GENETIC ANALYSIS OF MAIZE STREAK VIRUS DISEASE RESISTANCE ON TROPICAL MAIZE

BY

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Abstract

Maize streak virus (MSV) disease, transmitted by leafhoppers (*Cicadulina mbila*) is a major contributing factor to low maize yields in Africa. The disease threatens maize production in Zimbabwe, thus the importance of breeding Zimbabwe maize varieties that carry resistance to this disease. Cimmyt has developed inbred lines, good for other traits, for example, high general combining ability (GCA) effects for grain yield performance, but susceptible to MSV hence efforts are being made in CIMMYT to introduce MSV resistance genes from MSV resistant donor lines known to be good for important traits, especially yield, but bad for MSV resistance.

This study was designed to assess newly developed tropical maize inbred lines with complete resistance to MSV and to compare disease progression between the MSV susceptible and resistant inbred lines artificially infect plants with virulent leafhoppers. Breeders usually cross two resistant parents to exploit the potential contribution of beneficial resistance alleles originating from them to generate transgressive segregation that can lead the development of new maize inbred lines with much higher levels of resistance to MSVD and desirable agronomic traits. Twelve inbred lines from CIMMYT were evaluated to determine their level of resistance. Genotype effects on MSV scores were significant from week 1-6 as well as for the average ($p < 0.05$). Significant effects on MSV scores were also observed on each week interval, except for week 4. Broad-sense heritability (H2) estimates for MSV scores was high (<50%) on each week interval as well as for the average MSV score. Genotypic effects showed to be more important than the environmental variances on each week MSV recordings were taken.

This study showed that inbred line CML536 was highly resistant confirming previous observations made with artificial infection in Zimbabwe. Candidate lines CL1210634 and CL1210635 showed complete resistance to MSV meaning they may share the same major gene Msv1 with CML539 and CML536 check inbred. The data we obtained provide quantification of conclusions of visual observations: (a) some varieties bred for resistance are less affected by MSD than others, even when infected at the same stage; (b) early infection is more damaging than late infection, but resistant varieties differ in their response, and; (c) varieties carrying no resistance can be little damaged if infected late.

In order to avoid the over-dependence on Msv1, further studies should be carried out to identify a second gene for MSD resistance to compliment Msv1 gene in conferring enhanced and durable resistance to MSD. Enhanced resistance through additional phenotypic selection will also help prevent possible breakdown of Msv1.

Declaration

UNIVERSITY OF KWAZULU NATAL COLLEGE OF ENGINEERING AND SCIENCE.

I hereby declare the research project entitled **'Genetic Analysis of maize streak virus resistance on tropical maize'** project is submitted to the University of Kwazulu Natal college of Engineering and Science. This thesis is a record of my own research. The material has never been presented before in any academic institution for an academic award.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Maize (*Zea mays L.*) is a staple for over 100 million people and the most important cereal crop in sub-Saharan Africa where approximately 15 million ha are planted annually (Bosque-Pérez, 2000). It is the most important grain cereal crop cultivated and consumed at almost every household in Sub Sahara Africa (SSA) (Tembo et al., 2020). Maize forms the base of daily dietary supply of energy and nutrition elements for almost every household in in the region. Globally maize is the third crop largest crop after wheat and rice, in terms of production and consumption (Ramirez-Cabral et al., 2017). Regardless of its global importance, productivity of this crop is averagely low, particularly in sub-Saharan Africa (SSA), with yields <1.8 t ha which is among the lowest in the world (Masuka et al., 2017). Such low production is a result of a number of bottlenecks, including periodic drought, high incidence of biotic stresses (diseases, insect pests, and weeds), poor soil fertility, scarcity and high cost of irrigation, and farmers' inability to access and afford quality seeds and fertilizers (Masuka et al., 2017). These yield levels are not sufficient to meet up with the demand for maize grain at most households, thereby rendering them food insecure. Therefore, innovations that would cause increase in maize productivity in SSA ought to be encouraged as this will boost food security at household level in this region.

In SSA, maize production is constrained by multiple challenges, including the abiotic (especially, drought and heat stress) (Masuka et al., 2017) and the biotic (i.e., pests and diseases) (Nair et al., 2015). Maize streak virus (MSV), belonging to the genus Mastrevirus of the family Geminiviridae, is the most damaging viral pathogen of maize (*Zea mays L.*) in Africa. It is the causal agent of maize streak disease (MSD), which is endemic throughout sub-Saharan Africa (Oppong et al., 2014) It is the most widely studied, because of its ecological versatility and high yield loss potential. It also is a major factor contributing to the instability of maize production in Sub Sahara Africa (Taiwo et al., 2006). MSD is the most important disease in Zimbabwe where it occurs in every natural farming region. The disease is more prevalent in the Highveld (1219-1675m) above sea level and Middle veld (600-1200m) above sea level where farmers row maize and winter cereals in rotation (Karavina et al., 2014). Depending on the cultivar grown and the time of infection, yield losses caused by MSV have been reported to range up to 100%. Hence, effective and sustainable control strategies should always be deployed by the farmers in order to safeguard against loss of farming investments.

In research, MSV gained a lot of traction because it is very difficult to manage and this phenomenon is usually linked to: the variability exhibited by the virus; susceptibility of the locally adapted maize lines; and, the unpredictable vector migratory and survival pattern (Garcia-Oliveira et al., 2020). MSV incidences in the fields are unpredictable and varies between year to year resulting in up to 100% yield losses in epidemic years (Martin and Shepard 2009). Global yield losses of up to US$120-480 million, in- terms of lost income and higher maize prices due to MSV infestations were previously reported (Martin and Shepard, 2009), but the same authors indicated that at least half of such loss could be recovered if the disease is effectively controlled. MSV is widespread in tropical and sub-tropical Africa and for years, it was reported as the most damaging disease of maize in the African continent (Lorroki et al., 2017). The disease is generally considered to be an endemic viral disease in Africa and it is not known to occur in the western Hemisphere.

For decades, resistance breeding has been considered an economical, sustainable, eco-friendly and efficient method of control and prevention of yield losses due to plant diseases, including MSV(Martin and Shepherd, 2009). The development and use of resistant maize cultivars have been recognized as the most reliable, cost-effective, and socially acceptable means of controlling MSV. Presently, numerous breeding programs in Africa are incorporating resistant genes identified at the International Institute of Tropical Agriculture (IITA) into their local cultivars. Most MSV-resistant cultivars (Taiwo et al., 2006). In SSA, maize breeders have been continually selecting against MSV susceptibility and most of the released maize varieties exhibit some level of MSV resistance (Nair et al., 2015). The International Maize and Wheat Improvement Centre (CIMMYT), in collaboration with IITA successfully developed several maize lines with MSV resistance and drought tolerance, involved as parents in several hybrids released by many seed companies in SSA (Kim et al., 1989). Furthermore, several studies have been carried out in order to ascertain how MSV is genetically controlled and results indicated that, a few major genes code for resistance in maize inbred lines possessing complete resistance whereas partial resistance is controlled by several genes (Kyetere et al.,1999; Pernet et al., 1999). However, deployment of virus resistant hybrids and cultivars will be critical for disease control in areas where subsistence farmers rely on a continuous maize crop for food and have difficulty using insecticides (Jones et al., 2018).

1.2 Problem Statement

As part of its mandate, the International Centre for Maize and Wheat Improvement (CIMMYT) develops and deploys new maize inbred lines with desirable traits for both, abiotic and biotic stress tolerance or resistance (Masuka et al., 2017). In its breeding programs in Africa, lines confirmed to exhibit partial and complete resistance to MSV were released. But other inbred lines, good for other traits, for example, high general combining ability (GCA) effects for grain yield performance, but susceptible to MSV, developed in Africa and in Mexico, are also common in CIMMYT's Mid-altitude breeding programs. With the advent of molecular breeding tools, for example, marker-assisted selection (MAS) which have been proven to speed up the rate of product development in a plant breeding program. Efforts have been made in CIMMYT to introduce MSV resistance genes from MSV resistant donor lines, in some of the inbred lines known to be good for important traits, especially yield but bad for MSV resistance using MAS. But, for some of these newly developed lines, it is still yet to be confirmed phenotypically, if they harbour partial or complete resistance to MSV.

1.3 Justification

Exploring the nature in-which MSV resistance is expressed at the phenotypic level is the starting point to understanding how the line can be useful in hybrid development in maize breeding programs. As a general rule of thumb, in breeding for disease resistance, complete resistance is favoured compared to partial resistance because no visual symptoms are noticed. Inbred lines exhibiting complete resistance can also be potential targets for identification of other set of genes, apart from the commonly known, Msv1 gene in maize. This is so because, complete resistance is known to be present when a few major genes code for the MSV resistance.

1.4 Main Objective

Understanding the nature in-which MSV resistance is expressed at the phenotypic level in new tropical maize inbred lines developed using marker-assisted selection.

