at least) have to be linked to molecular markers; and (vi) not more than 20-30% of molecular markers have to be randomly dispersed or unlinked to QTLs. Thus low correlation between heterosis and genetic distance has been attributed to absence of linkage between molecular markers used to estimate divergence and genes controlling heterosis and random dispersion of markers across the genome (Drinic et al., 2002).

1.8 Genotype by environment interactions

The association of the environment (non-genetic factors, such as locations, seasons, years, rainfall, temperature) and the phenotypic (visible characteristics resulting from genetic makeup and environment) expression of a genotype constitute the genotype x environment (G x E) interaction (Fan et al., 2007). The presence of G x E interaction complicates the selection of superior genotypes for a target population of environments (TPE); the absence of G x E would imply that superior genotypes would be suitable in all environments in the TPE.

There are two possible strategies of developing varieties with low G x E interaction. The first is the stratification of the environment into homogenous regions with cultivars developed for specific sub-regions. This approach is costly and ineffective since some environmental variations cannot be reliably predicted (Allard and Bradshaw, 1964). The second strategy for reducing G x E interaction involves selecting cultivars with good stability and adaptability across a wide range of environments to better predict behaviour (Eberhart and Russell, 1966).

Genotype x environment interactions can either be qualitative (i.e., crossover type) or only quantitative (i.e., non-crossover type) (Singh et al., 1999). Changes in the genotype ranks across environments suggest the existence of crossover genotype by environment interactions. Breeders are concerned with crossover interactions as it results in changes of cultivar ranks across environments and makes it difficult to recommend a single best genotype for all environments (Fehr, 1987).

1.8.1 Stability and adaptability analyses

A stable genotype is idealized as having a predictable response across diverse environments. Yield stability is the measure of the ability of a genotype to maintain relative performance across a wide range of environments. An appropriate stable cultivar is capable of utilizing resources that are available in high yield environments, while maintaining above average yield in all other environments (Finlay and Wilkinson, 1963). Stability has been defined as static, where a genotype remains unchanged regardless of the environmental conditions, or dynamic where a genotype changes in a predictable manner across a wide range of environmental conditions (Becker and Leon, 1988). There are indications that static stability is most useful for qualitative traits such as diseases or stress resistance while for quantitative traits such as yield, the dynamic concept of stability is most practical (Norden et al., 1986). It is reported that grain yield stability is a heritable trait controlled by additive gene action and can be improved through selection (Lee et al., 2003). A major goal in many breeding programmes is selection of high yielding genotypes with wider adaptation.

1.8.2 Determination of G x E interactions

There are a variety of statistical procedures available for the determination of G x E in multi-environment trials. These are based on analysis of variance, linear regression or non-linear analysis, multivariate analysis, biplots and/or non-parametric statistics. Parametric methods have limitations including the need to satisfy the assumptions of normality, the homogeneity of variance, and the additivity (linearity) of the effects of genotypes and environment (Yue et al., 1997). Non-parametric models which are based on the relative classification of cultivars across different environments do not require these assumptions, are often viewed as good alternatives for parametric measurements (Huehn, 1990). Combinations of these groups have been used to identify the most suitable genotypes (Balestre et al., 2009b, Scapim et al., 2010). In the assessment of stability, association of different models helps breeders choose the best adjusted

and most informative stability parameter(s) to fit the static and dynamic concepts of stability (Scapim et al., 2010).

Multivariate statistical methods have been used to identify and group genotypes with similar environmental response. These include the additive main effect and multiplicative interaction (AMMI) analysis, which interprets the effect of the genotype (G) and sites (E) as additive effects plus the GE as a multiplicative component and submits it to a principal component analysis (PCA) (Zobel et al., 1988). A more recent modification of the AMMI analysis is the GGE analysis proposed by Yan et al. (2000) that instead pools together the genotype effect and GE (multiplicative effect) and submits these effects to PCA.

The GGE biplot method for graphical display of the multi-environment data was used in the present study. The GGE biplot method is useful for identifying genotypes with dynamic stability, since its principal component axis I (PC1) shows genotypes that are highly adapted while PC2 axis denotes genotype stability. In comparison, to other methods of estimating G x E, the GGE biplot has been considered superior to AMMI1 in explaining sums of squares of GE and G + GE and in predictive accuracy (Balestre et al., 2009a). The method is also highly correlated and provides similar results with other "traditional" stability methods such as Shukla's Stability variance and Eberhart-Russell regression model (Blanche et al., 2007). Thus, the GGE biplot is more versatile and allows easily comprehensible presentation of the data, since it includes both mean performance and stability.

1.9 Participatory approach for assessment of maize production constraints in Africa

Farmers have a long term and direct interaction with their environments and cropping systems and this experience can be used to improve crop breeding strategies(Banziger et al., 2000). Farmer field surveys have been instrumental in ascertaining the extent to which maize diseases occur within subsistence maize cropping systems in different parts of Africa. In addition, certain farmer agronomic practices are associated with increased disease severity and incidence for the various maize diseases. For example for ear rots, increased occurrence in farmers' fields has been associated with continuous monocropping and irrigation for green maize production, leaving crop residue on the soil surface and very early or very late planting (Mukanga et al., 2011). Governments through agricultural extension can use the information gathered in these surveys for mitigating disease spread to guide farmers for optimal production devoid of losses incurred from maize disease infections. The findings in such studies may have implications on

both food policy and crop improvement strategies for the small-scale commercial and subsistence farming sectors in tropical environments.

Participatory crop improvement allows a better incorporation of the perspectives from diverse end users of breeding products (new varieties), meeting the needs of resource poor farmers, breeding for ecosystem resilience and specific niche traits (Dorward et al., 2007). The wide application of participatory rapid appraisal (PRA) tools in a breeding programme result in a better understanding of farmer needs and direct priority setting (Weltzien et al., 1996). Participatory communication tools, such as semi-structured or informal interviews, focus group discussions, transect walks, time lines, mapping, classification and ranking exercises, can be extremely useful for providing a good basis for planning. The strength of these tools is that they facilitate direct interaction between farmers and researchers for a common understanding on constraints and needs (Christinck et al., 2005, Witcombe et al., 2005)

Farmers have been involved in ranking maize production constraints in various maize producing areas. For instance, in the eastern belt of Zimbabwe, farmers ranked drought first, followed by non-availability of seed and then low soil fertility (Derera et al., 2006). Non-adoption of suitable maize varieties was identified as the second most important constraint responsible for low maize yields in western Kenya (Salasya et al., 2007). Further, cultivar preferences have been found to be area specific. For example, Salasya et al. (2007) reported that farmers in the more productive areas showed high preference for grain weevil resistance, while those in marginal areas preferred cultivars with drought tolerance, among other traits. Some breeder preferences such as prolificacy, cob size, and good husk cover have been completely overlooked in farmer variety selection exercises (Salasya et al., 2007). Further, reasons for preference of either hybrids or landraces differ, hybrids being superior for tolerance to abiotic stress, drought and low soil fertility tolerance while landraces for superior taste and flint grain (Derera et al., 2006). Other factors influencing variety adoption have been socio-economic including farm size, education level of the farmer and locality specific characteristics (Salasya et al., 2007). Interaction with farmers can help in understanding the factors that influence the choice of variety and adoption. Varied opinions from farmers can be instrumental in developing selection indices for overall variety appeal and promotion of new varieties rather depending on single or few traits advantage.

1.10 Summary

From the review of literature, it is noted that maize yields are still very low in SSA and in Kenya, mostly less than 2 t ha^{-1}, falling too far below food requirements. Maize production can only be increased by intensifying production within the existing areas and through mitigation of many constraints that plague high maize productivity.

Maize streak virus (MSV) and northern leaf blight (NLB) are found across major maize growing regions in SSA. The conditions that exacerbate increased disease incidence and severity are likely to increase as maize production is intensified through irrigation and due to the effects of climate change. The environment further influences pathogenicity levels. Sources of resistance have been identified for these diseases but there is evidence of vulnerability of commercial varieties whenever epidemics occur. This suggests that resistance levels needs to be increased by additional sources of resistance. The mode of gene action for the main foliar diseases is reported as mostly additive and highly heritable implying simple breeding procedures. However, it is possible that non-additive effects play a major role in some instances complicating the process of selection. Further, the kinds of gene action involved depend on test germplasm and environment including the levels of disease pressure present. A thorough investigation on the kinds of gene action controlling resistance and their interaction will guide selection procedures especially where development of a variety with multiple disease resistance is envisaged.

Heterosis is a well-established approach to hybrid breeding and has helped achieve high yields in the developed countries. The mixed origin of tropical materials often hampers the level of heterosis that can be achieved. High heterosis can be achieved if genotype classifications are conducted with procedures that clearly identify heterotic patterns, including the use of molecular markers. It is also necessary to remove redundant germplasm that exist within breeding programmes that slow breeding progress and genetic gain.

Identification of stable cultivars in a breeding programme is a continuous process due to changing of environmental conditions and development of new hybrid combinations. Germplasm exhibiting dynamic stability for quantitative traits such as grain yield and static stability for qualitative traits such as disease resistance would be most beneficial for breeding and commercialization. The genetic control of yield stability is not widely researched but is noted to be under additive gene action. There are numerous methods of estimating G x E interaction and stability and a comparison among methods can be a useful strategy to identify superior varieties.

Farmers are end users of plant breeder's research efforts and their involvement in the breeding process is paramount. This will greatly influence adoption of new varieties. Farmers also provide the best gauge of changing environmental conditions and prevalence certain pests and diseases, as they are always in contact with their production environments. The slow replacement of old varieties with new ones in many SSA countries is a worrying trend, as farmers are often unaware of value-added traits that breeders introgress to create resilient, 'climate-ready' varieties. The involvement and concerted effort of key players in the maize industry will ensure increased and sustained maize yields for food security in SSA.

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CHAPTER 2

Maize streak and other maize production constraints based on farmer perceptions in central Kenya

Abstract

Small-scale maize farmers in the central midlands of Kenya live largely subsistence lives in which maize is by far the most important staple food commodity. A survey was carried out in maize growing zones of central Kenya between December 2010 and June 2011 to assess the current status of maize streak virus (MSV), among other constraints and varietal preferences with farmers. Data were collected from 100 farmsteads by use of questionnaires. Individual interviews with farmers and extension officers were conducted. Incidence of MSV, assessed from about 500 randomly picked plants, ranged from less than 1% to 100% infection among seven counties. Kiambu county had the highest incidence averaging over 40%, while Nyandarua had the least incidence at 5%. High MSV incidences were associated with susceptible varieties, alternative hosts and recycled hybrid seed. Many farmers categorised MSV as “serious” but they were not aware of its causative agent erroneously attributing it as “soil-borne”. Results also showed thatfarmers had a rather too wide a choice of up to 20 improved varieties, some claimed to be resistant to MSV although only a few farmers were aware of such claims. Varietal preference was based on many reasons including disease resistance, drought tolerance, early maturity, and high yields. Quality of seed contributes to slow replacement of old varieties with new varieties. It is concluded that maize streak virus is still a persistent problem in central Kenya with high disease incidence associated with growing susceptible varieties, recycling hybrid seed and presence of alternative hosts for insect vectors. Breeding for disease resistance hybrids should incorporate farmer desired characteristics including high yield and early maturity.

2.1 Introduction

Maize is an important food staple in Kenya although also becoming an important source of fodder in terms of dry matter stover, thinnings and green stover (Methu et al., 1996, Murdoch et al., 2003). Therefore, adequate amounts are required to feed the growing human population and an increasing need of maize as livestock feed. There has been a slight increase in national

production levels but that is attributed to an increase in maize production area, some of which are in marginal unsuitable areas (Olwade and Smale, 2012). Therefore the national yields in Kenya remain low, 1-2 t ha^{-1}, against a potential of 6 t ha^{-1} (Jaetzold et al., 2006). This has been attributed mostly to impoverished soils, unfavourable climatic conditions, pest and diseases (MoA, 2013). Global warming and its associated effects have changed weather patterns leading to erratic and unreliable distribution of rainfall, resulting in drought. While most of these constraints in maize growing zones in Kenya have been generalized, this study sought to understand the most important maize production constraints in Central Kenya.

Maize streak disease is one of the most damaging of all maize foliar diseases in many countries in the sub-Saharan region including Kenya. First described in Kenya by Storey in the 1920s, research to develop resistance to MSV has been ongoing for over three decades (ISAAA, 1999), and several resistant varieties have been released. MSV has however remained a persistent constraint in maize production system in Kenya midlands (Magenya et al., 2009) reducing both grain and forage yields (Murdoch et al., 2003). Despite being an established and researched problem, susceptibility and high vulnerability of commercial cultivars has been observed with high yield losses. This is evident in the recent reports of the ministry of Agriculture in Kenya, where MSV consistently features as a serious problem. Historically, MSV in Kenya is known to be restricted to the coastal lowlands, central highlands and the Lake Victoria region (KARI, 1997, Mwangi, 1998). Therefore the current study seeks to quantify the problem in central highlands and probably find out why improved varieties have not made any difference.

The overall percent of farmers growing hybrids in Kenya is about 80% (Olwade and Smale, 2012), but the slow pace of hybrid replacement is still a cause for concern. Poor adoption of varieties have mostly risen from the weak integration of variety requirements between breeders and farmers since in many cases the research agenda is driven by governments and/or donors (Mukanga et al., 2011). However, if varieties fail to meet the minimum criteria for any breeding objectives, it is likely that the varieties will not be adopted by farmers and consumers (Badu-Apraku et al., 2011). Farmers make rational decisions in variety choice and input use based on compelling economic considerations (Wambugu et al., 2012), therefore integration of farmers perceptions and preferences will contribute greatly in improving maize productivity. Since breeding of new varieties focuses on integration of traits to cater for changing environments, farmers need to be aware of this to facilitate the replacement of “old” varieties that could succumb to different stresses, especially MSV susceptible varieties.

It is necessary to conduct a survey and surveillance of the disease to get comprehensive information on disease distribution, level of incidence and severity, extent of spread and to locate hot spots for testing of genotypes, which are being developed by the maize programs in Kenya. This study was undertaken to determine the extent of different maize production constraints with a particular focus on maize streak virus (MSV) disease in central Kenya. Specifically, the study aimed to (i) assess farmers' perceptions of the maize streak problem in maize production; (ii) establish baseline information about the maize varieties grown by farmers and the criteria they use in choosing which variety to grow; (iii) determine maize production constraints and farmers' coping strategies for the control of maize streak and other important constraints; and (iv) investigate the new opportunities for breeding MSV varieties in the medium altitude areas of Kenya.

2.2 Materials and Methods

2.2.1 Study area

The districts in the KARI Embu and KARI Muguga mandate region, which fall within the upper and the lower midland agro- ecological zones, formed the sampling frame. The two areas fall under the lowland tropics according to the Kenya Agricultural Research Institute (KARI) classification system (Hassan, 1998). The survey was conducted during the short rains season (September to December) 2010 and the long rains season (April to August) 2011 in two districts of central Kenya, Kiambu and Nyeri. Field disease assessments for MSV were however also recorded in neighbouring districts, Embu, Murang'a, Kirinyaga and Nyandarua districts (Figure 2.1). The study area falls between latitudes 0º75' and 1º20' south of the equator and longitudes 36º54' and 36º85' east. The area experiences bimodal rainfall between March and May and in October and November with annual rainfall ranging from 900 to 1500 mm. There are four agro-ecological zones in the selected districts namely Upper Highland (UH), Lower highland (LH), Upper Midland (UM) and Lower Midland (LM) and maize is grown mainly in the LH 1-3, UM 1-3, LM 1-4 (numbers 1 to 4, represent classifications of moisture availability as 1= humid, 2= sub-humid, 3= semi-humid, and 4= semi-humid to semi-arid) (Sombroek et al., 1982).

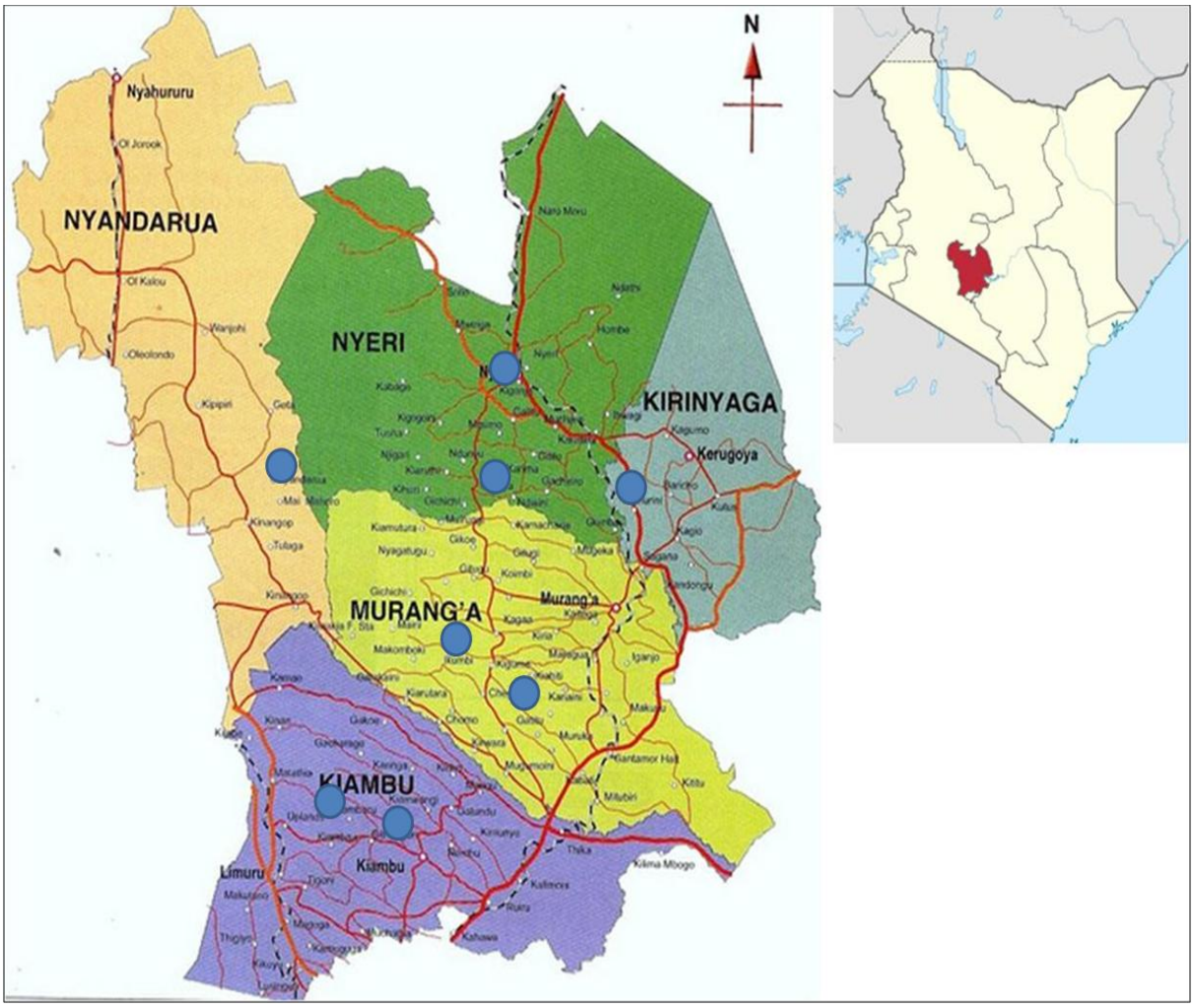

Figure 2.1. Survey area in Kenya midlands

Source: Jaetzold et al. (2006)

2.2.2 Sampling procedures, participants and data collection

A formal questionnaire type survey was carried out where 100 farmers were interviewed. A non-random purposive selection method was used to identify the farmers to be interviewed. The research team consisted of researchers from the Kenya Agricultural Research Institute (KARI) and government agricultural extension officers from the Ministry of Agriculture (MOA). First there were discussions with key informants (extension officers) followed by a formal interview of the farmers using an interview schedule containing open ended and closed questions. Different maize varieties grown by farmers, average yield, perceived constraints to maize production, inputs used in maize production, and other general information were obtained from the survey. In order to obtain homogenous data, only farmers who planted less than two ha of maize and

had no farm machinery formed the sampling frame. Most of the data was based on memory recall. Other participatory methodologies included focus group discussion, matrix scoring, pair-wise ranking and transect walks. The facilitators used diseased plant samples for common diseases to assist farmers in the discussion.

2.2.3 Field visits and observations

Maize streak virus infections were evaluated on site based on the symptoms or nature of damage. The survey was undertaken when maize crops were between tasseling and physiological maturity, a period within which MSV development is considered to be at its optimum. About 500 plants were randomly picked in each field and examined for MSV disease incidence and severity estimated. Disease severity of MSV in the sample was assessed using the disease rating of 1-5 as described by Beyene et al. (2012) where 1 = no symptoms on leaves, 2 = light symptoms on 20 to 40% leaf area, 3 = moderate symptoms on 40 to 60% leaf area, 4 = severe symptoms on 60% of leaf area, 5 = severe symptoms on 75% or more of leaf area, plants severely stunted, dying or dead. MSV incidence was assessed as $100\ (\frac{x}{N})$, where x is the number of infected maize plants with a rating of 2 or more; while N is the approximate number of plants. Portion of land allocated to maize was also calculated as a ratio of farm size and area under maize to estimate the importance of maize in farmsteads. Data were analysed using descriptive statistics of the Statistical Package for Social Sciences (SPSS).

2.3 Results

2.3.1 Information from key informants: Crop extension officers

Extension officers from three zones were available for discussions including Embu west, Mwea and Murang'a north. In Embu West, the areas surveyed fell under the UM1 and UM2 and a transition to UM3 while MSV was reported to be a problem in the UM3 agro-ecological zone. The most commonly grown maize varieties in Embu West were the "5 series", particularly H513. The crop officers reported the use of a local variety called "Kiembu" which was believed to be mixture of seed from a collection of improved varieties. Although Embu west experiences bimodal rainfall, farmers were reported to have a preference for the short rains (September-December) for growing maize since these rains were fairly better distributed than the long rains (March to May). It seems that farmers best monitor weather patterns for crop growth since the Kenya Meteorological Department has also confirmed that the long rains are becoming less reliable with low rainfall compared to short rain seasons (MoA, 2013).

The farmers grew maize in approximately one acre, where the yields currently averaged 15 bags acre^{-1} (or 3.4 t ha^{-1}). The extension officer in Embu west reported a high incidence of MSV during the long rain season of 2009. They attributed the increases in disease incidences to failure by the farmers to rotate (relay cropping) their crops. However, since farm sizes in central Kenya had drastically reduced due to high population pressure, rotation is not always feasible.

The agricultural officer in Mwea, the neighbouring county to Embu also reported high incidence of MSV approximating over 50% in the same season. The high occurrence of MSV in Mwea was attributed to the common practice of irrigated agriculture in the area for maize and other crops and thus creating a favourable environment for the leafhoppers that spread the disease.

In Murang'a north, the main maize growing area was in LM4 agro-ecological zone. This area records rainfall amounts ranging from 600 and 800 mm, annually. The extension officer listed the commonly grown maize varieties as the following: DH01, DH02, DH04, Duma 41, Duma 43, PH3253, H513, DK8031, PAN67, and PAN4M. The variety Duma 43 was reported by the officer to be the most preferred variety for having early maturity, large cobs, and drought tolerance. In the seasons where rainfall is low, the preferred varieties were DH01, DH02, PAN4M, and DK8031. Similarly as reported by extension officers in Embu and Mwea, the extension in Murang'a north also reported a high incidence of maize streak virus in the long rains season of 2009, approximating the incidence at about 45%. They added that incidences were high where farmers had grown variety H513, however, in areas grown with Duma 43 some tolerance to MSV was observed. Discussion with extension officers in the three districts established that there was an outbreak of MSV in central Kenya in the long rains of 2009 with high incidences associated with certain varieties and irrigation of maize crops.

2.3.2 Estimated yields, land allocation to maize and profitability of maize

It was noted that farmers in Murang'a north recorded very low maize yield in the last 3.5 years due to unreliable rainfall patterns, which were attributed to effects of climate change. Although the average maize acreage was 1.5 acres, maize yields in the last three seasons was recorded at five bags acre^{-1} (or 1.13 t ha^{-1}). In Nyeri district, farm sizes averaged at 0.9 ha and farmers allocated half of this land (0.4 ha) to maize production. Farmers were unable to estimate their yields (expected yields) until the cobs set. Most of the maize was used for home consumption and was consumed whilst in the field before the grain completely dried. Thus, it was difficult to estimate how much a farmer produced since maize was consumed as it matured and hardly remained for sale. However, most farmers in the study area apportioned 25% of the harvest as

green maize and 75% as dry maize. Most of the farmers also used part of the crop for fodder although at negligible amounts and mostly from thinnings and dry stover after harvest. Farmers reported to plant varieties that differ in maturity so as to have ready maize to consume for a long time in the growing season. The little surplus of home consumption was sold as dry maize. At least 75% of the farmers reported that maize was profitable and some female farmers referred to maize as the "man of the house". With sale of surplus, famers could purchase inputs, feed the family, which implies food security, and stover was used for animal feed. Where maize was found not to be profitable, it was attributed to high cost of producing the crop versus the low returns in the market.

2.3.3 Diversity of maize variety use in central Kenya

The common varieties grown by farmers in Nyeri and Kiambu districts are shown in Table 2.1, all of which were commercial hybrid varieties. Although the areas sampled were mostly in the mid-altitude areas, farmers grew varieties recommended for other agro-ecological zones such as highland and dryland areas. This indicated that some varieties were popular even in areas not bred for, indicating broad adaptation. It could also suggest that there exist pockets of areas within widely categorized mid-altitude areas that are drier or wetter suiting the production of different maturity maize varieties. The most common variety in Nyeri was H513 (58%) followed by H614 (51%) whereas in Kiambu, H614 (75%) was the most popular followed by H513 (67%). Across the two districts, the five most popular varieties were as follows: H614>H513>Duma 43>H624>PH3253.

Table 2.1. Maize varieties grown by farmers in Nyeri and Kiambu districts

Name of variety	recommended AEZ	% farmers growing the variety		
		Nyeri (n=50)	Kiambu (n=30)	Mean
H513	Mid-altitude	58	67	62
H614	Highland	51	75	63
H624	Transitional moist	20	33	27
Duma 43	Mid-altitude	18	50	34
PH3253	Mid-altitude	9	25	17
H629	Highland	9	17	13
PAN67	Mid-altitude	4	8	6
H628	Highland	2	8	5
H625	Highland	2	8	5
DH04	Dryland	11	_	
DK8031	Mid-altitude	18	_	
H511	Mid-altitude	7	_	
H515	Mid-altitude	4	_	
H516	Mid-altitude	33	_	
H520	Mid-altitude	7	_	
Olerai 22	Mid-altitude	9	_	
WH 403	Mid-altitude	2	_	

_= variety not mentioned

2.3.4 Varietal preference

The two most popular varieties, H513 and H614 were compared for preference (Table 2.2). It was noted that H513 was most preferred for early maturity while H614 was preferred for high yield. Thus in seasons with poor rainfall farmers tended to plant H513 and H614 when the season was favourable. It was also noted that palatability and/or sweet taste ranked highly for both varieties. The variety H513 was also attributed to have good seed viability. However, the farmers cited poor attributes of these two varieties as susceptibility to MSV in the case of H513 and low tolerance to drought for H614. Both varieties were reported to be particularly susceptible to head smut.

Table 2.2. Two most preferred maize hybrids, their good and poor attributes

H513		H614	
Preferred Attribute	Rank	Preferred Attribute	Rank
Early maturity	1	High yield	1
Palatability	2	Large cobs	2
High yield	3	Sweet taste	3
Good seed viability	4	High weight of grains	4
Cheaper seed	5	Good stover	5
H513		H614	
Poor attribute	Rank	Poor attribute	Rank
Susceptible to maize streak virus	1	Low tolerance to drought	1
Highly susceptible to weevils	2	Susceptible to head smut	2
Susceptible to head smut	3	Late maturity	3

Among the top five most popular maize varieties H624 and Duma 43, were fairly recently released, in 2004, compared to H513 and H614 which were released for production in 1995 and 1986, respectively. This suggested that H624 and Duma43 have had a rather quick adoption by farmers in the study area; probably due to some traits preferred by farmers as shown in Table 2.3. It was revealed that the two varieties H624 and Duma 43 were most preferred for early maturity and high yield. Notably, H624 which is bred for transitional moist areas which fall between highlands and midlands was exceptionally early but also high yielding. The variety H624 was also noted to be resistant to frost, which is a problem in some parts of central Kenya. The attribute of leafiness and thus good stover for H624 was a plus for most farmers in central Kenya who also keep dairy cattle. As regards Duma 43, besides earliness, which was a major attribute, an important feature of this variety was MSV resistance. This was confirmed by the reports by extension officers about MSV resistance of Duma 43. Good seed viability was also mentioned in the choice of Duma 43. It was noted in the discussions with farmers that seed viability and seed purity were significant problems in some of the varieties they grew. For poor attributes, however, both varieties were noted to be susceptible to weevils. Hybrid 624 was also noted to be susceptible to MSV. Stay green trait was found to be a negative trait in Duma 43 as farmers could not tell when the crop was ready for harvest.

Table 2.3. Attributes of two popular and newly adopted hybrid varieties in Nyeri and Kiambu

H624	Rank	Duma 43	Rank
Good attributes			
High yield	1	Early maturity	1
Early maturity	2	High yield	2
Good stover	3	MSV resistance	3
Large cobs	3	Sweet taste	4
Frost resistance	4	Good seed viability	4
Poor attributes			
Susceptibility to MSV	1	Susceptibility to ear rot	1
Susceptibility to weevils	2	Susceptibility to weevils	2
Susceptibility to head smut	3	Stay green	3

2.3.5 Desirable characteristics in an "ideal variety" for farmers in Nyeri

A group of 50 farmers, 20 women and 30 men, attending a field day at Wambugu (Nyeri) farmers training centre were asked to give desirable characteristics they sought in an 'ideal variety' (Table 2.4 and Fig 2.2). The women group was homogenous with a narrow range in age 35-50 years, averaging at 45. However, there was a greater diversity within the male farmers ranging from 22 to 80 years and averaging at 45 years. Consequently, the male farmers gave most detailed characteristics. Both male and female farmers indicated an ideal variety should be high yielding, disease resistant, insect resistant, early maturing and of high seed integrity (varietal purity). A higher proportion of women (19%) mentioned sweet taste compared to men (4%). A majority of male farmers (39%) however mentioned resilience, which is a vague description of a variety that could withstand changing climatic conditions and could be grown in areas where maize was not suited (one male farmer mentioning a variety suited to the coffee zone) and/or a low input variety. Only 6% of female farmers mentioned resilience. It appeared that frost was a problem as both female (6%) and male (11%) mentioned frost resistance as a desirable trait. In most cases, when farmers were referring to insect resistance, it was as regards field insects mainly stem borers. For storage pest, only 4% of male farmers mentioned resistance to weevils but this was not recorded for the female farmers. Overall across both groups, disease resistance had the highest percentage of importance (50%) followed closely by high yield (48%) and early maturity (28%). It was however noted that disease susceptibility was

not necessarily plants presenting pathogenic symptoms but for farmers any deviations from a healthy plant was termed a disease, which in some cases were symptoms of insect pest infestation or abiotic stress.

Table 2.4. Desirable traits for an "ideal" variety by a group of farmers in Nyeri during a field day

Desirable trait according to women (n=20)	%	Desirable trait according to men (n=30)	%	Mean (%)
High yield	63	High yield	32	48
Disease resistant	50	Disease resistant	50	50
Insect resistant	19	Insect resistant	21	20
Sweet taste	19	Sweet taste	4	12
Early maturity	13	Early maturity	43	28
Varietal purity	13	Varietal purity	11	12
Drought tolerant	6	Drought tolerant	21	14
Frost resistance	6	Frost resistant	11	9
Good standability	6	Good standability	7	7
Good stover	6	Good stover	4	5
Resilient varieties	6	Resilient varieties	39	23
High marketability	6	_		
High prolificacy	6	_		
Palatability	6	_		
_	_	Does well in high rainfall	4	_
_	_	Drooping ears	4	_
_	_	Good grain type	4	_
_	_	Good storability	7	_
_	_	Large cobs	4	_
_	_	Resistant to weevils	4	_
_	_	Stay green	4	_

_= trait not mentioned

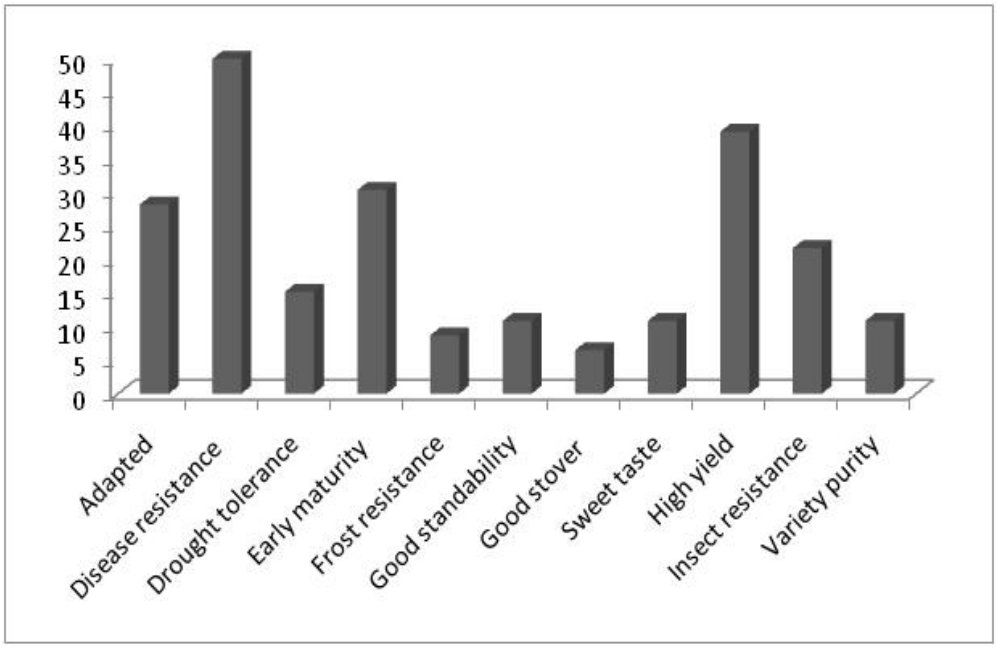

Figure 2.2. Desirable traits for ideal maize varieties by farmers in Central Kenya

2.3.6 Constraints to maize production

The constraints were first discussed broadly with farmers in the following categories: High cost of inputs, field diseases, field pests, storage pests, abiotic stresses and marketing policies as influencing maize production in central Kenya (Fig. 2.3). It was noted that although farmers believed diseases were a major problem only 23% attested to this, followed by field pests (18.9%) and high cost of inputs (18.1%). Very few farmers (5.3%) believed marketing policies affected maize production patterns and productivity.

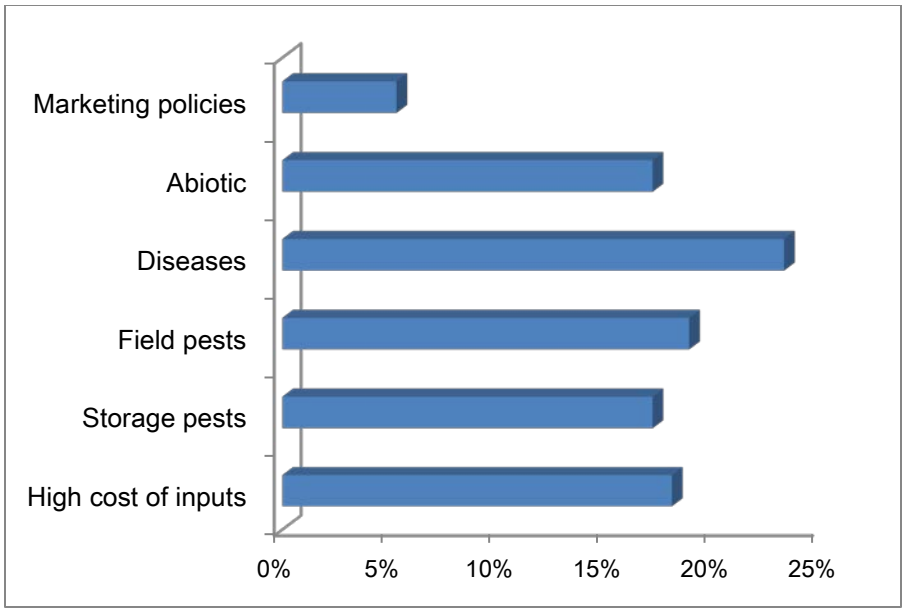

Figure 2.3. Constraints to maize production

The major constraints were then discussed in their specifics (Table 2.5, Fig. 2.4) during the individual farmer interviews and it was reported that among the inputs necessary for maize production, fertilizers were the most important and expensive to most farmers (65%), while labour was the least expensive since most farmers used family labour in the small fields of maize. Among field pests, almost all farmers (84%) reported the importance of weevils, while 50% of farmers found stem borers a major problem among field pests. In the disease category, MSV was the most important (47%) followed closely by common and head smuts (32%); most farmers did not know the differences between these two smuts. Drought was a major abiotic constraint (77%) compared to low soil fertility (23%). Thus, across all constraints the highest percentages of farmers recorded the importance of drought and weevil problems (Fig. 2.4).

Table 2.5 Most common constraints to maize production in Nyeri and Kiambu districts

Constraints	Specific type	Percent (%) (n=70)
High costs of inputs	Fertilizers	65.0
	Seeds	23.3
	Labour	11.7
Storage pests	Weevils	83.7
	Larger grain borer	6.1
	Moths	10.2
Field pests	Stem borer	50.8
	chaffer grub	5.1
	Cut worms	44.1
Diseases	MSV	46.7
	Grey leaf spot	7.8
	Northern leaf blight	6.7
	Common or head smut	32.2
	Stem rots	2.2
	Ear rots	4.4
Abiotic	Drought	76.6
	Soil fertility	23.4

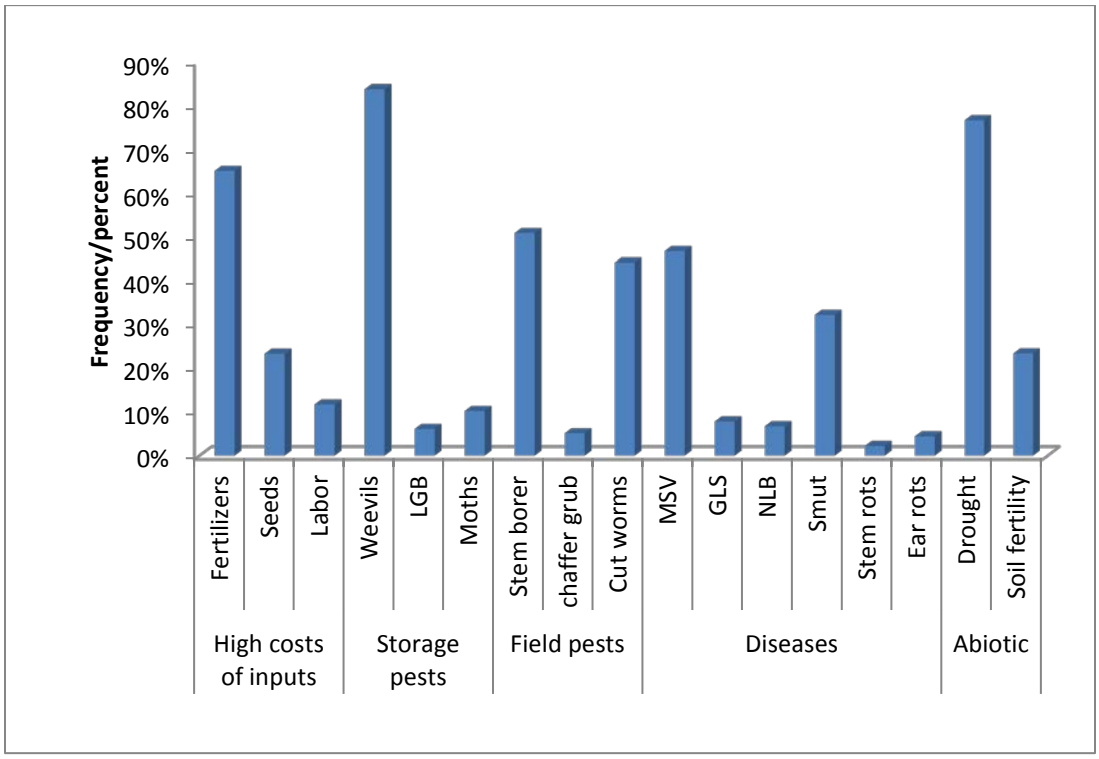

Figure 2.4. Maize production constraints faced by maize farmers in Central Kenya

2.3.7 Distribution and farmers' knowledge of maize diseases

Since diseases were highlighted as an important problem, it was important to establish which of these diseases was most wide spread in Kiambu and Nyeri districts and if farmers could identify disease symptoms and if there were local names for the diseases (Table 2.6). The study revealed that farmers had seen some of these diseases in their fields when presented with diseased plant samples in the individual interviews. Majority of the farmers could identify and had observed common smut (84%) in their fields while 65% had noticed head smut. It was therefore inferred that common smut was more prevalent than head smut in Nyeri and Kiambu districts. Both smuts were referred to as 'Ndutu' in the local language. When presented diseased leaves with GLS symptoms, very few farmers (12.9%) could associate these with a disease they had seen in their fields, suggesting low prevalence of this disease. Half of the farmers (52%) could identify MSV and had observed it in their fields. This disease is locally referred to as 'Gikware'. Very few farmers could identify with the symptoms of common rust (22%) and leaf blight (16%) but blight was referred to as 'baa' a name that is also used for frost damage. Most of the diseases were associated with poor weather conditions and climatic changes, poor seed and poor soils. Roguing was the most common control measure for all diseases while a few who could afford applied foliar feed to counteract these diseases.

Table 2.6. Distribution and farmers' knowledge of maize diseases in Nyeri and Kiambu districts

Sample	Knowledge		Local Name	Farmer associated factors	Control Measures
	Yes (%)	No (%)			
Common Smut	83.9	16.1	Ndutu	Spores/bad weather/poor seeds/soil	Cut & dispose/burn bury/apply ash/rogue
GLS	12.9	87.1	-		
Head smut	64.5	35.5	Ndutu	Spores/bad weather/soil/poor seeds/insects	Uproot, Cut & dispose/ burn/ bury/apply ash
MSV	52.0	48.0	Gikware	Poor/sick soils /leaf hoppers/poor seeds/ shortage of rain	Uproot/apply foliar feed
Rust	22.6	77.4	-	-	Apply foliar feed
Blight	16.1	83.9	Baa	Climatic changes	-

Field observations in fields in Kiambu and Nyeri were also made to assess the prevalence of the common diseases to corroborate the discussions with farmers. Maize streak virus was the highest in occurrence while blight was the least in prevalence (Fig 2.5). MSV and head smut were found to be the most common diseases in maize with each contributing to 36.5%, followed by common smut with 21.2%. Grey leaf spot (3.8%) and northern leaf blight (1.9%) were not common diseases at the time and area of study.

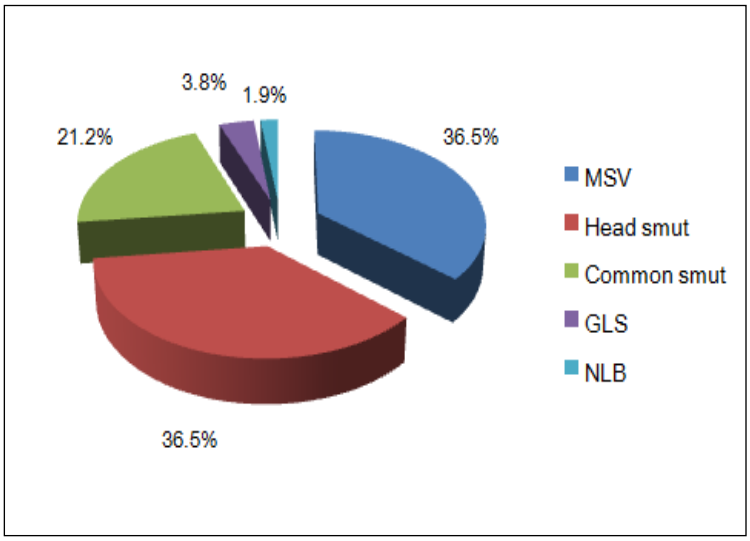

Figure 2.5. Prevalent maize diseases in farmers' fields during period of study

2.3.8 Maize streak virus

Field observations for MSV were extended to ten fields in neighbouring districts, i.e., Kiambu, Nyeri, Murang'a, Embu, Nyandarua, Kirinyaga and Meru to ascertain MSV incidence and severity (Table 2.7, Fig. 2.6). The number of MSV diseased plants was assessed from a plant population of about 500 plants. MSV occurred in all counties, was widespread in occurrence ranging from 5% in Nyandarua to 42% in Kiambu (Table 2.7). The highest severity of MSV on a scale of 1 (no symptoms) to 5 (highly infected) was observed in Nyeri and Meru with score 3.5 and the least severity was observed in Nyandarua with score 2.5.

Table 2.7. Maize streak virus disease incidence and severity in seven counties of central Kenya

district/county	% incidence	Severity-scale 1(symptomless) -5 (severely diseased)
Kiambu	41.9	3.0
Murang'a	37.5	3.0
Kirinyaga	34.5	3.0
Nyeri	31.7	3.5
Embu	26.8	3.0
Meru	25.9	3.5
Nyandarua	4.8	2.5
Mean	29.01	3.1

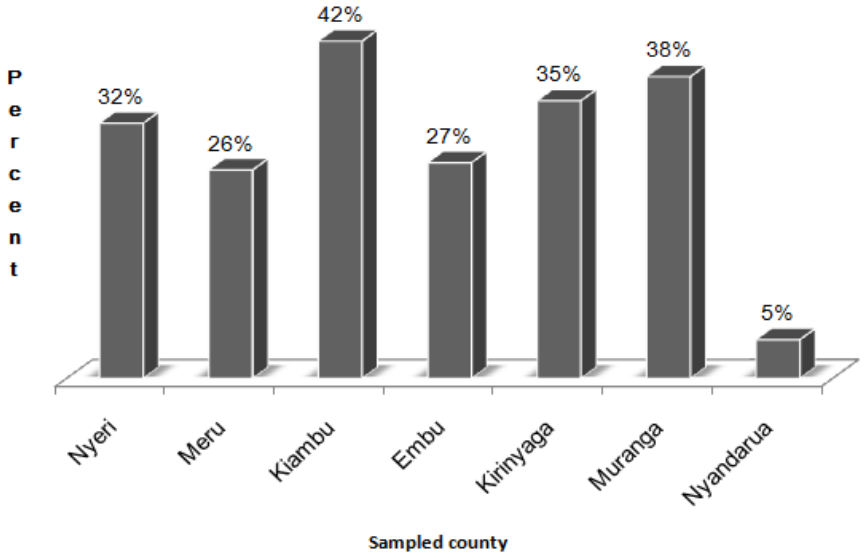

Figure 2.6. MSV incidence in seven counties of central Kenya

2.3.9 Seasonality differences in MSV incidences

About half of the farmers (48%) believed that MSV was most severe in the short rainy season, and about a third (17%) reported the incidence was more serious during the long rainy season, while the rest stated the problem was equally distributed between the two seasons (Fig. 2.7). Whereas distribution across seasons is attributable to conducive environment for disease and vector, the reasons for these discrepancies in farmer perceptions were unclear. It was noted, however, that there was a wide cultivar choice of diverse MSV disease response, which might

explain the seasonal differences. High incidences of MSV were observed in areas where farmers used recycled seed especially in Kiambu and Nyeri (Fig. 2.8). High incidences of MSV were also observed in maize fields adjacent to nappier grass suggesting this pasture grass, which is commonly grown in central Kenya for animal feed, may be alternative host to either the disease and/or vector. Further, MSV was mostly found on field edges and on roadsides than in the middle of the fields, probably due to movement patterns of the leafhopper vectors. Several farmers pointed out MSV was rare in well fertilized fields, therefore indicating that soil nutritional health could play a major role in mitigating MSV induced stress in maize.

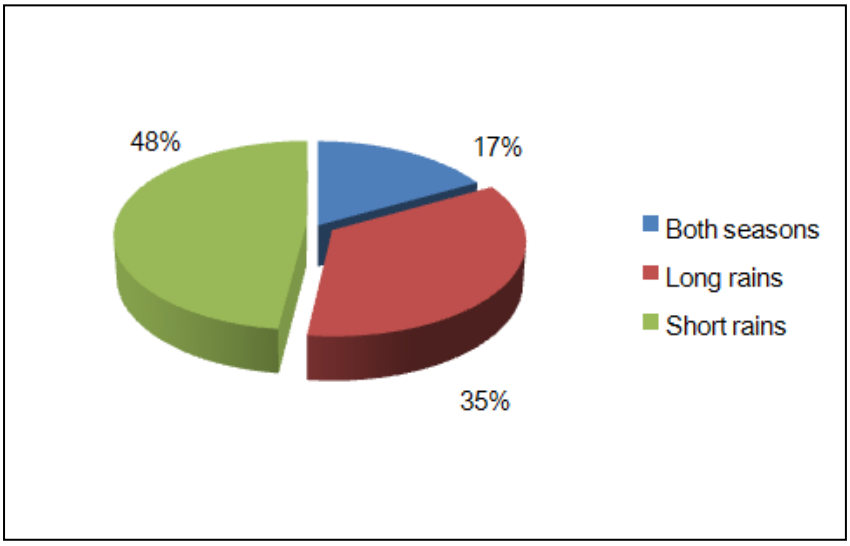

Figure 2.7. Farmers' observations for seasonality differences of MSV occurrences

Figure 2.8. MSV disease infections in a farmers' field with recycled seed in Kiambu county

Figure 2.9 Interview and discussions with a farmer in Embu west

2.4 Discussion

2.4.1 Diversity and adoption of maize hybrids in central Kenya

This study found that up to 20 maize hybrid varieties were grown in the two districts in central Kenya. This agrees with the survey of Tegemeo Institute in 2004 to 2010, where between 22 and 26 hybrids were grown in the lower and upper midlands of Kenya (Olwade and Smale, 2012). The diversification of maize varieties is one strategy of farmers managing production risk especially in difficult production environments (Smale et al., 2006). Farmers grow a wide range of improved maize varieties and the choice of variety by farmers is often hampered by the flooding of the market by many seed companies, similar to Ali-Olubandwa et al. (2011). However, liberalization of the seed industry in Kenya seems in some cases to have led to an increase in maize production due to a decline in the year to year variation of yields and total production (Mbithi and Van Huylenbroeck, 2000). It is argued that the percentage of farmers growing maize hybrids is no longer important in Kenya but what matters for national maize productivity is the dynamic replacement of older with newer materials as long as the newer materials truly represent an improvement compared to previously released hybrids (Olwade and Smale, 2012).

