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DECLARATION

CANDIDATE

I hereby declare that 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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Signature _____ Date 20-07-09

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision and submitted for examination with our approval.

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DEDICATION

To my wife Agatha Nunu and sons Walter Edupu and Eric Ekiru who have been my inspirati

and

To my deceased grandmother Maria Naduk

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LIST OF ACRONYMS

MSV - Maize streak virus

MSVD - Maize streak virus disease

GY - Grain yield

DTS - Days to silking

DTT - Days to tarsel

PH - Plant height

EH - Ear height

CL - Cob length

CIMMYT - *Centro Internacional de Mejoramiento de Maiz y Trigo*

CIRAD - *Centre International de Recherche Agronomiques pour le Development*

IITA - Internation Institute of Tropical Agriculture

KARI - Kenya Agricultural Research Institute

IRAT - Institute Recherche Agronomique Tropicale

CML - CIMMYT Maize Line

OSU - Ohio State University

GENERAL ABSTRACT

Maize streak virus disease (MSVD) is the most destructive viral disease of maize in the Sub-Saharan Africa. In Kenya, the disease results in reduction of crop dry matter and grain yields. Like most foliar disease, the disease is managed by means of quantitative partial resistance. Since breeding for durable resistance is an essential trait for improved maize varieties, it is thus important to understand genetic systems conditioning MSV resistance in diverse sources and also assess the yield damage caused by the disease. This study was designed to determine (i) Mode of gene action of two maize inbred lines, an MSV-tolerant inbred line CML202 and an MSV-immune inbred line Osu23i (ii) allelic relationships of MSV genes found in the two MSV-parental sources. (iii) The effect of MSV infection on dry matter and grain yields.

A set of six generation designated Parent1, Parent2. F1, F2, Backcross1:1 and Backcross1:2 derived from parental and biparental crosses of MSV susceptible parent EM11-133 and the MSV tolerant parent CML202 and another set derived from MSV susceptible inbred line EM11-133 and MSV immune parent Osu23i and a set of four generations designated P1, P2, F1 and F2 derived from both parental and biparental crosses of both MSV sources of resistance CML202 and Osu23i were planted in three randomized complete block design experiments. The Means and variances of MSV scores rated on individual plants were fitted onto Hayman's 1958 additive-dominance model to determine the mode of gene action of the two parental sources while allelic relationships of genes in the two sources were determined using means of MSV scores and graphic presentations. Eight varieties including three parentals EM11-133, CML202 and Osu23i,

three F1s from three respective generations, a MSV resistant check WH505 and a MSV susceptible check H614D were planted in a fourth split-plot experiment to assess maize crop yields damage caused by MSVD infection. One main plot was inoculated with MSV using viruliferous leafhoppers while the other main plot was a control experiment.

The mode of gene action results indicated MSV resistance in CML202 and Osu23i is controlled by additive gene effects with dominance x dominance epistatic interaction. The numbers of effective factors were estimated to be between 2-7 genes. It appears two separate genetic systems are involved in control of MSV; MSV is controlled through partial resistance in CML202 while complete resistance is responsible for control of MSV in Osu23i. Allelic relationship studies results revealed the two sources CML202 and Osu23i had different genes. Thus by utilizing CML202 and Osu23i MSV sources, a breeder can attain a robust oligenic or multigenic resistance systems which will be hard for destructive virus isolates to overcome. Results showed that MSV disease considerably reduced ($P<0.001$) stover dry matter and grain yields. Stover yields losses ranged from 19 -29% while that of grain ranged from 8 to 48 % The susceptible inbred line EM11-133 sustained large reduction in stover yields (29 %) and grain yields (48%). The susceptible check H614D and tolerant check WH505 sustained stover yield reduction of 22 and 25% and grain yield reduction of 25 and 19 % respectively. However, the yields of MSV immune parent Osu23i, tolerant parent CML202 and EM11-133xCML202 (F1) progeny were not affected. From the results of this study, it is evident that MSV resistance sources exist and that these sources can be utilized in the formation of hybrids which can be availed to farmers to solve the problem of forage and grain shortage.

CHAPTER ONE
GENERAL INTRODUCTION

1.0 Background

Maize (*Zea may* L.) is an important cereal crop for food and feed in many parts of the world (Asea, G, 2005). It is the third most important after rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Gordon and Thottapilly 2003). Since its introduction into Africa at the beginning of the 16th centaury, it has become the continent's second most important food crop, after cassava (*Manihot esculenta)* (DeVries and Toenniessen, 2001). Lack of the grain in Africa means famine, but increasing its production is considered essential for food security (Nderitu, 1999: CIMMYT, 2002: Salasya et al 1998). In Kenya, the cereal is the single most important food and it is the main source of income and employment for the majority of rural households (Salasya *et al.*, 1998). The crop contributes 20% of the total agricultural production, constitutes about 78% of total cereal consumption, 44% of total energy needs and 32% of the total protein requirement (Ruto, 1992).

Developing countries have more land area devoted to maize cultivation than developed countries, but yields in the latter are about four times higher. The average maize yields in industrialized countries is more than 8t/ha while in developing worlds it is slightly less than 3t/ha (Pingali and Pandey, 2001). The crop yields 2.3 to 4.7 tons lower per hectare in Africa (Nderitu 1999). In Kenya, annual average maize production currently is 2.7 million tons. This is slightly lower than 3 million tons consumed each year (ISAAA, 1999). The wide yield-gap difference between the developed world and developing countries is accounted by wide disparities in climatic conditions between tropical and temperate environments and lack

of farming technologies to address myriad production constraints some of which are endemic in developing countries.

Maize production in Africa is affected by numerous production constraints. DeVries and Toenniessen (2001) identified insect pests such as stem borers, grain borer and weevil, ear rots and the parasitic weed, *striga* as important constraints responsible for the maize yield loss. Other priority constraints according to CIMMYT (2002) include low Nitrogen, drought and low pH, while foliar diseases such as gray leaf spot (*Cercospora zeae-maydis)* , northern corn leaf blight

(*Exserohilum turcicum,)* southern corn leaf blight (*Bipolaris maydis*), common rust (*Puccinia sorghi)* and maize streak virus disease (maize streak virus) also feature prominently among major biotic constrains limiting maize production. Compared to other diseases, gray leaf spot, northern corn leaf blight and maize streak virus disease are regarded as the most persistent and destructive diseases of maize and are ranked highly in international and national maize research objectives (Pingali and Pandey, 2001).

The maize plant is infected by about 32 virus species out of which 7 have been reported in sub- Saharan Africa (Brunt *at el.,* 1990). The endemic nature of some of these viral diseases is one of the major factors responsible for low maize productivity (Thottappilly *at el* 1993; Nderitu, 1999). A survey carried out in 1990 in the Sub-Saharan Africa showed that MSV is one of the two most important biotic constraints affecting maize production in the continent (ISAAA, 1999). The disease has the most devastating effects because it can result in complete crop failure (Bosque-Perez, 2000). MSV infection have been reported in southern, eastern and western Africa covering an estimated 60% of the total land area (DeVries and Toenniessen, 2001) and has caused yield losses of up to 100% in some instances (Nderitu,

1999). MSV is an important disease particularly in the central highlands of Kenya including Kiambu, Murang'a and Maragua districts (Kiduyu, 1991; Njuguna, 1996).

1.2 Problem statement

Maize streak virus disease (MSVD) is widespread in tropical and sub-tropical Africa (Alegbejo et al., 2002; DeVries and Toenniessen, 2001). It has been reported as the most damaging disease of maize in the continent (Kim *et al.*, 1989; Thottappilly *et al.,* 1993). The disease is generally considered to be an endemic viral disease in Africa and it is not known to occur in the western Hemisphere (Bosque-Perez, 2000). Serious MSV epidemics have been reported in at least 20 African nations, including Kenya, Nigeria, Ethiopia, Sudan, Tanzania, Zimbabwe, Zambia, Angola, Mozambique, Malawi, Senegal, Ghana, Cameroon, Togo, Benin, Sao Tome and Burkina Faso, (ISAAA, 1999). Other countries where the disease is extensively spread include adjacent islands of Mauritius, Reunion, and Madagascar (Thottappilly *et al.,* 1993). According to reports by Bosque-Perez *et al.,* (1998) grain yield reductions exceed 70% in susceptible varieties. However, average yield losses associated to the virus infection ranges from 30 to 100% (Alegbejo *et al.*, 2002).

Maize streak virus disease is an important constraint to maize grain production in Kenya where yield losses of 24 – 63% have been reported (Guthrie, 1978). The highest incidence of 87 % was observed (Njuguna et al 1989) in Kiambu district of central Kenya. A survey conducted between 1994 and 1995 showed existence of MSVD in seven provinces and seventeen (17) districts. An incidence of 53 % was recorded in Kiambu district in 1994 (Njuguna, 1996). Further Studies by McLeod *et al* (2001) showed that MSVD is the most important disease of maize in central highlands of Kenya. Farmers rated the disease as having the highest impact on yields. The disease has been cited as the most important and difficult

disease to control in central Kenya (Mcleod et al., 2001). Other parts where serious yield losses due to the disease have been reported include the Coastal lowlands and around the Lake Victoria basin (Njuguga, 1996).

1.3 Justification

Although several methods such as integrated pest management (Alegbejo *et al.*, 2002), chemical control followed by roguing-out diseased plants and avoiding planting maize next to alternative hosts have been suggested (Rose 1978) for the control of MSV. The use of resistant varieties is the most cost effective method of control (Njuguna 1996). This is because the inherent ability of the plant to control the disease is conferred by genes which offer resistance to the infective strain of the pathogen.

Resistance to maize streak virus (MSV) is an essential trait of improved maize varieties in the Sub-Saharan Africa (Welz *et al.,* 1988). Thus, development of hybrids with higher level of resistance to MSV through introgression of genes from resistant donors requires better understanding of the genetics of MSV-disease resistance. The methods of selection to be used and the expected selection gain will be determined by the mode of gene action. The breeder or plant geneticist is interested in estimating the genetic effects, which will aid in formulation of the most advantageous breeding procedures.

Genetic inheritance studies of MSV disease resistance have so far suggested monogenic inheritance (Storey and Howland 1967), Oligogenic inheritance (Kim et.al., 1989) polygenic inheritance (Rose 1938; Gorter 1959 and Engelbrecht 1975) and multiple genetic systems (Rodier *et al.* 1995) as possible modes of gene action conferring resistance to MSV disease. However, interpretation of results from these earlier studies according to Kyetere *et al* (1999), remains difficult and could have been influenced by many factors. These are

leafhopper infestation (natural Verses controlled), parental genotypes or population structures (inbred lines Verses populations), disease rating scales, methods of statistical analysis, genotypes x environmental interactions and differences in MSV isolates.

There is, therefore, a need to study and confirm type of inheritance in popular sources of MSV resistance used in the country under local conditions, using adapted susceptible lines to help develop a better understanding of the mode of gene action involved. The main goal of this study is to contribute towards the development of MSV resistant varieties by determining the mode of inheritance of the MSV disease.

The specific objectives were:

(i) To determine the mode of inheritance of resistance to MSV among two inbred lines

(ii) To determine allelic relationship between resistant genes from two MSV parental lines

(iii) To assess the effect of MSV disease infection on yields of MSV resistant parental lines and their F1 single crosses in maize.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification, origin and importance of maize

Maize (*Zea mays* L.) is a coarse, annual grass belonging to the large and important family graminaeae tribe maydeae, genus *Zea* and species *mays*. The crop is native to the Americas (Gordon and Thottapilly 2003). where nearly one-half of the total world production is produced. It was cultivated by Indians as a principle food plants for many centuries before the settling of Europeans in America (Poehlman, 1987). It is believed to have originated in southern Mexico and Central America because of the great diversity of native forms found growing in cultivated fields in those regions. The cereal has two close wild relatives; *teosinte* and *tripsacum*. *Teosinte* has three species (*Zea mexicana*, *Zea perennis* and *Zea diploperennis*) while *Tripsacum* has nine species which differ in ploidy levels (Poehlman, 1987).

The maize plant is normally monoecious, the staminate (male) and pistilate (female) flowers are borne in separate inflorescence on the same plant (Onvueme and Sinha 1991). These unique nature of inflorescence characteristics allows the plant to cross pollinate and have been exploited by plant breeders in their attempt to make improved varieties. Virtually every part of the maize plant including the grain, leaves, cobs and stalks has economic value. Maize can be prepared and consumed in a multiple of ways. It can be roasted, boiled, baked, fried and fermented when it is either whole or ground. The grain of maize or maize germ is important as livestock feed while the stalks, leaves and immature ears are also used as fodder. Maize is industrially important chiefly for the production of starch, oil and alcohol. The

starch can be used as such or converted into dextrins, syrups and sugars. It is also extensively used as an industrial by-product for making alcohol (beer and whisky), industrial alcohols ethyl's and butyl alcohol and acetone. Oil, obtained from the germ, is used for cooking, making soaps and glycerine. (Onvueme and Sinha 1991). Maize is a staple human food in Kenya (Lukuyu, 2005). Two crops are grown each year in lowlands and medium elevations (Njuguna, 1996).

2.2 Maize streak virus disease (MSVD).

Maize streak virus disease occurs in most Sub-Saharan African countries (Kyetere et al., 1999). The epidemics of the disease have been reported in west, central, eastern and southern Africa south of the Sahara desert (Bock 1974; Rose1978; Kim et al 1989; Soto et al., 1982). The epidemics of maize streak disease are promoted by high temperature and low rainfall which increase vector populations and movement (Rose, 1978; Dabrowski, 1985). The occurrences of MSV have been erratic over the years and seasons and its effect range minimum damage to total devastation of the crops depending on climatic variation.

Recent severe epidemics occurred in 1982, 1983, 1984, 1986 and 1987 cropping seasons in many African countries (Kim et al., 1989). In 1988 and 1989 a widespread maize streak epidemic occurred in the central province of Kenya and caused an estimated 40% yield loss in maize (Theuri and Njuguga, 1988; Njuguga et al., 1989), other districts where the MSV disease was reported include Kiambu, Muranga, Kirinyanga, Nyeri, Embu, Kitui, Machakos, Meru, Kwale, Kilifi, Kajiado, laikipia, Nakuru, Narok, Kisumu, Busia, Bungoma and kakamega (Njuguga, 1996). Possible factors thought to have contributed to maize streak epidemic were increased cultivation of napier grass (*Pennisetum purpureum* schumach) in the

highlands, or a new virulent strain of MSV spreading in the highlands. Other factors included expansion of sugarcane farming in the lake region and possibly a large build-up of vector population (Njuguna, 1996).

All the popular maize hybrids such as H614 and H513 which are produced by the Kenya Seed Company are susceptible to MSV. Liberalization of the seed market has allowed other seed companies like Western Seed companies and Agriseed Company to introduce MSV tolerant varieties such as WH403, WH505, DUMA41 and DUMA43 into the Kenyan market (Njuguna et al 2006)

2.2.1 Host range of MSV

Many cereal crops and wild grasses serve as reservoirs of MSV (Njuguga, 1996). The virus infects a wide range of hosts within the gramineae family (Damsteegt, 1983). In addition to Maize, other crops such as oats (*Avena sativum*), sugarcane (*Saccharum officinarum*), and wheat (*Triticum aestivum* L.) have been reported to be naturally infected with MSV (Gorter, 1959: Rose 1978). The virus may also infect indigenous African crops such as pearl millet (*Pennisetum typhyoides*), sorghum (*Sorghum vulgure*) and finger millet (*Eleusine coracana*) (Rose, 1978). While among the wild grasses, *Axonopus compressus, Bracharia deflexa, B. Distichphylla, B. Mutica,* and *Panicum maximum* were reported to be infected by MSV (Thottappilly et al., 1993).

2.2.2 Transmission of MSV by Vectors.

Maize streak virus is not seed borne or sap transmissible (Gordon and Thottapilly, 2003). Experimentally, MSV or its cloned genomic DNA is transmissible to germinating maize seedling and maize kernels by vascular puncture inoculation (VPI) (Redinbaugh, 2003; Njuguna et al., 1997). Normally screening for resistance is done in field upon natural infection by leafhoppers. The virus is obligately transmitted by viruliferous leafhoppers of the genus *Cicandulina* spp (Kyetere et al., 1999). Studies conducted by Storey (1925) showed that the vectors are found in all occasions associated with the streak-disease outbreaks. The insects acquire the virus by sucking juices and picking the virus from infected maize, once infected, it can transmit the disease to other plants, since the virus persists in the leafhopper throughout it's life cycle (Storey, 1925: Gordon and Thottapilly, 2003).