1.5 Specific Objectives

To identify newly developed tropical maize inbred lines with complete resistance to MSV

To compare disease progression between the MSV susceptible and resistant inbred lines.

1.6 Hypotheses

At the phenotypic level, the new tropical maize inbred lines developed for MSV resistance using marker-assisted selection, will all exhibit complete resistance to MSV and the partially resistant inbred lines will show some degree of MSV disease symptoms during the early vegetative stages but symptoms disappear with age.

Chapter 2: LITERATURE REVIEW

2.1 Introduction

Maize (*Zea mays. L*) is one of the most frequently produced crops on the planet, producing food, animal feed, and biofuel (Yang et al., 2017). Almost every household in SSA grows and cultivates it as the most significant grain cereal crop (Wamaitha et al., 2018). The great majority of maize in Africa is grown by small-scale farmers with little resources. It is a staple in Zimbabwe and most of East and Southern Africa, where it provides the majority of the daily energy and nutrition requirements. Maize, after wheat and rice, is the world's third most popular crop in terms of production and consumption (Ramirez-Cabral et al., 2017). The yearly maize demand in Zimbabwe only, ranges from 17 to 1.8 million tonnes.

Many diseases harm the crop, reducing both the quality and quantity of produce. Grey Leaf Spot, caused by *Cercospora zea-maydis*, ear, kernel, and cob rots, Maize Streak Virus (MSV) illness, and Turcicum Leaf Blight (TLB), caused by *Helminthosporium turcicum*, are the most commercially important maize diseases in Zimbabwe (Karavina et al., 2014). In some parts of the world, virus infections in maize can result in substantial yield decreases, posing a danger to agricultural output and food supplies (Luis et al., 2014). Maize production in Sub-Saharan Africa (SSA) is beset by a slew of problems, the most serious of which are insect pests and disease outbreaks. MSV is one of the most economically important maize diseases, ranking third behind grey leaf sport (GLS) and Northern corn leaf blight (NCLB) among the most destructive maize diseases worldwide (Martin and Shepherd, 2009). MSV is spread by various species of leafhoppers (*Cicadulina spp*.), with *Cicadulina mbila* and Cicadulina triangular being the most important vector species (Bosque-Pérez, 2000). MSV occurrence in the fields is unpredictable and varies from year to year, with yield losses of up to 100% observed in epidemic years (Martin and Shepard 2009). Many factors influence the occurrence of maize diseases, including environmental conditions, host genotype and infection period (growth stage of the crop), crop production practices, and previous disease history (Yang et al., 2017). Infectious illness distribution, prevalence, and severity may change in unforeseen ways as a result of climate change in different places.

In Africa, there is also a low uptake of productivity-enhancing technology such as better seed since these technologies are far more expensive than low-income farmers can afford (Odendo et al., 2001). Low adoption of productivity-enhancing technology is also linked to a lack of awareness and understanding among farmers, with some still believing that these technologies are ineffective when used in conjunction with fertilizer application (Odendo et al., 2001). Farmers' preferences for variety should be taken into account while breeding for new enhanced varieties to maximize the likelihood of acceptance.

2.2 Literature review methodology

The recommended reporting items for systematic reviews and meta-analyses (PRISMA) standards (O'Dea et al., 2021) were followed in this study, as well as the ROSES flow-chart (Jordon et al., 2020). For the years 1993–2020, systematic literature searches were conducted in Scopus, Web of Science Core Collection, and Google Scholar. To find articles that reported on genetic analysis of MSV disease, the search method included a mix of phrases linked to "maize streak virus," "genetic analysis," "disease resistance," and "tropical maize." The databases yielded 686 publications, which were whittled down to 36 publications related to MSV disease resistance in tropical maize after screening. Screening was done by year of publication and old and duplicated literature were then excluded. Screening was also based on the relevance of the articles to study and relevance to the target study region The authors of the current study did all of the additional screening and coding detailed below done manually. We took into account all empirical data. Our goal was to determine the nature of MSV resistance at the phenotypic level in novel tropical maize inbred lines developed in Africa using marker-assisted selection (MAS), and we used both qualitative and quantitative research methods, according to the EPO database (Eppo, P. 2011). Studies were considered for this review if they matched all four criteria listed below: (a) The study focused on MSV disease (b) It assessed MSV resistance in tropical maize (c) It included genetic analysis (d) It reported quantitative or qualitative data (including more peripheral remarks or assertions) on any maize streak virus disease progression.

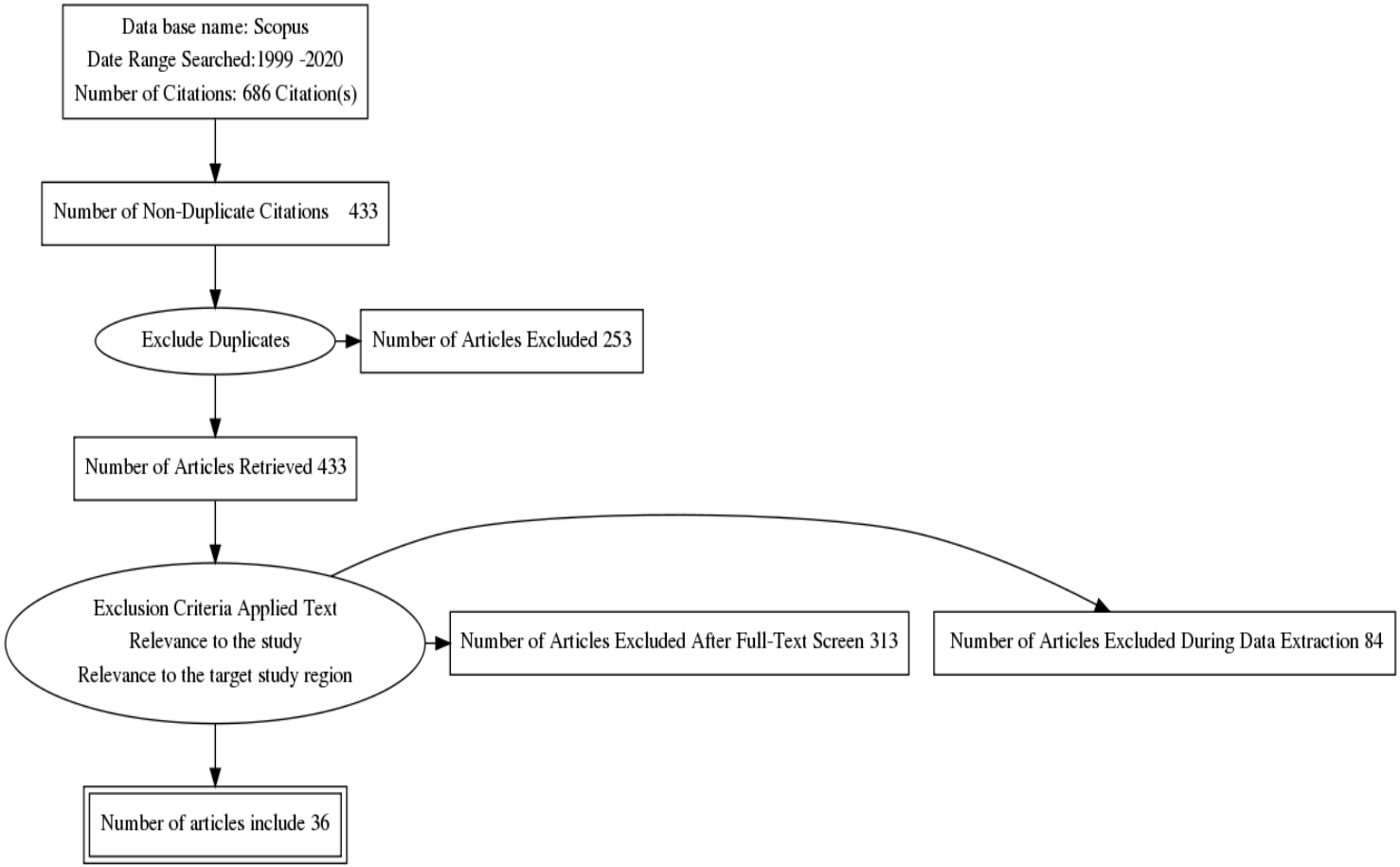

Fig 2.0: Prisma flow diagram for Analysis of maize streak virus disease resistance on tropical maize

2.3 Maize streak virus disease symptom

Maize streak virus (MSV) is a significant maize pathogen in Sub-Saharan Africa. Despite low levels of streak disease in some years, devastating epidemics with complete crop failure have occurred, often following droughts or irregular early rains (Welz et al., 1998). The effects of maize streak virus disease (MSD) on maize growth and yield are most severe when the plants are infected when they are young, with infection rates approaching 100% in plants infected less than one week after emergence (Page et al., 1999). Storey termed the condition 'maize streak' in 1925 after first describing it as 'maize variegation' (Bosque-Pérez, 2000). MSD incidence and severity differ by maize genotype and plant age at the time of infection (Charles, K. et al., 2014).