A spill over of varieties bred for high-potential environments into less favourable environments was observed in central Kenya. This has also been noted by other researchers working in different ecologies in Kenya (Hassan et al., 1998). In the present study, it was observed that the majority of farmers continue to grow the 'old' variety H614; a variety released nearly three decades ago, is still very popular in central Kenya. This is despite the dramatic increase in the number and range of hybrids as seed markets liberalize in Kenya. Although the overall percent of farmers growing hybrids in Kenya is about 80% (Olwade and Smale, 2012), the slow pace of hybrid replacement is still a cause for concern. Olwade and Smale (2012) provided several factors affecting the replacement of 'old' varieties with new ones in Kenya including the existence of counterfeit seeds in the market, inadequate promotion and marketing of new seed varieties, inadequate extension and/or information services and seed-to-grain price ratios.

The Kenya Plant Health Inspectorate Service (KEPHIS) conducts seed quality control services as stipulated in the Seeds and Plant Act (Cap 326) of the laws of Kenya (http://www.kephis.org, verified 6 March 2014). This ensures farmers receive quality seed input products. However, it would seem that farmers often fall victim of fraudulent traders. In the present study, concern was raised on the quality of seed quality in the market, that in fact good seed viability was an

attribute influencing variety choice. Nyoro (2002) also noted the reduction in the quality of maize seed in Kenya especially poor germination and low yield which may discourage adoption of new maize varieties infiltrating the market. Therefore, confidence of the seed quality affects adoption and not necessarily information about the existence of such varieties.

The value of hybrids however is not tapped if farmers use less than optimal levels of fertilizers. This study found that high cost of fertilizers was a major constraint to production in the category of high cost of inputs. Thus, most farmers used sub-optimal levels of fertilizer. Thus although adoption of modern varieties is at its peak in central highlands, the high costs of fertilizers continues to limit the full potential of maize production in smallholder farms in Kenya.

2.4.2 Criteria for varietal preferences

Although the survey was restricted to Kenya midlands, a large number of farmers were found to prefer highland varieties. Further, farmers in central Kenya, did not seem particularly interested in grain traits such as grain size or flintiness as their counterparts in Western Kenya (Anjichi et al., 2005, Wambugu et al., 2012) and Eastern Kenya (Leley, 2007). This was probably because most farmers in central Kenya grow certified improved varieties, even though sometimes as seed mixtures and recycled seed and did not identify with local varieties as reported in Western (Anjichi et al., 2005) and Eastern Kenya (Leley, 2007). The only known local variety 'Githigu' was grown by a negligible percentage of farmers. The existence and use of landraces has also been associated with strong cultures of seed exchange between farmers or traditional social networks of relatives as reported in western Kenya (Anjichi et al., 2005). Most of central Kenya is becoming highly urbanized leading to the erosion of indigenous practices including landraces. It is noted that wealth, income, and education level in communities are associated with greater richness and equitability in the spatial distribution of maize varieties (Smale et al., 2006).

The most popular varieties (H614, H513, H624 and Duma 43) were preferred mainly for high yield and early maturity, in addition to some specific traits in specific varieties such as sweet taste, good stover and large cobs. Studies conducted in Zambia (Mukanga et al., 2011) similarly reported the top three criteria used to choose which variety to grow as high yield potential, early maturity, and tolerance to drought. Yield potential and earliness are universally important criteria for farmers. Thus, farmers presented a challenge to breeders for early maturing and high yielding maize varieties. The newly adopted varieties in central Kenya, H624 and Duma 43 appeared to be meeting these criteria. Highland varieties are preferred in the medium areas almost certainly because they generate more foliage for use as animal feed.

2.4.3 Desirable characteristics for an 'ideal' maize variety

A good cultivar according to farmers in central Kenya would be high yielding, disease resistant, insect resistant, early maturing and of high seed integrity (varietal purity). In similar studies in South Africa, farmers provided characteristics of an ideal cultivar as high yield, good taste, low input variety, early maturity, disease resistant and tolerance to acid soils (Sibiya et al., 2013). It seems in many regions of sub-Saharan Africa, early maturity and disease resistance are ideal traits, besides high yield, which is universal.

It is possible that weevil resistance was minimally mentioned among farmers in Nyeri, since rarely do these farmers keep maize in granaries in large quantities and for a long time, partly due to small quantities harvested as dry grain. Varietal purity appeared low and could explain why farmers planted so many varieties since the repeatability of good stands and production with some of the varieties was poor. Owuor et al. (2010) also reported the thorny issue of adulterated agricultural inputs in Nyeri South district of Central Kenya.

Frost resistance was mentioned by both male and female farmers indicating the importance of this problem, which might be unique to certain regions in Kenya. In the reports of the Kenya food security steering group (KFSSG) in 2012, frost was cited as one of the causes of declining crop production in Nyeri (KFSSG, 2012). This therefore also presents an opportunity for breeders to select for frost resistance.

2.4.4 Agronomic practices associated with increased MSV incidences

Agronomic practices such as mono-cropping maize and use of irrigation to produce maize were associated with high MSV disease severity and incidence. The practice of growing maize all year-round was observed in Mwea contributing to high prevalence of the disease. This is because overwintering of the virus and vectors occurs primarily in grasses and irrigated maize during the dry season. The continuous presence of the maize crop and grass weeds or pasture crops such as nappier as noted in Nyeri has serious implication on MSV epidemiology as the MSV pathosystem lacks any temporal gap. This would also explain why there were hardly any seasonality differences in MSV occurrence. It seems therefore that both long rains and short rains support leafhopper populations equally, partly due to presence of alternative hosts during the dry seasons. Increasing epidemics of MSV and *Cicadulina* occurrences in Africa have been associated with a shift in cultural practices as monoculture, increased area under maize, and introduction of new susceptible genotypes (Bosque-Perez, 2000).

However, most farmers were able to associate particular varieties with high MSV incidences. The high incidence of disease in some of the commercial hybrid varieties gave an indication that the level of MSV resistance in most varieties is unsatisfactory. It is possible that most commercial varieties grown in Kenya have a narrow genetic base resulting in severe vulnerability to various disease infections. If these varieties are grown widely in the east African region, the problem with disease could be wide spread. Disease incidence and severity was higher where farmers used recycled seed indicating that hybrid vigour or dominance of resistance plays a general role in increasing resistance in hybrids.

Although there exist a wide cultivar choice (over 20), many claimed to be resistant/tolerant, high incidence levels were noted even among popular commercial varieties. There is urgent need to bring awareness to farmers on the existence of resistant maize varieties bred for different diseases that infect maize. Further, good crop husbandry helps MSV tolerance since MSV was infrequent in maize fields where manure and fertilizer application were properly applied. Further, MSV incidence and other foliar diseases were low in fields where variety choice was made carefully.

2.5 Conclusions

From this study, it was concluded that MSV is a common problem in temporal and spatial distribution and remains an important constraint to maize production in Kenya. High MSV incidences were associated with susceptible varieties, alternative hosts (irrigated maize and pasture grasses), and recycled hybrid seed. Other diseases such as head and common smut also need research attention. An ideal variety of maize was based on desirable characteristics such as, in order of importance, disease resistance, high yield, early maturity, resilience and insect resistance. Varieties with acceptable MSV resistance and high yields in addition to yield stability are required for these areas and should be promoted rigorously. It is also crucial to restore farmers confidence in value to new varieties bred for disease resistance.

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CHAPTER 3

Phenotypic characterization of maize inbred lines for maize streak virus resistance

Abstract

Maize streak virus (MSV) is a major maize disease in sub-Saharan Africa that critically curtails maize production. Most of the commercial varieties in the Eastern African region are still highly vulnerable to the disease due to reliance on a few resistant sources. Identification of additional sources is key for broadening the genetic base. This study was therefore conducted to evaluate resistance to MSV of maize inbred lines adapted to tropical and sub-tropical environments of Africa under artificial disease pressure and to quantify disease symptom expression over time. Forty inbreds were evaluated in long rain season of 2011 at Muguga and Embu in Kenya for MSV disease severity, area under disease progress curves (AUDPC) and some agronomic traits. Highly significant differences ($p<0.001$) were detected among the lines for MSV severity, AUDPC values, grain yield, and days to flowering. Disease development was rapid at Muguga compared to Embu resulting in different severity levels of MSV among the genotypes. Half of the inbreds evaluated were highly resistant with scores of ≤2 on a scale of 1 (symptomless) to 5 (highly susceptible). Nine inbreds were symptomless including Osu23i, MUL88, MUL1184, MUL114, MUL1182, C92, C238-14, C238-21, and CML539 representing highly resistant sources that could be used to improve resistance to MSV in Africa. Ranking of genotypes using AUDPC and MSV severity scores was generally similar based on significant and positive correlations between the two parameters. The AUDPC for disease severity was not affected by differences in maturity levels of the genotypes. Resistance was highly heritable implying that phenotypic selection would be effective in breeding for MSV resistance. The results clearly demonstrated that high levels of resistance were available in the regionally adapted germplasm and new sources of resistance including MUL88, MUL8814, MUL1182, C238-14, C238-21, and CML539 were identified.

3.1 Introduction

Maize streak, incited by maize streak mastrevirus (MSV) and transmitted by leafhopper (*Cicadulina mbila*) is a major disease in tropical and subtropical maize growing areas of Africa, severely limiting maize production. Losses from this disease may exceed 30% depending on environment and host genotype resistance (Bosque-Perez, 2000). Host plant resistance is considered the most effective control measure in mitigating significant losses due to the disease (Pratt and Gordon, 2006). Generally, the increased incidence and economic importance of the disease is linked to environmental conditions and use of susceptible varieties.

Sources of resistance to MSV have been identified in various germplasm. High levels of quantitative resistance have been demonstrated in inbred lines such as IB32 (Kim et al., 1989), CML202 (Welz et al., 1998), Tzi4 (Kyetere et al., 1999), D211 (Pernet et al., 1999a), and CIRAD390 (Pernet et al., 1999b). Genotypes possessing complete immunity to MSV have been scarcely reported and most breeders rely on polygenic quantitative resistance to this disease. Further, the reliance on a few sources of resistance in commercial hybrids increases the vulnerability of these cultivars to disease epidemics. Variations in maize streak virus development and susceptible reactions in resistant germplasm exposed to different strains has been reported (Njuguna, 1999, Rodier et al., 1995). It is also important to establish stability of resistance of the various sources obtained from the region.

In recent years, maize streak virus has repeatedly featured in the reports of the ministry of agriculture in Kenya as a key constraint curtailing maize production (MoA, 2013) indicating the level of resistance to MSV in commercial hybrids in Kenya is not adequate. This is the case after many decades of research on MSV indicating that the most appropriate sources of resistance have not been found. This study was therefore designed to screen putative sources of resistance that could be used against MSV. The specific objectives included (i) screening maize inbred lines adapted to the tropical and sub-tropical environments of Africa with varying levels of resistance to MSV (ii) studying the progress of MSV disease in the field, (iii) estimating heritability of MSV resistance.

3.2 Materials and methods

3.2.1 Germplasm sources

Germplasm screened included maize inbred lines obtained from the Kenya Agricultural Research Institute, the CIMMYT programme in Harare, Zimbabwe, and the International Institute of Tropical Agriculture. Forty (40) inbred lines were screened for MSV resistance. The variation among the lines is indicated by ear length, ear diameter, number of kernel rows and grain type (Table 3.1).

3.2.2 Experimental field design

Field trials were conducted during the 2011 long rain season in two environments in Kenya. The 40 entries were sown at Muguga on 23rd March 2011 and Embu on 19th April 2011. Muguga is located 25 km west of Nairobi at 1º13' South and 36º38' East at altitude of 2095 m asl. Embu is located 120 km northeast of Nairobi at 0º53' South and 37º45' East and altitude of 1508 m asl. The two sites experience bimodal rainfall at peaks between March-May (long rains) and October-December (short rains) with annual averages of between 900-1200 mm. Trials were planted during the long rains of 2011 with rainfall records of 310 mm and 480 mm for Muguga and Embu respectively during the March to August, 2011 growing season. Average maximum temperatures were 21.5ºC and 23.6ºC respectively for Muguga and Embu over the same period. The 40 inbred lines were sown in a 10 x 4 alpha (0,1) lattice design with two replications in the two locations. The plot size in both locations was two rows, 2.5 m long, with 0.75 m inter-row spacing and 0.25 m intra-row spacing. Two seeds per hill were planted and later thinned to one resulting in plant population densities of approximately 53,333 per hectare. Experimental management including fertilizer application and weed control followed standard practice for maize trials.

Table 3.1. Features of 40 maize lines evaluated for MSV disease resistance

Line code	Pedigree	EL	ED	NR	Source	GT
REGN36	EAREGNUR 36-1	14.8	3.7	14	KARI	FL, W
EC14	EC 573(R12)C_8-S_3-14	15.1	3.3	12	KARI	F, W
Z168	SZSYNKITII-F2	18.2	4.0	12	KARI	DL, W
Z419	SYN[Kitale/Tuxp-GLS]F2	13.4	5.0	16	KARI	I, W
Z426	[CML197/N3//CML206]-X-32-1-1-2-BB	10.8	4.2	12	KARI	F, W
REGN29	EAREGNUR 29-5-1	12.4	3.3	10	KARI	FL, W
S558	[EM12-210/CML197//E12-210/OSU23i]-x-58-2-2-3-1	15.3	3.6	12	KARI	FL, W
REGN99	EA REGNUR 99/96	14.0	4.3	14	KARI	I, W
MUL71	[EM12-210/CML 202]-X-71-1-2-1-1-3	15.4	3.7	12	KARI	F, W
MUL88	[EM11-133/OSU23i]-X-88-5-2-3-2	12.2	2.7	10	KARI	F, W
MUL104	[EM11-133/CML 197]-X-104-1-3-1-1-5	13.6	2.8	8	KARI	FL, W
OSU23i	[MSRXPOOL9] C1F2-205-1	17.1	3.2	8	CIMMYT	F, W
CML204	[7794]-SELF-4-1-S9-1-4-7-4-5-BBB	11.0	3.2	10	CIMMYT	FL, W
CML505	[92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	13.1	3.9	12	CIMMYT	I, W
CML202	ZSR923-S_4BULK-5-1-BBB	12.5	3.5	12	CIMMYT	FL, W
CML509	[92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB	12.6	3.4	10	CIMMYT	FL, W
MUL1184	[EM12-210/OSU23i]-X-118-1-1-3-4-1	10.8	3.4	12	KARI	FL, W
MUL114	[EM12-210/OSU23i]-X-114-2-2-1-3	13.0	3.4	8	KARI	F, W
MUL1182	[EM12-210/OSU23i]-X-118-1-1-3-1-2	20.0	4.9	12	KARI	I, W
C92	_	16.0	4.2	12	CIRAD	I,Y
C238-8	MAS [206/312]-159-2-3-4-1-B	14.0	3.8	16	CIMMYT	FL, W
C238-7	MAS [202/312]-20-1-1-4-2-B	11.4	3.5	14	CIMMYT	FL, W
C238-3	MAS [202/312]-20-1-1-1-1-B	6.9	3.3	12	CIMMYT	FL, W
C238-10	MAS [MSR/312]-119-3-1-1-2-B	13.4	3.5	12	CIMMYT	FL, W
C238-14	MAS [MSR/312]-119-5-1-1-3-B	12.7	3.4	12	CIMMYT	F, W
C238-21	MAS [MSR/312]-111-3-1-2-1-B	9.8	3.6	14	CIMMYT	F, W
C238-25	MAS [202/312]-20-1-1-4-1-BB	9.6	3.3	12	CIMMYT	F, W
C238-28	MAS [MSR/312]-X-119-5-1-3-2-BB	10.8	3.0	12	CIMMYT	F, W
CML206	[EV7992#/EVPO44SRBC3]#BF37SR-2-3SR-3-5-2-BB	12.6	3.4	12	CIMMYT	F, W
CML539	MAS [MSR/312]-117-2-2-1-B*4	12.3	3.4	12	CIMMYT	FL, W
C238-16	MAS [MSR/312]-119-5-1-3-2-B	12.6	3.3	10	CIMMYT	F, W
C238-9	MAS [MSR/312]-119-1-1-4-1-B	12.2	3.5	12	CIMMYT	F, W
TZM1736	_	11.5	3.9	14	IITA	FL, W
TZM1746	_	11.5	3.9	16	IITA	FL, W
TZM1750	_	10.4	4.2	16	IITA	FL, W
TZM1759	_	13.3	3.3	16	IITA	F, W
TZM1749	_	13.2	3.0	16	IITA	F, W
TZM1760	_	12.2	3.3	10	IITA	FL, W
A076	_	14.2	4.0	14	South Africa	FL, W
VHCY	_	17.6	4.5	14	South Africa	FL,Y

EL=Ear length, ED= Ear Diameter, NR= No. of kernel rows, GT=grain type (F-flint, I-Intermediate, FL-flint-like, DL=dent like, W=white, Y=yellow); germplasm sources: CIMMYT = International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, CIRAD = Centre de cooperation internationale en recherché agronomique pour le développement, KARI =Kenya Agricultural Research Institute

3.2.3 Artificial inoculation and disease assessment

Artificial inoculation for MSV disease expression was done using reared leafhopper (*Cicadulina mbila*, Naude) colonies in both environments. Disease infected maize plants collected from each region were used as source of inoculum. The screening was based on inoculation protocols slightly modified from Leuschner and Buddenhagen (1980). The leafhoppers, reared on pearl millet grown in glasshouses were given 48 hours of Acquisition Access Period (AAP) on MSV-infected maize plants. The viruliferous leafhoppers were then given a two-day Inoculation Access Period (IAP) at the 2-3-leaf stage of the maize plants to be infected. Inoculation was done on plants grown in the field by attaching a small plastic vial with three viruliferous leafhoppers onto the distal portions of the youngest leaf as shown in Fig 3.1.

Figure 3.1. MSV inoculation in the field at Muguga

Inoculation was done 21 days after planting, i.e., on 14th and 15th April 2011 at Muguga and 10th and 11th of May 2011 at Embu. At Muguga, however, the material was re-inoculated on 15th May 2011, when some lines of CIMMYT and KARI sources failed to acquire any symptoms. Nonetheless, even after the second inoculation, these lines were not infected and were assumed immune. The materials at Embu were not re-inoculated as similar patterns as those at

Muguga were noted. The no choice inoculation method of infecting each plant with three leafhoppers was to ensure uniformity in infection of all plants.

Maize streak virus (MSV) disease severity was assessed at seven-day interval, beginning at 21 days after inoculation, based on visual assessment of the whole plot. A 1-5 logarithmic rating scale with mid-points was used as described by Beyene et al. (2012) where 1 = no symptoms on leaves, 2 = light symptoms on 20 to 40% leaf area, 3 = moderate symptoms on 40 to 60% leaf area, 4 = severe symptoms on 60% of leaf area, 5 = severe symptoms on 75% or more of leaf area. The scores were used to define resistance types as follows: 1.0 (Immune), 1.1-1.4 (highly resistant), 1.5-2.4 (resistant), 2.5-2.9 (moderately resistant), and 3.0-5.0 (susceptible).

In reference to results and discussion, the term “mean scores” refers to average of 12 scores taken between 21 days after inoculation (DAI) and 99 DAI at Muguga. The term “final score” at Muguga thus refers to the rating taken at 99 DAI. At Embu, the mean score is the average of six disease ratings taken between 21 DAI and 56 DAI; subsequently the term “final score” at Embu refers to the score taken at 56 DAI.

3.2.4 Agronomic data

Agronomic data such as number of plants per plant at emergence and harvest, plant and ear height, days to 50% anthesis and 50% silking, and other disease scores were recorded on all plants within a plot, following the standard practice used at CIMMYT (CIMMYT, 1985). Grain yield was determined from ear mass from the whole plot adjusted to 12.5% moisture and converted to t ha^{-1}.

3.2.5 Data analysis

Area under disease progress curves (AUDPC) values were calculated for the disease severity scores using the following formula for AUDPC (Wilcoxson et al., 1975): $\mathrm{AUDPC} = \sum\left[\frac{(xi+xi+1)}{2}\right].(ti+1-ti)$, where xi is the disease rating on date$i$ and ti is the time (in calendar days) on which xi was recorded. Microsoft excel was used for the calculation of AUDPC and plotting graphs. Agronomic and disease data from individual environments and from combined environments were analysed using PROC GLM procedure in SAS 9.2 (SAS Institute, 2004). The data were subjected to ANOVA firstly by environment with genotypes as main effect, then a combined analysis across environments was conducted to analyse the effect of locations, genotypes and interaction.

Pearson's correlation coefficient values were calculated for selected parameters using the SAS procedure, PROC CORR (SAS Institute, 2004). The impact of MSV disease severity scores on grain yield of the inbred lines was determined through a simple linear regression analysis PROC REG (SAS Institute, 2004).

3.2.6 Heritability estimates

Variance component were calculated by equating the mean squares from the analysis of variance (ANOVA) estimates to the expectations of mean squares (EMS) (Table 3.2). Estimations were done on the combined environments using the following model (Singh et al., 1993): $Y_{ijk} = \mu + g_i + \alpha_k + \beta_{jk} + \delta_{ik} + \varepsilon_{ijk}$ where Y_{ij} is the response of i^{th} genotype grown in the j^{th} block (i = 1, 2, ….v), j^{th} block (j = 1, 2, ….b) over the k^{th} environment (k = 1, 2, ….l), μ = general mean, g_i= effect of the i^{th} genotype, α_k = effect of the k^{th} environment, δ_{ik} = interaction effect between the i^{th} genotype and the k^{th} environment and β_{jk} = effect of the j^{th} block within the k^{th} environment. The effects g_i, δ_{ik} and ε_{ijk} are assumed to be independently and normally distributed with zero mean and variances σ_g^2, σ_{gxe}^2 and σ_e^2.

Table 3.2. ANOVA and the expected mean square (EMS) used for estimating variance components

Source	df	Mean square	EMS	Variance component
Environment	$e-1$			
Rep (Env)	$e(r-1)$			
Genotype	$g-1$	M1	$\sigma_e^2 + r\sigma_{g*e}^2 + er\sigma_g^2$	$\sigma_g^2 = \frac{M1 - M2}{er}$
Genotype*Env	$(g-1)(e-1)$	M2	$\sigma_e^2 + r\sigma_{g*e}^2$	$\sigma_{g*e}^2 = \frac{M2 - M3}{r}$
Error	$e(r-1)(g-1)$	M3	σ_e^2	$\sigma_e = M3$

Heritability was then estimated as $h^2 = \sigma_g^2/(\sigma_g^2 + \frac{\sigma_{\text{gxe}}^2}{e} + \frac{\sigma_e^2}{re})$, where σ_g^2 is the genetic variance, σ_e^2 = environmental variance and σ_{gxe}^2 = variance of the genotype x environment interaction (Hallauer and Miranda, 1988).

3.3 Results

Analysis of variance for selected agronomic traits and MSV scores are presented in Table 3.3. Mean squares for genotypes were highly significant (p≤0.001) for all traits, i.e., MSV mean and final scores, EPO, DTA and GY. However, environment variance was significant for all traits except MSV final scores. In addition, genotype x environment interaction was significant for MSV mean scores, EPO, and EPO but not for MSV final scores, and DTA. The phenotypic correlation between mean scores and final scores was positive and highly significant (r=0.97, p<0.001).

Table 3.3. Combined analysis of variance for MSV disease severity scores for the 40 maize genotypes screened at Muguga and Embu in 2011

Source of variation	df	Mean squares				
		MSV		EPO	DTA	GY
		mean score	final score			
Environment	1	8.081***	0.225^{ns}	0.254***	6786.79***	6.091***
REP(Env)	2	0.713***	1.531***	0.006***	18.251^{ns}	1.178**
Entry	39	2.116***	3.781***	0.011***	128.307***	2.867***
Genotype x Env	39	0.245***	0.231^{ns}	0.00***	22.881^{ns}	0.295*
Error	78	0.073	0.156	0.001	16.955	0.174
CV (%)		14.393	17.666	6.597	5.001	23.988
$LSD_{0.05}$		0.419	0.716	0.041	5.727	0.623
Mean		1.879	2.238	0.925	82.340	1.741
R^2		0.947	0.929	0.441	0.907	0.909
Pearson correlation between MSV mean score and final score = 0.973***						

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05); EPO = ear position, DTA = days to anthesis, GY= grain yield (t ha^{-1})

3.3.1 Reaction of inbreds to MSV disease infection

The severity scores for the inbreds were variable and differences were significant (p = 0.05) across environments. The mean severity scores ranged from 1.0 to 3.3 across the two environments. The mean MSV score was higher at Muguga (2.1) than at Embu (1.65) (Table 3.4). Despite higher disease pressure at Muguga, 13 lines were symptomless at Muguga, while eight were symptomless at Embu (Table 3.4). These were either immune lines or escapes. Across environments, nine inbred lines were symptomless including C92 and Osu23i, four lines from KARI, Kenya, namely MUL114, MUL88, MUL1184, MUL1182 and three lines from CIMMYT Harare programme, i.e., C238-14, C238-21 and CML539. Some regionally

important sources of resistance CML202 and CML204 were resistant to MSV though at moderate levels, especially CML202. The most resistant source from IITA was TZM1750 with mean score of 1.6 across environments. The most susceptible lines included CML206 and three lines used in the KARI highland breeding program, Z419, Z168, EC14. Lines Z419 and Z168 are derived from Kitale synthetic while EC14 is derived from Ecuador 573 population. Most of the inbreds were consistent in their disease reaction in the two environments, except in a few cases, notably TZMI760 that scored 1.67 at Embu and 3.00 at Muguga.

Table 3.4. Means of MSV scores in 40 inbreds screened in 2011 in two locations

Genotype	Embu	Muguga	Across sites	Disease reaction ‡
	MSV mean scores			
MUL88	1.0	1.0	1.0	Immune
OSU23i	1.0	1.0	1.0	Immune
MUL1184	1.0	1.0	1.0	Immune
MUL114	1.0	1.0	1.0	Immune
MUL1182	1.0	1.0	1.0	Immune
C92	1.0	1.0	1.0	Immune
C238-14	1.0	1.0	1.0	Immune
CML539	1.0	1.0	1.0	Immune
C238-21	1.0	1.0	1.0	Immune
C238-10	1.1	1.0	1.1	HR
C238-28	1.1	1.0	1.1	HR
C238-16	1.1	1.0	1.1	HR
C238-9	1.1	1.0	1.1	HR
CML509	1.3	1.6	1.5	R
VHCY	1.4	1.5	1.5	R
TZM1750	1.7	1.5	1.6	R
CML204	1.8	1.5	1.7	R
CML505	1.6	1.9	1.7	R
TZM1759	1.5	2.3	1.9	R
TZM1749	1.8	2.2	2.0	R
TZM1736	1.8	2.2	2.0	R
TZM1746	1.5	2.6	2.0	R
C238-3	1.9	2.4	2.1	R
S558	2.0	2.4	2.2	R
C238-8	1.7	2.8	2.2	R
C238-7	1.9	2.6	2.3	R
CML202	1.9	2.7	2.3	R
TZM1760	1.7	3.0	2.3	R
MUL71	2.1	2.6	2.4	R
C238-25	2.0	2.8	2.4	R
REGN36	1.9	2.9	2.4	R
A076	2.0	3.0	2.5	MR
REGN99	2.1	3.0	2.5	MR
Z426	2.0	3.2	2.6	MR
REGN29	2.3	3.0	2.7	MR
MUL104	2.3	3.1	2.7	MR
CML206	2.6	3.3	3.0	S
Z419	2.6	3.6	3.1	S
Z168	2.5	3.8	3.1	S
EC14	2.7	3.9	3.3	S
Mean	1.65	2.10	1.88	
$LSD_{0.05}$	0.51	0.59	0.42	
CV (%)	11.90	17.50	15.80	

Disease reaction ‡ HR= highly resistant, R= resistant, MR = moderately resistant, S = susceptible

3.3.2 Area under disease progress curves for disease severity scores

Analysis of variance for area under disease progress curves are presented in Table 3.5. Mean squares for genotypes, environment, genotype x environment interaction were all highly significant ($p<0.001$) for MSVAUDPC. The AUDPC values in the two seasons were variable with values ranging from 78 to 309.1 at Muguga and 38.5 to 91 at Embu and 58.2 to 200.1 across environments (Table 3.6). Overall, the combined environment data for AUDPC were significantly different ($p=0.05$). Generally, AUDPC values were higher at Muguga since there was higher disease expression compared to Embu and further, disease was assessed over 12 disease ratings at Muguga while six ratings were taken at Embu. Higher AUDPC values were recorded on susceptible than resistant lines, for example the most susceptible inbreds EC14 and Z168 had AUDPC scores of 200 and 191, respectively, compared to AUDPC of 84.1 and 86.4 in resistant genotypes VHCY and CML509, respectively. Immune lines had AUDPC values of 58.2 across locations.

Correlation coefficient between AUDPC values for disease severity with final MSV scores in the two and across environments (Table 3.7) were highly significant ($p\leq0.001$) and positive ($r = 0.971$ to 0.983). Across environments, final MSV scores and AUDPC scores were not significantly correlated with either days to anthesis or days to silking. However, in the combined environments, days to silking was slightly correlated with AUDPC scores ($r = 0.329$, $p\leq0.05$). Grain yield (t ha^{-1}) was negatively and significantly correlated with MSV final scores ($r = -0.43$, $p\leq0.01$) and AUDPC ($r = -0.42$, $p\leq0.01$) at Embu but at Muguga and across environments, these correlations were not significant. In addition, regression analysis conducted at Embu showed a negative effect of disease severity and AUDPC on grain yield with adjusted R^2 values of 0.168 and 0.151, respectively (Table 3.7).

Table 3.5. Analysis of variance of AUDPC for MSV severity scores over two locations

Source	d.f.	Across sites	d.f.	Muguga	Embu
		Mean squares			
Environment	1	447957.23***			
Rep (env)	2	1501.81**	1	1884.22*	1119.38***
Genotype	39	8193.59***	39	11185.79***	554.53***
Genotype x env	39	3546.74***			
Error	78	237.46	39	388.07	86.85
mean		112.04		164.95	59.13
$LSD_{0.05}$		25.15		39.85	18.85
CV (%)		13.75		11.94	15.76
R^2		0.98		0.97	0.87

*, **, *** indicates the term is significant at $p\leq0.05$, $p\leq0.01$ and $p\leq0.001$, respectively, ns indicates non-significant ($p>0.05$);

Table 3.6. AUDPC for MSV scores taken over six ratings in two environments

Genotype	AUDPC			MSV score across sites at 56 DAI
	Muguga	Embu	Across sites	
MUL88	78.0	38.5	58.2	1.0
OSU23i	78.0	38.5	58.2	1.0
MUL1184	78.0	38.5	58.2	1.0
MUL114	78.0	38.5	58.2	1.0
MUL1182	78.0	38.5	58.2	1.0
C92	78.0	38.5	58.2	1.0
C238-14	78.0	38.5	58.2	1.0
CML539	78.0	38.5	58.2	1.0
C238-21	78.0	39.4	58.7	1.1
C238-10	78.0	42.9	60.4	1.1
C238-28	78.0	42.9	60.4	1.1
C238-16	78.0	42.9	60.4	1.1
C238-9	78.0	42.9	60.4	1.5
VHCY	116.5	51.6	84.1	2.4
CML509	125.6	47.2	86.4	1.9
TZM1750	116.7	59.5	88.1	1.9
CML204	117.0	63.9	90.4	2.1
CML505	146.8	56.0	101.4	2.3
TZM1749	175.1	62.1	118.6	2.1
TZM1736	172.2	65.6	118.9	2.3
TZM1759	181.9	56.0	118.9	2.4
C238-3	184.4	65.6	125.1	2.9
S558	184.4	70.0	127.2	2.6
TZM1746	201.4	54.3	127.8	2.6
C238-7	203.0	67.4	135.2	2.6
CML202	210.4	64.8	137.6	2.9
C238-8	218.8	59.5	139.1	2.8
MUL71	204.9	73.5	139.2	2.8
C238-25	218.0	68.2	143.1	2.9
REGN36	227.5	67.4	147.4	3.1
TZM1760	236.6	60.4	148.5	2.8
A076	233.0	70.9	151.9	2.8
REGN99	232.9	73.5	153.2	3.3
REGN29	235.8	81.4	158.6	3.1
Z426	251.6	70.9	161.2	3.3
MUL104	243.6	81.4	162.5	3.3
CML206	257.0	90.1	173.6	2.9
Z419	282.6	90.1	186.4	3.9
Z168	297.4	84.1	190.7	3.9
EC14	309.1	91.1	200.1	3.9
mean	165.0	59.1	112.0	2.2
$LSD_{0.05}$	39.8	18.8	23.2	0.7
CV (%)	11.9	15.8	14.7	22.7

Table 3.7. Pearson correlation coefficients among AUDPC values, final MSV scores and days to flowering for the 40 inbreds evaluated across two locations

Muguga	DTA	DTS	MSV	AUDPC	GY
DTA	1	0.955***	0.159^{ns}	0.212^{ns}	-0.321*
DTS		1	0.212^{ns}	0.271^{ns}	-0.362*
MSV			1	0.971***	-0.035^{ns}
AUDPC				1	-0.091^{ns}
GY					1
Embu	DTA	DTS	MSV	AUDPC	GY
DTA	1	0.065^{ns}	0.123^{ns}	0.175^{ns}	-0.483**
DTS		1	-0.031^{ns}	0.009^{ns}	-0.268^{ns}
MSV			1	0.971***	-0.428** †
AUDPC				1	-0.415** †
GY					1
Combined	DTA	DTS	MSV	AUDPC	GY
DTA	1	0.948***	0.114^{ns}	0.176^{ns}	-0.431**
DTS		1	0.256^{ns}	0.329*	-0.491***
MSV			1	0.983***	-0.219^{ns}
AUDPC				1	-0.189^{ns}
GY					1

† regression of GY on MSV y = 2.42-0.409x , R^2 = 0.163; GY on AUDPC y = 2.79 – 0.021x, R^2 = 0.151

DTA = days to anthesis, DTS = days to silking, GY= grain yield (t ha^{-1}); *, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05);

3.3.3 Disease progress curves for MSV severity

A selection of disease progress curves representative of the range of disease values recorded in the field are shown in Fig. 3.2 for Muguga and Fig. 3.3 for Embu. There were some marked differences in the severity of the disease development caused by MSV in the inbreds. Disease severity symptoms were taken from 21 days after inoculation (DAI) in both experiments; about 42 days after planting (DAP). The progress of disease at Muguga was slow at first between 21 DAI and 36 DAI followed by a substantial increase of about two disease levels between 36 and 43 DAI especially in the most susceptible genotypes EC14 and CML206. The disease levels at Muguga levelled off after 43 DAI. At Embu, the progress of the disease was different from Muguga. There was no distinct lag phase for most of the lines. The increase was gradual and did not level off even at 56 DAI as compared to Muguga.

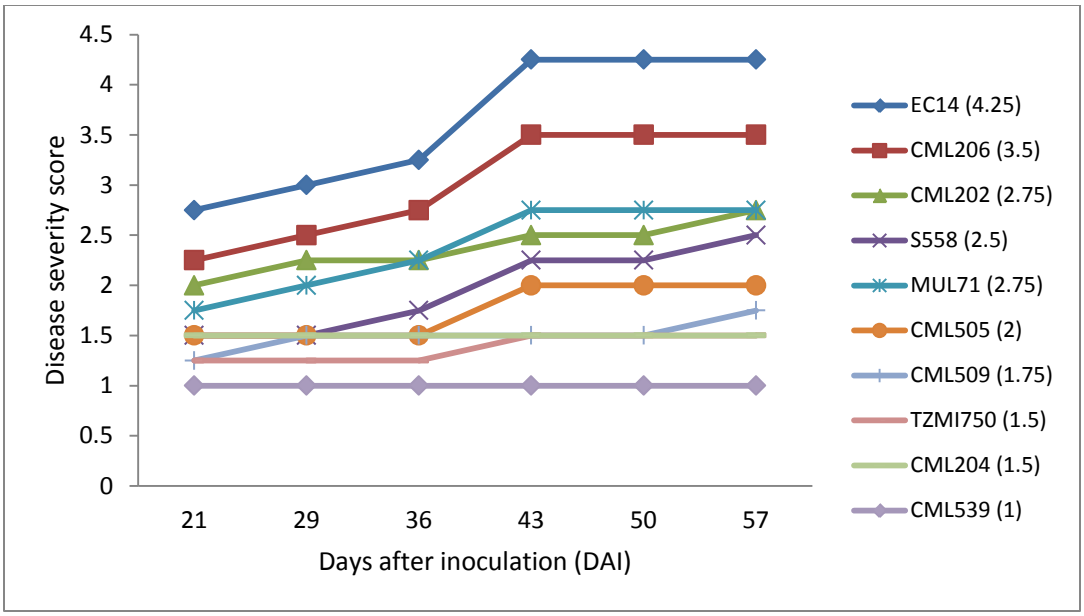

Figure 3.2. Disease progress curve based on severity scores of ten selected lines (final scores in parenthesis) at Muguga

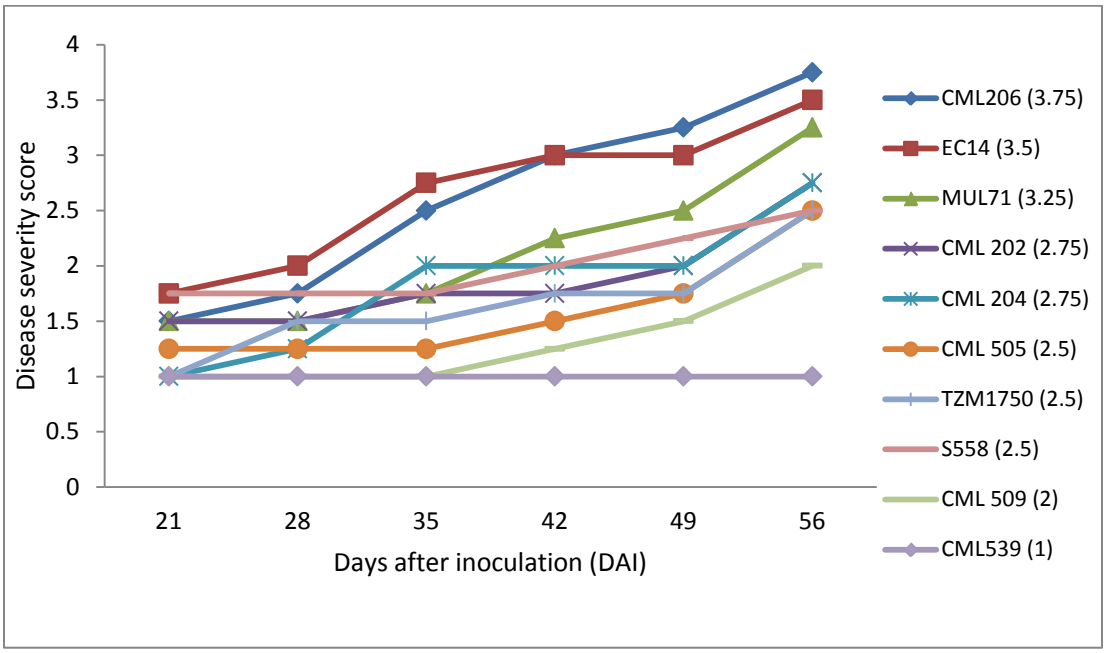

Figure 3.3. Disease progress curve based on severity scores of ten selected lines (final scores in parenthesis) at Embu

3.3.4 Variance components and heritability estimates

The variance components and heritability estimates based on mean scores and final scores are presented in Table 3.8. The heritability estimate was high for MSV reaction based on both the mean score (88%) and final score (94%). Grain yield, days to silking and ear position were also highly heritable in these materials (90, 82 and 73%, respectively). Heritability was however moderate for AUDPC (57%). The variance ratio $\frac{\sigma_g^2}{\sigma_{g\text{x}e}^2}$ increased from 10.9 in the mean score to 46.7 in the final MSV score indicating that genotypic differentiation was much better at the peak of the epidemic.

Table 3.8. Variance components and heritability estimates for MSV response and other traits

Component	MSV		AUDPC	EPO	DTA	GY
	mean score	final score				
σ_g^2	0.468	0.888	1161.713	0.002	26.356	0.643
$\sigma_{g\text{x}e}^2$	0.043	0.019	827.320	0.001	1.482	0.030
σ_e^2	0.018	0.039	59.365	0.000	4.239	0.044
σ_p^2	0.529	0.946	2048.398	0.003	32.077	0.717
Heritability (%)	0.88	0.94	0.57	0.73	0.82	0.90
$\frac{\sigma_g^2}{\sigma_{g\text{x}e}^2}$	10.88	46.74	1.40	4.00	17.79	21.43

EPO = ear position, DTA = days to anthesis, GY= grain yield (t ha^{-1}); $h^2 = \sigma_g^2/(\sigma_g^2 + \frac{\sigma_{g\text{x}e}^2}{e} + \frac{\sigma_e^2}{re})$

3.4 Discussion

Significant effects of environment and genotype by environment interaction were detected when mean scores were considered but these effects were not significant when final scores were considered. Therefore, based on final scores, MSV resistance of the genotypes was consistently ranked in the two environments, Muguga and Embu. Similarly, Welz et al. (1998) rated lines for MSV at 21 days after infesting (DAI) and 83DAI and reported detectable genotype x location interaction for scores taken at 21 DAI but negligible at 83 DAI. This suggests that at disease peak, disease symptoms stabilize and location differences become negligible. Non-significant G x E interaction for MSV severity also implied that single environment could be used in selecting for resistance for MSV provided there is adequate and uniform disease pressure.

The environment of the study and latent period of the isolates also determines the stage at which genotypic differences are evident. Further, considering that the two environments, Embu and Muguga could represent two geographically separated MSV isolates, non-significant G x E indicated that the different MSV isolates ranked resistance of the maize genotypes similarly. Several studies have reported parallel results that genotype x isolate interactions are not important for MSV (Martin et al., 1999, Mawere et al., 2006) and that all isolates rank genotypes in exactly the same order from least to most resistant. These results further imply homogeneity of MSV such that resistant cultivars of maize should be effective in most African countries (Mawere et al., 2006). However, based on the responses in the current study of some known sources of resistance, breeders should test all putative sources in their target environments for identification of the strongest resistant sources, since virus isolates differ in virulence. Some known sources such as CML202 were moderately resistant to MSV at Muguga and Embu suggesting that environmental differences in the virulence of the pathogen would greatly influence the choice of a source of resistance. Some studies have further indicated that maize varieties known to be MSV resistant in one ecological zone may show susceptibility to the disease in another environment (Bosque-Perez, 2000, Magenya et al., 2008).

The differences in the resistance levels among lines bred for resistance may be attributed to different number of genes conditioning resistance or to the influence of genetic background. Among the genotypes screened were high resistance levels with some indication that some parents had immunity to the disease, at least in the environments tested. Immunity was detected for Osu23i, a line widely used for MSV resistance and found in other studies to be immune to the disease (Njuguna, 1999). This indicated that symptomless KARI lines derived from crosses with Osu23i, namely MUL88, MUL1184, MUL1182 and MUL114 were similarly immune. Other symptomless lines were obtained from CIMMYT-Harare, C238-14, C238-21, and CML539 were derivations from MSR population (MSRxPOOL9]C1F2-205-1(OSU23i)-1-5-B-B-5-B). It is possible that all symptomless lines are related by pedigree having a common parent OSU23i. The greater MSV resistance of genotype MSR is reported in other studies (Mawere et al., 2006). It may be imperative however to test some of the immune lines identified in other environments to confirm these responses. The most susceptible lines were EC14 and Z168. These two lines are derived from Ecuador 573 and Kitale synthetic populations, respectively, which form the basis of highland maize breeding programme in Kenya. This highlights that susceptibility of these base populations could be transmitted to their hybrids and is a possible

explanation for susceptibility of some commercial hybrids in Kenya, which contain these lines as parents.

Significant and positive correlation coefficients between AUDPC values with MSV final scores were obtained implying that the ranking of genotypes for AUDPC and final MSV severity scores were generally similar. This suggests that a single score made at the peak of an epidemic would be adequate when screening large numbers of germplasm as is less laborious than the several assessments required to obtain AUDPC values.

It was noted that the disease level at Muguga levelled off before the materials attained flowering. On average the genotypes flowered at 91 days after planting (DAP) at Muguga while the disease levelled off at 43DAI (i.e., 64 DAP). This indicated that there was little influence of maturity on resistance levels. Further, across environments, AUDPC values for disease severity were not significantly correlated with either days to 50% anthesis or silking confirming that resistance levels were not affected by maturity. For other diseases, such as Phaeosphaeria Leaf Spot (PLS), the effect of days to flowering on AUDPC has been reported especially with early maturing genotypes (Sibiya, 2009).

There were differences in disease progress between the two environments with disease progress much faster at Muguga compared to Embu. There was only one rise in disease infection at Muguga between 36 and 43 DAI, which would be between 57 and 64 DAP. Disease progress levelled off at Muguga at 43 DAI but at Embu, disease progress was on the increase even at 56 DAI (or 77 DAP). This suggested that disease progress was much slower at Embu and could have been influenced by differences in temperature and differences in virulence of virus isolates in the two sites. Although Muguga is cooler than Embu, there was higher rainfall at Embu, which could have prolonged disease progress. It is also possible that the virus isolate used at Muguga is more virulent compared to that used at Embu. Some authors have noted a close relationship between weather conditions and infection rates in maize host for MSV (Atiri et al., 2000).

There was an influence of MSV on grain yield detected only at Embu as indicated by significant correlations of severity scores and AUDPC and grain yield. Further, regression analysis showed a negative effect of MSV severity and AUDPC at Embu of 16 and 15% respectively. It is possible that MSV was severe later in the season after disease rating had ceased (56 DAI or 77 DAP). The disease progress curves did not plateau at Embu during the period of disease assessment also suggesting that severity increased further on into the season. This could have

contributed to the negative effect of MSV on yield that was not detected at Muguga. It is noted therefore that due to differences in environments in affecting disease progress for MSV, readings should be taken when susceptible cultivars have reached the highest levels of the disease. In addition, in environments where disease progress was slow, the interval of the disease ratings can be longer. Thus, the seven-day interval used at Muguga did not suffice for Embu since disease progress was slow at Embu and had an eventual effect on yield. Bombom et al. (2012) noted that MSV symptoms are most stable just after flowering since at this stage no more new leaves develop. However, for QTL detection, (Pernet et al., 1999a) suggested that the best time for screening for MSV is between 14 DAI and 35 DAI to prevent masking effects of minor QTLs and avoid interferences from other abiotic and biotic stresses.

Heritability estimates were high for final MSV severity scores (0.94) indicating that genetic variance highly controlled the trait and phenotypic selection would be effective for improving resistance. Since inbred lines were evaluated, heritability in this case is in the narrow sense, hence additive effects highly controlled resistance, which can be fixed in selection. Heritability estimates were within the range reported for MSV (Gandiwa, 2007, Welz et al., 1998). A slightly lower heritability (0.88) was however obtained when mean scores were used in this assessment. Welz et al. (Welz et al., 1998) also found a higher heritability (0.93) with scores taken at 83 DAI and a lower score (0.62) with scores taken at 21 DAI. Heritability for MSV has therefore been found to increase with time after infection (Pernet et al., 1999a). Heritability estimates were however low for AUDPC (0.57) across environments. This implied the substantial influence of the environment in the variability of AUDPC. High heritability estimates for AUDPC have however been obtained in other maize diseases such as southern leaf blight (0.91) (Ali et al., 2012). Heritability was also high for grain yield (0.90) showing the potential use of this germplasm to improve other agronomic traits besides disease resistance. The high heritability estimates for MSV resistance also suggest that selection for MSV resistance can be based on the visual scoring system as used in this study.

3.5 Conclusion

There were high levels of resistance in most of the inbred lines evaluated indicating availability of potential sources of resistance from different regions. Correlation coefficients between AUDPC values for disease severity with MSV severity scores were significant and positive resulting in similar rankings of the genotypes. Therefore, a single assessment for the final disease severity would be adequate for MSV resistance, especially for screening large numbers

of germplasm. The AUDPC values for MSV disease severity were not correlated with flowering days (50% to anthesis and silking) therefore, maturity did not influence AUDPC values. Heritability estimates were high for MSV resistance indicating progress would be made from selection. The results demonstrated that high levels of resistance are available in locally and regionally adapted germplasm and additional sources of resistance to MSV have been identified, which will be used in controlling this important disease.

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CHAPTER 4

Molecular characterization of maize streak virus resistant germplasm using SSR markers

Abstract

Knowledge of genetic diversity of potential inbred line parents is important in maize breeding programs that emphasize F_1 hybrids. Such information is not available for programs in sub-Saharan Africa to devise strategies for breeding MSV resistant hybrids. Therefore, the objectives of this study were to investigate the levels of genetic diversity in tropical inbred lines with potential for use as parents for MSV resistant hybrids. A set of 40 tropical maize inbred lines were selected for this study. They included six lines from IITA, 17 from CIMMYT, one from CIRAD, two from Grains Crop Institute, South Africa, and 14 developed by KARI, Kenya. These MSV resistant inbred lines were assayed for polymorphism using 28 SSRs markers. In total, 135 SSR alleles were identified, with a mean of 4.8 alleles per locus ranging from 2 to 11. The polymorphic information content (PIC), a measure of gene diversity, ranged from 0.13 to 0.85.SSR markers sampled were highly polymorphic as 12 of them displayed PIC values greater than 0.6. Genetic distances (GD) among the 40 lines ranged between 0.12 and 0.72, with 60% of the 780 combinations having GD greater than 0.5.There were large GDs for the inbred lines from different programmes in the region. Inbred lines were clustered into seven groups consistent with genetic distances, available pedigrees and genetic background information. The information would be helpful in undertaking detailed genetic and molecular analyses of MSV resistance, besides utilization of promising genotypes in breeding for MSV resistance in tropical maize hybrids.

