Performance of Maize Hybrids and Inbred lines under Gray Leafspot (*Cercospora zea-maydis* L.) infestation in western Kenya

BY

Peter Okoth Mbogo

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Horticulture (Breeding)

Department of Horticulture and Botany
Maseno University

ABSTRACT

Gray leaf spot (GLS) caused by *Cercospora zea-maydis* is recognized as one of the most yield limiting diseases of maize world-wide. In susceptible genotypes, yield losses of up to 60 percent are not uncommon. The objectives of the study were to evaluate the response of diverse maize genotypes to Gray leaf spot (GLS) infestation in western Kenya, and to determine the genetics of GLS resistance in two maize inbred lines (CML312 and CML389). Another was to evaluate the relationship between GLS assessment methods, severity and lesion length. A total of 16 hybrids were evaluated for yield and yield components under artificial GLS infestation during the 2007/08 seasons at Maseno and at Bungoma. Other genotypes evaluated included, 13 inbred lines, 2 F_1 hybrids, and F_2 populations of crosses MSN21 and CML389 or CML312. Among the inbred lines, MSN21 was the most susceptible to GLS and had the highest disease severity rating. The inbred lines CML389 and CML388 and their F_1 hybrids showed high levels of GLS resistance. CML312 and CML384 showed tolerance to GLS. There was a very good correlation between the lesion length and severity ratings ($r=0.9$), suggesting that both could be used in disease damage assessment.

The best hybrid for GLS resistance was the experimental EH9; however, it had lower grain yields compared to others. The commercial hybrids PhB3253 and Kenya Seed H516 were the most susceptible to GLS. Fifty percent of the experimental hybrids performed better than the commercial check hybrids for grain yield under artificial GLS infestation. The best hybrid was the experimental EH10, with 32% yield advantage over the commercial checks. The frequency distribution of severity data for the F_2 population of a cross between MSN21 and CML312 was continuous, suggesting that GLS tolerance is influenced by quantitative genes. A similar frequency distribution data for F_2 population of a cross between MSN21 and CML389, showed 2 distinct peaks, and the genotypes within the 2 classes fitted a 9 to 7 ratio. This suggests that the resistance to GLS in CML389 may be conditioned by at least 2 major genes, with complementary epistatic interactions.

CHAPTER 1

INTRODUCTION

1.1 Background information

Maize (*Zea mays* L.) in its different processed forms is an important food for large numbers of people living in the developing world (FAO, 1998). It is the third most important cereal crop in the world, after rice and wheat, and mainly used as human food and animal feed (Saleh *et al.*, 2002). Maize is the main staple food of Kenya, averaging over 80 percent of total cereals (FAO, 1998). Maize production in Kenya is a highly relevant activity due to its importance as it is a dominant food crop (Mantel and van Engelen, 1997). It is wholly produced under rain fed conditions. The total land area under maize production in Kenya is about 1.6 million hectares with seventy to ninety percent from small-scale farms.

Gray leaf spot (GLS) caused by *Cercospora zea-maydis* (Tehon and Daniels, 1925), is one of the major yield limiting diseases of maize in sub-Saharan Africa (Ward *et al.*, 1999). It is recognized as one of the most yield limiting diseases of maize world-wide and is estimated to be spreading at a rate of 80-160 km each year (Ward *et al.*, 1999). It was first reported in Uganda in 1994 (Bigirwa *et al.*, 2001a) and has since become an important disease of maize throughout most of Eastern and Southern Africa (Pixley, 1997). The disease increased dramatically between 1991 and 1997 in Malawi (Ngwira *et al.*, 1998).

In Kenya, gray leaf spot causes significant yield losses of between 10 and 25 percent at any one season, especially in the western part of the country. In susceptible genotypes, yield losses of up to 60% are not uncommon (Ininda *et al.*, 2007).

The incidence of GLS in Africa has been associated with no-till or reduced tillage practices, coupled with continuous maize production and use of susceptible cultivars (Gevers *et al.*, 1994). In sub-Saharan Africa, small scale farming systems are heavily affected because of widespread multiple cultivations and favorable agro ecological conditions (Bigirwa *et al.*, 2001b). Yield losses due to GLS vary from 11 to about 70 percent (Ward *et al.*, 1999), with estimated losses as high as 100 percent when severe epidemics contribute to loss of photosynthetic area, increased stalk lodging, and premature plant death (Latterell and Rossi, 1983; Stromberg and Donahue, 1986; McGee, 1988).

1.2 Problem Statement

Popular Maize varieties in Kenya are susceptible to GLS and therefore resistant variety development should be a priority in the country. Hybrids such as H624 and H511 usually develop high levels of GLS disease (Bigirwa *et al.*, 2001, Ininda *et al.*, 2007), a fact which corroborates with earlier findings by Bigirwa *et al.*, 1999 that most hybrids from Kenya Seed Company tended to succumb. Concerted effort on breeding for gray leaf spot resistance is required for Eastern and Southern Africa region (Devries and Toeniesen, 2001). Host genetic resistance has been suggested to be the most appropriate method of reducing yield losses due to GLS in small holder farms in Africa (Menkir, 2005).

The current popular maize varieties in Kenya are susceptible to Gray leaf spot. Therefore, there is a need to identify sources and varieties with Gray leaf spot resistance. Sources of resistance to GLS have been identified in inbred lines in both public and private institutions. However, some of these lines with resistance lack local adaptation to target environments or are poor combiners with other elite germplasm. Therefore, there is need to introgress GLS resistance genes into elite breeding lines.

There have been very few studies conducted to evaluate mechanisms or genetics of GLS resistance. Previous studies have suggested that resistance to GLS in maize could be due to quantitative factors or a single gene (Gevers *et al.,* 1994). Quantitative resistance has also been reported. Therefore, a study elucidating the genetics of GLS resistance in specific inbred lines may be necessary in order to formulate appropriate breeding strategies.

1.3 Objectives

This study aimed at evaluating the response of diverse maize hybrids and inbred lines under Gray leaf spot (GLS) infestation in western Kenya, and to determine the genetics of GLS resistance in two inbred lines (CML312 and CML389).

The specific objectives of this investigation were,

1. To screen locally available and CIMMYT maize inbred lines for GLS response.
2. To evaluate the effect of GLS infestation on yield and yield components of maize hybrids.
3. To determine the inheritance of GLS resistance/tolerance in CML389 and CML312.
4. To evaluate the relationship between different GLS assessment methods (severity and lesion length).

1.4 Hypothesis

The objectives were based on the following hypotheses:

1. Different maize genotypes respond differently to GLS Infestation.

2. There is a correlation between GLS severity and the GLS lesion length.

CHAPTER 2

LITERATURE REVIEW

2.1. Global Importance of Maize

Maize is the third most important cereal grain in the world that provides nutrients to humans and animals (FAO, 1998). It plays a significant role in the nutrition of millions of people around the world and its importance is widely recognized. In East Africa, maize production, processing and utilization provide vital employment and income generation activities for a large cross section of people (Twumasi-Afriyie *et al.*, 2001). Globally, maize production has increased since the 1979, mainly as a result of increases in land area planted, genetic improvements on varieties grown, more efficient technological field practices as well as proper agronomic practices (FAO, 1988). Developing countries have more land area under maize compared to the developed countries though maize yields in developing countries have increased only slightly since 1981 (FAO, 1998). Maize production in these countries is constrained by among other factors several biotic and abiotic constraints hence the slight increases in maize yields. Gray leaf spot is one of the causes of poor maize yields in mid altitudes of eastern Africa.

2.2. The Gray Leaf spot Disease of Maize

Gray leaf spot (GLS) is caused by the fungus *Cercospora zeae-maydis* and thrives in humid environments that favor slow drying dews and late season fogs (Nelson *et al.*, 2006). The initial official report from the African continent was the occurrence of the pathogen in Kwa Zulu-Natal, South Africa in the late 1980s (Ward *et al.*, 1999). Gray leaf spot has the potential to threaten food security in many countries and is particularly significant in Africa, because maize is the main staple food crop for millions of people in the rural areas (Ward *et al.*, 1999). The disease is most severe and damaging when periods of extended high relative humidity occurs, caused by slow-drying dews and prolonged late season fogs (Beckman and Payne, 1983).