The leafhopper adult is a small insect measuring 2-3 mm in length; it has transparent wings and bears a brown longitudinal stripe. The head, thorax and abdomen are largely yellow with some dark brown markings on the dorsum, the eyes are dark brown. The adults may be found at rest on the upper surface of the young maize leaves forming the terminal cone on the plant (Hill, 1975). Two forms or biological races of the insect exist, an 'active' or viruliferous form capable of virus transmission, and an 'inactive' or non viruliferous form which is incapable of transmission. The active forms become infective 24 hours after feeding on a diseased plant and remain so for most their life cycles (CIMMYT, 2004).

Twenty two species of *Cicundulina* are known to exist and of these, only eighteen species occur in Africa. Eight including *Cicandulina mbila, C. similis, C. storeyi, C. arachidis, C. latens, C. bipunctata, C. ghaurii* and *C. parazeae* have been confirmed as vectors of MSV

(Storey, 1925; 1936; Okoth *et al.*, 1988 and Alegbejo *et al* 2002). The efficiency and ability of transmission varies from species to species and between individuals of the same species and is inherited, dominant and sex linked (Storey, 1932). *Cicandulina mbila* which has ability to transmit MSVD with up to 100% efficiency appears to be the most important vector of MSVD (Velders et al. 2001).

2.2.3. Symptoms of MSV disease.

The symptoms of maize streak include small, spherical, chlorotic spots that can develop into broken to continuous streaks along the veins of the leaves. In susceptible maize varieties, chlorotic spots and continuous stripes may start developing within 4 – 5 days after infection (Kiduyu, 1991). The symptoms first appear on inoculated or infected leaves and later on subsequent younger leaves since the virus is systemic (Thottappilly et al., 1993). Spots initially are scattered but eventually become more numerous and grouped together, as the plant continues to grow. The symptoms become severe and stripes may coalesce and form wider stripes fused together, producing both yellow and white lesions that cause an entire leaf to become chlorotic (Storey 1938). Severe chlorotic stripping results in stunted growth, poor ear formation and reduced seed setting. Intensity of striping may vary strongly according to genotype (Kiduyu, 1991). For instance, highly susceptible maize cultivars may show almost complete chlorotic streaking of all new leaves following infection (Bock et al. 1974). Sometimes, chlorosis is followed by progressive necrosis and premature plant death, particularly when infection occurs at an early stage of the plant growth (Bosque-Perez, 2000). Resistance to MSV as the case for most foliar diseases is expressed as scattered discountinous streaks and in some lines as immunity (Damsteeght, 1983; Asea, 2005).

Maize grain yield losses due to MSV disease depend on many factors, including cultivars and growth stage at the time of infection (Van Rensburg, 1981). Plants infected early are more severely diseased than plants infected when older and consequently, yields losses are directly proportional to the age of the plant at infection (Bosque-Perez, 2000). Maize plants are vulnerable from emergence to tarselling (Kiduyu, 1991). Symptoms may also vary depending on MSV isolate inciting the disease. Lesion colour varies from white to yellow with incident light, the white lesions being translucent in transmitted light (Pinner *et al.*, 1988). Some virus strains give red pigmentation on maize leaves and abnormal shoot and flower bunching in grasses (Pinner *et al.,* 1988).

2.2.4 Management of Maize streak virus disease.

Maize streak virus can be managed by various cultural practices and insecticides (Rose, 1978). The most important practices are timely planting, planting barrier crops between early and late-planted maize fields to disrupt vector movement. Crop rotation, avoidance of maize planting downwind from earlier planted susceptible cereal crops and removal of diseased plants are other recommended control measures. The leafhoppers vectors can easily be controlled by application of contact insecticides such as DDT and Carbonyl, spraying of systemic insecticides such as dimethoate and demeton-methyl and application of granular systemic insecticides disultoton, phorate, aldicarb and carbofuran into the soil at planting (Rose, 1978).

Use of insecticides, however, is known to be detrimental to the environmental. 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). Although integrated pest management (IPM) has been

recommended as a good viable option of control, the most appropriate, economically viable and practical method to minimize damage caused by maize streak is host resistance (Thottappilly et al., 1983: Alegbejo *et al* 2002).

2.3 The particle structure and strains of maize streak virus

Maize streak virus (MSV) is a member of single stranded DNA plant viruses belonging to the genus m*astervirus* and family geminiviridae. It is the causal agent of maize streak disease (Bosque-Perez, 2000). The virus was first purified by Bock et al., (1974), who found out that the virus had an unusual particle and appeared to be an association of two particles occurring in doublets, referred to as germinates leading to the term 'geminivirus'. It is represented by small particles approximately 20 x 30 nm in diameter and its nucleic acid was determined as a single-stranded DNA existing predominantly as closed circular molecule of 2.7 kilobases and mass 7.1 x 10^5 Daltons (Harrison et al., 1977; Harrison, 1985; Mullineaux *et al.*, 1984). The coat protein of MSV consists of a single protein subunit of molecular weight 28,000 Da (Harrison, 1985).

Distinct strains of MSV are known that vary in their degree of nucleotide sequence and ability to induce different degrees of disease severity (Mesfin et al., 1992; Martin et al., 2001). Maize streak virus isolates from various African grasses and other gramineous crops were designated as host-adapted isolates or strains of MSV (Gordon and Thottapilly 2003). Typical maize-infecting MSV was termed MSV-A while MSV strains that incite MSV in *pannicum* and sugarcane were denoted PanSV and SSV, respectively (Rybicki and Hughes, 1990). Virulent forms of the virus incite severe stunting and chlorosis on infected susceptible hosts, and subsequently drastically reduce yield (Bosque-Perez, 2000).

Diverse groupings of MSV strains include MSV-A. These strains and isolates have approximately 95% sequence identity, and induce more severe streak symptoms on maize (Gordon and Thottapilly, 2003). MSV-B includes strains from wheat and a number of wild grass species that shares 80% genome sequence identity with MSV-A isolates. Maize streak virus-C is currently represented only by MSV-Set, an isolate from a *Setaria* sp that shares 80% genome sequence identity with MSV-A and MSV-B (Gordon and Thottapilly, 2003). Different subtypes within the MSV-A strain of the virus appeared to predominate in different parts of Africa and certain of these subtypes including MSV-A1, MSV-A2 and MSV-A5 were determined to be more pathogenic in maize than others such as MSV-A3 and MSV-A4 (Martin et al., 2001). These differences are of potential importance in screening and deployment of resistant genotypes in specific geographic regions (Asea, 2005). The compete sequence of the genomes of three MSV-A isolates from geographically separate regions in Africa have been sequenced and published (Briddon et al., 1994). Nigerian isolate was sequenced by Mullineaux et al., (1984), Kenyan isolate by Howell, (1984) and South African isolate by Lazarowitz, (1988). Variability studies of these genomes showed that the sequences are 98% homologous (Briddon et al., 1994).

2.4: Previous and present attempts of breeding for resistance against MSVD.

The existence of resistant sources to MSV had been recognized in the early thirties in South Africa (Storey and Howland, 1967). Resistance was discovered in a variety entitled "Peruvian Yellow" which had satisfactory control to streak (Gorter, 1959). Storey and Howland (1966) 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 three-

way crosses showed great reduction of the disease (Storey and Howland, 1967). Storey's resistant lines were not maintained and so they were "lost".

Screening for resistant germplasm continued and in 1976 resistance was identified in a variety "revolution" which was collected by Institut Recherche Agronomique Tropicale (IRAT) in Reunion. The "Revolution" germplasm was used as a component of the MgA (Muguga A) maize population which was constituted in Muguga. MgA and MgB (Muguga B) maize populations were developed with a view to produce improved varieties suitable for central Kenya with resistance to maize streak, headsmut and *turcicum* blight (Manwiller, 1983). The two populations were later shelved because of low heterosis and poor adaptability (Njuguna et al', 1989).

In 1975, maize scientists at IITA found a new source of resistance in tropical *Zea* yellow population (TZ-Y) derived from crosses of a white grain "Tuxpeno Planta Baja' from CIMMYT in Mexico and unidentified yellow germplasm from East Africa (Kim et al., 1989; Ajala, 1999). Tropical *Zea* streak resistant –white (TZSR-W) and Tropical *Zea* streak resistant – yellow (TZSR-Y) were the first two populations with high MSV resistance. They were developed by crossing resistant plants from TZ-Y with TZPB, an MSV-susceptible white variety with high yield potential. A resistant inbred line IB32 was developed from TZ-Y in 1979 (Kim *et al.*, 1989), one S1 line derived from TZ-Y and designated TZ-Y 32 had all its plants rated as highly resistant. The line was advanced to S6 and designated as IB32 (Ajala, 1999).

Using the resistant sources IB32 and "revolution", scientists from IITA in collaboration with those from CIMMYT and National Programs in Africa have up to date developed more than

100 different maize varieties and hybrids adapted to the different ecologies (Kim *et al.*, 1989). In addition, many breeding programs also use resistant sources developed from international programs to incorporate resistance into their own locally developed varieties (Soto et al., 1982).

In late 1989, the Kenya Agricultural Research Institute (KARI) started a breeding program to develop streak resistant (SR) cultivars for mid-altitude and lowland areas of Kenya. Resistant sources namely MSR X Pool19aOP, 100MSRIL, MUAP 13 OP, MUAP 3 OP, MUAP 4 OP, KAAP 3 OP, MUVC 9014 SR VC and MUVC 9084 were obtained from CIMMYT- Harare whereas Tzi3, Tzi8, TziSYN, (S x Tzi15)F1, (F x Tzi3)F1 and (N x Tzi9)BC1 were obtained from IITA. Results showed that resistance sources from CIMMYT- Harare were more stable and agronomically acceptable compared to IITA sources (Muthamia et al., 1994). Further work conducted by Njuguna (1999) indicated that among 18 maize genotypes obtained from different programs and evaluated for streak, only two (Osu23i and Cirad's C390) sources showed resistance to MSV. Similar observation was reported by Ininda (1999) while investigating polymorphism and disease expression among different sources of MSV. The results showed that there was adequate polymorphism within CIMMYT's inbred lines Osu23i, CML202 and also IITA's inbred line Tzi3 and in the susceptible Kenyan inbred line EM11-133. Further, 69% inbred lines originating from Osu23i, 62% inbred lines from C390 and 42% inbred lines from CML 202 were classified as resistant (Ininda, 1999).

2.5: Genetics studies of inheritance to maize streak virus.

Studies on the inheritance of resistance to streak disease started in Kenya during the early 1960's and continued on to the 1980's (Muthamia *et al.*, 1994). Further studies have so far been conducted and there are varying reports in literature about the inheritance or mode of

gene of maize streak resistance. While addressing the participants of the advances in maize streak virus disease in Eastern and Southern African workshop, DeVries (ISAAA, 1999) pointed out that "MSV is known to be controlled by one major gene with several modifiers". Same or conflicting results have so far been reported. Storey and Howland, (1967) ascribed maize streak resistance to partial dominance conferred by a single gene. While Engelbrecht (1975) found that five dominant genes were involved, but Kim et al., (1989) reported that resistance in inbred IB32 was quantitatively inherited through additive gene action of two or three major genes and some modifiers. More recent studies on genetic control of MSV resistance in population CVR3-C3 have shown that multiple genetic systems for resistance may exist (Rodier *et al.*, 1995). Both unimodal and bimodal frequency distributions of symptom ratings were observed when progeny developed by self-pollination within resistant, partially-inbred lines were inoculated with MSV. The results suggested the possible existence of two different genetic systems controlling resistance. One system with major genes for complete resistance, the other with minor genes controlling partial resistance.

Genomic regions associated with MSV resistance have been identified in several studies using different germplasm and environments. A major Quantitative trait loci (QTL) was identified by Kyetere et al., (1995; 1999) in IITA inbred line Tzi4 on the short arm of chromosome 1 (1S - bin1.04) and was designated as msv1. The same locus was identified by Welz et al., (1998) in a population derived by crossing CML202, an MSV resistant inbred, and Lo951, a susceptible inbred. Other additional studies by Pernet (1999a and 1999b) identified a major QTL in the same genomic location on chromosome 1S, and proposed that MSV resistance was 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.

2.6 Estimation of genetic factors (genes) and determination of mode of gene action in generation mean analysis.

Analysis of phenotypic data for estimating genetic effects and heritability for quantitative traits is an important tool for plant breeders (Gusmini et al., 2007). In conventional plant breeding, The plant geneticist or breeder is interested in the estimation of gene effects in order to formulate the most advantageous breeding procedures for the improvement of the attribute in question (Gamble, 1961). However, analysis of these genetic effects influencing the traits is difficult since it is not possible to identify individual gene effects because each of the multiple genes influencing the traits expresses a small effect on the phenotype (Griffing, 1950).

A number of genes at different loci contribute to the expression of a quantitative character, thus interpretation of their effects on the some traits is not easy due to pooled or cumulative effects of the genes (Gamble, 1961). Because of this low order of effects of individual genes, it is necessary to study the action combined action of these genes together by statistical techniques (Griffing, 1950). This allows partitioning of total variance into genetic and environmental variances and the genetic variance into additive and dominance components and inter allelic interaction effects (Holland et al., 2003). The use of biometrical studies, based on generation means and variances of the studied traits, allow one to study the relative magnitude of additive, dominance and epistatic interaction as well as to estimate the effective

number of genes (Lande, 1981). This has been possible and has been facilitated by application of genetic models.

Typically, genetic studies of tolerance to MSV have attempted to fit disease severity assessment data of segregating progeny into phenotypic classes predicted by various models of gene number and dominance effects (Kyetere, 1999). A procedure is outlined for the separation, into six parameters, of gene effects affecting genetic variation of a quantitative trait. These parameters represent mean effects, additive and dominance gene affects, and the three types of digenic epistatic effects (Gamble, 1961). The most commonest genetic model is that of Anderson and Kemthorne, (1954) and Hayman, (1960). This six parameter model permits estimation of the additive, dominance, additive x additive, additive x dominance and dominance X dominance gene effects with less difficulty in their interpretation.

Anderson and Kempthorne (1954) showed in particular that all the information about additive, dominance and digenic epistatic variation available in the means of generations descended from two inbred lines is contained in just six parameters. This model was reviewed by Gamble (1961). Estimates of the parameters are obtained using the population means of two inbred lines, their cross, and descendants due to subsequent selfing and crossing. The relative importance of the different gene effects can be evaluated from the magnitude and significance of the estimates (Gamble 1961). The desirable characteristics of Anderson and Kempthorne (1954) and Hayman (1960) genetic model is that the parameters have genic interpretation, is applicable to any number of loci and is flexible with respect to increasing assumption. The model assumes that linkages and lethal genes are absent and there is constant viability for all genotypes (Anderson and Kempthorne 1954).

CHAPTER THREE

MODE OF GENE ACTION TO MSV IN MID ALTITUDE CIMMYT INBREDLINES CML202 AND OSU23I

3.1 Abstract

Maize streak virus is the most destructive viral disease of maize in Africa. Like most foliar disease, it is managed by means of quantative partial resistance (Asea 2005). Since breeding for durable resistance is an essential trait to improved maize varieties in the Sub-Saharan African (Welz et al 1998), it is important to understand genetic systems conditioning resistance in diverse sources. This study was designed to determine mode of gene action in parental inbred lines, CML202 and Osu23i and allelic relationships of genes found in both sources. The parents F1, F2 and backcross generations of a MSV susceptible parent EM11-133 and both sources of resistance CML202 and Osu23i were planted in three RCBD experiments in the short rains of 2007. The trials were inoculated twice. The maize streak virus score means and variances rated on individual plants were fitted onto an additive-dominance model (Hayman1958). Results indicated that resistance in CML202 and Osu23i is controlled by additive gene effects with dominance x dominance epistatic interaction. The numbers of effective factors were estimated to be between 2-7 genes. Based of frequency distribution of MSV scores in segregating population (BC1:1, BC1:2 and F2), two separate genetic systems appear to be involved in control of MSV. Maize streak virus is controlled through partial resistance in CML202 while complete resistance is responsible for control of MSV in Osu23i. Allelic relationship studies revealed that genes in two different sources were different. Utilization of both sources will be difficult for viruses to overcome.