4.1 Introduction

Knowledge of genetic diversity is pertinent for a systematic sampling of germplasm for breeding purposes (Cortese et al., 2010). When this information is obtained within and among different maize germplasm it provides a guide for predicting the degree of inheritance, level of variation, and amount of heterosis, that are essential for efficient breeding of maize (Duan et al., 2006). While the exchange of germplasm between institutions is becoming more frequent, it is advisable to assess their molecular diversity if redundant genotypes are to be eliminated (Yong-Jin et al., 2009). Germplasm introductions often represent a wide array of material that can be used in disease and pest resistance, nutritional quality, and other traits of interest to the

consumer (Liu et al., 2003). A wide array of germplasm with maize streak virus resistance has been developed in several programs in Africa (Bjarnason, 1986, Bosque-Perez, 2000, Kim et al., 1989, van Rensburg, 2005). Breeders in sub-Saharan Africa face challenges in selecting inbred lines for use in hybrids especially for programmes, which emphasize MSV resistance. This is because of the difficulty in selecting suitable genetic backgrounds for use as sources of MSV resistance. While designing hybrids, breeders need to find divergent sources of MSV resistance. Many lines have released based MSV resistance at CIMMYT, IITA and other programmes, but different genetic background of these lines is unknown.

Among the protocols that facilitate the assessment of molecular diversity, simple sequence repeats (SSRs) or microsatellite variation is the preferred system since it detects a large number of DNA polymorphisms with relatively simple technical complexity (Li et al., 2002, Yong-Jin et al., 2009). Simple sequence repeat (SSR) markers are PCR based, genetically co-dominant, robust, reproducible, hyper variable, informative and reasonably easy to use (Powell et al., 1996). Also, SSR markers are available in abundance in maize (http://www.maizegdb.org, verified 25 Nov 2013) and, in addition, they offer significant advantages in DNA fingerprinting, genetic diversity analysis, gene/QTL mapping, and molecular marker-assisted breeding in crops (Betrán et al., 2003, Reif et al., 2003a, Warburton et al., 2002). The choice of SSR markers is also because these markers are genome knowledge-based and therefore many molecular markers are not needed to assay genetic diversity among germplasm as long as the markers are selected from all the 10 chromosomes within the maize genome (Badu-Apraku et al., 2013). Therefore SSR markers being relatively easier to analyse by PCR and simple to interpret, have been shown to be superior to other DNA markers and are widely used in assessing genetic diversity, identifying germplasm, and characterizing population structure for systematic introgression of exotic germplasm (Aguiar et al., 2008, Choukan et al., 2006, Menkir et al., 2004, Reif et al., 2003a, Xu et al., 2004).

Linking genetic diversity of germplasm to the phenotype would greatly aid in hybrid development in the improvement of MSV resistance in maize. Moreover, if markers can help delimit heterotic groups within the sampled MSV resistant inbreds from different sources, breeding for MSV resistance could be done without interrupting the groupings that exist within germplasm. There have been few attempts made to characterize maize germplasm used in the eastern African region (Ininda et al., 2007, Legesse et al., 2007, Wende et al., 2012) but this has not been done specifically targeting at MSV resistant inbred lines.

Thus until now the genetic orientation and background of MSV resistant inbred source germplasm has not been established. The random use of resistance sources could compromise heterosis in maize hybrids because in many cases existing viable heterotic groups are disrupted. This can partly explain the yield plateau, which has not been broken since the time breeders started to emphasize MSV resistance in maize. This has led to conclusions that maize varieties resistant to MSV have low yield potential and are thus not commercially successful (Martin and Shepherd, 2009). Resistant germplasm has to be carefully sorted before deployment in hybrids. The present study was undertaken with the purpose of molecular profiling and analysing genetic diversity in a selected set of lines with distinct differences in response to maize streak virus (MSV) disease. The objectives were to (i) investigate the genetic diversity among tropical inbred lines and relationships among MSV resistant germplasm, (ii) associate genetic variation for phenotype with diversity grouping with molecular markers, and (iii) identify SSR markers effective for fingerprinting of MSV germplasm.

4.2 Materials and Methods

4.2.1 Germplasm

Seeds of 40 maize lines were accessed breeding programmes to represent MSV resistant germplasm used for breeding for disease resistance in Eastern, Western and Southern Africa. Fourteen (14) lines were obtained from the Kenya Agricultural Research Institute (KARI), six from the International Institute of Tropical Agriculture (IITA), 17 from the International Maize and Wheat Improvement Centre (CIMMYT), two from grains crops institute and one from CIRAD. The sources of resistance in these lines are varied but based on available pedigree they include CML202, Osu23i, CML197, and MSR. The pedigrees and seed sources of these materials are given in Table 4.1.

Table 4.1. Forty maize lines used in the study indicating pedigree and source

Entry	Code	Pedigree	Grain type[a]	Source
1	REGN36	EAREGNUR 36-1	FL	KARI
2	EC14	EC 573(R12)C_8-S_3-14	F	KARI
3	Z168	SZSYNKITII-F2	DL	KARI
4	Z419	SYN[Kitale/Tuxp-GLS]F2	I	KARI
5	Z426	[CML 197/N3//CML206]-X-32-1-1-2-B-B	F	KARI
6	REGN29	EAREGNUR 29-5-1	FL	KARI
7	S558	[EM12-210/CML197//E12-210/OSU23i]-x-58-2-2-3-1	FL	KARI
8	REGN99	EA REGNUR 99/96	I	KARI
9	MUL71	[EM12-210/CML 202]-X-71-1-2-1-1-3	F	KARI
10	MUL88	[EM11-133/OSU23i]-X-88-5-2-3-2	F	KARI
11	MUL104	[EM11-133/CML 197]-X-104-1-3-1-1-5	FL	KARI
12	OSU23i	[MSRXPOOL9] C1F2-205-1	F	CIMMYT/OSU
13	CML 204	[7794]-SELF-4-1-S9-1-4-7-4-5-BB	FL	CIMMYT
14	CML505	[92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	I	CIMMYT
15	CML202	ZSR923S4BULK-5-1-BB	FL	CIMMYT
16	CML509	[92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB	FL	CIMMYT
17	MUL1184	[EM12-210/OSU23i]-X-118-1-1-3-4-1	FL	KARI
18	MUL114	[EM12-210/OSU23i]-X-114-2-2-1-3	F	KARI
19	MUL1182	[EM12-210/OSU23i]-X-118-1-1-3-1-2	I	KARI
20	C92	_	I	CIRAD
21	C238-8	MAS [206/312]-159-2-3-4-1-B	FL	CIMMYT
22	C238-7	MAS [202/312]-20-1-1-4-2-B	FL	CIMMYT
23	C238-3	MAS [202/312]-20-1-1-1-1-B	FL	CIMMYT
24	C238-10	MAS [MSR/312]-119-3-1-1-2-B	FL	CIMMYT
25	C238-14	MAS [MSR/312]-119-5-1-1-3-B	F	CIMMYT
26	C238-21	MAS [MSR/312]-111-3-1-2-1-B	F	CIMMYT
27	C238-25	MAS [202/312]-20-1-1-4-1-BB	F	CIMMYT
28	C238-28	MAS [MSR/312]-X-119-5-1-3-2-BB	F	CIMMYT
29	C238-33	[EV7992#/EVPO44SRBC3]#BF37SR-2-3SR-3-5-2-BB	F	CIMMYT
30	C238-34	MAS [MSR/312]-117-2-2-1-B*4	FL	CIMMYT
31	C238-16	MAS [MSR/312]-119-5-1-3-2-B	F	CIMMYT
32	C238-9	MAS [MSR/312]-119-1-1-4-1-B	F	CIMMYT
33	TZM1736	_	FL	IITA
34	TZM1746	_	FL	IITA
35	TZM1750	_	FL	IITA
36	TZM1759	_	F	IITA
37	TZM1749	_	F	IITA
38	TZM1760	_	FL	IITA
39	A076	_	FL	South Africa
40	VHCY	_	FL	South Africa

[a] grain type: F=Flint, FL=Flint-like, I=intermediate, DL=Dent-like[a]: W=white, Y=Yellow, I=intermediate; germplasm sources, CIMMYT = International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, CIRAD = Centre de cooperation internationale en recherché agronomique pour le développement, KARI =Kenya Agricultural Research Institute

4.2.2 Phenotypic evaluation

The 40 inbred lines were evaluated for MSV disease reaction and other agronomic traits under artificial disease pressure at two sites, Muguga (2096 m asl; $1^0$13'S, $36^0$38'E) Embu (1508 m asl $0^0$53'S, $37^0$45'E) in long rain season (March-September) of 2011. The experimental design was an α- lattice of four blocks with ten genotypes each, replicated two times in each location. The experimental unit consisted of two 2.5 m rows spaced at 0.75-m with a plant density of 53333 plants ha^{-1}. Standard agronomic practices for maize experimental fields were followed.

In both locations, inoculation with MSV using viruliferous *Cicadulina mbila* hoppers was conducted 21 days after plant emergence using three leafhoppers placed in a plastic vial attached to distal portion of the lower leaf. The severity of MSV was rated as described by Beyene et al. (2012), where 1 = no symptoms on leaves, 2 = light symptoms on 20 to 40% leaf area, 3 = moderate symptoms on 40 to 60% leaf area, 4 = severe symptoms on 60% of leaf area, 5 = severe symptoms on 75% or more of leaf area, plants severely stunted, dying or dead. Resistance was classified as follows: 1.0 (Symptomless, Immune), 1.1-1.4 (highly resistant), 1.5-2.4 (resistant), 2.5-2.9 (moderately resistant), and 3.0-5.0 (susceptible).

4.2.3 Simple sequence repeats (SSRs) assays

Twenty-eight SSR primer pairs, distributed throughout the maize genome were chosen based on their repeat unit and base composition. Primers for these SSR markers were synthesized through Research Genetics Inc., USA using sequence information available in the public domain (http://www.maizegdb.org, verified 25 Nov 2013). Information regarding map position and repeat type for each of the SSR primers used can be found in Table 3.3. The SSRs were multiplexed for maximum efficiency. Polymerase chain reaction (PCR) was conducted in a volume of 10 ul in a 96-well microtiter plate, containing 20 ng of the template DNA, 10uM of forward primer, 10uM of reverse primer, 2mM of each dNTP, 25mM of $MgCl_2$, and 5U/ul of HotStarTaq polymerase. The amplification reaction began with 15 min at 95 ˚C and 1 min at 72 ˚C. The last cycle was repeated 35 times and terminated at 72 ˚C for 30 min. The PCR products were prepared for separation on capillary electrophoresis (Applied Biosystems, Foster City, USA).

4.2.4 Data analysis

An analysis of variance for the MSV response data was conducted using the following general linear model, tested for the genotypic differences: $Y_{ij} = \mu + \beta_i + \alpha_j + \varepsilon_{ij}$where: Y_{ij} = observations; μ= mean of experiment; β_i= effect ofi^{th}replicate; α_j= effect due toj^{th} genotype in thei^{th}replicate and ε_{ij} = experimental error.

The SSR bands were scored as present (1) absent (0), and missing (-1), each of which was treated as an independent character. Genetic similarities (GS) coefficients were calculated from SSR data for all possible pairs of inbreds by the following equation: $\mathrm{GS_{ij}} = 2\mathrm{N_{ij}}/(\mathrm{N_i} + \mathrm{N_j})$, where $\mathrm{N_{ij}}$ is the total number of bands common to lines i and j(Nei and Li, 1979). Genetic similarity values were converted into genetic distance: $\mathrm{GD} = 1 - \mathrm{GS}$. The GD values vary from 0 in identical profiles between two inbreds to 1, where there are no common bands. The average number of alleles (*A*) was estimated as follows: $A = \sum_{i=1}^{n} A_i \,/n$; where A_i is the number of alleles at the i^{th} allele. The expected heterozygosity (H_e), was calculated according to Nei (1978) using the equation: $H_e = n(n-1) * (1 - \sum p_i^2)$ where p_i is the frequency of alleles i in the analyzed trees and n is the number of alleles. Polymorphism information content (PIC), a measure of the allele diversity at a locus, was estimated for each of the polymorphic SSR loci detected in the present study using the following equation: $\mathrm{PIC} = 1 - \sum_{i=1}^{l} p_i^2 - 2\sum_{i=j+1}^{l}\sum_{j=1}^{l-1} p_i^2\, p_j^2$; where p_i and pj are the frequencies of the i^{th} and j^{th} alleles at a locus with l alleles in a population, respectively (Botstein et al., 1980).

Pairwise comparisons were made between the genotypes based on DICE (Dice, 1945) similarity coefficient. The resultant distance matrix data were used to construct a dendrogram using the agglomerative hierarchical un-weighted pair-group method with an arithmetic average (UPGMA) sub-programme of NYSTS (Rohlf, 1998).

4.3 Results

4.3.1 Phenotypic performance for MSV disease response

There were differences in resistance levels among the inbred lines with respect MSV disease (Table 4.2). The scores ranged from 1 to 3.3 across the two locations, Muguga and Embu. However, Muguga had higher disease scores with the most severe symptoms in line EC14 with score of 4. Lines with OSU23i in their pedigree including MUL88, MUL1182, MUL1184 and MUL114 had the lowest disease ratings, most having scores of 1. Other lines with high

resistance originated from CIMMYT Harare with MAS[MSR/312] background. Materials from IITA had moderate levels of resistance ranging from 1.6 for TZM1750 to 2.3 for TZM1760. Some of the lines that showed MSV susceptibility, scores>3, in Muguga were EC14, Z426, REGN99, REGN29, A076.

Table 4.2. Mean MSV scores of 40 maize lines evaluated under artificial infection in two locations in 2011

Line name	Muguga		Embu		Across sites	
	MSV	rank	MSV	rank	MSV	rank
MUL88	1.0	1	1.0	1	1.0	1
OSU23i	1.0	1	1.0	1	1.0	1
MUL1184	1.0	1	1.0	1	1.0	1
MUL114	1.0	1	1.0	1	1.0	1
MUL1182	1.0	1	1.0	1	1.0	1
C92	1.0	1	1.0	1	1.0	1
C238-14	1.0	1	1.0	1	1.0	1
C238-21	1.0	1	1.0	1	1.0	1
CML539	1.0	1	1.0	1	1.0	1
C238-10	1.0	1	1.1	10	1.1	10
C238-28	1.0	1	1.1	10	1.1	10
C238-16	1.0	1	1.1	10	1.1	10
C238-9	1.0	1	1.1	10	1.1	10
VHCY	1.5	14	1.4	15	1.4	14
CML509	1.7	17	1.3	14	1.5	15
TZM1750	1.5	16	1.7	19	1.6	16
CML204	1.5	15	1.8	23	1.7	17
CML505	1.9	18	1.6	18	1.7	18
TZM1759	2.3	22	1.5	17	1.9	19
TZM1736	2.1	19	1.8	23	2.0	20
TZM1746	2.6	24	1.5	16	2.0	22
TZM1749	2.2	20	1.8	22	2.0	21
S558	2.2	20	2.0	30	2.1	23
C238-3	2.4	23	1.9	25	2.1	24
CML202	2.7	27	1.9	25	2.3	28
C238-8	2.9	29	1.7	19	2.3	26
C238-7	2.6	24	1.9	27	2.3	25
TZM1760	2.9	30	1.7	19	2.3	27
REGN36	3.0	31	1.9	27	2.4	31
MUL71	2.6	26	2.1	33	2.4	29
C238-25	2.8	28	2.0	29	2.4	29
A076	3.0	32	2.0	30	2.5	32
REGN99	3.0	34	2.1	33	2.6	33
Z426	3.3	36	2.0	32	2.7	34
REGN29	3.0	33	2.3	35	2.7	35
MUL104	3.2	35	2.3	35	2.9	36
CM	3.3	37	2.6	38	3.0	37
Z168	3.9	39	2.5	37	3.2	39
Z419	3.7	38	2.6	38	3.2	38
EC14	4.0	40	2.7	40	3.3	40
Mean	2.14		1.65		1.89	
$LSD_{0.05}$	0.501		0.602		0.418	

4.3.2 SSR polymorphism

The twenty-eight SSR primers identified 135 alleles among the 40 maize inbred lines. Diversity at the gene level showed an average of 4.8 alleles per locus and a range of 2 to 11 alleles per locus (Table 4.3). Two markers (Phi 062, Umc1327) revealed two alleles, and the highest numbers of alleles (9-11) were detected at the phi227562, phi96100 and umc1545. The SSR di-nucleotide repeat units revealed a higher number of average alleles per locus (4.5) compared to tri-nucleotide repeat units (3.9). The tetra-nucleotide repeats, however, revealed the highest average number of alleles (6.4).

The discriminatory power of an SSR marker as defined by the number of alleles and the relative frequency of the alleles at a locus and in the population being studied is estimated in the PIC values (Senior et al., 1998). In the present study, the PIC for all loci ranged from 0.134 to 0.85 with a mean of 0.52 (Table 4.3). Twelve SSR markers (phi299852, phi123, phi076, phi079, phi374118, phi308707, phi056, phi96100, phi114, phi041, umc1545, and phi227562) manifested a PIC value of more than 0.6, reflecting their potential to detect differences between the inbred lines. The marker, phi227562 that detected the highest number of alleles, 11, and the highest PIC value of 0.85, had a tetra-nucleotide repeat. Low PIC values were however obtained for markers umc1367 (0.13), phi062 (0.15) and nc133 (0.18). Expected heterozygosity (He) values, as a measure of allelic diversity at a locus, varied from 0.0588 to 0.6275 with an average of 0.2168. The SSR loci showing high and low levels of heterozygosity were phi10228 (0.63) and phi299852 (0.06), respectively.

Table 4.3. Characteristics of the 28 SSR loci used in study including chromosomal location, repeat type and PIC estimates

Chromosome location	SSR locus	Repeat type	Bin no	No. alleles	He*	PIC value
Chromosome 1	phi056	CCG	1.01	5	0.106	0.677
	phi227562	ACCT	1.11	11	0.392	0.851
	phi308707	AGC	1.1	6	0.353	0.672
	umc1917	CTG(6)	1.04	3	0.125	0.31
Chromosome 2	nc133	GTGTC	2.05	3	0.059	0.183
	phi96100	ACCT	2.00-2.01	9	0.271	0.692
Chromosome 3	phi029	AG/AGCG***	3.04	4	0.255	0.487
	phi102228	AAGC	3.06	3	0.628	0.394
	phi374118	ACC	3.02	4	0.275	0.651
Chromosome 4	phi072	AAAC	4	4	0.163	0.591
	phi076	AGCGGG	4.11	5	0.098	0.631
	phi079	AGATG	4.05	5	0.061	0.635
Chromosome 5	nc130	AGC	5	4	0.438	0.406
	phi331888	AAG	5.04	5	0.157	0.487
Chromosome 6	phi031	GTAC	6.04	4	0.204	0.411
	phi075	CT	6	5	0.245	0.548
	phi123	AAAG	6.07	5	0.26	0.602
	phi299852	ACC	6.07	5	0.059	0.602
Chromosome 7	phi112	AG	7.01	4	0.118	0.437
	phi114	GCCT	7.03	6	0.229	0.724
	umc1545	AAGA(4)	7	9	0.255	0.767
Chromosome 8	umc1161	GCTGGG(5)	8.06	4	0.196	0.407
	umc1304	TCGA(4)	8.02	3	0.255	0.457
Chromosome 9	phi065	CACTT	9.03	5	0.225	0.414
Chromosome 10	phi041	AGCC	10	7	0.347	0.767
	phi062	ACG	10.04	2	0.061	0.153
	phi084	GAA	10.04	3	0.082	0.349
	umc1367	CTG(6)	10.03	2	0.157	0.134
			Mean	4.8	0.22	0.52
			Max	11.0	0.63	0.85
			Min	2.0	0.06	0.13

*He=expected heterozygosity

4.3.3 Genetic distances

Genetic distance (GD) estimates were expressed by Dice similarity coefficient for all 780 pairwise comparisons among the 40 inbreds. The largest GDs were obtained from contrasting groups (sources) while the smallest GDs were found within groups (Table 4.4). Thus, the smallest GD of 0.12 was found between lines C238-28 (CIMMYT) and C238-6 (CIMMYT) and the largest GD of 0.72 was obtained between MUL1182 (KARI) and TZM1759 (IITA). Genetic distance within each group of lines by source, CIMMYT, IITA and KARI was also examined. The genetic distance of the six IITA lines sampled averaged at 0.37, ranging from 0.19 between TZMI759 and TZMI749 to 0.56 between TZMI1736 and TZMI759. Seventeen (17) lines sampled from CIMMYT had average genetic distance of 0.43, ranging from 0.12 between C238-28 and C238-16 to 0.63 between CML505 and CML202. Among the 14 KARI lines sampled, genetic distance averaged at 0.52 ranging from 0.16 between MUL1184 and MUL1182 and 0.66 between EC14 and REG99. In general, 26 of the 780 combinations (3%) had genetic distance less than 0.3 while 62% of the combinations had genetic distances ≥ 0.50 (Fig. 4.1). A few combinations (12%) however had GD exceeding 0.61.

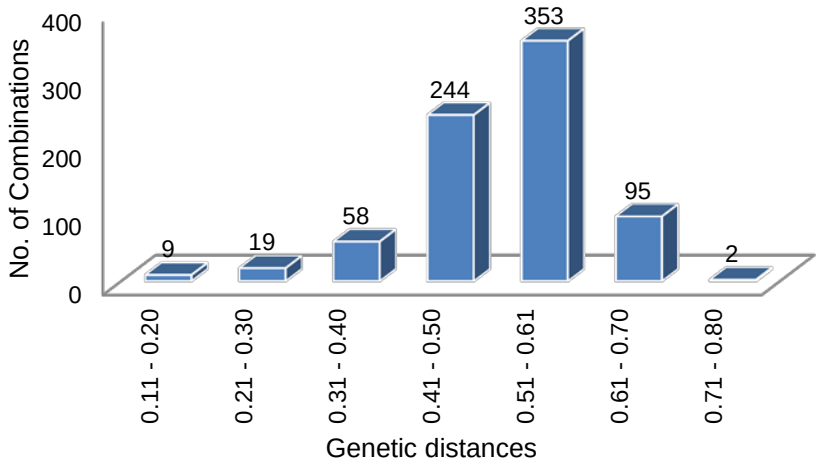

Figure 4.1. Genetic distances based on 28 SSR markers of the 40 lines in 780 combinations

Table 4.4. Genetic distances among 40 lines represented by top, middle, bottom ten divergence combinations

10 combinations with lowest GD	GD	10 combinations with moderate GD	GD	10 combinations with highest GD	GD
C238-28&C238-16	0.12	C238-21&A076	0.52	MUL1184&TZM1759	0.68
C238-7&C238-25	0.13	MUL104&C238-3	0.52	MUL1182&C238-16	0.68
C238-3&C238-25	0.13	CML202&TZM1746	0.52	MUL1182&TZM1749	0.68
C238-7&C238-3	0.16	C238-8&TZM1759	0.52	REGN29&C238-16	0.68
MUL1184&MUL1182	0.16	C238-3&CML539	0.52	Z168&TZM1736	0.69
S558&CML202	0.18	C238-10&TZM1760	0.52	REGN29&TZM1736	0.69
C238-10&C238-9	0.19	C238-21&C238-28	0.52	CML539&TZM1736	0.69
TZM1759&TZM1749	0.19	C238-21&TZM1759	0.52	REGN29&C238-28	0.70
C238-10&C238-28	0.20	C238-25&CML539	0.52	MUL1182&C238-9	0.71
CML505&CML509	0.21	C238-16&A076	0.52	MUL1182&TZM1759	0.72

Average GD = 0.51; Max 0.72, Min 0.12

Table 4.5. Genetic distances of ten most resistant lines, three lines from each major source with MSV response in parenthesis

Inbred line	C92(1.0)	MUL1184(1.0)	MUL114(1.0)	MUL88(1.0)	CML539 (1.0)	C238-9(1.1)	CML509(1.5)	TZM1759(1.9)	TZM1736(1.9)	TZM1750(1.6)
Source	CIRAD	KARI	KARI	KARI	CIMMYT	CIMMYT	CIMMYT	IITA	IITA	IITA
C92	-	0.56	0.52	0.47	0.60	0.51	0.50	0.49	0.57	0.45
MUL1184		-	0.49	0.48	0.53	0.68	0.56	0.68	0.67	0.61
MUL114			-	0.24	0.54	0.53	0.48	0.67	0.66	0.62
MUL88				-	0.53	0.51	0.46	0.62	0.61	0.50
CML539					-	0.50	0.55	0.51	0.69	0.63
C238-9						-	0.50	0.53	0.65	0.51
CML509							-	0.57	0.57	0.52
TZM1759								-	0.56	0.45
TZM1736									-	0.45
TZM1750										-

The genetic distances of the most resistant lines by source of origin are presented in Table 4.5. Among highly resistant lines across sources, the highest genetic distance (0.69) was found between CML539 (CIMMYT) and TZMI736 (IITA). Other high genetic distances (0.68) were found between MUL1182 (KARI) and C238-9 (CIMMYT) and between MUL1184 (KARI) and TZM1759 (IITA). Across these highly resistant sources, the least GD (0.45) was between C92 (CIRAD) and TZMI750 (IITA). Thus, except for one combination within KARI lines, MUL88 and MUL114 where GD was low (0.24), it was possible to find divergent sources among the highly resistant lines within and between sources.

4.3.4 Cluster analysis based on SSR markers

Genetic similarity based on the 135 alleles in the 40 accessions is presented in a cluster by the method of UPGMA (Fig. 4.2). The 40 lines were divided into seven clusters, except for lines REG99/96 and A076 that were not grouped in the clusters suggesting a weak relationship of these lines with the other germplasm (Fig. 4.2 &Table 4.6).

Cluster I consisted of seven lines, six of IITA origin and line REGN36-1 probably suggesting this line might have a background similar to IITA germplasm. Cluster II had seven lines mostly from CIMMYT having either CML202 or CML197 in their pedigree, in addition to line VHCY from South Africa. Cluster III had only CIMMYT lines from marker assisted selection (MAS) [MSR/312] background and all the seven lines had MSV scores of ~ 1 (Table 4.2) showing high levels of resistance. Cluster IV had three lines, all of different origin and background; CIMMYT, KARI and Regional nursery. Cluster V had four lines, three lines with highland (Kitale) origin except C92. Cluster VII had five lines, three of these lines had MSV resistant parent OSU23i in their pedigree while two lines were the closely related CIMMYT lines, CML505 and CML509. Lastly, Cluster VII had three KARI lines, all with a background of EMBU12-210. Two lines CML202 and OSU23i appeared in the same clusters with inbred lines derived from them. However, CML202 was found in cluster II whereas OSU23i was found in cluster VI. The two lines are commonly used as sources of MSV resistance in Africa.

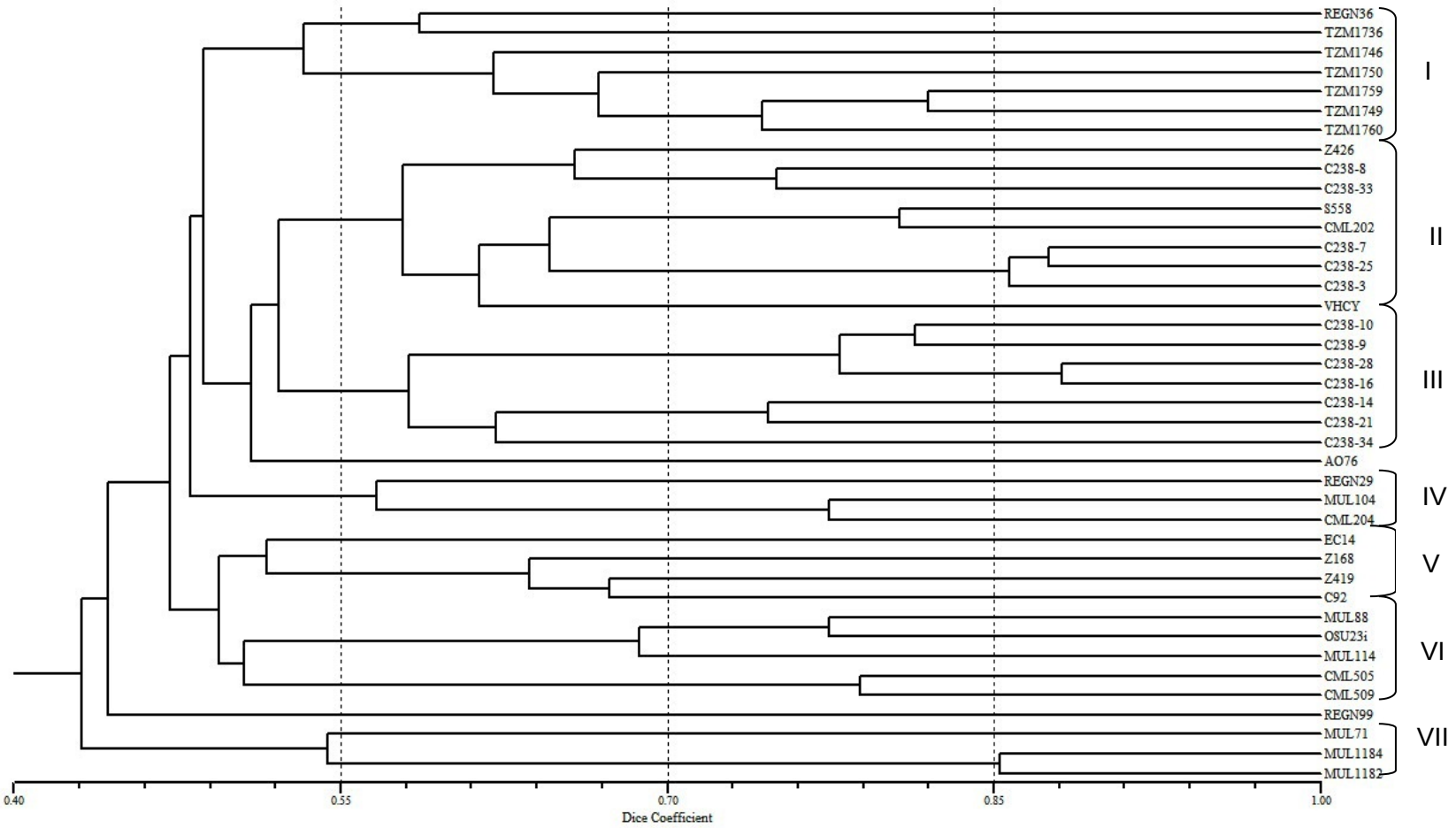

Figure 4.2. Dendrogram of cluster analysis of 40 maize lines tested with 28 SSRs. I-VII indicate the codes of the clusters

Table 4.6. Clustering pattern of 40 lines based on 28 SSR markers

Cluster	Genotype	Number of genotype
I	REGN36, TZMI736, TZMI746, TZMI750, TZMI759, TZMI749, TZMI760	7
II	Z246, C238-8, C238-33, S558,CML202, C238-7, C238-25, C238-3,VHCY	9
III	C238-10, C238-9, C238-28, C238-16, C238-14, C238-21, C238-34[a]	7
IV	REGN29, MUL104, CML204	3
V	EC14, Z168, Z419, C92	4
VI	MUL88, Osu23i, MUL114, CML505, CML509	5
VII	MUL71, MUL1184, MUL1182	3
nc[b]	AO76	1
nc[b]	REGN99	1

[a]C238-34- this line was recently released by CIMMYT with the name CML539; nc[b] these two lines were unclustered

4.3.5 Genetic structure of the maize lines

The results presented in Table 4.7 indicate modest genetic differentiation of 15% of estimated genetic variances among the lines according to the origin or source populations i.e., KARI, CIMMYT, IITA, and others. In addition, only 2% of individuals within a population were genetically differentiated while the largest variation, (83%) was within individual accessions. The genetic structure of the four populations was further analysed using principal component analysis (PCA) in GenAlEx and delivered similar results where the overall topology of the PCA plot was similar to the dendrogram constructed in NTSYS v.2.1 (Figure 4.3). Twenty-seven unique alleles were detected in 12 SSR loci across all 40 inbred lines analysed (Table 4.8). The highest number of alleles was detected in SSR loci phi10228 (3 out of 3), while five other loci had 3 out of 4 alleles as unique, namely: phi029, phi031, phi112, phi374118 and umc1161. Line C92 had the highest number of unique alleles.

Table 4.7. Analysis of molecular variance (AMOVA) for SSR variation among and within maize accessions by source

Source of variation	d.f.	MS	variance component	variation (%)
Among populations	3	348.8	14.521	15
Among individuals within a population	36	83.5	1.778	2
Within individuals	40	80.0	80.038	83
Total	79		96.336	100

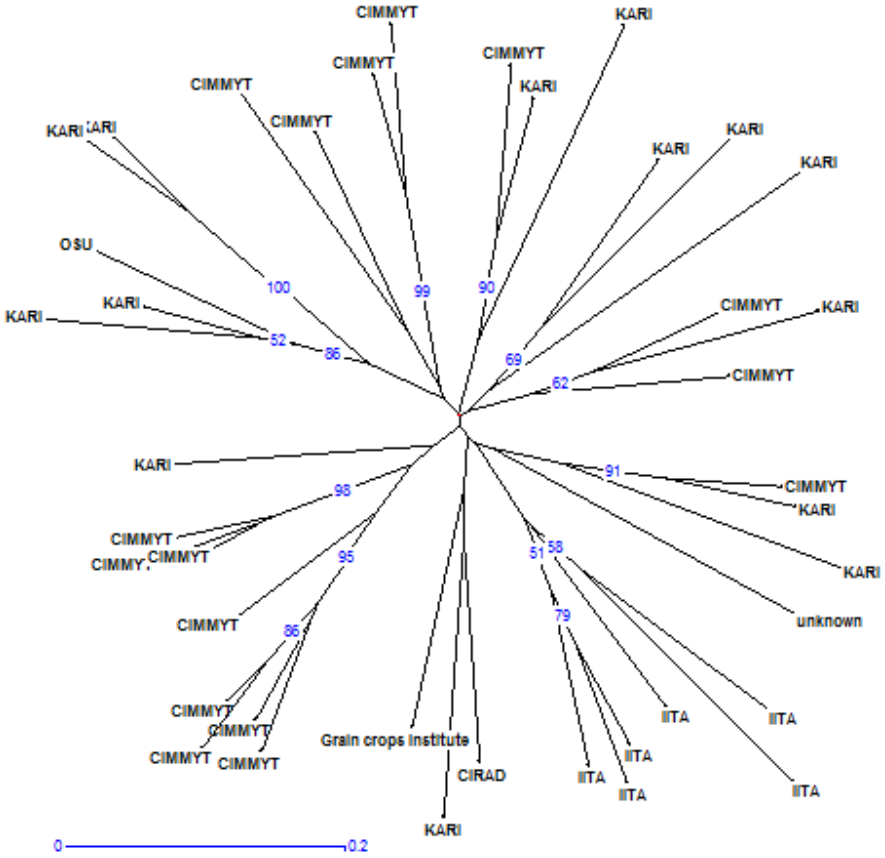

Figure 4.3. Cluster analysis of 40 inbred lines based on source of origin

Table 4.8. Unique alleles identified by 12 markers for specific maize lines

Locus	Allele size	Inbred allele is unique for	MSV response†	SSR data cluster
phi374118	206	REG36	R	I
phi374118	219	REG36	R	I
phi374118	226	REG36	R	I
umc1161	144	Z426	MR	II
umc1161	147	Z426	MR	II
umc1161	150	Z426	MR	II
phi029	148	C92	I	V
phi029	153	C92	I	V
phi029	160	C92	I	V
phi031	192	C92	I	V
phi031	204	C92	I	V
phi031	229	C92	I	V
phi96100	275	C92	I	V
phi96100	281	C92	I	V
phi96100	284	C92	I	V
phi056	252	C92	I	V
phi041	197	Z419	S	V
phi114	135	Z419	S	V
phi114	160	Z419	S	V
phi114	163	Z419	S	V
phi102228	116	Z168	S	V
phi102228	122	Z168	S	V
phi102228	127	Z168	S	V
phi112	152	MUL1182	I	VII
phi112	157	MUL1182	I	VII
phi112	159	MUL1182	I	VII
umc1545	66	REG99	MR	No cluster
umc1545	74	REG99	MR	No cluster
umc1545	79	REG99	MR	No cluster
nc130	153	REG99	MR	No cluster

†MSV response: I= Immune, R= resistant, MR=moderately resistant, S= susceptible

4.4 Discussion

4.4.1 Genetic diversity and new sources of MSV resistance

High variability of MSV response was observed among the 40 maize genotypes indicating the existence of promising and potentially useful germplasm for improving MSV resistance for breeding programmes. Some lines with scores of <1.5 should be explored for probable use in transferring resistance to adapted but MSV susceptible backgrounds. These were identified from different regions including MUL1182, MUL1184, MUL114, and MUL88 from KARI, VHCY from South Africa, C238-10, 14, 21, 28, 34, 16 and 9 from CIMMYT Harare germplasm, CIRAD's C92 and OSU23i. Notably, some of these outstanding MSV resistant lines could also be used for multiple disease resistance having tested favourably for GLS (Ininda et al., 2007) such as REG36 and S558. It is important to explore and identify resistance emerging from different regions to improve diversity of populations for breeding disease resistance that target stability of resistance across regions.

4.4.2 SSR polymorphisms

The 28 SSR markers used in studying the 40 maize inbred lines identified 135 alleles averaging at 5.0 alleles per locus. The average number of alleles per locus was close to that reported in several studies in maize (Lu and Bernado, 2001, Vaz Patto et al., 2004, Wende et al., 2012). Often, the number of alleles detected is influenced by differences in sample size, nucleotide nature of the repeat base microsatellite, and the broad genetic base of the genetic materials analysed (Badu-Apraku et al., 2013). The markers with tetranucleotide repeats revealed the highest average number of alleles. However other studies have mostly reported most alleles from dinucleotide repeats (Legesse et al., 2007, Li et al., 2002, Wang et al., 2011). It would also seem that increasing the number of SSR markers would not proportionately increase the number of amplified alleles per primer. The present study found an average of 4.8 alleles in 40 lines with 28 markers; whereas in the studies of Wegary et al. (2013) with 15 lines and 40 SSR markers, alleles averaged at 4.2.

Polymorphic information content values give the discriminatory power of a marker based on allele number and frequency of those alleles in the population of study. Thus, marker loci with a large number of alleles occurring at equal frequencies would have the highest PIC values (Senior et al., 1998). This was confirmed in the present study with markers, phi96100, phi041, umc1545, and phi227562, which had the highest number of alleles ranging from 7 to 11 and high PIC values ranging from 0.69 to 0.85. The primer phi96100, could be a reliable marker, as

it has been similarly shown to reveal high number of alleles (Wegary et al., 2013). Overall, the high discriminatory power of the four markers (phi96100, phi041, umc1545, and phi227562) suggests that they may be suitable for exploration of genetic diversity in large-scale and fine-scale analyses.

The average PIC value obtained of 0.52 was close to that of Legesse et al. (2007) (0.58) who used a similar number of maize lines and SSR markers. Lower values of PIC have been attributed to use of small number of accessions (Li et al., 2002), method of allele detection (Senior et al., 1998) and repeat unit of SSR markers (Xu et al., 2004). In this study, SSR markers with tetranucleotide repeats had the highest PIC values in the range of 0.6 to 0.85. This is in contrast with results of Xu et al. (2004) who found higher PIC values in SSR primers with more dinucleotide repeat. However, most of the markers had PIC values of more than 0.6, reflecting their potential to detect differences between the inbred lines.

4.4.3 Genetic distance

Genetic distance in this study ranged from 0.12 to 0.72 among the 780 pairwise comparisons of the 40 lines. Only 26 comparisons (3%) fell within a genetic distance less than 0.3 indicating lack of redundant lines. Further, these low genetic distance were obtained from within group sources, most of which were derived from the same populations. Whereas 60% of the comparisons had genetic distances ≥0.5, thus most of these lines can potentially contribute new alleles to a breeding program. The genetic distances obtained in this study compare to those of Drinic et al. (2002) who evaluated 12 lines of different origin with 21 SSR markers obtaining genetic distance in the range of 0.12 and 0.67.

4.4.4 Cluster analysis based on SSR markers

While clusters generally agreed with pedigree information of these lines as reported by several workers (Aguiar et al., 2008, Menkir and Ayodele, 2005, Semagn et al., 2012), there were discrepancies in the representation of heterotic groups in these clusters. For instance, CML202 and CML204 that are categorized in heterotic group B using test cross data (CIMMYT, 2004) fell in different clusters with molecular markers in the present study. However, in the studies of Ininda et al. (2007), these two lines, CML202 and CML204, clustered closely together. Similar discrepancies were observed with the clustering of EC573 and Kitale Synthetic backgrounds in the same group and also that of lines with EM12-210 and EM11-133 in the same clusters which conventionally belong to different heterotic groups (KARI, 1992). The mismatch in the results of molecular clusters and other conventional classification of germplasm have been attributed to

several factors such as selection and/or erroneous pedigree record (Senior et al., 1998) and the fact that DNA markers of identical status may not always have identical ancestry (Li et al., 2002). Difficulties of assigning lines into genetically diverse heterotic groups has also been attributed to mixed genetic constitution and further complicated by insufficient documentation during germplasm development (Wen et al., 2011). Therefore, classification of germplasm would be accomplished best with a combination of conventional and molecular methods, an opinion also expressed by Li et al. (2002) and Wen et al. (2011). This would include the use of pedigree information, heterotic grouping and ecological adaptation (Menkir et al., 2010).

Molecular markers were useful for identification of both the genetic background of a line, and in determining the influence of different germplasm in the pedigree of a line. For instance, in cluster V the line MUL114 was clustered together with OSU23i suggesting that MUL114, which is an inbred line extracted from a cross with OSU23i and EM12-210, had more genetic background from OSU23i compared to similar KARI lines in cluster VI. Xu et al. (2004) also noted that SSR data can accurately identify lines based on composition and pedigree relationships. Two lines CML202 and OSU23i which are MSV resistant parents appeared in the same clusters with inbred lines derived from them, similar to the findings of Ininda et al. (2007). In the studies of Semagn et al. (2012), using SNP markers, lines were also grouped into populations with common maize streak resistant parents, CML202 or OSU23i. Wen et al. (2011) suggested that lines closely related by pedigree or sharing environmental adaptation or program origins may be more accurately classified into corresponding subsets. In addition, lines were clustered according to their geographical distribution, similar to the findings of Meinie and Fourie (2013). While evaluating inbred lines differing in stress response, groupings have been largely based on the pedigree rather than on the reaction of the inbreds to stress (Badu-Apraku et al., 2013). The present study however found some clusters where lines grouped based on MSV response and also on their pedigree classification. The two lines (REG99 and A076) that were not clustered with other inbreds need to be studied further. Notably, Ininda et al. (2007) also found the line REG99 not in a cluster in other lines which is indicative that the line REG99 could have unique allele combinations.

4.4.5 Genetic structure of the population

Some modest genetic differentiation of 15% of estimated genetic variances was found among the four sources of origin or populations established i.e., KARI, CIMMYT, IITA, and while very little genetic differentiation (2%) within individuals in a population and the highest variation (83%) was found within individuals. Similarly, Vaz Patto et al. (2004) found most genetic

diversity among accessions within the different groups established. Wen et al. (2011) defined a subset of lines by pedigree information, environmental adaptation and breeding scheme, revealing much higher variation within subsets than among subsets. It is possible that the reduced differentiation between germplasm from different programmes is because most of the programmes in the region especially in breeding for MSV resistance have used the CIMMYT developed germplasm for the eastern and southern Africa, which include widely used CIMMYT Maize Lines (CMLs). However, the placement of CIMMYT and IITA germplasm were in different clusters provides evidence that maize germplasm from these regions are genetically diverse and can be introgressed into locally adapted germplasm to improve various traits.

There was amplification of more than one band per inbred line in 12 SSR primers used. Some of the markers that showed high rate of polymorphism, phi10228, phi029, phi031, phi112, phi374118 and umc1161, could be used for rapid fingerprinting of inbreds. The expression of these unique alleles has however been attributed to residual heterozygosity, contamination, or amplification of similar sequences in two separate genomic region (Senior et al., 1998). All lines in this study showing unique alleles, i.e., REG36, Z426, C92, Z419, Z168, MUL1182 and REG99, expressed some vigour. The highest number of unique alleles (10) was found in line C92, which is a resistant line with yellow endosperm. Mienie and Fourie (2013) also found the highest number of alleles in yellow maize breeding lines than white endosperm lines which might be attributed to mixed genetic origin. The twenty-seven unique alleles could represent unique alleles and untapped genetic potential for genetic improvement of maize germplasm for various traits.

4.5 Conclusions

The analysis of genetic divergence using 28 SSR markers estimated 135 alleles were detected among the 40 maize genotypes. A number of diverse pairs of MSV resistant lines were identified which were polymorphic at many SSR loci even among highly resistant lines. The SSR loci with high and low levels of heterozygosity were identified which was instrumental in choice of markers that offer highest differentiation among maize germplasm. Genetic distances between pairwise comparisons showed considerable variation among the inbred lines studied, which can systematically be used for breeding. The germplasm was grouped into seven clusters with the UPGMA clustering algorithm although there was little correlation between known heterotic groups and SSR data classifications. The results in this study however showed some separation of germplasm by breeding program and response to maize streak virus. Twenty-

seven unique alleles identified could represent untapped genetic potential for maize germplasm improvement. Information generated from this study can be used to select parents for MSV hybrid development to maximize yield potential. Information generated in this study would guide breeders in selecting useful combinations for hybrid breeding. This information is also crucial as a prerequisite in a marker-assisted breeding programme focusing on MSV resistance.

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Chapter 5

Gene action for grain yield and associated traits and prediction of maize single cross performance using SSR markers

Abstract

Genetic diversity among tropical maize germplasm is a safeguard against vulnerability and is critical for increasing yields. The relationship of genetic diversity and F_1 yield potential and yield heterosis would help to design breeding strategy and predict hybrid performance, if ascertained. Twelve maize inbred lines were crossed in a half-diallel pattern to elucidate gene action controlling grain yield and associated traits and to estimate correlations of SSR marker distance with F_1 yield performance. Sixty-six diallel crosses with four check hybrids were evaluated in a 10 x 7 α-lattice design in five locations in 2011 and 2012 rainy seasons, along with the 12 parental lines in adjacent trials. General and specific combining ability mean squares were important for grain yield and most of the other traits across environments. Parental line C92 was identified as the best general combiner for grain yield. The top ranking hybrid C92 x TZMI736 out-yielded the best commercial check, H624, by 6% across environments. The SSR marker-based genetic distance was positively and highly significantly correlated with grain yield (r = 0.53), with SCA (r = 0.62) and with heterosis (r = 0.68). These relationships showed that high grain yield and yield heterosis could be predicted from SSR marker based distances. Highest mid-parent heterosis (220%) was obtained in CML539 x TZMI736, a cross combination that also had the highest genetic distance by markers (0.692). Based on SCA effects, CML509 x C92 was the major heterotic pattern, whereas with both markers and heterosis CML539 x TZMI736 was the major heterotic pattern. Three to four heterotic groups were found using SCA estimates, SSR marker distances, and heterosis, which were dependent on the level of relatedness within the lines and some environmental influences. Preponderance of additive gene action for grain yield associated traits indicated their usefulness in recurrent selection programs of tropical maize.

5.1 Introduction

Maize is a significant component of food security across the world and improvement of yield potential for this cereal is the focus for genomics and breeding. Improvement of maize germplasm entails selection of superior parents and precise identification of heterotic patterns (Hallauer and Miranda, 1988). Moreover, the choice of selection methods for the improvement of traits in target germplasm will depend on the type of gene action involved (Gamble, 1962a). Relationships among germplasm can further be exploited through the identification of heterotic relationships. Heterotic groups are established to contain genotypes which unequivocally represent differences in allele frequency of the population (Aguiar et al., 2008). Information on genetic interrelationships makes it possible to develop productive hybrids without testing all possible hybrid combinations among potential parents available in a hybrid programme. The diallel cross analysis is widely used to determine the nature of gene functioning, i.e., the inheritance of important traits among a set of genotypes (Wright, 1985) and also in the identification of heterotic groups and prediction of traits in new populations (Hallauer and Miranda, 1988).

Gene action conditioning most complex traits such as grain yield cannot be generalized, as it is often variable between different test populations. Further, environments modify the expression of additive and non-additive effects for the different agronomic traits necessitating selection of parents with stable GCA and SCA effects to cater for environmental variations (Dehghanpour and Ehdaie, 2013). The genetic control of grain yield is often under non-additive variance than additive variance (Izhar and Chakraborty, 2013, Shahrokhi et al., 2013, Srdic et al., 2007) but in some cases additive effects have been reported to predominate (Legesse et al., 2009). The genetic inheritance of other agronomic traits is also varied in terms of the contribution of additive to non-additive genetic variation.

Molecular markers and combining ability data can provide information on genetic distances of parental materials and hybrid performance. While combining ability tests such as diallel crosses can be restrictive in the number of lines and crosses to be evaluated, molecular markers allow the inclusion of a greater number of lines. Further, testers or genotype by environment interaction do not influence discrimination of heterotic groups based on genetic distances obtained from molecular marker data (Aguiar et al., 2008). Marker based genetic distance could be invaluable where heterotic groups are not well established. Several studies have shown the usefulness of simple sequence repeat markers in the assignment of lines into heterotic groups

(Aguiar et al., 2008, Meinie and Fourie, 2013, Menkir et al., 2010, Srdic et al., 2007). Some researchers have reported similar groupings for germplasm using a combination of molecular markers and estimates of specific combining ability (Pinto et al., 2003).

Despite the fact that the magnitude of heterosis is proportional to the degree of divergence between parents (Hallauer and Miranda, 1988) there have been controversy in the prediction of yield and yield heterosis using molecular marker distances. Non-significant correlations between heterosis (Wegary et al., 2013), SCA effects (Parentoni et al., 2001) or yield of hybrids with genetic distance by molecular markers have led to the inference that it is not possible to deduce the behaviour of maize hybrids from genetic divergence between parental lines. Further that genetic diversity cannot be accurately estimated in highly diverged lines (Hamblin et al., 2007). In other cases, correlation of genetic distances with yield and heterosis were only detected when germplasm group divisions were considered (Lanza et al., 1997). Further, environmental effects on the performance of hybrids can alter the relationship between genetic distance and heterosis. Most of these studies however agree that molecular markers are most useful for placing lines into different heterotic groups and for eliminating negative heterosis and SCA.

