REACTION OF MAIZE GERMPLASM TO COMMON FOLIAR DISEASES AND VARIABILITY OF MAIZE STREAK VIRUS ISOLATES

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A thesis submitted in partial fulfilment of the requirements for the award of degree of Master of Science in Crop Protection

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2014

DECLARATION

This thesis is a presentation of my original research work and has not been presented for a degree in any other University.

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DEDICATION

To the Almighty God for his grace and strength every step of the way. My late father Charles who did what was considered then a taboo in our Meru culture, disposing land to educate a girl child. My mother Eunice for your prayers encouragement and making me what I am today.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to my supervisors Dr. W. M. Muiru, Prof. J. W. Kimenju and Dr. K. Njoroge for their excellent support and guidance throughout my studies. I am greatly indebted to Dr. Muiru and Prof. Kimenju for the provision of funds to do my research work.

I am grateful to Dr. S.T. Gichuki of Kenya Agricultural Research Institute (KARI) Biotechnology Centre for allowing me to do the molecular work at the centre. Great appreciation to all the laboratory staff at KARI Biotechnology Centre who worked with me all through especially Mr. Kenneth Monjero for his guidance in the molecular work. I thank Mr. Elias Thuranira of KARI Social Economics Department for his assistance in data analysis.

Thanks also to BecA- ILRI staff for their assistance in DNA sequencing. Lots of thanks goes to the staff in the Ministry of Agriculture in Kiambu, Embu and Nakuru Counties for their help in reaching the farmers.

I thank the University of Nairobi for giving me an opportunity to study. My great appreciation goes to the staff of the Department of Plant Science and Crop Protection. My heartfelt appreciation to Mr. Joseph Wagura for his assistance in the field work and data collection from day one to the end.

To my husband Dr. D. W. Miano for his encouragement, prayers and support all through my work. To my little angels Wangari, Gacheri and Wanjiku who had to endure my absence for so long. To all my brothers and sisters for their prayers and support. May the Lord richly bless all those who were directly and indirectly involved in this research work.

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ACRONYMS AND ABBREVIATIONS

AEZ	Agro ecological zones
ANOVA	Analysis of variance
BecA	Biosciences East and Central Africa
CAN	Calcium ammonium nitrate
CIMMYT	International Maize and Wheat Improvement Center
DAP	Diammonium phosphate
DNA	Deoxyribonucleic acid
DH01	Dryland hybrid 1
DH04	Dryland hybrid 4
DK8031	Delkab 8031
FAO	Food and Agricultural Organisation
GLS	Gray leaf spot
GPS	Global positioning system
H513	Hybrid 513
H516	Hybrid 516
H614	Hybrid 614
H624	Hybrid 624
H625	Hybrid 625
H628	Hybrid 628
H629	Hybrid 629
H6213	Hybrid 6213
ILRI	International Livestock Research Institute
KARI	Kenya Agricultural Research Centre
LH2	Lower highland 2
MSD	Maize streak disease
MSV	*Maize streak virus*
MLND	Maize lethal necrosis disease
NCLB	Northern corn leaf blight
NLB	Northern leaf blight
ORF	Open reading frame
PCR	Polymerase chain reaction
SSA	Sub-Saharan Africa
UM1	Upper midland 1
UM2	Upper midland 2
UM3	Upper midland 3
UM4	Upper midland 4

ABSTRACT

Maize is an important food crop that is grown in most regions of Kenya and it is consumed in various forms by over 80% of the population. Among the biotic constraints, foliar diseases cause heavy yield losses thus compromising food security in the country. This study was undertaken to determine the major foliar diseases infecting maize in different agro-ecological zones in Kenya and assess the reaction of various germplasm to these diseases. Isolates of *Maize streak virus* (MSV), a causal agent of one of the main maize diseases in the country was also characterized using molecular techniques.

A survey was conducted to determine the occurrence, incidence, severity and distribution of different diseases infecting the crop in different agro-ecological zones in Kiambu, Embu and Nakuru counties. The study focused on six diseases which were northern leaf blight (*Exserohilum turcicum),* common rust (*Puccinia sorghi),* maize streak disease (*Maize streak virus,* MSV), gray leaf spot (*Cercospora zea maydis,* (GLS), head smut *(Sphacelotheca reiliana)* and common smut *(Ustilago maydis).* Twenty maize varieties were also evaluated for their reaction to the different maize diseases in a field experiment at University of Nairobi Kabete Campus. During the survey, maize leaves with maize streak disease (MSD) symptoms were collected to study the variability of MSV. Degenerate primers for Geminiviruses were used to amplify C1/C2 regions of different isolates of MSV. Polymerase chain reaction (PCR) products were sequenced and nucleotides used to compare the Kenyan isolates within themselves and with other sequences from the GenBank.

Northern leaf blight, common rust and maize streak disease were found to be the three most prevalent and severe diseases in the different agro-ecological zones of the three counties. The same diseases were recorded in all the genotypes in the field evaluation. More efforts are therefore needed to manage the three diseases. Gray leaf spot, head and common smuts were also present, but were not widely distributed and had low incidence and severity both in the survey and field evaluations. These diseases should however not be ignored as their status may change with changes in climatic conditions.

The Kenyan isolates were highly similar to one another with 99 to 100% nucleotide and 95 to 100% amino acid sequence similarities. They were also closely related to others from the rest of the world with 98 to 100% nucleotide and 94 to 100% amino acid sequence similarities. They all belonged to the MSV-A strain, the main subtype infecting maize. The high percent sequence similarities indicate low variability within the sequenced C1/C2 region of the virus. This information is important to breeders since low virus diversity indicates that maize genotypes showing resistance to MSV may have wider areas where they can be grown without risk of infection by different virus strains.

CHAPTER 1

INTRODUCTION

1.1 World maize production

Maize (*Zeae mays* L.) is one of the major cereal crops and ranks third in production worldwide following wheat and rice (Faruq, 2008). In more than 20 developing countries in the world, maize is the single largest source of calories and protein for the poor and is a primary weaning food for babies. In sub-Saharan Africa maize is one of the most important staple foods, providing food and income to over 300 million resource-poor smallholders (Tefera *et al*., 2011). Over 650 million people consume on average 43 kg of maize per year (a 35% increase since 1960), reaching 85–140 kg in Kenya, Lesotho, Malawi, South Africa, Zambia and Zimbabwe (Lumpkin and Armstrong, 2009). Its cultivation spans the entire continent and is the dominant cereal food crop in many countries, accounting for 56% of total harvested area of annual food crops and 30-70% of total caloric consumption (Tefera *et al*., 2011).

1.2 Maize production in Kenya and its constraints

Maize is the most important staple food in Kenya (Muriithi and Mutinda, 2001), a widely consumed cereal by over 90% of the population (Muiru, 2008). A shortage of the commodity often leads to hunger. The major maize producing areas lie in the Rift Valley region and include Trans Nzoia and Uasin Gishu counties, although the cultivation is widespread. The bulk of the maize produced is consumed at household level. Kenya leads in maize consumption among East African countries with per capita annual consumption of 105 kg (Muiru, 2008) which translates to roughly 30 to 34 million bags (2.7 to 3.1

million metric tons) per year. Maize is also important in Kenya's crop production patterns, accounting for roughly 28% of gross farm output from the small-scale farming sector (Nyoro *et al*., 2004).

Rainfall failure or erratic rains is the principle course of low yields since maize is produced mainly under rain fed conditions (Nyoro *et al.*, 2004). In most parts of Kenya, maize is produced by smallholder farmers, majority of who are resource poor and can hardly afford the required farm inputs such as certified seeds. High costs and inadequate supply of certified seeds has led to underutilization of improved germplasm resulting in low productivity (Faruq, 2008). Soil fertility decline and weeds infestation also cause significant reduction in maize yields being estimated at 30-100% annually (Manyong *et al*., 2007; Karaya *et al.*, 2012).

Pests and diseases, both in the field and in storage, are the major biotic constraint limiting maize production in the country (Pandey and Pigali, 2000). Among the field pests, stem borers are estimated to cause annual yield losses of about 12 to 15% while the larger grain borer may result in 100% yield loss in storage (Tefera *et al*., 2011). Foliar diseases range from fungal, bacterial and viral and include maize rust, maize smut, northern leaf blight (NLB), ear rots, gray leaf spots (GLS), maize streak disease and the recently reported Maize lethal necrosis disease (Mwangi, 1998, Wangai *et al*., 2012). In storage, infection of maize by fungi results in production of toxins such as aflatoxins which result in deaths when infected grains are consumed (Njuguna *et al.*, 1992).

1.3 Problem statement and justification

Over time, national maize production has not kept pace with consumption. To bridge the ever-increasing gap between maize supply and demand, Kenya has been importing maize formally and informally across the border from Uganda and Tanzania in addition to large offshore imports from as far as South Africa, Malawi and United States of America (Nyoro *et al*., 2004). The low maize yields are due to disease and pests, among other factors. About 26 diseases caused by fungi, bacteria, nematodes, and viruses are reported to infect maize in Kenya (Njuguna *et al.,* 1992). Infection of maize by these diseases cause high yield losses (up to 100%) leading to decline in agricultural and per capita food production. In the recent years, no comprehensive study has been undertaken to document the incidence, distribution and the severity of these diseases in the maize growing areas, the last one having been done over a decade ago (Mwangi, 1998). There are many genotypes that have been introduced in the country and are not yet evaluated on how they react to the major maize diseases. Changes in climate may also have led to creation of conditions conducive for various diseases, resulting in introduction of diseases to new areas, and therefore changes in occurrence and distribution. New diseases that may not have been reported previously may have emerged, creating the need to screen the available maize germplasm for their reaction to these new diseases.

Maize streak disease, caused by Geminivirus *Maize streak virus* (MSV), is a major constraint to maize production in Kenya and leads to significant yield losses (Algbejo *et al.,* 2002). Whereas extensive characterization of MSV in different parts of the region

such as Uganda has shown limited variability between isolates, little has been done to show the virus diversity in Kenya.

1.4 Objectives

The broad objective of the study was to determine the reaction of different maize genotypes to foliar diseases and study the variability of *Maize streak virus* isolates in Kenya.

The specific objectives were;

(i) To determine the distribution and levels of foliage diseases of maize in diverse agro-climatic regions in Kenya.

(ii) To assess the reaction of different maize genotypes to the major maize diseases in Kenya.

(iii) To characterize *Maize streak virus* isolates using molecular techniques based on DNA sequences.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and world production of maize

Corn (*Zea mays),* also known as maize, is a member of the family *Poaceae* or *Gramineae*. It is indigenous to Mesoamerica and was domesticated in Mexico some 9000 years ago, then spread throughout the American continent and now cultivated all over the world (Hasanudin *et al*., 2012). Its diffusion to the rest of the world from the centre of origin was due to the discovery of maize cobs and phytoliths in South America (Hufford *et al*., 2012).

Maize was introduced into Africa by the Portuguese in the 16th Century in the Eastern and Western Africa coast and slowly moved inland through the incursion of slave traders who valued maize as a storable and easily processed grain (Miracle, 1966; Wambugu and Wafula, 2000). Its cultivation spans the entire continent and is the dominant cereal food crop in many African countries, accounting for 56% of total harvested area of annual food crops and 30-70% of total caloric consumption (Tefera *et al*., 2011). Maize consists mainly of starch with about 82% carbohydrates, 10% fats and 8% protein. It is also a good source of B vitamins, folate, vitamin C, beta-carotene and fiber (Tefera *et al*., 2011).

2.2 Maize production in Kenya

Maize is the most important staple food in Kenya and about 90% of the Kenyan population depends on the crop directly or indirectly in terms of food, feed, labour and income (Ali-Olubandwa *et al*., 2011; Ouma and De-Groote, 2011). The crop is grown in

all Provinces but the Rift Valley Province produces approximately 50% of the country's total maize production (Lukuyu, 2000; SDP, 2000; Ouma *et al*., 2002). The other key production areas include Western, Nyanza and Eastern regions which produce an average of 14% each while Central province produces about 6%. In Kenya, maize production is entirely dependent on rainfall (Wokabi, 1997).

2.3 Maize production constraints in Kenya

Maize production is constrained by both abiotic and biotic factors. Rainfall failure or erratic rains is one of the main causes of low yields (Wokabi, 1997; Nyoro *et al.*, 2004). In most parts of Kenya and Africa in general maize is produced by smallholder farmers, majority of who are resource-poor (Faruq, 2008). High costs and inadequate supply of certified seed and fertilizer has led to underutilization of improved germplasm resulting in low productivity (Faruq, 2008). The increase in population has resulted in land pressure, leading to continuous cultivation and therefore low soil fertility (De Groote *et al*., 2010; Karaya *et al.,* 2012). Where fertilizers have been used for long, the soils become acidic and improved maize germplasm and landraces used by farmers are generally sensitive to acid soils (Kisinyo *et al*., 2013).

Weeds like *Striga hermonthica* L., which is parasitic to cereals, is a major constraint to maize production in Western Kenya. Striga causes 30-100% loss of maize yield annually (Manyong *et al*., 2007; Odhiambo *et al*., 2011). Pests and diseases are also another biotic constraint that limits maize production (Pandey and Pigali, 2000). The crop is attacked by both field and storage pests. Some of the important ones in the field are stem borers while storage pests include grain weevils, larger grain borer and rodents. Among pests, stem

borers reduce maize yield in Africa through damaging the leaves, stem, ears, and kernels. In Kenya, they cause significant annual losses in maize estimated at 12.9% and through crop loss trials at 13.5% (Tefera *et al*., 2011) worth US $91 million. Diseases range from fungal, bacterial and viral and include maize rust, maize smut, northern leaf blight (NLB), ear rots, gray leaf spots (GLS), maize streak disease (Mwangi, 1998) and the recently reported maize lethal necrosis disease (Wangai *et al*., 2012).