3.2 Introduction.

Maize streak virus (MSV) is caused by a virus that is transmitted by viruliferous leafhoppers of the genus *Cicandulina* (Asea 2005). The disease contributes to the problem of extremely low African maize yields (Owor et al., 2007). Management of maize streak virus disease (MSVD) is difficult partly due to the variability of the virus, and partly due to the susceptibility of the locally adapted maize lines and unpredictable vector migratory and survival pattern (Rodier, 1995; Danson et al., 2006). Although various cultural practices and insecticides are effective in managing MSVD (Rose, 1978), the development and deployment of resistant varieties is the most appropriate and cost effective approach to controlling MSVD (Fraser, 1992; Danson et al., 2006; Nguguna, 1996) since the inherent abilities of the plant to control the disease is conferred by genes which offer resistance to the infective strain of the pathogen.

Fulfilling the growing need for increased and sustainable maize production will depend on preventing yield losses and maximizing yield potential of the crop (DeVries and Toenniessen, 2001). Thus, development of hybrids with higher level of resistance to MSV through introgression of genes from resistant donors requires better understanding of the genetics of MSV-disease resistance since the methods of selection to be used and the expected selection gain will be determined by the mode of gene action. The breeder or plant geneticist is therefore interested in estimation of the genetic effects, which will aid in formulation of the most advantageous breeding procedures (Gamble, 1961; Hayman, 1958). This objective of this study was to investigate the genetic systems conditioning resistance to MSV among two commonly used sources as well as understand the relationship between alleles found in them.

3.3 Materials and Methods

3. 3. 1 Description of the three parental inbred lines used in the study.

An MSV susceptible inbred line EM11-133 from KARI and two MSV resistant inbred lines, one MSV tolerant inbred line CML 202 and an MSV immune inbred line Osu23i from CIMMYT were used in this study (Table 3.1).

EM11-133 is a susceptible line from KARI. It **was** extracted from Embu 11 (EM11) population through pedigree breeding. EM11 is a white endosperm dent breeding population maturing in 150 days at Embu (Odongo et al., 1995) it was a seed parent derived from diverse high altitude Kitale maize programme (H621, Inbred lines F and G) and Katumani dryland maize programme (Kat. VI and Kat. V) germplasm. The population is an advanced generation of crosses between (H621 x Kat. IV) (FXG) and (Kat V.) (Eberhart, 1989). Although EM11-133 is of desirable agronomic character, it is susceptible to MSV. The parental population, EM 11, was used in the formation of a commercial hybrid H511 (Odongo et al., 1995)

Osu23i refers to Ohio State University line 23 which is immune to MSV (Gibson et al., 2005) the immune line was obtained from CIMMYT as [MSRxPool9] CIF2-205-1(OSU23I). Both glasshouse and field MSV screening conducted in Kenya showed that the line was immune to MSV (Njuguna, 1999). This line is well adapted for mid –altitude maize growing zones.

Table 3.1: Origin, genetic and agronomic characters of plant material

Inbred line	Germplasm resource	Country of Origin	Mid-silk days	Grain Color	Grain Type	Inbreeding level	MSV Reaction
EM11-133	KARI	Kenya	85	white	Flint	S4	Susceptible
CML202	CIMMYT	Zimbabwe	89	White	Semi-dent	S4	Resistant
Osu23i	CIMMYT	Zimbabwe	89	white	Flint	S4	Immune

S4= indicates that the inbred line has been selfed four times and is almost homozygous

CML 202 inbred line has white, semi-dent kernels. It was developed by International Maize and Wheat Improvement Center (CIMMYT) Harare station. It was derived from the bulk population ZSR 923 'S4 bulk' originating from Cameroon 87' in West Africa. Genetic studies as confirmed that the inbred line has a relatively high level of partial resistance to *Exerohilum turcicum* and MSV (Welz et al., 1988; Schechert et al., 1999). The line is late maturing and generally well adapted to growing conditions in the humid mid-altitude zones of Eastern and Southern Africa. It is widely used in many tropical breeding programs for production of hybrids and new inbred lines due to its excellent combining ability for disease resistance and yield (Schechert et al., 1999).

3.3.2. Site Description

Generation of crosses, increase of parental lines and field experiments were conducted at Kenya Agricultural Research Institute- Muguga South Research Centre which is in Kiambu District, Central Province. The station is located 27 Km west of Nairobi City at an altitude of 2095 m, latitude 36° 34-36° 39`S and longitude 1° 11`-14`E. The mean annual rainfall is

946 mm. The soil types are Nitisols according to FAO/UNESCO classification (KARI, 2007). The planting of the trials were staggered to allow for the build-up of leafhopper (*Cicandulina mbila*) vector populations which were used to successfully inoculate all the plants.

3.3.3. Generation of crosses

3.3.3.1 Generation of F1 and F2

During the long rains (LR) of 2006, initial crosses were made between a MSV susceptible parent EM11-133 (P1) as a female and each of the sources MSV resistant parents CML202 and Osu23i as males to generate EM11-133x CML202 (F1) and EM11-133xOsu23i (F1) . The MSV resistant parents were further crossed among themselves to generate Osu23i x CML202 (F1). The parentals (EM11-133, CML202 and Osu23i) and resultant F1's progenies were grown in a separate crossing nurseries in 2006 short rains where parental seed of EM11-133, CML202 and Osu23i were increased by random mating and the F1 were selfed to produce three F2 namely EM11-133xCML202/EM11-133xCML202 (F2), EM11-133xOsu23i/EM11-133xOsu23i (F2) and Osu23i x CML202/ Osu23i x CML202 (F2)

3.3.3.2 Generation of backcrosses

During the short rains of 2006, EM11-133 (P1) CML202 (P2) and EM11-133x CML202 (F1) were planted in a nursery consisting of eight row plots to generate two backcrosses. EM11-133x CML202 (F1) was crossed EM11-133 (P1) to generate backcross EM11-133xCML202/EM11-133 (BC1:1) and EM11-133x CML202 (F1) again crossed to CML202 (P2) to generate EM11-133xCML202/CML202 (BC1:2).

In another crossing block, EM11-133(P1), Osu23i (P2) and EM11-133xOsu23i (F1) were planted in eight row plots to generate other two backcrosses. EM11-133xOsu23i (F1) was crossed to susceptible parent EM11-133 (P1) to generate backcross EM11-133xOsu23i/EM11-133(BC1:1) and EM11-133xOsu23i (F1) was crossed immune parent Osu23i (P2) to generate backcross EM11-133xCML202/Osu23i (BC1:2).

Every ear shoot was covered by a shoot bag soon after tassel emergence and before silk emergence. Pollination was done where silks had emerged under the shoot bag for 1-2 days and measuring more than two centimetres. Tassels were bagged a day prior to pollination when first anther dehiscence was observed. Pollination was carried out the following day early in the morning to avoid contamination. In all crossing nurseries, parental plants not involved in crossing were random-mated to increase parental seed. Generation of F2's was carried by collecting pollen from tassels of respective F1 and using it to self pollinate silks of same F1's. For F1's and backcrosses, pollen was collected from the tassels of the designated male parent and this pollen was dusted on the silks of the designated female parent. All information such as dates of bagging and intended pollination (selfing, random mating and crossing) undertaken were recorded on the tassel bags. After pollination, ears were covered using the tassel bags and clipped so as to hold firmly around the stem. Seeds from the different nurseries were sorted out and bulked after harvesting. The seeds were preserved in the cold room and planted for field testing during the short rains of 2007.

3.3.4 Determination of mode of gene action conferring MSV resistance and allelic relationships between resistant genes

To determine mode of gene in inbred line CML202 and Osu23i, two sets of six basic generations (P1, P2, F1, BC1:1, BC1:2 and F2) derived from crossing nursery one and two were planted in two separate experiments at onset of short rains of 2007. The experimental design in each case was a randomized-complete block, with three replications. Plots consisted of three rows for parents P1, P2 and F1, eight rows for backcrosses BC1:1 and BC1:2 and twenty rows for F2. A set of four generations (P1, P2, F1 and F2) derived in crossing nursery three were planted in a third experiment to determine allelic relationships between resistance genes. The experimental design was a randomized-complete block, with three replications. Plots consisted of three rows for parental P1, P2 and F1 and ten rows for F2. The number of rows in the backcross and F2 were many because segregation patterns is determined using those generations. For all three experiments, rows consisted of approximately 11 plants. The spacing was 75cm between rows and 30 cm within rows. Two seeds were sown per hill and later thinned to one before MSV infestation.

Crop husbandry practices for all experiments involved application of phosphatic fertilizer (Diammonium Phosphate- DAP 18:46:0) at the rate of 125 kg per hectare at planting and side-dressing was effected with 125 kg per hectare of nitrogenous fertilizer (Calcium ammonium Nitrate – CAN 26%N) at 6 weeks after planting. Bull dock, 0.05 GR (Beta-cyfluthrin) was applied to the whorl of each plant to control stalk borers. The insecticide was applied after inoculation, early application could have killed or cause leafhoppers to be ineffective. The crop was kept weed-free by two to three hand weeding and occasional spot weeding.

3.3.5. Generation of MSV inoculum and inoculation.

Transmission of MSV into the test maize plants in the fields was effected using leafhoppers of *Cicadulina mbila* species. The *C. Mbila* colony used was a direct descent from that used by Storey and Howland (1967), and Bock *et al.* (1974). This colony is also used routinely in the maize improvement programme at KARI-Muguga South.

The populations of nonviruliferous leafhoppers were maintained on clean pearl millet (*Pennisetum americanum*) grown in glasshouses, maintained at 25°C by means of an electric fan heater. Two days before inoculation, adult leafhoppers were transferred to insect proof cages containing young maize plants exhibiting very severe MSV disease symptoms and allowed 48 hours acquisition access period (AAP). These infected plants were collected from MSV hot spots within Kiambaa, Limuru, Murag'a and Maragua. This means that screening was thus carried out against a mixture of aggressive isolates. After the two days exposure, two to three veruliferous leafhoppers were placed in small cellulose acetate plastic vials and the vials containing the leafhoppers were attached on distal portions of the youngest leaf of maize which is at two-three leaf stage and allowed two-day inoculation access period (IAP).

For all trials, inoculation was done twice in order to obtain severe and uniform expression of the disease on all test plants. First inoculation was done fourteen days after emergence while the second was done 40 days after emergence.

3.3.6 Disease assessment and determination of physiological characteristics.

Maize streak virus (MSV) scores ratings were based on a five point scale (1-5) similar to one employed by IITA and CIMMYT and described by Kim *et al.*, (1989) and Ajaga (1999), where 1 = no or very few streak symptoms on lower leaves (highly resistant); 2 = light streak

symptoms on most leaves below ears with few symptoms above the ear (light infestation); 3 = moderate or mild streak symptoms on most leaves (tolerance); 4 = abundance symptoms on all leaves (about 60-80% of the leaf area- moderate infestation) and 5 = severe streak on all leaves (over 75-80% of the leaf area) is highly susceptible. Mid points (0.5) on the 1-5 scale was included.

For all the three experiments, MSV resistance ratings were done four times and were based on visual evaluation of disease symptoms on individual test plants. MSV disease ratings were done at 58, 72, 87 and 101 days after first inoculation.

The Area under disease progress curve (AUDPC) which measures the degree of MSV resistance or susceptibility was determined as follows:

$$AUDPC = \sum_{i}^{n} \left(\frac{Yi + Yi - 1}{2} \right) + (ti + 1 - ti)$$

Where n = no of assessment times

Yi = disease severity at ith assessment time

t i = time in days at the ith assessment time

Standardization of AUDPC was done as shown below in order to compare the levels of AUDPC of various generations under study.

$$AUDPC = \frac{\left\{ \sum_{i}^{n} \left(\frac{Yi + Yi + 1}{2} \right) + (ti + 1 - ti) \right\}}{tn - ti}$$

where ti = time at the start of epidemic

tn = time at the end of epidemic

Other foliar diseases like common rust (*Puccinia sorghi)* and northern leaf blight (*Exserohlum turcicum)* symptoms were rated using a scale (1-5) were 1 = clean, no infection and 5 = severely diseased. Days to pollen shed (DTT) and days to silking (DTS) were measured as number of days after planting when 50% of the plants per plot have shed pollen and 50 % of the plants per plot have 1 cm or more silk exposed respectively.

Plant height (PH) and ear height (EH) were measured in centimeters as height between the base of a plant to the insertion of the first tassel branch of the same plant and height between the base of a plant (ground level) to the node bearing the upper (top) ear respectively. Measurements were randomly collected per plot based on plot size and population; measurements were randomly obtained on 15 plants for the parentals and F1 progenies, 40 plants for the backcrosses and 100 plants for F2. Cob lengths (CL) were measured in centimetres by randomly measuring ten cobs per plot. Grain yield (GY) was calculated from field weight per plot and moisture content. Fresh weight (Kg) was determined by weighing the harvested ears per plot while the grain moisture content was determined by sampling a few cobs per harvested plot. Grain yield was transformed to tonnes per hectare assuming 80% shelling percentage as follows:

Yield = [Field Weight/ Plot size] X [(100- MC %) / 87.5] X 0.8 X 10

(Vivek et al 2003)

3.2.7 Statistical and Genetic data analysis

Analysis of variance (ANOVA) was done for each experiment separately using Genstat 8th edition software version 8.1. Analysis of variance for MSV and foliar diseases (rust and blight) scores were based on the rating of individual plants. For other traits such as plant height (PH), ear heights(EH) , days to pollen shed (DTT) and days to silking (DTS) , cob lengths (CL) and grain yield (GY), analysis of variance were based on plot means across replication.

Genetic analysis such as approximation of numbers of genes (alleles) conferring resistance in MSV sources were determinate using Mather's and Lande's 11 methods Mode of gene action was determined using Hayman (1958) Additive-dominance model (Figure 3.1) as indicated in calculations shown below:

Mather Method

$$\text{Number of genes} = \frac{(\mu P1-\mu p2)^2}{2} / \{2 \times \sigma^2_{F2} - (\sigma^2_{B1} + \sigma^2_{B2}\}$$

Where $\mu P1$ = parent 1 MSV scores Means $\mu p2$= parent 2 MSV scores means

σ^2_{F2} = F2 MSV scores variance σ^2_{B1}= backcross 1 MSV scores variance

σ^2_{B2} = Backcross 2 variance

Lande's Method 11

$$\text{Number of genes} = (\mu P1-\mu p2)^2/8 \times [(2 \times \sigma^2_{F2}) - (\sigma^2_{B1} + \sigma^2_{B2})]$$

Where $\mu P1$ = Parent 1 MSV scores means $\mu p2$= Parent 2 MSV scores means

σ^2_{F2} = F2 MSV score variance σ^2_{B1}= backcross 1 MSV score variance

σ^2_{B2} = backcross 2 variance.

Figure. 3.1. Hayman 1958 additive and dominance model.