Introduced germplasm for improving various stresses needs to be classified into existing or new heterotic groups for development of efficient hybrids. This would take advantage of the broad genetic diversity and potential heterotic groups that are available within tropical germplasm. However, one challenge of dividing lines into heterotic groups occurs when lines are developed from the same original pool without regard to heterotic pattern (Semagn et al., 2012). The new populations that can be developed from introduced disease resistant germplasm should be heterotic to each other so that they could be successfully exploited. The genetic potential of disease resistant germplasm from the region for grain yield and heterotic relationships has not been adequately studied. Information of genetic distances and their relationship to yield in MSV resistant germplasm is limited. Therefore, the objectives of this study were to determine the estimate the heterotic relationships and correlations of SSR marker distances with hybrid performance, heterosis and SCA in MSV resistant germplasm.

5.2 Materials and methods

5.2.1 Germplasm

Twelve maize parental lines constituting diverse sources of MSV resistance were obtained from various sources: the Kenya Agricultural Research Institute (KARI), the International Maize and Wheat Improvement Centre (CIMMYT) - Zimbabwe and the International Institute for Tropical Agriculture (IITA) (Table 5.1). The parental lines were crossed in a 12 x 12 half-diallel mating scheme in 2011 to form 66 F_1 progenies. The reciprocal seed was bulked with F_1 seed since agronomic traits of interest were not expected to be controlled by maternal effects.

5.2.2 Experimental design and management

The diallel evaluation experiments were conducted at four locations in 2011 and five locations in 2012 in Kenya, including Kiboko (975 m above sea level [asl]), Mwea Tebere (1159 m asl), Kakamega (1530 m asl), Muguga (2095 m asl) and Kabete (1826 m asl). These locations have a bimodal rainfall pattern with the exception of Kiboko, which is mainly irrigated. In Muguga, the experiments were grown under artificial MSV disease inoculation with viruliferous leafhoppers. Eighty-four (84) entries consisting of 66 F_1 hybrids, four hybrid checks, 12 parental lines, two inbred checks were sown in all trials. The hybrids were arranged in a 10 x 7α- lattice design, in double rows of 2.5 m at spacing of 75 cm between rows and 25 cm between hills. To minimize inter-plot competition and shading from the hybrids, the 12 parental lines and two inbred checks were grown in separate but adjacent trials in randomized complete blocks with two replications in each location. Experimental plots for parental lines were similar to those used on hybrids. Twenty-two seeds were sown in each row, and the seedlings were thinned at five-leaf stage to 11 plants per row to obtain a population density of 53,333 plants ha^{-1}. Fertilizer was applied to all trials at the rate of 120 kg ha^{-1} of P and 60 kg ha^{-1} of N at planting with an additional 60 kg ha^{-1} of N as a top-dress at four weeks after planting. The trials were kept weed-free by hand weeding. The experiments were irrigated as and when required until one week before maturity.

Table 5.1. Pedigree, source and grain characteristics of 12 parental lines used in diallel crossing and two inbred checks

Code	Pedigree	Source[b]	Grain type[a]
Z419	SYN[Kitale/Tuxp-GLS]F2	KARI	I, W
S558	(EM12-210/CML197//EM12-210/OSU23i)-x-58-2-2-2-1	KARI	FL, W
MUL 71	[EM12-210/CML 202]-X-71-1-2-1-1-3	KARI	F, W
Osu23i	[MSRXPOOL9] C1F2-205-1	OSU	F, W
CML505	[92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	CIMMYT	I, W
CML509	[92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB	CIMMYT	FL, W
MUL114	[EM12-210/OSU23i]-X-114-2-2-1-3	KARI	F, W
C92	na	CIRAD	I, Y
CML539	MAS [MSR/312]-117-2-2-1-B*4	CIMMYT	FL, W
TZMI736	na	IITA	FL, W
TZMI746	na	IITA	FL, W
VHCY	na	Grains crop Institute	FL, Y
CML202 (check 1)	ZSR923S4BULK-5-1-BB	CIMMYT	FL, W
CML312 (check 2)	S89500F2-(Sn)B*5	CIMMYT	DL, W

[a]Grain type: F-Flint, I-Intermediate, FL-Flint-like, DL-Dent-like, W-White, Y-Yellow; [na]-pedigree information not available; [b]Germplasm sources: CIMMYT = International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, CIRAD = Centre de cooperation internationale en recherché agronomique pour le développement, KARI =Kenya Agricultural Research Institute

5.2.3 Data collection

Data for the following agronomic traits were recorded on a plot basis at all locations as described by Magorokosho et al. (2009). Days to anthesis (DTA) and days to silking (DTS) were recorded as the number of days from planting to when 50% of plants in a plot had shed pollen and emerged silks, respectively. Anthesis-silking interval (ASI) was calculated as the interval in days between DTS and DTA. Plant Height (PH) and ear height (EH) were measured in cm as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Ear position (EPO) was taken as the ratio of EH to PH. Ear aspect (EASP) was scored on a scale of 1 to 5, where 1 = clean, uniform, large and well-filled ears and 5 = rotten, variable, small and poorly-filled ears. The total number of plants and ears were counted in each plot at the time of harvest. The number of ears per plant was then calculated as the proportion of the total number of ears at harvest divided by the total number of plants at harvest. All ears harvested from each plot were weighed and representative samples of ears were shelled to determine percent moisture. Grain yield adjusted to 12.5% moisture was computed from ear weight and grain weight assuming a shelling percentage of 80% based on the following formula: Grain weight ($t\ ha^{-1}$) = (Ear weight (kg)/area (m^2) x ((100-moisture)/87.5) x 10 x 0.80).

5.2.4 Molecular analysis

The 12 parents used in this study, were assayed for genetic diversity using molecular markers along with 18 other lines that were not included in the diallel. As part of 40 lines grown in a screen house at KARI, Muguga, young leaves were harvested at V5 stage from three seedlings of each parent. The leaf discs were shipped to DNA LandMarks molecular laboratory (Canada) for SSR analyses. Twenty-eight SSR markers were randomly selected from the maize database (www.maizegdb.org, verified 25 Nov 2013), with two to four markers per chromosome, with the exception of chromosome nine, which had one marker. The protocols followed for DNA extraction were based on Stange et al. (1999) while SSR polymerase chain reactions were performed following internal protocols at DNA LandMarks Inc.

5.2.5 Statistical analyses

Analyses of variance (ANOVA) to detect differences existed among the 84 entries (66 F_1, four hybrid checks, 12 parents and two inbred checks) for the typical plant parameters were separately performed on the data collected across environment (years and location combinations) with PROC GLM in SAS (SAS Institute, 2004). Combined ANOVA was then done

across nine environments for grain yield, DTA, DTS, PH, EH, EASP and EPP. All the effects, genotypes, environments and replicates were considered as fixed effects and means were separated using LSD. Excluding the checks, GCA effects of the parents and SCA of the crosses, as well as their mean squares in each environment were estimated in the 12 x 12 half-diallel crosses following Griffing's 4 model 1 (fixed parental effects) (Griffing, 1956). This was done using DIALLEL-SAS program developed by Zhang et al. (2005) adapted to the SAS software version 9.2 (SAS Institute, 2004). The statistical model for the combined diallel analysis across environments is as follows:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + l_k + gl_k + s_{ij}l_k + \varepsilon_{ijk}$$

Where Y_{ijk} is the observed measurement of the ij^{th} cross grown in the k^{th} environment, μ is the grand mean; g_i and g_j are the GCA effects; s_{ij} is the SCA effects; gl_k is the interaction effect between GCA and the environment; $s_{ij}l_k$ is the interaction effect between SCA and the environment, and ε_{ijk} is the error term associated with the ij^{th} cross evaluated in the k^{th} replication and l^{th} environment (Hallauer and Miranda, 1988). The restrictions $\sum g_i$=0 and $\sum s_{ij}$=0 were imposed on the combining ability effects. A t-test was used to test significance of GCA and SCA effects. The standard errors of the GCA and SCA effects were estimated as the square root of the GCA and SCA variances (Griffing, 1956).

Band profiles generated by SSR markers as 1 for presence, 0 for absence and -1 for missing, were used to estimate Euclidean distances among all possible pairs of the 12 inbred lines. Genetic similarities (GS) coefficients were calculated using 135 alleles identified as raw data by the following equation: $\mathrm{GS_{ij}} = 2\mathrm{N_{ij}}/(\mathrm{N_i} + \mathrm{N_j})$ where $\mathrm{N_{ij}}$ is the total number of bands present in line i and $\mathrm{N_j}$ is the number of bands present in line$j$(Nei and Li, 1979). The GS values were converted into genetic distance (GD): $\mathrm{GD} = 1 - GS$. The GD estimates vary from 0 (identical profile for all markers in two inbreds) to one (no common bands).

The matrix of specific combining ability estimates was considered a divergence matrix, where the larger the SCA, the larger the divergence between the lines. The SCA matrix was positively defined, where a constant (c=10) was added to the SCA estimates, thus transforming negative values into positive ones, without altering the relative position of the SCA estimates, according to Pinto et al. (2003).

Heterosis was also used to examine heterotic relationships among the lines. Heterosis (MPH) determined using the mid-parent (MP) was calculated as: $\mathrm{MPH} = \frac{\mathrm{F_1} - \mathrm{MP}}{\mathrm{MP}} \mathrm{x}\, 100$ where $\mathrm{F_1}$ is the

performance of hybrids, $\mathrm{MP} = \frac{(\mathrm{P_1}+\mathrm{P_2})}{2}$ in which $\mathrm{P_1}$ and $\mathrm{P_2}$ are the performance values of parents, respectively. Heritability in the broad-sense heritability $\left(\mathrm{h_b^2} = \frac{\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}{\hat{\sigma}^2\mathrm{phenotypic}}\right)$ and narrow-sense heritability $\left(\mathrm{h_n^2} = \frac{\hat{\sigma}^2\mathrm{GCA}}{\hat{\sigma}^2\mathrm{phenotypic}}\right)$ were calculated as indicated. Coefficient of genetic variation (CVg) was calculated as $\frac{\sqrt{\sigma^2}gca+\sigma^2 sca}{\mu}$ while coefficient of error variation (CVe) was computed as $\frac{\sqrt{\sigma^2}e}{\mu}$.

Unweighted pair-group using arithmetic average (UPGMA) cluster analyses were conducted on the SCA estimates, SSR divergence matrices and mid-parent heterosis. Through visual inspection of the dendrograms, lines that clustered together were put into the same heterotic group.

Simple correlations were calculated for different combinations of yield, SCA, heterosis and genetic distance using the PROC CORR statement in SAS 9.2 (SAS Institute, 2004). Simple linear regression was used to assess the relationship among genetic distance between pairs of lines and F_1 hybrid performance using GD as the independent variable and the corresponding hybrid performance as dependent variable.

5.3 Results

The combined ANOVA of the diallel crosses showed highly significant effects for environments, replications within environments, genotype x environment and entries for all measured traits (Table 5.2). The mean square for entry x environment interaction was significant for all traits with the exception of number of ears per plant (EPP). With the exception of EPP, broad sense heritability was high for all traits ranging from 0.86 to 0.97 (Table 5.3). Narrow sense heritability estimates were low for grain yield (0.27) and EPP (0.22) and moderate to high (0.65-0.77) for other traits. The ratio CVg/CVe was greater than one except for EPP indicating phenotypic differences from genotype were superior to experimental errors. Partitioning of the crosses into components revealed that GCA and SCA mean squares were significant for all measured traits, except the SCA estimates for EPP (Table 5.3). The interaction effects of GCA and SCA variances with environment were significant, except SCA x environment for EPP. Except for grain yield and EPP, the proportions of GCA effects for other traits were larger than SCA effects across test environments. In most cases, GCA effects reflected the values of the parental means (Table 5.5).

Table 5.2. Combined analysis of variance of a 12 x 12 diallel cross for grain yield and associated traits over nine environments

Source of variation	df	GY	DTA	DTS	PH	EH	EPO	EASP	EPP
ENV	8	276.79***	32500.76***	30832.44***	10367.47***	45799. 06***	0.335***	13.676***	1.076***
REP(ENV)	9	7.118***	40.952***	46.31***	977.048***	521.74***	0.007***	0.197ns	0.161ns
ENTRY	65	17.463***	132.851***	133.41***	5507.013***	4367.061***	0.021***	1.459***	0.137*
ENV*ENTRY	520	2.213***	8.624***	8.932***	289.768***	146.893***	0.0015***	0.222***	0.107ns
GCA	11	50.09***	672.44***	667.03***	25964.07***	22546.94***	0.114***	7.078***	0.358***
SCA	54	10.816***	22.936***	24.715***	1339.84***	663.75***	0.003***	0.315***	0.092ns
GCA x ENV	88	5.228***	24.85***	28.069***	652.74***	246.52***	0.002***	0.455***	0.142**
SCA x ENV	432	1.598***	5.318**	5.034***	215.83***	126.6***	0.001***	0.175**	0.101ns
Error	585	1.122	4.261	3.716	164.128	94.253	0.001	0.135	0.099
h^2_B		0.92	0.95	0.96	0.96	0.97	0.93	0.86	0.17
h^2_N		0.27	0.74	0.72	0.65	0.77	0.77	0.68	0.22
CVg (%)		40	9	9	19	33	16	25	9
CVe (%)		11	2	2	4	6	5	10	21
CVg/CVe		3.6	4.5	4.5	4.8	5.5	3.2	2.5	0.4
Bakers ratio		0.448	0.874	0.860	0.807	0.885	0.907	0.883	-
Mean		6.55	71.14	72.3	224.96	113.32	0.5	2.67	1.09

*, **, ***, and ^{ns} indicate significance at 0.05, 0.01, 0.001 probability, and non-significant, respectively, GY: grain yield, DTA: days to anthesis, DTS: days to silking, PH: plant height, EH: ear height, EPO: ear position, EASP: ear aspect,; h^2_B = Broad-sense heritability, h^2_N= Narrow-sense heritability, CVg = coefficient of genetic variation, CVe = coefficient of error variation; Bakers ratio = $\frac{2\sigma^2_{gca}}{2\sigma^2_{gca}+\sigma^2_{sca}}$

5.3.1 Grain yield

The yield performance of selected hybrids is shown in Table 5.3 where the top yielding ten hybrids did not differ significantly ($p \leq 0.05$) from the commercial check H624. The overall grain yield for nine environments was 6.6 t ha^{-1}, ranging from 7.92 t ha^{-1} in C92 x TZMI736 (7.92) to 2.11 t ha^{-1} in CML505 x CML509. The combined analyses of variance of the diallel crosses showed highly significant values ($p<0.01$) for both general and specific combining ability effects for grain yield (Table 5.3). The proportion of GCA variance to SCA variance according to Baker (1978) was 0.45 in the case of grain yield. Both GCA and SCA effects interacted significantly with the environments. The GCA effects for grain yield ranged from 0.63 t ha^{-1} to -0.95 t ha^{-1} (Table 5.4). Four lines had significant ($p<0.05$) and positive GCA effects for grain yield, namely C92, CML539, Z419, and MUL114 (0.63, 0.58, 0.50 and 0.32, respectively). In contrast, VHCY, CML505 and CML509 had highly significant ($p<0.01$) negative GCA estimates (-0.95, -0.89, and -0.45 respectively).

5.3.2 Maturity indices

The days to anthesis among the top ten yielding crosses ranged from 66 to 74 days and were significantly ($p \leq 0.05$) earlier than the best yielding check, which had 77 DTA (Table 5.3). Most parents were significantly ($p=0.05$) later flowering than their respective crosses. Combining ability analysis showed highly significant effects for GCA and SCA variances for DTA and DTS (Table 5.2); these effects interacted significantly with environments. A ratio of GCA to SCA variance of 0.87 was obtained for both DTA and DTS. Parent TZMI746 had the highest days to anthesis and 50% silking (81 and 83 days, respectively) and VHCY the lowest number of DTA and DTS (66 and 67 days, respectively) (Table 5.4). Consequently, parent TZMI746 had the largest significant ($p<0.001$) GCA effects for later anthesis and silking emergence with a significant value ($p<0.05$) of 2.5 days delay. In contrast, MUL71 had significantly ($p<0.001$) the largest GCA effects for early anthesis and silk emergence of about 3.8 days. Notably, the earliest parent VHCY had non-significant GCA effects.

Table 5.3. Means of top 20 and bottom 5 hybrids over nine environments sorted by grain yield

Cross	GY	DTA	DTS	PH	EH	EPO	EASP	EPP
C92 X TZMI736	7.92	72.4	73.7	241.3	146.0	0.55	2.6	1.20
Z419 X MUL114	7.86	72.3	73.7	251.7	139.4	0.51	2.9	1.09
CML509 X C92	7.81	71.3	71.8	235.2	136.9	0.54	3.0	1.18
MUL71 X OSU23i	7.78	66.3	67.4	235.9	138.9	0.51	2.6	1.12
S558 X MUL114	7.74	73.6	74.8	245.6	131.0	0.5	2.7	1.13
MUL71 X MUL114	7.71	68.1	69.4	239.2	133.3	0.48	2.7	1.12
Z419 X CML539	7.69	69.8	71.0	224.8	113.6	0.48	2.4	1.01
C92 X CML539	7.69	69.0	69.9	230.3	131.6	0.51	2.4	1.15
CML539 X TZMI736	7.62	71.8	72.7	207.0	153.9	0.48	2.3	1.14
OSU23i X TZMI736	7.55	72.4	74.1	230.0	114.4	0.51	2.6	1.07
H624 (check)	7.48	76.7	77.9	274.0	112.1	0.56	2.3	1.06
OSU23i X TZMI746	7.48	72.5	73.7	251.6	121.2	0.55	2.4	1.03
S558 X CML539	7.42	70.4	71.6	212.2	129.8	0.47	2.4	1.16
Z419 X TZMI736	7.39	74.4	76.0	221.6	153.5	0.52	2.5	1.08
Z419 X C92	7.39	72.2	73.6	252.6	124.2	0.56	2.9	1.14
MUL71 X CML539	7.30	64.8	66.2	210.2	116.2	0.47	2.5	1.04
Z419 X MUL71	7.28	66.8	68.4	227.3	136.3	0.49	2.6	1.01
CML539 X TZMI746	7.17	69.7	71.1	216.2	159.7	0.5	2.1	1.02
Z419 X OSU23i	7.17	69.9	71.2	239.6	131.2	0.53	2.9	1.02
Z419 X S558	7.14	72.1	73.6	232.8	128.0	0.5	2.6	1.07
S558 X VHCY	5.06	69.9	71.8	214.6	100.2	0.44	2.6	1.01
TZMI736 X TZMI746	5.00	79.0	79.4	206.1	105.2	0.52	2.4	1.02
OSU23i X MUL114	4.80	75.4	76.9	232.2	103.2	0.53	3.3	1.15
CML505 X VHCY	4.55	69.2	69.8	196.3	93.0	0.45	3.1	0.97
CML505 X CML509	2.11	73.4	74.6	159.3	76.6	0.42	3.4	1.02
Experimental mean	6.55	71.3	72.4	226.1	119.7	0.5	2.7	1.08
LSD	0.72	1.4	1.4	26.8	19.8	0.02	0.2	0.27
CV%	16.30	2.9	2.7	5.7	8.6	6.4	13.7	12.80

[a] Experimental mean, and least square difference (LSD) are based on 70 entries; GY: grain yield (t ha^{-1}), DTA: days to anthesis, DTS: days to silking, PH: plant height (cm), EH: ear height (cm), EPO: Ear position (Ear height/Plant height), EASP: ear aspect (1=good-5=bad), EPP: number of ears per plant

5.3.3 Plant stature

Nine of the top yielding crosses with plant height values ranging from 207 to 246 cm were significantly shorter ($p \leq 0.05$) than the check variety (H624) which had 274 cm (Table 5.3). Ear height averaged at 80.3 cm for parents and 119.7 cm in the crosses. The effects of GCA, SCA and their interactions with environment were highly significant for PH, EH and EPO. While GCA variance for PH and EH accounted for 81 and 89% of total genetic variation, 92% of the genetic variation for EPO was attributed to GCA effects (Table 5.2). The largest significant ($p<0.001$)

GCA values for plant height were found in parents C92, MUL114 and Z419 (22.69, 17.26 and 6.89, respectively) (Table 5.4). The same three lines had the highest positive GCA effects for ear height. In contrast, CML505, CML509 and CML539 had highly significant ($p<0.001$) negative GCA estimates for plant height and ear height.

5.3.4 Ear aspect

Ear aspect values among the top 20 hybrids ranged from 2.11 to 3.0 with only six hybrids having statistically lower values ($p\leq0.05$) than the experimental mean of 2.68 (Table 5.3). The best three hybrids for ear aspect were CML539 x TZMI746 (2.11), CML539 x TZMI736 (2.33) and check variety H624 (2.33). Although both GCA and SCA were highly significant, the variance for GCA effects accounted for 89% while SCA effects accounted for 11% of total hybrid variation (Table 5.2). Positive significant ($p<0.001$) GCA effects for ear aspect were detected for lines CML505 and CML509 (0.22 and 0.20, respectively) while negative GCA effects were detected for TZMI746 (-0.39), CML539 (-0.2) and TZMI736 (-0.2) (Table 5.4).

5.3.5 Ear prolificacy

Entries in this study differed minimally for number of ears per plant with values ranging from 1.01 to 1.2 among the top 20 hybrids (Table 5.3). The top three hybrids for EPP, S558 x CML539 (1.16), CML509 x C92 (1.18) and C92 x TZMI736 (1.20) had presumably four extra ears to the number of plants harvested. Variance due to GCA effects was highly significant but SCA variance was not significant ($p>0.05$) for EPP (Table 5.2). Despite the insignificance ($p>0.05$) of SCA variation, GCA effects accounted only for 44% of total hybrid variance. Parent S558 had the highest significant ($p<0.01$) and positive GCA values (0.078) for EPP while parents VHCY, Z419 and TZMI736 had significant ($p<0.05$) negative GCA values (-0.081, -0.047 and -0.047, respectively) (Table 5.4).

Table 5.4. The general combining ability (GCA) effects of parents and their mean values for grain yield and other secondary traits

Parents	Grain yield (t ha^{-1})		Plant height (cm)		Ear height (cm)		Ear aspect (1-5)		Days to anthesis		Days to silking		Ears per plant	
	mean	GCA	mean	GCA	mean	GCA	mean	GCA	mean	GCA	mean	GCA	mean	GCA
Z419	5.35	0.498***	189	6.891**	90.45	3.617*	3.06	0.048^{ns}	73.2	-0.060^{ns}	74.4	0.042^{ns}	1.02	-0.047*
S558	4.61	0.078^{ns}	173.1	-2.939^{ns}	75.21	-4.043**	2.61	-0.115**	74.1	1.328^{ns}	75.2	1.442^{ns}	1.18	0.078**
MUL71	2.56	0.038^{ns}	148.8	-5.434*	67.42	-6.951***	3.19	-0.098**	68.9	-3.871***	70.2	-3.746***	0.94	0.015^{ns}
OSU23i	3.44	0.095^{ns}	194.6	9.482***	101.21	9.251***	3.39	0.181***	74.4	-0.576^{ns}	76.3	-0.501^{ns}	1.11	-0.009^{ns}
CML505	3.24	-0.894***	141.2	-15.199***	57.31	-12.030***	3.19	0.226***	73	0.189^{ns}	73.8	-0.141^{ns}	1.01	0.010^{ns}
CML509	2.56	-0.453***	145.5	-10.989***	62.84	-8.319***	2.83	0.201***	73.2	0.256^{ns}	73.9	-0.057^{ns}	1.02	-0.022^{ns}
MUL114	2.77	0.322*	158.8	17.257***	77.12	10.969***	3.39	0.140***	78.7	1.645^{ns}	80.2	1.853^{ns}	1.18	0.022^{ns}
C92	6.46	0.625***	223.5	22.686***	125.62	23.321***	3.03	0.098**	72.3	0.812^{ns}	73.1	1.009^{ns}	1.22	0.048^{ns}
CML539	2.51	0.584***	142.3	-10.715***	63.75	-10.55***	2.92	-0.204***	70.6	-2.310*	71.3	-2.357*	1.19	0.011^{ns}
TZMI736	2.24	0.172^{ns}	143.4	-8.194***	70.57	-3.749*	2.67	-0.201***	80.2	2.151*	81.3	1.942^{ns}	0.96	0.023^{ns}
TZMI746	3.18	-0.122^{ns}	168.4	4.088^{ns}	92.54	9.486***	2.19	-0.395***	80.7	2.506*	82.9	2.542*	1.03	-0.047*
VHCY	3.37	-0.951***	184.9	-6.933**	79.29	-11.000***	2.78	0.118**	65.7	-2.071^{ns}	66.7	-2.029^{ns}	0.92	-0.081***
Mean	3.52		167.79		80.28		2.94		74.16		75.50		1.07	
$LSD_{0.05}$	1.55		27.61		19.63		0.79		1.62		1.67		0.27	
CV (%)	23.52		8.45		11.53		12.18		3.27		3.35		15.75	

*, **, ***, and ^{ns}indicate significance of GCA effects at 0.05, 0.01 and 0.001 probability and non-significant, respectively

5.3.6 Heterosis and correlation analysis

While the performance of the F_1 hybrids was significantly ($p \leq 0.001$) and positively correlated with mid-parent values for secondary traits, grain yield and ASI had non-significant ($p > 0.05$) correlations (Table 5.5). The highest correlation of F_1 hybrid performance and mid-parental values was obtained for ear position ($r = 0.89$, $p < 0.001$). The correlations of F_1 means with SCA were also significant ($p \leq 0.001$) and positive for all traits and had higher value for grain yield compared to the correlation of F_1 performance to the mid-parental values (Table 5.5).

Mean mid-parent heterosis for grain yield (94.43%) was high relative to plant height and ear height, which had moderate heterosis levels (35 and 42%, respectively). Some traits such as DTA, DTS and EASP had negative mid-parent heterosis values showing a general reduction in number of days from planting to flowering and EASP scores in the F_1 hybrids relative to their parents.

Table 5.5. Pearson's correlations of F_1, SCA and mid parent heterosis for grain yield and other agronomic traits

	GY	PH	EH	EPO	DTA	DTS	ASI	EASP	EPP
r (F_1, MP)	0.241^{ns}	0.674***	0.829***	0.890***	0.792***	0.771***	0.237^{ns}	0.649***	0.406***
r (F_1, MPH)	0.441***	0.161^{ns}	0.118^{ns}	0.315**	0.286*	0.232^{ns}	-0.775***	0.691***	0.681***
r (F_1, SCA)	0.72***	0.449***	0.357**	0.351**	0.379**	0.392**	0.786***	0.434***	0.718***
r (Het, SCA)	0.444***	0.499***	0.594***	0.771***	0.574***	0.549***	-0.726***	0.543***	0.733**
MPH (%)	94.44	34.71	42.07	5.84	-3.51	-3.49	0.61	-9.04	2.59

*, **, ***, and ^{ns} indicate significance of correlation at 0.05, 0.01 and 0.001 probability and non-significant, respectively, MP: Mid-parent, Het=mid-parent heterosis; GY: grain yield, PH: plant height, EH: ear height, EPO: ear position, DTA: days to anthesis, DTS: days to silking, ASI: anthesis silking interval, EASP: ear aspect, EPP: number of ears per plant

Simple correlations among individual traits (Table 5.6) showed positive and highly significant but moderate correlations between grain yield and plant height ($r = 0.45$, $p < 0.001$) and between grain yield and ear height ($r = 0.45$, $p < 0.001$). High positive correlations were obtained between plant and ear height ($r = 0.96$, $p < 0.001$).

However, grain yield was negatively correlated to ear aspect ($r = -0.31$, $p < 0.001$). Positive correlations were also found between ear height and days to silking and between ear height and

number of ears per plant. Flowering traits, DTA and DTS were not significantly (p>0.05) correlated with grain yield in this study.

5.3.7 Correlation of genetic distance with yield, heterosis and SCA

The correlation coefficient between genetic distance and grain yield across environments was positive and highly significant (r = 0.531, p≤0.001) (Table 5.6). However, these distances were inversely correlated with ear aspect (r = -0.559, p≤0.001) and not significantly (p>0.05) correlated with the other traits measured. The correlations coefficient of genetic distance and SCA was positive and significant (r=0.617, p<0.001). Genetic distance was strongly correlated with mid-parental heterosis (0.678, p<0.001). The relationship between GD and SCA (r = 0.617) and GD and heterosis (0.678) were stronger than that of GD and F_1 hybrid performance (r = 0.531) in the case of grain yield (Table 5.6). Further, heterosis for grain yield was positively and significantly correlated with SCA effects although moderately so (r = 0.455, p<0.001). Coefficient of determination (R^2) for regression of heterosis on genetic distance (Fig 5.1) and SCA on genetic distance (Fig. 5.2) were 46 and 38%, respectively.

Table 5.6. Correlations of genetic distance (GD) and F_1 performance for grain yield and other related traits

	GD	GY	PH	EH	DTT	DTS	ASI	EASP	EPP
GD	**1**	**0.531*** **	**0.201^{ns}**	**0.181^{ns}**	**-0.205^{ns}**	**-0.225^{ns}**	**0.166^{ns}**	**-0.559*** **	**0.109^{ns}**
GY		1	0.446***	0.406***	-0.045^{ns}	-0.035^{ns}	-0.068^{ns}	-0.312*	0.346**
PHT			1	0.963***	0.121^{ns}	0.161^{ns}	-0.306^{ns}	-0.112^{ns}	0.241^{ns}
EHT				1	0.221^{ns}	0.254*	-0.261*	-0.125^{ns}	0.253*
DTT					1	0.991***	-0.002^{ns}	0.001^{ns}	0.131^{ns}
DTS						1	-0.136^{ns}	-0.001^{ns}	0.124^{ns}
ASI							1	0.021^{ns}	0.043^{ns}
EASP								1	0.012^{ns}
EPP									1

Grain yield= r (GD, MP) = -0.397**, r (GD, MPH) = 0.678***, r (GD, SCA) = 0.617***, r GD, F_1) = 0.531***, r (MP, MPH) = -0.741***, r (MP, SCA) = 0.001^{ns}, r (MPH, SCA) =0.455***, r (MP, F1) = 0.241^{ns}

*, **, ***, and ^{ns} indicate significance of correlation at 0.05, 0.01 and 0.001 probability and non-significant, respectively, MP: Mid-parent, MPH=mid-parent heterosis; GY: grain yield, PH: plant height, EH: ear height, DTA: days to anthesis, DTS: days to silking, EPO: ear position, ASI: anthesis silking interval, EASP: ear aspect, EPP: ears per plant

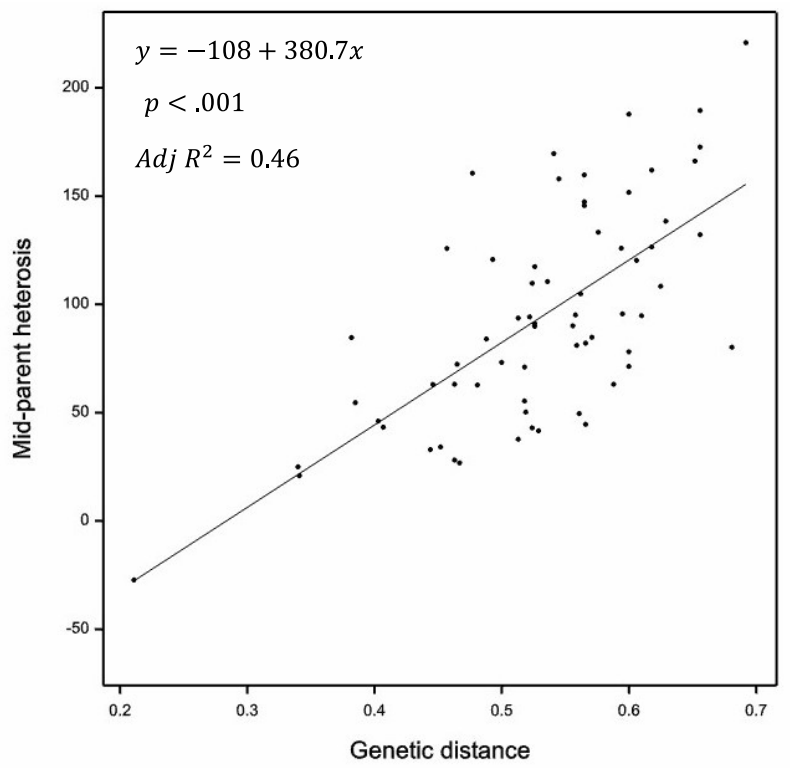

Figure 5.1. Regression analysis of genetic distance vs. mid parent heterosis

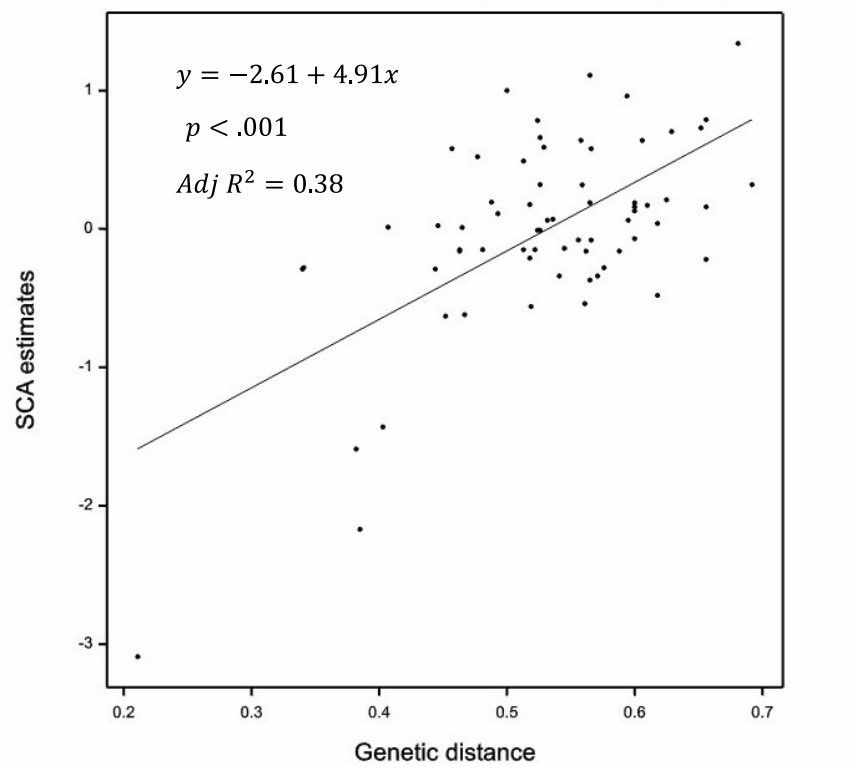

Figure 5.2. Regression analysis of genetic distance vs. SCA estimates

The average heterosis of the 66 F_1 hybrids was 94.4% (Table 5.7). The highest heterosis for yield was obtained in the combination CML539 x TZMI736 (220.8%) while the lowest was determined in the combination CML505 x CML509 (-27.3%). Generally about 40% of the crosses showed above 100% mid-parent heterosis values for grain yield. Genetic distance among the parents based on SSR markers ranged from 0.211 for cross CML505 x CML509 to 0.69 for cross CML539 x TZMI736 (Table 5.8) with an average of 0.53. Notably, single cross CML539 x TZMI736 had the highest genetic distance and highest average heterosis, and similarly the lowest GD and least heterosis in cross CML505 x CML509. This indicated that GD was strongly correlated with heterosis. Three crosses had a combination of high yield (> 7.5 t ha^{-1}), high SCA (>0.7), high heterosis (>150%) and high GD (≈0.6) were MUL71 x MUL114, MUL71 x OSU23i, and OSU23i x TZMI736. These crosses consisted of lines that were probably most divergent in this set of germplasm.

Table 5.7.Grain yield, mid-parent heterosis, SCA effects for 66 crosses and genetic distances by SSR markers

Combination	Yield	Mid-parent heterosis	SCA	GD	Combination	Yield	Mid-parent heterosis	SCA	GD
Z419 x 558	7.14	43.3	0.01	0.41	OSU23i x C92	6.64	34.1	-0.63	0.45
Z419 x MUL71	7.28	84.1	0.19	0.49	OSU23i x CML539	6.94	133.3	-0.28	0.58
Z419 x OSU23i	7.17	63.1	0.02	0.45	OSU23i x TZMI736	7.55	166.2	0.73	0.65
Z419 x CML505	6.14	43.0	-0.01	0.52	OSU23i x TZMI746	7.48	125.9	0.96*	0.59
Z419 x CML509	6.77	71.1	0.18	0.52	OSU23i x VHCY	5.54	62.8	-0.15	0.48
Z419 x MUL114	7.86	93.7	0.49	0.51	CML505 x CML509	2.11	-27.3	-3.09***	0.21
Z419 x C92	7.39	25.0	-0.29	0.34	CML505 x MUL114	6.62	120.3	0.64	0.61
Z419 x CML539	7.69	95.6	0.06	0.6	CML505 x C92	6.87	41.6	0.59	0.53
Z419 x TZMI736	7.39	94.7	0.17	0.61	CML505 x CML539	6.35	120.8	0.11	0.49
Z419 x TZMI746	6.38	49.6	-0.54	0.56	CML505 x TZMI736	6.53	138.4	0.7	0.63
Z419 x VHCY	5.80	33.0	-0.29	0.44	CML505 x TZMI746	5.72	78.1	0.19	0.6
558 x MUL71	5.24	46.1	-1.43***	0.4	CML505 x VHCY	4.55	37.7	-0.15	0.51
558 x OSU23i	6.56	63.1	-0.16	0.59	CML509 x MUL114	6.94	160.6	0.52	0.48
558 x CML505	7.07	80.2	1.34**	0.68	CML509 x C92	7.81	73.2	1.09**	0.5
558 x CML509	6.49	81.1	0.32	0.56	CML509 x CML539	6.54	158.0	-0.14	0.55
558 x MUL114	7.74	109.8	0.78	0.52	CML509 x TZMI736	5.89	145.6	-0.37	0.57
558 x C92	7.09	28.1	-0.16	0.46	CML509 x TZMI746	6.05	110.5	0.07	0.54
558 x CML539	7.42	108.4	0.21	0.63	CML509 x VHCY	5.78	95.1	0.64	0.56
558 x TZMI736	6.65	94.2	-0.15	0.52	MUL114 x C92	6.94	50.3	-0.56	0.52
558 x TZMI746	6.36	63.1	-0.15	0.46	MUL114 x CML539	7.12	169.6	-0.34	0.54
558 x VHCY	5.06	26.8	-0.62	0.47	MUL114 x TZMI736	6.83	172.7	-0.22	0.66
MUL71 x OSU23i	7.78	159.7	1.11**	0.57	MUL114 x TZMI746	6.91	132.2	0.16	0.66
MUL71 x CML505	5.36	84.8	-0.34	0.57	MUL114 x VHCY	5.84	90.1	-0.08	0.56
MUL71 x CML509	6.33	147.4	0.19	0.57	C92 x CML539	7.69	71.3	-0.07	0.6
MUL71 x MUL114	7.71	189.5	0.79	0.66	C92 x TZMI736	7.92	82.1	0.58	0.57
MUL71 x C92	7.01	55.4	-0.21	0.52	C92 x TZMI746	6.97	44.6	-0.08	0.57
MUL71 x CML539	7.30	187.8	0.13	0.6	C92 x VHCY	5.94	20.9	-0.28	0.34
MUL71 x TZMI736	6.28	162.0	-0.48	0.62	CML539 x TZMI736	7.62	220.8	0.32	0.69
MUL71 x TZMI746	6.50	126.5	0.04	0.62	CML539 x TZMI746	7.17	151.7	0.16	0.6
MUL71 x VHCY	5.63	89.8	-0.01	0.53	CML539 x VHCY	6.02	104.8	-0.16	0.56
OSU23i x CML505	5.76	72.4	0.01	0.47	TZMI736 x TZMI746	5.00	84.7	-1.59***	0.38
OSU23i x CML509	6.77	125.8	0.58	0.46	TZMI736 x VHCY	6.09	117.4	0.32	0.53
OSU23i x MUL114	4.80	54.6	-2.17***	0.39	TZMI746 x VHCY	6.26	91.2	0.66	0.53

Average heterosis = 94.4% [Max 220.8%, Min -27.25%]; Average GD = 0.53 [Max 0.69, Min 0.21];
SCA: Max 1.34**, Min=3.09***

5.3.8 Heterotic grouping using SCA estimates

Specific combining ability effects for most crosses were not significantly different from zero with only eight significant SCA effects for grain yield across environments. Four of these were positive effects: S558 x CML505 (1.34), MUL71 x Osu23i (1.11), CML509 x C92 (1.09) and Osu23i x TZMI746 (0.955) (Table 5.7). Except CML509 x C92, which had a poor x good GCA effects, the other three crosses had either bad or non-significant GCA effects.

A choice was made among the positive SCA effects for separating parental lines into heterotic groups based on significant and contrasting GCA effects of constituent parents and good yielding ability, besides positive and significant SCA, according to Pswarayi and Vivek (2008). The cross CML509 x C92 met these criteria and the two parents presumably belonged to different heterotic groups were used to separate the lines. Therefore, lines with positive SCA effects with either CML509 or C92 were placed into opposite heterotic groups while those exhibiting negative SCA effects were placed in the same group, as described by Vasal et al. (1992b). Heterotic grouping of lines having negative SCA effects with both testers was regarded as undetermined (neither heterotic group) whereas those having positive SCA effects with both testers were assigned to both testers, and according to Warburton et al. (2002) could be placed in a new group.

Separation in heterotic groups was conducted based on SCA estimates obtained in the lowest yielding environment, Kakamega 2011 and the highest yielding environment, Kakamega 2012 and across all nine environments (Table 5.8) using the cross CML509 x C92 as the major heterotic pattern. CML505 was designated heterotic group A while C92 was designated heterotic group B. In the lowest yielding environment, two lines showing negative SCA effects with CML509 were placed in heterotic group A, five lines showing negative SCA effects with C92 were placed in heterotic group B, whereas two lines, TZMI736 and VHCY, which had positive SCA effects with both testers were assigned heterotic group A/B. Parent S558 had negative SCA effect with both testers and was not placed into either group; naturally such lines would be discarded from the programme unless a third tester is found. In the highest yielding environment, three lines (S558, CML505 and CML539) showing negative SCA effects with CML509 were placed in heterotic group A; three lines (Z419, Osu23i and C92) showing negative SCA effects with C92 were placed in heterotic group B, while two lines MUL71 and TZMI746) which had positive SCA effects with both testers were assigned to heterotic group A/B. Two lines, MUL114 and TZMI736 were not placed in either group as they had negative

SCA with both CML509 and C92. Across the nine environments, two lines CML505 and TZMI736 showing negative SCA effects with CML509, were placed in heterotic group A, seven lines showing negative SCA effects with C92 (Z419, S558, MUL71, Osu23i, MUL114, TZMI736, and TZMI746) were placed in heterotic group B. Line CML539 with negative SCA effect with both testers was not placed into either group. In all the three cases, Osu23i and Z419 were consistently placed in heterotic group B while CML505 was consistently placed in heterotic group A.

5.3.9 Cluster analysis

The dendrogram based on SCA estimates (Fig. 5.3) showed three groupings: Z419, MUL71, S558 and CML539 formed one group, CML505 and CML509, the second group, while Osu23i, C92, VHCY and MUL114 formed the third group, but TZMI736 and TZMI746 did not seem to group with the other lines. The dendrogram constructed based on genetic divergence with SSR markers (Fig. 5.4) revealed three clusters: Z419, C92, and VHCY constituted the first group, S558, MUL71, TZMI736 and TZMI746 the second, while Osu23i, MUL114, CML505, CML509 and CML539 formed the third group. Clusters based on heterosis for grain yield (Fig. 5.5) displayed four groups: first group consisted of Z419, C92, S558, and VHCY, the second group consisted of CML505, CML509, the third group had MUL71, TZMI736 and TZMI746, while Osu23i, MUL114 and CML539 formed the fourth group. Based on clusters using GD and heterosis, CML539 seemed set apart from other lines and presumably had unique allele frequencies from the other lines in this study. Considering groups with SCA and SSR distances, some pairs of lines were consistently tightly clustered: MUL71 and S558, CML505 and CML509, Osu23i and MUL114, C92 and VHCY, and TZMI736 and TZMI746. Since these pairs represent 10 lines of 12 used in the diallel (with the exception of lines CML539 and Z419), it would seem that SCA and SSR gave similar patterns although not identical groupings. Heterosis gave similar grouping with SSR distances supporting the high correlations between these two parameters.

Table 5.8. Heterotic grouping of lines across different environments using cross CML509 x C92 as heterotic pattern

Lowest yielding environment KAK11[a]				Highest yielding environment KAK12[b]				across environments			
Parent	CML509 (A)	C92 (B)	Heterotic group	Parent	CML509 (A)	C92 (B)	Heterotic group	Parent	CML509 (A)	C92 (B)	Heterotic group
Z419	0.735	-0.336	B	Z419	1.578	-2.045	B	Z419	0.176	-0.286	B
558	-0.228	-0.005	Neither	558	-0.338	0.536	A	558	0.318	-0.16	B
MUL71	0.01	-0.381	B	MUL71	0.404	0.249	A/B	MUL71	0.196	-0.202	B
OSU23i	0.223	-0.063	B	OSU23i	0.851	-0.346	B	OSU23i	0.58	-0.628	B
CML505	-2.36	0.07	A	CML505	-5.065	0.682	A	CML505	-3.09	0.592	A
MUL114	0.86	-0.43	B	MUL114	-0.463	-1.323	Neither	MUL114	0.522	-0.563	B
CML539	0.608	-0.357	B	CML539	-0.133	0.392	A	CML539	-0.135	-0.068	Neither
TZMI736	0.157	0.082	A/B	TZMI736	-1.140	-0.022	Neither	TZMI736	-0.376	0.575	A
TZMI746	-0.265	0.577	A	TZMI746	0.276	0.586	A/B	TZMI746	0.073	-0.077	B
VHCY	0.081	0.236	A/B	VHCY	2.545	-0.193	B	VHCY	0.641	-0.278	B

Average yield across nine environments = 6.55 t ha^{-1}, Lowest yielding environment [a]Kakamega 2011= 3.86 t ha^{-1},
Highest yielding environment [b]Kakamega 2012=8.61 t ha^{-1}

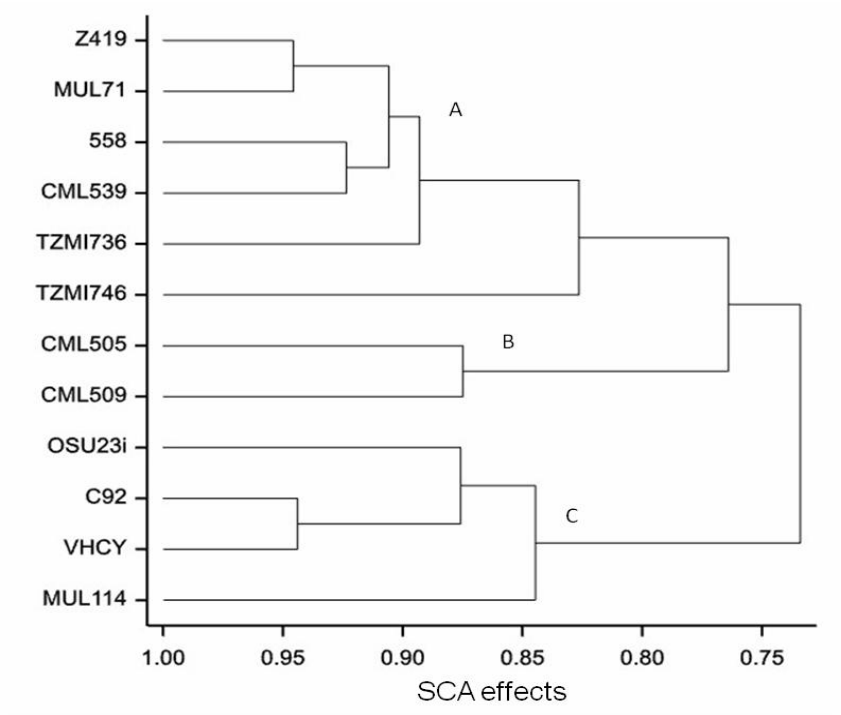

Figure 5.3. Cluster analysis of the 12 parental lines based on SCA effects of their 66 cross combinations. Letters A, B and C are indication of line groupings

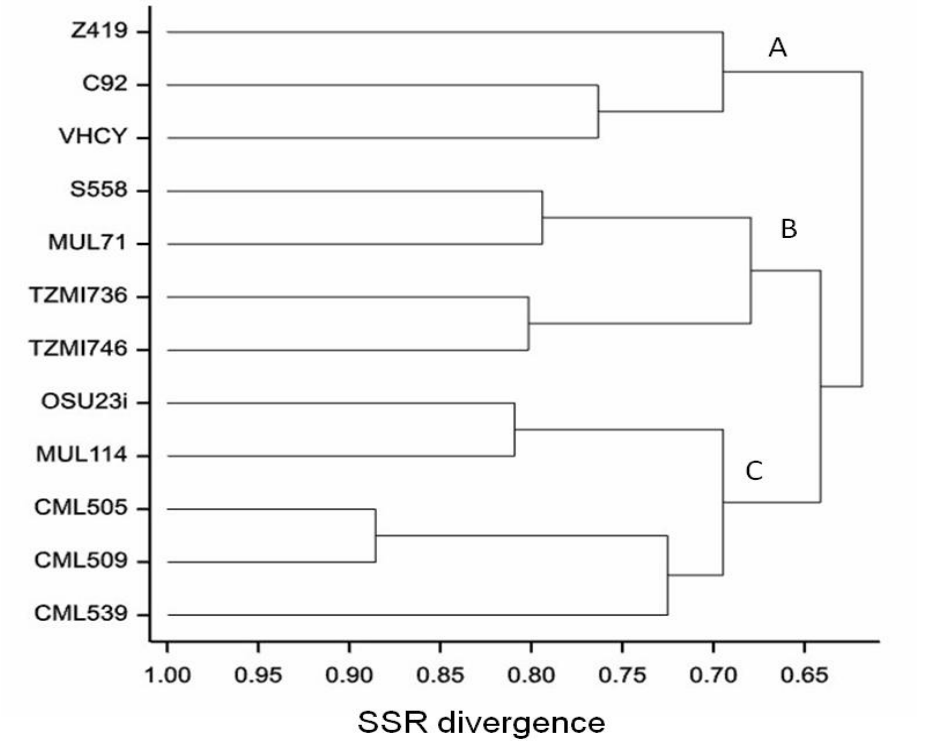

Figure 5.4. Clusters of 12 lines based on SSR marker-based genetic distances of 66 diallel combinations. Letters A, B, C are indication of line groupings

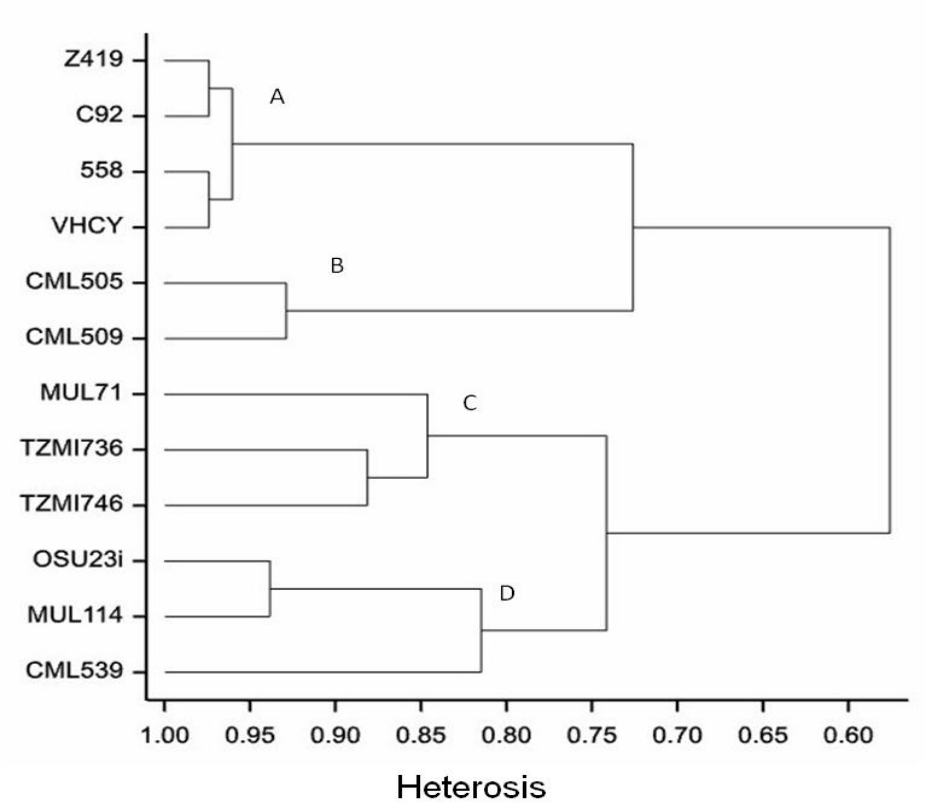

Figure 5.5. Cluster analysis of the 12 parental lines based on mid-parent heterosis values of their 66 cross combinations. Letters A to D are indication of line groupings

5.4 Discussion

High broad-sense heritability estimates except number of ears per plant showed that experimental errors and environmental influences were minimal for most traits measured. Selection would be more efficient for traits such as maturity indices, plant stature and ear aspect with high narrow-sense heritability estimates (>0.6) compared to grain yield and number of ears per plant which had low estimates.