Maize streak disease (MSD), caused by a Geminivirus *Maize streak virus* (MSV), is one of the major constraint to maize production throughout all temperate and tropical regions south of the Sahara (Martin and Shephered, 2009). Globally MSD is regarded as the third most serious disease of maize after NLB and GLS (Pratt and Gordon, 2006). In Africa, however, where maize is the staple food of the world's most malnourished people, MSD is a bigger problem than both NLB and GLS (Pingali and Pandey, 2000). MSD causes up to 100% yield losses in infected maize crops (Wambugu and Wafula, 2000; Alegbejo *et al*., 2002; Lagat *et al*., 2008).

2.4 Diseases affecting maize in Kenya

2.4.1 Gray leaf spot of maize

Gray leaf spot (GLS) is caused by the fungus *Cercospora zeae-maydis* (Pandey and Pingali, 2000). *Cercospora zeae-maydis* is composed of two genetically distinct butmorphologically similar species referred to as group I and group II. Additionally, an assumed variant of C. *sorghi* has been associated with GLS lesions and has been referred to as C. *sorghi* var. *maydis* (Kinyua *et al*., 2010). *Cercospora zeae-maydis* only affects maize (Ward *et al*., 1999). The disease is expressed in form of necrotic lesions, which

may coalesce and cause extensive blighting of leaves, thereby reducing the photosynthetic area of maize plants. Consequently, there is poor grain filling, which leads to low maize yields (Kinyua *et al*., 2010).

Gray leaf spot was not known to occur in Kenya before the first official report in 1995 and since then the diseases has spread to other maize agro-ecological zones of Rift valley, Central, and Eastern regions (Danson *et al*., 2004; Kinyua, 2004). Yield losses caused by GLS are estimated to be in the range of 30-50% in the country (Kinyua *et al*., 2010) making it a serious threat to the food security in Kenya. No-tillage practice encourages GLS development. When maize is planted in fields with infested maize residues remaining on the soil surface and with favourable environmental conditions for GLS, disease epidemics usually progress faster (Bigirwa *et al*., 2001). Removal or reduction of the amount of initial inoculum from the previous season's crop has been recommended for GLS management (Lipps *et al*., 1998). GLS epidemics are to a great extent due to various farming components leaving previous season's stover on the soil surface, type of maize variety, continous maize cropping and planting of maize in coffee or banana plantation mulched with infested stover (Bigirwa, 2001).

Cultural methods and use of fungicides such as benzimidazole and triazole have been used for GLS management (Ward *et al*., 1997) but have not been effective because fungicide application is costly and not practical in most operations for the resource-poor farmers and also unpredictable weather and the environmental side effects (Bigirwa *et al*., 2001; Danson *et al*., 2008). Most hybrids currently being produced in Kenya are

susceptible to GLS. Availability and adoption of resistant hybrids would provide a cost-effective means of controlling GLS (Ininda *et al.*, 2007). In Kenya development of improved lines with resistance to GLS and other foliar pathogens has commenced with resistance sources from IITA, CIMMYT and South Africa (Danson *et al*., 2008).

2.4.2 Common rust of maize

Common rust of maize is caused by *Puccinia sorghi* (Gutam and Stein, 2011) which is an obligate pathogen and occurs in all areas where maize is grown. In Kenya, it is prevalent on medium to high altitude zones including Rift Valley and Central regions (Danson *et al*., 2008). The disease is favoured by frequent rains, drizzle or dew with cool temperature and high humidity (Raid and Kacharek, 2006). Common rust may cause extensive yellowing and premature desiccation of maize foliage, resulting in leaf necrosis, and complete destruction of photosynthetic areas. In extreme cases, heavy rust infestations may result in stunting, incomplete ear tip fill, and pustules on ear husks, reducing marketability and yield. Yield loss varies depending on the percentage of leaf area infected and the host growth stages. Estimates of reduction in grain weight range from 3-8% for each of the total leaf area infected (Gautam and Stein, 2011). Yield loss in Kenya due to common rust can go upto 40% (Danson *et al*., 2008).

Use of cultural and chemical methods to control common rust has been inhibited by unpredictable weather patterns. A study carried out by Pataky *et al*. (1998) to show varietal reaction to diseases involving 36 open pollinated cultivars and commercial hybrids showed that the hybrids were more resistant than the open pollinated cultivars.

Breeding for resistance has been identified as a better method of managing the common rust disease.

2.4.3 Northern leaf blight of maize

Northern leaf blight of maize, also known as northern corn leaf blight, is caused by *Exserohilum turcicum* (pass), also referred to as *Helminthosporium turcicum*, *Dreschslera turcica* or *Trichometasphaeria turcica*. Generally, the disease is favoured by cool, wet weather, and high relative humidity (Muriithi and Mutinda, 2001) and is reported to be distributed all over Kenya especially highlands (Mwangi, 1998). Symptoms can range from cigar-shaped lesions on the lower leaves to complete destruction of the foliage, thereby reducing the amount of leaf surface area available for photosynthesis (Li and Wilson, 2013). A reduction in photosynthetic capability leads to a lack of carbohydrates needed for grain fill, which impacts grain yield (Li and Wilson, 2013). Heavily infected leaves appear dry as though affected by drought. The disease is more aggressive in young susceptible plants but the fungus is capable of infecting maize plants at all the stages of crop growth right from seedlings to maturity (Muiru, 2008). Northern leaf blight can cause yield loss of up to 70% (Muiru, 2008). The disease also causes qualitative changes in the seed resulting to decreased sugar content, germination capacity and severely infected plants are predisposed to stalk rot.

Host resistance is the cheapest and most effective way to control northern leaf blight and other blight diseases because chemical treatments are expensive, often ineffective and sanitation practices in crops search as maize are difficult to apply (Muriithi and Mutinda, 2001). A study carried out by Muiru (2008) to investigate the reaction of different

genotypes to northern leaf blight showed that a wide range of genotypes have varying levels of resistance to the disease. Resistance to northern leaf blight (NLB) of maize may be inherited monogenically or phylogenically (Pataky *et al*., 1986). Five dominant genes, *Ht*, *Ht2*, *Ht3, Htm1* and *Htn1*, control resistance to specific races of *E. turcicum* (Welz and Geiger, 2000).

2.4.4 Common smut

Common smut is caused by the fungus *Ustilago maydis* (Pataky and Snetselaar, 2006). The characteristic symptom of common smut is formation of galls or tumours on above ground parts of maize plant (CIMMYT, 2004). The galls are first covered by a shinning whitish green membrane. As the galls enlarge, they expose a powdery black mass. Throughout most of the world, common smut is considered to be a troublesome disease of corn (Pataky *et al*., 2006). The first record of common smut in Kenya was made in 1977 (Kedera, 1998). Since then, sporadic outbreaks have been observed in the central and western parts of the country. Yield losses associated with common smut are normally 10% over large areas. Though the yield loss is generally low, the disease is a potential threat to maize production and its frequency has been increasing over the years (Wang *et al*., 2006). Chemical and agronomic approach to management of the disease is inefficient, increases cost of production and also an environmental concern. Like many other diseases, host resistant is the most preferred solution for common smut (Pataky and Richter, 2007). Host resistance compared to other approaches is durable and eco-friendly to reduce the losses caused by common smut.

2.4.5 Head smut

Head smut is caused by the fungus *Sphacelotheca reiliana* (Njuguna, 2001). The disease mainly affects ears and causes significant damage to maize production. The most conspicuous symptoms are abnormal development of tassels, which become malformed and overgrown, black masses of spores that develop inside individual male florets and masses of black spores in place of the normal ear leaving the vascular bundles exposed and shredded (CIMMYT, 2004). The disease has been reported to occur in Australia, Mexico, France, Germany, Brazil, Russia, China and other maize growing areas of the world (Li *et al*., 2012). The disease can lead to significance yield loss in main maize growing regions of the world. The yield loss has been estimated to 0.3 million tonnes translating to 10-15% (Wang *et al*., 2012). Management of the disease depends on the field management practices and chemicals that have been in use. This is becoming time consuming and an environmental concern. The most favoured method for disease control is use of resistant varieties. However, a big challenge with this is lack of resistant germplasm (Wang *et al*., 2002).

2.4.6 Maize streak disease

Maize streak disease (MSD) is caused by *Maize streak virus*, MSV (Fuller, 1901; Storey, 1925). The disease is considered to be one of the major problems facing maize farmers in Kenya and other African nations (Magenya *et al.,* 2008; Martin and Shepherd, 2009) and is found wherever the crop is grown (Wambugu and Wafula, 2000). It is transmitted by leaf hoppers (*Cicadulina mbila and C. bipunctella zeae*) (Magenya *et al*., 2008).

Maize streak virus has a geminate, isometric particles measuring 20 nm in diameter occurring in pairs of 30 x 20 nm with a sedimentation coefficient of 54 and 76S and the particles contain single-stranded, predominantly circular, DNA with a molecular weight of $0.7x10^6$ daltons and exists as a single component (Lagat *et al*., 2008). The virus particles accumulate in the nucleus of the host cell producing large aggregates (Lagat *et al*., 2008), causing white to yellowish streaks on the leaves. The streaks are very narrow, more or less broken and run parallel along the leaves (Magenya *et al*., 2008). The reductions in yields depend on time of infection. Plants infected at early stage usually do not produce any cobs. Outbreaks of MSD have been experienced in different parts of Africa resulting in yield losses of up to 100% (Algbejo *et al*., 2002).

Different maize genotypes respond differently to MSD, making it difficult to estimate the average yield loss expected per infected plant given average timing of infection (Wambugu and Wafula, 2000). Some of the economic benefits that would be gained by eradication of MSD are lower and more stable maize prices for maize consumers and increased income for small scale farmers (Martin and Shepherd, 2009). While eradication of MSD is technically infeasible, it is potentially possible to produce maize genotypes that are completely immune to the disease.

As with most vector borne virus diseases, the epidemiology of MSD is extremely erratic. The erratic nature is certainly due to its being the product of interaction between multiple environmental and ecological factors that converge every 3 to 10 years to produce conditions conducive to the spread of the disease (Martin and Shepherd, 2009). A study

carried out in West Africa showed that the leafhopper populations build up with rains (Alegbejo *et al*., 2002). The number of leaf hoppers caught was very low at the onset of the rains, generally rising and reaching its maximum before the rains stopped. This strong association between MSD incidences, climate (temperature, rainfall, relative humidity) and leaf hopper population densities may ultimately make it possible to predict forecast of MSD epidemics (Reynaud *et al*., 2008). Certain farming practices are also associated with increased MSD incidence. For example, wherever multiple maize plantings are made during a growing season, the later plantings experience generally much higher incidence than earlier ones (Martin and Shepherd, 2009). Another important factor in fluctuations in MSD incidence is the density of leafhopper populations of the *Cicadulina* spp. mainly because it has a wider geographical range and a greater capacity to transmit MSV than other leaf hopper species.

2.4.6.1 Symptoms of maize streak disease

The maize plant is susceptible to MSV disease from emergence to flowering. The specificity of MSV infection in maize tissues shows that the virus occurs only in vascular tissues and does not invade the apical meristems within the shoot apex (Magenya *et al*., 2008). Infected maize plants with streak disease mainly manifest a minute pale circular spot on the lower exposed portion of younger leaves (Magenya *et al.,* 2008; Shepherd *et al*., 2009). As the disease progress new leaves emerge containing streaks up to several mm in length along the leaf veins with primary veins being less affected than secondary and tertiary veins (Shepherd *et al*., 2009). The highly sensitive maize varieties develop chlorosis of the entire leaf lamina, followed by plant death particularly if infection occurs in an early stage of plant growth (Magenya *et al*., 2008). Reduced photosynthesis and

increased respiration usually lead to reduction in leaf length and plant height; thus maize plants infected at an early stage become severely stunted producing undersize misshapen cobs or giving no yield at all. Yield loss in susceptible maize is directly related to the time of infection; infected seedlings produce no yield or are killed whereas plants infected at later times are proportionately less affected (Shepherd *et al*., 2009).

2.4.6.2 Management of maize streak disease

The maize adapted strain of MSV (MSV-A) that causes the most severe forms of MSD is moving far more rapidly throughout Africa than the less severe grass adapted strains of the virus. Although the cause of this increased mobility might be that the maize-adapted strain has a broader host range than its grass-adapted relatives, it is also possible that human trafficking of infected material may be responsible (Martin and Shepherd, 2009). Since MSD is not seed transmissible (Martin and Shepherd, 2009) and can only be transported by humans either within insects or symptomatic plants it might be in future prudent for African governments to regulate the movement of maize leaf material and insects between countries (Martin and Shepherd, 2009).

Resistance is by far the most attractive MSD management option (Martin and Shepherd, 2009). One problem associated with it is that the source of the MSV resistance tend to be fairly unappealing from an agronomic perspective and has proved difficult to remove undesirable traits associated with resistance genes. However, maize varieties with resistance to MSV have been developed (Alegbejo *et al*., 2002; Magenya *et al*., 2008) and many breeding programmes in Africa use these for incorporation into their varieties.