2.2.1. Symptoms, Cycle and transmission

Early symptoms are pin-point spots surrounded by a yellow halo which turns tan later in the season. These lesions are easily observed when the leaf is held against the light. Lesions take about 7 days to elongate and develop into the typical rectangular lesions symptomatic of GLS. Mature lesions are sharply rectangular, long and narrow, run parallel to the leaf veins and are grey to tan in color. Under severe disease pressure, lesions may coalesce and blight the whole leaf (Ward *et al.*, 1999).

The *Cercospora zeae-maydis* can only survive from one season to the next if maize debris is left on the soil surface. The fungus in the debris produces conidia in the spring, following periods of high humidity. Conidia are the primary source of inoculum and are wind-dispersed to the newly-planted maize crop (Ward *et al.*, 1999). Germinating spores produce appresoria over leaf stomata before penetrating the host tissue (Beckman and Payne, 1982). Primary infections usually develop on the lower maize leaves and when lesions mature, conidia are wind-dispersed to infect upper leaves. Unlike most fungi, *Cercospora zeae-maydis* can remain dormant during unfavorable environmental conditions (hot, dry weather) and resume rapid development as soon as favorable weather conditions return. Under prolonged favorable conditions, and especially after the canopy has closed, developing lesions may coalesce, resulting in extensive blighting and necrosis of leaf tissue. The disease usually develops from the time of tasselling. Under favorable environmental conditions, (>90% RH; 22-30°C) (Thornson and Martison 1993), especially in monoculture maize, it may occur before tasselling (Ward *et al.*, 1999). If GLS infected debris remains on the soil surface from winter to spring, spores will be produced which will infect the new maize crop. In this way, the disease cycle is repeated.

2.2.2 Economic importance of GLS and disease severity assessment

Losses associated with gray leaf spot occur when photosynthetic leaves are rendered non functional due to lesions and/or the blighting of leaves. The blighting and premature death of leaves severely limit radiation interception as well as production and translocation of photosynthates to developing kernels (Ward *et al*., 1999). This is especially true for the upper eight or nine leaves which contribute 75-90% of the photosynthate for grain fill (Beckman and Payne, 1982). Leaves of susceptible hybrids may become severely blighted or killed as early as 30 days prior to physiological maturity (Jenco, 1995; Ward, 1996). Additional losses are incurred when photosynthate is diverted from the stalk to the roots, which then predisposes the tissue to stalk and root rots resulting in stalk lodging. Losses due to lodging are often worse when maize is mechanically harvested, as opposed to hand-harvested fields (Ward *et al*., 1999).

Gray leafspot has resulted into significant losses in income to grain producers and adversely affected yield and quality of silage (Ward and Novell, 1994; Donahue *et al*., 1991). The components of yield which are mostly affected by GLS are number of kernels per ear and kernel size.

Percent yield loss is defined as the ratio of difference between the actual yield and attainable yield. (Nutter *et al*., 1993). Using this definition the yield losses caused by GLS infestation have been reported (Ayers *et al*., 1984; Beckman *et al*., 1981 Donahue *et al*., 1991). The losses vary from 10 to 100 percent of the yield potential in endemic areas but can be much higher. Gevers *et al*., (1994) observed up to 40 percent whereas Ward and Nowell (1994) and Ward *et al*. (1997) indicated yield losses between 50-65 percent with reduction in both yield and quality of silage. Latteral and Rossi (1983) reported the loss due to GLS approaching 80 to 100 percent in epiphytotics. It is clear from literature that GLS causes enormous maize crop losses in both endemic and epidemic situations.

Disease severity is unpredictable and might vary from year to year, field to field and from one cultivar to another. The pathogen can also cause extreme water loss from the plant. Sugar production is affected, resulting in reduced ear size, lower grain yields and sometimes premature death. Maize grown for silage is also affected by the lower nutritive value of the crop. Disease is most severe in warm (20-28°C), humid areas and is favored by prolonged overcast, rainy or misty days which provide enough free moisture on the leaves essential for disease development (Rupe *et al*.,1982).

Disease severity is often assessed using various methods that utilize scales, keys, visual estimations and measurements (Adipala *et al.*, 1993, Horsfall and Barratt 1945, Saghai Maroof *et al.,* 1993, Slopeck 1989, James 1968, Solomonovitz, Levy and Pataky 1992,). The GLS disease severity rating is done on a scale of 1 to 5, (Saghai Maroof *et al*., (1993). Where 1 = no visible infection, 2 a few scattered lesions on leaves below the ear, 3 = many lesions on leaves below the ear, with a few lesions above the ear, 4 = severe lesions on all but uppermost leaves, which may have a few lesions, and 5 = abundant lesions on all leaves with most of the leaf tissue being necrotic. Susceptibility is expressed in terms of incidence, severity and lesion type (Pratt *et al*., 2000).

Other GLS disease severity assessment indices are those that quantify disease response over the season as area under disease progress curve (Elwinger *et al*., 1990, Huff *et al*., 1988). This is particularly applicable when there is multiple disease assessments (Saghai Maroof *et al*., 1993).

Various researchers have assessed GLS severity based on whole plant or plot visual estimate of disease intensity (Saghai Maroof *et al*., 1993). Others have used the percentage of individual plant leaf area blighted in assessing disease severity.

2.2.3. Control and Management Practices

Although early findings suggested high plant populations were conducive to creating high humidity micro-climates for disease, this has more recently been disputed as it was found that in dense populations disease decreased, possibly because their canopies provide more of a shield to wind-borne spores than provided by open canopies (Ward and Nowell,1998).

2.2.3.1 Cultural Practices

Tillage operations aimed at complete burial of debris are one way of managing GLS (Ward *et al*., 1997). Disk ploughing provides insufficient burial of residues and ploughing can leave as much as 10% residue on the land surface. This could, provide sufficient inoculum to start an epidemic; subsequent tillage would have to bury residual debris (Ward and Nowell, 1998).

Mold board plowing does, but it may not be advisable in some fields because of increased soil erosion potential. Burial of infested debris however may not provide an effective means of reducing grey leaf spot inoculum in regions where widespread use of conservation tillage is practiced because the pathogen may blow into a field from adjacent fields (Latterell and Rossi, 1983). Removal of the crop for silage does reduce inoculum carry-over (Ward and Nowell, 1998).

Crop rotation as a cultural practice has also been emphasized by several studies (Smith and White, 1987; Ward and Nowell, 1994; Thornson, 1989; Latterall and Rossi, 1983). They said that avoiding inoculum by rotation to another crop would reduce the level of inoculum in the soil. Thus crop rotation can be a promising alternative control method because (a) the pathogen does not survive beyond a year in infected maize debris (Latterell and Rossi, 1983) and (b) because it is host specific and so rotating maize with soybeans, dry beans and cereals is feasible. Unfortunately, rotations are not always economically attractive (Ward and Nowell, 1998). The potential of herbicide carryover may restrict the selection of crops in the rotation scheme (Ward *et al*., 1999).

2.2.3.2 Water Regime

It has been shown that there is a significant increase in severity of GLS in maize grown under irrigation compared to dry land production. It is thought that irrigation extends the leaf wetness period therefore enhancing disease development (Ward and Nowell 1998).