Broken to practically continuous chlorotic streaks, concentrating initially on the tertiary leaf veins, characterize MSD symptoms. These grow into rectangular tan-coloured lesions that run parallel to leaf veins later. The lesions coalesce as the disease develops, causing the entire leaf to be blighted. The photosynthetic area, growth, and yield of the plant are all reduced as a result of this (Charles, K. et al., 2014). These short lines run mostly along the leaf laminae's veins and are evenly dispersed across the leaf surface (Figure 2.1; (Magenya et al., 2008). The presence of the virus is shown by a streaking pattern on the leaves, but the density of the streaking is determined by varietal susceptibility.

In very sensitive types, chlorosis of the entire lamina can result from chlorotic streaking (Bosque-Pérez, 2000, Fajemisin, 2003). Affected maize plants may become stunted in development, have fewer grains and ears, and have smaller cobs (Rodier et al., 1995; Shepard et al., 2010). Chlorosis causes the plant to die prematurely, especially if the infection occurs early in the plant's life cycle. MSD has the greatest impact on grain yield when it infects young plants, and this effect fades as the plant becomes older (Bosque-Pérez, 2000). In severe situations, the leaves become completely chlorotic, resulting in acute necrosis and the plant's early death before blossoming.

Fig 2.1: Maize streak disease symptoms (Martin and Shepherd, 2009).

2.4 Damage caused by maize streak virus disease

Maize plants are vulnerable to the MSV from emergence to tasselling. Infection of a maize crop at the first three weeks after planting, often results in 100% yield loss (Magenya et al., 2008). If infection occurs at seedling stage, it results in no ear formation (Magenya et al., 2008). Infection at a later stage result in undersized and poorly filled ears (Fajemisin, 2003), whereas infection at the 6–8-week stage after planting has little effect on vigour of the plant (Fajemisin, 2003). Yield loses of up to 70% or more occurs in susceptible varieties depending on the stage of plant growth when infection occurs (Magenya et al., 2008). Maize plants that are infected at the third leaf stage become severely stunted producing abnormal cobs or giving no yield at all (Karavina et al., 2014). Consequently, effective control measures against MSV must be found to secure high yields.

2.5 Maize streak virus biology

Literature suggests that the maize plant is infected by about 32 virus diseases, seven of-which are common only in SSA (Taiwo & Hughes et al., 2006). These are MSV, maize stripe virus (MStV), maize chlorotic dwarf virus (MCDV), maize mottle virus, maize eye spot virus, maize dwarf mosaic virus (MDMV), and the Guinea grass mosaic virus (GGMV). Of these seven viruses, the leafhopper-vectored MSV is considered the most important (Bosque-Pérez, 2000). The MSV disease is the most widely studied because of its high yield loss potential (Bosque-Perez, 2000, Blankson et al., 2018). The endemic nature of some of these viral diseases is one of the major factors responsible for the low maize productivity predominant in SSA (Thottappilly et al., 1993).

MSV is a species of the genus Mastrevirus of the family Geminiviridae (Zhang et al., 2001). MSV is a single-stranded DNA geminivirus obligately transmitted by *Cicadulina spp*. leaf hoppers. (Mafu, 2013). The leafhoppers (Figure 2.2) of the genus *Cicadulin*a, feeds on over 80 species of monocotyledonous plants belonging to the Poaceae family (Bosque-Perez et al., 2000, Harkins et al., 2009). These viruses have single-component single-stranded circular DNA genomes of 2.7 kb in size (Figure 2.3), and also by characteristic 'twinned' or geminate particles (Martin et al., 1999).

Figure 0.1: Maize leaf hopper (*Cicadulina mbila*) on a maize leaf (Mabhungu et al., 2019)

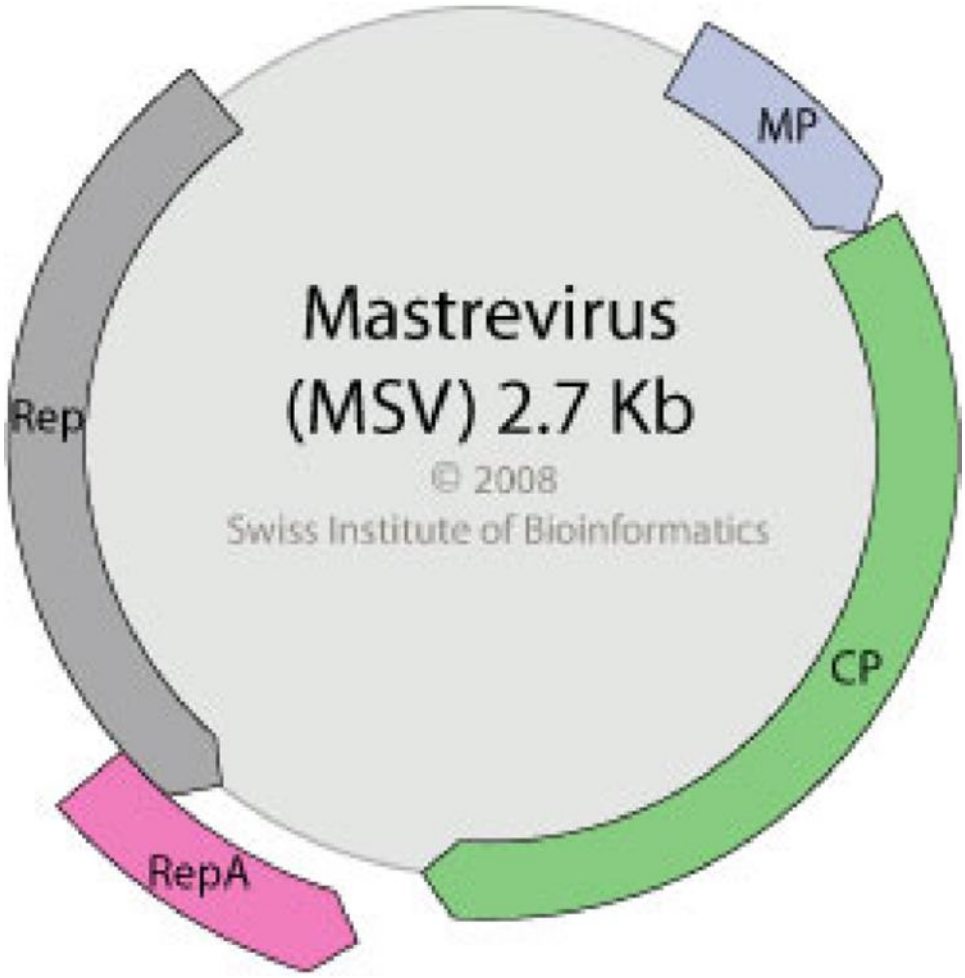

Figure 0.2: The genome of the genus Mastrevirus (*Source*: https://viralzone.expasy.org/110)

2.6 Maize streak virus disease geographic distribution

MSV is accepted as an endemic African virus that is confined to the African continent and its neighbouring islands (Bosque-Pérez, 2000). The virus is widely distributed in sub-Saharan Africa from Sudan to South Africa and Kenya to Senegal and in adjacent islands, including Mauritius, Reunion, Madagascar, Sao Tom ´ e and ´ Principe (Bosque-Pérez et al., 1998). However, no studies have been conducted in West Africa to evaluate the effect of MSV disease on the yield and growth of maize, or to compare different varieties of maize for their reaction to MSV disease under controlled condition (Bosque-Pérez et al., 1998).

2.7 Management of maize streak virus disease

The occurrence of epidemics of maize streak is closely related to the population dynamics of the vector, which in turn is influenced by rainfall, temperature, and availability of alternate host plants. Maize streak virus (MSV) may be an increasing threat in Africa, especially as maize area increases and agricultural production intensifies (Mawere et al., 2006). Irrigated winter wheat crops facilitate the over-wintering of both the virus and the vectors. (Mawere et al., 2006) also suggested that the spread of MSV between crops could be facilitated by successive cropping and the presence of wild grasses, which act as reservoir for both virus and vectors.

2.7.1 Cultural Practices

Management of MSV has been difficult owing partly to the unpredictability and sporadic nature of disease appearance and also due to the susceptibility of locally adapted maize cultivars (Martin & Shepherd, 2009). Since MSD is not seed transmissible and can only be transported by humans either within insects or symptomatic plants, it might in future be prudent for African governments to regulate the movement of maize leaf material and insects between countries (Martin & Shepherd, 2009). The most effective control practices known to date are timely planting as well as, planting barrier crops between early and late-planted maize fields in order to disrupt vector movement. Apart from these measures, crop rotation, avoidance of maize planting downwind from earlier planted susceptible cereal crops and removal of diseased plants are the other recommended cultural control measures against MSV. Cultural practices such as deep residue burial, crop rotation, irrigation and plant density manipulation can be used in effective management of MSV (Charles,K. et al., 2014). However, the control of diseases using tillage practices destroys the soil structure and exposes the soil to erosion, leading to siltation of water bodies. Crop rotation implementation is limited by land size in the smallholder sector. Farmers do not have the luxury of leaving a piece of land fallow even for a season. In Southern Africa, for example, many smallholder farmers use multiple planting dates over extended periods, a practice that results in greater exposure to maize streak, to ensure that at least part of their crop successfully avoids periods of severe drought stress (Mawere et al., 2006).