The resistance to MSV has been noted to be controlled by two genes but Kyetere *et al*. (1999) went further and demonstrated the presence of a single major gene (designated as *msv 1*) that controls MSV tolerance. Pathogen derived resistance has also been achieved where the pathogen in the plant is manipulated to protect it from the virus (Shephered *et al*., 2007). Plants infected with mild strain could be protected against infection by severe isolates or strains of that virus. This is also called cross protection. The process of introducing MSV resistance into commercially viable maize genotypes can be simplified by use of genetic engineering that provides a single dominant resistant gene (Martin and Shepherd, 2009).

Cultural practices aimed at reducing leaf hopper movement and subsequent spread of MSD between farms include incorporating barriers of bare ground between early and late-planted maize fields (Bosque-Perez, 2000), avoiding planting of maize downward from older cereal crops, adjusting planting dates to avoid infestation of young plants, use of crop rotation and intercropping (Magenya, 2008; Martin and Shepherd, 2009). Crop rotations and intercropping are considered a possible means of control by disrupting leaf hopper mating behaviour. However, studies in Uganda (Owor, 2008) showed that these methods have no discernable impact on MSD incidence, and do not necessarily improve yield. Despite this Owor (2008) also found that over 80% of farmers practiced intercropping probably not to manage MSD but to maximize their overall food production. Maize plants infected less than a week after germination produced no yield, at three weeks produced 5% yield and eight weeks produced almost full yield (Alegbejo *et al*., 2002; Magenya *et al*., 2008). Thus late planting of maize can achieve better yield

in MSV infected areas. However Shepherd *et al*. (2009) has recommended planting early in the season when inoculum is low.

Carbamate insecticides such as carbofuran applied to the planting furrow at 0.2g active ingredient per metre (ai/m) or seed coating was shown to significantly suppress leafhopper populations and reduce the incidence of MSD in the field by killing the leaf hoppers (Alegbejo *et al*., 2002; Magenya *et al*., 2008; Martin and Shepherd, 2009). Absolute protection against MSD is not achievable with insecticides, and unless an insecticide is able to kill leaf hoppers on contact (i.e. before they are able to feed) it could only be used to partially control MSD. For example, it is not uncommon for crops treated with carbofuran to experience disease incidence of up to 50%. In general repeated insecticide applications are often necessary to control new influxes of migrant leaf hoppers (Magenya *et al*., 2008). Besides controlling leaf hoppers it may be possible to control MSD through chemical control of wild grasses within and around maize fields which are a reservoir for MSV.

The potential of utilizing natural enemies (predators and parasitic) and entomopathogenic microbes for the control of leaf hoppers has been demonstrated in Asian countries. A number of parasitoids, predators and entomopathogens of important cicadellid pests including *Cicadulina* spp that occurs in India have been documented (Magenya *et al*., 2008).

2.4.7 Diversity of *Maize streak virus* isolates infecting maize

Viruses belonging to mastrevirus species are subdivided into strains containing isolates sharing >91% genome-wide sequence similarity and subtypes containing isolates sharing >98% similarity (Martin *et al*., 2001; Farauq *et al*., 2008). Eleven distinct MSV strains, classified as MSV-A to MSV-K, have so far been identified, of which only MSV-A are adopted to infecting maize, while majority of the rest are adopted to infecting wild grass species (Willment *et al*., 2001; Monjane *et al*., 2011). MSV-A has further been subdivided into five subtypes, MSV-A_1, MSV-A_2 MSV-A_3 MSV-A_4 and MSV-A_6, each being reported in different parts of sub-Saharan Africa (Varsani *et al*., 2008; Monjane *et al*., 2011).

Owor *et al*. (2007) used amplified fragment length polymorphism markers to characterize MSV isolates from Uganda and did polymerase chain reactions using degenerate primers. They reported that MSV-A was the predominant subtype in Uganda. Other scientists have done MSV characterization in other parts of the world using different methods. Studies on MSV characterisation have been carried out and sequenced isolates obtained from severely symptomatic maize and shared greater than 95% sequence identity (Peterschmitt *et al*., 1996). Full genomic sequences of MSV isolates obtained from wild annual grass species and wheat shared less than 90% identity with those of the isolates obtained from maize (Schnippenkoetter *et al.,* 2001). Minimal work has been done to characterize MSV isolates from Kenya.

CHAPTER 3

OCCURRENCE OF COMMON MAIZE DISEASES IN DIVERSE AGROCLIMATIC REGIONS IN KENYA

3.1 Abstract

Maize is an important food crop that is grown in most regions of Kenya. A survey was conducted to determine the occurrence, incidence, severity and distribution of different diseases infecting the crop in different agro-ecological zones in Kiambu, Embu and Nakuru counties. The study focused on six diseases which were northern leaf blight (*Exserohilum turcicum),* common rust (*Puccinia sorghi),* maize streak (*Maize streak virus*, MSV), gray leaf spot (*Cercospora zea maydis*, (GLS), head smut *(Sphacelotheca reiliana)* and common smut *(Ustilago maydis).* Northern leaf blight, common rust and maize streak were the three most prevalent and severe diseases in the different agro-ecological zones of the three counties. More efforts are therefore needed to manage the three diseases. Even though other diseases such as gray leaf spot, head and common smuts were present, they were not widely distributed and also had low incidence and severity. These diseases should however not be ignored as their status may change with changes in climatic conditions.

3.2 Introduction

Maize (*Zea mays* L.) is one of the major cereal crops worldwide and ranks third in production after wheat and rice (Muiru, 2010). It is important as a staple food in sub-Saharan Africa, providing food and income to over 300 million resource-poor smallholder farmers (Tefera *et al*., 2011). The crop provides high yields per unit of land,

making it a key crop in ensuring food availability and security for the consumers (Mboya *et al*., 2011).

Maize is a major staple in Kenya, with an average per capita consumption of 103 kg per year (De Groote, 2005; Tumusiime *et al*., 2010; Ouma and De-Groote, 2011). However, production is constrained by both abiotic and biotic factors (Wambugu and Wafula, 2000). Abiotic factors include climatic conditions, such as rainfall. The cultivation of the crop is almost entirely dependent on rainfall (Wokabi, 1997). Therefore, the production of maize is subject to sharp weather related fluctuations. Maximum crop production in a good season is about 34 million tons and it can drop to 18 million tons during drought years (EPZA, 2005).

Gray leaf spot (GLS) caused by *Cercospora-zeae maydis,* common rust of maize caused by *Puccinia sorghi,* northern leaf blight (NLB) caused by *Exserohilum turcicum* and maize streak are the most important maize diseases in Kenya (Mwangi, 1998; Muiru, 2008). *Exserohilum turcicum* is considered a serious pathogen where climatic conditions are cool with relative high humidity. Gray leaf spot is recognized as one of the yield limiting diseases with yield losses ranging from 10 to 70%. Losses of 90 to 100% have also been reported during times of GLS epidemics (Nzuve *et al*., 2013). The disease is most severe and damaging during high relative humidity and prolonged late season rain. Increased severity in Africa is associated with continuous cultivation of maize and use of susceptible maize cultivars (Danson *et al*., 2008). *Maize streak virus* (MSV) causes yield losses that range from a trace to almost 100% (Kyetere *et al*., 1999; Alegbejo *et al*.,

2002). Mwangi (1998) conducted a survey and found that the major maize diseases were widely distributed in the country and reported that MSV was severe in Eastern region while common rust was abundant in the Rift Valley Region.

Knowledge on the distribution of different diseases affecting maize production is crucial in crop protection. However, the most recent survey to determine the distribution of maize diseases in Kenya was carried out over a decade ago (Mwangi, 1998). No comprehensive study has been undertaken lately to document the incidence, distribution and the severity of these diseases in the maize growing areas as a tool to aid in prioritization of research in maize disease management country wide. Due to the introduction of new germplasm in the market and climate change, new pathogens are likely to be introduced into the country. This study was carried out to assess the incidence and severity of the major maize diseases in different agroecological zones in three different maize growing Counties of Kenya.

3.3 Materials and Methods

3.3.1 Survey to determine occurrence of maize diseases in different maize growing regions of Kenya

A survey was conducted in three different maize growing agro-ecological zones (AEZ) of Kenya represented by Kiambu Upper Midland (UM1, UM2 and UM4), Embu Upper Midland (UM1, UM2 and UM3), and Nakuru Lower Highland (LH2) and Upper Midland (UM3 and UM4). In each AEZ in the three counties, six diseases were assessed and these included northern leaf blight, common rust, gray leaf spot, head smut, common smut and maize streak disease. Sampling was carried out in four fields per AEZ approximately two

kilometres apart selected at random. Background information was obtained from farmers using a structured questionnaire (Appendix 1). The information captured in the questionnaire included size of the farm, area under maize, varieties planted, source of planting materials, cropping systems (pure or mixed stand / inter cropping), stage of crop growth when disease was first noticed, farmers perception of the diseases and measures employed by the farmers to manage the diseases.

Materials showing maize streak disease symptoms were collected from different plants along x-shaped transect stretching between opposite corners of each field for molecular characterization of the virus. The distance within sampled plants ranged between 0.5 to 5 metres depending on the size of the farms. These materials were placed in polythene bags and taken to the laboratory where they were stored at -80ºC awaiting further analysis. The stage of maize when survey was conducted was tasseling stage. Scoring for disease incidence and disease severity was done for northern leaf blight, common rust, gray leaf spot, maize streak disease, while for head smut and common smut, only disease incidence were taken. Identification of the diseases was based on visual symptoms as described in the CIMMYT field disease identification guide (CIMMYT, 2004). Disease severity was assessed using specific scoring keys described below (Section 3.3.2).

3.3.2 Assessment of disease prevalence, incidence and severity

Disease prevalence was assessed by determining the number of fields where a particular disease was recorded in relation to the number of fields sampled in different AEZ and counties. Disease incidence for all diseases assessed was determined by calculating the percentage of infected plants out of 10 plants taken along x-shaped transects.

Severity for NLB was rated using a modification of a scoring scale of 0 - 5 described by Elliots and Jenkins (1946) as follows; 0 = indicates no symptoms, 0.5 = very slight infection (one or two restricted lesions on lower leaves), 1 = slight infection (a few scattered lesions (3-8) on lower leaves, 2 = light infection (moderate number of lesions (9-15) on lower leaves), 3 = moderate infection (abundant lesions [>16] on lower leaves and a few on middle leaves), 4 = heavy infections (lesion abundant on lower and middle leaves and extending to the upper leaves), 5 = very heavy infection, lesions abundant on all leaves, plants may be killed.

Common rust was assessed using a key adopted from Danson *et al*. (2008) as follows; 1 = no symptoms, 2 = a few lesions corresponding to less than 1% of the leaf area with symptoms, 3 = several lesions, but not linked together corresponding to 1-5% infected leaf area, 4 = many lesions some linked together to form a necrotic (dead) area corresponding to 6-20% infected leaf area, 5 = necrotic areas linked together and a few leaf tips are dead corresponding to 21-50% infected leaf area, 6 = 50% of the leaf tips are dead corresponding to more than 50% leaf area with symptoms, 7 = most of the leaves are dead or the plant is dead.

Gray leaf spot was assessed using a modified scale by Danson *et al*. (2008) as follows; 1= no symptoms, 2 = moderate lesion below the leaf subtending the ear, 3 = heavy infestation on and below the leaf subtending the ear with few lesions above it, 4 = severe lesion on all but the uppermost leaves which may have a few lesions, 5 = all leaves dead.

Maize streak disease severity rating was done using a scoring key modified from Gichuru (2011) and Danson *et al*. (2008) as follows; 1 = no symptoms, 1.5 = very few streaks on leaves, 2 = light streaks on old leaves gradually decreasing on young leaves, 2.5 = light streaking on old and young leaves, 3 = moderate streaks on old and young leaves, 3.5 = moderate streaks on old and young leaves and slight stunting, 4 = severe streaking on 60% of leaf area, plants stunted, 4.5 = severe streaking on 75% of leaf area, plants severely stunted, 5 = severe streaking on 75% or more of the leaf area, plants severely stunted and or dying.

3.3.3 Data analysis

The data on disease incidence and severity was analysed using Genstat 13th Edition statistical program. Analysis of Variance (ANOVA) was used to test for significant differences and means separated using Fischer's Protected least significant difference at P=0.05

3.4 Results

3.4.1 Maize production practices in Embu, Kiambu and Nakuru counties

The background information collected using the questionnaires are summarized in Table 3.1 and Appendix 4. All the farmers interviewed in Embu County during the survey indicated that diseases were more severe where maize was planted in pure stand, while those in Kiambu and Nakuru indicated that the diseases were more severe both in pure and mixed cropping (Table 3.1). A high percentage of farmers in Embu (71%) managed the diseases while their counterparts in Embu and Nakuru were at 14% each. Out of those

who practised disease management in Embu, 80% sprayed, 50% rogued and 20% did not apply any control measures. In Kiambu the farmers did not spray any chemicals while 50% uprooted and 35% did nothing. In Nakuru 20% sprayed, none uprooted while 44% did nothing. Most of the farmers planted hybrids. The highest number of farmers who planted local varieties was in Kiambu at 70% while Embu had a much lower percentage of 30 and Nakuru had none (Appendix 4). Some of the hybrids found in Nakuru like KH 500 9A and Lentet are not found in any other region (Appendix 4).