5.4.1 Grain yield

There were significant differences among crosses in particular, the crosses from closely related parents produced low yield. Both GCA and SCA were highly significant denoting the importance of additive and non-additive effects for grain yield; this is a common finding (Dehghanpour and Ehdaie, 2013, Estakhr and Heidari, 2012, Gafishi et al., 2012, Haddadi et al., 2012). The GCA

to SCA ratio of 0.45 showed predominance of non-additive gene action in the inheritance of grain yield, and this agrees with other findings (Srdic et al., 2007, Unay et al., 2004). The intermediate contribution of SCA to hybrid variation implied it would be difficult to predict hybrid performance based on the GCA effects alone. Therefore, it would be necessary to test the parental lines in combination with multiple testers to identify superior hybrids (Hallauer and Miranda, 1988). The contrasts in the results of the mode of gene action controlling grain yield may be attributed to the fact that diversely sourced parents represent a wide range of germplasm where genes with different mode of genetic control may be important.

Significant mean squares for GCA x ENV and SCA x ENV indicating the effect of environment on additive variances was noted for grain yield, similar to other studies (Badu-Apraku and Oyekunle, 2012, Badu-Apraku et al., 2013, Gakunga et al., 2012). This would create difficulties in selection due to the presence of genotype by environment interaction effects emphasizing the need to test inbreds in dissimilar environments for stable performance and productivity in hybrids.

The favourable general combiners for grain yield, C92, CML539, Z419, and MUL114 could contribute to favourable alleles for the development of new varieties. Based on GCA effects, the best combiner for grain yield, CML539 would contribute to yield by 0.6 t ha^{-1}. The large grain yield GCA effect of these five lines indicates their value as testers in selection for yield. Several lines were noted as poor combiners for yield, notably VHCY would reduce yield by up to 0.9 t ha^{-1}. This line, VHCY, with a different set of lines was found to reduce grain yield by a similar value of 0.95 t ha^{-1} (Gichuru et al., 2011). Some parents such as MUL71 and Osu23i had non-significant GCA effects but positive and significant SCA effect when crossed. This behaviour has been attributed to complementary gene effects or “nicking” effects (Dehghanpour and Ehdaie, 2013).

5.4.2 Maturity indices

Additive gene action was most substantial in the controlling flowering traits, DTA and DTS. This concurred with other conclusions (Badu-Apraku et al., 2013, Bello and Olaoye, 2009, Legesse et al., 2009). Parent TZMI746 had positive values for GCA effects for DTA and DTS and contributed to late maturing hybrids. On the other hand, MUL71, TZMI746, CML539, and TZMI736 had highly significant negative GCA effects for both days to anthesis and days to silking and can be used to breed early flowering hybrids. Line MUL71 would offer reduction in DTA by up to 4 days. Early cultivars are capable of escaping drought stress at flowering and

grain filling periods and could possess genes for drought tolerance (Badu-Apraku and Oyekunle, 2012, Vivek et al., 2009). Further, maize breeders are often faced with the challenge of improving grain yield while maintaining earliness (Pswarayi and Vivek, 2008). Lines combining negative GCA effects for flowering traits and positive GCA effects for grain yield could be used for increasing grain yield while maintaining earliness. In the present study, line CML539 was identified as having positive GCA for grain yield and negative GCA for days to anthesis and 50% silking and thus a good combiner for grain yield and earliness. This has significant implications for breeding because such hybrids are rare.

5.4.3 Plant stature

General combining ability variance was large indicating that additive gene effects played an important role in determining plant height, ear height and ear position, in conformity with other findings (Glover et al., 2005, Legesse et al., 2009, Zare et al., 2011). Parent C92 had the highest mean ear height and GCA effect contributing to a 23.3 cm increase in ear height of its crosses. On the other hand, CML 505 had lowest parental mean ear height and largest GCA effect in the downward direction, decreasing ear height by 12 cm. There could also be a yield penalty in the reduction of plant stature in lines CML505 and CML509, which had negative GCA effects because these lines were among the least yielding in hybrids. Moreover, plant and ear heights need to be moderated for the adverse effects of lodging and influence of plant density.

5.4.4 Ear aspect

Additive effects indicated by significant GCA variance for EASP were found to be important in the inheritance of this trait, in agreement with other studies (Olakojo and Olaoye, 2005). The significance of SCA and SCA x environment interactions for EASP implied inconsistent performance of hybrids in the varying environments and has been similarly reported (Badu-Apraku and Oyekunle, 2012). Inbred lines CML505 and CML509 had positive GCA effects for ear aspect and were poor combiners when the objective is to improve EASP, which is scored on a scale of 1 (good) to 5 (poor). Thus, line TZMI746 that could improve EASP by up to 0.4 units should be considered for improving ear aspect.

5.4.5 Ear prolificacy

Prolificacy, the ability of a maize plant to produce more than one ear receives little attention, since the sub-apical ear is viewed to be antagonistic to the apical ear and thus adversely affects grain yield. Some studies have however shown that these sub-apical ears can make significant contribution to total grain yield especially during the wet seasons (Brathwaite and Brathwaite,

2002). Prolificacy in this study was however low, similar to several studies (Oliboni et al., 2012) suggesting selection against this trait. Significant GCA effects showed that additive effects were important in determining number of ears per plant (EPP). Variance due to SCA effects for EPP lacked significance indicating that gene frequencies for the trait were probably low in this set of parental lines. Thus implying that non-additive effects played a minor role compared to additive effects. Further, the lack of significance in SCA x environment interactions for EPP indicated that hybrids expressed the trait consistently in the different environments. However, only one good combiner for EPP was identified S558, based on positive GCA effect for this trait. Selection for EPP would be effective when applied in crosses involving this line.

5.4.6 Correlation, regression analysis and heterosis

Grain yield was positively correlated with plant height, ear height, and ear position, but negatively correlated with ear aspect. The positive correlation between grain yield and height traits has been previously reported (Badu-Apraku et al., 2011) and expected since size of the plant is usually in positive correlation with length of the vegetative stage and dry matter yield. Flowering traits were not significantly correlated with grain yield. Mostly, days to flowering are expected to be positively correlated to grain yield (Hallauer and Miranda, 1988) such that late maturing hybrids have higher grain yield. This was not the case in this study, confirming the presence of high yielding early maturing F_1 hybrids. Thus, the F_1 hybrids would have utility in programs that emphasize early maturity.

Due to dominance effects for grain yield, mid-parental (MP) values were not correlated with grain yield, but positive correlations of MP values were noted for other traits particularly ear position. This showed the importance of additive effects in the control of secondary traits and less for the control of grain yield. Further, the F_1 performance for grain yield was highly correlated with the SCA effects showing that SCA effect is more efficient in predicting hybrid performance compared to per se performance of inbred lines. This showed the importance of dominance for the control of grain yield. There was general agreement in the results in support of this since the single crosses with the most favourable SCAs, MUL71 x Osu23i, CML505 x C92, Osu23i x TZMI736 with the exception of S558 x CML505 were ranked among the top yielding crosses with over 7 t ha^{-1}. However, most individual SCA estimates were not significantly different.

The average heterosis for grain yield of 94.4% obtained in this study was generally lower than that obtained in other studies, for instance, Meseka et al. (2006) found 129% and 114% average

heterosis in low N and high N conditions, respectively. Other studies have shown higher heterosis in stressed environments than optimal conditions (Beck et al., 1991). Trials in this study were optimally managed therefore heterosis was mostly affected by differences in vigour in the lines used in the crosses. Minimal heterosis was detected in crosses with vigorous parents such C92, Z419, S558, VHCY. Desirable negative mid-parental heterosis for days to anthesis and silking showed that hybrids were earlier than their parental inbred lines, which is consistent with other reports (Wegary et al., 2013). Moderately high positive heterosis for plant and ear height indicated predominance of dominance effects among parental lines for taller plant stature and has been similarly reported (Legesse et al., 2008).

5.4.7 Correlation of genetic distance with yield, heterosis and SCA

Molecular analysis revealed moderate genetic diversity averaging at 0.53 and ranging from 0.211 between CML505 and CML509 to 0.69 between CML539 and TZMI736. The low to moderate genetic distance between pairs of lines indicated that the genetic base of maize germplasm sampled in this study was fairly narrow and could be broadened by introducing new and adaptive germplasm. A negative heterosis was found by crossing line CML505 and CML509. This is because of a low genetic complementarity of loci with non-additive effects since these two lines have a high degree of parental relationship. The lines exhibiting the highest genetic divergence, CML539 and TZMI736 had the highest mid-parent heterosis and the cross was the ninth highest yielding F_1 hybrid across all environments suggesting these two lines were most genetically divergent.

There was a positive correlation between genetic distance (GD) and F_1 yield performance (0.53, $p<.001$), however, higher correlations were detected between GD and SCA (0.62, $p<.001$), GD and heterosis (0.68, $p<.001$). The results are in sharp contrast with previous findings. Some studies have reported that molecular marker based distances are either low or non-predictive for F_1 yield (Xu et al., 2004), SCA for yield (Chigeza, 2003) and heterosis (Wegary et al., 2013). Drinic et al. (2002) reported a high correlation between genetic distance and SCA (0.63) although this was within a subset of the germplasm sampled. Xu et al. (2004) noted that a significant genotype x environment interaction for grain yield affects the correlation between SSR markers and yield or yield heterosis. In the current study, yield was averaged over nine environments, and SSR markers based genetic distances were shown to be effective predictors of heterosis and SCA effects in the set of materials studied. This is probably due to not only genetic distance but also genetic complementation between these lines. The SSR markers used were also highly efficient in establishing these relationships. This therefore

agrees with reports that the level of correlation between genetic distance and hybrid performance and heterosis depends on germplasm used (Betrán et al., 2003, Melchinger, 1990) and various types of gene effects (Drinic et al., 2002).

Genetic distance using SSR markers could predict 46% of mid-parent heterosis for grain yield, which is exceptionally high compared to previous findings. Xu et al. (2004) reported only 9% prediction of genetic distance for yield heterosis noting difficulty of predicting heterosis in maize using SSR markers. These authors advanced possible explanations for the poor prediction of heterosis at the DNA level as the following: Heterosis is influenced by environmental conditions which may not affect SSR markers, secondly SSR alleles are distributed evenly on the whole genome and on QTL loci which may not be related to yield heterosis and lastly, there is a relationship between gene expression and hybrid heterosis and such gene loci screened out at DNA level may not be expressed at RNA level. Since the present study showed a positive correlation between SSR-based genetic distances and F_1 grain yield, it can be expected that some of the SSR-markers are linked to QTLs controlling yield; the determination of linkage disequilibrium of markers with QTLs is however beyond the scope of this study.

5.4.8 Cluster analysis and heterotic grouping

Specific combining ability has been used to determine heterotic patterns and to assign inbred lines into heterotic groups (Badu-Apraku and Oyekunle, 2012, Badu-Apraku et al., 2013, Legesse et al., 2009, Vasal et al., 1992b). Besides highly significant positive SCA and high yield performance in cross CML509 x C92, its two parents had divergent GCA values, indicating the presence of relatively high genetic variation between them. There were differences in the placement of lines to either heterotic group across the different environments using this single cross especially when the highest and lowest yielding environments were considered. For example, under stress environment, KAK2011, five lines were assigned to C92 and two lines to CML509 whereas in the non-stress environment, KAK2012, three lines were assigned to C92 and three lines to CML509. This suggested the significant contribution of C92 under stress to performance of hybrids while the clustering of fewer lines with CML509 under stress was probably due to lower combining ability and lower heterosis of hybrids with this line. Therefore, in stress environments few groupings could be made due to diminished heterosis and low genetic differentiation. Other workers have reported environmental influence on heterotic grouping, for instance, Vivek et al. (2009) found three heterotic groups under stress conditions and six groups under optimal conditions. Moreover, even for screening purposes, different testers should be used for selecting best lines for different locations (Fan et al., 2010). Thus,

stress reduces the ability to discriminate hybrids based on phenotypic means because differences are small due to large error mean squares.

Despite the incongruities, line CML505 was consistently placed in the same group as CML509 group across all environments while lines MUL71 and Osu23i were consistently placed in the C92 heterotic group. Cross MUL71 x Osu23i would be a good candidate for a single cross tester for mid-altitude maize germplasm since the single cross was high yielding and the parental lines were consistently placed in the same heterotic group. However, the two constituent lines, MUL71 and MUL114 lines did not have significant GCA effects for grain yield across environments, therefore lacking this attribute of a good tester, among the characteristics stipulated by Pswarayi and Vivek (2008) of a single cross good tester. Li et al. (2007) compared four testers representing different heterotic groups and concluded the best testers have large genetic distance and lower coefficient correlation for grain yield implying different genetic alleles in the control of grain yield. The greater genetic variability present in tropical maize populations often causes difficulty in assigning inbred lines into heterotic groups. However, when lines with mixed origin are not placed into known heterotic groups, they can be used to identify new groups (Meinie and Fourie, 2013).

Heterotic grouping can also be influenced by the method used in assigning these groups (Menkir et al., 2003). There were few consistencies in the placement of lines using SCA estimates, SSR divergence and heterosis using cluster and PCO analyses, especially with lines CML509 and CML505 and with line MUL114 and Osu23i and also with TZMI746 and TZMI736. These pairs of lines were similar in their source populations, thus consistent placement into the same groups using SCA estimates probably arose from the lack of expression of high heterosis due to relatedness when these pairs of lines were crossed. These lines therefore showed the lowest SCA values and genetic distance by molecular markers. Some studies have shown that molecular marker data may not group lines following known heterotic groups (Semagn et al., 2012). This has been attributed to the use of lines developed from same original pool without regard of genetic origin or heterotic pattern, low selection within each heterotic group and that maximum heterotic response between groups has not yet been achieved (Warburton et al., 2002). It is suggested that many generations of reciprocal recurrent selection are required to ensure lines from each heterotic groups are significantly diverged (Xia et al., 2005).

Clustering of the 12 maize lines using SSR markers was similar to clustering based on heterosis data, which agreed well with known pedigree information. This result is in line with previous reports (Pinto et al., 2003, Xu et al., 2004). The highest yielding hybrids were obtained from

crossing lines found in different clusters. For instance, high yields were obtained from cross of Z419 and MUL114 which were distantly placed in dendrograms. In addition, the lines with the highest genetic distance had the highest heterosis for grain yield, and similarly lines with lowest genetic distance, the lowest heterosis. This agrees with the results of Xu et al. (2004) where lines clustered into families consistent with yield heterotic response.

5.5 Conclusions

Except for grain yield where SCA variance was relatively important, the high estimates of GCA variance to total genetic variance indicate that GCA is a useful guide to hybrid performance in general and evaluations based on a single representative tester should be sufficient for initial hybrid selections. The importance of SCA to grain yield indicates testing parental germplasm with multiple testers to identify superior hybrids. Environments played a significant role in modifying GCA and SCA effects for most of the traits measured and in the placement of lines to heterotic groups.

The SSR marker-based genetic distance was positively and highly correlated with grain yield and negatively correlated with ear aspect, (a component of grain yield). This shows that high grain yield can be predicted from SSR markers determined distances of the parents. The grouping of lines using SCA estimates, heterosis and genetic distance with markers was fairly consistent and dependent of relatedness and/or pedigree of the source populations. Relationship between genetic distance and yield/heterosis was high in the current study compared to previous findings, indicating that the data could be used to design hybrids in this set of germplasm.

Desirable heterosis found for grain yield and earliness shows the potential of inbred lines for hybrid development. Among the lines tested, CML539 had the highest potential for breeding based on large GCA effects for most agronomic traits including increasing grain yield, decreasing plant and ear height, decreasing days to anthesis and silking and improving ear aspect. The promising hybrids identified in this study C92 x TZMI736, Z419 x MUL114, CML509 x C92 and MUL71 x Osu23i, should be tested extensively in multi-location trials for commercialization to improve food security. This study confirms that MSV resistant lines are divergent and have favourable genes for improving grain yield.

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CHAPTER 6

Genetic analysis and role of epistasis in resistance to maize streak virus and northern leaf blight in tropical maize lines

Abstract

Maize streak virus (MSV) and northern leaf blight (NLB) are important diseases in sub-Saharan Africa and commonly occur in together severely limiting maize production. This study was conducted to determine the mode of inheritance of MSV and NLB resistance in germplasm accessed from the region and the possibility of combining resistance to the two diseases in hybrids. Twelve inbreds representing a range in responses to maize streak and northern leaf blight were crossed in a 12 x 12 half-diallel mating scheme. The resultant 66 F_1 crosses, parental lines and their controls were evaluated in two locations using 10 x 7 alpha lattice design with two replications in 2011/12 seasons under artificial and natural disease infection for MSV and NLB, respectively. Data were analysed using Griffing's and Hayman diallel analyses in SAS. General and specific combining ability effects were important in determining resistance to both diseases, with a preponderance of GCA variance based on Griffing analyses. The most resistant crosses for MSV and NLB resistance involved MUL114 and CML509, respectively, as parents. Non-significant ($p>0.05$) correlations were obtained between GCA effects for the two diseases, presenting difficulties of finding common good general combiners. However, the high level of resistance in inbreds MUL114 and CML509 would be bred into elite germplasm that lack resistance to both diseases. Hayman analysis detected the importance of epistasis for MSV resistance based on significant deviation from unity of regression coefficient on Wr (covariances) on Vr (variances) of the parental arrays and significant Wr-Wr analysis. Exclusion of four arrays having three resistant (S558, CML509 and Osu23i) and one susceptible parent (Z419) removed non-allelic interactions. This is possibly the first time genetic analysis of resistance to MSV using diallel analyses has reported the role of epistasis.

6.1 Introduction

Adequate production of maize is hampered by the prevalence of foliar diseases among other factors. The maize streak virus (MSV) and northern leaf blight (NLB) [caused by *Exserohilum turcicum* (Pass) Leonard & Suggs] are the major foliar diseases affecting maize throughout sub-Saharan Africa (SSA) (Muiru et al., 2008, Okori et al., 1999, Tefferi et al., 1996). Yield reductions up to 70% have been estimated for MSV (Bosque-Perez et al., 1998) and NLB in

susceptible genotypes(Pataky, 1992). These diseases may appear concomitantly in recurring epidemics favoured by continuous maize cropping, use of susceptible varieties and weather conditions (Pratt and Gordon, 2006).These conditions are prevalent in SSA thus MSV and NLB present a threat to food security in the region.

Some commercial varieties in the region may have some level of resistance, which may not be considered adequate for plant protection. It is important that stable and new sources of resistance be identified for these diseases, which are able to withstand the vagaries of tropical environments. To develop multiple disease resistant maize, breeding programmes must evaluate germplasm for resistance to each disease, or to the sub-set of diseases that are of highest priority for their target environments (Vivek et al., 2010). It would be useful to assess the possibility of stacking multiple resistances in a single elite cultivar. However, crucial genetic information is required to devise the most appropriate breeding strategy.

Estimates of genetic parameters offered by many tools of quantitative genetics provide a guide for breeders who want to select for various traits of economic interest such as grain yield and diseases resistance (Hallauer and Miranda, 1988). Obtaining parents that have good complementation of traits of disease resistance is important in developing resistant hybrids where these diseases occur. There is huge climatic variation across SSA and diseases may not occur or express with equal consistency. A multiple disease-resistant cultivar would provide farmers the insurance against seasonal changes in disease incidence and severity.

Genetic control of disease traits in maize as reported in the literature is often applicable to specific germplasm and test environments. Per se resistance of parents may not always reflect good or poor combiners necessitating combining ability analyses. High heritability and greater importance of additive gene control for foliar diseases are favourable for breeding for disease resistance (Brito et al., 2012). Previous studies have indicated that resistance to MSV is conditioned mostly by additive gene action (Gandiwa, 2007, Gichuru et al., 2011) while resistance to NLB involves both additive and dominance gene components (Chaudhary and Mani, 2010). The genetic variance that is apportioned to general combining ability effects have been high for most foliar diseases: 72% for GLS (Sibiya et al., 2012), 74% for MSV (Vivek et al., 2010), 84% for NLB (Njoroge and Gichuru, 2013). The effects of general (GCA) and specific combining ability (SCA) are also influenced by disease pressure, in some cases SCA effects increasing with high disease pressure (Schechert et al., 1997, Vieira et al., 2012), especially in the case of NLB. When additive gene effects are predominant and not interactive with environments, breeding progress for disease resistance can be achieved through selection at a

few hot-spot sites (Sibiya et al., 2012) for deployment in other environments in which they are adapted. The levels of resistance to MSV and NLB and the mode of inheritance have not been evaluated in the set of lines used in the current study, especially when lines are combined in a diallel cross. Inbred line resistance per se might be different from the resistance in hybrids (inter se). Therefore, it is prudent to test for the levels of MSV and NLB in the diallel cross, and devise a strategy for stacking the resistance.

Several procedures are used in analysis of data from diallel progeny involving the parental lines, mostly, Griffing's numerical approach and Hayman graphical approach. There is a general agreement from these two procedures in ascertaining the genetic control of inheritance to several traits in maize including disease resistance. Hayman's graphical approach gives symmetry of genes with positive and negative results and also gives an indication of the presence of non-allelic interaction (Hayman, 1954) strengthening the understanding of inheritance for various traits. For instance, Whythe and Gevers (1988) found concordance in the results of Griffing II and Hayman graphic analysis for the main factors controlling resistance to *Sphacelotheca reiliana* as additive effects. These two approaches have also been used to understand the inheritance of sorghum downy mildew resistance in maize where additive and non-additive genetic variance were detected (Geetha and Jayaraman, 2002). For grain yield traits, however, Srdic et al. (2007) found disagreement with the two approaches where combining ability ratio indicated importance of additive effects for 1000-kernel weight, while graphical analysis revealed dominance. They suggested more certainty is achieved by the regression analysis than with estimation of GCA and SCA effects.

If the mode of inheritance is similar and parents controlling resistance are common, populations can be obtained with multiple resistances and a common breeding strategy can be employed to obtain highly resistant populations to diseases, in this case MSV and NLB. Incorporating new sources of resistance can enhance the levels of MSV and NLB resistance in local germplasm. Varieties for deployment in Kenya and elsewhere in SSA where these two diseases occur together should carry both MSV and NLB resistance genes. This study investigates the usefulness of the sources of resistance to MSV and NLB available in selected maize genotypes for the development of new resistant hybrids. Specifically, the study examined (i) gene action and role of epistasis for control of resistance to MSV and NLB, and (ii) correlations of genetic effects for the two diseases.

6.2 Materials and methods

6.2.1 Germplasm

Twelve tropical inbred lines were variably sourced to represent important lines used for MSV breeding in the region (Table 6.1). The seed of these lines was evaluated for agronomic adaptation and those lines with medium maturity were chosen for diallel crossing. Four lines were included from the Kenya Agricultural Research Institute (KARI), i.e., Z419, S558, MUL71, and MUL114. Four lines originated from the International Maize and Wheat Improvement Centre (CIMMYT): CML505, CML505, CML539 and Osu23i. Two lines from the International Institute of Tropical Agriculture (IITA): TZMI736 and TZMI746, C92 from CIRAD and VHCY from the grains crop institute in South Africa. Two inbred lines CML202 and CML312 were included as resistant and susceptible inbred checks, respectively, for both MSV and NLB. The 12 inbred lines were crossed in a half-diallel design without reciprocal crosses.

6.2.2 Experimental design and management

The 66 F_1 progeny and their parents were evaluated for response to MSV at Muguga (2095 m, 1º15'S) and NLB at Kakamega (1550 m, 0º16'N). The evaluations were conducted in 2011 short rain season (September to December) and 2012 long rain season (April to June). The field at Muguga was artificially inoculated with MSV in both seasons, while in Kakamega fields were left to natural infection of disease without artificial inoculations. The 66 F_1 hybrids and four control hybrids were laid out in the field in two replications using a 10 x 7 alpha lattice design in each locality. The 12 inbred parents and two controls were planted in trials adjacent to the hybrids in a randomized complete block design with two replications. Plot sizes for the hybrids and the parental lines in each site were double rows of 2.5 m, with 0.75 m inter-row spacing and 0.25 m intra-row spacing. Two seeds per hill were planted and later thinned to one resulting in population densities of 53,333 plant ha^{-1}approximately.

6.2.3 MSV disease inoculation at Muguga

Artificial inoculation for MSV disease expression was conducted using reared leafhopper (*Cicadulina mbila*, Naude) colonies. The screening was based on inoculation protocols from Leuschner and Buddenhagen (1980). The leafhoppers reared on pearl millet grown in glasshouses were given 48 hours of Acquisition Access Period (AAP) on MSV-infected maize plants. The viruliferous leafhoppers were then given a two-day Inoculation Access Period (IAP)

at the 2-3-leaf stage to maize plants grown in the field. This was done by attaching a small plastic bottle with three viruliferous leafhoppers onto the distal portions of the youngest leaf.

A scale of increasing chlorosis coupled with an assessment of stunting was followed for MSV based on the protocol described by Beyene et al. (2012) where 1 = no symptoms on leaves, 2 = light symptoms on 20 to 40% leaf area, 3 = moderate symptoms on 40 to 60% leaf area, 4 = severe symptoms on 60% of leaf area, 5 = severe symptoms on 75% or more of leaf area, plants severely stunted, dying or dead. Rating was done on the entire plot at interval of 10-14 days, four times during the period of crop growth. The scores were used to define resistance types as follows: 1.0 (Immune), 1.1-1.4 (highly resistant), 1.5-2.4 (resistant), 2.5-2.9 (moderately resistant), and 3.0-5.0 (susceptible).

6.2.4 NLB disease infection

Kakamega is a hot spot for fungal foliar diseases of maize, mostly northern leaf blight and grey leaf spot as it is characterized by temperatures of 22 to 30°C, high air humidity, continuous cropping (inoculum survives on infected crop residues) and irrigation of maize crops. These are favourable conditions for NLB disease. The disease expressed highly in 2011 short rain season where rainfall (August to November) ranged from 400-500 mm, temperatures averaged at 22°C with air humidity of about 60% (Kenya Meteorological Department, 2011). Disease symptoms were observable in the trials at the V5 stage of crop growth. Data for leaf blight severity response were taken immediately the symptoms were noted to grain filling stage following procedures described by Badu-Apraku et al. (2012) on a 1-5 rating scale with mid-points as follows: 1 = 0%, 1.5 = <1%, 2.0 = 1-3%, 2.5 = 4-6%, 3.0 = 7-12%, 3.5 = 13-25%, 4.0 = 26-50%, 4.5 = 51-75%, and 5.0 = 75-100%. Disease resistance for NLB was rated as follows: 1.0 (immune), 1.1-1.9 was highly resistant, 2.0-2.4 (resistant), 2.5-2.9 (moderately resistant), 3.0-3.4 (moderately susceptible) and 3.5-5.0 (susceptible).

Table 6.1. Characteristics of inbred lines used in the study

Parent	Pedigree[e]	Origin[d]	Grain type[a]	MSV[b]	NLB[c]	GCA grain yield
Z419	SYN[Kitale/Tuxp-GLS]F2	KARI	W, I	S	S	good
S558	(EM12-210/CML197//EM12-210/OSU23i)-x-58-2-2-2-1	KARI	W, FL	R	R	poor
MUL71	[EM12-210/CML 202]-X-71-1-2-1-1-3	KARI	W, F	MR	MR	poor
OSU23i	[MSRXPOOL9] C1F2-205-1	CIMMYT/OSU	W, F	R	MR	poor
CML505	[92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	CIMMYT	W, FL	R	MR	poor
CML509	[92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB	CIMMYT	W, FL	R	R	poor
MUL114	[EM12-210/OSU23i]-X-114-2-2-1-3	KARI	W, F	HR	MR	good
C92	na	CIRAD	Y, I	HR	MS	good
CML539	MAS [MSR/312]-117-2-2-1-B*4	CIMMYT	W, FL	HR	R	good
TZMI736	na	IITA	W, FL	R	MR	poor
TZMI746	na	IITA	W, FL	R	MR	poor
VHCY	na	S.Africa	Y, FL	MR	S	poor
CML202 (check 1)	ZSR923S4BULK-5-1-BB	CIMMYT	W, FL	R	HR	-
CML312 (check 2)	S89500F2-(Sn)B*5	CIMMYT	W, DL	S	MS	-

[a]Grain type: W=white, Y=Yellow, I=intermediate, F=flint, FL-flint-like, DL= Dent-like; [b & c]:S= susceptible, MS=moderately susceptible, MR=moderately resistant, R= resistant; HR=Highly resistant; [e] na= pedigree not available; [d]Germplasm origin- CIMMYT = International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, CIRAD = Centre de cooperation internationale en recherché agronomique pour le développement, KARI =Kenya Agricultural Research Institute

6.2.5 Data analysis

Analysis of variance was performed for disease traits using PROC GLM procedure in SAS 9.2 (SAS Institute, 2004) using a fixed effects model. Excluding the checks, GCA effects of the parents and SCA of the crosses, as well as their mean squares in each environment were estimated in the 12 x 12 half-diallel crosses following Griffing's 4 model 1 (fixed parental effects (Griffing, 1956). This was done using DIALLEL-SAS program developed by Zhang et al. (2005) adapted to the SAS software version 9.2 (SAS Institute, 2004). The statistical model for the combined diallel analysis across environments is as follows:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + l_k + gl_k + s_{ij}l_k + \varepsilon_{ijk}$$

Where Y_{ijk} is the observed measurement of the ij^{th} cross grown in the k^{th} environment, μ is the grand mean; g_i and g_j are the GCA effects; s_{ij} is the SCA effects; gl_k is the interaction effect between GCA and the environment; $s_{ij}l_k$ is the interaction effect between SCA and the environment, and ε_{ijk} is the error term associated with the ij^{th} cross evaluated in the k^{th} replication and l^{th} environment (Hallauer and Miranda, 1988). The restrictions $\sum g_i$=0 and $\sum s_{ij}$=0 were imposed on the combining ability effects. A t-test was used to test significance of GCA and SCA effects (Griffing, 1956).

Genetic parameters were calculated as follows: (i) component of genetic variance on account of GCA effects $(\hat{\sigma}^2\mathrm{GCA})$: $\frac{\mathrm{MSGCA-MSSCA}}{2(\mathrm{p-2})}$ where MS is the mean square and p is the number of inbred lines; (ii) component of genetic variance of SCA effects $(\hat{\sigma}^2\mathrm{SCA})$: $\frac{\mathrm{MSSCA-MSE}}{2}$ where E is the experimental error; (iii) component of error variance $(\hat{\sigma}^2\mathrm{error})$: $\frac{\mathrm{MSE}}{\mathrm{n}}$ where n is the number of replications; (iv) component of phenotypic variance $(\hat{\sigma}^2\mathrm{phenotypic})$: $\hat{\sigma}^2$error + total genetic variance) where total genetic variance is $\hat{\sigma}^2\mathrm{GCA} + \hat{\sigma}^2\mathrm{SCA}$; (v) Baker's (1978) ratio $\frac{2\sigma^2\mathrm{GCA}}{2\sigma^2\mathrm{GCA}+\sigma^2\mathrm{SCA}}$; (vi) broad-sense heritability$\left(\mathrm{h}_\mathrm{b}^2\right)$: $\frac{\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}{\hat{\sigma}^2\mathrm{phenotypic}}$ and (vii) narrow-sense heritability $\left(\mathrm{h}_\mathrm{n}^2\right)$: $\frac{\hat{\sigma}^2\mathrm{GCA}}{\hat{\sigma}^2\mathrm{phenotypic}}$.

The 84 entries (66 F_1 hybrids, four hybrid checks, 12 parental lines and two inbred checks) in each environment were also ranked based on their reaction to the disease using PROC RANK statement in SAS 9.2. The smallest rank of one was given to the most resistant genotype and rank 84 to the most susceptible. Pearson correlation coefficients between combining ability effects and genotypic mean values for the two diseases were calculated using the PROC CORR

statement in SAS 9.2 (SAS Institute, 2004). Regression analysis was used to assess the relationship among inbred *per se* and hybrid performance using mid-parent values of inbreds *per se* as the independent variable and the corresponding hybrid performance as dependent variable.

6.2.5.1 Graphical analysis

Genetic analyses, based on an analysis of array variances (Vr) and covariances (Wr) followed the procedures developed by Jinks (1954). Adequacy of the additive-dominance model of gene action was tested by a joint regression of Wr on Vr and analyses of the Wr-Vr (Mather and Jinks, 1982). The test for epistasis was provided by regression coefficient estimate deviation from unity and significant Wr-Vr analyses. The distance between the origin and intercept on the Wr- axis provided a measure of average degree of dominance. Partial dominance was indicated by a positive intercept, complete dominance occurs when the regression line passed through the origin while overdominance was shown by a negative intercept. The statistical analyses were carried out using GenStat (Payne et al., 2011) and SAS (SAS Institute, 2004) software.

6.3 Results

6.3.1 Performance for hybrids and inbreds for MSV and NLB

6.3.1.1 Maize streak virus

In addition to artificial inoculation there was a higher disease pressure for MSV in 2012 at Muguga (average score 2.14) than in 2011 (score 1.82), therefore 2011 season was considered low disease pressure (LP) environment and 2012 high disease pressure (HP) in this study (Table 6.2). The type of resistance expressed in the single crosses for the MSV varied widely (Table 6.2).

Of the 66 F_1 crosses, based on HP season, six were symptomless (immune), eight were highly resistant (score 1.1 to 1.4), 37 were resistant (1.5 to 2.4), 15 were moderately resistant (2.5 to 2.9) while three were susceptible (>3.0) (Table 6.7). Among the parents, one line MUL114 was immune, two lines CML539 and C92 were highly resistant, six parents were resistant (CML505, CML505, S558, TZMI736, Osu23i, and TZMI746) (Table 6.2). Two lines VHCY and MUL71 were moderately resistant, while Z419 was susceptible. The resistance of inbred check CML202 (2.4) and susceptibility of CML312 (3.9) to MSV was confirmed.

Parent MUL114 was common in the six immune crosses (Table 6.2). Among the top ten crosses, one S x R cross (Z419 x MUL114) was resistant suggesting dominance of resistance for parent MUL114. None of the other resistant parents was able to completely mask the susceptible parent alleles. Moreover, three R x S crosses, Z419 x CML539, Z419 x MUL71 and Z419 x VHCY, were susceptible. This showed dominance of susceptibility or recessive alleles for resistance in the parents CML539, MUL71 and VHCY.

6.3.1.2 Northern leaf blight

As regards NLB, 2011 (average score 2.9) had higher disease pressure than 2012 (score 2.3) and would be considered high (HP) and low disease pressure (LP) environments, respectively, in subsequent results (Table 6.3). Based on the HP environment, of the 66 single crosses, nine were resistant (2.0 to 2.4), 27 were moderately resistant (2.5 to 2.9), 26 were moderately susceptible (3.0 to 3.4) while four were susceptible (Table 6.8). One R x S cross, CML509 x VHCY, was resistant showing dominance of resistance for parent CML509 (Table 6.3). Among the parents, one line S558 was highly resistant and four lines were moderately resistant (CML505, CML509, CML539 and TZMI746). Five lines MUL114, TZMI746, MUL71 and Osu23i were moderately susceptible while Z419 and VHCY were susceptible. The resistance and susceptibility of inbred checks CML202 (2.3) and CML312 (3.9), respectively, to NLB was confirmed. Considering both MSV and NLB simultaneously, one cross (OSU23i x CML539) was found among the top ten hybrids with score 1.1 for MSV and 2.0 for NLB.

Table 6.2. Performance of top and bottom ten F_1 hybrids and parental lines for MSV response across two seasons under artificial infection

Genotype	Muguga2011 (LP)		Muguga2012 (HP)		Across seasons	
	score	rank	score	rank	score	rank
Z419 x MUL114 (S x HR)	1.0	1	1.0 (I)	1	1.0	1
OSU23i x MUL114 (R x HR)	1.0	1	1.0 (I)	1	1.0	1
CML505 x MUL114 (R x HR)	1.0	1	1.0 (I)	1	1.0	1
CML509 x MUL114 (R x HR)	1.0	1	1.0 (I)	1	1.0	1
MUL114 x C92 (HR x HR)	1.0	1	1.0 (I)	1	1.0	1
MUL114 x CML539 (HR x HR)	1.0	1	1.0 (I)	1	1.0	1
MUL114 x TZMI736 (HR x R)	1.0	1	1.1 (HR)	8	1.0	8
MUL114 x TZMI746 (HR x R)	1.0	1	1.1 (HR)	9	1.1	9
MUL71 x MUL114 (MR x HR)	1.1	15	1.1 (HR)	9	1.1	10
OSU23i x CML539 (R x HR)	1.0	1	1.2 (HR)	12	1.1	11
MUL71 x VHCY (MR x MR)	2.3	66	2.8 (MR)	73	2.6	69
Z419 x TZMI746 (S x R)	2.4	71	2.8 (MR)	68	2.6	70
MUL71 x OSU23i (MR x R)	2.5	72	2.7 (MR)	66	2.6	71
Z419 x TZMI736 (S x R)	2.5	72	2.8 (MR)	68	2.6	72
558 x CML539 (R x HR)	2.7	78	2.6 (MR)	63	2.7	73
Z419 x 558 (S x R)	2.7	78	2.8 MR)	71	2.7	75
Z419 x CML539 (S x R)	2.3	66	3.1 (S)	80	2.7	76
Z419 x OSU23i (S x R)	2.8	81	2.9 (MR)	75	2.8	79
Z419 x MUL71 (S x R)	2.5	72	3.2 (S)	82	2.9	80
Z419 x VHCY (S x MR)	2.8	82	3.1 (S)	80	3.0	81
PH3253 (hybrid check) (S)	3.0	83	3.0 (S)	78	3.0	82
Parents						
MUL114	1.0	1	1.0 (I)	1	1.0	1
CML539	1.0	1	1.3 (HR)	14	1.1	13
C92	1.4	20	1.4 (HR)	16	1.4	18
CML509	1.6	28	1.7 (R)	23	1.7	24
CML505	1.6	28	2.0 (R)	32	1.8	28
558	1.6	28	2.1 (R)	37	1.9	31
TZMI746	1.5	24	2.3 (R)	45	1.9	32
OSU23i	1.5	24	1.5 (R)	21	1.5	21
TZMI736	2.0	46	2.4 (R)	57	2.2	57
VHCY	2.1	58	2.5 (MR)	60	2.3	59
MUL71	2.4	70	2.6 (MR)	62	2.5	67
Z419	3.0	83	3.0 (S)	78	3.0	77
CML202 (resistant inbred check)	1.6	28	2.4 (R)	56	2.0	45
CML312 (susceptible inbred check)	2.7	80	3.9 (S)	84	3.3	83
mean	1.81		2.13		1.97	
$LSD_{0.05}$	0.31		0.44		0.26	
CV (%)	8.60		10.3		9.70	

Resistance descriptions: I = immune, HR = highly resistant, R = resistant, MR = moderately or intermediately resistant, S = susceptible

Table 6.3. Performance of top and bottom ten F_1 hybrids and parental lines for NLB response across two seasons under natural infection

Genotype	Kakamega 2011 (HP)		Kakamega 2012 (LP)		Across seasons	
	score	rank	score	rank	score	rank
558 x CML505 (HR x MR)	2.0 (R)	2	1.9	15	2.0	5
OSU23i x CML539 (MS x MR)	2.1 (R)	3	1.8	11	2.0	2
558 x CML509 (HR x MR)	2.3 (R)	4	1.6	3	2.0	2
CML509 x TZMI736 (MR x MR)	2.3 (R)	4	1.7	5	2.0	6
MUL71 x CML505 (MS x MR)	2.5 (MR)	12	1.6	3	2.0	7
CML509 x C92 (MR x MS)	2.4 (R)	10	1.7	5	2.1	8
CML505 x CML509 (MR x MR)	2.5 (MR)	12	1.8	8	2.1	9
CML509 x CML539 (MR x MR)	2.6 (MR)	18	1.7	5	2.1	11
OSU23i x CML509 (MS x MR)	2.8 (MR)	30	1.5	1	2.1	9
CML505 x VHCY (MR x S)	2.3 (R)	4	2.1	41	2.2	17
Z419 x MUL71 (S x MS)	3.3 (MS)	72	2.6	66	3.0	71
OSU23i x VHCY (MS x S)	3.5 (S)	77	2.5	59	3.0	73
Z419 x MUL114 (S x MS)	3.6 (S)	79	2.5	59	3.0	75
Z419 x C92 (S x MS)	3.0 (MS)	44	3.1	81	3.1	76
Z419 x TZMI746 (S x MR)	3.3 (MS)	72	2.8	75	3.1	77
Z419 x CML539 (S x MR)	3.2 (MS)	61	3.0	79	3.1	78
MUL71 x TZMI746 (MS x MR)	3.4 (MS)	74	2.8	75	3.1	79
MUL71 x VHCY (MS x S)	3.5 (S)	77	3.1	80	3.3	80
Z419 x VHCY (S x S)	3.7 (S)	80	3.6	82	3.6	82
PH3253 (hybrid check)	3.9 (S)	82	2.0	17	3.0	72
Parents						
558	1.9 (HR)	1	2.0	17	2.0	4
CML509	2.8 (MR)	35	2.0	17	2.4	30
CML539	2.8 (MR)	35	2.0	17	2.4	30
CML505	2.5 (MR)	12	2.5	59	2.5	41
MUL114	3.0 (MS)	44	2.1	37	2.5	43
OSU23i	3.2 (MS)	61	2.0	17	2.6	47
TZMI736	3.1 (MS)	52	2.3	53	2.7	54
MUL71	3.2 (MS)	61	2.3	49	2.7	56
TZMI746	2.9 (MR)	40	2.8	75	2.9	64
C92	3.4 (MS)	74	2.6	68	3.0	74
Z419	3.8 (S)	81	3.8	84	3.8	83
VHCY	4.2 (S)	84	3.6	83	3.9	84
CML202 (resistant inbred check)	2.3 (R)	4	1.5	1	1.9	1
CML312 (susceptible inbred check)	3.9 (S)	82	2.8	75	3.4	81
mean	2.97		2.25		2.58	
$LSD_{0.05}$	0.47		0.31		0.39	
CV (%)	8.20		6.70		7.80	

Resistance descriptions: I = immune, HR = highly resistant, R = resistant, MR = moderately or intermediately resistant, MS = moderately susceptible, S = susceptible

6.3.2 General and specific combining ability

Results of individual and combined analysis for genetic control of MSV and NLB are presented in Table 6.4. In the different disease pressures, GCA and SCA effects were highly significant ($p<0.01$) for resistance to MSV and NLB. Across seasons, GCA effects contributed for most (82%) of the sums of squares for crosses while SCA contributed 18%. For NLB, GCA effects accounted for 68% of the variation in crosses while SCA contributed 32%. The effects of GCA and SCA significantly ($p<0.01$) interacted with environments for both diseases. The contribution of GCA x Env to hybrid x Env variation was 28% while the contribution of SCA x Env was 72% in the case of MSV. For NLB, GCA x Env accounted for 35% of the hybrid x Env sums of squares while SCA x Env accounted for 65%.

Table 6.4. Individual diallel and joint analysis for resistance to MSV and NLB over two seasons using Griffing method 4

Source of variation	df	MSV			NLB		
		across seasons	2011	2012	across seasons	2011	2012
		Mean squares					
Env	1	5.991***			29.197***		
Hybrids	65	1.265***	0.572***	0.769***	0.501***	0.277***	0.326***
Hybrids x Env	65	0.077***			0.102***		
GCA	11	6.152***	2.481***	3.801***	2.011***	0.866***	1.357***
SCA	54	0.269***	0.184***	0.152***	0.194***	0.157***	0.117***
GCA x Env	11	0.129***			0.213***		
SCA x Env	54	0.066***			0.081**		
Error	65	0.032	0.015	0.049	0.041	0.062	0.021
GCA to hybrids SS (%)		82			68		
SCA to hybrids SS (%)		18			32		
GCA x E to G x E (%)		28			35		
SCA x E to G x E (%)		72			65		
Mean		1.915	1.764	2.065	2.532	2.865	2.199
CV (%)		9.365	6.974	10.736	8.092	8.703	6.714
R^2		0.957	0.974	0.941	0.926	0.817	0.937

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

6.3.3 Genetic parameter and combining ability estimates

6.3.3.1 Maize streak virus

Genetic parameters for MSV showed relatively high genetic variances under high disease pressure (HP) than under lower disease pressure (LP) (Table 6.5). Subsequently, total genetic variance denoted by $\hat{\sigma}^2\mathrm{GCA} + \hat{\sigma}^2\mathrm{SCA}$, was higher (0.234) under HP and than under LP (0.199). The proportion of GCA over SCA variance was 2.5 times greater under HP than under LP. However, heritability in the broad sense under HP and LP were comparable (91 and 96%, respectively). Narrow sense heritability was considerably lower under LP (56%) compared to HP (71%). Thus under HP, more of the additive effects were captured for MSV. Moreover, Baker's (1978) ratio was greater than 0.5 in both cases, 0.87 in HP and 0.73 in LP, indicative of higher influence of additive effects.

The estimates of GCA effects for parents for MSV across the different seasons are presented in Table 6.6. Generally, the negative signs of GCA effects are related with resistance and positive effects with susceptibility. Five parents CML505, CML509, MUL114, C92 and CML539 had the significant ($p<0.05$) and negative GCA effects for MSV in individual seasons and across seasons with the exception of CML505 in HP season. Parent MUL114 had the highest negative GCA effects across environments (-0.96, $p<0.01$), followed distantly by CML539 (-0.27, $p<0.001$). In contrast, six parents, Z419, S558, MUL71, Osu23i, TZM1736, and VHCY displayed significant ($p<0.05$) positive GCA effects for MSV across seasons. Although some of these lines were actually MSV resistant lines, based on mean scores (Table 6.2), positive GCA effects showed they produced higher MSV means in their crosses in relation to those with negative GCA effects. However, the highest positive GCA effect across seasons was obtained in line Z419 (0.611, $p<0.001$), which was a susceptible parent.

Nineteen (19) of the 66 single crosses had significant SCA effects (Table 6.7). (Five hybrids with the lowest SCA effects for MSV (indicating better than predicted resistance) were MUL114 x VHCY (-1.04), OSU23i x CML539 (-0.67), Z419 x MUL114 (-0.62), CML505 x C92 (-0.45) and MUL71 x CML539 (-0.38) (Table 6.7). In these crosses, at least one or both parents had corresponding negative GCA effects. Six hybrids had positive SCA estimates, five of which had at least one parent or both parents having a positive GCA effect. One cross however, C92 x CML539, had a positive SCA effect (0.328***) and yet both parents had negative GCA effects and rated as highly resistant.