The exact Fit of Hayman (1958) additive and dominance model

$$m = \bar{F}_2$$

$$d = \bar{B}1 - \bar{B}_2$$

$$h = \bar{F}_1 - 4\bar{F}_2 - (1/2)\,\bar{P}_1 - (1/2)\,\bar{P}_2 + 2\bar{B}_1 + 2\bar{B}_2$$

$$i = 2\bar{B}_1 + 2\bar{B}_2 - 4\bar{F}_2$$

$$j = \bar{B}_1 - 1/2\,\bar{P}_1 - \bar{B}_2 + 1/2\bar{P}_2$$

$$l = \bar{P}_1 + \bar{P}_2 + 2\bar{F}_1 + 4\bar{F}_2 - 4\bar{B}_1 - 4\bar{B}_2$$

Where ***m, d, h, l, j*** **and l** refer to mean, additive, dominance, additive x additive, additive x dominance and dominance x dominance genic effects respectively

$$V_m = \bar{(F2)}$$

$$V_d = \bar{V}\,(B_1) - \bar{V}\,(B_2)$$

$$V_h = \bar{V}\,(F_1) - 16\,\bar{V}\,(F_2) + 1/4\,\bar{V}\,(P1) + 1/4\bar{V}\,(P_2) + 4\bar{V}(B_1) + 4\bar{V}(B_2)$$

$$V_i = 4\bar{V}(B_1) + 4\bar{V}(B_2) + 16\,\bar{V}\,(F_2)$$

$$V_j = 4\,\bar{V}\,(B_1) + 1/4\bar{V}(P1) + 4\bar{V}(B_2) + 1/2(P_2)$$

$$V_l = \bar{V}\,(P_1) + \bar{V}\,(P_2) + 4V(F_1) + 16\bar{V}(F_2) + 16\bar{V}(B_1) + 16\bar{V}(B_2)$$

Where V_m, V_d, V_h, V_i, V_j and V_l refer to mean, additive, dominance, additive x additive, additive x dominance and dominance x dominance genetic variances respectively

Calculaltion of Standard error of Means S.E

S.E $(_m)= (V_m)^{1/2}$

S.E $(_d)=(V_d)^{1/2}$

S.E$(_h)=(V_h)^{1/2}$

S.E $(_i)= (v_i)^{1/2}$

S.E$(_j)=(V_j)^{1/2}$

S.E$(_l)=(V_l)^{1/2}$

Where S.E $(_m)$, S.E $(_d)$, S.E$(_h)$, S.E $(_i)$, S.E$(_j)$ and S.E$(_l)$, refer to mean, additive, dominance, additive x additive, additive x dominance and dominance x dominance genetic variances respectively

Estimatation of 't' values

t$(_m)$ = m/ S.E $(_m)$

t$(_d)$ (d) = d/ S.E $(_d)$

t$(_h)$= h/ S.E$(_h)$

t $(_i)$= i/ S.E $(_I)$

t$(_j)$= j/ S.E$(_j)$

t$(_l)$= l/ S.E$(_l)$

Where Where t$(_m)$, t$(_d)$, t$(_h)$, t $(_i)$ t$(_j)$ and t$(_l)$ values represent the magnitude (significance) of respective genetic effects

Model adapted from: Singh R.K & Chaudhary 1995

3.4 Results.

3.4.1. Disease reaction among parents, F1, F2 and backcrosses of three generations evaluated during the short rains of 2007

Highly significant differences ($P<0.001$) were observed among the six generations (P1, P2, F1, F2, BC1:1 and BC1:2) derived from EM11-133 (P1) and CML202 (P2) for MSV, rust and blight severity scores (Table 3.2). The intensity of streak symptoms varied on the individual segregating generations. The susceptible parent EM11-133 (P1) had the highest MSV scores while the tolerant CML202 (P2) had the lowest MSV scores. The MSV scores average rating of F1 was 13% more than the tolerant parent CML202 MSV scores. The F2 had MSV scores which is similar to the mean MSV score value of the EM11-133 and CML202 (mid-parental value) while the MSV scores of BC1:1 and BC1:2 were comparable to the mid-parental value. The MSV reactions of the crosses (F1, BC1:1, BC1:2 and F2) deviated little or were similar to mid-parent MSV scores. The MSV susceptible parent EM11-133 had the highest AUDPC percentages compared to the MSV tolerant parent CML202 which had lower AUPDC. The F1, BC1:1, BC1:2 and F2 had AUDPC of 40-44 percent which are intermediate between the AUDPC of the parents.

The rust scores of the MSV susceptible parent EM11-133 (P1), MSV tolerant CML202 (P2) BC1:2 and F2 were significantly lower compared to those of the F1 and BC1:1. BC1:1 which had the highest rust scores was the most affected by rust while the F1 which had 7.5% decrease in rust scores compared to BC1:1 ranked second. The blight scores of EM11-133 and CML202 were significantly different to those of F1, BC1:1, BC1:2 and F2. The susceptible parent EM11-133 had the highest blight scores compared to CML202 which had 36 % decrease in blight. F1, BC1:1, BC1:2 and F2 were moderately affected by blight

Table 3.2. Disease scores of parents, F1, F2 and backcross populations derived from EM11-133 and CML202

	MSV Scores rating interval					AUDPC (%)	Diseases scores	
Generation	58 DPI	72DPI	87DPI	101 DPI	Means		Rust	Blight
P1	2.90^{c}	3.18^{d}	3.15^{c}	3.12^{d}	3.09^{c}	54.59	1.54^{a}	2.49^{c}
P2	1.99^{a}	1.92^{a}	1.94^{a}	1.92^{a}	1.94^{a}	34.12	1.48^{a}	1.82^{a}
F1	2.25^{b}	2.23^{b}	2.33^{b}	2.39^{b}	2.30^{b}	39.98	1.73^{b}	2.08^{b}
BC1:1	2.35^{b}	2.40^{c}	2.48^{b}	2.51^{c}	2.44^{b}	42.46	1.86^{c}	2.13^{b}
BC1:2	2.31^{b}	2.36^{c}	2.42^{b}	2.38^{b}	2.37^{b}	41.44	1.59^{a}	1.97^{b}
F2	2.41^{b}	2.48^{c}	2.54^{b}	2.59^{c}	2.51^{b}	43.67	1.64^{a}	2.04^{b}
Grand Mean	2.37	2.43	2.49	2.49	2.44		1.64	2.09
LSD	0.05	0.09	0.09	0.11	0.10		0.06	0.07
CV%	15.40	14.60	14.30	15.40	14.93		13.80	11.70

Means followed by the same letter within column do not doffer at P<0.001 probability level
LSD = Least significant differences DPI = days post inoculation
CV = Coefficient of variation AUDPC = Area under disease progress curves
P1= EM11-133 **P2**= CML202 **F1**= EM11-133xCML202 **BC1:1** = EM11-133 X CML202/EM11-133
BC1:2 = EM11-133 X CML202/CML202 **F2**= EM11-133 X CML202/EM11-133 X CML202

Six generations derived from the susceptible parent EM11-133 (P1) and the immune parent Osu23i (P2) showed varied (P<0.001) reactions to maize streak virus (Table 3.3). The susceptible parent EM11-133(P1) had 37% higher MSV infection compared to the immune parent Osu23i (P2) which had the lowest MSV infection. MSV Score rating of F1, BC1:1, BC1:2 and F2 were close to MSV scores of the immune parent Osu23i. The Areas under disease progress curves (AUDPC) of the susceptible parent EM11-133(P1) was 37% higher compared to that of the immune parent Osu23i AUDPC. The F1's and three (F2, BC1:1 and

BC1:2) segregating population had AUDPC which was closer to AUDPC of the immune parent OSU23i (Table 3.3)

BC1:2 was least susceptible to rust and blight while F1 was highly susceptible for both rust and blight (Table 3.3). The immune parent Osu23i (P2) had 30% decrease in rust and blight infection compared to BC1:2 was the second worse hit. All other generations including MSV susceptible parent EM11-133, BC1:1 and F2 had intermediate infection for both rust and blight (Table 3.3).

Table 3.3 Disease scores of parents, F1, F2 and backcross population derived from EM11-133 and Osu23i

	MSV Scores rating interval					AUDPC (%)	diseases scores	
Generation	58 DPI	72DPI	87DPI	101 DPI	Means		Rust	Blight
P1	2.70^{c}	3.04^{c}	3.17^{d}	3.14^{d}	3.01^{c}	53.35	2.13^{b}	2.46^{b}
P2	1.01^{a}	1.01^{a}	1.00^{a}	1.01^{a}	1.00^{a}	16.70	2.49^{c}	2.44^{b}
F1	1.07^{a}	1.08^{a}	1.11^{a}	1.09^{a}	1.09^{a}	18.10	2.70^{d}	2.67^{c}
BC1:1	1.41^{b}	1.37^{b}	1.56^{c}	1.59^{c}	1.48^{b}	18.10	2.13^{b}	2.48^{b}
BC1:2	1.04^{a}	1.06^{a}	1.06^{a}	1.05^{a}	1.05^{a}	18.40	1.93^{a}	1.87^{a}
F2	1.30^{b}	1.31^{b}	1.32^{b}	1.34^{b}	1.32^{b}	22.98	2.10^{b}	2.45^{b}
Grand Mean	1.42	1.48	1.54	1.54	1.49		2.25	2.40
LSD	0.15	0.15	0.15	0.16	0.16		0.08	0.07
CV%	39.50	38.30	38.70	40.20	39.18		12.6	10.3

Means followed by the same letter within column do not differ at P<0.001 probability level

LSD = Least significant difference DPI = days post inoculation

CV= Coefficient of variation AUDPC = Area under disease progress curves

P1 = EM11-133 P2= Osu23i **F1**= EM11-133 x Osu23i **BC1:1** = EM11-133 x Osu23i / EM11-133

BC1:2 = EM11-133 x Osu23i/ Osu23i **F2** = EM11-133 x Osu23i/ EM11-133 x Osu23i

The four generations derived between the two sources of resistance Osu23i and CML202 exhibited significant differences (P<0.001) in terms MSV, rust and blight severity scores (Table 3.4) The immune parent Osu23i was least susceptible to MSV while the tolerant parent CML202 which had 46% more MSV infection compared to the immune parent Osu23i was the most susceptible . The F1 and F2 had MSV scores close to that of the immune parent Osu23i. The MSV tolerant parent CML202 had the highest AUDPC while the immune parent Osu23i and the F1 had the lowest AUDPC percentage. The F2 had intermediate AUDPC. In terms of rust and blight reactions, the score rating of four generation derived from MSV sources Osu23i and CML202 shown diverse expression (P<0.001). The immune parent Osu23i was the most susceptible for both rust and blight. The tolerant parent CML202 was the least susceptible for rust and ranked second for blight reaction. The F2 showed moderate reactions for rust but was most affected by blight.

Table 3.4. Disease scores of parents, F1, F2 populations derived from Osu23i and CML202

	MSV Scores rating interval					AUDPC (%)	Diseases score	
Generation	58 DPI	72DPI	87DPI	101 DPI	Means		Rust	Blight
P1	1.03[a]	1.01[a]	1.00[a]	1.00[a]	1.01[a]	17.70	2.17[d]	1.87[c]
P2	1.93[c]	1.96[c]	1.94[c]	1.86[c]	1.92[c]	33.77	1.48[a]	1.76[b]
F1	1.01[a]	1.01[a]	1.01[a]	1.00[a]	1.00[a]	17.58	2.09[c]	1.68[a]
F2	1.24[b]	1.21[b]	1.21[b]	1.22[b]	1.22[b]	21.34	2.00[b]	1.90[c]
Grand Mean	1.30	1.30	1.29	1.27	1.29		1.94	1.80
LSD	0.08	0.08	0.08	0.09	0.08		0.06	0.07
CV%	22.60	23.50	23.20	25.10			11.30	13.80

Means followed by the same letter within column do not differ at P<0.001 probability level
LSD = Least significant differences DPI = days post inoculation
CV = Coefficient of variation AUDPC = Area under disease progress curves

P1= Osu23i **P2**= CML202 **F1**= Osu23i x CML202 **F2**= Osu23i x CML202/Osu23i x CML202.

3.4.2 Agronomic characteristics of the parents F1, F2 and backcrosses of the three generations evaluated during the short rains of 2007.

Significant difference (P<0.001) were observed on selected agronomic traits recorded on six generations derived from EM11-133 (P1) and CML202 (P2) (Table 3.5). The days to tarsel (DTT) and days to silk (DTS) for the generations were within those recommended for the medium altitude of 100 days. Except for the MSV tolerant parents CML202 (P2) and F2 progeny which flowered and silked 3 to 17 days later than the recommended days, the rest of the generations including the susceptible parent EM11-133 (P1), F1 and backcross progenies flowered and silked earlier with an average of 100 DTT and 101 DTS. The parental inbred lines EM11-133(P1), CML202 (P2) and F2 progenies were significantly shorter than the F1, and backcross progenies by 20 – 29%.

The generations varied significantly (P<0.001) in terms of grain yields. The parents EM11-133 (P1), CML202 (P2) and F2 being inbred, had significantly lower yields Compared to the backcrosses. The highest yielding backcross BC1:1 had 64% more yields compared to lowest yielding parent EM11-133, the second and third yielding crosses F1 and BC1:2 had yield advantage of 56% and 43% respectively compared to EM11-133. Except for the susceptible parent EM11-133(P1) which had 12 % shorter ears Compared to EM11-133, the rest of the generations including the tolerant parent CML202 F2, BC1:1 and BC1:2 had longer ears. Grain yield was highly and moderately correlated With plant height, ear height, and ear length (Appendix 6).

Table 3.5 Agronomic traits on the parents , F1, F2 and backcross populations derived from EM11-133 and CML202

Generations	DTT(days)	DTS(days)	PH(Cms)	EH (Cms)	G Y(t/ha)	CL(Cm
P1	101.00[a]	102.33[a]	144.80[a]	77.20[b]	2.33[a]	11.70[a]
P2	115.67[c]	117.67[c]	142.40[a]	60.70[a]	2.37[a]	13.14[b]
F1	99.67[a]	100.67[a]	172.80[b]	82.80[b]	5.30[c]	14.54[b]
BC1:1	100.33[a]	101.33[a]	183.90[b]	93.50[c]	6.53[d]	14.57[b]
BC1:2	99.33[a]	100.33[a]	170.60[b]	77.10[b]	4.13[b]	14.24[b]
F2	103.33[b]	106.00[b]	150.10[a]	69.80[b]	2.97[a]	14.13[b]
Mean	103.22	104.72	160.77	76.85	3.94	13.72
LSD	1.62	3.32	15.69	7.77	0.94	1.42
CV%	0.90	1.70	5.40	5.60	13.10	5.70

Means followed by the same letter within the same column do not differ at P<0.001 probability level

LSD = Least significant difference CV = Coefficient of variation DTT = days to tarsel, DTS = days to silking PH = Plant height, EH = ear height GY= Grain yield CL = cob length

P1 = EM11=133 **P2** = CML202 **F1**= EM11-133 x CML202 **BC1:1** = EM11-133 x CML202/EM11-133 **BC1:2** = EM11-133 x CML202/CML202 **F2** = EM11-133 x CML202/EM11-133 x CML202

The six generations derived from EM11-133 (P1) and Osu23i (P2) showed significant differences (P<0.001) in flowering dates and grains yields (Table 3.6). No differences were observed in plant heights and cob lengths. The susceptible parents EM11-133 (P1) was earliest to flower and silk, it was followed by the F1 which flowered and silked 10 days later, the P2, BC1:1, BC1:2 and F2 were the last to flower within the recommended range of 100 days. Excellent knicking ability was noted for the susceptible parent EM11-133 (P1), this generation tarselled and silked within the same day.

Table 3.6 shows that the parents, F1, F2 and backcross progenies differed significantly (P<0.001) in terms of grain yield, the yields of the crosses were comparably better (P<0.001) to those of the parents. The parents EM11-133 (P1) and Osu23i (P2) being inbred, were low yielding compared to crosses. The MSV immune inbred line Osu23i (P2) was lowest yielding among all the generations; this was followed by the MSV susceptible parent EM11-133 (P1) and the F2, both generations had 55% and 50% yields lower than F1 and BC1:1. both the F1 and BC1:1 had the highest yields (Table 3.6).

Table 3.6 Agronomic traits on the parents , F1, F2 and backcross populations derived from EM11-133 and Osu23i

Generations	DTT(days)	DTS(days)	PH(Cms)	EH (Cms)	G Y(t/ha)	CL(Cms)
P1	89.00[a]	89.67[a]	148.00	89.30	3.10[b]	13.74
P2	101.67[c]	102.67[b]	177.00	86.60	1.99[a]	14.13
F1	98.67[b]	99.67[a]	168.00	115.00	6.93[d]	16.70
BC1:1	100.67[c]	102.00[b]	212.00	109.80	6.93[d]	15.98
BC1:2	103.33[c]	104.33[d]	229.00	113.40	5.61[c]	16.48
F2	102.00[c]	104.67[d]	181.00	85.10	3.48[b]	15.60
Grand Mean	99.22	100.50	185.83	99.87	4.67	15.44
LSD	1.40	1.29	85.40	8.06	0.93	2.61
CV%	0.80	0.70	25.30	4.40	10.90	9.30
Level of significance	P<.001	P<.001	P<0.376	P<.001	P<.001	P<.134

Means followed by the same letter within column do not differ at respective probability level
LSD =Least significant difference. CV = Coefficient of variation DTT = Day to tarsel
DTS = days to silking, PH = plant height, EH= ear height GY= Grain yield CL=cob length
P1 = EM11=133 **P2** = Osu23i **F1**= EM11-133 x Osu23i **BC1:1** = EM11-133 x Osu23i/EM11-133
BC1:2 = EM11-133 x Osu23i/Osu23i **F2** = EM11-133 x Osu23i/EM11-133 x Osu23i

There were diversity (P<0.001) in flowering, plant height, ear heights and grain yield among four generations derived from Osu23i and CMl202 . However, there were no variations in cob lengths of all the generations.