2.7.2 Chemical Practices

The leafhoppers vectors can easily be controlled by application of: contact insecticides like Aldicarb, carbofuran, carboslfan, endoslfan and dimethoate (Charles, K. et al., 2014) which can be applied as seed dressing in planting furrows or as conventional sprays. There are however several challenges associated with insecticides like aldicarb and carbofuran which are extremely poisons to both humans and the environment (Charles, K. et. al., 2014). MSV incidence can lead up to 100% yield losses in susceptible maize cultivars if MSV outbreak occurs in the presence of drought or irregular early rains (Garcia-Oliveira et al., 2020). The unpredictable vector survival and migratory patterns makes breeding for MSVD resistance, as well as its management under field conditions, extremely difficult (Garcia-Oliveira et al., 2020).

Fungicides kill beneficial and non-targeted organisms and therefore adversely affect the food chain. Fungicides also need to be repeatedly sprayed and as a result may contaminate air and water bodies, and increase the cost of crop production (Karavina, 2014). Most resource-limited farmers can neither afford or are often reluctant to commit expensive inputs to small fields of crops that could, depending on the circumstances outside their control, fail completely (Asea, 2005). Therefore, the use of disease resistant varieties is relevant to the protection of the environment. It is also a sustainable and economically viable disease management option. Although integrated pest management (1PM) has been recommended as a viable option for disease control.

Given the dynamic nature of the environment tending to favour pest and disease development, a number of diseases can co-infect a crop. While research into the disease-resistant variety development has been going on, the main focus has been developing varieties with monogenic resistance. As a result, most varieties currently on the market are tolerant to a single disease, leaving them vulnerable to other diseases (Karavina et al., 2014).

Resistance breeding is considered as an economical, eco- friendly and efficient method of control and prevention of yield loss due to MSV infestations (Magenya et al., 2009; Martin and Shephard, 2009).

Whenever satisfactory disease resistant varieties are available, they are preferred over other means of disease control because the resistance is inbuilt within the plant and is ready to provide protection whenever the disease threat comes into play.

2.8 Previous and present attempts of breeding for resistance against MSD

Resistance to maize streak virus (MSV) is an essential trait of improved maize varieties in sub-Saharan Africa. Resistance breeding is perceived as the most practical solution for disease control. Breeding for resistance has long been an effort of the International Institute of Tropical Agriculture (IITA) and maize varieties that combine resistance to MSV with other desirable characters have been developed at the Institute. Many of IITA open pollinated varieties and hybrids exhibit reduced virus severity combined with low field disease incidence (Bosque-Pérez et al., 1998). Studies on the genetics of resistance to MSV have indicated that maize lines possessing complete resistance are controlled by few major genes and inheritance of this is simple while partial resistance is controlled by several genes and are quantitatively inherited with additive gene action (Ladejobi et al., 2018). Resistance in maize to MSV is controlled by a major gene, with two, three, or "few" modifying genes (Mawere et al., 2006). (Kyetere et al., 1999) were first to map this major gene to chromosome 1, and several authors have subsequently confirmed the importance of this chromosome segment, which has a major effect on resistance to MSV. Existence of resistant sources to MSV has been recognized in the early thirties in South Africa (STOREY & HOWLAND, 1967). Resistance was discovered in a variety known as "PeruvianYellow". (STOREY & HOWLAND, 1967) attempted to transfer resistance derived from a cross of South African maize Peruvian yellows and Hickory king into Kenyan maize. The Peruvian yellow was crossed with Hickory King, and by selection, resistant "P x H" lines were developed that gave white seeds. Later, "P x H" was crossed with a range of high yielding but susceptible lines, the resulting 3-way crosses showed great reduction of the disease (STOREY & HOWLAND, 1967). Storey's resistant lines were not maintained and so they were "lost".

Rodier et al., (1995) reported variations in maize streak development, and in some cases resistant germ plasm became susceptible when exposed to different strains of MSV. The most elegant study was probably that of (Martin et al., 1999)., who used Agrobacterium tumefaciens–mediated inoculation with constructs for MSV isolates collected in Kenya, Nigeria, Reunion, South Africa, and Zimbabwe, to quantify differences for relative susceptibility of maize genotypes to different MSV isolates. They found three categories—mild, moderate, and severe—of MSV isolate severity, but all of the mild isolates originated from alternate hosts (not maize) for MSV. Although substantial differences were recorded for maize streak symptom severity (54 to 75% chlorotic leaf area) induced on three maize

genotypes (very susceptible, moderately susceptible, and moderately resistant) by seven moderate or severe MSV isolates, all of these isolates ranked the maize genotypes in exactly the same order from least to most resistant. Whereas a mild MSV isolate only induced maize streak symptoms on very susceptible maize genotypes, one severe and one moderate MSV isolate similarly classified maize genotypes into most resistant to most susceptible thirds, but were not entirely consistent in ranking genotypes within each of those three categories (Mawere et al., 2006). Agroinoculation may produce different results than *Cicadulina mbila*–vectored inoculation for at least two important reasons: (i) any host-plant genetic or physical barriers to insect feeding, including possible insect non preference for a host genotype, are obviated by agroinoculation; and (ii) the constitutive nature of MSV replication by the agroinfectious MSV constructs would obviate any viral replication rate-reducing resistance mechanisms of the host genotype (Mawere et al., 2006).

In 1975, maize research scientists at IITA found a new source of resistance in the tropical Zea yellow population (TZ-Y) derived from crosses of a white grain "Tuxpeno Planta Baja' from CIMMYT in Mexico and an unidentified yellow germplasm from East Africa (Ladejobi et al., 2018). Maize varieties that combine resistance to MSV with other desirable characters have been developed at IITA and, more recently, at the Harare station of the International Maize and Wheat Improvement Centre (CIMMYT). Both high yielding varieties and varieties traditionally grown in various countries in Africa were converted into MSV-resistant ones at IITA in cooperation with CIMMYT and National Programs in Africa (Bosque-Pérez, 2000). Several national programmes in Africa are breeding for resistance to MSV. The emphasis has increased over the past few years, following severe epidemics across the continent. By the 1990s, streak-resistant maize germplasm had been released and was grown by farmers, was recommended for use, or was being considered for release in Benin, Burkina Faso, Cameroon, Chad, Congo, Coˆte d'Ivoire, Democratic Republic of Congo (formerly Zaire), Ethiopia, Gabon, Ghana, Guinea, Mali, Mozambique, Niger, Nigeria, Senegal, Sierra Leone, South Africa, Sudan and Togo (Kim et al., 1989).

Resistance lines express very few streak symptoms (< 5–30% of the leaf area compared to susceptible lines with streak symptoms > 75%) or the resistant plants initially produce severe symptoms (streaks on > 75% of the leaf lamina) but leaves emerging post infection show

symptom remission, termed as recovery resistance (Ladejobi et al., 2018). Many breeding programs use resistant sources developed from international programs to incorporate resistance into their own locally developed varieties.

2.9 Breeding and use of MSV disease resistant maize varieties

Breeding for disease resistance to MSV in maize is an efficient control measure that is reliable and cost-effective. It is based on the identification and incorporation of major resistance genes into economically important varieties (Wisser et al., 2006). The two types of resistance that are recognized are qualitative and quantitative (Wisser et al., 2006). Their mechanisms are diverse in their specificity and durability and interactions with the virus in the host plant (Mafu, 2013).

Use of resistant cultivars is probably the most economically viable approach to reducing losses that result from MSV (Bosque-Perez, 2000; Mawere et al., 2006). In practice, most of the commercially available maize hybrids are at best moderately tolerant to MSV disease (Martin and Shepherd, 2009). Collaborative efforts by several international and regional maize breeding programmes CIMMYT) and IITA) have produced a large collection of germplasm with improved MSV disease resistance (Welz et al., 1998, Bosque-Perez, 2000). Resistance to infection by MSVD has been identified in the CIMMYT inbred line CML202, which is adapted to the mid-altitude tropics (Welz et al., 1998).