Table 6.5. Estimates of genetic parameters for resistance MSV and NLB over two seasons

Genetic parameters	MSV			NLB		
	2011 (low pressure)	2012 (high pressure)	across season	2011 (high pressure)	2012 (Low pressure)	across season
$\hat{\sigma}^2\text{GCA}$	0.115	0.182	0.294	0.035	0.062	0.091
$\hat{\sigma}^2\text{SCA}$	0.084	0.051	0.118	0.047	0.048	0.077
$\hat{\sigma}^2\text{GCA} + \hat{\sigma}^2\text{SCA}$	0.199	0.234	0.413	0.083	0.11	0.167
$\hat{\sigma}^2\text{error}$	0.008	0.025	0.016	0.031	0.011	0.021
$\hat{\sigma}^2\text{phenotypic}$	0.207	0.258	0.429	0.114	0.121	0.188
$\frac{\hat{\sigma}^2\text{GCA}}{\hat{\sigma}^2\text{SCA}}$	1.361	3.548	2.484	0.748	1.303	1.188
$\frac{2\hat{\sigma}^2\text{GCA}}{2\hat{\sigma}^2\text{GCA}+\hat{\sigma}^2\text{SCA}}$ †	0.732	0.877	0.833	0.598	0.721	0.703
$\%\hat{\sigma}^2\text{GCA}$	58	78	71	43	56.6	54
$\%\hat{\sigma}^2\text{SCA}$	42	22	29	57	43.4	46
h_b^2 (%)	96	91	96	73	91	89
h_n^2 (%)	56	71	69	31	52	48

† Bakers (1978) ratio

Table 6.6. General combining ability estimates for the resistance to MSV and NLB over two seasons of different disease pressure

Parents	general combining ability estimates					
	MSV			NLB		
	2011 (LP)	2012 (HP)	across env	2011 (HP)	2012 (LP)	across env
Z419	0.559***	0.661***	0.611***	0.291***	0.448***	0.369***
S558	0.176***	0.233***	0.205***	-0.251***	-0.013^{ns}	-0.132^{ns}
MUL71	0.301***	0.311***	0.306***	0.123*	0.055^{ns}	0.089^{ns}
OSU23i	0.151***	0.105*	0.128**	0.041^{ns}	-0.182***	-0.071^{ns}
CML505	-0.098***	-0.093^{ns}	-0.096*	-0.259***	-0.326***	-0.292***
CML509	-0.173***	-0.143**	-0.158***	-0.234***	-0.407***	-0.321***
MUL114	-0.823***	-1.105***	-0.964***	0.073^{ns}	-0.082*	-0.004^{ns}
C92	-0.256***	-0.135*	-0.196***	0.015^{ns}	-0.026^{ns}	-0.005^{ns}
CML539	-0.231***	-0.299**	-0.265***	-0.218***	-0.057^{ns}	-0.137*
TZMI736	0.068*	0.151**	0.109*	-0.026^{ns}	-0.001^{ns}	-0.013^{ns}
TZMI746	0.093***	-0.013^{ns}	0.039^{ns}	0.091^{ns}	0.136***	0.113^{ns}
VHCY	0.234***	0.328***	0.281***	0.356***	0.455***	0.406***

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively, ^{ns} non-significant data

6.3.3.2 Northern leaf blight

Genetic parameters for NLB showed a different pattern from those of MSV. Variance of GCA and SCA were slightly higher under LP than under HP (Table 6.5). Total genetic variance was 0.11 under LP and 0.08 under HP. Further, Baker's (1978) ratios were moderate (0.6) under HP but higher (0.72) under LP. Heritability in the broad sense was notably higher (91%) under LP than under HP (73%). Subsequently, low narrow sense heritability estimates were obtained under HP (31%) compared to moderate estimates in LP (52%) season. Thus, SCA effects were most pronounced for NLB in the HP season while GCA effects were pronounced in the LP season.

General combining ability estimates across seasons for NLB are given in Table 6.6. Unlike MSV, there were discrepancies in the detection of GCA effects between HP and LP seasons, some parents appearing in one season and not another. In the HP season, four parents, S558, CML505, CML509 and CML539 had negative and highly significant ($p<0.001$) GCA effects of nearly similar magnitude (-0.21 to -0.26). In the LP season, three parents Osu23i, CML505 and CML509, had significant ($p<0.001$) negative GCA effects (-0.18, 0.33, and 0.41, respectively). Thus, CML505 and CML509 had negative GCA consistently across seasons. In contrast, two parents Z419 (0.37, $p<0.001$) and VHCY (0.41, $p<0.001$) had significant positive GCA effects for NLB in individual and across seasons.

Only four crosses had significant SCA effects and were all negative (Table 6.8). Two of the crosses C92 x TZMI746 (-0.5) and C92 x VHCY (-0.7) were composed of a common parent C92, which did not have a significant GCA effect, while the second parents, TZMI746 and VHCY, had positive GCA effects. The two other crosses Z419 x CML509 (-0.46), and CML505 x VHCY (-0.97) however, had at least one parent with negative GCA effect for NLB. In fact, the latter two crosses were R x S crosses, further showing that CML509 and CML505 were not only good combiners for NLB but also have resistant genes that displayed dominance.

Considering both MSV and NLB, one line CML509 had significant ($p<0.001$) negative GCA effects in LP and HP seasons for the two diseases. Some lines such as S558 and Osu23i had positive GCA effects for MSV but negative effects for NLB. There were no lines, which had negative GCA effects for MSV but positive GCA effects for NLB. Parents VHCY and Z419 had positive GCA effects across seasons for both MSV and NLB.

The means of severity scores of MSV and NLB for the 66 crosses were plotted against the mid-parent values and the regression line fitted (Figure 6.1a,b). The regression model provided a

better fit for MSV (r^2 = 0.67) compared to that of NLB (r^2=0.45). The regression coefficient for MSV was 1.334 $\pm$ 0.115, p<0.001) while that of NLB was 0.562 $\pm$ 0.077, p<0.001).

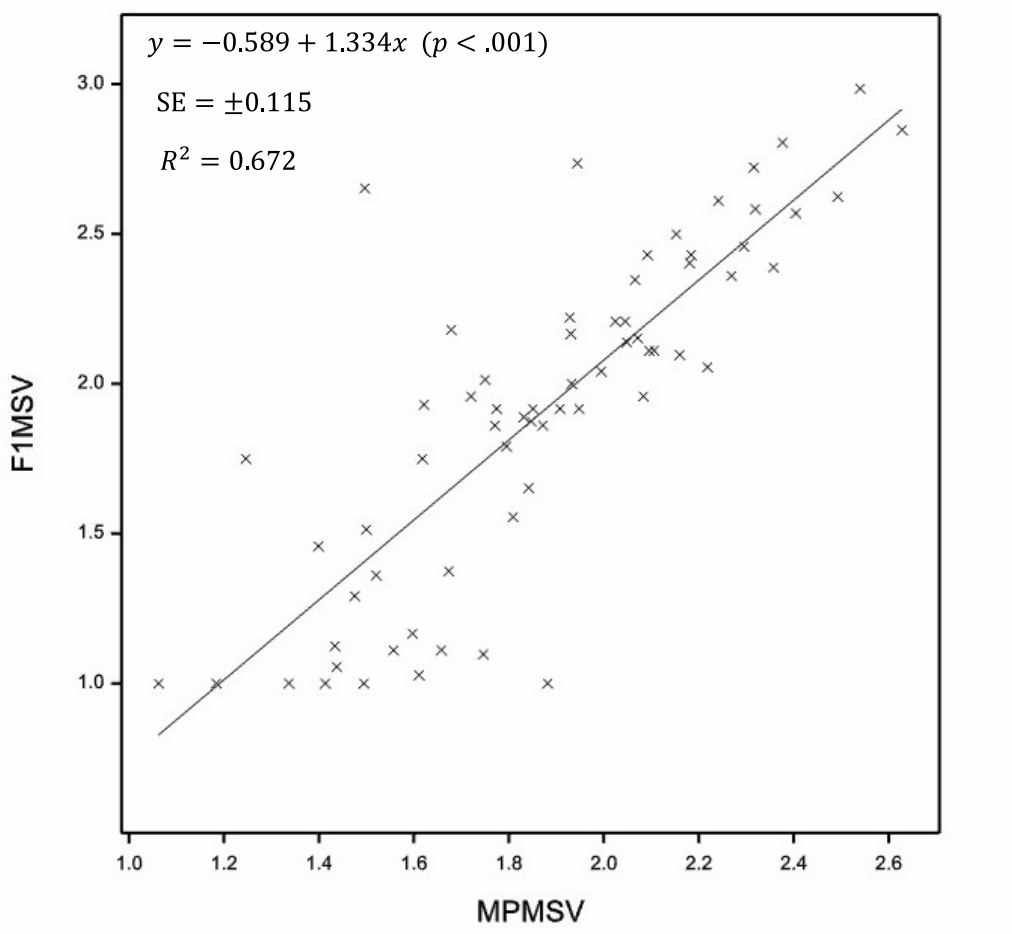

Figure 6.1a. Regression of the F_1 hybrid on mid-parent value for the severity of MSV of 66 crosses from a diallel of 12 maize lines

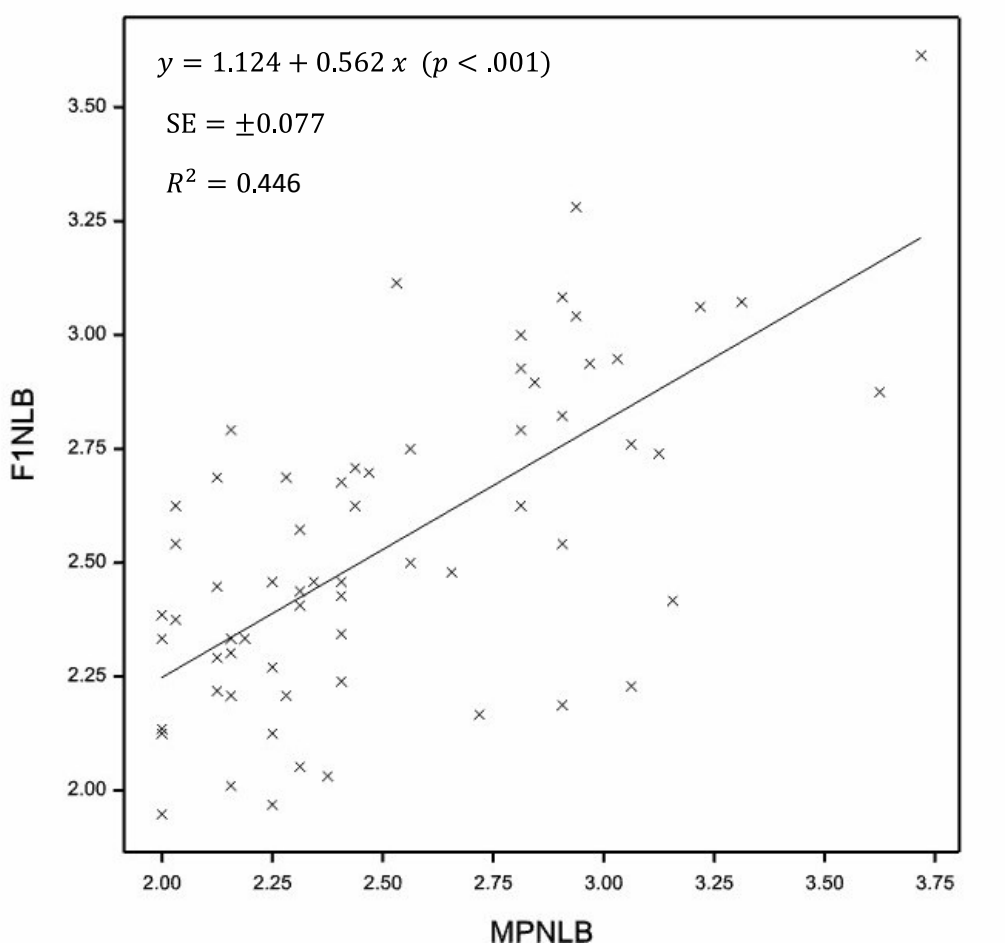

Figure 6.1b. Regression of the F_1 hybrid on mid-parent value for the severity of NLB of 66 crosses from a diallel of 12 maize lines

Table 6.7. Estimates of SCA (above diagonal) and means of F_1 hybrids in HP season (below diagonal) for MSV scores

Parent	Z419	S558	MUL71	OSU23i	CML505	CML509	MUL114	C92	CML539	TZMI736	TZMI746	VHCY
Z419	-	0.058	0.046	0.195	0.049	-0.289*	-0.617***	0.059	0.526***	0.011	0.056	0.533**
S558	2.8	-	0.014	0.024	-0.122	-0.071	-0.080	-0.126	0.855***	0.007	-0.253*	0.461*
MUL71	3.1	2.5	-	0.276*	-0.037	-0.111	-0.244	0.155	-0.378**	0.051	0.179	0.112
OSU23i	2.0	2.4	2.7	-	-0.027	0.010	-0.151	0.358**	-0.632***	-0.037	-0.061	0.356
CML505	2.8	2.1	2.4	1.8	-	0.336**	0.05	-0.454***	-0.251*	0.261*	0.056	-0.029
CML509	2.3	2.1	2.0	1.9	2.2	-	0.114	-0.195	-0.02	0.035	0.122	-0.052
MUL114	1.0	1.2	1.1	1.0	1.0	1.0	-	0.171	0.249*	-0.127	-0.012	-1.036***
C92	2.7	2.3	2.2	2.4	1.3	1.6	1.0	-	0.328**	-0.034	0.192	-0.192
CML539	3.1	2.6	1.4	1.2	1.6	1.5	1.0	2.0	-	-0.372**	-0.146	0.278
TZMI736	2.8	2.3	2.8	2.4	2.3	2.2	1.1	2.1	1.7	-	0.075	-0.013
TZMI746	2.8	2.1	2.5	2.2	2.1	2	1.1	1.9	1.6	2.1	-	-0.002
VHCY	3.1	2.7	2.8	2.8	2.3	2.3	1.2	1.9	1.9	2.7	2.2	-

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

Table 6.8. Estimates of SCA (above diagonal) and means of F_1 hybrids in HP season (below diagonal) for NLB scores

Parent	Z419	S558	MUL71	OSU23i	CML505	CML509	MUL114	C92	CML539	TZMI736	TZMI746	VHCY
Z419	-	0.057	-0.065	-0.327	-0.26	-0.459*	0.122	0.103	0.284	-0.166	0.027	-0.207
S558	3.1	-	0.245	0.035	-0.138	-0.128	0.025	0.048	0.157	-0.054	-0.132	0.359
MUL71	3.3	3.0	-	-0.254	-0.323	-0.105	-0.264	0.071	-0.029	0.084	0.392	0.21
OSU23i	3.1	2.7	2.6	-	0.248	-0.055	0.172	0.08	-0.384	0.333	-0.121	-0.09
CML505	2.8	2.0	2.5	2.9	-	0.137	-0.053	0.199	0.13	-0.028	0.092	-0.969**
CML509	2.5	2.3	2.5	2.8	2.5	-	0.311	-0.219	0.025	-0.226	0.32	-0.521
MUL114	3.6	2.8	2.7	3.3	2.7	3.1	-	-0.086	-0.009	-0.177	-0.005	-0.091
C92	3.0	2.8	3.4	3.1	3.0	2.4	2.7	-	-0.017	0.148	-0.503*	-0.696*
CML539	3.2	2.3	2.6	2.1	2.7	2.6	2.8	2.8	-	-0.181	-0.082	-0.201
TZMI736	2.8	2.4	3.3	3.1	2.7	2.3	2.7	3.1	2.7	-	0.114	-0.21
TZMI746	3.3	2.5	3.4	2.9	2.8	3.2	3.0	2.3	2.7	3.0	-	-0.319
VHCY	3.7	3.2	3.5	3.5	2.3	3.0	3.2	3.2	3.1	3.3	3.3	-

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

6.3.4 Correlations among genotypic means and genetic effects

There was a moderate relationship (0.42, $p<0.01$) between the performance of F_1 hybrids for MSV and NLB (Table 6.9). The performance of F_1 hybrids and SCA effects for NLB had a positive but low correlation (0.32, $p<0.01$), however for F_1 performance for MSV was not correlated with SCA effects. A low positive correlation (0.26, $p<0.05$) was also found between the SCA effects of MSV and those of NLB.

Some parents that were resistant to MSV had resistance to NLB as revealed by the moderate positive coefficient (0.59, $p<0.05$) between the parental means. This was only detected in the across season analysis but in the individual seasons, correlations of parental means to the two diseases were insignificant ($p>0.05$). It was further noted that the GCA effects for MSV and NLB were not significantly ($p>0.05$) correlated. In general, the best combiners for MSV were not necessarily the best combiners for NLB. Nonetheless, the parental means for the individual pathogens correlated highly with their GCA effects, MSV (0.93, $p<0.01$) and NLB (0.83, $p<0.01$). The mid-parental values were also highly correlated with the F_1 performance across seasons for both diseases, although the coefficient r (F_1, MP) was higher for MSV (0.822, $p<0.01$) than for NLB (0.674, $p<0.01$).

Table 6.9. Pearson correlation coefficients for genotypic means and genetic effects for MSV and NLB

Parameter	Correlations		
	Low disease pressure	High disease pressure	Combined
r (F_1 NLB, F_1MSV)	0.469***	0.27*	0.419***
r (F_1 NLB, SCA NLB)	0.196^{ns}	0.385**	0.315**
r (F_1 NLB, SCA MSV)	0.098^{ns}	0.033^{ns}	0.05^{ns}
r (F_1 MSV, SCA NLB)	-0.156^{ns}	0.038^{ns}	-0.053^{ns}
r (F_1 MSV,SCA MSV)	0.018^{ns}	0.124^{ns}	0.063^{ns}
r (F_1 NLB, MPNLB)	0.691***	0.631***	0.674***
r (F_1 MSV, MPMSV)	0.741***	0.812***	0.822***
r (SCA NLB, SCA MSV)	0.265*	0.192^{ns}	0.264*
r (PMSV,PNLB)	0.515^{ns}	0.321^{ns}	0.589*
r (PMSV,GCAMSV)	0.864***	0.888***	0.927***
r (PMSV,GCANLB)	0.471^{ns}	0.486^{ns}	0.562^{ns}
r (PNLB,GCAMSV)	0.533^{ns}	0.282^{ns}	0.553^{ns}
r (PNLB,GCANLB)	0.824**	0.867***	0.827***
r (GCA MSV, GCA NLB)	0.542^{ns}	0.329^{ns}	0.486^{ns}

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively, ^{ns} non-significant data; P=parental means, MP=mid-parental values ((p1+p2)/2)

6.3.5 Graphical analysis using Hayman's procedures

Hayman's analysis of variance showed high significance ($p<0.001$) of "a" (additive) and "b" (dominance) variance for the control of MSV and NLB resistance (Table 6.10). The "b_1" component of dominance was significant ($p<0.05$) for both MSV and NLB in the 12 x 12 diallel showing directional dominance thus dominance effects at various loci were predominantly in one direction. High significance ($p<0.001$) of "b_2" component for both diseases showed that some parents had more dominant alleles than others implying asymmetry of gene distribution. The significance of the "b_3" item ($p<0.001$) implied residual dominance effects caused by dominance x additive effects or dominance x dominance effects for MSV and NLB.

Table 6.10. Hayman analysis of variance for MSV and NLB response

Source of variation	MSV (12 x 12)			MSV (8 x 8)			NLB (12 x 12)		
	d.f.	s.s.	m.s.	d.f.	s.s.	m.s.	d.f.	s.s.	m.s.
a (additive)	11	75.168	6.833***	7	31.4433	4.492***	11	33.436	3.039***
b (dominance)	66	20.278	0.307***	28	6.4788	0.231***	66	15.448	0.234***
b_1	1	0.251	0.251*	1	0.1234	0.123^{ns}	1	2.515	2.516*
b_2	11	5.456	0.496***	7	1.9	0.271*	11	2.455	0.223***
b_3	54	14.571	0.269***	20	4.4554	0.223***	54	10.476	0.194***
total	77	95.446	1.239	35	37.922	1.084	77	48.884	0.635
a to total SS (%)		79			83			68	
b to total SS (%)		21			17			32	
b_1 to b SS (%)		1			2			16	
b_2 to b SS (%)		27			29			16	
b_3 to b SS (%)		72			69			68	
a SS/ b SS		3.7			4.9			2.2	

*, **, ***= significant at 5%, 1%, and 0.1% probability respectively; ns=non-significant data

Analysis of variance showed that the array of parental order of dominance Wr + Vr and the difference of arrays for Wr – Vr were highly significant ($p>0.05$) for MSV and NLB (Table 6.11). The joint regression of Wr (covariances) on Vr (variances) for MSV with regression of 0.57 ± 0.14 SE (Fig.6.2) was significantly different from unity. In the model of diallel analysis proposed by Jinks and Hayman (1953) a "β" regression coefficient estimate different from 1 indicates the presence of epistasis, otherwise its absence. This observation supported a major role of epistasis effects in controlling MSV resistance based on two methods, heterogeneity of Wr-Vr and regression of Wr on Vr.

Table 6.11. Analysis of variance for MSV and NLB resistance for Wr+Vr and Wr-Vr estimates in 12 x 12 diallel

	Source of variation	d.f.	MSV		NLB	
			s.s.	m.s.	s.s.	m.s.
Wr+Vr	Rep	3	0.294	0.098	0.025	0.008
	Wr+Vr	11	1.034	0.093***	0.494	0.044***
	Residual	33	0.178	0.005	0.332	0.010
	Total	47	1.506		0.852	
Wr-Vr	Rep	3	0.027	0.009	0.022	0.007
	Wr-Vr	11	0.169	0.015***	0.040	0.004*
	Residual	33	0.042	0.001	0.047	0.001
	Total	47	0.238		0.109	

*, **, ***= significant at 5%, 1%, and 0.1% probability respectively

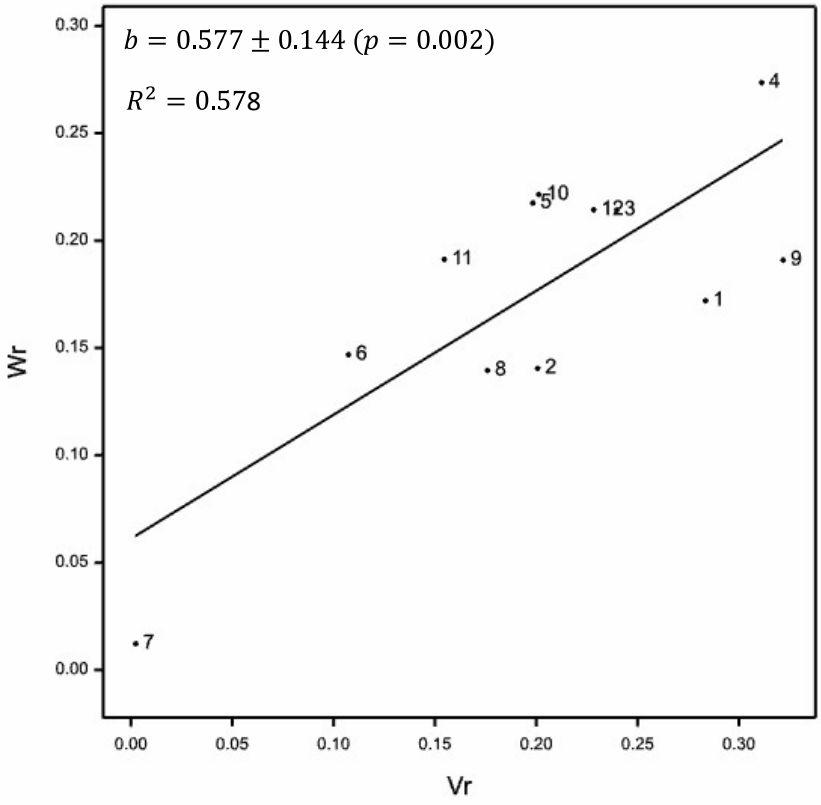

Figure 6.2. Linear regression of Wr on Vr for MSV showing distribution of parental lines in the 12 x 12 diallel

1 = Z419, 2 = 558, 3 = MUL71, 4 = OSU23i, 5 = CML505, 6 = CML509, 7 = MUL114, 8 = C92, 9 = CML539, 10 = TZMI736, 11 = TZMI746, 12 = VHCY

The joint regression of Wr on Vr for NLB with regression coefficient of 0.829± 0.121 was however not significantly (p>0.05) different from unity. Therefore, these results did not provide evidence for the role of epistasis in conditioning resistance in NLB and that the analysis of the 12 x 12 half-diallel showed adequacy of additive-dominance model of gene action. Based on the Figure 6.3, partial dominance to complete dominance was found for NLB. Parents closest to the origin (CML505 and CML509), had the highest proportion of dominant alleles for NLB, while VHCY and Z419, which were furthest from the origin has the maximum number of recessive alleles. This also corresponded with resistance patterns of CML505 and CML509 and susceptibility of VHCY and Z419 to NLB.

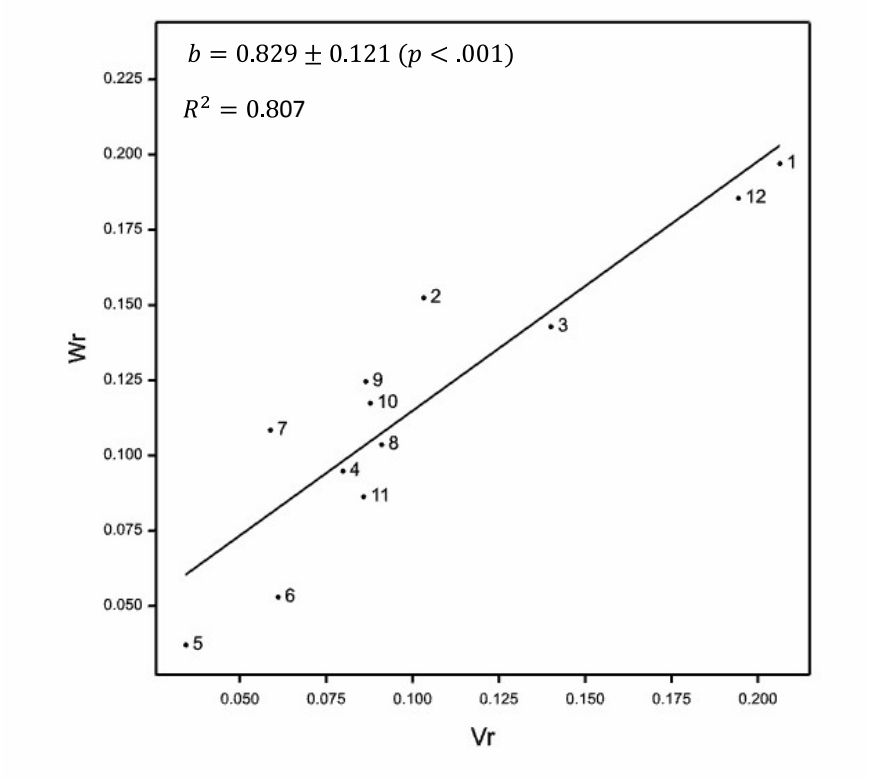

Figure 6.3. Linear regression of Wr on Vr for NLB showing distribution of 12 parental lines
1 = Z419, 2 = 558, 3 = MUL71, 4 = OSU23i, 5 = CML505, 6 = CML509, 7 = MUL114, 8 = C92, 9 = CML539, 10 = TZMI736, 11 = TZMI746, 12 = VHCY

Further assessment of genetic analysis of MSV resistance was conducted since additive-dominance model of gene action was not satisfactory in the 12 x 12 half-diallel. This required the removal of arrays (parents) contributing most to interaction followed by analysis of the resultant reduced diallel to establish that an additive-dominance model fitted the data (Hayman,

1954). This objective was accomplished by excluding four parents in turn which contributed most to heterogeneity of Wr-Vr analysis. First, parent 2 (S558) was eliminated, followed by parent 4 (Osu23i), 6 (CML509) and 1 (Z419). The first three were resistant parents and the forth parent eliminated was a susceptible line (Table 6.12).

Analysis of the 8 x 8 model was thus found adequate for additive-dominance model of gene action (Table 6.12 and Fig. 6.4). This was based on two techniques: regression coefficient of Wr on Vr was not significantly different from unity and analysis of variance of Wr-Vr was not significant with the reduced 8 x 8 model. During parent elimination for the best fit, the two methods differed in sensitivity. Regression could only detect epistasis at the 12- and 11-parent diallels, while Wr-Vr method could detect epistasis up to the 9-parent diallel.

The magnitude of regression coefficients increased from 0.58 to 0.98 and the regression intercept decreased from 0.06 to 0.02 as parents contributing most to epistasis were eliminated. However, the intercept values did not differ significantly from zero, an indication that the loci controlling resistance to MSV showed near complete to complete dominance. The mean potence ranged from 0.08 to -0.07 between the 12 x 12 diallel and 8 x 8 diallel (Table 6.12) showing that the mostly hybrids were not superior to inbreds in MSV resistance. There was a strong positive correlation between the mean of the array and corresponding Wr+Vr values ($r = 0.934$, $p<0.001$) showing that among the eight lines, resistance to MSV was dominant to susceptibility.

The dispersion of array points in the graphical analysis was not consistent between models. Notably, parent 9 (CML539) which was found distant from the origin in the 12 x 12 model was intermediately positioned in the 8 x 8 model. However, the position of parent 7 (MUL114) array point on the both the full (Fig. 6.2) and reduced (Fig. 6.4) models was unchanged indicating that MUL114 carried more dominant genes for MSV resistance than any of the other lines included in this trial and that it transmits this resistance to its progeny. This was shown in the mean performance of the top ten hybrids for resistance, where MUL114 was a common parent. In contrast, parents 3, 5 and 11 (MUL71, CML505, and TZMI736) were located on the upper portion of the straight line; these parents, therefore, contained a high proportion of recessive alleles. Parents 8, 9 and 11 had intermediate values when compared to other parents, suggesting that they has a higher proportion of dominant alleles than parents 3, 5 and 11 but lower than in parent 7. Based on the distribution of the other arrays on the Wr/Vr graph there seemed to be more recessive alleles than dominant ones.

Table 6.12. Tests for non-allelic interaction for MSV response in 12 x 12 and reduced F_1 diallels

Array	Mean potence	Correlation Wr+Vr vs. array mean	H_0:$\alpha = 0$ Intercept	Regression Coefficient	S.E	t value	H_0:$\beta = 1$ t prob	Mean squares Wr-Vr	Parents eliminated			
12	0.078	0.694*	0.061^{ns}	0.577	0.144	2.938	0.002	0.014***				
11	0.044	0.771**	0.053^{ns}	0.694	0.138	2.217	0.013	0.011***	S558			
10	0.054	0.742*	0.042^{ns}	0.759	0.148	1.628	0.052	0.011***	CML509	S558		
9	-0.016	0.858**	0.045^{ns}	0.826	0.175	0.994	0.161	0.003**	OSU23i	CML509	S558	
8	-0.071	0.934***	0.016^{ns}	0.979	0.079	0.266	0.395	0.003^{ns}	Z419	OSU23i	CML509	S558

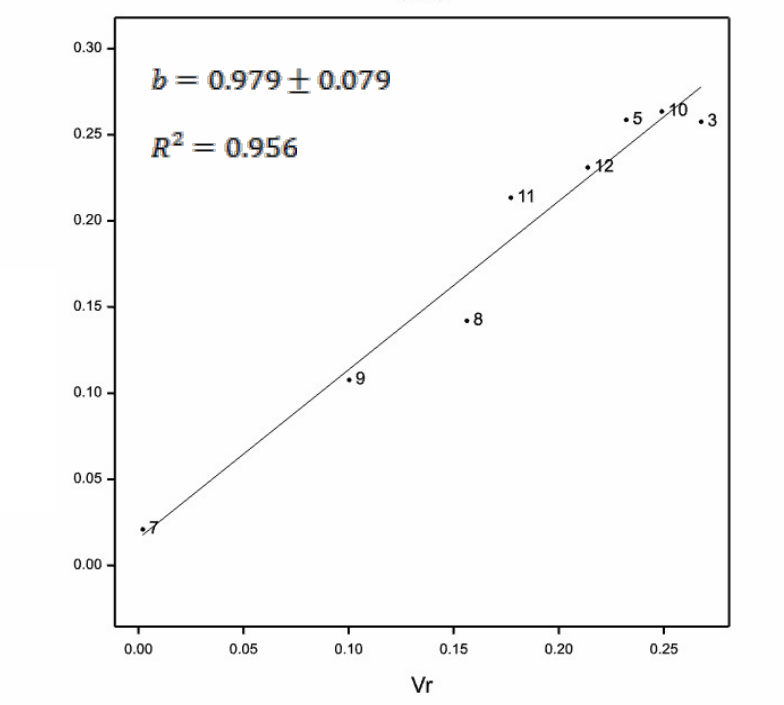

Figure 6.4. Linear regression of Wr on Vr for MSV in the reduced 8 x 8 model:

3 = MUL71, 5 = CML505, 7 = MUL114, 8 = C92, 9 = CML539, 10 = TZMI736, 11 = TZMI746, 12 = VHCY

The contribution of additive and dominance did not vary much between the full model and the reduced 8 x 8 diallel (Table 6.10). Additive contributed to 79% of total variation in the 12-parent diallel and 83% in the 8 x 8 diallel. Dominance variance contributions were similarly close, 21% in the full model and 17% in the reduced model. Nonetheless, the "b_1" component of dominance variance was not significant ($p>0.05$) in the reduced 8 x 8 model (Table 6.9) indicating that the nature dominance changed from directional in the 12 x 12 diallel to ambi-directional. Thus, whereas all genes showed dominance in one direction in the full model, dominance was in different directions in the 8 x 8 model.

6.4 Discussion

6.4.1 Mean performance of crosses and parental lines

The results showed that in comparison to the check varieties, the diallel genotypes evaluated were superior in resistance to the checks. One cross, Osu23i x CML539, was highly resistant to both diseases and would be most useful for the extraction of lines combining resistance to the two diseases. Parent MUL114 occurred most frequently in the most resistant MSV crosses indicating that this line conferred its resistance to these crosses. This is unlike the case of NLB where the highest *per se* resistance of parent S558 did not feature in the most resistant NLB crosses indicating that it did not transmit the resistance to its progenies and would not be a good source of resistance for NLB. Some authors have similarly reported the contribution of susceptible inbreds to heterosis for resistance in single crosses than resistant inbreds (Lim and White, 1978). Further, heterosis for disease resistance can occur because of dominance for resistance and also because of an indirect effect of general hybrid vigour (Hung and Holland, 2012). Parent S558 probably derives its resistance to NLB from CML197 in its pedigree, which has been found to be a good source for NLB resistance (CIMMYT, 2001). Inbred check CML202 was the most resistant line to NLB. The CIMMYT line has been shown to provide quantitative resistance to NLB (Schechert et al., 1999), a characteristic that was confirmed in this study.

In the case of MSV, two crosses showed dominance of resistance, i.e., S x R (Z419 x CML509) and S x HR (Z419 x MUL114). This suggested that best sources for MSV resistance would be MUL114 and CML509. Whilst for NLB, two R x S crosses (CML505 x Z419 and CML505 x VHCY) had resistant responses suggesting that CML505 was the strongest NLB

source in these materials. There was no cross between two susceptible lines that resulted in a hybrid with good resistance. It is noted that for optimal resistance in hybrids, with the exception of line MUL114 and CML509, which had dominant resistance over the susceptible lines, hybrids made with the other MSV resistant lines will require that both carry resistance. For NLB, adequate resistance in hybrids will require that both parents carry resistant genes, with the exception of CML505, which had dominance over susceptibility.

6.4.2 General and specific combining ability

Highly significant GCA and SCA effects implied that both additive and non-additive gene effects, respectively, for both MSV and NLB were important in determining resistance to these diseases. The contribution of GCA to the crosses sums of squares were 82 and 68% respectively for MSV and NLB suggesting predominance of additive effects, expressed by superiority of GCA to crosses sums of squares. The relatively higher contribution of GCA to MSV compared to NLB was similarly reported in the studies of Vivek et al. (2010), where GCA effects contributed 74% and 61% to the hybrid sums of squares for MSV and NLB, respectively. The predominance of additive effects associated with MSV (Gichuru et al., 2011) and NLB (Njoroge and Gichuru, 2013, Wisser et al., 2011) have been reported. The ratio $\hat{\sigma}^2\mathrm{GCA}/\hat{\sigma}^2\mathrm{SCA}$ indicated that GCA effects were more important for MSV but SCA effects were relatively more important for NLB under high disease pressure. Since SCA effects were important for NLB, initial screenings to identify lines with superior resistance should be followed by evaluation of these lines crossed to multiple testers to assess the concept of hybrid resistance. Other studies have also reported the predominance of non-additive genetic variance for NLB under natural severe disease pressures (Tembo et al., 2012, Vieira et al., 2012). It has however been demonstrated in several studies that additive and non-additive genes act together in conditioning resistance to NLB (Vieira et al., 2012, Vivek et al., 2010). Further, $\frac{2\hat{\sigma}^2\mathrm{GCA}}{2\hat{\sigma}^2\mathrm{GCA}+\hat{\sigma}^2\mathrm{SCA}}$ ratio was greater than 0.5 indicating preponderance of additive genes over non-additive genes and was confirmed in the graphical analysis.

Broad sense heritabilities (h_B^2) were consistent across disease pressures for MSV, however for NLB h_B^2 was higher in low disease pressure than higher disease pressure. Narrow sense heritability was higher under high MSV disease pressure while for NLB, h_N^2 was higher under lower disease pressure. This indicated that additive effects increased with increase in MSV pressure but non-additive effects increased with increase in NLB pressure. The narrow sense

heritabilities were moderate for MSV (0.69) and NLB (0.48) across seasons suggested the significant role of the dominance and environment effects in modifying these traits.

Genetic variances differed across seasons with higher values being obtained under relatively higher disease pressure for MSV indicating that selection of MSV resistant genotypes should be conducted under high disease pressure environments. There was however a shift for NLB as there was a slightly higher genetic variance under low than under high disease pressure This could mean that differences between genotypes were not very distinct under high pressure than they were under low disease pressure. It is noted that natural disease pressure as was the case for NLB is highly variable and can greatly affect the precision and accuracy of conclusions in genetic estimates for diseases (Vivek et al., 2010). Vieira et al. (2012) however found higher genetic variance to NLB under high disease pressure although based on partitions of phenotypic variation they suggested that breeding assessments for NLB may be performed similarly under low and high disease pressure.

The lines CML509, MUL114, C92 and CML539 had negative GCA estimates for MSV in both seasons and were therefore responsible for decreasing overall MSV mean values of the diallel crosses. These lines therefore contributed favourable alleles for MSV resistance levels in their crosses. For NLB two lines CML505 and CML509 maintained negative GCA estimates across seasons thus these lines promoted reduction of NLB severity and tended to produce hybrids with resistance to the disease. In the contrary, Z419 and VHCY that had positive GCA effects across seasons were associated with NLB susceptible hybrids.

In summary, according to the estimates of the general combining ability effects, the lines MUL114, C92, CML509, and CML539 may be used in intrapopulation breeding with focus on MSV resistance, whereas CML505 and CML509 may be used for resistance to NLB in intrapopulation breeding programmes. Further, parents for improving MSV and NLB simultaneously based on GCA effects were CML505, CML509 and CML539. The good combiners for MSV, C92, MUL114 and CML539 were reported in a previous chapter in this study (Chapter 5) to have good GCA effects for grain yield. In comparison, the good combiners for NLB, CML505 and CML509 were poor combiners for grain yield. Therefore, lines for improving MSV and grain yield simultaneously were identified but no lines combining good GCA for grain yield and NLB were identified in these studies. This presents a challenge to breed NLB in elite lines.

Hybrid combinations with favourable SCA effects and with, at least one parental line of good GCA are appropriate for inter-population breeding. Significant negative SCA effects associated with reduced MSV disease levels were obtained in some hybrids where one parent was resistant, for instance, Z419 (S) x MUL114 (HR). In most cases however, negative SCA effects were detected when both parents were resistant, such as CML539 x Osu23i and CML505 x C92. Therefore, susceptible or moderately resistant parents in combination with highly resistant parents could form resistant hybrids. In such cases, the other parent can be chosen for another trait of agronomic importance such as high yield. For instance Z419, was found to be a good combiner for grain yield in a previous study, and in combination with MUL114, a good combiner for MSV can produce a highly productive hybrid. For northern leaf blight, a desirable SCA effect was reported in cross CML505 x VHCY, where the CML505 had desirable GCA for the disease. Thus, this cross can be used for the improvement of resistance to NLB.

It must be emphasized however that the estimates of SCA and GCA reported here are only relative to one another and specific to the selected group of inbred lines involved in this study. Therefore, the large GCA negative effects of MUL114 and CML509 for MSV and NLB respectively may have arisen because these lines were much more resistant than the other lines with which they were tested. The higher values of GCA variance compared with SCA indicated the greater relative importance of genes with additive effects. However, there was some evidence for the presence of epistasis and dominance, as shown by the greater SCA variance than the GCA variance for NLB under high disease pressure. Although GCA x Env was highly significant, the lower values of the variance components of GCA x Env as compared to those of GCA across seasons indicated consistent responses of genotypes in different environments. Therefore, preliminary selection for genotype resistance to MSV and NLB may be efficient without multi-location tests.

6.4.3 Correlations among genetic effects

The F_1 performance of hybrids and SCA effects correlated positively for NLB but not for MSV indicating that SCA can be used as predictor for performance of hybrids for NLB but not MSV. Further, there was a positive but low correlation between the SCA effects of both diseases suggesting that non-additive gene effects for the two diseases acted positively and unidirectional agreeing with positive correlation of hybrid performance for the two diseases.

There were highly significant and positive correlations between GCA effects and *per se* performance of the lines demonstrated homogeneity of 'potence' according to Gilbert (1958). The high correlations between GCA and the corresponding parental effects suggest that parent per se evaluations would be sufficiently reliable indications of hybrid resistance and good reference for selecting parents or suitable inbred line testers.

The correlations between mid-parental values and hybrid resistance in both diseases were high. This would permit breeders to reduce population sizes by selection for MSV and NLB in inbred generations before generating testcrosses for hybrid evaluations. The positive correlations of inbred per se performance with F_1 hybrid disease response suggested the presence of additive gene action in the parental lines. There are similar reports of high correlations between inbred and hybrid resistance for traits such as Fusarium ear rots in maize (Hung and Holland, 2012). Regression analysis further showed the presence of additive effects although explaining 67% and 45% for MSV and NLB variation, respectively. There were thus moderate predictions of hybrid disease resistance from the parent per se performance.

There were non-significant correlations between the GCA effects of MSV and GCA effects of NLB in the individual seasons and across seasons. This presented the challenge of finding good combiners and identifying resistant sources common to the two diseases. Thus, different additive genes acted for resistance to each disease. This could probably be solved by developing suitable recombinant inbred lines from segregant generations of the hybrids that showed resistance to both diseases. Vieira et al. (2012) examined the possibility of combining NLB and GLS resistance and reported similar difficulties since lines with negative GCA effects for NLB had positive GCA effects for GLS. Vivek (2010) also reported non-significant correlations between GCA effects of several maize diseases, including MSV and NLB. The populations from this diallel can be exploited to develop lines that show resistance and combining ability for both diseases. While studying the breeding strategy for ear rots, combining of single resistances as opposed to multiple resistance was recommended as the appropriate breeding strategy (Tembo et al., 2012). Recombining inbred lines that have complementary resistances to different diseases, such as MSV and NLB, can be extremely useful as source germplasm to quickly develop adapted hybrids or synthetic composites for direct use by farmers.

6.4.4 Graphical analysis using Hayman procedures

Among the assumptions for the use of the diallel, independence of gene action, multiple alleles and unrelated distribution of genes among parents are difficult to fulfil in advance and can only be tested following the completion of a diallel. Analyses of Hayman diallel indicated that both additivity and dominance were involved in the inheritance of MSV and NLB resistance. The predominance of additive gene effect was amply demonstrated by highly significant “a” values. The effect of dominance at some of the loci was also obvious from the highly significant “b” values. The additive effect however had much greater than the dominance effect.

The graphical analysis has the advantage that it can detect epistasis and isolate the parents contributing to it. From the regression of Wr on Vr, it was found that the additive-dominance model was not adequate for data analysis and was confirmed by the analysis of variance of Wr+Vr and Wr-Vr. This suggested the presence of non-allelic interactions in the control of MSV resistance. The existence of non-allelic interaction for MSV has not been widely reported. In a generation mean analysis study, Lorroki (2009) found the control of CML202 and Osu23i under the dominance x dominance type of epistatic interaction. Epistasis can be exploited in a breeding program where the F_1 hybrids are superior to their corresponding parental genotypes. This was not the case with MSV since F_1 were inferior to parental lines in most instances. The pattern of MSV inheritance in some genotypes expressing epistasis may be difficult to fix and thus progress in selection using some resistant backgrounds (such as CML509, Osu23i, and S558) could be inherently slow. It was noted that since the contribution of dominance to total variation in the full model and reduced model differed by only 4 units; detection of epistasis in this study did not cause an overestimation of dominance. This therefore suggests that non-allelic interaction did not contribute significantly to the genetic control of MSV. It was further noted that the b_1 component of dominance, which shows the direction of dominance, was not significant in the 8 x 8 model suggesting ambi-directional nature of dominance. Thus, alleles with increasing and decreasing effects appeared to be dominant and recessive to the same extent in the 8 x 8 model.

In the case of MSV, crosses with MUL114 showed dominance of high resistance, which was depicted in the Wr/Vr graphs as this parent had the maximum number of dominant genes. The parents with maximum number of recessive genes in the reduced 8 x 8 diallel were CML505, TZMI736 and MUL71, which were rated as resistant and moderately resistant for MUL71. Therefore, resistance in these parents is probably due to recessive genes, which may not be adequate to give resistant hybrids when combined with susceptible parents. For instance, cross

Z419(S) x MUL71 (MR) gave a susceptible hybrid since Z419 could not complement resistance as it possibly lacked any alleles for resistance.

On the other hand, for NLB, regression analysis of Wr on Vr found the additive-dominance model appropriate for NLB as the regression coefficient was not different from unity. Despite the heterogeneity of Wr-Vr, the evidence for epistasis for NLB was not convincing, as was the case for MSV. A review of classical studies of NLB resistance by Welz and Geiger (2000) concluded that epistatic gene action for NLB was rare. Partial to complete dominance was indicated as the intercept of the regression line cut the Wr axis near the origin. Although CML505 was rated as moderately resistant for NLB, this parent had the maximum number of dominant genes according to the Wr/Vr graph. All eleven crosses with this line (CML505) exhibited dominance of resistance and in fact, four of these crosses showed over-dominance. However, two lines VHCY and Z419 had the maximum number of recessive genes, and had been rated as susceptible to NLB. Crosses with these two lines showed additivity of many genes or dominance of susceptibility except with CML505, where there was over-dominance. Overall, since partial to complete dominance and additive gene effects were indicated for MSV and NLB, hybrids would show high levels of resistance when both parents are contributing the alleles.

6.5 Conclusion

Based on predominant additive effects and partial dominance for resistance, combinations of inbred lines resistant to MSV with inbred lines resistant to NLB, may produce hybrid maize varieties resistant to both diseases. The study identified lines with good resistance to the two diseases, which can serve as breeding parents, while those with less resistance can serve as appropriate testers for future evaluation of top-crosses to develop elite inbred lines for resistance. The insignificant correlations of MSV and NLB GCA effects indicated that each reaction was influenced by a separate genetic system. Breeding efforts to increase resistance to MSV and NLB will consequently require a good choice of parents. Parental lines MUL114 and CML505 (or CML509), which displayed highly negative GCA effects and maximum number of dominant alleles can be used as sources of MSV and NLB resistance genes to improve maize germplasm in Kenya and in the region. This study reported for the first time the importance of epistasis for MSV resistance in a diallel cross. Most of the epistasis was associated with parents CML509, Osu23i, S558 and Z419. This result indicates the difficulty of selecting for resistance in hybrid genotypes. However, the overall importance of additive gene action suggests that selection would be an effective procedure to develop material with resistance to maize streak

virus and northern leaf blight, which can be done by utilizing segregating populations of hybrids identified in the study.

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CHAPTER 7

Yield stability analysis of a 12 x 12 maize half-diallel cross

Abstract

A total of 66 F_1 maize hybrids developed from a 12 x 12 half-diallel was evaluated at four locations in Kenya over two seasons to investigate genotype x environment interactions effects for grain yield. The genotype and genotype by environment interaction (GGE) biplot method was used for graphical display of the data. The eight environments (location-year combinations) were examined for representativeness, discriminating ability and repeatability. Stability of combining ability effects was examined using rank variance and Wrickes' ecovalence. The effect of environments as a source of yield variability was moderate (44%). The magnitude of G x E interaction (23%) and that of genotype effect (21%) was almost equal. The eight environments were divided into two mega-environments, the first representing medium-transitional locations while the second mega-environment had medium-late locations. Hybrid 6 (Z419 x MUL114) and hybrid 22 (MUL71 x Osu23i) were the winning genotypes for the first and second mega-environments, respectively. Hybrid 47 (CML509 x C92) was the most stable and highest yielding hybrid across environments and can be recommended to medium-transitional and medium late environments. The inbred line CML539 displayed the most stable general combining ability. Effects of SCA were highly variable and mostly contributed to SCA x E interaction with equal magnitude. There was presence of crossover interaction signifying the need to breed for both broad and specific adaptation.

7.1 Introduction

Genotype x environment (G x E) interaction is problematic in breeding and farming communities as it results in the failure of genotypes to respond consistently in variable environmental conditions. Production environments for maize in sub-Saharan Africa are highly variable, not only based on edaphic and climatic conditions but also diverse biotic and abiotic stress factors. The complex environment results into genotype x environment interaction effects for various traits of economic importance such as grain yield and disease resistance (Sibiya et al., 2012, Vivek et al., 2010). The ultimate goal of maize breeding programs focusing on various stress breeding is to increase and stabilize grain yield production. Eastern Africa is divided into mega-environments based on maize regional trials. Kenya has diverse agro-ecological zones and maize varieties are bred for different zones (KARI, 1992). However, maize is grown in almost all

ago-ecological regions of the country, which are highly variable in rainfall, temperature and soil characteristics.