Contrary to the findings of the first two studies, the F1 was the earliest to tarsel and silk. This was followed secondly by the immune parent Osu23i and the F2 which flowered in 2 to 3 days later. The tolerant parent CML202 was the last to flower and silk. The varieties differed in plant height. The MSV tolerant inbred line CML202 which was 79% shorter than the tallest variety F1 was the shortest variety, the second and third tallest varieties were the immune inbred line Osu23i and the F2 which were 35% and 22% shorter than the tallest variety F1. The grain yield and cob lengths were significantly different (Table 3.7); both parental inbred lines Osu23i and CML202 were the lowest yielding and had short cob lengths. The third highest yielding was the F2; this variety had almost half yields of the highest yielding variety. The F1 was the highest yielding variety (Table 3.7)

Table 3.7 Agronomic traits on the parents , F1 and F2 populations derived from Osu23i and CML202

Generations	DTT(days)	DTS(days)	PH(Cms)	EH (Cms)	G Y(t/ha)	CL(Cms)
P1	95.67^{b}	96.00^{a}	181.20^{b}	85.70^{b}	2.31^{a}	13.46^{b}
P2	115.67^{c}	118.67^{b}	137.20^{a}	58.90^{a}	1.89^{a}	12.01^{a}
F1	94.00^{a}	95.00^{a}	245.20^{d}	115.70^{d}	9.92^{c}	18.61^{d}
F2	96.67^{b}	97.33^{a}	200.80^{c}	95.90^{c}	4.81^{b}	15.94^{c}
Grand Mean	100.50	101.75	191.10	89.05	4.73	15.01
LSD	1.29	1.97	14.37	7.38	1.37	1.02
CV%	0.60	1.00	3.80	4.10	14.50	3.40
Level of significance	P<.001	P<.001	p<0.001	P<.001	P<.001	P<.0.134

Means followed by the same letter within column do not differ at respective probability level
LSD =Least significant difference. CV = Coefficient of variation DTT = Day to tarsel
DTS = days to silking, PH = plant height, EH= ear height GY= Grain yield CL=cob length
P1 = Osu23i P2 = CML202 F1 = Osu23i x CML202 F2 = Osu23i x CML202/Osu23i x CML202

3.4.3: Mode of gene action conferring MSV resistance in inbred lines CML202 and Osu23i and allelic relationships of genes found in two sources.

Scale test using Hayman's (1958) additive-dominance model revealed that the additive gene effects were quite important in the inheritance of MSV resistance in parent CML202 (P2). Estimates of dominance gene effects were of low (negative) magnitude. The dominance x dominance gene effects were more important than additive x additive and additive x dominance genic effects (Table 3.8a) The numbers of effective factors (genes or "allele") conferring MSV resistance in CML202 (P2) ranged from 2 and 7 genes according to Mathers and Lande11 Methods respectively (Table 3.8b).

Individual plants of BC1:1, BC1:2 and F2 segregating populations exhibited a range of symptoms on the scoring scale (Figure 3.2). Some plants had scores which were different from those of either of the parents. The number of plants falling in different disease category for each segregating populations resulted generally in a uni-modal distribution with high percentage of intermediate symptoms ratings compared to low and high symptoms rating which were quite variable (Figure 3.2).

Table 3.8a Additive-dominance model (Hayman1958) scale test of tolerant line CML202

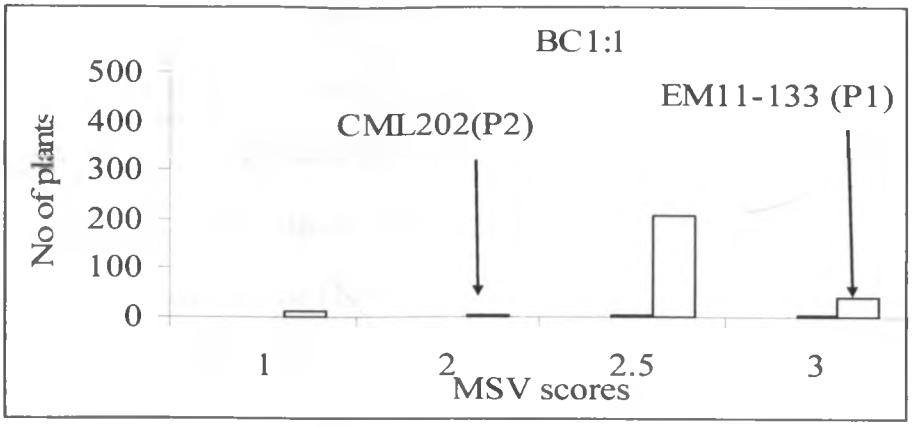
MSV scores

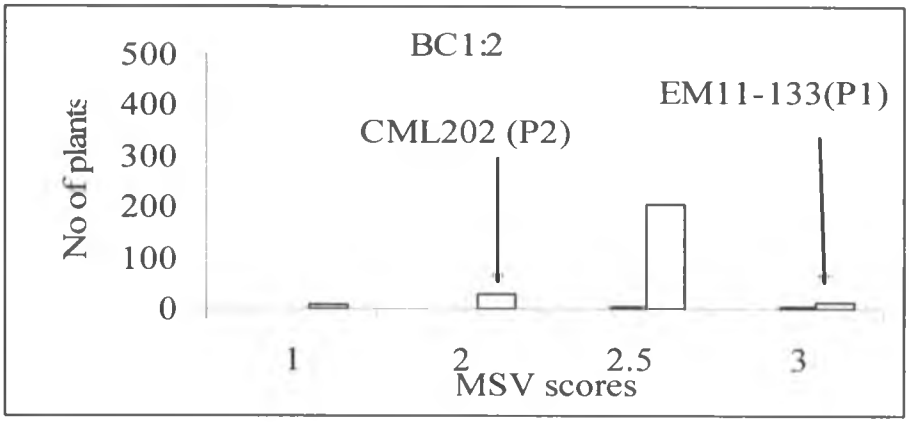
main and epistatic genic factors	Genetic effects	Variances	Standard Error of means	significance test 't'
m	2.565	0.0002	0.014	181.91
d	0.095	0.0004	0.02	4.75
h	-0.641	0.002	0.048	-13.38
I	-0.466	0.006	0.08	-5.825
j	-2.44	0.004	0.063	-38.85
l	0.462	0.025	0.159	2.906

m = Mean, ***d*** = additive, ***h*** = dominance, ***I*** = additivex additive, ***j***= additive x dominance
l = dominance X dominance

Table 3.8b Effective factors and magnitude of variances conferring resistance in CML202

Method	Number of genes
Mather's	6.947
Lande's 11	1.736





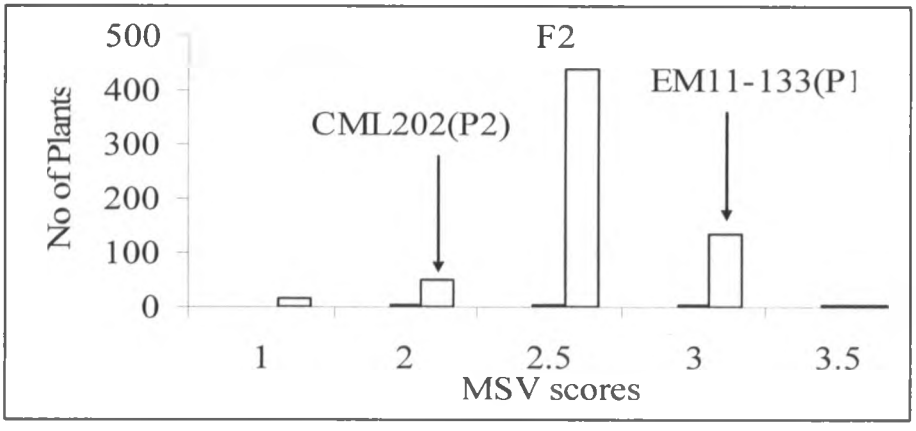


Figure 3.2: Frequency distribution of Segregating population BC1:1, BC1:2 and F2 derived from EM11-133 and CML202 during the short rains of 2007.

Scale test using Hayman's (1958) additive-dominance model revealed that the additive gene effects were quite important in the inheritance of MSV resistance in immune parent Osu23i (Table 3.9a) Estimates of dominance gene effects were of low (negative) magnitude. The dominance x dominance gene effects were more important than additive x additive and additive x dominance genic effects (Table 3.9a) The numbers of effective factors (genes or "allele") conferring MSV resistance in Osu23i ranged from 2 and 6 genes according to , Mather's and Lande's 11 Methods respectively (Table 3.9 b)

Analysis of intra-generation distribution of plants exhibiting different symptoms in segregating populations revealed two distributions patterns (Figure 3.3). For BC1:1 and F2 the frequency distribution was bimodal showing quite variable proportions. There was a high percentage of symptom-free plants compared to intermediate percentage of intermediate symptoms ratings. The BC1:2 had strictly left skewed unimodal frequency distribution with no completely susceptible lines appearing (Figure 3.3)

Table 3.9a Additive-dominance model (Hayman1958) Scale test of immune line Osu23i MSV scores

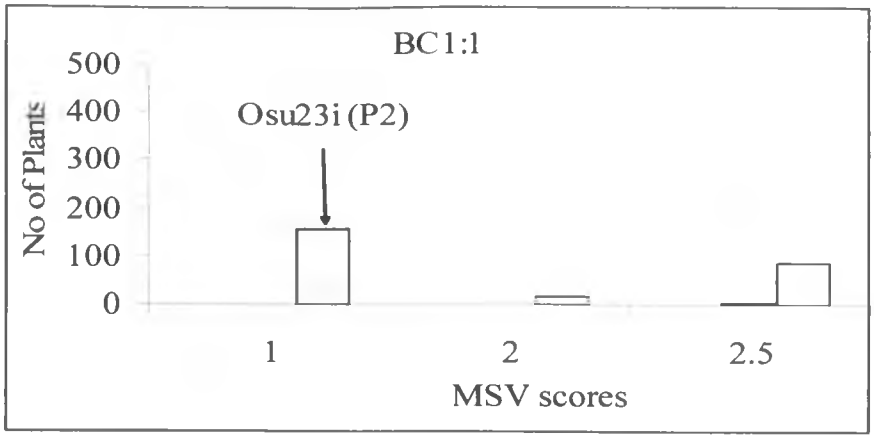
Main and Epistatic genic factors	Genetic effects	Variances	Standard Error of means	significance test 't'
m	1.33	0.0007	0.026	50.378
d	0.518	0.002	0.042	12.392
h	-0.981	0.021	0.145	-6.762
I	-0.052	0.018	0.136	-0.382
j	-0.557	0.002	0.049	-11.311
l	1.137	0.053	0.230	4.943

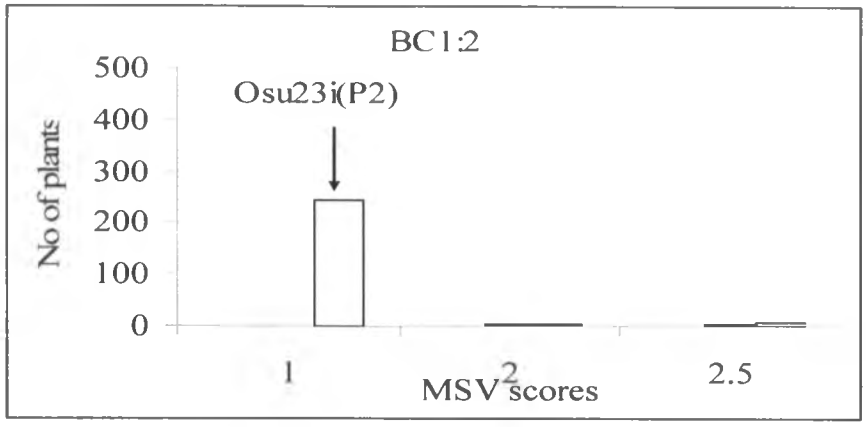
m = Mean, ***d*** = additive, ***h*** = dominance, ***I*** = additive x additive, ***j***= additive x dominance

l=dominancexdominance

Table 3.9b Effective factors and magnitude of variance conferring resistance in Osu23i

Method	Number of genes
Mather's	6.326
Lande's 11	1.582





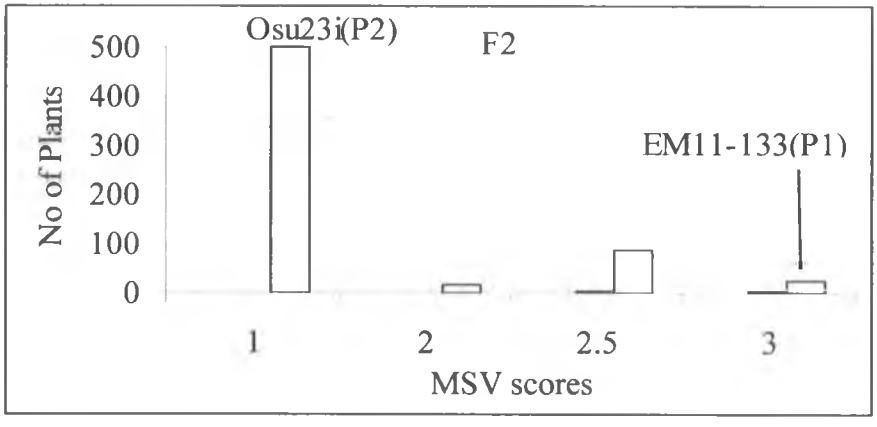


Figure 3.3: Frequency distribution of Segregating population BC1:1, BC1:2 and F2 derived from EM11-133 and Osu23i during short rains of 2007

Individual plants of F2 segregating populations derived from both parental sources exhibited a range of symptoms on the scoring scale; some plants had MSV scores which were different from those of either of the parents. The numbers of plants in different categories are shown in Appendix 5. Analysis of intra-generation distribution of plants exhibiting different symptoms in F2 segregating population reveal a distinct bimodal distributions patterns (Figure 3.4), There was a high percentage of symptom-free plants and very low percentage of intermediate symptoms ratings..

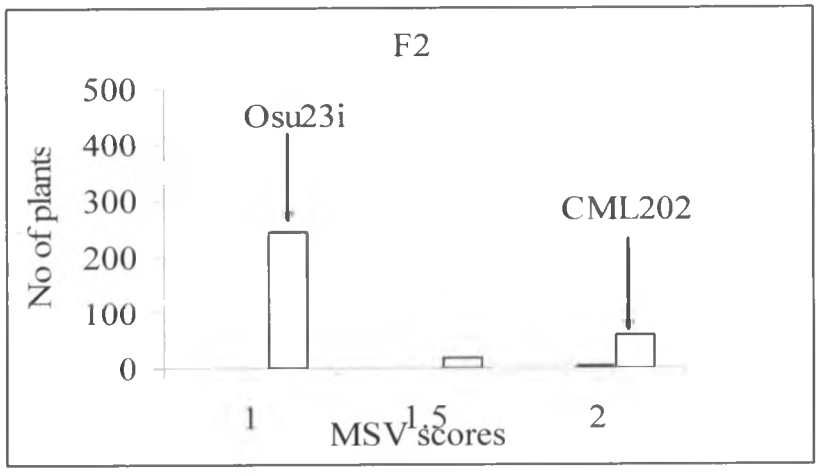


Figure 3.4: MSV scores segregation pattern of F2 population derived from Osu23i x CML202 cross during the short rains of 2007

3.5 Discussions

3.5.1 Disease reaction among parents, F1, F2 and backcrosses of three generations evaluated during the short rains of 2007.