Other sources of resistance include C390 from the Agricultural Research Centre for International Development (CIRAD), IITA's Tzi3 and Tzi4, CIMMYT's18 OSU231, PANNAR's A076 and Embu11 from the Kenya Agricultural Research Institute (KARI) (James, 1999). The development and use of resistant maize cultivars have been recognized as the most reliable, cost-effective, and socially acceptable means of controlling MSV. Presently, numerous breeding programs in Africa are incorporating resistant genes identified at the International Institute of Tropical Agriculture (IITA) into their local cultivars (Taiwo et al., 2006). In order to improve MSV resistance in hybrids, heterosis breeding is recommended. Using sets of germplasm bred for MSV resistance in distinct programs, productive MSV resistant hybrids can be created (Maphumulo et al., 2021)

2.10 Genetics studies of inheritance to maize streak virus

Resistance to maize streak virus disease is an essential trait required in breeding for improved maize varieties targeted to regions in Africa. Resistance is measured as partial and complete resistance (Rodier et al., 1995). Studies on genetics of resistance to MSV have indicated that maize lines possessing complete resistance are controlled by few major genes and inheritance of this is simple while partial resistance is controlled by several genes and are quantitatively inherited with additive gene action (Ladejobi et al., 2018). Resistance also depends on the number of genes involved, in sugarcane two major genes were involved in conferring resistance to sugarcane mosaic virus, Smv1 and Smv2. One gene can work alone to confer resistance while on the other hand, 2 or 3 genes can work together to confer resistance. The phonological stage of the crop also takes part in resistance. Some genes provide resistance at all developmental crop stages while others confer resistance at a later stage of crop development (Redinbaugh et al., 2018). Virulence also contributes to resistance, the more virulent the virus is the more devastating the disease will be.

2.11 Qualitative Resistance

Qualitative resistance is also referred to as vertical resistance in that it is either present or absent, and there are no intermediates (Mafu, 2013). This type of resistance is usually race-specific and because it is usually based on a single dominant gene, it confers a high level of resistance (Wisser et al., 2006). It is easier to work with qualitative resistance in crop genetic studies and in plant breeding as the genes follow a Mendelian pattern. However, this resistance is not stable because it is usually matched by virulent races of polygenic pathogens within 3-5 years. In contrast, quantitative resistance, also known as horizontal resistance, is often more useful in an agronomic context hence it is generally recommended for the small-scale and subsistence farmers (Wisser et al., 2006).

Quantitative resistance is usually assessed in the field and is considered to have a generally higher durability and broader specificity since it is controlled by multiple genes with small continuous phenotypic effects (Fajemisin, 2003). Quantitative resistance can occur at every level between a minimum and a maximum level (Fajemisin, 2003); Wisser et al., 2006). Environmental and gene-for gene interactions play important roles in the phenotypic

expression of quantitative resistance (Wisser et al., 2006). Extensive field-testing is, therefore, required for assessment of quantitative resistance under multiple environments and also at different growth stages (Fajemisin, 2003). Wisser et al. (2006) stated that "the majority of disease resistance deployed in elite maize varieties in the field is quantitative in nature" and because breeding for resistance can be a long and tedious method, it is most cost effective to breed for quantitative resistance, which is likely to provide long-term, durable protection (Mafu, 2013).

2.12 Nature and mechanism of MSV disease resistance

Kyetere et al., (1999) and Mawere et al. (2006) reported a major quantitative trait locus (QTL) on the short arm of chromosome 1 (1S - bin1.04) and designated it msv1. Mawere et al. (2006) stated that "resistance in maize to MSV is controlled by a major gene, with two, three or 'few' modifying genes". The same locus was identified by (Welz et al., 1998) in a population derived by crossing CML202, an MSVD resistant inbred, and Lo951, a susceptible inbred. Although most of the resistance was explained by the locus on chromosome 1, with the major MSV resistance gene being identified as msv1 (Welz et al., 1998; Kyetere et al., 1999; (Pernet et al., 1999), minor QTL effects have been detected at bins 3.06, 5.03 and 8.07 (Asea et al., 2008). Pernet et al. (1999) investigated QTL responsible for resistance to MSVD and showed that the resistance was quantitatively inherited. They detected at least five significant QTL on chromosomes 1, 2, 3, and 10 in resistant cultivar D211.

MSV resistance is thus under the control of two genetic systems, one arising from a major gene on the short arm of chromosome 1 and the other conditioned by minor genes on chromosomes 2, 3 and 10, that confer quantitative resistance (Mafu, 2013). Virus resistance is associated with one or two major resistance loci in most cases, which facilitates MAS, but resistance genes have been found to cluster in the maize genome (Jones et al., 2004).

2.13 Marker-assisted selection in plant breeding for MSV disease resistance

Marker-assisted selection (MAS) in plant breeding refers to the use of molecular markers, usually DNA-based for the selection of plants with a region of DNA involved in the expression of a trait of interest (Abalo et al., 2009). Markers are tightly linked to agronomically important genes to assist in the selection of elite lines for the next generation crosses in crop improvement programmes, thus the marker is used to identify the gene (Mafu, 2013). Marker-assisted

selection involves exploiting the presence or absence of a marker to facilitate phenotypic selection (Abalo et al., 2009). It is a more efficient and reliable approach than conventional plant breeding methodology as it is unaffected by environmental factors (Abalo et al., 2009). This development has opened up a new realm of possibilities in agriculture towards improvement of economically important crop varieties. The advantage of MAS is that genotypes can be identified at the seedling stage, eliminating the time needed for plant maturation and reducing population sizes (Abalo et al., 2009). Conventional breeding methodology on the other hand, relies on phenotypic evaluation, which does not always accurately reveal the basic genomic information of the plant (Mafu, 2013). Therefore, MAS allows for a greater degree of selection precision whilst still greatly reducing the time required to achieve a particular breeding objective (Abalo et al., 2009).

2.14 Application of markers for screening for disease resistance

In the case of disease resistance, marker-based selection is valuable for simplifying the pyramiding of several major resistance genes into one genetic background (Welz et al., 1998). It is particularly useful in the screening for one resistance gene that interferes with the ability to screen for another, a common problem in disease resistance breeding (Welz et al., 1998). Efficient gene development and deployment can thus be accelerated through the use of marker-assisted breeding. Consequently, QTL from diverse donors can be rapidly introgressed into a desirable genetic background of commercial cultivars (Welz et al., 1998); (Mafu, 2013). In most cases, virus resistance is associated with one or two major resistance loci, which facilitates MAS (Jones et al., 2004). To make use of MAS, virus resistance must first be identified in maize germplasm and then mapped to specific regions of the maize genome. To aid in the identification of MSV or other virus resistance sources, identification and mapping of genes or QTL for virus resistance using markers must be available. This provides information on the number of genes or regions that must be transferred by breeding programmes (Jones et al., 2004). A study was conducted by (Garcia-Oliveira et al., 2020) to determine the usefulness of molecular markers linked to consensus QTL controlling partial-resistance systems for NCLB, GLS and MSV in maize. The NCLB disease resistance QTL in chromosomal bins 3.06, 5.04 and 8.06; GLS QTL in bins 2.09 and 4.08; and a consensus MSV QTL in bin 1.04 were examined for selection in improving host resistance levels and pyramiding resistance loci of these diseases. Evaluations for each disease were done in a population of 410 F2:3lines derived from hybridisation between inbred line CML202 with known resistance to NCLB and MSV,

and VP31, a breeding line with known resistance to GLS. The study concluded that markers linked to major resistance loci can facilitate pyramiding of resistance against multiple diseases during early generation selections. The major locus conferring resistance to MSV on chromosome 1 was significant ($P<0.05$) for resistance across seasons and phenotypic values indicated that QTL in bin 4.08 for GLS, bin 1.04 for MSV and bins 3.06 and 5.04 for NCLB significantly reduced disease severity.

CHAPTER 3: IDENTIFICATION OF NEWLY DEVELOPED TROPICAL MAIZE INBRED LINES WITH COMPLETE RESISTANCE TO MSV AND COMPARISON OF DISEASE PROGRESSION BETWEEN THE MSV SUSCEPTIBLE AND RESISTANT INBRED LINES.

3.1 Introduction

The maize streak virus (MSV) is one of the major biotic constraints in maize throughout Sub Sahara- Africa. MSVD outbreaks often coincide with drought periods or irregular early rains complete crop failure resulting in complete crop losses may occur (Nair et al., 2015). Maize is the important grain cereal crop grown and cultivated in Zimbabwe by almost every household. MSV is obligately transmitted by as many as six leaf hopper species in the genus *Cicadulina*, and mainly by *C. mbila* and *C. storeyi*. MSV epidemiology is related to environmental influences on the vector species, leading to erratic epidemics in every 3–10 years (Nair et al., 2015). MSV infection results in chlorotic streaks parallel to the veins due to the destruction of the chloroplast in the leaf lamina resulting in necrotic stripes and wilting of affected portions. In severe cases leaves become totally chlorotic leading to severe necrosis and premature death of the plant before flowering. Affected maize plants may become stunted in growth and have reduced cod size with smaller grains and ears (Rodier et al 1995; Oppong et al., 2014). MSV incidences in the fields is unpredictable and varies between year to year resulting in up to 100% yield losses in epidemic years (Martin and Shepard 2009) They also reported losses of uptoUS$120-480 million in terms of lost income and higher maize prices due to MSV but indicated that at least half of such loss could be recovered with the effective control MSV.