Identifying widely adapted and stable genotypes across a wide range of environments is the most ideal way to minimize (G x E) interactions although the presence of G x E interaction can be exploited by selecting superior genotypes for specific target environments (Ceccarelli, 1989). Stable and widely adapted genotypes are identified when G x E is apportioned into stability indices assigned to each genotype across environments. Although analysis of variance procedure indicates the existence and magnitude of G x E interactions, the variance components alone do not provide a satisfactory explanation for G x E. Several statistical models have been advanced to interpret patterns of G x E interactions. Increasingly, researchers have used the genotype and genotype- by-environment (GGE) biplot (Alwala et al., 2010, Fritsche-Neto et al., 2010, Oliveira et al., 2010) proposed by Yan et al. (2000) for interpretation of G x E interactions.

The GGE biplot is based on the site regression (SREG) model of multi-environment data where the first two principal components from the data display a graph of genotype main effect (PC1-primary effects) and genotype-by-environment interaction (PC2-secondary effects) (Yan and Hunt, 2001). Further, this analysis helps to determine whether the cropping region is homogenous or should be divided into mega-environments (Samonte et al., 2005, Yan and Rajcan, 2002). The GGE biplot is also suited to describe the concept of dynamic stability since it portrays genotypes based on their yields as well as their stability on a two-dimensional display (Alwala et al., 2010). The GGE methodology is thus a useful tool for identifying environments that optimize cultivar performance and also environments with close association for reducing testing cost under limited resources (Beyene et al., 2012).

When variation attributable to hybrid x environment is partitioned into GCA x E and SCA x E, both components are frequently significant for grain yield and other agronomic traits (Badu-Apraku and Oyekunle, 2012) indicating that GCA and SCA rankings of parental inbred lines change across environments. Besides identifying high stable single crosses, it is therefore important to identify parental lines that not only possess high mean grain yield but also have high stability for GCA and SCA effects to adjust to environmental changes. A superior inbred line should be vigorous, have stable and significant positive GCA and SCA in cross combination across environments, and contribute relatively little to GCA × E interactions (Dehghanpour and Ehdaie, 2013).

There is a great environmental variation in Kenya and thus large G x E interactions are expected. There have been a several studies to analyse the effects of G x E interactions and to characterize Kenyan environments for maize adaptation (Beyene et al., 2011, Beyene et al., 2012, Nzuve et al., 2013). However, stability assessment of MSV resistant germplasm and deployment strategy of these germplasm in different regions where the disease occurs is required. Stability of new elite varieties could help enhance farmer acceptability, as these varieties will efficiently utilize resources in their environments for high productivity. Information on G x E interactions would guide selection strategy for either specific or broad adaptation. Further, the description of the relationship between the stability of parental lines and their F_1 hybrids across diverse environments would guide line breeder on whether or not to focus on improving grain yield performance or yield stability in early generations of breeding. The overall goal is to develop products that combine high productivity in yield and disease resistance with high stability. The objectives of the present study were therefore to (i) identify the most productive and stable hybrids under diverse environments (ii) identify the best sites among those used for discriminating genotypes for grain yield and (iii) to quantify the stability of GCA and SCA effects of inbred lines and hybrids, respectively, and identify parents with minimum contribution to combining ability x environment interactions. When found, such parents would contribute to development of productive and stable hybrids.

7.2 Materials and methods

7.2.1 Germplasm

Sixty-six single cross hybrids were derived from a half-diallel cross of 12 medium maturing lines, obtained from diverse sources, three from CIMMYT, two from IITA, five from KARI, one from CIRAD and one from South Africa (Table 7.1). Two sets of germplasm were evaluated, one trial comprising 66 F_1 diallel crosses and four hybrids checks and the second, the 12 parental lines and two inbred checks.

7.2.2 Description of locations

The study was conducted in four research sites representative of different maize growing agro-ecological zones. Muguga and Mwea are located in the medium transitional zones characterized by altitude of 1000 to 1700 m above sea level (asl) with rainfall amounts between 750 and 1000 mm. Kakamega is located in the medium-late maize zone also referred to as moist-transitional zone, characterized by rainfall patterns of 1000-1800 mm, although with

similar altitude as the medium transitional area. Kiboko is found in the dryland agro-ecozone, where the altitudes are 500-1000 m asl; varieties grown in Kiboko have a rainfall requirement of 350-500 mm. Additional information on the experimental sites is given in Table 7.2.

7.2.3 Experimental design and management

The hybrid trials with 70 entries were laid out as a 10 x 7 alpha lattice design with two replications in all locations. The 14-entry-inbred trial was laid out as randomized complete block design with two replications. Plots were made up of two rows of 2.5 m long with 0.75 m inter-row and 0.25 m intra-row spacing. The maize seedlings were thinned to one plant per hill giving a stand of ≈53,333 plants ha^{-1}. Fertilizers were applied at the rate of 60 kg N and 60 kg P_2O_5 ha^{-1} for all experiments. Nitrogen was applied in two splits: at planting and knee stage. The fields were kept free of weeds by hand weeding.

The data for grain yield and other agronomic traits were taken following the standard practice for maize trials used at CIMMYT (Magorokosho et al., 2009). The following traits were measured: number of days to anthesis, number of days to silking, plant and ear height, ear aspect and number of ears per plant. For grain yield, ears were harvested on a whole plot basis and fresh weight (kg) determined. The field weight was then used to estimate the grain yield (t ha^{-1}) which was adjusted to 12.5% grain moisture content.

Table 7.1. Inbred lines used in the half-diallel including their reaction responses to MSV and NLB, grain type, and source of origin

Parent	Pedigree[d]	Origin[e]	Grain type[a]	MSV[b]	NLB[c]
Z419	SYN[Kitale/Tuxp-GLS]F2	KARI	W, I	S	S
S558	(EM12-210/CML197//EM12-210/OSU23i)-x-58-2-2-2-1	KARI	W, SF	R	R
MUL71	[EM12-210/CML 202]-X-71-1-2-1-1-3	KARI	W, F	MR	MR
OSU23i	[MSRXPOOL9] C1F2-205-1	OSU/CIMMYT	W, F	R	MR
CML505	[92SEW2-77/[DMRESR-W]EarlySel-#I-2-4-B/CML386]-B-11-3-B-2-#-BB	CIMMYT	W, SF	R	MR
CML509	[92SEW1-2/[DMRESR-W]EarlySel-#L-2-1-B/CML386]-B-22-1-B-4-#-1-BB	CIMMYT	W, SF	R	R
MUL114	[EM12-210/OSU23i]-X-114-2-2-1-3	KARI	W, F	HR	MR
C92	na	CIRAD	Y, I	HR	MS
CML539	MAS [MSR/312]-117-2-2-1-B*4	CIMMYT	W, SF	HR	R
TZMI736	na	IITA	W, SF	R	MR
TZMI746	na	IITA	W, SF	R	MR
VHCY	na	S.Africa	Y, SF	MR	S
CML202 (check 1)	ZSR923S4BULK-5-1-BB	CIMMYT	W, SF	R	HR
CML312 (check 2)	S89500F2-(Sn)B*5	CIMMYT	W, SD	S	MS

[a]Grain type: W=white, Y=Yellow, I=intermediate, F = Flint, FL= Flint-like, DL = Dent-like; [b & c]:S= susceptible, MS=moderately susceptible, MR=moderately resistant, R= resistant; HR=Highly resistant; [d]na= pedigree not available; [e] = germplasm sources, CIMMYT = International Maize and Wheat Improvement Centre, IITA = International Institute of Tropical Agriculture, CIRAD = Centre de cooperation internationale en recherché agronomique pour le développement, KARI =Kenya Agricultural Research Institute

Table 7.2. Four test locations used for evaluating 66 F_1 hybrids and 12 parental lines in 2011 and 2012

Location	Altitude (m asl)	Latitude	Longitude	Rainfall (Sep 11-Feb 12)	Rainfall (Mar 12-Aug12)	Max, Min (Sep 11-Feb 12)	Max, Min (Mar 12-Aug12)	Soil texture	Agro-ecology
Kiboko (KIB)	975	2º 15' S	37º75' E	269.7	294.2	32.1, 17.3	30.7, 16.1	Sandy loam	Dryland
Mwea (MWE)	1159	0º37' S	37º21' E	451.5	511.5	28.7, 15.6	28.2, 15.0	Sandy clay loam	Medium- transitional
Muguga (MUG)	2095	1º15' S	36º39' E	498.4	760.9	23.9, 14.3	24.5, 14.1	Sandy loam	Medium- transitional
Kakamega (KAK)	1583	0º16' N	34º46' E	899.18	1069.5	28.9, 16.4	25.8, 15.3	Sandy loam	Medium-late

The agro-climatic zones in Kenya are based on a moisture index (Sombroek et al., 1982) using annual rainfall. Maize agro-ecology refers to a specific geographical region with similar rainfall, temperature, soil type and altitude for recommending different maize cultivars.

7.2.4 Statistical analyses

The yield data and other agronomic traits were subjected to analysis of variance using individual plot data for each environment separately and then combined across all eight environments. In this study environment is a two-year x four-location combination. The following model was used: $Y_{ijkl} = \mu + G_i + r_k + E_j + GE_{ij} + \varepsilon_{ijkl}$; where Y_{ijkl} is the observed measurement of the i^{th} genotype in the k^{th} replication at the j^{th} location, μ is the overall mean, G is the main effect of genotype i where i = 1…66,r is the effect of replication k,E is the main effect of environment j where j = 1…8,GE_{ij} is the genotype-by-environment effect, and ε_{ijkl} is error term for the Y_{ijkl} observation. The environments and replications within environments were considered random and therefore tested against the residual error term. The genotype x environment interactions terms were used to test the significance of the corresponding genetic effects. The same model was used for the inbred trials.

Grain yield data across environments were graphically analysed and interpreted using the genotype and genotype x environment (GGE biplot) based on the principal component analysis (PCA) of environment-centred data (Yan, 2002, Yan et al., 2000) to explain variation due to genotypes and genotypes x environments (G x E). The model is denoted as follows: $Y_{ij} = \mu + \beta_j + \lambda_1\xi_{i1}\eta_{j1} + \lambda_2\xi_{i2}\eta_{j2} + \varepsilon_{ij}$; where: $Y_{ij}$is the mean yield of i^{th}hybrid in j^{th}environment, μ is the grand mean, $\beta_j$is the maineffect of environment j, $\mu + \beta_j$is the mean yield across all hybrids in environment j, $\lambda_1$and $\lambda_2$are the singular values (SV) for the first and second principal component (PC1 and PC2), respectively, $\xi_{i1}$and $\xi_{i2}$are the eigen vectors of hybrid i for PC1 and PC2,respectively, η_{j1} and η_{j2} are the eigen vectors ofenvironment j for PC1 and PC2, respectively and ε_{ij}isthe residual associated with hybrid i in environment j.

To visualize the performance of the genotypes in each environment and groups of environments, a polygon view (Yan, 2002) was drawn by connecting hybrids that were furthest from the biplot origin such that all hybrids were enclosed within the polygon. The biplot was also used to explore the interrelationships among environments by constructing lines (environment vectors) from the biplot origin to markers for the environments. The cosine of the angle between environments corresponds to the degree of correlation between environments. An angle of zero indicated a correlation of +1, while an angle of 90 or -90, a correlation of zero, and an angle of 180, a correlation of -1 (Yan, 2002). The length of the vectors was also used to determine the discriminating ability of each of the test environments, with a shorter vector implying that the environment was not well represented by PC1 and PC2 (Yan et al., 2007).

The GCA and SCA effects were estimated according to Griffing's (Griffing, 1956) Method 4, Model 1, using the DIALLEL-SAS05 program (Zhang et al., 2005).The GCA effects of each parent were ranked per environment to estimate stability of GCA for grain yield where the largest positive GCA was ranked 1 while the largest negative GCA was ranked 12. Then, rank sum and rank variance were calculated for each inbred line across the environments. Inbred lines with low rank sums and low rank variances were identified as having high stability for GCA effects. Rank sum method by Kang (1988) was suggested to quantify stability of grain yield in different genotypes.

The contribution of each inbred line to sum of squares of GCA x environment was calculated using Wricke's (1962) ecovalence as follows: $W_i^2 = \sum(R_{ij} - \overline{R}_{i.} + \overline{R}_{.j} + \overline{R}_{..})$, where R_{ij} is rank of i^{th} inbred line in j^{th} environment, $\overline{R}_{i.}$ is mean rank of i^{th} inbred line, $\overline{R}_{.j}$ is mean rank of j^{th} environment, and $\overline{R}_{..}$ is the grand mean. The same procedures were adopted to study the pattern of stability for SCA for grain yield, with ranking ranging from 1 (largest) to 66 (smallest) SCA in each environment. A superior inbred line and a single-cross hybrid should have a significant positive GCA and SCA effects for grain yield with high stability in the combining ability effects across environments and relatively small contribution to GCA × E and SCA × E interactions, respectively (Dehghanpour and Ehdaie, 2013). Stability of GCA effects and SCA effects across environments was determined by rank variance.

7.3 Results

7.3.1 Analysis of variance

7.3.1.1 Hybrids

The ANOVA results presented in Table 7.3 indicated that genotype (hybrids), location and year main effects were significant (p≤0.01) for grain yield and other traits implying a substantial variation among genotypes, locations as well as years. The genotypes responded differently to locations and years as depicted by the significant (p≤0.01) G x L, G x Y and G x Y x L interactions. The highest percentage of total variation for grain yield was explained by environment (location, year and location x year) main effects (44%), while genotype and G x E interactions together, contributed 44%. The interaction G x L (11%) however contributed more variation than G x Y (3%) and G x L x Y (9%) interactions. Among the other agronomic traits, environment main effect controlled >90% of the variation in days to anthesis and silking

(Table 7.3) although most of the variation was due to differences in locations. Genotypic variance was higher than G x E for plant height, ear height, ear position, and days to anthesis and silking. However, G x E was higher than genotypic variance for grain yield, ASI, ear aspect and ears per plant. The existence of genotype x environment interaction raises the need to identify stable and high yielding genotypes.

7.3.1.2 Inbred lines

In the parental line evaluation, genotype and location main effect for grain yield were significant ($p \leq 0.01$), however year main effect and genotype x year interaction were not significant ($p > 0.05$), thus parental line yield performance was consistent over the two years (Table 7.4). The environment effect (location plus location x year) controlled 38% of the variation, while genotype (parental lines) and G x E jointly accounted for 56%. Similar to the hybrids, locations contributed the highest variation (≈80%) in days to anthesis and days to silking among the parental lines.

Table 7.3. Analysis of variance for grain yield and other agronomic traits of 66 F_1 hybrids and four checks tested in four locations in 2011 and 2012 and the contribution of different main and interaction effects to the total sums of squares

Source of variation	Mean squares								
	d.f.	GY	PH	EH	EPO	DTS	DTA	ASI	EA	EPP
Hybrid (G)	69	15.872***	4961.1***	4102.3***	0.020***	117.704***	120.76***	4.29***	1.367***	0.066***
Location (L)	3	167.998***	196423.2***	88530.6***	0.342***	70564.477***	75031.251***	321.896***	17.044***	2.885***
Year (Y)	1	150.055***	165595.7***	10538.5***	0.272***	526.629***	108.751***	253.651***	37.705***	0.750***
Hybrid x Location	207	2.728***	412.3***	194.9***	0.001***	12.609***	11.499***	2.377***	0.284***	0.035***
Hybrid x Year	69	2.285***	199.8^{ns}	142.1*	0.001*	5.94*	6.235*	1.711^{ns}	0.258***	0.017^{ns}
Location x Year	3	555.167***	43098.5***	38248.7***	0.332***	7780.093***	7866.451***	352.003***	4.039***	0.137***
Hybrid x Location x Year	207	2.296***	215.6*	128*	0.001***	6.337***	6.717***	2.138**	0.200***	0.031***
Residual	559	1.111	172.9	101.3	0.001	4.02	4.701	1.634	0.137	0.019
Contribution of main and interaction effects to total sums of squares (%)										
Genotype (Hybrids)		21	23	35	27	3	3	7	24	11
Environment		44	60	48	25	94	94	50	26	25
Location		9	40	33	20	85	85	21	13	22
Year		3	11	1	5	0	0	6	10	2
Location x year		32	9	14	19	9	9	23	3	1
G x E		23	10	9	15	2	2	24	31	37
G x L		11	6	5	7	1	1	11	15	18
G x Y		3	1	1	2	0	0	3	5	3
G x Y x L		9	3	3	6	1	1	10	11	16
CV (%)		15.1	5.7	8.4	6.5	2.7	2.9	-109.1	14.1	30.3
mean		6.62	225.3	115.4	0.51	70.86	69.67	-0.613	2.64	1.067

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05); GY = grain yield (t ha^{-1}), PH= plant height (cm), EH= ear height (cm), EPO = ear position, DTS = days to 50% silking, DTA = days to 50% anthesis, ASI = anthesis silking interval, EA = ear aspect EPP = number of ears per plant

Table 7.4. Analysis of variance for grain yield and other agronomic traits of 12 parental lines and two inbred checks tested in four locations in 2011 and 2012 and the contribution of different main and interaction effects to the total sums of squares

Source of variation	Mean squares									
	df	GY	PH	EH	EPO	DTS	DTA	ASI	EA	EPP
Lines (G)	13	26.7441***	9275.2***	5417.9***	0.038***	360.264***	303.365***	8.812***	2.291***	0.234***
Location (L)	3	68.285***	17457.5***	7504.43***	0.055***	17536.802***	17054.798***	39.04***	13.871***	0.673***
Year (Y)	1	0.035^{ns}	36195.9***	4826.49***	0.019***	13.504^{ns}	111.446***	47.362***	1.697**	0.325***
Lines x location	39	3.276***	596.2***	268.74***	0.003**	51.357***	44.958***	4.694***	0.246^{ns}	0.088***
Lines x year	13	0.776^{ns}	505.8***	326.85***	0.002^{ns}	14.533***	12.177***	4.362*	0.205^{ns}	0.053*
Location x year	3	53.837***	9288.4***	4001.75***	0.033***	2203.302***	1941.815***	76.754***	6.263***	0.641***
Lines x location x year	39	1.409***	344**	129.03^{ns}	0.001^{ns}	11.241***	12.315***	3.075^{ns}	0.356***	0.045*
Residual	111	0.605	172.2	93.98	0.002	4.635	4.512	2.215	0.1688	0.028
Contribution of main and interaction effects to total sums of squares (%)										
Lines		36	40	50	41	7	6	10	22	19
Environment		38	39	28	23	88	89	35	45	26
Location		21	17	16	14	78	80	10	30	12
Year		0	12	3	2	0	0	4	1	2
Location x year		17	9	9	8	10	9	20	14	12
G x L		13	8	7	11	3	3	16	7	21
G x Y		1	2	3	3	0	0	5	2	4
G x Y x L		6	4	4	5	1	1	11	10	11
CV (%)		23.7	9.9	12.2	6.4	4.4	4.6	-112.4	12.94	16.1
mean		3.55	166.12	80.21	0.48	74.57	73.25	-1.32	2.96	1.04

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05); GY = grain yield (t ha^{-1}), PH = plant height (cm), EH = ear height (cm), EPO = ear position, DTS = days to 50% silking, DTA = days to 50% anthesis, ASI = anthesis silking interval, EA = ear aspect, EPP = number of ears per plant

7.3.2 Stability and adaptability

Stability and adaptability was assessed based on the values of PC1 and PC2 scores of the biplot (Table 7.5) as there was high correlation of genotypic effects and PC1 scores ($r=0.99$, $p\leq0.01$). High PC1 scores denote high genotypic means, while close to zero PC2 scores, denote stable genotypes (Yan et al., 2000). Thus, PC1 scores indicate genotype adaptability especially when highly correlated with yield rankings. This was confirmed in the current study. Due to a high correlation between mean yield and PC1, the top five hybrids using both parameters were similarly ranked as follows: 16>22>6>37>58 based on yield and 22>61>16>6>58 based on PC1 scores (Table 7.6). Further, yield ranks and PC1 ranks identified the same low yielding hybrids as 39<45<33<21<64. The top five parental lines by mean yield ranks were 8>1>2>4>12 while using PC1 ranks they were 8>1>2>12>4 (Table 7.5). The bottom five parental lines identified by mean yield ranks were 10<9<6<14<13 whereas using PC1 scores they were 10<14<9<6<13.

The grain yield across the years and locations ranged from 7.94 t ha^{-1} to 2.14 t ha^{-1} for S558 x MUL114 and CML505 x CML509, respectively (Table 7.5). Two hybrids combined high PC1 scores and close to zero PC2 scores, 47- CML509 x C92, and 36-OSU23i x TZMI736, showing high yield potential and high stability. These crosses probably had the least contribution to G x E interaction, although they ranked 6th and 12th for grain yield across environments. Some hybrids on the other hand had high PC1 scores and high PC2 scores (6-MUL71 x OSU23i and 22- Z419 x MUL114), therefore these had high yielding potential but adapted to specific environments. The two hybrids, 6 and 22, were most responsive in their environments indicating dynamic stability. Low yielding (negative PC1 scores) but stable (PC2 scores close to zero) hybrids were also identified, hybrid 21- S558 x VHCY and 12- S558 x MUL71. There were also some low yielding (negative PC1 scores) and unstable (high PC2 scores) hybrids, 39- CML505 x CML509, 11- Z419 x VHCY, and 30- MUL71 x VHCY. The latter three hybrids ranked 70, 58 and 59 for grain yield among the 70 hybrids evaluated.

In the parental evaluation unstable yield performance was detected since none of the PC2 scores were close to zero. However, high PC1 scores and least PC2 scores (0.82) were obtained for one line C92, the highest yielding and stable parental line (Table 7.6). Two high yielding (high PC1 scores) and unstable (high PC2 scores) lines, Z419 and S558 were also identified. Lines CML202 and CML539 showed low yield potential (low PC1scores) but highly stable performance (PC2 values close to zero). Two parents TZM1736 and CML312 were low yielding (low PC1scores) and highly unstable (high PC2 scores). Disregarding yield

performance for the inbred lines, the most stable lines based on the least PC2 scores were TZMI746, CML202, CML539, C92, CML505 and CML509, while the least stable lines were MUL71, Osu23i, MUL114, Z419, and TZMI736. Following this, the specifically adapted hybrids 6-MUL71 x OSU23i was constituted by unstable x unstable lines while 22- Z419 x MUL114 was formed from unstable x unstable lines. On the other hand, the broadly adapted hybrids 47- CML509 x C92, was composed of stable x stable line but 36-OSU23i x TZMI736 was formed from unstable x unstable lines.

Table 7.5. Mean yield, PC1 andPC2scores and ranks for top 20 and bottom 15 F_1 hybrids evaluated in eight environments

Entry	Hybrid	GY	GY rank	PC1	PC1rank	PC2	PC2rank
16	S558 x MUL114	7.94	1	3.63	3	0.27	25
22	MUL71 x OSU23i	7.94	2	4.33	1	-1.47	62
6	Z419 x MUL114	7.94	3	3.62	4	2.49	4
37	OSU23i x TZMI746	7.91	4	3.57	8	-0.53	45
58	C92 x TZMI736	7.91	5	3.62	5	0.55	19
47	CML509 x C92	7.84	6	3.39	9	-0.07	31
8	Z419 x CML539	7.83	7	3.05	11	2.34	5
61	CML539 x TZMI736	7.83	8	3.69	2	-0.79	51
25	MUL71 x MUL114	7.79	9	3.60	6	0.55	19
57	C92 x CML539	7.79	10	3.59	7	-0.61	48
27	MUL71 x CML539	7.74	11	3.12	10	0.61	18
36	OSU23i x TZMI736	7.60	12	2.82	12	-0.15	32
18	S558 x CML539	7.58	13	2.61	13	-0.59	47
2	Z419 x MUL71	7.50	14	2.20	15	1.17	13
9	Z419 x TZMI736	7.39	15	2.18	16	1.28	12
68	H624 (check)	7.39	16	2.32	14	0.63	17
62	CML539 x TZMI746	7.37	17	2.05	18	-0.49	42
1	Z419 x S558	7.31	18	1.66	22	1.39	11
35	OSU23i x CML539	7.21	19	1.90	20	-0.35	40
53	MUL114 x CML539	7.20	20	1.91	19	-0.31	38
56	MUL114 x VHCY	5.96	56	-2.09	57	0.48	21
31	OSU23i x CML505	5.94	57	-1.59	54	-1.38	59
30	MUL71 x VHCY	5.83	58	-2.81	62	1.96	7
11	Z419 x VHCY	5.83	59	-3.58	63	4.77	2
60	C92 x VHCY	5.83	60	-2.47	61	0.80	16
44	CML505 x TZMI746	5.80	61	-2.41	59	-1.40	60
51	CML509 x VHCY	5.77	62	-2.34	58	-0.22	34
38	OSU23i x VHCY	5.73	63	-2.46	60	-0.01	28
12	S558 x MUL71	5.27	64	-4.06	65	0.42	22
23	MUL71 x CML505	5.12	65	-4.06	64	-1.02	55
64	TZMI736 x TZMI746	5.06	66	-4.83	66	-0.26	35
21	S558 x VHCY	4.93	67	-4.91	67	-0.16	33
33	OSU23i x MUL114	4.73	68	-5.26	68	-0.88	52
45	CML505 x VHCY	4.61	69	-5.85	69	-0.33	39
39	CML505 x CML509	2.14	70	-12.59	70	-2.42	69

Mean GY = 6.5 (t ha^{-1}); Pearson correlation grain yield (GY) vs. PC1= 0.993 ($p<0.001$); r (GY, PC2)= 0.099^{ns}

Table 7.6. Mean yield, PC1, and PC2 scores and ranks for 12 diallel parents and two inbred checks evaluated in eight environments

Entry	Parent name	GY	GY rank	PC1	PC1 rank	PC2	PC2 rank
8	C92	6.714	1	9.118	1	0.893	6
1	Z419	5.685	2	7.027	2	-2.036	14
2	S558	4.74	3	2.674	3	2.959	1
4	OSU23i	3.696	4	0.337	5	1.837	3
12	VHCY	3.548	5	0.444	4	-1.982	13
5	CML505	3.384	6	-0.667	6	0.95	5
11	TZMI746	3.303	7	-0.813	7	-1.15	9
7	MUL114	2.872	8	-1.747	8	-1.629	11
3	MUL71	2.833	9	-1.812	9	-1.268	10
13	CML202 (check)	2.787	10	-2.196	10	-0.188	8
6	CML509	2.707	11	-2.988	12	1.09	4
9	CML539	2.549	12	-3.04	13	0.476	7
14	CML312 (check)	2.537	13	-2.541	11	-1.959	12
10	TZMI736	2.392	14	-3.797	14	2.009	2

Mean GY = 3.6 (t ha^{-1}); Pearson correlation grain yield (GY) vs. PC1= 0.994 ($p<0.001$); r (yield, PC2)= 0.099^{ns}

7.3.3 Polygon view of GGE biplot analysis of F_1 hybrid evaluation

From the GGE biplots, the first two PCs explained 64.83% (PC1=50.91 and PC2=13.92%) of the total GGE variation for grain yield. Results of the polygon view for hybrid evaluation are presented in Fig. 7.1. The polygon view of the GGE biplot provides a good visualization of crossover G x E interactions (Yan and Kang, 2003). When environments fall into different sectors of the polygon, there are specifically high yielding cultivars for each sector (Yan et al., 2007). This shows crossover G x E and thus environments could be divided into mega environments.

The rays of the biplot divided the plot into six sectors with the eight environments appearing in two sectors and the 70 hybrids falling in all the six sectors (Fig 7.1). The environments fell in two sectors thus there were two mega-environments and an indication of presence of crossover interactions. The first mega-environment was composed of five environments (MWE12, MWE11, KIB11, KIB12 and MUG11) with vertex hybrid 6- (Z419 x MUL114) as the winning genotype. Hybrid 22 (MUL71 x Osu23i) was the winning genotype in the second mega-environment composed of KAK11, KAK12 and MUG12. Vertex hybrids 17, 39 and 67 were not definitive of any of the environments but had the long vectors from the origin indicating they were poorest and/or most unstable. Most of the other hybrids located near the plot origin, were

less responsive, and thus lacked dynamic stability compared to the vertex genotypes, hybrid 6 and 22.

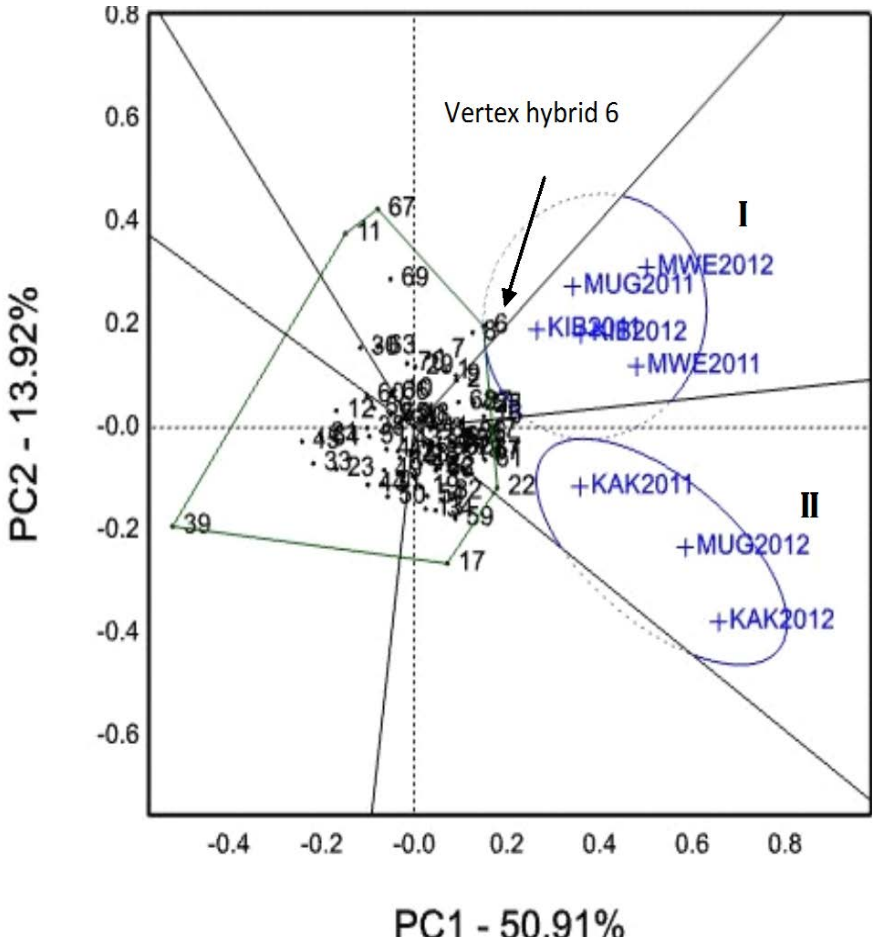

Figure 7.1. Polygon view of the GGE biplot based on grain yield (t ha^{-1}) for eight environments (location x year) for hybrids. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

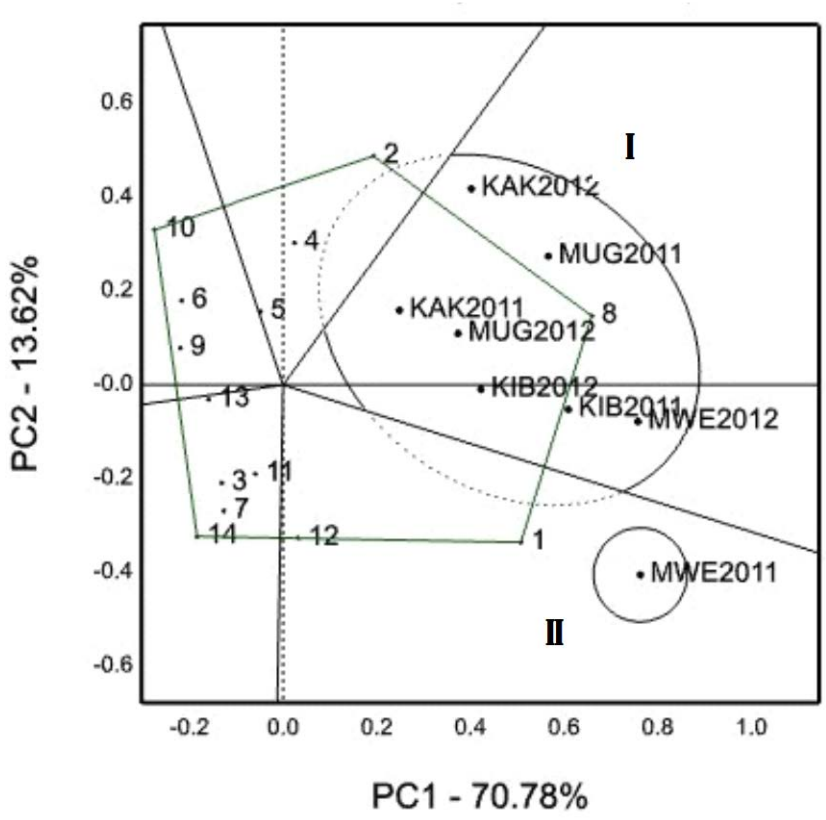

Figure 7.2. Polygon view of the GGE biplot based on grain yield (t ha^{-1}) for eight environments (location x year) for parental lines. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

A hypothesis was formulated based on the mega-environment patterns detected from the hybrid biplot to statistically test the crossover pattern of hybrid 6 and hybrid 22, the vertex genotypes in the two mega-environments, according to Yan et al. (2010). Numerical contrast of within-group means showed that hybrid 6 and 22 yielded 8.7 and 7.8 t ha^{-1}, respectively, in mega-environment 1 and 6.6 and 8.2 t ha^{-1}, respectively, in mega-environment 2. These two hybrids switched ranks showing that 6 was specifically adapted to mega-environment I while 22 is specifically adapted to mega-environment II.

7.3.4 Polygon view of GGE biplot analysis of diallel parental line evaluation

In the GGE biplot representing parental lines of the diallel, the first two principal components (PCs) explained 84.4 (PC1=70.78 and PC2=13.62) of the total GGE variation for grain yield (Fig. 7.2). The rays of the biplot divided the plot into five sectors, and the environments fell into two sectors and parental lines were found in all the sectors. There were thus two mega-

environments and an indication of crossover interaction. The vertex genotypes represented the highest yielding parents for the two mega-environments; parent 8 (C92) for the first mega-environment composed of seven environments and parent 1 (Z419) for the second mega environment which consisted of one environment. Most of the other parents were located on the negative side of PC1 since they were low yielding inbred lines compared to C92 and Z419.

7.3.5 Relationship among test environments in hybrid evaluation

Environmental vectors were drawn from the biplot origin to connect the environments for hybrid evaluation based on environment focused scaling to estimate the pattern of environments (Fig 7.3). All locations had positive PC1 scores indicating good yield discriminating ability; however, five environments had positive PC2 scores, while three had negative PC2 scores. Relationship among test environments was based on the theory that the cosine of an angle between the vectors of two environments approximates the genetic correlation between them and allows similarity and dissimilarity between environments in ranking genotypes (Yan, 2002, Yan, 2011). According to the theory, acute angles (less than 90) indicate high and positive correlation, obtuse angles (greater than 90) show negative correlation while right angles indicate no correlation (Yan and Kang, 2003). The angles between all environments were less than 90 indicating high correlation amongst them. Although the angles between all environments were acute, the smallest cosine was found between environments KIB11 and MUG11 and between KAK11 and MUG12 indicating higher correlations for these environments. The largest angle was found between MUG11 and KAK12 showing these two environments were not as highly correlated.

Spearman rank correlation coefficients among the eight environments for hybrid and parental line evaluation are presented in Table 7.7 and 7.8, respectively. The existence of positive and significant correlations showed that the information obtained between pairs of environments was very similar indicating that those environments would have ranked genotypes in a similar fashion. Significant and positive spearman rank correlations confirmed the acute angles between environments in the GGE biplot.

The highest positive and significant coefficient was between KAK11 and KAK12 ($r=0.661$, $p<0.001$) (Table 7.6), which were both placed in the mega-environment II. Correlations larger than 0.5 were found between environments placed in the same mega-environment, for instance KAK12 and MUG12 and KIB12 and MWE11. Two environments MUG11 and KAK12 had the

largest angle in the GGE biplot (Fig. 7.3) and corresponding low and non-significant correlation (Table 7.7) indicating that these had different interaction with genotypes.

Environments with the longest vectors from the biplot origin would be the most discriminating of the hybrids. The most discriminating environment in mega-environment I was MWE12 and KAK12 in mega-environment II (Fig. 7.3). Across all environments however, KAK12 supported the highest discrimination of hybrids according to yield potential and was the highest yielding environment. On the other hand, KIB11 and KAK11 were the least discriminating environments in mega-environment I and II, respectively. Overall, across environments, KIB11 had the shortest vector and was the most non-discriminating for the hybrids. Therefore, in terms of discriminating ability of F_1 hybrids for grain yield, the environments were ranked as follows: KAK12>MUG12>MWE12>MWE11>KIB12≈KAK11>MUG11>KIB11.

The degree of correlation between years within locations is a measure of the repeatability of that location (Yan et al., 2011). The smaller the angle between years of a test location, the more repeatable the test location. Based on this prerequisite, the most repeatable test location was KIB, which had the smallest acute angle between years, followed KAK (Figure 7.3). The least repeatable test locations were MUG for the crosses. Based on repeatability therefore, the environments were ranked as follows: KIB>KAK>MWE>MUG.

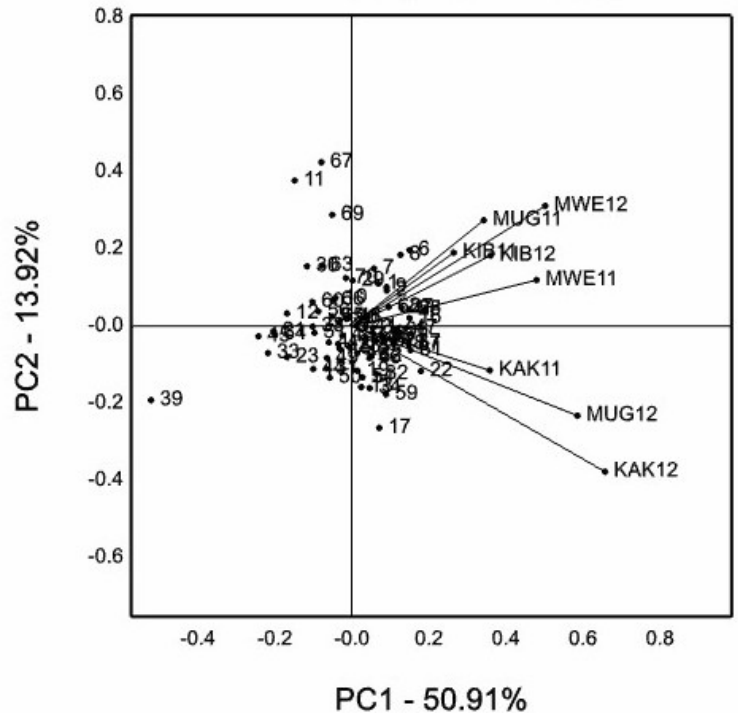

Figure 7.3.GGE biplot based on grain yield for eight environments showing the relationship among the environments for hybrids. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

Table 7.7. Spearman's rank correlation coefficients among eight environments in hybrid evaluation

Environment	KAK12	KIB11	KIB12	MUG11	MUG12	MWE11	MWE12
KAK11	0.661***	0.186^{ns}	0.286*	0.317**	0.502***	0.471***	0.365**
KAK12		0.261**	0.314**	0.205^{ns}	0.532***	0.445***	0.376**
KIB11			0.355**	0.318**	0.207^{ns}	0.143^{ns}	0.38**
KIB12				0.133^{ns}	0.181^{ns}	0.561***	0.381**
MUG11					0.231^{ns}	0.303*	0.357**
MUG12						0.443***	0.462***
MWE11							0.449***

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05); The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

The GGE biplot for ranking of environments based on discriminative ability and representativeness across environments relative to yield performance is shown in Figure 7.4. A test environment that has smaller angle with the average environment coordinate (AEC) is more representative of the other test environments (Yan and Tinker, 2006). A representative location is the average location that would be used as a reference or a standard. Thus, MWE11 was the most representative environment (Fig. 7.4) whereas KIB11 with large deviation from AEC was the least representative. The ideal test environment (the centre of concentric circles) should be both highly discriminating (ability to differentiate genotypes) and most representative of the target environments (Yan and Tinker, 2006). This ideal environment rarely exists under natural conditions but could be used as a reference. Thus, MWE11 was closest to the ideal test environment where the best hybrids could be most easily identified.

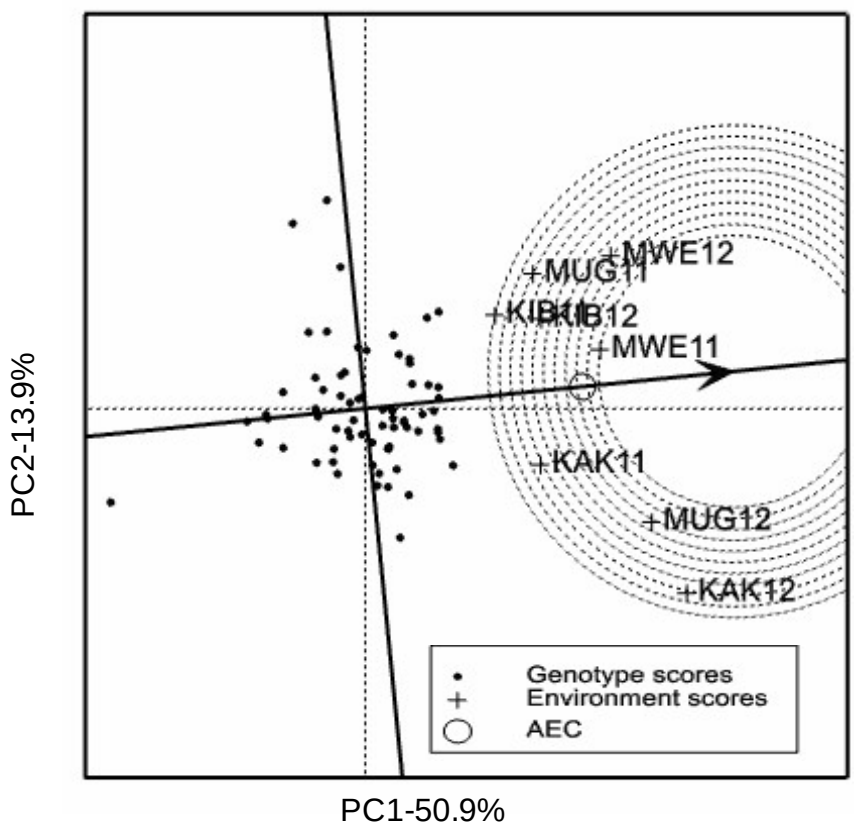

Figure 7.4. GGE-biplot based on environment-focused scaling for comparison of environments for hybrids. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

7.3.6 Relationship among test environments in evaluation of parental lines

Environmental vectors drawn from biplot origin for parental genotypes (Fig 7.5) showed that all environments had positive PC1 scores and they provided good discriminating ability for the parental lines based on yield data. The angles between all environments were, similar to hybrids, less than 90 indicating high correlation amongst the eight environments. The spearman correlations among parental lines were mostly non-significant indicating distinct environments (Table 7.7).

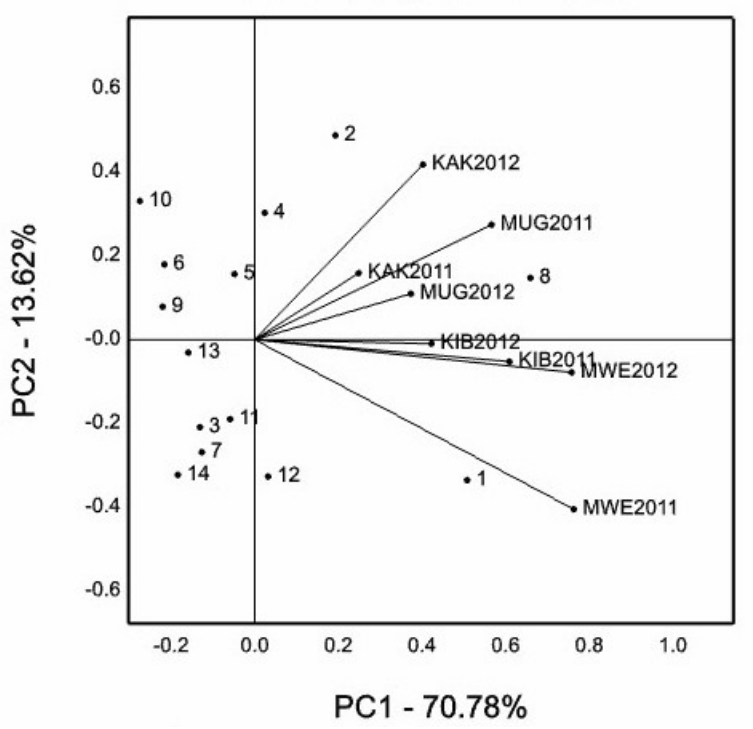

Figure 7.5. GGE biplot based on grain yield for eight environments showing the relationship among the environments for parental lines. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

Table 7.8. Spearman rank correlation coefficients among eight environment in evaluation of 12 parental lines and two inbred checks

Environment	KAK12	KIB11	KIB12	MUG11	MUG12	MWE11	MWE12
KAK11	0.512^{ns}	0.529^{ns}	0.441^{ns}	0.353^{ns}	0.283^{ns}	0.213^{ns}	0.476^{ns}
KAK12		0.314^{ns}	0.169^{ns}	0.432^{ns}	0.323^{ns}	-0.094^{ns}	0.446^{ns}
KIB11			0.815***	0.705**	0.494^{ns}	0.446^{ns}	0.661*
KIB12				0.556*	0.626*	0.639*	0.542*
MUG11					0.582*	0.204^{ns}	0.538*
MUG12						0.265^{ns}	0.384^{ns}
MWE11							0.534*

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ns indicates non-significant (p>0.05); The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko evaluated in 2011 and 2012

Although MWE11 formed a separate mega-environment II, a significant correlation was found between this environment and KIB12 (Table 7.8). Two environments, KAK12 and MWE11, which were far apart on the GGE biplot and thus had a large angle between them, (Fig. 7.5) had corresponding low negative and non-significant correlation (Table 7.8). The most discriminating environments for inbred line evaluation by the length of the vectors from the origin were MWE11 and MWE12. Environment KAK11 had the shortest vector and provided the least discrimination of parental genotypes for grain yield. In terms of discriminating ability among parental genotypes, the environments were ranked as follows: MWE12≈MWE11>KIB11>MUG11>KIB12>KAK12>MUG12>KAK11 (Fig 5).

For grain yield performance among the parental lines, the most repeatable test location was KIB (Kiboko) (Fig. 7.5), similar to the observation made for hybrids, as the two Kiboko seasons had the smallest acute angle between them. The second most repeatable location was Muguga, unlike hybrids where Muguga was the least repeatable. For the parental line evaluation, the repeatability of the environments was ranked as follows: KIB>MUG>KAK>MWE.

The most representative environment for inbred line evaluation was MWE12 based on a smaller with the average environment coordinate (AEC) (Fig. 7.6). The least representative environment was KAK11 as it had a large deviation from AEC. The ideal environment that was most discriminating and most representative (found at the centre of concentric circles) was MWE12 for parental line evaluation.

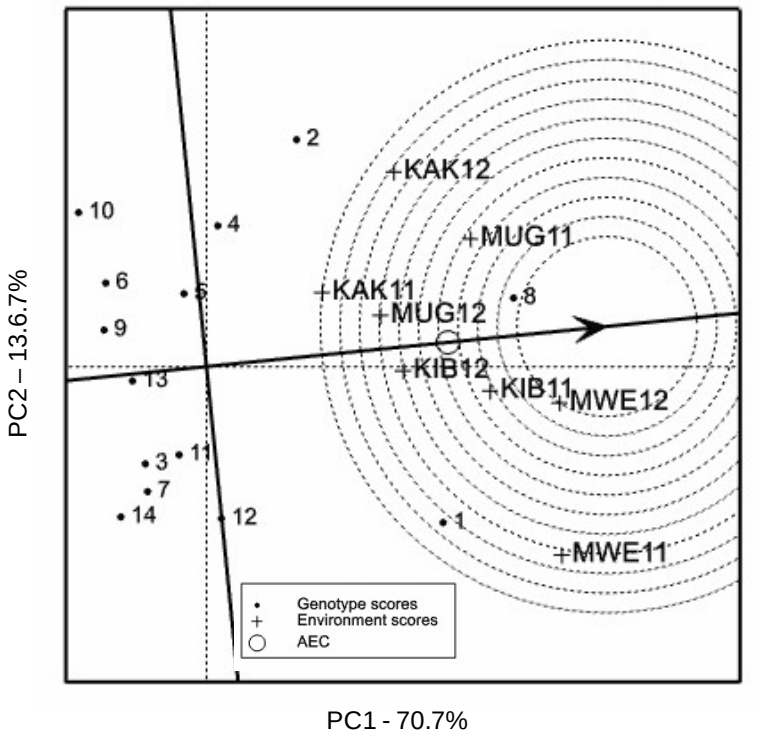

Figure 7.6. GGE-biplot based on environment-focused scaling for comparison of environments for parental lines. The environments are KAK=Kakamega, MUG=Muguga, MWE=Mwea, KIB=Kiboko in 2011 and 2012

7.3.7 Stability of combining abilities for grain yield

The combined analysis of variance across environments indicated highly significant ($p<0.001$) GCA x E and SCA x E interactions for grain yield and other agronomic traits suggesting changes in rank of GCA and SCA across environments (Table 7.9). The contribution of GCA x E to G x E was in the range of 22 to 54% for the various traits, while SCA x E to G x E was in the range of 48 to 72%. The contribution of GCA x E to G x E for grain yield was 41% while SCA x E contributed 59%.