Table 3.1 Background information gathered from maize farmers in three counties of Kenya

	Counties		
Parameters	Embu	Kiambu	Nakuru
Mode of cultivation			
Pure stand	0.0	0.0	100.0
Mixed stand	62.5	12.5	25.0
Both	29.5	31.7	39.0
Season when disease are more severe			
Rainy season	42.9	42.9	14.3
Dry season	50.0	22.2	27.8
Both	0.0	100.0	0.0
Not observed	0.0	33.3	66.7
Farmers perception on diseases			
Serious	60.0	30.0	10.0
Moderately serious	50.0	0.0	50.0
Not serious	17.2	34.5	48.3
Disease control/management done?			
Yes	71.4	14.3	14.3
No	21.2	36.4	42.4
Not applicable	0.0	0.0	100
Measures taken to manage			
Spray	80.0	0.0	20.0
Uproot	50.0	50.0	0.0
Not observed	20.6	35.3	44.1

3.4.2 Prevalence of maize diseases in different agro-ecological zones in Kiambu, Embu and Nakuru counties

Northern leaf blight, common rust and maize streak diseases were the three most common diseases in the different agro-ecological zones of the three counties (Table 3.2). There were significant (P=0.05) differences in disease prevalence for the three diseases between the different agro-ecological zones in the different counties. Northern leaf blight had the highest prevalence in UM3 (Embu) at 97.5% and lowest in UM2 (Kiambu) at 26%. Common rust had the highest prevalence value of 100% in UM1 in Kiambu and also the lowest prevalence in UM2 at 16% in the same county. Maize streak disease had lower prevalence compared to both common rust and NLB with the highest value at 45% UM1and the lowest at 2.5% in UM4. Gray leaf spot and the smuts were not common and had low prevalence across the counties where reported.

Table 3.2 Disease prevalence (%) for northern leaf blight, common rust, gray leaf spot, maize streak and smut in different agro-ecological zones in Kiambu, Embu and Nakuru Counties

		Disease					
County	AEZ	NLB	Rust	MSD	GLS	Head Smut	Common Smut
Embu	UM1	95.0a	70.0cd	45.0a	0	0	0
	UM2	92.5a	57.5d	15.0bc	0	0	2.5a
	UM3	97.5a	75.0bcd	12.5bc	5.0a	0	0
Kiambu	UM1	95.0a	100.0a	35.0ab	7.5a	0	5.0a
	UM2	26.0b	16.0e	22.0bc	0	4.0a	6.0a
	UM4	87.5a	57.5d	22.5abc	0	0	0
Nakuru	UM3	82.5a	97.5a	15.0bc	0	0	0
	UM4	72.5a	90.0abc	2.5c	0	0	0
	LH2	87.5a	92.5ab	15.0bc	0	1.3a	0
	p-value	<.001	<.001	0.039	0.017	0.251	0.014
	LSD	23.4	18.4	20.8	4.3	3.2	3.8
	CV	23.1	20.1	83.4	280.7	348.0	207.0

Key: AEZ = Agro-Ecological Zones, UM = Upper Midland, LH = Lower Highland, NLB = Northern Leaf Blight, GLS = Gray Leaf Spot, MSD = Maize streak disease
Means followed by the same letters along the columns within each of the AEZs are not significantly (P=0.05) different.

3.4.3 Incidence of maize diseases in different agro-ecological zones in Kiambu, Embu and Nakuru counties

Northern leaf blight, common rust and maize streak diseases had the highest percent incidence across the three counties (Table 3.3). There was a significant difference ($p<0.05$) in northern leaf blight incidence between the counties with Embu having the highest at 95% and the lowest being in Kiambu at 66.2%. There was also a significant difference in common rust incidence between counties with the highest percentage

incidence of 93.1% in Nakuru and lowest in Kiambu at 54.6%. A significant (P=0.05) difference in maize streak disease incidence was observed across the counties. Gray leaf spot was recorded in Embu and Kiambu, head smut in Nakuru and Kiambu and common smut recorded in Embu and Nakuru all with percentage incidence of less than 4.

Table 3.3 Mean disease incidence of common maize diseases in Kiambu, Embu and Nakuru counties

	Disease					
County	NLB	Rust	MSD	GLS	Head smut	Common smut
Embu	95.0a	67.5b	24.2ab	1.7a	0	0.8b
Nakuru	82.5ab	93.1a	11.9b	0	1.5a	3.9a
Kiambu	66.2b	54.6b	26.2a	2.3a	0.6a	0
p-value	0.027	<.001	0.081	0.278	0.347	0.009
LSD	19.9	19.2	14.1	3.1	2.0	2.5
CV	31.7	33.9	90.5	325.1	359.5	221.5

Key: NLB = Northern Leaf Blight, MSD = Maize streak disease, GLS = Gray Leaf Spot. Means followed by the same letters along the columns within each of the counties are not significantly (P=0.05) different.

Northern leaf blight, common rust, maize streak and common smut diseases all had a significant (P=0.05) difference in incidence across the agro-ecological zones (Table 3.4). However, there was no significant (P=0.05) difference between gray leaf spot and head smut. Disease incidences of GLS, head smut and common smut were below 10% across the agro-ecological zones. An observation was made in Kiambu zone UM2 where the disease incidence for northern leaf blight, common rust and maize streak disease was generally low compared to other agro-ecological zones.

Table 3.4 Mean disease incidence of common maize diseases in different agro-ecological zones in Kiambu, Embu and Nakuru Counties

		Disease					
County	AEZ	NLB	Rust	MSD	GLS	Head smut	Common smut
Embu	UM1	95.0a	70.0cd	45.0a	0	0	0
	UM2	92.5a	57.5d	15.0bc	0	0	2.5a
	UM3	97.5a	75.0bcd	12.5bc	5.0a	0	0
Kiambu	UM1	95.0a	100.0a	35.0ab	7.5a	0	5.0a
	UM2	26.0b	16.0e	22.0bc	0	4.0a	6.0a
	UM4	92.5a	57.5d	22.5abc	0	0	0
Nakuru	UM3	82.5a	97.5a	15.0bc	0	0	0
	UM4	72.5a	90.0a	2.50c	0	0	0
	LH2	87.5a	92.5ab	15.0bc	0	1.3a	0
	p-value	<.001	<.001	0.039	0.017	0.251	0.014
	LSD	23.4	18.4	20.8	4.3	3.2	4.4
	CV	23.1	20.1	83.4	280.7	348.0	207.5

Key: AEZ = Agro-ecological zones, UM = Upper midland, LH = Lower highland, NLB = Northern leaf blight, GLS = Gray leaf spot, MSD = Maize streak disease, Means followed by the same letters along the columns within each of the AEZs are not significantly (P=0.05) different.

Disease incidence for GLS, head smut and common smut were below 10% across agro ecological zones (Figure 3.1). Disease incidence was generally low in UM2 compared to other agro-ecological zones. There was a significance difference between the three main diseases, NLB, common rust and MSD in UM2. In UM1 there was a significance difference between MSD and common rust but no significant (P=0.05) difference between NLB and common rust. A similar trend was observed in UM3, UM4 and LH2.

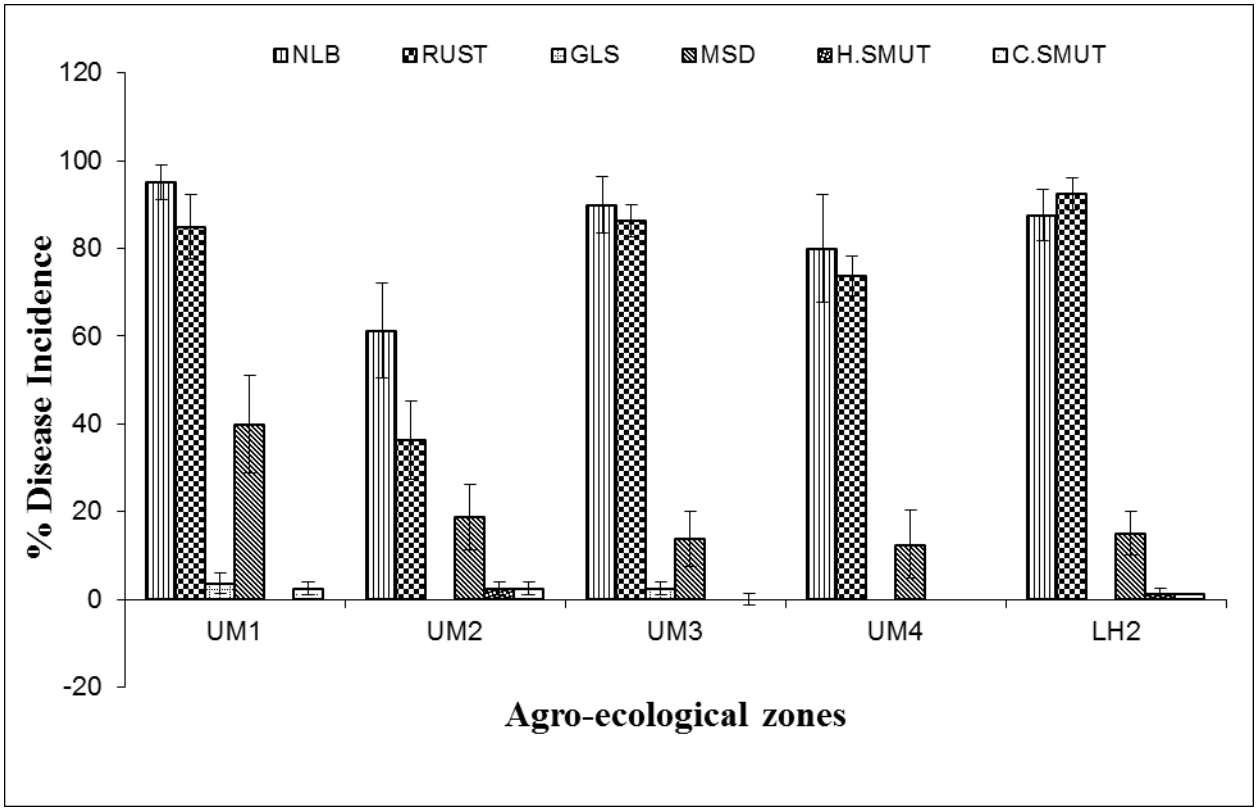


Figure 3.1 Incidence of the most common diseases of maize in different agro-ecological zones of Nakuru, Kiambu and Embu counties.
Key: AEZ = Agro-ecological zones, UM = Upper midland, LH = Lower highland, NLB = Northern leaf blight, GLS = Gray leaf spot, MSD = Maize streak disease, H.Smut=Head smut, C.Smut=Common smut

3.4.4 Severity of maize diseases in different agro-ecologizal zones in Kiambu, Embu and Nakuru counties

Four diseases (NLB, GLS, MSD and common rust) were assessed for severity (Table 3.5). There was no significant (P=0.05) difference observed across the three counties for NLB whereas differences were significant (P=0.05) for common rust and MSD. Gray leaf spot was only recorded in Embu and Kiambu with no significant (P=0.05) difference between the two counties.

Table 3.5 Disease severity of northern leaf blight, common rust, gray leaf spot and maize streak disease in Kiambu, Embu and Nakuru counties

	Disease			
County	NLB	Common rust	MSD	GLS
Embu	0.6a	1.7b	1.2ab	1.05a
Kiambu	0.5a	1.6b	1.3a	1.03a
Nakuru	0.5a	2.2a	1.1b	0
P-value	0.592	<.001	0.014	0.396
LSD	0.1871	0.2687	0.1784	0.760
CV	46.4	18.8	19.4	9.6

Key: - NLB = Northern Leaf Blight, MSD = Maize streak disease, GLS = Gray Leaf Spot. Means followed by the same letters along the columns within each of the counties are not significantly (P=0.05) different.

Northern leaf blight, common rust and MSD had a significant (P=0.05) difference across the agro-ecological zones (Table 3.6) while GLS was observed in only two zones, one zone in Kiambu (UM1) and one zone in Embu (UM3). The difference in GLS between the two zones was not significant (P=0.05).

Table 3.6 Disease severity of northern leaf blight, common rust, gray leaf spot and maize streak disease in different agro-ecological zones in Kiambu, Embu and Nakuru Counties

		Disease			
County	AEZ	NLB	Common rust	MSD	GLS
Embu	UM1	0.5b	1.8b	1.3b	0
	UM2	0.6abc	1.6b	1.2b	0
	UM3	0.7ab	1.8b	1.1b	1.2a
Kiambu	UM1	0.7ab	2.0ab	1.6a	1.1a
	UM2	0.2bc	1.2bc	1.3b	0
	UM4	0.8a	1.5bc	1.2b	0
Nakuru	UM3	0.4c	2.2ab	1.1b	0
	UM4	0.3cd	1.9b	1.0c	0
	LH2	0.6ab	2.3a	1.1b	0
	p-value	0.001	< .001	0.041	0.072
	LSD	0.2276	0.3618	0.2786	0.1110
	CV	35.0	15.7	18.8	8.7

Key: AEZ = Agro-ecological zones, UM = Upper midland, LH = Lower highland, NLB = Northern leaf blight, GLS = Gray leaf spot, MSD = Maize streak disease, Means followed by the same letters along the columns within each of the counties are not significantly (P=0.05) different.

Common rust had the highest severity score across the agro-ecological zones (Figure 3.2). In UM1, UM2, UM3, UM4 and LH2 there is a significance difference between the four diseases, NLB, common rust, MSD and GLS. There was a significance difference in UM3 between common rust and NLB. No significant (P=0.05) difference was observed between MSD and GLS.