Management MSVD has been difficult due to unpredictability of vector migratory and survival pattern, variability of the virus and also due to the susceptibility of locally adapted maize lines (Martin and Shepherd, 2009) Resistance Breeding has been considered an economical, sustainable, eco-friendly and efficient method of control and prevention of yield losses due to MSV (Magenya et al 2009; Martin and Shepard,2009).

Breeders' efforts to develop germplasm with tolerance/resistance to multiple stress tolerance in the SSA often involves crossing MSV-resistant lines with other breeding materials that may not have resistance to MSV (although these may have other adaptive traits), and hence, require

an efficient trait-tracking mechanism during the advancement of segregating generations (Nair et al., 2015).

3.2 Materials and Methods

3.2.1 Evaluation sites and germplasm

A set of 12 inbred lines (i.e., 5 susceptible + 2 partially resistant + 3 completely resistant + 2 candidates for MSV complete resistance) developed by CIMMYT (Table 3.1) were evaluated under artificial MSV infestations, in an open field (OF) as well as in a greenhouse environment (GE). The OF experiment was established at the CIMMYT-Muzarabani site (Altitude =432.00m/1417.32ft; Average annual temperature = 40^{o}C maximum; Average annual rainfall = 600-800mm) during the summer season of 2019-20.

Table 0.1: Description of the MSV resistant and susceptible inbred lines evaluated under artificial infestations in open field and under a greenhouse environment

Line Number	Name	Attributes	MSV status
1	CML312	MSV susceptible line bred in Mexico	Susceptible
2	CZL0618	MSV partially resistant line	Partially resistant
3	CML202	MSV partially resistant line	Partially resistant
4	CZL0815	MSV resistant line	Completely resistance
5	CL1210634	Marker-assisted selection from MSV resistance donor line and CML312 {Candidate Line}	Candidate line
6	CML549	MSV Susceptible line	Susceptible
7	CL1210635	Marker-assisted selection from MSV resistance donor line and CML312 {Candidate Line}	Candidate line
8	CML373	CML373 MSV susceptible line	Susceptible
9	CML539	Marker-assisted selection from MSV resistance donor line and CML312 and confirmed for MSV1	Completely resistance

10	CML311	MSV susceptible line bred in Mexico	Susceptible
11	CML536	MSV resistant check	Completely resistance
12	CML338	MSV susceptible check	Susceptible

3.2.2 Experimental Layout

The 12 inbred lines were laid out under both, the OF and GE, using the generalized lattice design replicated three times, with three incomplete blocks, with a block size of four nested within each replication. The of experiment consisted of plots with twelve rows, which were 2m long. A gross plot size of 24 m^2 was used (i.e., 12 rows of 2 m length), with in-row spacing of 0.75m and in-row spacing of 0.30cm.

For the GE experiment, pots were three quarter filled with soil and two seeds were planted in each pot and the seed was not treated with fungicides to avoid deaths of leaf hoppers.

3.2.3 Agronomic Management

Fertilizer regimes recommended under optimal maize management were applied for both, the OF and the GE experiments. A mixture of Super dash (Emamectin benzoate 20 g/l + Acetaprimid 50 g/l), Ampligo 150EC (Lambda cyhalothrin 15 g/l) and Lambda at a rate 20 ml/knapsack was used to control fall armyworm (*Spodoptera frugipeda*), while thionex granules were applied for stalk borer (*Busiola fusca*) control, five weeks after crop emergence.

3.2.4 Artificial Infestation

The earlier the plants are infested with MSV the more severe the symptoms (Dekker et al., 1988). Inoculation of plants with MSV was do

ne by infesting them with viruliferous leafhoppers. Leafhoppers, *C. storeyi* (= triangular) China, were anesthetized with CO2 prior to dispensing them into the whorls of plants as described by (THOTTAPPILLY et al., 1993). Insects were obtained from a colony maintained at CIMMYT and reared on pearl millet *Pennisetum americanum* (L.) K. Schum. (THOTTAPPILLY et al., 1993). This colony ´ has been selected for a high proportion of virus transmitters (50 to 60% of individuals) and insects are capable of transmitting MSV following an inoculation access period as short as 2 hours. Artificial infestation was done on the same day.

3.2.5 Symptom Rating

Incidence of MSV disease was based on visual symptoms; assessments were carried out six times during the season. Severity of symptoms was assessed using a 0 to 9 scale modified after Soto et al. (1982), where zero represents healthy plants, 1 = 1 to 5% of the leaf area covered by streak symptoms (very few broken lines mostly around the midrib of the upper leaves), and 2, 3, 4, 5, 6, 7, and 8 represent approximate streak coverage of 6–15, 16–30, 31–45, 46–60, 61–75, 76–90, and 91–100%, respectively. Nine represents 100% of the leaves extensively streaked and the plant showing necrosis and stunting, indicating imminent plant death.

A scale of 1-9 was used to visually rate MSV symptoms (Table 3.2).

Table 0.1: Rating MSV disease symptoms visually on a scale of 1-9

Score	Description
0	no symptoms
1	few and widely
2	Slight streaks on small area of leaves
3	multiple sports and slight streaking
4	Moderate Streaking on 40% of leaf area
5	moderate streaking; very light stunting
6	streaking on 50% leaf area causing general yellow appearance of plant

7	Severe streaking on 75% leaf area
8	Severe streaking over entire leaf area resulting in chlorotic plants
9	Badly stunted plants

3.2.6 Data collection and analysis

MSV scores were taken on a weekly basis for six weeks. Gathered data was subjected to across site analysis of variance (ANOVA) using Genstat Software, 17th Edition (Yang et al., 2017), but this was done after log transformation in order to avoid errors. Genotypic and environmental variance as well as the broad-sense heritability estimates was predicted in the Multi Environment Trial Analysis with R (META-R) software v2.1 (Beyene et al., 2019). In order to identify candidate lines with complete resistance and to visually assess disease progression for each of the 12 inbred lines, heat maps with a dendrogram were graphed using the 'heatmap.2' function in the gplots R package (Matsuoka et al., 2009). Briefly, for disease progression assessment, a dissimilarity matrice between the MSV scores taken for six weeks were calculated using the 'Manhatan' method (Yang et al., 2017) and the clustering was done based on the 'Ward.D' method (Chavent & Lechevallier, 2006). Similarly, identification of candidate lines with complete resistance to MSV, was done using similar procedures for generating dissimilarity matrices and clustering of inbred lines with similar genetic attributes.

3.3 Results

3.3.1 Inbred line performance under greenhouse and open field conditions

Artificial inoculation was successful both in greenhouse and Open field and streak development was as expected in the tested and check inbreds. At the time score 2 was taken, the susceptible checks were extremely affected, especially in the open field. The CIMMYT inbred line CML536 was highly resistant to MSV in this experiment, confirming previous observations made with artificial infection in Zimbabwe. Genotype effects on MSV scores were significant from week 1-6 as well as for the average ($p < 0.05$). Significant inbred line x site effects on MSV scores were also observed on each week interval, except for week 4. Significant variation was also observed among lines tested and the disease incidence on all

lines evaluated. Broad-sense heritability (H2) estimates for MSV scores was high (<50%) on each week interval as well as for the average MSV score. Also, on each week MSV recordings were taken, genotypic effects showed to be more important than the environmental variances (Table 3.1). Symptom severity of MSV also varied significantly among the different inbred lines ($P < 0.01$). There was a general increase in severity over time (Table 3.3), although some lines showed relatively smaller marginal increase in severity over time. Changes in both disease incidence and severity were consistent over time; hence, neither incidence × time nor severity × time interaction effects were observed ($P > 0.05$).