Table 7.9. General and specific combining ability variances for grain yield and contribution to G x E

Source of variation	Mean squares					
	GY	PHT	EHT	EPO	DTA	EA
GCA	56.05***	25964.1***	22546.9***	0.113***	672.44***	7.07***
SCA	38.12***	1339.8***	663.7***	0.003***	22.94***	0.31***
GCA x E	5.69***	652.7***	246.5***	0.002***	24.85***	0.45***
SCA x E	1.79***	215.8**	126.6***	0.001***	5.31***	0.17***
GCA to Entry SS (%)	48	78	86	86	86	83
GCA to Entry SS (%)	57	22	14	14	14	17
GCA x E to G x E SS (%)	41	38	28	22	52	34
SCA x E to G x E SS (%)	59	62	72	78	48	66

*, **, *** indicates the term is significant at p≤0.05, p≤0.01 and p≤0.001, respectively, ^{ns} indicates non-significant (p>0.05) data
GY = grain yield (t ha^{-1}), PHT = plant height (cm), EHT = ear height (cm), EPO = ear position, DTA = days to 50% anthesis, ASI = anthesis silking interval, EA = ear aspect

The magnitude of GCA for grain yield for the parental inbred lines and their ranks in eight environments are presented in Table 7.10. The rank sum computed varied from 22 for CML539 to 91 for CML505. The rank variance ranged from 0.55 (CML505) to 12.86 in two lines, Z419 and TZMI746. Lower values of rank sum and low rank variance were associated with high and stable GCA effects across environments. Parental lines were separated into three groups based on rank sum, the lowest values (20-40) were found in lines CML539, Z419, C92, and MUL114, intermediate values (41-60) in Osu23i, S558, MUL71, TZMI736 and TZMI746 and high values (61-91) in VHCY, CML505 and CML509. The rank variances of parents CML505 (0.55), CML539 (2.2) and TZMI736 (3.1) were relatively low compared to the maximum variance of 12.86, indicating that these three lines had stable GCA effects across the eight environments. However, only CML539 had low rank sum and low rank variance required for high positive GCA effects and high stability for grain yield. The contribution of the GCA effects of the parental lines to GCA x E interaction ranged from 1% in CML505 to 17% in two lines Z419 and TZMI746. Four parents namely CML505, CML509, CML539 and TZMI736 contributed least (1% to 4%) to GCA x E interaction supported by the fact that they had low rank variances. The parent CML539 was identified as the most stable inbred line with positive GCA effects in all environments and minimal contribution (3%) to GCA x E interaction.

The ranks of the SCA effects among the 66 F_1 hybrids across the eight environments (Table 7.11) varied widely such that the lowest rank sum was 80 in hybrid 14 (S558 x CML509)

out of a possible minimum rank sum of eight (i.e., having rank 1 in all eight environments). The highest rank sum of 574.6 was obtained in hybrid 39 (CML505 x CML509) out of a maximum possible rank sum of 528 (i.e., having rank 66 in all eight environments). The rank variance ranged from 0.6 in cross 39-CML505 x CML509 to 574.6 in cross 55- MUL114 x TZMI746. The cross that combined both low rank sum and low rank variance was hybrid 14 (S558 x CML509) with rank sum 80 and rank variance of 41.4. Six hybrids with the lowest rank variance ranging from 0.6 to 68.2 had zero contribution to SCA x E interaction. The hybrid 55 (MUL114 x TZMI746) with the largest rank variance 574.6 also had the highest contribution to SCA x E interaction, although this was only 4%. There were low contributions of individual SCA effects to SCA x E interaction among the 66 hybrids ranging from 0% to 4%.

The GGE biplot of SCA (Fig. 7.7) and GCA for grain yield (Fig. 7.8) confirmed the rank sum and rank variance patterns noted among the diallel genotypes. Hybrid 14 had the most stable SCA since it had PC2 values close to zero (Fig. 7.7). Vertex hybrids, 22, 47, 11, 33, 39 and 59, had the most unstable SCA effects. There were three mega-environments for SCA effects characterized by environments MUG12, MWE12 and KAK11 in mega-environment I, KAK12 for mega-environment II and KIB11, MUG11, MWE11 and KIB12 for mega-environment III. The most stable SCA effect was found in mega-environment II. Based on GCA effects, the eight environments were also separated into three mega-environments, and the most stable GCA effect, parent 9 (CML539) was found in mega environment II characterized by MWE11 (Fig 7.8).

Table 7.10. General combining ability effects on grain yield of 12 maize lines in eight environments, their ranks (bold in parentheses), ranks sum, and rank variance and their contribution of GCA x E interaction

Entry	Parent	KAK11	KAK12	KIB11	KIB12	MUG11	MUG12	MWE11	MWE12	Rank sum	Rank variance	GCA x E (%)
1	Z419	0.166 **(5)**	-0.181 **(10)**	0.729 **(1)**	0.881 **(1)**	0.811 **(1)**	-0.361 **(8)**	0.263 **(5)**	1.409 **(1)**	32	12.86	17
2	558	0.712 **(2)**	0.211 **(6)**	0.135 **(6)**	-0.411 **(10)**	0.034 **(6)**	0.171 **(6)**	-0.461 **(9)**	-0.039 **(8)**	53	5.98	8
3	MUL71	-0.087(**8)**	0.045 **(8)**	0.206 **(5)**	-0.275 **(9)**	-0.057 **(7)**	-0.273 **(7)**	0.305 **(4)**	0.838 **(2)**	50	5.64	8
4	OSU23i	0.036 **(7)**	0.709 **(2)**	0.004 **(7)**	-0.243 **(8)**	-0.342 **(9)**	1.058 **(3)**	-0.010 **(6)**	0.233 **(5)**	47	5.84	8
5	CML505	-0.341 **(10)**	-0.876 **(11)**	-0.903 **(12)**	-1.054 **(12)**	-0.436 **(11)**	-1.148 **(11)**	-1.361 **(12)**	-1.588 **(12)**	91	0.55	1
6	CML509	-0.121 **(9)**	-0.024 **(9)**	-0.818 **(11)**	-0.133 **(7)**	-0.351 **(10)**	-0.659 **(9)**	-0.487 **(10)**	-1.127 **(11)**	76	1.71	2
7	MUL114	-0.404 **(11)**	0.391 **(4)**	0.339 **(3)**	0.139 **(5)**	0.097 **(4)**	1.191 **(2)**	-0.137 **(7)**	0.756 **(4)**	40	8.00	11
8	C92	0.197 **(4)**	0.752 **(1)**	-0.303 **(10)**	0.689 **(2)**	0.212 **(3)**	1.427 **(1)**	1.167 **(2)**	0.219 **(7)**	30	10.21	14
9	CML539	1.087 **(1)**	0.361 **(5)**	0.394 **(2)**	0.252 **(4)**	0.569 **(2)**	0.771 **(4)**	1.234 **(1)**	0.787 **(3)**	22	2.21	3
10	TZMI736	0.158 **(6)**	0.473 **(3)**	0.271 **(4)**	0.111 **(6)**	-0.084 **(8)**	0.252 **(5)**	-0.171 **(8)**	0.219 **(6)**	46	3.07	4
11	TZMI746	0.327 **(3)**	0.209 **(7)**	-0.047 **(9)**	0.621 **(3)**	-0.492 **(12)**	-0.814 **(10)**	0.536 **(3)**	-0.708 **(9)**	56	12.86	17
12	VHCY	-1.733 **(12)**	-2.072 **(12)**	-0.007 **(8)**	-0.575 **(11)**	0.039 **(5)**	-1.615 **(12)**	-0.878 **(11)**	-1.001 **(10)**	81	6.13	8
	S.E.	0.109	0.252	0.233	0.197	0.198	0.276	0.352	0.143			

Table 7.11. Specific combining ability (SCA) effects and their ranks (bold in parentheses) for grain yield for top 10 and bottom 10 F_1 hybrids sorted by rank variance and contribution to SCA x E interaction

Entry	cross	KAK11	KAK12	KIB11	KIB12	MUG11	MUG12	MWE11	MWE12	Rank sum	Rank variance	SCA x E (%)
55	7 x 11	-0.18 **(41)**	2.39 **(2)**	1.24 **(6)**	1.25 **(5)**	-0.68 **(48)**	-2.40 **(65)**	0.60 **(21)**	-0.28 **(47)**	235	574.6	4
11	1 x 12	-0.82 **(61)**	-1.92 **(61)**	1.16 **(9)**	0.63 **(22)**	1.39 **(9)**	-1.35 **(58)**	0.19 **(29)**	-1.50 **(61)**	310	571.6	3
65	10 x 12	0.23 **(21)**	1.20 **(10)**	-1.74 **(64)**	0.82 **(14)**	-1.12 **(57)**	0.15 **(32)**	1.17 **(9)**	1.66 **(3)**	210	526.2	3
59	8 x 11	0.58 **(12)**	0.59 **(21)**	-1.26 **(60)**	-0.65 **(53)**	-0.92 **(53)**	1.33 **(7)**	-0.32 **(42)**	0.82 **(10)**	258	485.1	3
46	6 x 7	0.86 **(6)**	-0.46 **(48)**	-1.58 **(63)**	0.09 **(31)**	0.60 **(18)**	1.40 **(6)**	2.09 **(1)**	0.24 **(29)**	202	481.6	3
18	2 x 9	1.25 **(2)**	-0.02 **(35)**	0.49 **(18)**	-0.82 **(58)**	0.41 **(23)**	0.97 **(11)**	-1.28 **(58)**	0.80 **(11)**	216	460	3
66	11 x 12	1.00 **(4)**	0.53 **(25)**	0.14 **(30)**	-0.72 **(54)**	0.71 **(15)**	0.77 **(15)**	-1.22 **(57)**	1.77 **(1)**	201	443.8	3
29	3 x 11	-0.34 **(51)**	-0.98 **(57)**	-0.66 **(54)**	-0.07 **(34)**	0.63 **(17)**	-0.49 **(46)**	1.49 **(5)**	0.61 **(14)**	278	412.5	3
7	1 x 8	-0.34 **(50)**	-2.05 **(62)**	0.05 **(33)**	-1.27 **(61)**	0.59 **(19)**	0.61 **(20)**	-1.69 **(61)**	0.57 **(17)**	323	412	3
48	6 x 9	0.18 **(26)**	-0.13 **(40)**	-0.35 **(42)**	-0.60 **(51)**	1.84 **(6)**	-1.25 **(57)**	-1.85 **(63)**	0.57 **(16)**	301	403.7	2
24	3 x 6	0.01 **(34)**	0.40 **(27)**	0.03 **(34)**	0.63 **(21)**	0.36 **(25)**	-0.92 **(53)**	-0.02 **(35)**	0.39 **(24)**	253	102.3	1
37	4 x 11	1.06 **(3)**	0.47 **(26)**	1.17 **(8)**	1.74 **(2)**	1.67 **(8)**	2.12 **(2)**	0.33 **(26)**	0.63 **(13)**	88	99.7	1
12	2 x 3	-1.13 **(63)**	-3.10 **(64)**	-1.26 **(61)**	-0.18 **(38)**	-1.71 **(63)**	-1.87 **(62)**	-1.10 **(55)**	-1.52 **(62)**	468	76.3	0
16	2 x 7	0.81 **(7)**	1.04 **(13)**	1.27 **(5)**	0.36 **(25)**	2.43 **(2)**	0.53 **(23)**	0.80 **(16)**	0.60 **(15)**	106	68.2	0
32	4 x 6	0.22 **(22)**	0.85 **(16)**	1.12 **(10)**	1.30 **(4)**	0.30 **(27)**	1.10 **(10)**	0.49 **(22)**	0.46 **(18)**	129	59	0
21	2 x 12	-0.43 **(55)**	-0.99 **(58)**	-0.63 **(52)**	-0.72 **(55)**	-2.10 **(65)**	-0.15 **(43)**	-0.83 **(51)**	-0.19 **(43)**	422	54.5	0
10	1 x 11	-0.20 **(42)**	-0.61 **(53)**	-1.20 **(59)**	-0.54 **(48)**	-0.79 **(49)**	-0.93 **(55)**	-1.14 **(56)**	-0.15 **(41)**	403	42.8	0
14	2 x 5	0.36 **(19)**	1.18 **(11)**	0.80 **(16)**	0.87 **(11)**	2.80 **(1)**	1.90 **(3)**	0.91 **(14)**	1.18 **(5)**	80	41.4	0
33	4 x 7	-1.54 **(65)**	-4.15 **(65)**	-2.48 **(66)**	-1.11 **(60)**	-1.49 **(61)**	-3.12 **(66)**	-1.84 **(62)**	-3.26 **(66)**	511	6.1	0
39	5 x 6	-2.36 **(66)**	-5.06 **(66)**	-1.96 **(65)**	-3.10 **(66)**	-4.57 **(66)**	-2.36 **(64)**	-2.73 **(66)**	-2.31 **(65)**	524	0.6	0
S.E.		**0.324**	**0.773**	**0.695**	**0.589**	**0.591**	**0.824**	**1.052**	**0.426**			

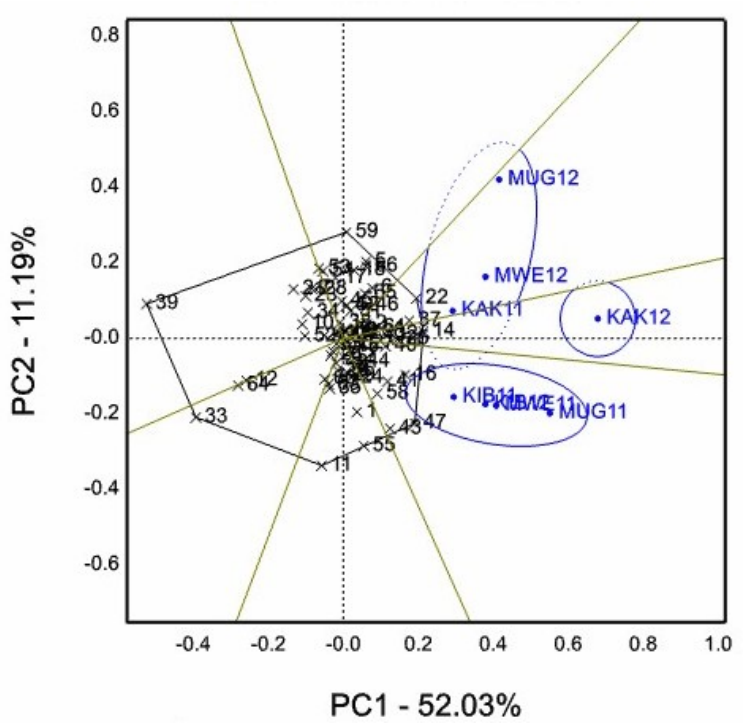

Figure 7.7. Polygon view of the GGE biplot based on SCA effects of 66 single crosses for grain yield across eight environments

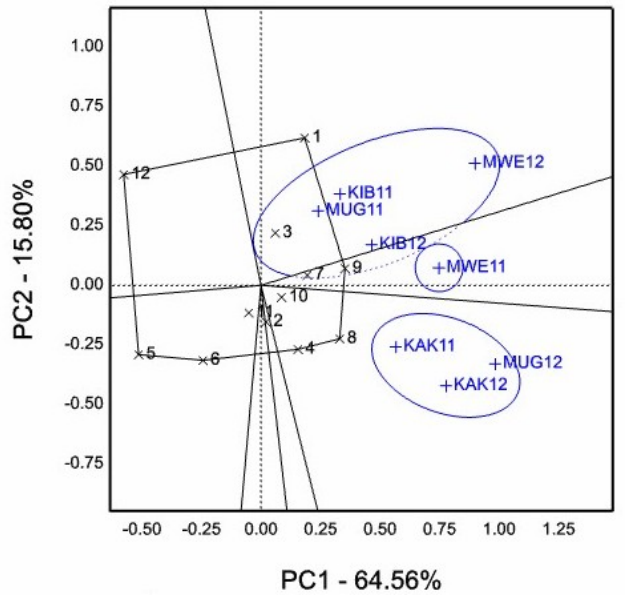

Figure 7.8. Polygon view of the GGE biplot based on GCA effects of 12 parents for grain yield across eight environments

7.4 Discussion

7.4.1 Combined analyses of variance

Highly significant ($p \leq 0.01$) variance due to environment and G x E interaction indicated that hybrids interacted differentially with environments. Environments (locations, years and location x year interaction) controlled 44% of total variation for grain yield, a value that was lower compared to others who have reported high (>80%) variation due to environment main effects (Admassu et al., 2008, Fritsche-Neto et al., 2010, Tonk et al., 2011). This could suggest similarity of environments and consistency of performance for some of the genotypes evaluated. The variations due to location main effects and G x L were larger than variation due to years main effects and G x Y for grain yield. In this situation, where G x L is the dominant portion of G x E, it is possible to exploit dynamic stability (or specific adaptation) since genotypes would be more responsive over predictable features (such as locations) but stable over unpredictable features (such as different years). This is in accordance with the theoretical concepts described by Fox et al. (1997). Homogeneous regions can thus be identified that minimize G x E within regions and form uniform domains for release and recommendation of genotypes.

The relatively larger G x E interaction relative to G effect in this study for grain yield indicated the presence of remarkable G x E interaction and the possible existence of different mega-environments with different top-yielding genotypes. Some traits such as grain yield, anthesis-silking interval, ear aspect and number of ears per plant had a higher G x E variance than genotypic variance, therefore it is essential to evaluate genotypes for these traits in different environments. A higher G x E variance compared to genotypic variance for maize grain yield is commonly reported (Beyene et al., 2012, Tefera et al., 2013, Tonk et al., 2011). For traits with higher genotypic variance than G x E variance, such as plant height, ear height, ear position, days to anthesis and days to silking, selection for these traits can be done in a few target environments.

7.4.2 Stability and adaptability

Pearson correlation coefficient between the PC1 scores and the genotype effects was high (0.99), suggesting that although G x E existed and crossover genotypes were detected, the patterns of G x E were not very complex. This could also suggest that the crossover interactions could be occurring within a few varieties. A “near-perfect correlation ($r=0.95$) between genotype PC1 scores and genotype main effect occurs when genotypes contribute 40% or more of GGE (Yan et al., 2001). This was attained in the current study since the correlation was 0.993

(p≤0.001) and genotypes accounted for 47% of GGE. The high correlation further indicates that the which-won-where pattern analysis (Yan et al., 2007) would provide a good estimate of ideal genotypes and test locations.

Selection for yield stability involves identification of promising genotypes that combine high yield potential (high PC1 scores) and minimal G x E interaction (close to zero PC2 scores). The genotypes showed considerable variability for stability and adaptation to different locations and seasons that could be exploited in crop improvement. Scores PC1 and PC2 identified cultivars with wide adaptation and those with specific adaptation. Two hybrids displayed high yield potential and high stability 47- CML509 x C92, and 36-OSU23i x TZMI736 and can be promoted for wide adaptation in the medium and medium-late environments though testing in additional environments is required. It was also noted that hybrid 47- CML509 x C92 was a combination of stable x stable lines but 36-OSU23i x TZMI736 was from unstable x unstable lines. Therefore, parental lines per se performance may not have contributed yield potential and stability in these hybrids.

Hybrids 6- MUL71 x OSU23i and 22- Z419 x MUL114 were most responsive in their environments and therefore can be adopted according to their specific adaption, medium and medium-late locations, respectively. The adaptation responses of genotypes based on nominal yield can also be used identify appropriate check genotypes for all environments (general check) or for specific environments (specific check) (Samonte et al., 2005). In this regard, hybrid 47- CML509 x C92, and 36-OSU23i x TZMI736, which were broadly adapted, can be used as general checks, while hybrids 6- MUL71 x OSU23i and 22- Z419 x MUL114, would be applicable as specific checks for mega-environments I and II respectively. In the current study. Inbred lines CML202 and CML539, which are elite breeding lines in Eastern and Southern Africa, displayed high stability. Improved adaptiveness and stress tolerance of CIMMYT line 202 in diverse environments has been shown for several stresses including MSV resistance (Welz et al., 1998), NLB resistance (Schechert et al., 1999) and drought tolerance (Gebre, 2005). The line CML539 is also reported as broadly adapted and MSV tolerant (Cairns et al., 2013).

7.4.3 Polygon view of GGE biplot analysis

Visualization of the “which won where” pattern of multi-environment data is necessary for studying the possible existence of different mega-environments and identifying genotypes potentially suitable to specific mega-environments. A mega-environment refers to a group of fairly homogeneous environments that consistently share the best genotypes (Yan and Rajcan,

2002). In this study, the high yielding hybrid 6- Z419 x MUL114 was most suited in MUG11, MWE11, MWE12, KIB11, and KIB12 classified as mega-environment I. Hybrid 22- MUL71 x Osu23i was most suited in KAK11, KAK12 and MUG12, classified as mega-environment II. The rank order changes in the ranks of genotypes under these environments were used in selecting specifically adapted hybrids 6 and 22.

In terms of location versus season grouping, both seasons at a location were placed in the same mega-environment, except MUG11, which was found, is mega-environment I and MUG12 in mega-environment II. This could have been attributed to several genotypes changing ranks in the two seasons at Muguga. Although both seasons were artificially inoculated for MSV, there was higher disease pressure in 2012, which could have resulted in higher disease infection in some genotypes with a significant and differential reduction in yield affecting stability for yield potential. Jalata (2011) also noted that large G x E interactions can occur when genotypes vary widely in resistance to stresses or when environments differ widely in incidence to the same stresses.

Despite the fact there was a crossover with MUG11 and MUG12, the other environments were grouped together in accordance with their agro-ecology, Kiboko, Mwea and Muguga together and while Kakamega was set apart. Kiboko is found in a dryland agro-ecological zone, but is an irrigation site, which possibly creates a micro-environment for materials to behave as in the mid-altitude areas such as Mwea and Muguga. Kakamega is medium late environment and although the genotypes were disease and drought stressed in 2011, making KAK11, the least discriminating and low yielding environments, both seasons were placed in the same mega-environment. In 2012 in Kakamega, the season was more optimal with higher rainfall and low disease pressure, thus the highest average yields were recorded in KAK12, making this environment the most discriminating. Therefore, differences among genotypes could easily be detected in KAK12.

The variation explained by PC1 and PC2 scores of GGE biplot was 64.8% in the G x E variation for hybrid evaluation. This was higher than the variance captured by G and G x E in ANOVA (44%). Therefore, GGE biplot was efficient in representing variation due to G and G x E compared to ANOVA. Differences have been reported on the variation captured by the GGE biplot, although these do not seem to relate to number of environments and genotypes used. For instance, Alwala et al. (2010) evaluated 24 hybrids in 24 locations and the GGE biplot explained only 32.7% of the G x E variation while Setimela (2007) evaluated 34 hybrids in 38 locations in four years and obtained PC1 and PC2 joint scores of 93.1%. Similar to the present

study, Tonk et al. (2011) used four location x two year environment combination for the evaluation of 17 hybrids and found PC1 and PC2 joint scores of 61.2%. It is reported that GGE captures more variation when genotypes are evaluated in similar environments since in such situations the 'noise' in the sums of squares of (G + GE) and GE is reduced, as compared to where very different sites in terms of soil and climatic features are used (Oliveira et al., 2010). Therefore, biplot analysis may not be sensitive to the number of genotypes studied although it was noted in present study that the biplot provided best visualization for genotype stability prediction when a smaller number of genotypes are evaluated, similar to Rose et al. (2008). The GGE biplot can thus be used in advanced stages of testing where small number of genotypes are tested over a wide range of environments for recommendation in certain domains as well as in early stages of testing when a large number of genotypes are evaluated in fewer locations where the primary objective is to discard inferior genotypes.

7.4.4 Interrelationships among test environments

The vector length of an environment measures the discriminating power of its ability to differentiate the cultivars (Yan et al., 2010). KAK12 and MWE11 had longer vectors than other environments for hybrids and parental lines respectively, indicating that these two environments were best for genetic differentiation of the genotypes. The contrast of KAK12 as the most discriminating and KAK11 as the least discriminating, was due to a better yielding condition mostly due to higher rainfall and lower disease pressure at Kakamega in the 2012 than in 2011 cropping season. Since performance and stability of germplasm requires test environments that are representative and discriminatory (Yan et al., 2007), MWE11 and MWE12 combined these attributes in the evaluation of hybrids and parents, respectively. Therefore, the environment in Mwea was most efficient for genotype differentiation and is a representative of target environments (medium-transitional) for the cultivars to be released. Although this environment is largely used for rice production in Kenya, maize breeders could consider Mwea as an alternative site for testing mid-altitude and transitional germplasm for yield potential and yield stability.

Repeatability analysis assesses the representation of the location for the target environment which should be repeatable so that genotypes selected in each year will have superior performance in future years (Yan et al., 2011). Kiboko was identified as the most repeatable location for both hybrids and parental line evaluation. The repeatability of Kiboko for crosses and parental material and may not be surprising as this site is irrigated throughout the growing

season therefore the performance of genotypes is not affected by seasonality differences such as rainfall distribution.

7.4.5 Stability of combining ability effects for grain yield

The hybrid by environment interaction was partitioned into significant GCA x environment and SCA x environment interaction effects. This indicated that the rankings of both GCA and SCA effects changed across different environments similar to several findings (Machida et al., 2010, Makanda et al., 2010, Makumbi et al., 2011). Ranking of GCA and SCA effects for grain yield across environments showed different patterns for the parental lines, concurring with other findings (Dehghanpour and Ehdaie, 2013, Machado et al., 2009). Parents CML539 had high stability for GCA effects across environments for grain yield potential, and it contributed only 3% to GCA x E interaction. This implies that this parent has high utility for breeding programmes that emphasize stability of hybrids.

The high rank sum values among SCA effects showed high instability of the SCA effects of the crosses among environments. The lowest rank sum of 80 was not satisfactory for high positive SCA effects across environments since ideally, the cross with the most stable and favourable SCA effect would have a rank sum of eight, i.e., having a rank one (best rank) in each of the eight environments. It was also noted that crosses with large SCA in the negative direction had the most stable SCA effects based on lower variance (<5.0), for example, hybrid 33 (Osu23i x MUL114) and 39 (CML505 x CML509). It could be hypothesized that stability of SCA effects is affected by their magnitude.

Hybrid 14, which had the lowest sum rank and lowest rank variance, was not among the top 20 high yielding hybrids thus stability of SCA effects was not reflected in stability for grain yield. The top yielding hybrid across environments, hybrid 16-S558 x MUL114, however, had rank sum of 106 and rank variance of 68.2, values that were close to those of hybrid 14, indicating that high productivity of hybrid 16 could have been contributed, though minimally by stability of SCA effects. Therefore, the most productive hybrids could not be predicted by stability of SCA because most of the crosses had highly variable SCA effects across environments.

Overall, there was little relationship between stability of SCA effects and stability of F_1 hybrids since most of the SCA were highly variable across environments and small in magnitude (most not significantly from zero). This was due to a relatively large contribution of additive effects to grain yield in some environments. Further, the contribution of each SCA effects to variation due to SCA x E interaction was small and of similar magnitude (0% to 4%). The low contribution of

individual SCA effects to SCA x E interaction seems to be a common trend. For instance, Machado et al. (2009) in 55 single crosses from a diallel mating design reported values of 0.2% to 4%, while Dehghanpour and Ehdaie (2013) reported values of 0% to 7% in a 9 x 9 half-diallel. Difficulties of identifying parents having stable SCA effects associated to good performance in hybrid combinations has also been reported (Dehghanpour and Ehdaie, 2013).

7.5 Conclusions

The application of GGE biplots facilitated the visual comparison and identification of superior genotypes and environments for breeding decisions in variety selection and recommendation in different environments. Two mega-environments were identified among the hybrids showing distinct Kenyan environments for mid-altitude varietal differentiation. Hybrid CML509 x C92 was the best hybrid in combining stability with high grain yield and wide adaptability. The irrigation-managed site, Kiboko, was the most repeatable although it was not very discriminating. The most discriminative and representative environment, Mwea, should be used more often by breeders to facilitate hybrid selection to target environments. It was possible to select parent CML539 with high stability for combining ability, high GCA value and minimal contribution to GCA x E. However most SCA effects were highly variable and contributed to SCA x E with small effects of equal magnitude. Stability analyses suggest that maize breeding programs in Kenya could improve grain yield by using maize inbred line CML539.

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CHAPTER 8

Overview of research findings

8.1 Introduction

The research focus for this study was to explore breeding source germplasm for use in developing MSV resistant maize hybrids. Therefore, breeding investigations were pursued in order to develop a viable strategy for generating MSV resistant hybrids that will be accepted by farmers in target environments in Kenya. It is crucial that heterosis is not compromised in breeding for high level of MSV resistance in hybrids. Therefore maize germplasm lines must be carefully selected to avoid disruption of existing heterotic groups. To this effect germplasm of diverse genetic background was characterized to assess its utility for improving hybrids for MSV resistance; and to determine the germplasms' potential for developing productive and stable maize hybrids. A combination of high productivity, high stability and high resistance to MSV, is key for new maize hybrids that are targeted to the highly variable production environments in sub-Saharan Africa. The work accomplished by this study is reported in the foregoing chapters. This overview gives a summary of the main findings and thoughts for further research.

The following hypotheses were tested in the study:

1. Farmers are aware of the most important production constraints in their production zones and can provide succinct focus for agricultural development and the breeding research agenda.
2. Selected inbreds have genes for resistance to MSV which can be transmitted effectively to progeny and that disease progress is uniform among genotypes
3. The inbreds have substantial genetic divergence that can be exploited for breeding for MSV disease resistance and grain yield performance.
4. The diverse maize accessions have potential of improving grain yield and its associated traits and that heterotic patterns can be delimited through diallel mating system and SSR marker based genetic distances
5. Inheritance of MSV resistance is controlled by simple mechanisms that can be introgressed into susceptible backgrounds and/or stacked into an elite line for hybrid formation

6. Hybrids with high MSV resistance are high yielding and have stable yield performance and wide adaptation. The selected inbred lines have stable performance across the target regions, which can be transmitted to stable and highly adapted F_1 hybrids.

8.2 Summary of the major findings

8.2.1 The status of MSV in central Kenya and farmers preferences

A survey and participatory rural appraisal (PRA) conducted in Central Kenya, mainly in Kiambu and Nyeri districts established that maize yields are still very low and several factors continue to limit these yields. The highlights of the study were:

- Extension officers reported an outbreak of MSV in the long rains of 2009 attributed to susceptible varieties and continuous maize cropping.
- Maize hybrids have been highly adopted in central Kenya and farmers have a wide range of these varieties, often changing variety with season; growing both medium altitude and highland varieties and alternating these between seasons with low and high rainfall.
- Farmers continue to grow “old” varieties, despite numerous varieties at their disposal.
- Popular varieties were preferred for early maturity, sweet taste, and high yield.
- Desirable characteristics for an ideal variety were in order of importance: disease resistance, high yield, early maturity, resilience, insect resistance, drought tolerance, sweet taste, varietal purity, frost resistance, good standability and good stover.
- Production constraints in order of importance were diseases, field pests, abiotic stresses, high cost of inputs, storage pest and marketing difficulties.
- Fertilizers were most important in terms of high cost of inputs, weevils among storage pests, stem borers in field pests, MSV among diseases and drought among abiotic stresses.
- Prevalence of disease in farmers fields in order of highest to lowest were MSV≈head smut>common smut>GLS>NLB.
- MSV incidence was highest in Kiambu district (42%) and lowest in Nyandarua (5%) and ranged between 27% and 38% in other districts.
- MSV appeared in both long rains and short rains seasons.

- High levels of MSV in farmers' fields were associated with hybrid seed recycling, pasture grasses and low soil nutrition.

8.2.2 Phenotypic characterization of maize inbred lines for MSV resistance

A set of 40 diverse lines adapted to tropical and sub-tropical environments were evaluated for MSV response under artificial disease pressure in two environments. Disease symptom expression was monitored over time.

- Half of the inbred lines tested were highly resistant with scores of less than 2.0 on a scale of 1 (immune) to 5 (highly susceptible), indicating that there is opportunity for selection of lines that would combine high levels of yield heterosis and MSV resistance.
- Across locations, the following nine lines were symptomless indicating immunity for MSV: MUL88, MUL1182, MUL1184, C92, Osu23i, C238-14, C238-21 and CML539, qualifying them as the most important donors for use in breeding MSV resistance in hybrids.
- AUDPC values for disease severity were positively and significantly correlated with MSV final disease scores, indicating that they were equally effective for discriminating germplasm lines according to resistance to MSV.
- High heritability estimates were obtained for the mean MSV scores (84%) and final MSV scores (94%), indicating that the final rating would be the most effective for discriminating germplasm according to resistance to MSV.

8.2.3 Molecular characterization of maize germplasm for MSV resistance

The levels of genetic diversity in a set of40 tropical maize inbred lines with potential for use as parents for MSV resistant hybrids was examined using 28 SSRs markers.

- The study identified a set of 28 SSR markers, which was effective for discriminating the MSV resistant lines according to their genetic backgrounds. They detected 135 SSR alleles averaging at 4.8 per locus ranging from 2 to 11.They were very polymorphic with PIC ranging from 0.13 to 0.85, average of 0.52. At least 12 of them displayed PIC values greater than 0.6. The tetranucleotide repeat SSR markers revealed the highest number of alleles and highest PIC. Twenty seven (27) unique alleles were detected in 12 SSR loci. This set of

28 SSR markers will be recommended for use in breeding programmes that emphasise MSV resistance.

- The MSV resistance breeding source germplasm was effectively discriminated by genetic distance in a manner that is consistent with pedigree data. Genetic distance (GD) ranged between 0.12 and 0.72, with 60% of the lines having >0.5 GD between them, confirming that they are divergent. There were large GDs for the inbred lines from different programmes in the region. In this regard, the lowest GDs were obtained for germplasm of the same origin: e.g., GD of 0.12 between C238-28 and C238-6 (both from CIMMYT, Zimbabwe); while highest GDs were obtained for germplasm of different origin: e.g., GD of 0.72 between MUL1182 (from KARI, Kenya) and TZMI759 (IITA, Nigeria) lines.
- Cluster analysis showed a similar trend with GD data, because the clusters were mostly constituted by lines from the same origin, genetic background and in some cases of similar MSV reaction. The 40 lines were grouped into 7 clusters, except for two lines.

8.2.4 Gene action and heterotic grouping of MSV resistant inbred line

A 12 x 12 half-diallel cross was evaluated to determine heterotic patterns, combining ability, gene action and heterosis: with a view to fit the lines into heterotic groups for effective utilization of the germplasm lines in breeding MSV resistant hybrids. The results indicate significant variation as follows:

- Grain yield ranged from 7.92 t ha^{-1} (C92 x TZMI736) to 2.11 (CML505 x CML509). The top ten hybrids were statistically comparable with yield performance of the best commercial check hybrid, indicating that stacking of MSV resistance genes will not necessarily compromise productivity of hybrids. This is underlined by high average mid-parent heterosis for grain yield of 94%. Therefore, there is potential to develop productive hybrids among these MSV resistant lines.
- Both GCA and SCA effects were important for grain yield potential, days to flowering, plant stature and ear aspect. However, the GCA and SCA effects interacted with environments for grain yield and other traits, indicating that multi-location trials would be required to validate performance of MSV resistant hybrids.
- The study also identified lines with great potential for use in programs that emphasize MSV resistance in hybrids: the best general combiners were C92, CML539, Z419 and MUL114 for grain yield potential; MUL71 and CML539 (earliness); CML505, CML509

and CML539 (short stature); and TZMI746, TZMI736 and CML539 (ear aspect and grain texture).

- With the exception of number of ears per plant, heritability in the broad sense (H_b) was high ranging from 0.86 to 0.97, with grain yield having H_b of 0.92. Narrow sense heritability was however low for grain yield (0.27), indicating that non-additive effects were very influential hence the hybridization strategy will be pursued to obtain MSV resistant varieties.
- The study also reveals crucial information that can be used to design MSV resistant hybrids. Grain yield was positively correlated with plant stature and negatively correlated with ear aspect due to preference of flinty types.
- It would also be possible to predict hybrid performance based on GD because the GD of SSR markers was significantly and positively correlated with F_1 yield means ($r = 0.53$, $p<0.001$), with SCA data ($r = 0.62$, $p<0.001$) and with mid-parent heterosis ($r = 0.68$, $p<0.001$).Mid-parental values were highly correlated with F_1 performance for MSV resistance ($r = 0.82$, $p<0.001$) and NLB ($r = 0.67$, $p<0.001$), indicating that resistance of hybrids could be predicted based on their hybrids.
- The diallel cross evaluation was also effective for grouping the inbred lines into heterotic groups based on the exceptional CML509 (A) x C92 (B) heterotic pattern. Two lines which had negative SCA with CML509 were assigned to group A and seven lines which displayed negative SCA with C92 were allocated to group B, and one line, CML539, which did not show significant SCA with both testers was not classified.
- Taking both exceptional heterotic pattern and SSR-marker data into consideration, the germplasm lines could be fitted into three broad groups for convenience of managing breeding programs, as follows: Group A lines are in the same cluster with CML509 and consisted of lines Osu23i, MUL114, CML505, CML509 and CML539; Group B lines were in the same cluster with C92 and consisted of the lines Z419, C92 and VHCY; Group C lines were not in the same cluster with C92 and CML509. The C group is constituted by S558, MUL71, TZMI736, and TZMI746. This has implications for breeding because new MSV resistant lines will be developed by crossing lines in the same cluster, while hybrids will be created by crossing lines from different genetic.
- Use of both SCA and SSR data was actually effective for classifying lines into heterotic groups, because 10 out of the 12 lines were clustered in the same heterotic groups using SCA and SSR clusters, with the exception of Z419 and CML539. This was

possible because the study indicated that SCA and GD data were actually highly correlated, and the clustering of germplasm with SSR marker data was consistent with pedigree data.

8.2.5 Role of epistasis in conferring MSV resistance in maize hybrids

The 12x12 diallel cross was also evaluated to determine the genetic effects responsible for conferring MSV resistance in hybrids.

- In order to validate the additive-dominance model, the assumption of no epistasis was tested with the aim of checking whether epistasis play any influential role for MSV resistance. The diallel cross was evaluated using the Hayman analysis which detected non-allelic interaction effects for MSV but not NLB. Therefore, the diallel analysis was reduced from 12 x 12 to 8 x 8 after elimination of four arrays of S558, CML509, Osu23i and Z419 as common parents, which were responsible for conferring epistasis for MSV resistance. This is the first time that epistasis has been found to be influential for MSV resistance in maize hybrids. Previous genetic models ignored epistasis therefore the implication is serious because epistasis was confounded with dominance gene effects, which inflated the levels of SCA. According to Hallauer and Miranda (1988) the diallel mating design is the most widely used and abused. Observation of epistasis also explains why it has been so difficult to develop productive MSV resistant hybrids compared to other diseases such as grey leaf spot.
- In the reduced diallel model (8x8), the parent MUL114 revealed the maximum number of dominant genes while CML505, TZMI736, MUL71 had the largest proportion of recessive genes for MSV resistance.
- Effectively the lines managed to transmit their resistance to hybrids, because out of 66 hybrids, 51 were resistant with scores of 1.0 to 2.4 for MSV disease. One immune parent (MUL 114) successfully transmitted MSV resistance to six single cross hybrids where it is involved.
- The inbred line MUL114 showed dominance for resistance when crossed with MSV susceptible lines.
- Occasionally MSV occurs together with NLB, therefore the diallel cross was also evaluated for NLB resistance: nine crosses were resistant, CML509 displayed

dominance for NLB resistance when crossed with susceptible lines. The cross Osu23i x CML539 had resistance to both MSV and NLB with scores 1.1 and 2.2, respectively.

- For NLB, the full model (12 x 12) was valid because epistasis was not detected. The lines CML505 and CML509 displayed the highest frequency of dominant genes while Z419 and VHCY had the maximum number of recessive genes.

8.2.6 Diallel cross analysis for yield stability

A 12 x 12 half-diallel was evaluated in four locations in Kenya over two seasons to investigate genotype x environment interactions effects for grain yield.

- The study confirmed significant genotype x environment interaction (44%) for grain yield of hybrids. Cross-over interactions were detected and the eight environments grouped into two mega-environments, namely medium-transitional locations where MUL71 x Osu23i were specifically adapted; and medium-late environments where Z419 x MUL114 was specifically adapted. Two hybrids CML509 x C92 and Osu23i x TZMI736 showed high yield potential and high stability.
- The first two principal component axes of the GGE biplot explained 79% to the total GGE variation.
- The contribution of GCA x E to G x E was 41% and that of SCA x E to G x E was 51%. Parent CML539 had the most stable GCA effect and a low contribution to GCA x E (3%). Although SCA effects were highly unstable, they showed a small contribution to SCA x E.

8.3 Implications of the findings for breeding MSV resistant hybrids

8.3.1 Survey and participatory rural appraisal

A high MSV incidence and severity was observed in Kiambu and Nyeri districts making these areas the most promising targets for MSV resistant varieties. A high adoption rate of hybrid varieties was noted, therefore modern varieties developed incorporating farmer and breeder traits would easily be adopted. One way to incorporate farmer desired traits especially sweet taste and resilience would be to perform participatory variety selection and on-farm trials. On-farm trials, which are conducted with progressive farmers, may not create the right representation for resilience. Thus, on-farm trials should preferably be conducted on farms

beleaguered with stresses as pointed out, including frost, drought, disease pressure and poor soils to represent the actual environment where MSV resistant hybrids will be grown.

Further, breeders in the public institutions need to work very closely with seed companies that market and promote their varieties to ensure the seed integrity is maintained and that farmers are aware of the value added traits in these varieties including MSV disease resistance. It was noted that farmers could quickly adopt new varieties provided they are thoroughly promoted, especially at field days, demonstrations and in the media. However, the knowledge of existence of the new varieties is not the main bottleneck to adoption but that new varieties are able to compete with old and existing varieties and contain the required end-user traits. In addition, these new varieties need to be stable in performance over time and space to ensure continuous use since most smallholder farmers are experimental, and will grow numerous varieties at their disposal. The consistence in growing a variety will depend greatly on seed integrity and stability of grain yield under stress.

Among the top most constraints highlighted by farmers some can be tackled through breeding while others will require policy interventions. Most importantly among abiotic constraints there is a breeding opportunity for drought tolerant varieties; while among field and storage pests, stem borers and weevils are major areas of research. Breeding for MSV resistance remains a priority as revealed by its prominence among diseases in addition to head smut.

Among the desirable traits, high yield potential remains a universal preference and as maize breeders improve resistance to various stresses, such as diseases, the agronomic appeal of hybrids has to be maintained. This is amidst the effects of changing climates thus development of “climate-ready” varieties is a major challenge. Selection of high yielding, early maturing, palatable (sweet taste) and MSV disease resistant hybrids will enhance fast adoption of new varieties in central Kenya.

8.3.2 Screening of diverse inbred lines for MSV and disease progress

The study on screening lines for MSV demonstrated high levels of disease resistance including immunity are available in regionally and locally adapted germplasm. The additional sources of resistance will be made available to breeding programmes focusing on MSV resistance. The sources have to be tested in other hybrid combinations and in different environments to evaluate efficiency of resistance. The best sources can then be used to improve the susceptible but adapted germplasm. It is crucial for streak resistant versions of inbred lines to be developed

that form the basis of hybrid breeding in Kenya. This will enhance the levels of resistance in commercial hybrids, reducing vulnerability to unforeseen MSV disease outbreaks.

Disease progression was variable based on environment and prevailing weather conditions. The timing of disease symptoms to capture greatest genetic differentiation is important for MSV. Later in the season, symptoms are masked by other biotic and abiotic stresses. The optimal time for symptom assessment would be between 42 and 56 days after inoculation in environments where disease progress is fast. However, where disease progress is slow symptoms can be taken around flowering. In this case, the influence of disease on yield potential can also be determined. Moreover, the high correlation that exists between AUDPC and final scores shows that a single score, properly timed can eliminate the need for several ratings especially when evaluating large numbers of germplasm. Another consideration for timing disease symptoms is assessment of heritability as this increases with time after infection so rating must be delayed to effectively differentiate genotypes for MSV resistance.

Unlike other diseases of maize, especially fungal diseases, days for flowering were not associated with disease progress with MSV. It is possible that the MSV pathosystem is not associated with host maturity; therefore, this disease can affect early maturing and late maturing varieties alike, complicating further MSV disease epidemiology. Also, selection for earliness does not improve response to MSV stress, as would be the case for other traits such as drought stress.

8.3.3 Genetic diversity based on SSR markers

The study on molecular diversity of germplasm with SSR markers identified the diverse and closely related lines within a pool of lines used for breeding MSV resistance in hybrids. Some of these lines have been used indiscriminately without regard of their molecular structure and heterotic grouping. This has led to the assertion that MSV resistant hybrids are not agronomically acceptable. If the right combinations of resistant germplasm are used it is possible to attain high yield potential. Further, breeders can also use germplasm within programmes that are most diverse and useful for inbred line extraction instead of introgressing diverse lines of mixed origin, which may not provide complementation of genes for resistance or agronomic performance.

There were high genetic distances among most pairs of lines indicating uniqueness of the majority of these lines and their utility for MSV breeding programs that emphasize hybrids. The results would also be useful in identifying pairs of lines suitable for QTL analysis. This study

involved a set of carefully selected 28 SSR markers, which could be replicated in other studies to reveal clear diversity patterns. It is likely that this set of markers could increase evidence that certain regions in the maize genome harbour genes of interest for plant improvement.

8.3.4 Gene action for grain yield and prediction of F_1 performance using SSR marker distances

Integrating the right germplasm in a breeding program and understanding the gene action involved in the key sources for the development of new disease resistant hybrids is generally a low cost technology. This has potential to contribute to improved yields for smallholder and resource poor farmers, and to food security in the region.

Except for grain yield and number of ears per plant, genetic factors for the other traits including plant stature, maturity and ear aspect were of additive nature, therefore prediction of hybrid performance for these traits can solely be based on the effect of the general combining ability. The preponderance of additive gene action in explaining variations in the agronomic characters indicates that genetic improvement can be achieved by accumulating favourable alleles from parents with highly negative GCA values in target genotype using conventional breeding methods such as recurrent selection and backcross breeding. Further, additive genes coupled with high narrow-sense heritability in the inheritance of these traits suggests that selection should be effective in early segregation generations. Lines with larger estimates of GCA for grain yield such as C92, CML539, Z419, and MUL114 can be used in the constitution of new populations aiming at attainment of higher genetic progress. Nevertheless, pedigree crosses must be made within identified heterotic groups to avoid disrupting hybrid development system.

In a traditional breeding program, thousands of crosses have to be done and F_1 grain yield has to be evaluated in experimental designs. This study showed that SSR-based genetic distances could be used to help in the choice of the crosses to be made among tropical maize lines derived from a broad genetic base population, in this way reducing the number of single cross hybrids to be evaluated.

8.3.5 Genetic analysis for MSV and NLB

The identification of superior hybrid combinations is crucial for breeding programmes that aim at improving resistance of MSV in maize cultivars. The ratio of non-additive gene effects

(dominance and/or epistasis) to the additive variance indicates the level of difficulty that may be encountered in improving a trait. Resistance to MSV and NLB was found to be additive based on high GCA effects compared to SCA effects and regression of mid-parental values on F_1 hybrid performance. However, heritability in the narrow sense were moderate to low strongly suggesting that non-additive effects controlled inheritance of MSV and NLB resistance as well. It was noted that for NLB, non-additive effects increased with disease pressure. While high disease pressure is required for effective selection of MSV resistance, selection for NLB could be achieved under low and high pressure. However if disease pressure is too high, genetic variance seem to be reduced. This study has identified a few sources that can be used for multiple disease resistance and stacking of resistance genes but additional sources from different genetic backgrounds are required to further broaden the genetic base.

There would be an interest on genotypes such as MUL114 and CML509, for MSV and NLB with lower severity of the disease, which also contributed to diminished disease presence and consequently showed negative estimates of GCA effects. Resistance to MSV has been reported to be controlled by a few major genes with minor modifiers (Rodier et al., 1995; Pernet et al., 1999a). The present study showed, for the first time, existence of epistatic effects, which should be considered in genetic studies of MSV resistance. Epistasis was important in three lines with MSV resistance CML509, Osu23i and S558. Moreover, recessive genes mostly conditioned disease response in these lines. Therefore, hybrid combination with these three lines will require that the other parent carry dominant genes for resistance for adequate complementation. However, since GCA effects constitutes a large component of the genetic variability for MSV and NLB resistance, a recurrent, backcross or pedigree selection programme to develop cultivars with higher resistance should be effective.

The study found few hybrids with superior resistance to parental lines for MSV resistance suggesting that selection for superior inbred lines should be more effective for improving MSV resistance than development of hybrids. When hybrids are required for disease resistance, however, the dominance for MSV resistance found in some parents (such as MUL114) may prove advantageous, particularly in environments conducive to MSV. This study showed thus that with the exception of strong sources of resistance with complete dominance, breeding hybrids with high levels of resistance would require that both parents carry resistance genes.

8.3.6 Genotype x environment analysis

Genotype x environment interaction complicates testing and selection of superior genotypes but may be overcome by a better understanding of genotype adaptation. Cross-over interactions are particularly important to plant breeders as they imply changes in ranking of genotypes across environments. In the presence of cross-over interactions as detected in this study, identification of specifically adapted genotypes would be the best approach for increasing genetic gains.

The GGE biplot was a useful tool for evaluating mean performance and stability within the set of genotypes and environments used in this study. Based on multiple options as regards genotype or environment scaling, it is a tool of choice for breeders interested in cultivar stability evaluation, identifying discriminating environments, partitioning multiple environments into mega-environments (thus cross-over interactions), and identifying winning genotypes for each mega-environment.

Hybrids with specific adaptation (MUL71 x Osu23i and Z419 x MUL114) and broad adaptation (CML509 x C92 and Osu23i x TZMI736) were identified; most importantly, hybrids resistant to MSV suited to specific regions were identified as the hybrids aforementioned rated highly for resistance. This study also identified lines (CML539 and C92) with stable GCA effects therefore these inbred lines are expected to transmit their MSV resistance gene effects consistently across environments. Further, stability of inbred lines for grain yield and combining ability could represent highly adapted lines, which can be used effectively in hybrid development. This is also important since these lines carry genes for improvement of MSV resistance.

Conclusion

The foregoing shows that the study was successful in identifying the best inbred lines for use in breeding new maize hybrids with MSV resistance. It was equally successful to establish for the first time the nature of gene effects, in particular the role of epistasis, and G x E in conditioning MSV resistance in hybrids. Results indicate serious implications for previous models that ignored epistasis in studying MSV resistance in maize. Above all, it is shown that SSR genetic distance data can be used to predict performance of hybrids, especially when the correct set of markers is used. Many previous studies have not found a significant relationship between GD and heterosis, due to G x E and using a wrong set of markers. The study also established the important heterotic groups, which will be exploited for effective and efficient development of

MSV resistant hybrids. These strategies will be recommended to breeding programs that emphasize MSV resistance in maize hybrids.

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