2.2.3.3 Chemical Control

Fungicide sprays are necessary to maintain maize yield potentials in most circumstances. Fungicides delay leaf blighting, especially during grain fill. Combinations of products belonging to the triazole and benzimidazole chemical groups have been registered for use (Perkins *et al*., 1995). The reason for use of combination fungicides is part of resistance management strategies aimed at preventing or delaying pathogen-resistance build-up to the fungicides used. The possibility of development of pathogen resistance is much greater if fungicides of a single chemical group (such as the benzimidazoles) are applied alone. Research has shown that the best time for initial spraying is when disease severity levels reach 2 to 3% of the leaf area blighted and when lesions are restricted to the basal five leaves of the maize plant. Highest grain yields are achieved with treatments providing disease controls until the crop is physiologically mature (Ward *et al.*, 1999). This option is however not appropriate for small scale farmers because they lack the financial means to use fungicides to control the disease in maize (Ward *et al.*, 1999).

2.2.3.4 Host plant Resistance

Host resistance is considered one of the best options for managing GLS (Bubeck *et al.*, 1993). Losses from gray leaf spot can be reduced by planting hybrids less susceptible or more tolerant to this disease. In Kenya, there is no maize hybrid currently available to the grower that is immune to gray leaf spot.

2.3 Genetic Analysis and Breeding for Resistance

Genetic resistance appears to be the best means of reducing yield loss from GLS in Africa, more so to the resource poor small scale farmers (Ward *et al.*, 1999). Most sources of resistance to GLS identified and used in maize breeding have genes for resistance inherited in a quantitative manner (Manh, 1977; Thompson *et al.*, 1987; Ulrich *et al.*, 1990; Gevers *et al.*, 1994). In South Africa, an unexpectedly high frequency of quantitative resistance to GLS has been found already present within commercial hybrids. In addition, a single gene conferring qualitative resistance to GLS has been found in one South African maize genotype (Ward *et al.*, 1999). International Institute of Tropical Agriculture (IITA) developed maize inbred lines with quantitative resistance to GLS from diverse sources but the genetic basis of these new inbred lines has not been identified (Menkir *et al.*,2005). Predominantly, additive genetic effects have been shown to influence resistance to GLS and other traits in maize (Derera *et al.*, 2008). Most of the crosses with one or more resistant parents produced resistant hybrids, whereas most crosses between susceptible lines generated susceptible hybrids (Menkir *et al.*, 2005).

CIMMYT maize breeding programme at Harare, Zimbabwe have developed several maize inbed lines with resistance to GLS. Some of these include CML386, CML388, CML389, CML390, CML390IR and CML391, all of which have EV7992 in their pedigree (Table1).

Table 1: CIMMYT Maize Inbred lines that are resistant to GLS and have population EV7992 in pedigree.

(***Source: CIMMYT, http:/www.cimmyt.org/english/wps/obtain_seed/germplas.htm***)

Inbred Line	**GLS Severity* (Scale1-5)**	**Pedigree**
CML386	1.5	[EV7992#/EVPOP43-SRBC3]#b#bsr-118-2-2-5-7-B-1-1-B*4
CML388	1.5	[EV7992#/EV8449-SR]C1F2-334-1(OSU9i)-8-2(I)-B-1-2-B*4
CML389	1.5	[EV7992#/EV8449-SR]C1F2-334-1(OSU9i)-8-6(I)-B-B-3-B*4
CML390	1.3	[EV7992]C1F2-430-3-3-3-B-7-B*4
CML390IR	1.3	[EV7992]C1F2-430-3-3-3-B-7-B*4.....IR
CML391	1.5	[EV7992]C1F2-430-3-3-B-1-B*4

*GLS severity scores based on report of work done at CIMMYT, Zimbabwe.

2.3.1. Maize Hybrids Selection Criteria

In practical hybrid advancement processes, selection among hybrids is often based on their performance on a number of agronomic traits. Independent culling, where genotypes meeting certain minimum levels of performance are selected (Bernardo, 2002), plays a crucial role in these hybrid advancement processes. In independent culling, minimum hybrid performance values for agronomic characters are set based on check hybrid performances, the importance of the trait, farmer preferences, and organization resources. Farmer preferences play a significant role in the selection criteria because farmers are the end users of the hybrids. Thus their preferences are incorporated in the selection criteria in order to increase the rate of product uptake should the hybrid be commercialized in future. Independent culling leads to selection of genotypes that are exceptional for traits under consideration (Bernardo, 2002). Genotypes meeting the set criteria are selected and advanced to the next cycle of testing whilst the rest are rejected.

2.3.2 Correlation of Traits in Maize

There are three main causes for correlation between traits, pleiotropy, linkage, and environmental influence (Aastveit and Aastveit, 1993). The association between traits is an important aspect to deal with in breeding programs, because genetic change in a given trait may change positively or negatively other traits (Vencovsky and Barriga, 1992). In addition, in most breeding programs the strategy is based on selection for several traits simultaneously and, therefore, knowledge on the genetic association between traits is inevitably useful for the establishment of selection criteria (Saleh *et al.*, 2002). The basic causes of genetic correlation are pleiotropy, and linkage disequilibrium (Falconer, 1964; Vencovsky, 1978; Hallauer and Miranda Filho, 1995).

One important application of the genetic correlation in breeding programs refers to indirect selection for traits of low heritability with low direct response to selection. Selection for another trait may result in indirect response of the lowly heritable trait, provided the following conditions are satisfied: i) traits under consideration must be highly correlated genetically; ii) heritability of the secondary trait must be higher than the trait of higher interest (Falconer, 1964; Vencovsky and Barriga, 1992). In maize, for example, two secondary traits identified for indirect selection to increase yield were prolificacy (Paterniani, 1981) and tassel size (Geraldi *et al.*, 1985).

Manh, 1977 reported a relationship between hybrid maturity and GLS severity; observing that early maturing hybrids appeared to be more susceptible to GLS than late maturing hybrids. In a study of corn hybrids and inbreds in Tennessee, Hilty *et al.*, 1979 noted that initial disease symptoms coincided with silk emergence, and that the number of GLS lesions tended to increase as plants reached senescence. Rupe *et al.*, 1982) later observed that initial disease symptoms did not appear until plants were near anthesis, and that a three week delay in planting resulted in a three week delay in symptom appearance.

2.3.3. Correlations between Inbred Lines and Hybrid Traits

In plant breeding, breeders inevitably incorporate new inbred lines for testing and introgression into their breeding programmes. The lines are evaluated for obvious weaknesses before they can be used in crosses. This is done in order to reduce costs associated with the running of yield trials. Therefore, any information on inbred lines that predicts the performance of their progenies is desirable so as to reduce the number of crosses and save costs (Hallauer and Miranda, 1988). Expenses can be cut by investigating methods that reduce the testing of inbreds in hybrid combinations and determine if the performance of hybrids can be predicted from inbred performance (Hallauer and Miranda, 1988). Even though the final analysis must be based on the performance of lines in crosses it is obvious that the capacity is not normally there to test all the crosses possible (Hallauer and Miranda, 1988).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Sites.

Field experiments were conducted at the Maseno University farm and Bungoma Agricultural Training Center (Mabanga) in Western Kenya over two seasons. These sites both have a bimodal type of rainfall where the first peak falls between March and June and the second peak between October and January. The short rains season (October to January), however are sometimes unreliable.

Maseno University, site lies along the Equator at latitude 0° , longitude 34° 30'E and at an altitude of 1515m a.s.l. The soils at Maseno are well drained, extremely reddish brown and friable clay. The soils vary in colour, consistence and texture. They are classified as dystric nitisols (Jaetzold and Schmidt, 1982). It experiences mean minimum and maximum temperatures of 15.4^{0}C and 29.9^{o}C respectively with an average annual rainfall of 1250mm. Bungoma Agricultural Training Centre lies at latitude of 0^0 35.98'N, longitude 34^0 53.45'E at an altitude of 1490m a.s.l. The soils are well drained loam on a gentle sloping land. The average annual rainfall is 1650mm per annum.

3.2 Materials

3.2.1 Experiment I: Inbred lines and F_1 hybrid screening for GLS

This was done during the long rains seasons of 2007 and 2008 at Maseno University Farm.