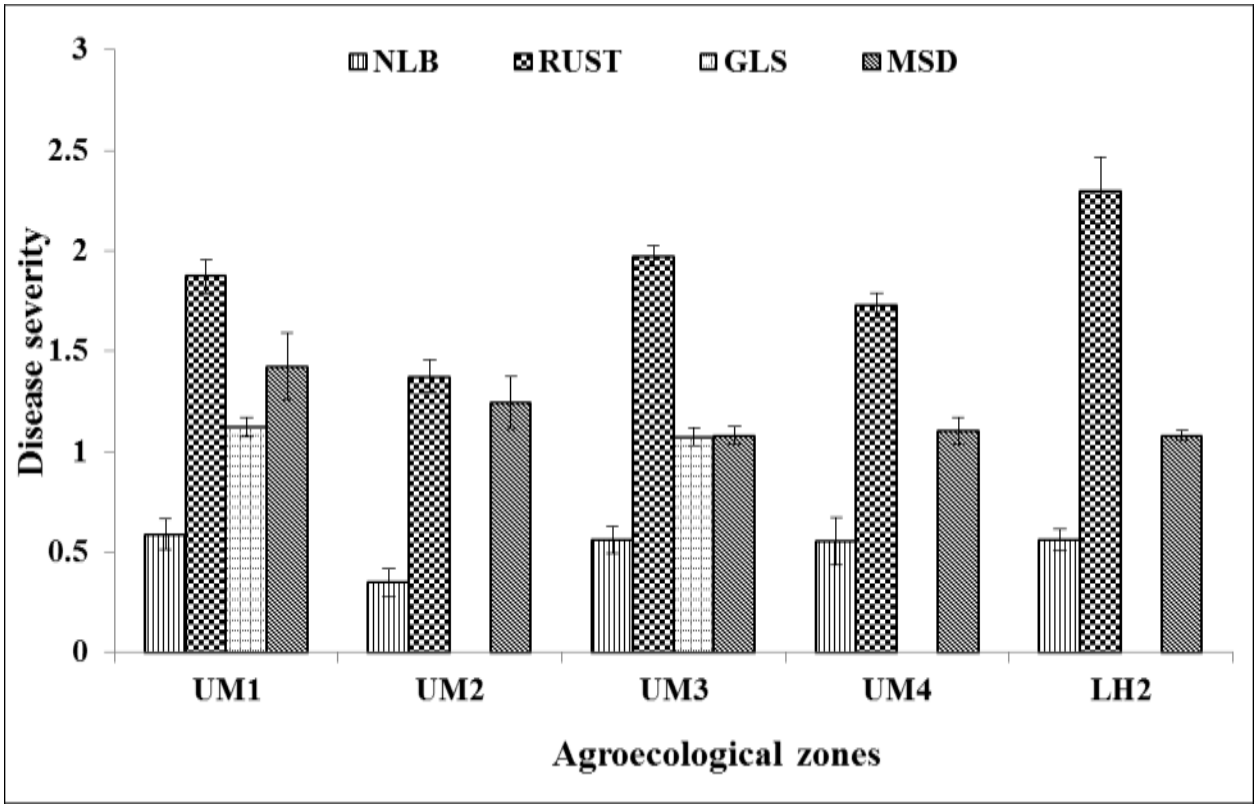


Figure 3.2 Severity of the most common maize diseases in different agro ecological zones.
Key: AEZ = Agro-ecological zones, UM = Upper midland, LH = Lower highland, NLB = Northern leaf blight, GLS = Gray leaf spot, MSD = Maize streak disease.

3.5 Discussion

Information generated from interviews with the farmers indicated that maize farming in Nakuru is different from Embu and Kiambu. Farmers in Nakuru (which is in the Rift Valley region) planted more hybrids and had higher adoption of certified seeds than farmers in the other two counties. Rift Valley is a net supplier of maize with its annual consumption at eight million bags (Business Daily, August 23rd 2013). In Nakuru the farms are generally large with some as big as 100 acres per farmer hence they practice mechanized farming, while farms in Kiambu and Embu are much smaller. Kiambu

County had the highest number of farmers planting local varieties of maize probably because of the size of the farms. On the management of the diseases half of the farmers interviewed in Kiambu and Embu managed the diseases by rogueing compared to Nakuru where they don't rogue but spray with chemicals. The differences in farming practices are expected to have an impact on incidence and severity of diseases.

The most important foliar diseases from the study were found to be northern leaf blight, common rust and maize streak diseases. Earlier studies identified the same three diseases as important across the country including the counties of Kiambu, Embu and Nakuru (Mwangi, 1998). The earlier study by Mwangi (1998) was carried over a decade ago, indicating that interventions carried out over the period did not have a major impact on the management of northern leaf blight, common rust and maize streak disease.

Northern leaf blight, common rust and maize streak diseases were distributed in all counties surveyed with high incidences and were not restricted to any particular agro-ecological zone. Kinyua (2004) and Muiru (2008) found NLB to be common in all the regions surveyed confirming that the disease has a wide ecological distribution. These are the areas with high annual rainfall of about 1100mm, high humidity and cool temperatures of between 11 to 27ºC. These conditions create ideal environment for infection and dispersal of inoculum (Mwangi, 1998).

Common rust was also most serious in all agro-ecological zones of Nakuru County and in zone UM1 of Kiambu. Mwangi (1998) also reported high common rust incidences of between 91% and 98% in Nakuru. The disease infects the leaves and sheath and is severe

on late planted maize. Maize streak disease was highest in Kiambu. This could be attributed to the farming systems in Kiambu where the land for farming is small. Farmers in the county practice alternate, successive and relay cropping of maize. Such farming practices and the presence of other hosts such as wild grasses increase MSD epidemics (Magenya *et al*., 2008). McLeod *et al*. (2002) reported that farmers in Kiambu ranked MSD as the most difficult problem of maize to manage. This may be due to the fact that farmers are unaware of the new cultivars that are resistant to MSD and also due to the non-availability of the seed in local stores. Hence, farmers resorted to planting the local cultivars which happen to be susceptible to the disease. The advantage of MSD tolerant cultivars is mainly because they reduce the effect of MSD (Bosque Perez *et al*., 1998).

In this study, gray leaf spot was only reported in two agro-ecological zones of Kiambu (UM1) and Embu (UM3). The disease was not known to occur in Kenya before 1995. However, Danson *et al*. (2004) and Kinyua (2004) reported that the disease has spread to other maize agro-ecological zones of Rift valley, Central and Eastern regions. No-tillage practice encourages GLS development especially when infected maize residues remain on the soil surface and environmental conditions are favourable like in coffee or banana plantations (Bigirwa *et al*., 2001). Kiambu and Embu are both in coffee and banana growing zones and relay cropping may have promoted the presence of GLS in these zones unlike in Nakuru where maize is planted in pure stand and for only one season a year.

Head smut and common smut were both recorded in all counties but in different agro-ecological zones. The two diseases are mainly spread through infected seeds and the use

of certified seeds is important in their management. The presence of the disease in a particular region may therefore be dependent on where the farmers obtained their seeds.

This study identified northern leaf blight, common rust and maize streak as the three most important diseases of maize in the different agro-ecological zones of Nakuru, Kiambu and Embu counties. Although there have been a lot of research efforts over the years to come up with management strategies to contain the diseases, their importance still remains high. It would be important to establish why the technologies that have been developed for their management have not had an impact. Other diseases such as gray leaf spot, head smut and common smut were present but not in high intensities. These diseases should not be ignored as they have the potential to change to epidemic levels especially with change in climatic conditions.

CHAPTER 4

REACTION OF MAIZE GERMPLASM TO FOLIAR DISEASES

4.1 Abstract

An experiment to determine the reaction of 19 different maize genotypes to different maize diseases was conducted at Kabete Field Station, University of Nairobi. The experiment was carried out in two seasons, one in the short rains (December to April 2010) and the other one in the long rains (April to July 2011). Monitoring and scoring for disease incidence and severity was done on weekly basis for seven weeks. All the genotypes screened were infected with two or more diseases. Northern leaf blight (NLB), common rust and maize streak disease (MSD) were the main diseases, both in incidence and severity in the two seasons. Gray leaf spot and the smuts had the lowest mean incidence. Disease severity in the different genotypes was generally low for all the diseases with none going above moderate infection of a score of two for GLS or a score of three for all other diseases. Three diseases namely northern leaf blight, common maize rust and maize streak disease had the highest severity observed in both seasons in all the 19 varieties screened. Gray leaf spot was minimal with means across all the genotypes being less than two. Hybrid varieties had generally higher MSD incidence and severity. Variety Pannar 4m-19 performed better against common rust while variety Pannar was more tolerant to MSD. These varieties are good sources of tolerance against the two diseases and should be included in breeding programs.

4.2 Introduction

Diseases are serious constraints to maize production in Kenya (Mwangi, 1998). The most economical way to manage the diseases is through use of resistant varieties. Different studies have been carried out in the past regarding the reaction of maize germplasm to different foliar diseases. Muriithi and Mutinda (2001), using commercial hybrids, indicated that only four out of 30 genotypes tested were susceptible to northern leaf blight. Mwangi (1998) carried out a study on status of northern leaf blight, *Phaeosphaeria maydis* leaf spot, southern leaf blight, rust, *Maize streak virus* and physiological specialization of *Exserohilum turcicum* in Kenya and reported that ideal temperature and moisture conditions coupled with susceptible genotypes could result in yield losses approaching those in the 50's. Evaluations done by Adipala *et al*. (1993) on Ugandan maize germplasm for resistance to *E. turcicum* showed that all were susceptible to the disease when inoculated with races of the pathogen. Mwangi (1998) also found out that even the hybrids that have been developed for maize improvement program were susceptible to two or three different pathogens.

In the recent past, different maize germplasm have been introduced into the country and some of them may not be well adapted to some of the agro ecological zones in Kenya. It is important to establish the reaction of these germplasm to the common maize diseases found in the country. The study was undertaken with the aim of screening the different germplasm for resistance to common maize diseases in Kenya. This is crucial given that there are new maize varieties in Kenya which may not have adapted to the ecological

conditions and disease pressure and hence their reaction to the diseases needs to be ascertained.

4.3 Materials and methods

4.3.1 Field evaluation of maize genotypes for reaction to common diseases

The field plots were established at Kabete Campus, University of Nairobi where the field was prepared by disc ploughing and harrowing to obtain a fine tilth. Two experiments were carried out in two seasons, season one in the short rains (December 2010 to April 2011) and season two in the long rains (April 2011 to July 2011).

A total of 19 different maize lines were planted at a depth of 5cm and DAP fertilizer applied at the rate of 10 g per hill. The trial was laid out in a randomized complete block design. The rows represented the experimental plots and these were replicated four times. Each row had 20 hills with inter row spacing of 75cm and 25cm between the hills and the blocks were spaced two metres apart. Two seeds were sowed per hill and thinning to one plant per hill was done when the maize seedlings attained a height of 15 cm.

The plants were exposed to naturally occurring populations of the different pathogens and two spreader rows of hybrid H6213 were planted in two rows all-round the blocks. These acted as sources of secondary inoculum. Watering was done using overhead irrigation to promote conditions that are favourable for disease development. Top dressing was done using calcium ammonium nitrate (CAN) fertilizer at the rate of 10 g per hill when the plants were about 40 cm in height. The crop was protected from stem borers using Beta-

cyfluthrin 0.5g/kg granules, which is a systemic insecticide and a synthetic pyrethtroid marketed as Bulldock 0.5 GR.

Seven plants were chosen at random from each row and tagged for identification purposes and data collection. Monitoring and scoring for reaction of different varieties to common diseases namely northern leaf blight (NLB), common rust, maize streak disease (MSD), gray leaf spot (GLS), common smut and head smut, was done weekly for seven weeks on the tagged plants. Severity scores based on percent leaf area affected were assessed per variety using the disease scales described in section 3.3.2 above. Disease incidence was calculated as a percentage of the plants infected out of the 20 plants per row.

4.3.2 Data analysis

The data was analysed using Genstat 13th Edition statistical program. Analysis of Variance (ANOVA) was used to test for significant differences and means separated using Fischer's Protected least significant difference at P=0.05.

4.4 Results

4.4.1 Incidence of different foliar diseases infecting maize in the field

Three major diseases NLB, common rust and MSD were present in the two seasons and all the varieties were susceptible to these diseases (Table 4.1). In season one, common rust had the highest incidence at an average mean of 14.29 for the variety DH04. In season 2, northern leaf blight (NLB) had the highest mean at 19.21 for the variety

Kinyanya Makueni and common rust followed closely with 18.32 for the variety Katumani. NLB was recorded throughout the growing period.

Gray leaf spot and the smuts had the lowest mean incidence ranging between 0 and 0.25 for common smut and 0 and 2.6 for GLS. The means for northern leaf blight were significantly ($p = 0.05$) different within the varieties with the most susceptible variety Katumani having incidence of 4.786 and the least susceptible Hybrid 625 with a mean of 2. The incidence of common rust was highest in DH04 with 14.29 and lowest in Pannar 4m-19 with a mean of 6.36 in season one. Season two had higher means where the most susceptible maize variety was Hybrid Katumani with 18.32 and the lowest incidence being observed in Pannar 4m-19 with a mean of 15.32.