Table 0.1: Across site analysis of variance (ANOVA) of the experimental maize inbred lines evaluated under open field and greenhouse conditions of Zimbabwe during the 2019–2020 cropping season

Source of variation	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Average Score	
	MS	p-value	MS	p-value	MS	p-value	MS	p-value	MS	p-value	MS	p-value	MS	p-value
Site	0.186	<.001	0.054	0.002	0.185	<.001	0.129	0.005	0.207	<.001	0.543	<.001	0.194150	<.001
Replication (Site)	0.033	0.008	0.015	0.056	0.006	0.114	0.020	0.267	0.033	<.001	0.012	0.048	0.009911	0.009
Block (Replication x Site)	0.011	0.119	0.013	0.030	0.014	<.001	0.008	0.762	0.012	0.007	0.017	0.002	0.015467	<.001
Inbred line	0.146	<.001	0.152	<.001	0.112	<.001	0.099	<.001	0.064	<.001	0.095	<.001	0.086298	<.001
Inbred line x Site	0.027	<.001	0.012	0.020	0.025	<.001	0.014	0.468	0.007	0.048	0.022	<.001	0.010332	<.001
Heritability	0.8401		0.9269		0.754		0.8782		0.9118		0.8044		0.9196	
Genotype variance	0.0216		0.0247		0.0153		0.0171		0.0113		0.0151		1.3982	
Error variance	0.0054		0.0051		0.0022		0.0139		0.0033		0.0038		0.1784	
G X E variance	0.0064		0.0022		0.0093		1.00E-04		0.0011		0.0061		0.1849	
CV	16.5302		12.3946		6.9181		16.8757		7.9076		8.5825		8.8533	
LSD	0.1437		0.1394		0.0922		0.2314		0.113		0.1208		0.828	
Grand mean	0.4435		0.5738		0.6796		0.6995		0.7292		0.7184		4.7714	

3.3.2 Determining candidate lines with complete resistance to MSV

Cluster analysis identified the two candidate lines (i.e., CL1210634 and CL1210635) as closely related with CML539 and CML536, which all have complete resistance to MSV. Surprisingly, CML373 demonstrated some levels of MSV resistance, as it clustered together with the resistant lines. Inbred known to be susceptible and those known to exhibit partial resistance to MSV showed the most severe MSV symptoms (Figure 3.1).

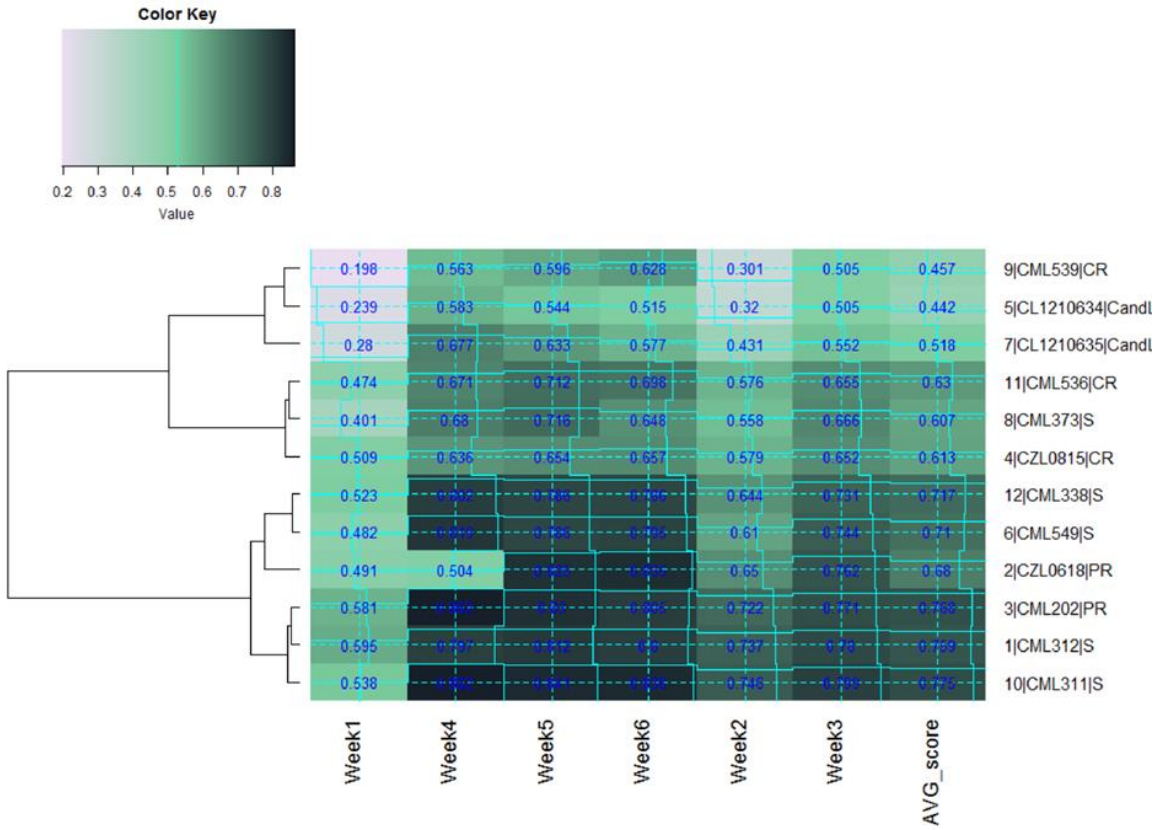

Figure 0.1: A heat map showing candidate inbred lines with similar attributes with the well-known lines with complete resistance to MSV

3.3.3 Disease progression assessment with maize growth

Disease symptoms on the susceptible genotypes seemed to have constantly increased from week 1 up to week 3. Symptoms were mostly severe at week 4, but started diminishing at week 5. The susceptible lines (e.g., CML311 and CML312) and the partially resistant lines (e.g., CML202) showed the most severe MSV symptoms and in most cases, symptoms seemed to be most severe at week 4. With the exception of CZL0618 (a partially resistant line), which showed to be clean during its early stages of grown and symptoms started to show up during week 5

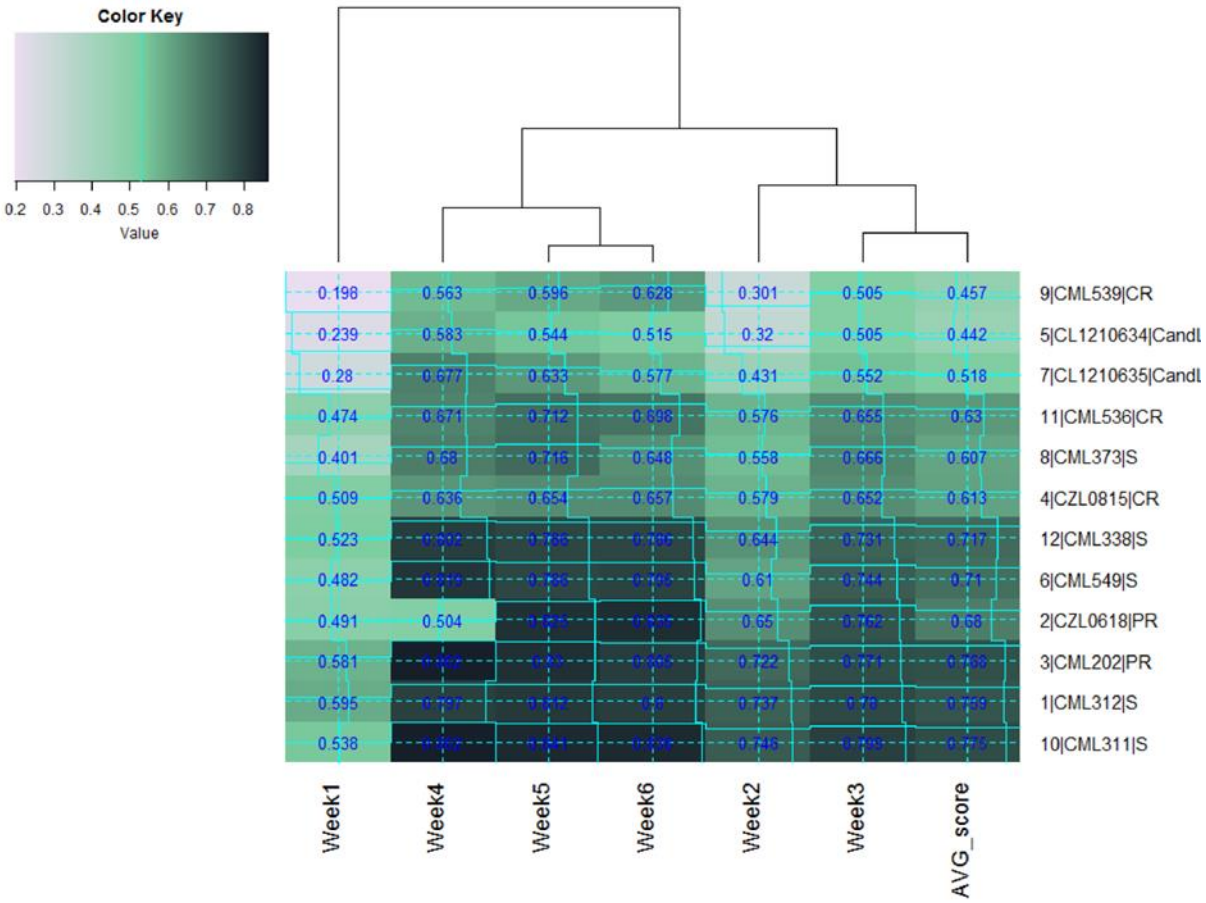

Figure 0.2: A heat map showing disease progression from week 1 of disease inoculation up to 6 weeks after infection

3.4 Discussion

Resistance to maize streak virus disease is a very important trait required in breeding for improved maize varieties targeted for SSA (Bosque-Pérez, 2000); Masuku et al., 2017). The form of resistance in several resistant sources has been found to be polygenic with both major and minor genes of varied effects (Nair et al., 2015) This study focused on developing new tropical inbred lines with complete resistance to MSV and also comparing disease progression between the MSV susceptible and resistant inbred lines.