3.2.2 Plant Materials.

The lines evaluated were obtained from either CIMMYT or Maseno University (Dr. M. Dida's inbred lines). The lists of materials evaluated are in Table 2.1. In 2007, five inbred lines and two F_1 hybrids were evaluated. In 2008, eleven inbred lines and one three way cross hybrid were evaluated. Inbred lines and F_1 hybrids used in the experiment included susceptible, tolerant and resistant materials to GLS. The inbred lines were developed using the pedigree method of breeding and they were highly homozygous and phenotypically uniform. The F_1 hybrids were obtained by crossing MSN21 and CML 389 or CML 312.

Table 2.1. List of inbred lines and F_1 hybrids screened for GLS at Maseno in 2007 and 2008 long rains Seasons.

Entry	Material	Source/Status	GLS response statusф
1	CML 389*¶	CIMMYT	Resistant
2	CML 312*¶	CIMMYT	Tolerant
3	CML 384*	CIMMYT	Tolerant
4	CML388*	CIMMYT	Resistant
5	MSN21*¶	MASENO	Susceptible
6	MSN21 x CML 389F_1*	F_1 HYBRID	Unknown
7	MSN21 x CML 312F_1*	F_1 HYBRID	Unknown
8	CML218¶	CIMMYT	Unknown
9	CML387¶	CIMMYT	Resistant
10	CML321¶	CIMMYT	Unknown
11	CML442¶	CIMMYT	Unknown
12	M112¶	MASENO	Susceptible
13	CML389/CML388//M112¶	HYBRID	Unknown
14	EX87/02-3¶	MASENO	Unknown
15	EX87/02-1¶	MASENO	Unknown
16	EX44/42-1D¶	MASENO	Unknown

*** Lines/Hybrids evaluated 2007**

¶ Lines/Hybrids evaluated 2008

ф Gray leaf spot status is based on either published CIMMYT documents or preliminary work done at Maseno University.

3.2.3 Agronomic Practices

Land preparation was done using a disc plough and harrowed before planting. A pre marked twine and hoes were used to mark planting stations. Plantings were done on 22nd of April 2007 and 1st April 2008. The inbred lines and hybrids were planted in single row plots of 5 meters long at spacing of 75 cm between rows and 25 cm between plants in a row. Fertilizer was applied at 60 and 128 kg N and P_2O_5 ha^{-1}, respectively, at planting in the form of di-ammonium phosphate (18-46-0) to ensure reasonable maize development. Two seeds were planted per hole and thinned to one plant after emergence. Standard cultural practices, including hand weeding were followed.

3.2.4 Inoculation

The GLS inoculum was obtained from dry infected leaves of maize harvested from the previous season. The leaves were ground to fine dust with a hammer mill and stored in a refrigerator at 4^0C. Approximately 3 g of GLS inoculum was placed in the whorl of each plant to augment natural infestation. This artificial inoculation was done at the 8-leaf stage of growth and repeated after 10 days.

3.2.5 GLS Severity Assessments

GLS scores were taken only once, at near physiological maturity on a scale of 1-5 (Saghai Maroof *et al*., 1993) on a whole plot basis. Where 1 = no visible infection, 2 a few scattered lesions on leaves below the ear, 3 = many lesions on leaves below the ear, with a few lesions above the ear, 4 = severe lesions on all but uppermost leaves, which may have a few lesions, and 5 = abundant lesions on all leaves with most of the leaf tissue being necrotic.

For the 2008 inbred evaluation, GLS severity was also assessed based on lesion length. This was measured using a ruler on mid leaves with fully expanded lesions of at least 5 randomly selected plants per plot (Plate1).

Plate 1: GLS lesion length measurement at Maseno University Farm

3.3 Experiment II: F_2 Hybrid Evaluation for GLS response

3.3.1 Plant Materials

The F_1 hybrids of MSN21xCML389 and MSN21xCML312 were advanced to F_2 generation by self pollination of individual plants. This was done at Maseno during the long rains season of 2008. This experiment was planted on the 16th of April 2008. The F_2 seeds of (MSN21xCML312) were planted on 4 rows, 5 meters long, and MSN21WxCML 389 were planted on 10 rows of 5 meter long, all at spacing of 0.75meters between rows by 0.25 meters intra row. The former were planted on fewer rows due to the limited amount of seed compared to the latter. Two seeds were planted per hill and later thinned to one. .

3.3.2 Agronomic Practices

All agronomic practices were done in similar way as described in section 3.2.2. GLS inoculation was done in a similar way as previously described in section 3.2.3.

3.3.3 GLS Severity Assessments

A total of 66 F_2 plants of the MSN21xCML312 were evaluated for GLS severity towards physiological maturity on a scale of 1-5, as outlined in Section 3.2.4. Ratings were on individual plant basis. A total of 184 surviving plants of the MSN21xCML389 F_2 populations were evaluated for GLS severity on a scale of 1 to 5. In addition, fully developed GLS lesions lengths were measured, on at least 5 middle leaves per plant. Lesion length measurement was done only on the MSN21xCML389 F_2 population which appeared interesting based on segregation pattern of severity ratings.

3.3.4 Data Analysis

For each F_2 population, individual plant disease severity scores were plotted into frequency distribution diagrams. Mean lesion length for each individual F_2 plant of MSN21x389 population were also plotted. Pearson's correlation analysis was done for the GLS severity scores and lesion length for the MSN21xCML389 F_2 population. Based on the shape of frequency distribution of the MSN21xCML389 F_2 population, an empirical classification of susceptible and resistant genotypes was done. To test for the goodness of fit of this data to a complementary gene action (9:7), Chi-square analysis was done.

3.4 Experiment III: Hybrid Evaluation

3.4.1 Plant Materials

The hybrid materials evaluated consisted of experimental hybrids designated as EH1- EH12 and 4 commercial check hybrids (Table 2.2). The experimental hybrids were 3 way crosses from the breeding programme at Maseno University. The commercial hybrids included varieties from seed companies (H516 and H515 from Kenya Seed Company., Phb3253 from Pioneer, and WH505 from Western Seed Co.). The evaluation was done under combined natural and artificial inoculation of GLS in the disease hotspot zones at Bungoma and Maseno during the short and long rains of 2007 and 2008.

Table 2.2. List of maize hybrids evaluated during the short rains season of 2007 and long rains season of 2008.

Experiment 1		
Entry	**Hybrid**	**Hybrid Type**
1	EH1	Experimental
2	EH2	Experimental
3	EH3	Experimental
4	EH4	Experimental
5	EH5	Experimental
6	EH6	Experimental
7	EH7	Experimental
8	EH8	Experimental
9	EH9	Experimental
10	EH10	Experimental
11	EH11	Experimental
12	EH12	Experimental
13	H515	Commercial
14	Phb3253	Commercial
15	H516	Commercial
16	WS505	Commercial

3.4.2 Experimental Design

Experimental design was alpha (0, 1) lattice (Patterson and William, 1976) with 16 treatments including 4 commercial checks replicated twice. The randomization was done using the computer software Fieldbook (Banziger and Vivek, 2007).

Hybrids were evaluated for two seasons at two sites (Bungoma and Maseno). Evaluation trial at Bungoma during the 2007 short rains season was planted on the 20th September 2007. Whereas, the long rains season trial was planted on the 3rd April 2008. The Maseno hybrid evaluation trials were planted on the 15th of September 2007 and on the 15th March 2008. Each variety was planted at each location in a 5-m row plot spaced at 75 cm apart with 50 cm between plants within each row. Three seeds were planted per hill and thinned to two plants after emergence to attain a population density of 53,333 plants ha^{-1}.