Table 4.1 The incidence of different maize diseases at the Kabete Field Station, University of Nairobi

	Season one (short rains)					Season two (long rains)			
Variety/Disease	**NLB**	**Rust**	**MSD**	**GLS**	**C Smut**	**NLB**	**Rust**	**MSD**	**GLS**
Hybrid Katumani	4.78 a	14.11ab	1.96bc	0.67b	0.101a	18.75a	18.32a	10.68 cde	0
Kinyanya (Mak)	4.35ab	14.21ab	2.46bc	0.42b	0.17a	19.21a	17.50a	9.04 de	0.21ab
Kikamba	4.321ab	13.21ab	1.85bc	1.14b	0.14a	16.68a	15.68a	12.07cd	0.10ab
Pannar 4m-19	4.03 abc	6.36e	1.92bc	2.67a	0.03a	17.93a	15.32a	11.82 cd	0
Dry Highland 01	4.00 abc	12.39abc	1.67c	2.21ab	0.14a	18.46a	17.36a	13.64 abc	0
Hybrid 513	4.00 abc	12.39abc	3.50ab	1.21ab	0.10a	18.39a	16.32a	16.50 abc	0
Kisakwa Kitune	3.89 abc	11.68abcd	1.67b	0.28b	0.03a	-	-	-	-
Dry Highland 04	3.71 abc	14.29a	2.46bc	1.53ab	0.25a	18.54a	17.54a	15.25 abc	0
Pioneer	3.67 abc	13.21ab	1.28c	2.00ab	0	19.21 a	18.04a	13.00 bcd	0.35a
DK 8031	3.28 abc	12.96ab	2.75bc	1.75ab	0	17.96a	17.89a	8.21 e	0.14ab
Duma 43	3.17 abc	12.43abc	1.50c	1.14b	0	18.68a	17.61a	5.50 e	0.03b
Hybrid 624	3.17 abc	9.29cde	1.85bc	0.64b	0.07a	18.11a	16.21a	14.57 abc	0.14ab
Githigu	3.10 abc	12.21abcd	3.53ab	1.64ab	0	17.82a	16.64a	13.64abc	0
Hybrid 628	2.57 abc	8.64de	3.03ab	1.10b	0.10a	17.50a	16.68a	16.64 abc	0
Pannar	2.53 bc	12.82ab	1.50c	1.07b	0.25a	18.25a	16.93a	6.50 de	0.03b
Hybrid 629	2.46 bc	9.14cde	3.92ab	0.75b	0.25a	18.82a	17.64a	17.46 ab	0
Hybrid 614	2.42 bc	10.29bcd	5.14a	1.60ab	0	17.04a	15.54a	17.89 a	0
Hybrid 516	2.21 bc	10.64bcd	4.10ab	0.85b	0.21a	16.25a	16.79a	15.96 abc	0
Hybrid 625	2.00 c	10.79bcd	2.60bc	1.25ab	0.10a	17.86a	16.04a	14.32 abc	0
Hybrid 6213	-	-	-	-	-	17.07a	15.36a	17.32 ab	0
p-value	0.11	0.001	0.001	0.77	1.00	0.36	0.18	1.00	1.00
LSD	2.23	3.61	2.38	1.50	0.46	3.33	3.25	4.78	0.36

Key: NLB:-Northern leaf blight, GLS:- gray leaf spot, MSD:-Maize streak disease, CSmut- common smut, _ the variety was not planted in this particular season. Means are calculated as averages of 20 plants. Means bearing the same letters along the columns are not significantly (p=0.05) different.

4.4.2 Severity of different foliar diseases infecting maize in the field

Disease severity in the different genotypes was generally low for all the diseases with none going above moderate infection of a score of two for GLS or a score of three for all other diseases (Table 4.2). Three diseases namely northern leaf blight, common maize rust and maize streak disease had the highest severity observed in both seasons in all the 19 varieties screened. All diseases had higher severity scores in season one (short rains) except maize streak disease which had higher scores in season two (long rains). Gray leaf spot was minimal with means across all the genotypes being <2. There was no significant (P=0.05) difference for GLS in all the varieties screened for season two while there was a significant (P=0.05) difference for season one. All genotypes had a severity score of less than two for NLB and therefore the disease was not severe in any of the genotypes. The best performing variety against common rust was Pannar 4m-19 with severity scores of 1.96 in season one and 1.79 in season two. Severity scores for MSD were significantly (P=0.05) higher in hybrid varieties compared to other genotypes especially in season two where scores were greater than two. Variety Pannar was the best performer against MSD compared to all other varieties.

Table 4.2 Severity of different diseases infecting maize at Kabete Field Station, University of Nairobi during the short and long rain seasons

	Season one (short rains)				Season two (long rains)			
Variety/Disease	GLS	MSD	NLB	RUST	GLS	MSD	NLB	RUST
Kikamba	1.65a	1.35c	1.44ab	2.53b	1.01a	2.46a	0.92ab	2.25a
Githigu	1.64ab	1.62a	0.93c	2.24bcd	1.01a	2.52a	0.71bc	1.95abc
Pioneer	1.61ab	1.27c	0.95c	2.21cd	1.02a	1.76bc	0.67bc	2.04a
Duma 43	1.60ab	1.65ab	0.95c	2.39bc	1.01a	1.36cd	0.59c	1.94abc
Hybrid 614	1.58ab	1.65ab	0.75d	2.07cd	1.01a	2.45a	0.74bc	1.78c
Dry Highland 04	1.55ab	1.60a	1.03cd	2.40bc	1.02a	2.10a	0.72bc	1.93abc
Hybrid Katumani	1.53abc	1.30c	1.61a	2.52b	1.07a	2.22a	1.03ab	2.05a
Hybrid 516	1.51abc	1.49bc	0.87cd	2.11cd	1.02a	2.34a	0.79bc	1.77c
Hybrid 628	1.51abc	1.66a	0.80d	2.00cd	1.04a	2.49ab	0.66bc	1.82c
Hybrid 513	1.51abc	1.40bc	1.01cd	2.37bc	1.00a	2.31a	0.72bc	2.06a
Pannar 4m-19	1.49abc	1.39bc	1.02cd	1.96d	1.00a	1.79bc	0.60c	1.79c
Kinyanya Makueni	1.46abcd	1.40bc	1.23bc	2.49bc	1.05a	1.64bcd	1.14a	2.03a
Hybrid 624	1.45abcd	1.36c	0.97cd	2.12d	1.01a	1.90bc	0.92ab	1.90bc
Dry Highland 01	1.45abcd	1.20c	1.79a	2.53b	1.06a	1.93bc	1.04ab	2.24a
Hybrid 629	1.40abcd	1.82a	0.80d	2.15d	1.00a	2.23a	0.75bc	2.14ab
Pannar	1.38bcd	1.31c	0.94cd	2.23cd	1.02a	1.16d	0.65bc	1.81c
DK 8031	1.28cd	1.38c	0.92cd	2.37bc	1.07a	1.86bc	1.12a	1.96abc
Hybrid 625	1.26cd	1.47bc	0.63d	2.14cd	1.00a	2.29a	0.87ab	1.95abc
Kisakwa Kitune	1.20d	1.49bc	0.95c	2.83a	-	-	-	-
p. value	1.00	1.00	0.001	0.001	1.00	1.00	0.004	0.735
LSD	0.28	0.27	0.36	0.29	0.13	0.57	0.29	0.33

Key: NLB=Northern leaf blight, GLS= gray leaf spot, MSD=Maize streak disease. -The seeds for variety Kisakwa Kitune were not enough for season two. Means bearing the same letters along the columns are not significantly (p=0.05) different.

4.5 Discussion

All the 19 maize genotypes screened for reaction to different diseases were infected with two or more diseases. However, the disease pressure was generally low for all diseases during the two seasons, and therefore none of the genotypes had severe infection for any of the diseases. Season two (April to July) was planted in an adjacent field and prior to harvesting of maize from season one (December to April). Such relay cropping would generally provide enough inoculum that is critical in build up for the subsequent cropping

(Pedersen and Oldham, 1992; Mwangi, 1998). However, the inoculum build up (and therefore disease severity) was not as high as anticipated, due to the prevailing climatic conditions. Pan evaporation, mean temperatures and mean rainfall were higher in season one compared to season two hence the high disease levels in season one (Appendix 2).

Three diseases namely common rust, northern leaf blight and maize streak disease had higher incidences and severity in both seasons, with season two showing higher scores for maize streak disease. Northern leaf blight is favoured by mild temperature and high humidity (Muiru, 2008). Heavy dews, cool temperature and frequent rains create good sustained environmental conditions for NLB development (Mwangi, 1998). *Puccinia sorghi* Schw. the causal agent of common rust is a monocyclic heteroecious obligate pathogen of maize that occurs wherever maize is grown. The uredospores from season one act as the primary source of inoculum for season two and secondary spread. The disease gets severe on late planted maize (Seem, 1990; Mwangi, 1998). The first experiment was planted late in December 2010 long after the rains had set off. This explains the higher severity means in season one compared to season two. Another factor that could have contributed to higher severity means is the weather conditions during the time of the experiment. In season one the rainfall, temperature and pan evaporation were generally higher compared to season two making it favourable for the pathogens to thrive (Appendix 2). Studies have shown that higher incidence levels may not mean higher severity levels. Dillard and Seem (1990) carried out an experiment on incidence-severity relationship for common rust that showed that severity levels increased at low rates until

the third year when they managed to attain similar rates as incidence. Pannar 4m-19 had the lowest rust disease.

Maize streak disease is the only disease that showed higher incidence and severity in season two compared to season one. This can be attributed to a number of factors. Alternate and successive cropping of maize (Magenya *et al*., 2008) contributes to increased infections. Other studies have also shown that *Cicadulina mbila*, the vector for *Maize streak virus*, is more successful in acquiring MSV from maize than from other hosts (Bosque- Perez, 2000; Alegbejo *et al*., 2002). Given that at the time season two experiments was started season one experiment was still in the field and in a neighboring plot hence the vector could easily acquire and transmit the virus to the new crop. Once the virus is acquired it persists in the vector throughout its lifespan (Bock, 1974). Hence the virus acquired by the vector in season two continued infecting the crop the following season. Hybrid varieties with the higher MSD incidence and severity are popular varieties in the Kenyan Highlands and have been reported to be susceptible to MSV (Magenya *et al*., 2008).

All genotypes reacted the same to GLS infection even though incidence and severity reduced between season one and season two. This too was due to the fact that the environment for the growth of the fungus was conducive in season one where there was higher amounts of rainfall and higher temperatures.

All the recommended varieties of 600, 500 series, Hybrid Katumani, varieties from outside the country like Pannar from South Africa, Duma 43 and all landraces were found to be susceptible to two or more of the pathogens observed. This is a clear indication that maize varieties grown in Kenya are susceptible at various degrees to different pathogens infecting maize in Kenya.

Although the disease pressure was generally low, a few varieties had lower disease compared to others. Variety Pannar 4m-19 performed better against common rust while variety Pannar was more tolerant to MSD. These varieties may be good sources for tolerance to the two diseases and should be incorporated in breeding programs.

CHAPTER 5

CHARACTERIZATION OF MOLECULAR DIVERSITY OF MAIZE STREAK VIRUS ISOLATES FROM SELECTED MAIZE GROWING REGIONS OF KENYA

5.1 Abstract

A survey was conducted in different agro-ecological zones in Kiambu, Embu and Nakuru counties to determine the occurrence of common diseases infecting maize. During the survey, samples showing symptoms similar to those caused by *Maize streak virus* (MSV) were collected for molecular characterization of the virus. Out of 30 samples collected, eight tested positive for the presence of the MSV. Polymerase chain reaction (PCR) products from the C1/C2 region of the virus were sequenced and nucleotide and amino acid sequences used to compare the Kenyan isolates with themselves and with other sequences from the GenBank. The Kenyan isolates were highly similar to one another with 99 to 100% nucleotide and 95 to 100% amino acid sequence similarities. When compared to other MSV isolates from the rest of the world, the Kenyan isolates had 98 to 100% nucleotide and 94 to 100% amino acid sequence similarities. They all belonged to the MSV-A strain, the main subtype infecting maize. The high percent sequence similarities indicate low variability within the sequenced C1/C2 region of the virus. This information is important to breeders since low virus diversity indicates that maize genotypes showing resistance to MSV may have wider areas where they can be grown without risk of infection by different virus strains.

5.2 Introduction

Maize streak disease (MSD) was first documented in Natal, South Africa by Fuller (1901). However, its causal agent, the *Maize streak virus* (MSV; genus *Mastrevirus*, family *Geminiviridae*), was described later by Storey (1925). The virus is distributed throughout the African continent and surrounding islands (Monjane *et al.*, 2011). It is one of the most economically significant members of the *Geminiviridae* family (Bosque-Perez, 2000). Despite being restricted to Africa and its neighboring islands, globally MSD is regarded as the third most serious disease of maize after northern leaf blight (NLB) and grey leaf spot (Pratt and Gordon, 2006). In Africa, however, MSD is a bigger problem than both NLB and GLS (Martin and Shepherd, 2009).

The reductions in yields due to MSV depend on time of infection. Plants infected at early stage usually do not produce any cobs. Epidemics resulting in economic losses of up to 100% have been reported in at least 20 African countries including Nigeria, Ghana, Sudan, Cameroon, Zimbabwe, Tanzania, Togo, Benin, Bukina Faso, Sao Tome, Uganda and Ethiopia (Lagat *et al*., 2008). In East Africa MSV has been identified as a major constraint to maize production in Kenya (Magenya, 2008; Martin and Shephered, 2009; Gichuru *et al*., 2011) and Uganda (Owor, 2008). The disease is more serious in mid-altitude areas and mid highland zones (Lagat *et al*., 2008).