When comparing resistance to MSV based on field observation of disease incidence and severity, it is important to subject all the maize lines to the same disease pressure and at the same time. Any differences observed in disease incidence and symptom severity are most likely due to the genetic potential of the plants. In this study, all the plants were artificially inoculated with viruliferous leafhoppers with 100% transmission rate. Chances of having escapes were minimized and, hence, the low incidences of disease observed were attributed to resistance.

Disease symptoms on the susceptible genotypes seemed to have constantly increased from week 1 up to week 3. Symptoms were mostly severe at week 4, but started diminishing at week 5. The susceptible lines (e.g., CML311 and CML312) and the partially resistant lines (e.g., CML202) showed the most severe MSV symptoms and in most cases, symptoms seemed to be most severe at week 4. With the exception of CZL0618 (a partially resistant line), which showed to be clean during its early stages of grown and symptoms started to show up during week 5 (Figure 3.2).

Two candidate lines were identified by cluster analysis (i.e., CL1210634 and CL1210635) as closely related with CML539 and CML536, which all have complete resistance to MSV. Surprisingly, CML373 demonstrated some levels of MSV resistance, as it clustered together with the resistant lines. Inbred known to be susceptible and those known to exhibit partial resistance to MSV showed the most severe MSV symptoms (Figure 3.1). two candidate findings demonstrate that MSV disease significantly affects the growth of maize plants, although this is also a function of the relative level of susceptibility/resistance of the maize variety as reported by Bosque-Pérez, (2000). The data we obtain, provide quantification of conclusions of visual observation: (a) some lines bred for partial resistance were severely affected by MSD than others even when infected at the same stage; (b) early infection is more damaging than late infection, but resistant lines differ in their response, and (c) lines carrying no resistance can be little damaged if infected late.

From this study, the new tropical maize inbred lines developed for MSV resistance using marker-assisted selection all exhibited complete resistance to MSV and the partially resistant inbred lines show some degree of MSV disease symptoms during the early vegetative stages but symptoms disappear with age.

Developing improved maize cultivars with genetic resistance to MSV is an important component of sustainable crop management strategy in Sub-Saharan Africa. International institutions, such as CIMMYT and IITA, have partnered with several regional and national institutions to develop and deploy several maize hybrids and OPVs with higher levels of resistance in SSA, primarily through conventional breeding (Semagn et al., 2015).

Although there is significant success, phenotype-based selection strategies require robust artificial epiphytotic conditions in the target ecologies which are resource-intensive and time consuming. Improved tropical and sub-tropical maize germplasm developed at CIMMYT in Mexico, are routinely deployed in SSA (especially at the maize breeding hubs of Kenya, Zimbabwe and Ethiopia) and vice versa. Adoption of maize varieties in SSA is mostly conditional upon reasonable levels of MSV resistance, along with high grain yield.

Following equal challenge conditions, varieties were found to differ in subsequent disease incidence, and this changed with plant age. Thus, as plants become older, they are either more difficult to infect or disease response is delayed. This might be due in part to leafhopper preferences as *C. storeyi* has been found to prefer maize at early growth stages than older maize plants (Mafu, 2013). In addition, highly resistant varieties are more difficult to infect than susceptible varieties.

CHAPTER 4: OVERALL CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusion

Undoubtedly, MSD remains an important disease in sub-Saharan Africa and is one of the major biotic stresses that the maize breeding programs in SSA routinely screen for. In spite of no major, widely reported epidemics having occurred in the last two decades, the disease is there to stay in the region. With climate change and more land being utilized for cereal cropping, it is most likely that the next major epidemic is just around the corner. In this study inbred line CML536 was highly resistant confirming previous observations made with artificial infection in Zimbabwe. Candidate lines CL1210634 and CL1210635 showed complete resistance to MSV meaning they may share the same major gene Msv1 with CML539 and CML536 check inbred. The data we obtained provide quantification of conclusions of visual observations: (a) some varieties bred for resistance are less affected by MSD than others, even when infected at the same stage; (b) early infection is more damaging than late infection, but resistant varieties differ in their response, and; (c) varieties carrying no resistance can be little damaged if infected late. Several methods exist that can be used to control MSD, but the use of resistant cultivars was identified as the most efficient and economic, particularly for subsistence farmers in SSA. Our findings demonstrate that conventional breeding alone is not sufficient for effective plant breeding programmes that aim for highly adapted elite lines in a shorter space of time as evidenced by the disease progression assessment on the growth of maize during the experiment. The use of molecular marker technology (MAS) can greatly assist by reducing generation times nearly by half. Markers are able to detect diversity at DNA sequence level, thereby informing the breeder of any desirable traits or genes.

The smallholder farmer who is mainly dependent on cereal crops for food is more exposed to such epidemics when they occur. In the meantime, an integrated approach to MSD management is the best way of dealing with the disease. Climate change is likely to impact SSA in a significant manner and consequently intensive efforts are being made by institutions like CIMMYT in SSA to develop and deploy climate resilient germplasm with a range of abiotic and biotic stress tolerance (Masuka et al., 2017). CIMMYT and IITA, in partnership with several institutions in SSA, have developed an array of donor lines with resistance to various abiotic and biotic stresses; this germplasm is very widely distributed and used by both public and private sector institutions. Breeders' efforts to develop germplasm with tolerance/resistance to multiple stress tolerance in the SSA often involves crossing MSV-

resistant lines with other breeding materials that may not have resistance to MSV (although these may have other adaptive traits), and hence, require an efficient trait-tracking mechanism during the advancement of segregating generations.

The objective of the original MSV resistance breeding strategy developed at IITA in 1975 was to develop maize with horizontal resistance that would be stable over time and operational over geographic space. We believe it should be the aim of maize breeding (Nair et al., 2015) programs in Africa to follow a challenge and select methods that will result in the incorporation of multiple MSV resistance factors in their new maize varieties.

4.2 Recommendations

One of the most sustainable ways of managing the MSV disease is through host plant resistance. Maize breeders in SSA have been continually developing resistant varieties that are routinely deployed in the region. Resistant varieties have been developed and have a major role to play in reducing the threat posed by MSV. Although extensive studies have been conducted on the virus and its vectors, it is still not clear how easily a new virus isolate that is more virulent on such resistant varieties and arising in a single plant, would be disseminated widely within a season (Bosque-Pérez, 2000). There is a need for further research to answer these and other important questions on MSV, its epidemiology and evolution. Rodier et al. (1995) reported both major and minor genes/loci governing MSV resistance, with complete to partial dominance of resistance. IITA and CIMMYT have developed several lines with MSV resistance (Kim et al. 1987; Wambugu et al., 1999), which are widely used by various institutions in SSA.

4.3 Future Perspectives

Breeding for MSV resistance is done by the private sector, international research centres and national programmes (Nair et al., 2015). The International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) are the major international research centres involved in breeding for MSV resistance in Africa. They have identified germplasm that has high tolerance to MSV, with the Msv1 gene responsible for conferring the resistance (Kyetere et al., 1995). Private companies and government

programmes involved in breeding MSV-resistant hybrids get germplasm from these international research centres. CIMMYT has regional offices in Harare (Zimbabwe) and Nairobi (Kenya) servicing southern and East Africa, respectively. In view of the severity of MSV in sub-Saharan Africa, all maize breeding programmes incorporate resistance to MSV. To date, several MSV-tolerant cultivars have been released throughout the sub-Saharan region. In Zimbabwe, for example, Seed Co (a private company) has released the cultivars SC403, SC411, SC621, SC713 and SC719 which are being marketed in a number of countries in the region (Garcia-Oliveira et al., 2020). The yield and quality of the tolerant cultivars are comparable to those of susceptible cultivars. Msv1 has been reported in almost all MSV-resistant lines identified so far and is generally considered an essential prerequisite for reasonable levels of MSV resistance. The deployment of maize cultivars resistant to MSV is the most preferred approach in SSA where approximately 55 million smallholder farmers depend on its cultivation. Thus, identification of new loci associated with MSV resistance in maize is highly relevant, because the over dependence on a single locus (Msv1) can pose a major threat to continue to develop maize cultivars with durable resistance to the virus in the SSA (Garcia-Oliveira et al., 2020). In order to avoid the over-dependence on Msv1, further studies should be carried out to identify a second gene for MSD resistance to compliment Msv1 gene in conferring enhanced and durable resistance to MSD. Enhanced resistance through additional phenotypic selection will also help prevent possible breakdown of Msv1-mediated resistance in the long term, as multiple strains of MSV-A are reported to sporadically co-occur with the most virulent strain, MSV-A1 in different parts of SSA (Shepherd et al. 2010).

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