3.4.3 Agronomic Practices

Land was disc ploughed and harrowed before planting. A pre-marked twine and hoes were used to mark planting stations. All the trials were planted by hand. Fertilizer was applied at 60 and 128 kg N and P_2O_5 ha^{-1}, respectively, at planting in the form of di-ammonium phosphate (18-46-0) to ensure reasonable maize development. Three seeds were planted per hole and thinned to two plants after emergence. Standard cultural practices, including hand weeding were followed. At four weeks after crop emergence dipterex (endosulfan) one percent granules were applied in the crop funnels at 4 kg/ha to control stalkborer. A spot application against the stalk borers was done at six weeks after crop emergence.

3.4.4. Inoculation

The GLS inoculum was obtained from dry infected leaves harvested from the previous season. These leaves had been ground with a hammer mill and stored at 4^{0}C. Approximately 3 g of GLS inoculum was placed in the whorl of each plant to augment natural infestation. This artificial inoculation was done at the 8-leaf stage of growth and repeated after 10 days.

3.4.5 GLS Severity Assessment and other Data collected

The data were collected on whole plot basis. The primary traits recorded were GLS scores and grain yield in t ha $^{-1}$. GLS severity scores were taken once; at near physiological maturity (65 days after inoculation) on a whole plot basis (see section 3.2.5). Additional traits recorded were plant stand count, the number of days to 50 percent silk emergence and anthesis, ear and plant heights in centimeters. Anthesis and silking notes were taken daily from the date of onset of flowering. Also scored were plant and ear aspects, and these were rated using a scale of 1 to 5, with 1 being excellent overall phenotypic appeal; and 5, poor phenotypic appeal.

3.4.6. Data Analysis

All the data were subjected to analysis of variance using CIMMYT Alpha software (Banziger and Vivek, 1997) and significant means separated using Least Significant Differences (LSD).

CHAPTER 4

RESULTS

4.1. Inbred lines and F_1 hybrid screening for GLS at Maseno during the long rains season of 2007.

The overall mean GLS score for inbreds and F_1 hybrids evaluated at Maseno was 2.0 (Table 3.1). GLS severity scores ranged from 1.0 to 4.5. Inbred lines CML389 and CML388 did not show any signs of GLS attack (Plate2) and were both rated with severity scores of 1.0. The lines CML312 and CML384 had GLS severity scores of 2.8 and 2.5, respectively (Table 3.1). These inbred lines showed tolerance to GLS attack. Though their leaves eventually succumbed and developed some lesions, full development took long (they remained tan longer, Plate 3). The susceptible MSN21 had a severity score of 4.5 (Table 3.1, Plate 4). The F_1 hybrid of MSN21 and CML389 had a severity score of 1.0 and was clean just like the latter parental line (CML389). The F_1 hybrid of MSN21 and CML312 had a severity rating of 1.5.

Table 3.1: GLS severity scores and flowering data for inbred lines and F_1 hybrids and at Maseno long rains season, 2007.

ENTRY	INBRED LINE/F1 HYBRID	GLS SEVERITY RATINGS	DAYS TO POLLEN SHED	DAYS TO SILKING
1	MSN21	4.5	58	62
2	CML389	1.0	74	77
3	CML312	2.8	75	79
4	CML384	2.5	77	79
5	CML388	1.0	72	75
6	MSN21xCML389 F_1	1.0	72	75
7	MSN21xCML312 F_1	1.5	65	66
Mean		2.0	70.5	73.2
SEM		0.5	2.5	2.5

Key: SEM-standard error of mean

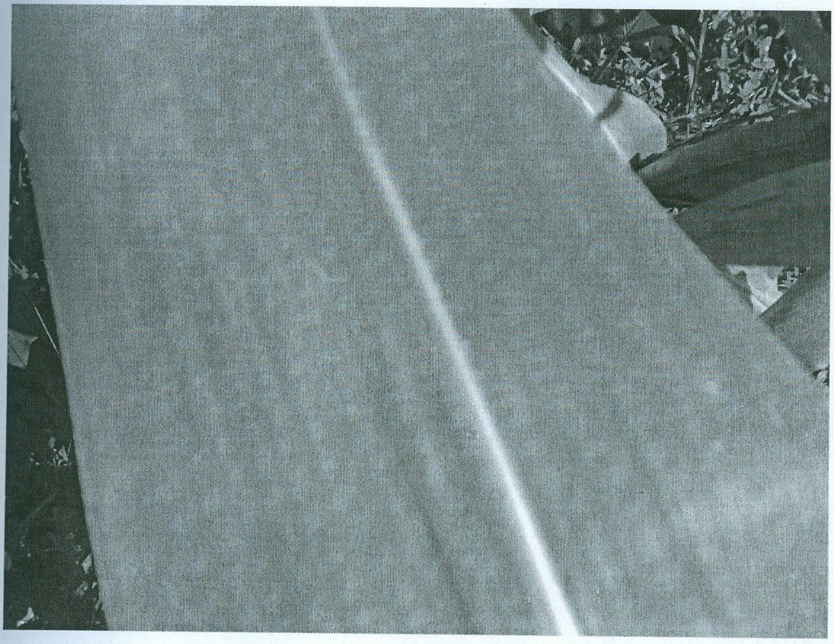

Plate 2: Leaf of a resistant inbred line CML389 at Maseno University Farm (65 days after artificial GLS infestation).

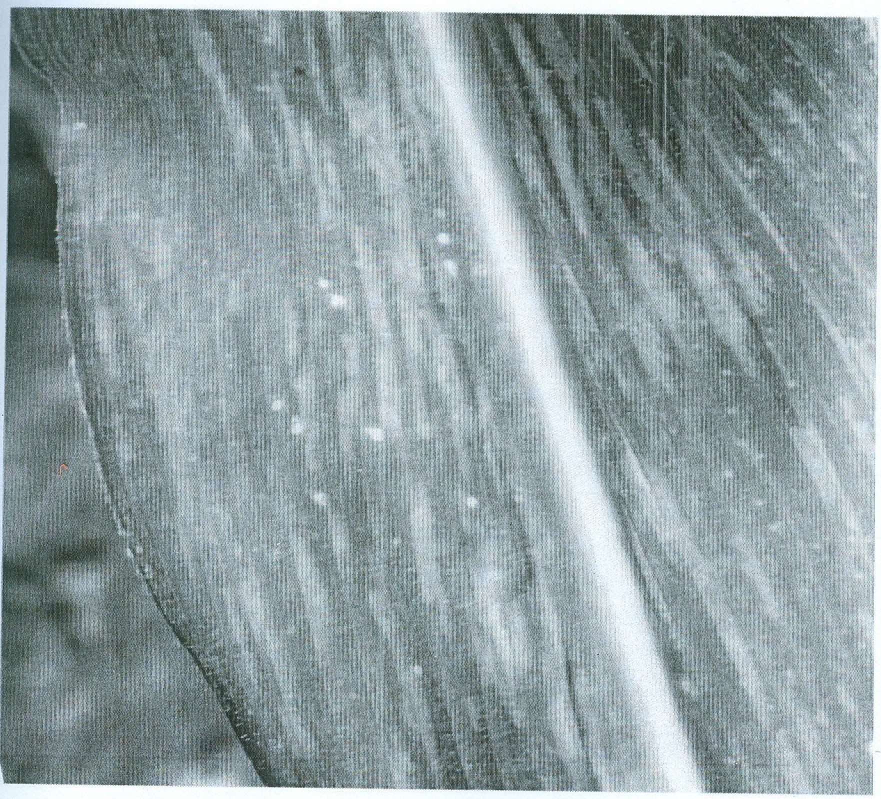

Plate 3: Leaf of a tolerant inbred line CML384 at the Maseno University (65 days after artificial GLS infestation)

Plate 4: Leaf of a highly susceptible inbred line MSN21 at Maseno University (65 days after artificial GLS infestation)

4.2 Inbred Lines and F_1 hybrid screening for GLS at Maseno during the long rains 2008

GLS severity scores ranged from 1.0 to 4.0 (Table 3.2) with a mean value of 2.3. Inbred line CML389 had the lowest severity score of 1.0 and also very short lesion length of 1.0 cm. Inbred line CML312 showed GLS incidences and had a severity score of 2.5 and an average lesion length of 2.4 centimeters. Other entries 5, 7, 10, 11 and 12 also showed moderate levels of GLS resistance. MSN21 was the most susceptible with a severity index score of 4.0 and mean lesion length of 2.8 cm. The lines CML 321(entry 6), CML 218 (entry 4) and M112 (entry 8) appeared to be susceptible with severity rating between 3.0 to 3.5. The 3-way cross hybrid (entry 9) was resistant with a GLS score of 1.0 and lesion length of 0.9 cm.