Viruses belonging to mastrevirus species are subdivided in to strains containing different isolates (Martin *et al*., 2001; Farauq *et al*., 2008). Eleven distinct MSV strains, classified as MSV-A to MSV-K, have been identified, of which only MSV-A are adopted to

infecting maize, while majority of the rest are adopted to infecting wild grass species (Willment *et al*., 2001; Monjane *et al*., 2011). MSV-A has further been subdivided into five subtypes, MSV-A_1, MSV-A_2 MSV-A_3 MSV-A_4 and MSV-A_6, each being reported in different parts of sub-Saharan Africa (Varsani *et al*., 2008; Monjane *et al*., 2011). MSV-A is the predominant subtype in Uganda (Owor *et al*., 2007). However, limited work has been done to characterize MSV isolates from Kenya. This study aimed at characterizing different MSV isolates collected from different maize growing regions of Kenya using molecular technique.

5.3 Materials and Methods

5.3.1 Sample collection and nucleic acid extraction

Young maize leaves were collected from plants showing MSD symptoms were collected in Kiambu, Embu and Nakuru counties. Symptoms included chlorosis with broken yellow streaks along the veins, contrasting with the dark green color of normal foliage. The samples were transported to the Kenya Agricultural Research Institute (KARI) Biotechnology Centre laboratories in Kabete, in a cool box. Total DNA was isolated from leaf tissue using Dellaporta extraction method (Dellaporta *et al*., 1983). About 200 mg of leaf tissue was ground using a motor and pestle. Five hundred microlitres (500 µl) of Dellaporta buffer was added twice to the leaf sample and the mixture crushed into fluid state. The fluid was transferred into labeled microcentrifuge tubes, where 140 µl of 10% Sodium dodecyl sulphate (SDS) was added and the tubes gently inverted in a rack. The samples were incubated in a water bath at 65^{o}C for 20 minutes with gentle inversion after which 250µl of 8M potassium acetate was added and tubes gently inverted. The samples were placed on ice for 10 minutes and then spinned at maximum speed of 14000 rpm for

10 minutes. The supernatant was collected and transferred into sterilized labeled micro centrifuge tubes. The samples were resuspended in 600µl cold isopropanol (–20^{o}C). Spinning was done at a speed of 14000rpm for 5 minutes and the supernatant discarded and the pellets were washed in 200µl of 70% ethanol. Spinning was done once again at a speed of 14000rpm for 3 minutes. The ethanol was discarded and the pellets within the micro centrifuge tubes air-dried in a lamina flow hood on dry clean paper towels for 20 minutes. The pellets were resuspended in 70µl molecular water and stored in -80^{0}C until PCR was done.

5.3.2 Amplification of *Maize streak virus* by Polymerase chain reaction

The extracted genomic DNA was amplified by Polymerase Chain Reaction (PCR) using Geminivirus degenerate primers G4F (5'-AGB KKK KBC ATC GST TCG T-3') and G6R (5'-CTG TAC ATC CTC GGG CCA ACA AGA AC-3'). These primers were used to anneal to regions of open reading frame (ORF) C2/C1 as described by Van Antwerpen and Rutherford (2008). The primers were expected to amplify a 900 bp fragment. PCR was performed using a Gene Amp PCR system 9700. Amplifications were performed in 25µl reaction volumes containing 2µl of the DNA extract, 1.25µl and 1.87µl of forward and reverse primers respectively, 0.625µl of 10mM dNTP mix, 0.16µl of Taq DNA polymerase, 2.5µl reaction buffer, 3.5µl of 25mM $MgCl_2$. PCR was run at 35 cycles of 94°C for 5min, 60°C for 1 min, 72°C for 2 min and 72°C for 10 min. PCR products were assessed by electrophoresis in 1.2 % agarose gels in TBE buffer, stained with ethidium bromide, and viewed under ultraviolet (UV) light.

5.3.3 Purification and sequencing of PCR products from *Maize streak virus* isolates

Polymerase chain reaction products from four samples with the strongest bands when viewed under the UV light were purified for sequencing using Qiagen kit following manufacturer's instructions (QIAGEN Inc., Valencia, CA). Five (5) volumes of binding buffer (PB) was added to one volume of PCR products (100µl to 20µl) and transferred to Qia-quick column in provided 2ml collection tube. The sample was applied to the column and spinned for 1min. The flow through was discarded and the column returned back in the same tube. Then 0.7ml wash buffer (PE) was added to the Qia-quick column and spinned for 1min at 13000 rpm. The flow through was discarded and placed back to the column in the collection tubes. A short spin was performed to remove residual wash buffer. The columns were placed in clean 1.5ml micro centrifuge tube, 30µl elution buffer (buffer EB) or molecular grade water (pH7) was added to elute DNA and spinned for 1 minute at 13000 rpm. The eluted DNA was used for sequencing. Nucleotide sequences were determined at Biosciences for East and Central Africa (BecA), ILRI.

5.3.4 Data analysis

The obtained sequences from ORF C1/C2 were compared with corresponding sequences of other MSV infecting maize obtained from the GenBank. Relationships between the different sequences from the Kenyan isolates were compared to each other and with others obtained from GenBank. The sequences and their accession numbers are listed in Table 5.1. Percent nucleotide identities were determined using pairwise global alignment. The alignments were used to determine the percent nucleotide sequence identity using ClustalX version 1.83 procedure (Jeanmougin *et al*., 1998). Multiple sequence alignments

and phylogenetic analysis using neighbor-joining and bootstrap option (1000 replicates) were carried out using MEGA4 (Tamura *et al*., 2007).

Table 5.1 GenBank accession numbers for MSV sequences used in this study.

Isolate	GenBank accession number	Origin
MSVA_CM_Baf1_Cam11	HQ693319	Cameroon
MSVA_CF_Bim1_Car16	HQ693305	Central African Republic
Bambui_MB1K1	FM210279	Cameroon
UBush_53	EF547075	Uganda
UMasin_149	EF547098	Uganda
MSVA_MZ_Pem4_Moz40	HQ693365	Mozambique
MSVA_CM_Baf4_Cam19	HQ693322	Cameroon
MSVA_CF_Yal1_Car32	HQ693317	Central African Republic
MSVA_BF_Lou1_BF5	HQ693282	Burkina Faso
MSVA_ZW_Mas4_Mic6	FJ882145	Zimbabwe
MSVA_ZM_Chi1_Z6	HQ693450	Zambia
MSVA_KE_Nye1_Ken11	HQ693332	Kenya
MSVA_KE_Nan2_Ke2	HQ693330	Kenya
MSVA_KE_Nan1_Ke3	HQ693329	Kenya
MSVA_CM_Baf6_Cam23	HQ693324	Cameroon
MSVA_NG_Eji3N29a	HQ693371	Nigeria
MSVA_NG_Ile_N34	HQ693382	Nigeria
k40	Test isolate	Kenya
k39	Test isolate	Kenya
k38	Test isolate	Kenya
k37	Test isolate	Kenya
MSV-K_Zw-Mic23	EU628644	Zimbabwe

5.4 Results

5.4.1 Polymerase chain reaction amplifications

Out of a total of 30 samples collected, eight tested positive for MSV using primers G4F/G6R and produced the expected 900 nucleotide PCR fragment. No amplification products were generated from some of the maize samples even though they had symptoms similar to those observed in maize infected with MSV. The bands from four of

the positive samples were weak, indicating low quantities of PCR products and therefore could not be used for sequencing. The remaining 4 samples with strong bands (one from Nakuru and three from Kiambu counties) were purified and submitted for sequencing. Figure 5.1 shows the DNA bands after purification of PCR products.

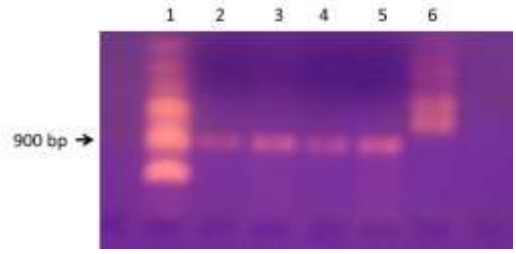


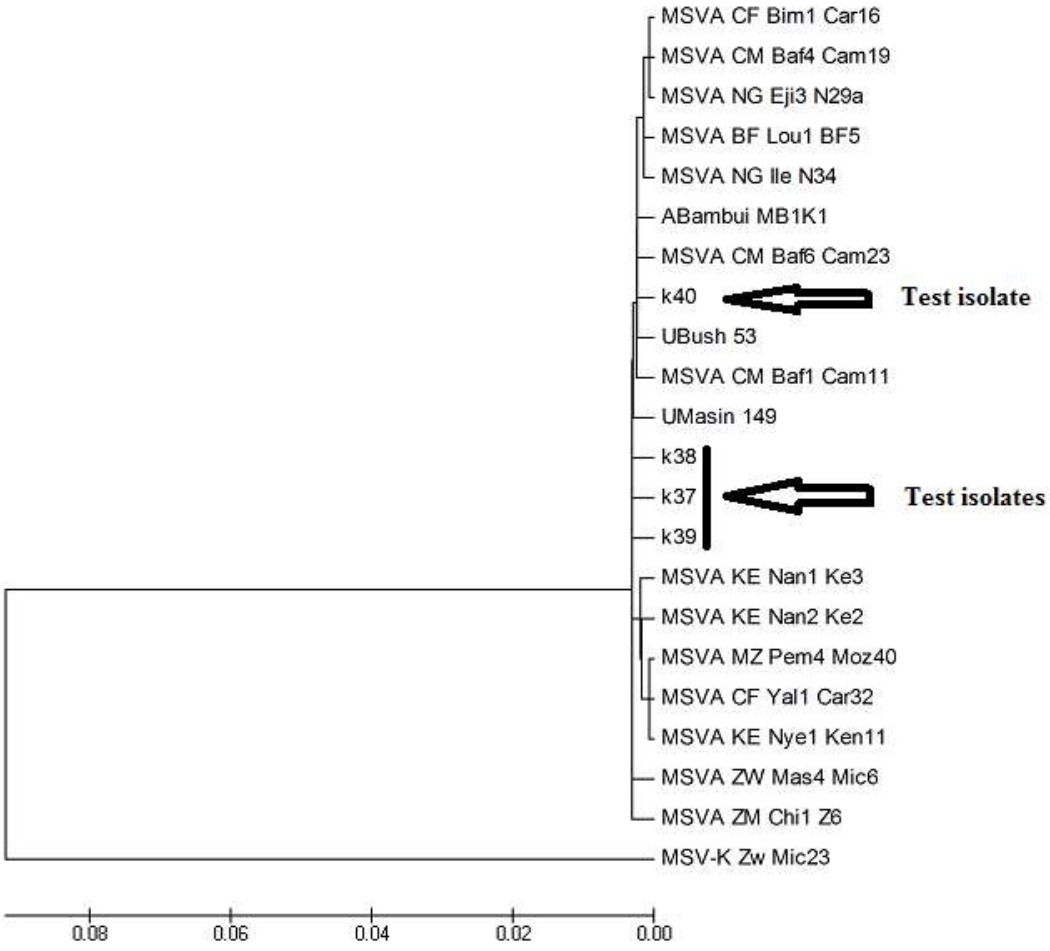
Plate 5.1 Purified DNA from Polymerase chain reaction amplification products generated from maize showing maize streak disease using *Maize streak virus* primers G4F/G6R (Van Antwerpen and Rutherford, 2008). Lane 1: molecular weight marker, Lane 2-5: purified DNA from PCR products generated from maize samples collected from different maize growing regions of Kenya, Lane 6: water control

5.4.3 Results from sequence analysis and comparisons

Pairwise comparisons of nucleotide sequences from the four Kenya isolates gave identities ranging from 99 to 100% while amino acid identities ranged from 95 to 100%, indicating that they are isolates of the same virus (Table 5.2). Comparisons with MSV nucleotide sequences from other parts of the world gave 98 to 100% nucleotide and 94 to 100 amino acid sequence similarities to the MSV-A strains. Phylogenetic analysis using nucleotide sequences resulted in only one group of isolates being visually distinguished (Figure 5.1).