The different generations significantly differed in reaction to MSV with EM11-133 being highly susceptible and CML202 being partially resistant. The standard term "partial resistance' according to Rodier et al., (1995) refers to plants that exhibit symptoms, but to a lesser extent than that showed by the susceptible parental line. The immune parent Osu23i was symptomless indicative of immunity or complete resistance. The standard term "complete resistance" according to Rodier et al., 1995 refers to symptom-free plants in which virus multiplication is totally prevented.

Unique segregation pattern and expression of resistance to streak were observed among the three generations evaluated during the short rains of 2007. The six generation derived from the susceptible parent EM11-133 (P1) and CML202 (P2) showed varied segregation pattern. The generation segregated into three main categories. EM11-133 was severely streaked and rated highly for MSV; CML202 had few streaks and low MSV scores, the rest of the generations including F1, F2, BC1:1 and BC1:2 had moderate streaking to MSV and had MSV scores close to or similar to mean MSV scores of EM11-133 and CML202 (Mid parental value) indicating that co-dominance or partial dominance controlled resistance in the tolerant parent CML202. Similar findings were observed by Storey and Howland (1967) who observed that heterozygotes between resistant and susceptible lines reacted to infection in a manner intermediate between the parents. Kyetere et al., (1999) also found an intermediate reaction for F1 while using Tzi4. More recent studies conducted by Gichuru (2008) showed that the reactions of the F1 for disease resistance in crosses generated from five different

MSV sources deviated little or not at all from the mid-parental value showing that MSV resistance in the study material was co-dominant or partially dominant. The results of the area under disease progress curves were comparable to those of MSV scores, the AUDPC of the F1 and the segregating generations including the F2, BC1:1 and BC1:2 crosses were either intermediate or closer to the AUDPC of the tolerant parent CML202 than the susceptible EM11-133 further showing that there was improvement for resistance in these crosses arising from MSV superior alleles donated by tolerant parent CML202. This also indicates importance of dominance over susceptibility for resistance among parental sources.

The segregation pattern of six generation P1, P2, F1, BC1:1, BC1:2 and FC2 derived from the susceptible parents EM11-133 and Osu23i exhibit a different pattern compared to those derived from EM11-133 and CML202. The mean MSV crosses of the crosses deviated little or were similar to immune parent Osu23i MSV scores hence indicative of complete dominance of the resistant parent Osu23i over the susceptible parent EM11-133. Similar findings were observed by Rodier et al., (1995) while investigating mode of gene action in lines extracted from CVR3-C3 (Composite Viroses Resistant 3 –cycle 3) population. Similarly, the area under disease progress curves (AUDPC) of the F1 and three segregating populations including the F2, BC1:1 and BC1:2 crosses were closer to the AUDPC of the immune parent Osu23i than the susceptible EM11-133. Improvement of MSV resistance observed in the crosses could be due to donation of MSV resistant genes by the immune parent Osu23i which suppressed severe expression of MSV among the crosses.

The four generations derived from the two MSV sources Osu23i and CML202 exhibited different MSV scores segregation pattern (Table 3.4). Most judgment on allelic relationship are made by examining the ratings of MSV scores of the F1 and F2, if the scores are similar,

alleles controlling the trait are similar but if the scores in the F1 and F2 are different, alleles controlling the trait are different (Fraser 1992), The F1 and F2 generations showed MSV scores that were closer to that of the immune parent Osu23i indicating that the immune parent Osu23i is more dominant than the tolerant parent CML202 and therefore alleles in the two sources are different.

The disease expression of CML202 and Osu23i confirms the lines as tolerant and immune respectively. This was also reported in other studies (Njuguna 1999, Clerget *et al.,* 1996) that similar sources of resistance such as C92 exist. According to Fraser (1992), fully recessive alleles may be associated with immunity. CML202 and Osu23i contributed best to lowering the disease expression of the F1 and backcrosses generations, this shows that dominance is influencing resistance, which could be due to the existence of a major gene for resistance (Pernet et al., 1999).

3.5.2 Agronomic characteristics of the parents F1, F2 and backcrosses of the three generations evaluated during the short rains of 2007.

The various generations significantly differed ($P<0.001$) in days to 50 percent pollen shed and silking, plant height and ear heights, grains yields and cob lengths. This suggests presence of sufficient diversity which can be exploited for breeding work. However, the great diversity observed might be due to combined evaluation of both parental inbred lines and biparental crosses.

The generations had between 99 and 100 days to pollen shed and silking indicating that they are adaptable to the mid altitude agro-ecological zones. The knicking ability showed by the generations is also a desirable trait targeted by breeders during development of varieties since

a shorter anthesis-silking interval (ASI) normally maximizes seed sets. Generally, the susceptible parents EM11-133 matured earlier than the tolerant parents CML202 and the immune parents Osu23i, early maturity of the susceptible parent EM11-133 was perhaps a survival mechanism to counteract effect of MSVD infection. Kiduyu (1991) noted that plants exhibiting severe MSV symptoms tend to mature faster and have shorter plant and ear length. Moderate and high negative correlations were noted between DTT and DTS with foliar diseases scores, plants and ear height, this suggests that the effect of MSV disease was more on generations which flowered earlier than those which flowered late.

In general, the mean grains yields of the F1 and backcross generations were better compared to those of inbred lines and this is because taller crosses normally yield more as a result of heterosis compared to inbred lines which often are shorter due to inbreeding. Correlation analysis shows that grain yield was highly correlated with plant height and Ear height while cob lengths also correlated highly with both plant and ear height. The high grain yields of the F1 and backcross generations suggest that very good materials exist among the test materials for exploitation in breeding and commercialization (Gichuru, 2008). These highest yielding crosses, in addition to being used either directly as cultivars or as parents could also be utilized in inbred line development through pedigree breeding.

3.5.3 Mode of gene action conferring MSV resistance in inbred lines CML202 and Osu23i and allelic relationships of genes found in two sources.

Combined analysis of maize streak intensity, area under disease progress curves (AUDPC), and scale testing using the Hayman 1958 additive-dominance model of means of MSV scores from six generations derived from parental lines EM11-133 and CML202 suggests that MSV resistance in the tolerant parental line CML202 is controlled additively by

relatively few (2-6) genes expressed in dominant manner. Genetic studies of six generations derived from EM11-133 and Osu23i suggests that MSV resistance in the immune parental line Osu23i is controlled additively by relatively few (2-7) genes expressed in a completely dominant manner. Both of these results concur with those of Storey and Howland, (1967) who reported that a dominant gene mainly controlled MSV resistance in Peruvian yellow x Arkell's Hickory inbred line, they reported deviations from the theoretical Mendelian segregation and attributed it to modifying genes. Therefore, presence and importance of modifying genes can not be ruled out since they could be contributing to marked variations of symptoms observed among the BC1:1, BC1:2 and F2 populations. Soto et al., (1982) found resistance to be simply inherited and fixable rapidly through breeding. The finding also agrees with those of Rodier et al., (1995) who found that major and minor genes were responsible for resistance of S1 and S2 lines derived from population CVR3-C3. Further studies by Kim et al., (1989) found out that resistance in IB32 inbred line from IITA is controlled quantitatively, mainly additively, with relatively small (2-3) number of genes involved. Studies conducted by Pixley et al (1997) showed that additive effects are important for resistance.

Quantitative trait loci (QTL) for resistance to MSV were mapped by Welz et al., (1998) in a population of F2:3 lines derived from a cross CML202 and a susceptible inbred line, major QTL was identified in chromosome 1 and three minor QTL were identified in chromosome 2, 3, and 4. Further genetic studies using line Tzi4 were conducted by Kyetere et al., (1999) using molecular markers, they identified a single major gene in chromosome 1 similar to that reported by Welz et al., (1998). The findings however, differ with those of Gorter (1959) who found resistance to MSV to have a quantitative kind of inheritance and

Caulifield (1997) who reported that resistance to MSV to be largely additive but could be controlled polygenically.

The analysis of allelic relationship was carried out by observing the segregation pattern of streak intensity scores of the F1 and F2 crosses derived from Osu23i and CML202. The F2 frequency distribution of individual plants exhibiting different symptoms displayed a unique bimodal segregation pattern. These findings suggest that two separate genetic systems similar to that reported by Rodier et al., 1995 confer resistance in different sources (CML202 and Osu23i) investigated in this study. Thus, the two sources seem to have two different genes based of different MSV scores of the parents CML202 and Osu23i and also MSV scores of the F1and the F2 which resulted to bimodal distribution pattern.

In conclusion, useful sources of MSV resistance such as CML202 and Osu23i exists which can be utilized by breeders to introgress MSV resistant genes into adaptable high yielding but susceptible hybrids, However, The MSV-immune parent Osu23i resistance should be used over short term and medium period in creating inbredlines and formation of hybrids since it offers complete resistance which is easy for viruses to break down while resistance in CML202 used over long term periods but should be backed up by recurrent selection.

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CHAPTER FOUR

EFFECT OF MAIZE STREAK VIRUS DISEASE ON GRAIN AND STOVER DRY MATTER YIELDS OF ADAPTED MID-ALTITUTE MAIZE INBRED LINES AND THEIR F1 SINGLE CROSSES.

4.1 Abstract

Maize streak virus disease (MSVD) is an important constraint to maize grain production in Kenya. An experiment was set up at KARI Muguga during the short rains of 2007 to investigate the effect of MSV infection on dry matter and grain yields of locally adapted germplasm. The test material included three parents namely EM11-133, Osu23i and CML202, three F1 single crosses derived from these parental lines and two local checks H614D and WH505. The experimental design was a split plot with inoculation levels as main plots and varieties as sub plots. Results showed that MSV disease considerably reduced ($P<0.001$) stover dry matter and grain yields. Stover yields losses ranged from 19 -29% while that of grain ranged from 8 to 48 % The susceptible inbred line EM11-133 sustained large reduction in stover yields (29 %) and grain yields losses(48%). The susceptible check H614D and tolerant check WH505 sustained stover yield reduction of 22 and 25% and grain yield reduction of 25 and 19 % respectively. However, the yields of MSV immune parent Osu23i, tolerant parent CML202 and EM11-133xCML202 (F1) progeny were not affected, thus confirming existence of resistance to MSV among parental sources. The use of resistant varieties if adopted by farmers will offer a cost effective solution to the problem of forage and grain shortage experienced by farmers in the mid-altitude areas.

4.2 Introduction

Maize is the staple food crop and most popular cereal in Kenya and where rainfall permits, farmers grow two crops per year (Staal et al., 1997). In the crop and livestock mixed farming systems of central Kenyan highlands where dairying is the most important agricultural activity, maize is not only an important source of food but also forage for livestock (Lukuyu et al., 2000; Staal, 1997). In this medium altitude regions, farming is becoming more intensive as the population grows and land pressure increases. Average farm sizes are small, ranging from 1.1 to 2.0 ha per household (Gitau et al., 1994; Staal et al., 1997). Due to the intensification, smallholder dairy cattle feeding is increasing based on maize forage, as thinning and stover therefore making production of sufficient forage for dairy cattle increasingly difficult for farmers (Lukuyu, 2005).

A survey in central highlands showed that low dry matter intake is one of the most important constraints to dairy production (Omore et al., 1996). During the dry seasons, milk production is drastically reduced following scarcity of forage. Studies by Methu et al., (1997) have showed that there is a positive correlation between stover intake and milk yield. To cope with the feed problem, the residues obtained from maize as thinning, green stover and dry stover following harvest constitute the main basal diet for livestock in the both wet and dry seasons when ruminant animals can barely gather sufficient feed from natural grazing and browsing (Adugna et al., 1999; Lukuyu, 2000). Many farmers in Kiambu stall-feed dairy and use thinning, green and dry stover to provide about 24% of forage needs (Mcleod et al., 2001)

Despite the significant importance of this crop, the yield of stover and grain yields are threatened by disease epidemics. Maize streak virus disease appears to be the most common

and potentially damaging of the diseases in Kiambu district and farmers do not have a reliable control method (McLeod et al., 2001). The disease causes yellowing of the leaf, and when it strikes early, it results in severe stunting or destroys plants. If infection occurs nearer to tarselling, it causes yellowing and may reduce palatability or feed value. Studies conducted by Lukuyu (2005) showed that if the crop is infected 14 days after emergence reduced the thinning dry matter yields by 29%. Yield losses of such magnitudes are serious and warrant further investigation to estimate the actual yield losses suffered by farmers. Breeders are keen to note the yield advantage that will accrue by planting a resistant variety compared to a susceptible check. They are also interested in estimating the yield gaps that will be observed in disease and no-disease situations in order to avail to farmers cost effective and alternative measures of MSV control and increase yields. This study was therefore designed to assess the expression and effect of MSV disease on yields of adapted mid-altitude maize parental inbred lines and their F1 single crosses.

4.3. Materials and methods

4.3.1 Generation of crosses and increase of parental seed

Initial single crosses were made during 2006 short rains by crossing the MSV susceptible parent EM11-133 from KARI to immune parent Osu23i to form EM11-133xOsu23i (F1) and to tolerant parent CML202 to form EM11-133XCML202 (F1). The two MSV sources of resistance Osu23i and CML202 were crossed among themselves to form Osu23ixCML202 (F1). A few ears of each of the three parental EM11-133, Osu23i and CML202 were random mated to increase the seed of the parents

4.3.2 Experimental design and layout

The three parental lines namely EM11-133, Osu23i and CML202 and the resultant three F1's (EM11-133xOsu23i, EM11-133xCML202 and Osu23ixCML202) together with a susceptible check H614D from Kenya seed and MSV tolerant hybrid WH505 from western seed (Table 4.1) were planted in a split plot design in KARI Muguga during the short-rains growing season of 2007. The field was divided into two main blocks. Each main plot had three replication and each replication had eight plots where the eight varieties were randomly assigned. One main plot was inoculated with MSV using viruliferous leafhoppers while the other main plot served as a control.

Five barrier rows were planted between the two main plots to minimize natural infection in the control plot by preventing movement of leafhopper vectors between main plots. In the subplots all the eight varieties were planted in uniform eight row plots, each row had 11 hills. Two seeds were planted per hill and later thinned to one before MSV infestation. The spacing between rows was 75 cm while within row spacing was 30 cm. At planting, 125 kg of Diammonium phosphate (DAP) was applied ha^{-1} and fields were top dressed with calcium ammonium nitrate (CAN) at 125 kg ha^{-1} seven weeks after planting. Inoculation of one of the main plots was done fourteen days after emergence. The experiment was harvested at physiological maturity.

Table 4.1 List of germplasm used for grain yield and dry matter assessment

Variety	Genetic constitution	Source
EM11-133 (susceptible parent)	Inbred line	KARI
CML 202 (Tolerant parent)	Inbred line	CIMMYT
Osu23i (immune parent)	Inbred line	CIMMYT
EM11-133/CML202 (F1)	Single cross	KARI
EM11-133/Osu23i (F1)	Single cross	KARI
Osu23i/CML202 (F1)	Single cross	KARI
H614D (Local popular Check)	Hybrid	Kenya seed Co.
WH505 (resistant Check)	Hybrid	Western seed Co

4.3.3 Preparation of inoculums and inoculation

Transmission of MSV into the test maize plants in the fields was done 14 days after emergence using leafhoppers (*Cicadulina mbila*). The populations of nonviruliferous leafhoppers were fed on clean pearl millet (*Pennisetum americanum*) grown in glasshouses, maintained at 25°C by means of an electric fan heater. Two days before inoculation, adult leafhoppers were transferred to insect proof cages containing young maize plants with severe disease symptoms and allowed acquisition access period (AAP) of 48 hours. Two days after exposure, two to three veruliferous leafhoppers were placed in small cellulose acetate plastic vials and the vials containing the leafhoppers were attached on distal portions of the youngest leaf of maize which is at two-three leaf stage and allowed an inoculation access period (IAP) for two days.