Table 3.2: GLS Severity scores for inbred lines and a hybrid at Maseno in the long rains season, 2008

ENTRY	INBRED LINE/HYBRID	SOURCE	GLS RATINGS	GLS lesion Length(cm)
1	MSN21	MASENO	4.0	2.8
2	CML389	CIMMYT	1.0	1.0
3	CML312	CIMMYT	2.5	2.4
4	CML218	CIMMYT	3.5	2.6
5	CML387	CIMMYT	1.3	0.9
6	CML321	CIMMYT	3.0	1.8
7	CML442	CIMMYT	1.8	1.4
8	M112	MASENO	3.5	2.8
9	CML389/CML388//M112	MASENO	1.0	0.9
10	EX87/02-3	MASENO	2.0	2.0
11	EX87/02-1	MASENO	2.0	2.4
12	Ex44/42-1D	MASENO	2.0	2.0
Mean			2.3	1.9
SEM			0.3	0.2

Key: SEM-standard error of mean

4.3 Evaluation of the F_2 population response to GLS at Maseno during the long rains season of 2008.

4.3.1 Variation in GLS scores for MSN21xCML 312 F_2 population

The GLS severity scores for the MSN21xCML312 F_2 population showed a continuous distribution (Figure 4.1). The scores ranged from a score of 1.5 to 5.0. There was a slight skew in distribution towards the right (tolerant parental value).

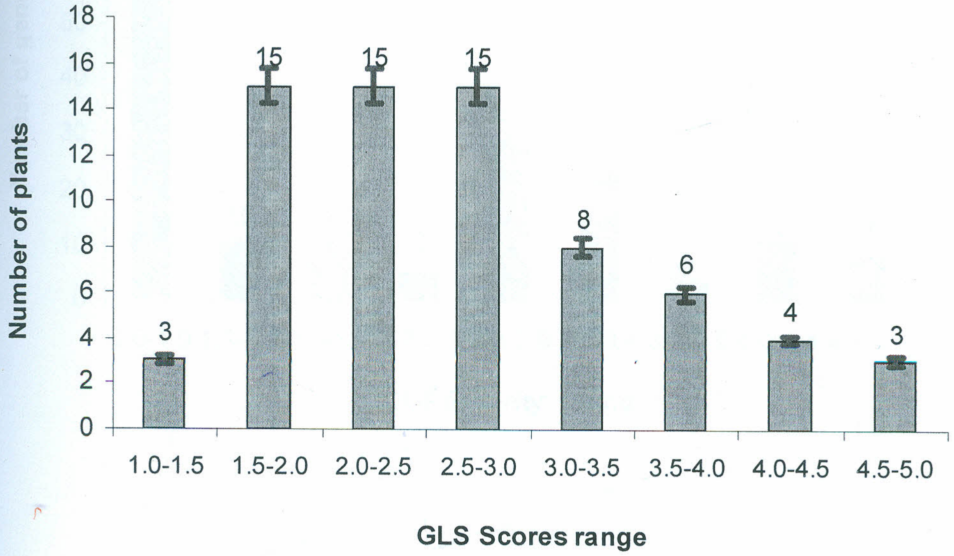

Fig 4.1: Frequency distribution of GLS severity rating of MSN21xCML312 F_2 population

4.3.2 Variation in GLS severity rating for MSN21 x CML 389 F_2 populations

The MSN21xCML389 F_2 population GLS severity ranged from 1 to 4.5. The frequency distribution figure showed two peaks (Fig. 4.2). Taking individuals with mean severity scores of 1 to 2 as resistant and greater than 2 as susceptible, 105 genotypes were classified as resistant and 79 as susceptible. This nicely fitted a 9:7 ratio (Chi Square test, P>0.05).

For the same F_2 population, the distribution in lesion length data also showed a similar topology, with two major peaks (Fig. 4.3). Taking genotypes with mean lesion length of less than 2 as resistant and above as susceptible, 107 were classified as resistant and 77 as susceptible. This also fitted a 9 to 7 ratio (P>0.05).

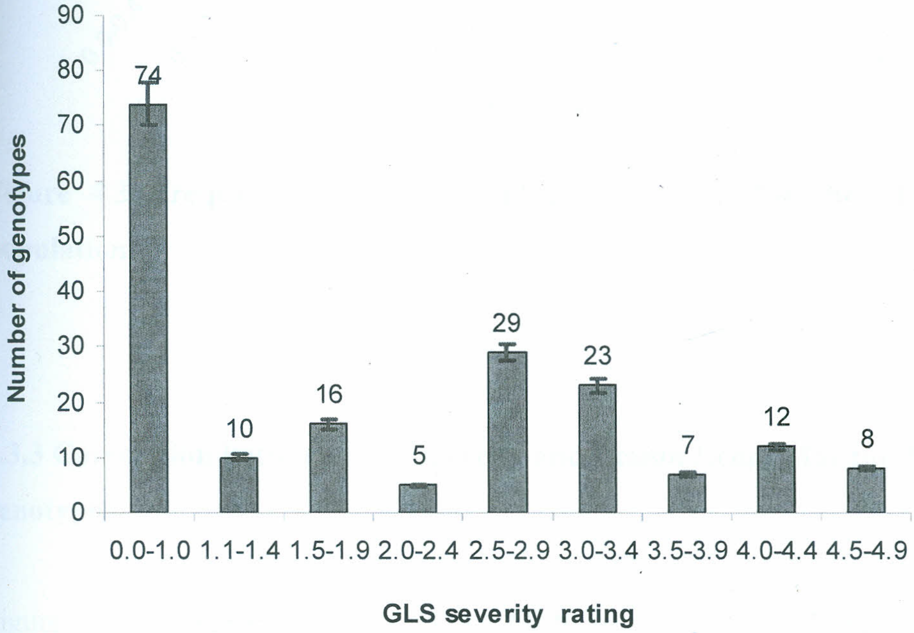

Figure 4.2 Frequency distribution of GLS severity rating of 184 MSN21 x CML389 F_2 population

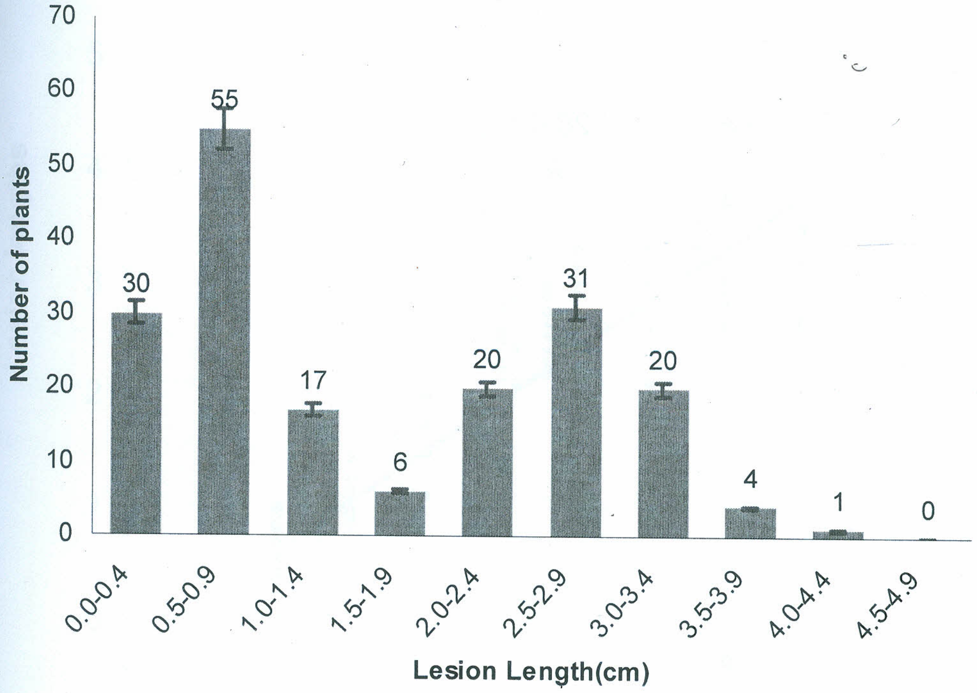

Figure 4.3 Frequency distribution of lesion length for the MSN21WxCML389 F_2 population