Table 5.2 Percent nucleotide (upper diagonal) and amino acid (lower diagonal) sequence identities for *Maize streak virus* isolates from Kenya and other parts of the world

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	MSVA_CM_Baf4_Cam19		99	99	99	99	99	99	99	99	99	99	98	99	99	98	99	98	98	98	98	98	83
2	MSVA_BF_Lou1_BF5	100		99	99	99	99	98	99	99	99	99	98	98	98	98	98	98	98	98	98	98	83
3	MSVA_NG_Eji3_N29a	100	100		100	99	99	99	99	99	99	99	98	99	99	99	99	99	99	99	99	99	84
4	MSVA_CF_Bim1_Car16	100	100	100		99	99	99	99	99	99	99	98	99	99	99	99	99	99	99	99	99	84
5	MSVA_NG_Ile_N34	100	100	100	100		99	99	99	99	99	99	98	99	99	99	99	99	99	99	99	99	84
6	ABambui_MB1K1	100	100	100	100	100		99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	84
7	MSVA_CM_Baf6_Cam23	99	99	99	99	99	99		98	99	99	99	98	98	98	98	98	98	98	98	98	98	84
8	k40	99	98	99	99	99	99	98		99	99	99	98	99	99	99	99	99	99	98	98	98	83
9	UBush_53	100	99	99	100	99	100	99	99		99	99	99	99	99	99	99	99	99	99	99	99	83
10	MSVA_CM_Baf1_Cam11	99	99	99	99	100	99	98	99	100		99	99	99	99	99	99	99	99	99	99	99	84
11	UMasin_149	99	98	98	99	99	99	98	98	99	99		98	99	99	99	99	99	99	99	99	99	84
12	k38	95	94	95	95	95	95	94	95	95	95	94		99	99	99	99	99	99	99	99	99	83
13	k37	98	98	98	98	98	98	98	98	99	98	98	95		99	99	99	99	99	99	99	99	84
14	k39	98	98	98	98	98	98	98	98	99	98	98	95	99		99	99	99	99	99	99	99	84
15	MSVA_ZW_Mas4_Mic6	98	98	98	98	98	98	97	98	98	98	98	95	99	99		99	99	99	99	99	99	84
16	MSVA_MZ_Pem4_Moz40	98	98	98	98	98	98	98	98	99	98	98	96	99	99	100		99	99	99	99	99	84
17	MSVA_ZM_Chi1_Z6	98	98	98	98	98	98	98	98	99	98	98	96	99	99	100	100		99	99	99	99	84
18	MSVA_CF_Yal1_Car32	98	98	98	98	98	98	98	98	99	98	98	96	99	99	100	100	100		99	99	99	84
19	MSVA_KE_Nan1_Ke3	98	98	98	98	98	98	97	98	98	98	98	95	99	99	99	100	99	100		99	99	84
20	MSVA_KE_Nan2_Ke2	98	98	98	98	98	98	97	98	98	98	98	95	99	99	99	100	100	99	99		99	84
21	MSVA_KE_Nye1_Ken11	98	97	98	98	98	98	97	97	98	98	97	95	98	98	99	99	99	99	99	99		84
22	MSV-K_Zw_Mic23	71	71	71	71	71	71	70	70	71	71	70	68	71	72	71	72	70	72	72	72	72	



Key: ← Kenyan isolates

Figure 5.1 Phylogenetic relationships of *Maize streak virus* isolates from Kenya and others from other parts of the world. *Maize streak virus* MSV-K from Zimbabwe was used as an outgroup.

5.5 Discussion

Geminivirus degenerate primers yielded the expected PCR amplicons of approximately 900bp for eight of the thirty samples analyzed. There was no amplification in 22 symptomatic samples, indicating that either the primers were not broad enough to

amplify some of the viruses present, or the symptoms were caused by other viruses different from MSV. Recent outbreaks of maize lethal necrosis (Wangai *et al*., 2012) indicate the presence of other viruses that are not related to MSV but may cause similar symptoms. There is need to confirm which viruses could have caused the symptoms in the 24 symptomatic samples. The primers used in this study targeted only a specific region of the virus (ORF C1/C2) and thus may have missed to amplify viruses with different sequences in this region. A wider panel of primers or other amplification techniques such as rolling circle amplification (RCA) with ability to capture more diversity should be employed in future studies.

The amplified fragments represented partial sequences from open reading frame C1/C2 of Geminiviruses. The PCR products from four isolates (one from Nakuru and three from Kiambu Counties) were sequenced and used for comparative studies. The four sequences generated were aligned together with each other and also with 18 other sequences previously described from different parts of the world. Phylogenetic analysis indicated that all of the Kenyan MSV sequences were closely related to each other. When the four sequences were compared with other MSV sequences from the rest of the world, they were all related to the maize-adapted MSV-A type identified previously in a 1999 survey of African MSV diversity (Martin *et al*., 2001). Similar studies carried around the East African region had concluded that the main strain of MSV in the region belong to the MSV-A subtype (Owor *et al*., 2007).

The four MSV isolates were collected from infected maize. However, other grasses are also known to be hosts of different strains of MSV (Willment *et al*., 2001). Monjane *et al*. (2011) indicated that the strains infecting the grasses are different from those infecting maize. The different virus strains are known to recombine and may result to more virulent virus strains. There is need to determine the diversity between MSV strains infecting the different grasses in the country and map out their distribution. Such studies will be important in determining if there is possible recombination occurring within the geminiviruses infecting grasses and their possible impact to maize production in the future. Information generated will also be used to inform regulators on possible restrictions of infected materials to avoid introduction of new strains into new areas.

The East Africa region is known to be a hot spot for MSV (Owor *et al*., 2008). Monjane *et al.* (2011) suggested that regular analysis of MSV-A genome within such diversification hot spots should be used to monitor the emergence of future MSV-A lineages that could affect maize cultivation in Africa. An extensive survey covering different regions of maize streak virus and involving more grasses is needed and more sequences done to comprehensively establish the diversity and strain distribution in Kenya.

The four Kenyan isolates of MSV were found to be highly similar to one another and to others infecting maize from different regions of the world. This may be an indication of low MSV diversity within the amplified region and a possibility of limited recombination with other geminiviruses infecting other grasses in the country. However, only 4 partial

sequences were done and this cannot give conclusive information on what may be happening in the different maize growing regions. Low virus diversity is good for breeders since developed resistant varieties can be adopted over a wider region and possibility of emergence of resistant breaking strains is also low.

CHAPTER 6

GENERAL DISCUSSION

The most important maize foliar diseases from the survey and the screening study were northern leaf blight, common rust and maize streak diseases. The three diseases were distributed in all counties surveyed and were not restricted to any particular agro-ecological zone. All the genotypes screened were also infected with the three diseases. Earlier studies had identified the same three diseases as important across the country (Mwangi, 1998). The incidence for the three diseases was high, both in farmers' fields and in Kabete field station during the two seasons. This may be an indication that despite the efforts being put by researchers to manage the three diseases, true resistance has not been found. However, severity for the three diseases was generally low across all the sites, an indication that most of the varieties had some level of tolerance to the diseases. Changes were observed in terms of importance of disease between seasons, with common rust being more important in season one (December to April) while NLB was higher in season two (April to July). Disease intensity varied between seasons, indicating that weather changes affected amount of disease. Season one with low rainfall and high temperatures was conducive for common rust while high rainfall and low temperatures favoured NLB, an observation that was also noted by Mwangi, (1998) and Muiru (2008).

Northern leaf blight was equally common in all the regions surveyed, confirming that the disease has a wide ecological distribution as reported earlier (Kinyua, 2004 and Muiru, 2008). Heavy dews, cool temperature and frequent rains create good sustained environmental conditions for NLB development (Mwangi, 1998). Common rust

incidence and severity was higher in the different agro-ecological zones of Nakuru County and in zone UM1 of Kiambu.

Gray leaf spot, head smut and common smut were recorded during the surveys and in the field but not at high intensities. The disease was reported to occur in different agro-ecological zones of Rift valley, Central and Eastern regions and no-tillage practice are known to encourage disease development (Danson *et al*., 2004; Kinyua, 2004). Head smut and common smut are mainly spread through infected seeds and the use of certified seeds is important in their management.

Maize streak disease was highest in Kiambu County. In the field trials, MSD incidence and severity was higher in hybrid varieties which are popular in the Kenyan Highlands such as Kiambu (Magenya *et al*., 2008). The partial sequences of four MSV isolates studied indicated that the viruses have high degree of genetic similarity to isolates infecting maize in other parts of the world and that the variability of the virus in the maize growing regions visited during the survey is low, which is important for breeding programs since the developed resistant varieties may be useful over wide maize growing regions.

Hybrid varieties were generally more susceptible to two or more of the pathogens observed. Development of resistant maize hybrids will remain a priority to manage the diseases and prevent future outbreaks.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Maize is affected by a host of different pathogens despite its importance as the major staple crop in Kenya. During the survey all the diseases assessed (northern leaf blight, common rust, maize streak disease, gray leaf spot, common smut and head smut) were present in all counties. Northern leaf blight and common rust had the highest incidence, maize streak disease the most widely distributed and most severe, while incidences of GLS and head smut were low. The same diseases were also found at the Kabete Field Station, though at different levels of incidence and severity between the different varieties evaluated. Unfortunately most of the farmers are not able to identify and differentiate these diseases in their farms and associate them with cold or heat. The diseases therefore continue to affect the maize causing significant yield loss in the country. The maize germplasm available to farmers, including the hybrids, are all susceptible to the disease, though at different levels. There is likelihood that the status of these diseases can change to epidemic levels especially with climate change.

The four partial sequences of MSV isolates indicated the viruses are similar to isolates infecting maize in other parts of the world and that the variability of the virus in the maize growing regions covered in the survey is low. Low virus diversity is good for breeders since the developed resistant varieties may be useful over wide maize growing regions. However, only a small region of the virus was sequenced and variability may

differ in other regions. Sequencing of the full virus genome is therefore needed to confirm whether the low diversity is extended to other sections of the virus genome.

6.2 Recommendations

1. Northern leaf blight, common rust and maize streak disease were found to be the three most prevalent and severe diseases in the different agro-ecological zones of the three counties. These diseases infected all genotypes evaluated in the field. More efforts are needed to develop management strategies to minimize losses that may be associated with the three diseases.

2. Gray leaf spot, head and common smuts were also present, but were not widely distributed and had low incidence and severity both in the survey and field evaluations. These diseases should however not be ignored as their status may change with changes in climatic conditions.

3. Variety Pannar 4m-19 had low incidence and severity scores for common rust while variety Pannar was more tolerant to MSD. These two varieties may be good sources for tolerance to the two diseases and should be incorporated in breeding programs

4. The nucleotide and amino acid sequences of Kenyan isolates of *Maize streak virus* were highly similar to one another, closely related to others from the rest of the world, and belonged to the MSV-A strain, the main subtype infecting maize.

The high percent sequence similarities indicate low variability within the sequenced region of the virus. This information is important to breeders since low virus diversity indicates that maize genotypes showing resistance to MSV may have wider areas where they can be grown without risk of infection by different virus strains. However, only a small segment of the virus was sequenced. Full genome sequence is recommended to confirm the low diversity reported in this study.

CHAPTER 8

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CHAPTER 9

APPENDICES

APPENDIX 1 Questionnaire used to collect data in farmers' fields

Enumerator to introduce himself to the famer and gather the following information

1) Name of the farmer……Farm No……Date……..

2) District…….Division……Village…….

3) Size of the whole farm ……..Acres

4) Area under maize……Acres

5) Varieties of the maize he/she plants…….

6) Varieties not affected by the diseases
- Northern Leaf Blight
- Common Rust
- Gray Leaf Spot
- Maize Streak Disease
- Head Smut
- Common Smut

7) The Varieties most affected by the diseases……..
- Northern Leaf Blight
- Common Rust
- Gray Leaf Spot
- Maize Streak Disease
- Head Smut
- Common Smut

8) At what stages are the diseases so severe…….
- Northern Leaf Blight
- Common Rust
- Gray Leaf Spot
- Maize Streak Disease
- Head Smut
- Common Smut

9) Mode of cultivation either pure stand or mixed stand…….

10) Are these diseases more severe in pure or mixed stand…….

11).In which season are the diseases most severe (rainy or dry)…….

12. Source of the planting material (a) Own (b) Neighbours (c) Local Market (d) Certified seed

13) Farmers perception of the diseases (a) Serious (b) Moderately serious (c) Not serious

Northern Leaf Blight
Common Rust
Gray Leaf Spot
Maize Streak Disease
Head Smut
Common Smut

14) Do you control the diseases……..

15) List the measures taken……..

16) For Enumerator record.

(a) Longitude…….. (b) Latitude……
(c) Altitude……. (d) Disease prevalence
(e) Disease incidence (DI) 10 plants……
(f) Disease severity (DS) 10 plants…….

Appendix 2 Meteorological observations recorded during crop growth period at Upper Kabete Field Station

Month	Mean Temperature(°C)		Rainfall(mm)	Pan Evaporation
	Maximum	Minimum		
December 2010	23.7	13.8	74.5	144.0
January 2011	25.3	13.3	4.2	184.2
February 2011	26.5	13.6	66.3	173.1
March 2011	25.7	14.6	147.7	163.0
April 2011	24.0	15.3	80.7	143.1
May 2011	23.3	14.7	93.9	97.4
June 2011	23.2	13.5	47.8	72.4
July 2011	23.4	11.3	14.3	100.3
August 2011	21.2	12.7	26.9	72.2

Source: Kabete Metrological station.

Appendix 3 Extraction buffer used in nucleic acid extractions

Stock concentration	Working concentration	Final concentration(100ml)
1M Tris	100Mm	10ml
0.5M EDTA	50mM	10ml
5M Nacl	500mM	10ml
14M BME	10mM	2ml
dH_20		78ml

Appendix 4 Percentage of farmers that plant different maize varieties in the major maize growing areas

Variety	Embu	Kiambu	Nakuru
H513	26.3	42.1	31.6
DH04	0	25	75
Pioneer	25	37.5	37.5
Local	30	70	0
H614	36.8	15.8	47.4
Pannar	0	71.4	28.6
Nduma 43	50	50	0
DK8031	60	40	0
H625	0	100	0
H629	0	100	0
H624	33.3	33.3	33.3
DK21	100	0	0
DK3831	100	0	0
H628	50	0	50
H522	100	0	0
H6213	0	0	100
KH Lentet	0	0	100
KH 500 9A	0	0	100
KH 22	0	0	100
KDVT 90031	0	0	100
Yellow maize	0	0	100