4.3.4 Disease assessment

Maize streak virus scores ratings were based on a five point scale (1-5) similar to one employed by IITA and CIMMYT and described by Kim *et al.*, (1989) and Ajaga (1999), where 1 = no or very few streak symptoms on lower leaves (Highly resistant); 2 = light streak symptoms on most leaves below ears with few symptoms above the ear (light

infestation); 3 = Moderate or Mild streak symptoms on most leaves (tolerance); 4 = abundance symptoms on all leaves (about 60-80% of the leaf area- Moderate infestation) and 5 = severe streak on all leaves (over 75-80% of the leaf area) highly susceptible. Mid points (0.5) on the 1-5 scale was included. MSV score rating were done separately for the control and infected main plots. The numbers of infected plants in all eight control subplots were counted in order to estimate disease incidence due to natural infection. For the inoculated subplot, MSV score rating was done once at flowering when the disease had fully developed and symptoms fully expressed. Other foliar diseases like common rust (*Puccinia sorghi)* and northern leaf blight (*Exserohilum turcicum)* symptoms were rated using a scale (1-5) were 1 = clean, No infection and 5 = severely diseased (Kim et al., 1989)

4.3.5 Dry Matter and yield assessment.

Samples used for dry matter assessment were randomly sampled from any four rows in each subplot. The yields of stover thinning were destructively sampled four times at monthly intervals at 31 days after infestation (DAI), 62 DAI, 92 DAI and 156 DAI respectively. During each sampling, 5 plants were randomly picked and chopped into small pieces and placed in paper bags and fresh weight determined. The Chopped stover was placed in an oven and allowed to dry at 60 -70 ^{o}C until a constant weight was attained.

The remaining four rows in each plot were trial was harvested at physiological maturity for grain yield determination. Harvesting was done on two inner rows to avoid edge effects. Both plants and cobs weights (field weights) harvested from the inner rows were recorded in kilograms. A few cobs from each plot were shelled to obtain the moisture content.

Other agronomic data was collected following guidelines described by Vivek et al., (2005) included days to 50% pollen shed (DTT) included days to 50% Silking (DTS). Plant height (PH) and ear height (EH) were measured in centimeters as height between the base of a plant to the insertion of the first tassel branch of the same plant and height between the base of a plant (ground level) to the node bearing the upper (top) ear respectively. Cob lengths (CL) was measured in centimetres by randomly measuring ten cobs per plot. Grain yield (GY) was calculated by weighing harvested ears per plot to give the fresh weight while grain moisture content was sampled from a few cobs per harvested plot. Grain yield was transformed to tonnes per hectare assuming 80% shelling percentage as follows:

Yield = [Field Weight/ Plot size] X [(100- MC %) / 87.5] X 0.8 X 10

(Vivek et al 2003)

4.3.6 Statistical data analysis.

Analysis of variance for MSV and foliar disease scores data was based on the rating of individual plants. For other traits such as plant height (PH), ear heights (EH), days to pollen shed (DTT) and days to silking (DTS), cob lengths (CL) and grain yield (GY), analysis of variance was based on plot means across replication. The usual procedure of statistical analysis suggested for split plot was followed during analysis of data of all traits except for MSV scores where randomized complete block design was followed since MSV score rating was done on inoculated subplots. Genstat 8th edition software version 8.1 (Lawes Agricultural Trust, 2002) was used for analysis. Correlation analysis was carried for all traits.

4.4. Results.

4.4.1 Reactions of varieties to MSV rust and blight

The eight varieties inoculated with MSV differed significantly (P<0 .001) in streak symptoms intensity. The most susceptible variety was EM11-133; this variety had the highest MSV scores compared to the immune parent Osu23i which had lower MSV scores. WH505 and EM11-133/CML202 which had 21% and 35% less MSV infection compared to the susceptible variety EM11-133 showed moderate streak infection. The susceptible check H614D from Kenya seed had lower mean MSV scores similar to those of those of the immune parent Osu23i, Osu23i/CML202 and EM11-133/Osu23i. No significant differences were observed between the inoculated and the control in terms of rust and blight infection (Table 4.2).

Table 4.2 Mean severity scores of foliar diseases of varieties tested during 2007 mid season

Varieties	MSV scores	Rust scores			Blight scores		
		Control	Inoculated	Variety	Control	Inoculated	Variety
CML202	1.49[b]	1.26	1.41	1.34	1.31	1.25	1.28
EM11-133/CML202	2.18[c]	1.61	1.59	1.60	1.67	1.65	1.66
EM11-133	3.36[e]	2.15	2.05	2.10	1.57	1.55	1.56
EM11-133/Osu23i	1.00[a]	2.12	1.74	1.93	1.39	1.31	1.35
H614D	1.00[a]	1.19	1.10	1.14	1.26	1.30	1.28
Osu23i/CML202	1.00[a]	2.20	9.05	2.07	1.08	1.14	1.11
Osu23i	1.00[a]	2.67	2.47	2.57	1.11	1.16	1.14
WH505	2.64[d]	1.46	1.38	1.42	1.63	1.54	1.59
Mean	1.71	1.82	1.71	1.77	1.38	1.36	1.37
LSD Control Vs inoculated			0.53			0.32	
LSD Control Vs inoculated means			0.62			0.04	
LSD Variety	0.11		0.40			1.25	
CV%	7.90		18.90			15.10	

Means followed by the same letter do not differ at P<0.001 probability level

4.4.2. Effect of maize streak virus infection on dry matter and grain yields.

Significant differences ($P<0.05$) were noted in the mean dry matter yields of inoculated and non inoculated main plots 31, 62 and 92 days after inoculation. The dry matter yields of the inbred lines were lower significantly ($P<0.001$) compared to those of the crosses. The MSV resistant sources Osu23i and CML202 had lower yields compared to those of the EM11-133 X CM202, EM11-133xOsu23i, and Osu23i X CML202 (F1 progenies) and two checks H614D and WH505 at 31 days after inoculation . Although no significant differences were noted between the yields in the control and inoculated subplots 31 days after inoculation, dry matter yields of some varieties were reduced significantly ($P<0.001$) by MSV infections at 62 and 92 days after inoculation. The varieties whose yields were affected include the susceptible parent EM11-133 (34 and 27%) and the two checks H614D (23 and 12%) and WH505 (59 and 37%). The dry matter yields of immune parent Osu23i, tolerant parent CML202 and EM11-133 x CML202 were not affected throughout the three sampling periods (Table 4.3)

The reduction in stover yields was comparable to that of grain yields. The varieties whose stover yields were significantly reduced ($P<0.001$) include susceptible parent EM11-133 (28%), susceptible check H614D (22%), Osu23i/CML202 (19%), tolerant check WH505 (25%). While grain yields reduction ($P<0.05$) of the four varieties including EM11-133, H614D, Osu23i /CML202 and WH505 was reduced 48%, 25%,8% and 20% respectively. The stover and grain yields of the resistant parents CML202, Osu23i and EM11-133 x CML202 single cross progenies were not significantly affected by MSV infection. EM11-133 sustained large reduction in both grain and stover yields while Osu23i x CML202 sustained least reductions (Table 4.4 and 4.5)

Table 4.3: Mean dry matter yields (t/ha) of thinning samples harvested 31, 62 and 92 DAI during mid season of 2007

Varieties	31 DAI			62 DAI			92 DAI		
	Ctrl	Inocul	Var	Ctrl	Inocul	Var	Ctrl	Inocul	var
CML202	0.43	0.33	0.38[a]	3.08	2.54	2.81[a]	7.24	7.24	7.56[a]
EM11-133/CML202	1.38	1.35	1.36[c]	6.54	6.11	6.32[c]	11.40	10.48	10.96[b]
EM11-133	0.88	0.69	0.78[b]	6.06	3.96**	5.01[b]	8.28	6.04**	7.16[a]
EM11-133/Osu23i	1.50	1.40	1.45[c]	10.10	9.00	9.55[c]	11.80	9.50**	10.65[b]
H614D	1.30	1.37	1.33[c]	7.68	5.89**	6.78[c]	14.40	16.17**	15.25[c]
Osu23i/CML202	1.25	1.09	1.17[c]	9.21	7.69**	8.84[c]	12.10	9.93	11.03[b]
Osu23i	0.43	0.45	0.46[a]	3.39	2.49	2.94[a]	9.03	7.82	8.43[a]
WH505	0.86	0.83	0.85[b]	9.71	3.97**	5.34[b]	13.80	8.68**	11.22[b]
Mean	1.01	1.94*	0.92	6.60	5.21*	5.90	11.00	9.57**	10.28
LSD Control Vs Inocul		0.24			1.33			1.56	
LSD Control Vs Inocul means		0.04			0.18			0.18	
LSD Variety		0.19			0.97			1.06	
CV%		16.3			13.9			8.8	

Ctrl = Control Inocul = Inculated Var = variety

Means followed by the same ltter within columns do not differ at P<0.001 probability level

* = P<0.05 and ** = P<0.001

Table 4.4 Mean yields (t/ha) of stover and grain yields sampled at harvest during the mid season of 2007

Varieties	Stover DM (t/ha)			Grain Yield (t/h)		
	Control	Inoculated	Variety	Control	Inocul	Variety
CML202	6.24	6.86	6.56[a]	2.86	2.81	2.84[a]
EM11-133/CML202	9.46	8.35	8.90[b]	8.47	8.87	8.67[c]
EM11-133	8.22	5.84**	7.03[a]	5.51	2.85*	4.18[a]
EM11-133/Osu23i	8.19	7.50	7.85[a]	6.69	4.06*	5.38[b]
H614D	12.79	9.99**	11.39[c]	10.23	7.70*	8.97[c]
Osu23i/CML202	10.56	8.59**	9.58[b]	9.24	8.5*	8.87[c]
Osu23i	6.94	6.42	6.68[a]	3.37	3.43	3.40[a]
WH505	11.67	8.73**	10.20[b]	10.19	8.20*	9.20[c]
Mean	9.26	7.79*	8.52	7.07	5.80	6.44
LSD Control Vs inoculated		1.52			1.54	
LSD Control Vs inoculated means		0.82			1.30	
LSD Variety		1.11			1.06	
CV%		11			14	

Means followed by the same letter within columns do not differ at P<0.001 probability level
LSD = Least significant difference CV = Coeffiecient of variation
*Indicates significance at 0.05 percent probability level;
** Indicate significance at 0.001 percent probability level

Table 4.5 Yield losses of stover and grain yields during the short mid season of 2007

	Stover (t/ha)			Grain Yield (t/ha)		
	Control	Infected	%(reduction)	Control	Infected	% (Reduction)
EM11 -133(susceptible parent)	8.22	5.84**	28.95	5.51	2.85*	48.28
Osu23i x CML202	10.56	8.59**	18.65	9.24	8.50*	8.00
H614D (susceptible check)	12.79	9.99**	21.89	10.23	7.70*	24.73
WH505 (Tolerant check)	11.67	8.73**	25.19	10.19	8.20*	19.53

*Indicates significance at 0.05 percent probability level;
** Indicate significance at 0.001 percent probability level

The eight varieties differed significantly ($P<0.001$) in DTT and DTS (Table 4.6). The earliest varieties to flower were two F1 including EM11-133/Osu23i and Osu23i/CML202. the rest of the varieties including the parentals CML202, EM11-133, Osu23i, EM11-133/Osu23i, Osu23i/CML202 and WH505 tarselled in an average of 98 -106 days and silked in an average of 101.33-105 days (Table 4.6)

The inbred lines were generally shorter than the checks and the hybrids, the parents CML202 and Osu23i were 47% shorter that the tallest variety H614D, they were followed Closely by EM11-133 and WH505, these two varieties were 38% shorter than the tallest variety H614D. The tallest varieties were the susceptible check H614D, EM11-133/ Osu23i and EM11-133/CML202. The plant height corresponded with ear height, CML202 and Osu23i being inbred lines had the shortest ear heights, the two varieties had 49% and 51% lower ear heights compared to varieties with tallest ear height, they were followed closely by WH505 and EM11-133 which are varieties adapted to the mid altitude areas. The varieties that had the tallest ear height include the EM11-133/CML202, Osu23i/CML202, EM11-133/Osu23i and H614D. Plant height was highly correlated with dry matter of thinning, stover and grain yields (Table 4.7). The varieties produced yields corresponding to their heights.

Table 4.6 variations in selected agronomic traits recorded on eight entries tested during the mid-season of 2007

Variety	Days to tarsel			Days to silk			Plant height (cms)			Ear heights (cms)		
	Cont	Inocul	Variety	Cont	Inocul	Variety	Cont	Inocul	Variety	Cont	Inocul	Variety
CML202	105.33	103.00	104.17[b]	106.33	104.00	105.17[b]	131.50	137.70	134.67[a]	59.33	59.00	59.17[a]
EM11-133/CML202	100.00	96.00	98.00[b]	102.33	100.33	101.33[b]	248.83	243.20	246.00[c]	118.17	111.67	111.70[d]
EM11-133	107.33	105.33	106.33[b]	109.00	106.33	107.67[b]	165.67	153.80	159.75[b]	85.50	86.17	85.83[c]
EM11-133/Osu23i	93.33	89.00	91.17[a]	95.67	91.00	93.33[a]	240.33	246.80	243.58[c]	117.83	118.17	118.00[e]
H614D	101.33	103.33	102.33[b]	103.00	105.00	104.00[b]	259.67	254.00	256.83[d]	119.00	117.33	118.20[e]
Osu23i/CML202	95.33	92.33	93.83[a]	97.67	95.67	96.67[a]	251.00	247.30	249.17[c]	114.67	111.33	113.00[d]
Osu23i	104.33	95.33	99.33[b]	106.33	98.67	102.50[b]	132.83	137.00	134.92[a]	57.83	56.17	57.00[a]
WH505	103.00	100.33	101.67[b]	105.33	103.33	104.33[b]	159.50	156.20	157.83[b]	78.67	73.67	76.17[b]
Mean	101.25	98.08	99.67	103.21	100.54	101.88	198.67	197	197	93.88	91.69	92.78
LSD Control Vs Infected		2.12			5.06			4.55			4.11	
LSD Control Vs Infected Means		4.34			4.58			3.47			2.17	
LSD variety		3.20			3.42			3.38			3.08	
CV%		2.70			2.80			3.00			5.7	

Note: Means followed by the same letter within columns do not differ at $P<0.001$
Key : Cont = Control Inocul = Inoculated

Table 4.7: Phenotypic correlation for foliar disease and recorded agronomic traits of eight entries tested in 2007 short rain season

	DM1	DM2	DM3	DMS	GY	RS	MSV score	BT	DTS	DTT
DM 2	0.79***	1								
DM3	0.617***	0.523***	1							
DMS	0.512***	0.522***	0.78***	1						
GY	0.632***	0.559***	0.704***	0.797***	1					
RS	-0.186	-0.021	-0.372**	-0.418**	-0.3*	1				
MSV score	-0.038	0.028	0.025	0.192	0.146	0.002	1			
BT	0.122	-0.007	0.033	-0.011	0.173	-0.12	0.398**	1		
DTS	-0.493***	-0.52***	-0.109	-0.035	-0.16	-0.17	0.415**	0.236	1	
DTT	-0.339*	-0.301*	0	0.131	0.098	-0.16	0.218	0.007	0.51***	1
PH	0.903***	0.794***	0.647***	0.53***	0.631***	-0.2	-0.186	-0.01	-0.5***	-0.33*

*Sign*ificance level *= P<.0.05, **=P<0.01 and ***P<0.001

DM1= Dry matter at 31 days after inoculation (DAI) , DM2= Dry Matter at 62 DAI

DM3= Dry matter at 92 DAI, DMS= stover dry matter, GY=Grain yield

RT= Rust, MSV= Maize streak virus, BT=Blight, DTS=Days to silk, DTT= Days to tarsel PH=Plant height

4.5 Discussion.

In this study, highly significant differences ($P<0.001$) were observed among the entries in MSV severity scores. The susceptible parent EM11-133, single cross EM11-133/CML202 and tolerant check WH505 were infected fairly heavily compared with EM11-133/Osu23i, Osu23i/CML202, H614D and MSV sources of resistance CML202 and Osu23i. The two CIMMYT inbredlines CML202 and Osu23i were highly resistant to MSV, confirming previous observations made with artificial infection in Zimbambwe (welz et al., 1998) Although all popular maize hybrids which are produced by the Kenya seed company are susceptible to MSV (Njuguna 1996) , a popular hybrid H614D tested in this experiment showed high resistance to MSV, further work is needed to confirm existence of MSV resistance in H614D which was previously reported as susceptible. It will be important to find out if MSV resistant genes have been introgressed into the variety

Among the three single crosses tested in this study, MSV severity scores of EM11-133 x Osu23i and Osu23i / CML202 crosses were significantly different ($P<0.001$) compared to those of EM11-133/CML202 progeny. MSV source of resistance Osu23i is a parent in the first two single crosses i.e EM11-133 x Osu23i and Osu23i / CML202 which were highly resistant but the last single crosses EM11-133/CML202 which was moderately resistant to MSV had CML202 as a parent , this suggests that Osu23i as a parent was capable of completely suppressing the expression of the MSV disease compared to CML202, thus indicates that alleles for MSV resistance in Osu23i were dominant while those in CML202 are partially dominant.