4.3.3 Correlation between GLS severity and Lesion Length for the MSN21xCML 389 F_2 genotypes

Figure 4.4 is a correlation plot of the GLS lesion length and GLS severity data of an F_2 population of a cross between the susceptible MSN21 and the resistant line CML389.

The correlation was positive (r = 0.932) and highly significant ($P < 0.001$). This shows that GLS severity is a significant factor of lesion length, that is, as lesion length increases then GLS severity is high. The co-efficient of determination of the relationship is 0.87, that is, GLS severity accounts for 87% of the variance in lesion length.

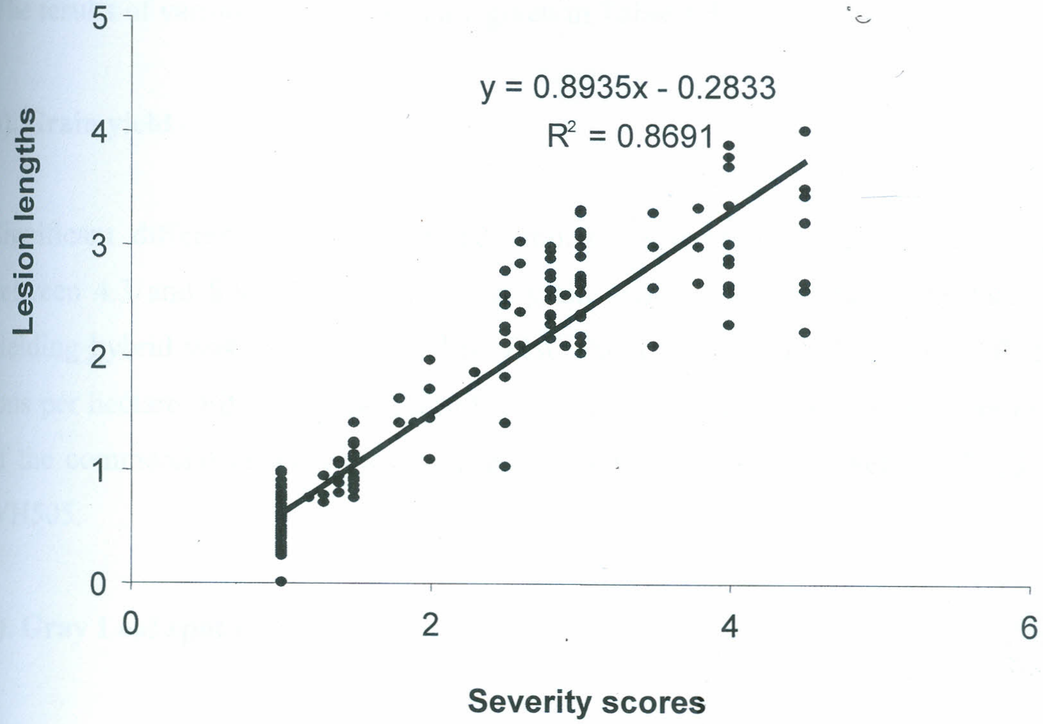

Figure 4.4 Correlation plot of GLS lesion length and GLS severity for the MSN21x CML389 F_2 population

4.4 The Performance of Hybrids Evaluated at Maseno during short rains season of 2007

The results of various traits studied are given in Table 3.3

a). Grain yield

Significant differences were observed among the hybrids for grain yield. Yields varied between 4.3 and 8.8 tons/ha. The mean grain yield was 6.3 tons per hectare. The highest yielding hybrid was EH10 followed by EH6.The average yield of commercial checks was 6 tons per hectare. Fifty eight percent of the experimental hybrids yielded more than the mean of the commercial check varieties. The lowest yielding hybrids were EH9, EH7, EH8 and WH505.

b). Gray Leaf spot scores

There were significant differences amongst the hybrids for GLS severity scores. The GLS scores ranged from 1.3 to 3.0. The average GLS scores for all the hybrids was 1.8. The best hybrids with lower severity scores were EH9, EH8, WS505 and EH6. They had a score of 1.3. The worst entry was H516 with a score of 3.0. The highest yielding hybrid EH10 had a score of 2.3

Table 3.3: Mean values for grain yield, GLS severity scores and other agronomic characters measured on maize hybrids evaluated at Maseno during short rains 2007

ENTRY	HYBRID	GRYD (tons/ha)	GLS Score	DAY SHD	DAY SLK	PLT HT (CM)	EAR HT (CM)	PLANT ASP	EAR ASP	PLT STD (%)
10	EH10	8.81	2.3	70.5	74.5	214.5	129.5	1.0	2.0	83.0
6	EH6	8.45	1.3	71.0	73.0	212.0	119.5	1.8	2.0	73.9
5	EH5	7.40	1.5	71.0	74.0	203.0	101.5	2.3	2.0	72.7
2	EH2	7.28	1.8	70.5	73.5	211.0	103.0	2.3	2.3	84.1
4	EH4	6.54	2.0	71.0	73.0	213.5	99.0	1.5	2.3	93.2
14	PHB3253	6.54	2.0	70.5	73.5	208.5	97.0	2.0	2.3	88.6
15	H516	6.50	3.0	70.5	74.5	221.0	102.5	3.0	2.5	95.5
11	EH11	6.49	1.8	69.5	74.5	208.0	89.5	1.8	2.0	90.9
1	EH1	6.48	2.0	70.5	74.5	200.5	99.0	2.3	1.8	86.4
13	H515	6.20	2.0	70.5	73.5	193.0	101.5	3.3	2.3	95.5
12	EH12	5.48	2.3	69.5	72.5	202.0	101.5	2.3	2.3	86.4
3	EH3	5.41	2.0	70.0	74.0	212.0	106.0	2.8	2.0	95.5
16	WS505	4.92	1.3	70.5	74.5	206.5	101.0	2.5	2.5	86.4
8	EH8	4.63	1.3	70.0	74.0	192.0	94.0	2.5	2.3	76.1
7	EH7	4.51	1.5	71.0	75.0	200.0	89.0	4.0	3.0	81.8
9	EH9	4.29	1.3	70.5	73.5	199.5	99.0	3.5	2.8	54.5
MEAN		6.25	1.8	70.4	73.9	206.1	102.0	2.4	2.3	84.0
LSD (5%)		2.70	1.5	1.6	1.8	30.9	36.3	1.3	0.8	26.1
CV (%)		19.85	38.3	1.1	1.1	7.0	16.7	25.7	15.7	14.5
SEM		1.0	0.5	0.5	0.6	10.2	12.0	0.4	0.3	8.6
P		*	*	*	*	NS	*	*	*	*