Highly significant differences (P<0.001) were observed among the entries in grain yields. Among the eight entries tested, four varieties including the susceptible check EM11-133, single cross Osu23i/CML202 and two checks H614D and WH505 sustained grain yield reduction of between 8 – 48%, EM11-133 sustained the largest grain yield reduction of 48% while the single cross Osu23i/CML202 sustained the least grain yield reduction of 8%. The results of grain yield losses due to MSV disease reported in this study are comparable to yield reductions reported elsewhere, for instance. In Kenya, Guthrie (1978) reported grain yield losses of 24 – 63% after caging infective leaf-hoppers on selected plants. Further studies by Bosque-Perez *et al.,* 1998., showed that MSV disease decreased grain yield of resistant hybrid 8321-21 by 10%, and of moderately resistant hybrid 8329-15 by 17%. Yield of susceptible variety TZB Gusao was reduced significantly more, by 71%. In Mauritius, higher grain yield reduction of 91% was recorded in plants infected with MSV (Roca de doyle et al 2008). Grain yield reduction reported in this study are within average grain yield losses associated to the virus infection which were estimated to be between 30 to 100% by Alegbejo *et al.* (2002).

The eight varieties tested differed significantly (P<0.001) in stover yields. Four varieties Including the susceptible check EM11-133, single cross Osu23i/CML202 and two checks H614D and WH505 sustained stover yield reduction of between 19 – 29%, this finding are similar to recent studies conducted by Lukuyu (2005) in Kenya, which showed that infecting the crop 14 days post emergence reduced the thinning dry matter yields by 29% .

Findings from these study demonstrate that MSV disease significantly reduce dry matter and grain yields although this is a function of susceptibility/resistance levels of varieties. The grain and stover of four out of eight varieties were not affected by MSV, these includes the two sources of resistance CML202 and Osu23i, two single crosses EM11-133/CML202 and EM11-133 /Osu23i. These single crosses had better yield performance than the parental inbred lines and checks for instance, F1 progeny EM11-133xCML202 seemed to have constant yields indicating resistance. These F1 progenies can be used to make hybrids which will have both high grain and stover yields. This will help to alleviate the problem of forage shortage experienced by farmers who are unaware of the epidemiology of the disease and have no method to control it (Mcleod et al., 2001).

Breeding for dual purpose maize using inbred lines extracted from popular MSV resistance sources Osu23i and CML202 have yielded three-way cross hybrids MU03-017 and MU03-036 which have early maturity, high grain and stover yields advantage. These varieties were entered in National Performance Trial (NPT) in 2005 and 2006 and were fully released by the National Performance Release Committee (Ininda et al., 2006). Efforts are currently underway to popularize these varieties in the mid-altitude areas of Central Kenya where MSV have caused untold suffering to farmers. The use of resistant varieties if adopted by farmers will offer a cost effective solution to the problem of forage and grain shortage experienced by farmers in the mid-altitude areas.

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CHAPTER FIVE

GENERAL DISCUSSION AND RECOMMENDATION

A series of four experiments were conducted in order to investigate mode of gene action controlling MSV resistance among two sources CML202 and Osu23i both from CIMMYT. The results of scale testing using the Hayman (1958) additive- dominance model of means of MSV scores from six generations derived from EM11-133 and CML202 suggests that MSV resistance in the tolerant parental line CML202 is controlled additively by relatively few (2-6) genes expressed in partial dominance manner while genetic studies of six generations derived from EM11-133 and Osu23i suggests that MSV resistance in the immune parental line Osu23i is also controlled additively by relatively few (2-7) genes expressed in a completely dominant manner.

The segregation and expression of resistance in F1, BC1:1, BC1:2 and F2 crosses were affected by the genetic background of the resistant parents CML202 and Osu23i. Both mean MSV scores and AUDPC of the four generation derived from the tolerant parent CML202 and EM11-133 were intermediate between means MSV scores of the tolerant parent CML202 and the susceptible parent EM11-133. Those of the four generation F1, BC1:1, BC1:2 and F2 derived from Osu23i and EM11-133 were closer to the mean MSV scores and AUPDC of the immune parent Osu23i than the susceptible parent EM11-133. This indicates that partial dominance is responsible for control of MSV resistance in tolerant parent CML202 while MSV resistance in

the immune parent is controlled in dominant manner. The findings of these study on mode of gene action in CML202 and Osu23i based on the Hayman 1958 scale tests and segregations patterns of plants exhibiting diverse symptoms conforms with the reports of most earlier investigators who reported that mode of gene action conferring resistance to MSV is controlled by a major gene with minor or modifiers genes.

The mean grains yields of the F1 and three segregating populations F2, BC1:1, BC1:2 were better compared to those of inbred lines Osu23i and CML202. This is because taller crosses normally yield more as a result of heterosis compared to inbred lines which often are shorter due to inbreeding. These high grain yields of the F1 and backcross generations suggest that very good materials exist among the test materials for exploitation in breeding and commercialization. The highest yielding crosses, in addition to being used either directly as cultivars or as parents could also be utilized in inbred line development through pedigree breeding.

The findings of the study conducted to investigate stover and grain yield losses showed that MSV inoculation did not affect the dry matter of thinning, Stover and grain yields of MSV sources of resistance CML202 and Osu23i but MSV inoculation drastically reduced the yields of MSV susceptible inbred line EM11-133, susceptible check H614D, tolerant parent WH505 and Osu23i x CML202. The Stover yield reduction was between 19-29% while grain yield reduction was between 8 - 48%. These yield reduction are similar or within range to those reported by earlier investigators for instance in Kenya, Guthrie (1978) reported grain yield losses of 24 – 63. Further studies by Bosque-Perez *et al.,* 1998., showed that MSV disease decreased grain yield

by between 10 -71%, while average grain yield losses associated to the virus infection were estimated to be between 30 to 100% by Alegbejo *et al.* (2002). Recent studies conducted by Lukuyu (2005) in Kenya, showed that infecting the crop 14 days post emergence reduced the thinning dry matter yields by 29%. The finding of this thus confirms the usefulness of MSV resistant sources as useful parents in breeding for MSV resistant hybrids; these hybrids can be availed to farmers in the medium altitude areas of Kenya where MSV has caused great yield reduction.

Based on the results of this study, the following is recommended:

1. Since both sources of resistance have been used for a wide exploited in formation of varieties, diversification is urgently needed to ensure the durability of resistance in released varieties. Major genes should be identified and included to create varieties with environmentally stable and long lasting resistance.

2. Among the test materials, several single crosses (F1's) and backcrosses were identified which had better yield performance and MSV disease resistance. These single crosses can be used be used as such or crossed to form double cross hybrids or combined with the parents to form three-way hybrids to be release to farmers.

3. The high yielding single crosses EM11-133 x CML202 and Osu23i x CML202 with yields of more than 8 t ha^{-1} can be investigated further for possible release to farmers.

4. The parental line Osu23i which is a very good immune line for MSV should be improved for rust to combine both resistance to both diseases to make it a good parent in further breeding work.

5. The MSV-immune parent Osu23i resistance should be used over short term and medium period in creating hybrids while that in CML202 over long term periods but should be backed up by recurrent selection. Recurrent selection will improve and accumulate genes for resistance thus making it difficult for a new virus isolate to overcome it.
6. Both lines CML202 and Osu23i can be crossed in order to pyramid genes found in both sources into one background, through pedigree breeding lines can be extracted that have combined resistance.
7. Farmers in the mid altitude areas where MSV is menace should be encouraged to adopt or plant MSV resistant hybrids.

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Appendices

Appendix 1. No of plants, means variances and variances of mean of MSV scores for EM11-133 and CML202 Parents, their F1, F2 progenies and backcrosses tested in 2007.

Generations	No of plants	Means	Variance	Variance of mean	S.E	SD
P1	96	3.136	0.167	0.0018	0.0413	0.404
P2	91	1.934	0.054	0.0006	0.0246	0.233
F1	98	2.36	0.168	0.0017	0.0412	0.409
BC1:1	262	2.496	0.145	0.0006	0.023	0.343
BC1:2	253	2.401	0.059	0.0002	0.0202	0.32
F2	640	2.565	0.154	0.0002	0.155	0.391
Total	**1440**					

S.E = standard errors SD= standard deviations

P1= EM11-133, P2= CML202, F1=EM11-133/CML202, BC1:1=EM11-133/CML202XEM11-133 BC1:2= EM11-133/CML202XCML202 and F2 EM11-133/CML202 X EM11-133/CML202

Appendix 2. No of plants mean, variance and variances of mean of MSV scores for EM11-133 and Osu23i Parents, their F1, F2 progenies and backcross tested in 2007.

Generations	No of plants	Means	Variance	Variance of mean	S.E	SD
P1	94	3.152	0.095	0.0007	0.026	0.253
P2	94	1.003	0.002	0.000	0.003	0.026
F1	93.5	1.099	0.216	0.0015	0.038	0.37
BC1:1	258.5	1.576	0.768	0.002	0.045	0.719
BC1:2	253	1.058	0.115	0.0003	0.017	0.269
F2	631.5	1.33	0.624	0.0007	0.026	0.653
Total	1425					

S.E = standard errors SD= standard deviations
P1= EM11-133, P2=Osu23i, EM11-133/Osu23i=F1, EM11-133XOsu23i/P1(BC1:1), EM11-133XOsu23i/P2 =BC1:2 and F2=EM11-133/Osu23i XEM11-133/Osu23i

Appendix 3. Frequency distribution of MSV scores in BC1:1, BC1:2 and F2 segregating populations derived From EM11-133 and CML202 parents

MSV scores	BC1:1 No. plants	BC1:2 No. plants	F2 No .plants
1	12	8	14
2	5	28	51
2.5	207	204	438
3	39	13	134
3.5			4

Appendix 4. Frequency distribution of MSV scores in BC1:1, BC1:2 and F2 segregating populations derived From EM11-133 and Osu23i parents

MSV scores	BC1:1 No .plants	BC1:2 No. plants	F2 No . plants
1	156	242	498
2	15	4.5	17
2.5	86	6.5	88
3			27

Appendix 5. Frequency distribution of MSV scores in F2 segregating populations derived from MSV-tolerant parent CML202 and MSV-immune parent Osu23i

Scores interval	MSV scores rated 87 DAI	MSV scores rated 101 DAI	Mean MSV scores
1	251	241	246
1.5	18	17	18
2	58	61	60

Appendix 6. Phenotypic correlations for foliar disease and recorded agronomic traits of parents, F1, F2 and backcross derived from EM11-133 and CML202.

	SC1	SC2	S3	SC4	RST	BLT	PH	EH	DTT	DTS	GY	CL
SC1	1***											
SC2	0.877***	1										
SC3	0.963***	0.863***	1									
SC4	0.942***	0.866***	0.986***	1								
RST	-0.031	0.137	0.009	0.088	1							
BLT	0.882***	0.898***	0.912***	0.931***	0.115	1						
PH	-0.182	-0.147	-0.14	-0.08	0.741***	-0.06	1					
EH	0.236	0.266	0.31	0.377	0.735***	0.419	0.833***	1				
DTT	-0.52*	-0.564*	-0.59**	-0.63**	-0.53*	-0.53*	-0.57*	-0.738***	1			
DTS	-0.494*	-0.541*	-0.57**	-0.6**	-0.53*	-0.51*	-0.58*	-0.742***	0.989***	1		
GY	-0.217	-0.054	-0.15	-0.1	0.819***	-0.04	0.836***	0.811***	-0.51*	-0.53*	1	
CL	-0.404	-0.186	-0.37	-0.33	0.658**	-0.34	0.491	0.29	-0.2	-0.17	0.56*	1

Significance level *= P<.0.05, **=P<0.01 and
***P<0.001
SC1= MSV scores rated 58 days after inoculation (DAI) , SC2= MSV scores rated 72 DAI
SC3 = MSV scores rated 87 DAI, SC4 =MSV Scores rated 101 DAI, RST = Rust scores
BLT scores = Bloight scores , PH=plant Height EH=Ear height DTS=Days to silk, DTT= Days to tarsel, GY+garin yield, CL=Cob length

Appendix 7. Phenotypic correlation for foliar disease and recorded agronomic traits of parents,

F1, F2 and backcrosses derived from EM11-133 and Osu23i.

	SC1	SC2	S3	SC4	RST	BLT	PH	EH	DTT	DTS	GY	CL
SC1	1											
S2	0.877***	1										
S3	0.963***	0.863***	1									
S4	0.94***	0.866***	0.986***	1								
RST	-0.031	0.137	0.009	0.088	1							
BLT	0.882***	0.898***	0.912***	0.931***	0.115	1						
PH	-0.182	-0.147	-0.142	-0.08	0.741	-0.06	1					
EH	0.236	0.266	0.31	0.377	0.735***	0.419	0.833***	1				
DTT	-0.52*	-0.564*	-0.594**	-0.63**	-0.53*	-0.53*	-0.572*	-0.738***	1			
DTS	-0.494	-0.541*	-0.566*	-0.6**	-0.526*	-0.51*	-0.58*	-0.742***	0.989***	1		
GY	-0.217	-0.054	-0.149	-0.1	0.819***	-0.04	0.836***	0.811***	-0.514*	-0.531*	1	
CL	-0.404	-0.186	-0.37	-0.33	0.658**	-0.34	0.491*	0.29	-0.196	-0.167	0.561*	1

Significance level *= P<.0.05, **=P<0.01 and ***P<0.001

SC1= MSV scores rated 55 days after inoculation (DAI) , SC2= MSV scores rated 69 DAI

SC3 = MSV scores rated 87 DAI, SC4 =MSV Scores rated 99 DAI, RST = Rust scores

BLT scores = Bloight scores , PH=plant Height EH=Ear height DTS=Days to silk, DTT= Days to tarsel, GY+garin yield, CL=Cob length

Appendix 8. Phenotypic correlations for foliar diseases and recorded agronomic traits of parents , F1 and F2 derived from Osu23i and CML202.

	SC1	SC2	SC3	SC4	RST	BLT	DTT	DTS	PH	EH	GY	CL
SC1	1											
SC2	0.995***	1										
SC3	0.993***	0.999***	1									
SC4	0.957***	0.96***	0.968***	1								
RST	-0.96***	-0.95***	-0.947***	-0.894***	1							
BLT	-0.04	-0.09	-0.091	-0.089	0.251	1						
DTT	0.491	0.512	0.511	0.539	-0.41	0.03	1					
DTS	0.498	0.52	0.519	0.551	-0.42	0.01	0.999***	1				
PH	-0.8*	-0.8*	-0.798*	-0.735*	0.687*	-0.2	-0.27	-0.25	1			
EH	-0.84***	-0.84***	-0.836***	-0.769**	0.758**	-0.1	-0.32	-0.31	0.989	1		
GY	-0.55ns	-0.54	-0.52	-0.476	0.414	-0.4	-0.13	-0.11	0.89***	0.86***	1	
CL	-0.69*	-0.67*	-0.668*	-0.624*	0.571	-0.3	-0.18	-0.17	0.953***	0.933***	0.94***	1

Significance level *= P<.0.05, **=P<0.01 and ***P<0.001
SC1= MSV scores rated 58 days after inoculation (DAI) , SC2= MSV scores rated 72 DAI
SC3 = MSV scores rated 87 DAI, SC4 =MSV Scores rated 102 DAI, RST = Rust scores
BLT scores = Blight scores , PH=plant Height EH=Ear height DTS=Days to silk, DTT= Days to tarsel, GY=garin yield, CL=Cob length