Key: NS – non-significant, * - significant ($P<0.05$), SEM – standard error of mean. GRYD-Grain yield DAY SHD-Days to 50% pollen shed; DAY SLK-Days to 50% silking; HT (cm)-Plant height in centimeters; ASP-Aspect; PLT STD-Plant stand. GLS-Gray leafspot scores

c). Number of days to 50 percent anthesis

There were significant differences amongst the hybrids for the number of days to 50 percent anthesis. The average number of days to pollen shed was 70 whilst the pollen shedding period ranged from 69 to 71 days. The earliest hybrids to flower were EH11 and EH12, they took about 69 days. Hybrids that took longest to flower were EH6, EH5, EH4 and EH7. They flowered in 71 days after planting. The checks on average flowered in about 71 days.

d). Number of days to 50 percent silk emergence

There were significant differences amongst the hybrids for the number of days to 50 percent silk emergence. The average number of days to 50 percent silk emergence was 74 whilst the silking period ranged from 73 to 75.0 days. The latest silking hybrids was EH7and it was not significantly different from most of the entries. The earliest silking hybrids were EH12 and EH6 at 73 days after planting. The checks had a mean of 74 days to 50 percent silking.

e). Plant height

There were no significant differences amongst the hybrids for plant height. The mean plant height for all the genotypes was 206 cm. Plant heights ranged from 192.0 cm to 214 cm.

f). Ear height

There were significant differences amongst the hybrids for ear height. The mean ear height for the genotypes was 102 cm. Ear heights ranged from 89.0 cm to 129.5 cm. EH10 had the highest ear placement of 129.5 cm whilst the hybrid EH7 had the lowest ear placement of 89.0cm.

g). Plant Aspect

There were significant differences amongst the hybrids for plant aspect scores. The overall mean plant aspect for all the hybrids was 2.4. The plant aspect scores ranged from 1 to 4. The hybrid EH10 had the best score of 1.0.The worst hybrid was EH7 with a score of 4.0.The best commercial hybrid check was PhB3253 with a score of 2.0.

h). Ear Aspect

There were significant differences amongst the hybrids for ear aspect scores. The overall mean ear aspect for all the hybrids was 2.3. The ear aspect scores ranged from 1.8 to 3.0. The best hybrid was EH1 with a score of 1.8.The worst entry was hybrid EH7 with a score of 3.0.The mean ear aspect for the check varieties was 2.4

i). Plant Stand Percent

There were significant differences amongst the hybrids for the plant stand percentage. The overall mean number of plants per plot was 84 percent. The plant stand percentage ranged from 55 to 96 percent. EH9 had the lowest plant stand of 55 percent. The hybrids with the highest plant stands were EH3, H515 and H516.

4.5. Performance of Hybrids Evaluated at Bungoma during the short rains season of 2007

The results of various traits studied are given in Table 3.4

a). Grain yield

Significant differences were observed amongst the hybrids for grain yield. Grain yields ranged from 2.52 to 4.89 tons/ha with overall mean of 3.53 tons/ha. The highest yielding hybrid was EH10, whilst the lowest yielding hybrid was EH9.Other hybrids with low yields were PhB3253 and EH1. The average yield of commercial checks was 3.6 tons per hectare. Forty two percent of the experimental hybrids yielded more than the mean of the commercial check varieties.

b). Gray Leaf spot scores

There were highly significant differences ($P < 0.001$) amongst the hybrids for GLS severity scores. The GLS scores ranged from 1.3 to 2.8. The average score was 1.7. The susceptible check hybrids PhB3253 had a severity score of 2.3 while, EH2 had the highest score of 2.8. The most resistant hybrids with the best GLS scores were EH4, EH6, EH9, H515 and H516 with mean scores of 1.3

c). Number of days to 50 percent anthesis

There were no significant differences for the number of days to 50 percent anthesis. The average number of days to pollen shed was 71 whilst the pollen shedding period ranged from 70 to 74 days.

d). Number of days to 50 percent silk emergence

There were no significant differences amongst the hybrids for the number of days to 50 percent silk emergence. The average number of days to 50 percent silk emergence was 75 whilst the silking period ranged from 73 to 79 days.

e). Plant height

There were significant differences amongst the hybrids for plant height. Plant heights ranged from 125.5 cm to 180 cm. The mean plant height for all the genotypes was 151.9 centimeters (cm). Hybrid EH4 had the highest plant height (180cm). The commercial checks had an average plant height of 151cm.

Table 3.4: Mean grain yield, GLS severity scores and other agronomic characters of maize hybrids evaluated at Bungoma during the short rains season of 2007

ENTRY	HYBRIDS	GRAIN YIELD(tons/ha)	GLS	DAY SHD	DAY SLK	PLT HT(CM)	EAR HT(CM)	PLANT ASP	PLT STD (%)
10	EH10	4.89	1.8	73.5	76.0	141.5	53.0	1.3	71.6
2	EH2	4.77	2.8	72.5	76.0	160.5	73.5	1.8	78.4
15	H516	4.47	1.3	70.5	73.5	142.0	66.5	2.3	40.9
7	EH7	4.37	2.5	70.5	73.5	160.5	76.0	1.8	54.5
16	WS505	3.96	1.8	74.0	78.0	169.0	83.0	2.0	54.5
6	EH6	3.80	1.3	72.0	75.0	157.5	83.5	3.0	68.2
4	EH4	3.80	1.3	71.0	75.0	180.0	80.5	3.3	78.4
8	EH8	3.59	1.5	74.0	79.0	145.0	68.0	1.8	53.4
13	H515	3.51	1.3	72.0	77.0	168.0	73.0	1.3	61.4
11	EH11	2.96	1.5	70.5	72.0	147.0	71.0	3.3	71.6
12	EH12	2.91	1.8	73.5	78.0	148.5	74.5	3.3	67.0
3	EH3	2.87	1.5	72.5	77.0	156.5	69.5	2.8	63.6
5	EH5	2.72	2.5	71.5	73.5	148.0	60.0	2.5	75.0
1	EH1	2.66	1.8	73.5	75.0	131.5	70.5	3.0	62.5
14	PHB3253	2.59	2.3	70.5	75.0	125.5	59.0	2.8	65.9
9	EH9	2.52	1.3	71.5	73.5	149.0	76.0	3.0	73.9
MEAN		3.53	1.7	72.1	75.4	151.9	71.1	2.4	65.1
LSD (5%)		1.15	0.8	4.9	6.3	35.0	35.4	1.7	22.7
CV (%)		14.93	21.7	3.2	3.9	10.8	23.4	33.3	16.4
SEM		0.6	0.3	1.6	2.1	11.6	11.8	0.6	7.5
P		*	***	NS	NS	*	NS	*	*

Key: *** - highly significant ($P < 0.001$), ** - significant ($P < 0.01$),*- Significant ($P<0.05$), NS – non-significant, SEM – standard error of mean. DAY SHD-Days to pollen shed; DAY SLK-Days to silking; HT (cm)-Plant height in centimeters; ASP-Aspect; PLT STD-Plant stand. GLS-Gray leafspot score

f). Ear height

There were no significant differences amongst the hybrids for ear height. The mean ear height for the genotypes was 71.1 cm. Ear heights ranged from 53.0 to 83.5 centimeters.

g). Ear Aspect

The ear aspect parameter was excluded from this table because of very high co-efficient of variability of data.

h). Plant Aspect

There were significant differences amongst the hybrids for plant aspect scores. The overall mean plant aspect for all the hybrids was 2.4. The plant aspect scores ranged from 1 to 3. The hybrids EH10 and H515 had the best score of 1.3. The worst hybrids were EH4, EH11 and EH12 with scores of 3.3. The commercial hybrid checks had an average score of 2.1.

i). Plant Stand Percent

There were significant differences amongst the hybrids for the plant stand percentage. The overall mean number of plants per plot was 65 percent. The plant stand percentage ranged from 40.9 to 78.4 percent. Commercial check hybrid H516 had the lowest plant stand of 40.9 percent. EH2 and EH5 had significantly higher plant stand percent compared to H516.

4.6 Hybrid Evaluation at Bungoma for the long rains season of 2008.

The results of various traits studied are given in table 3.5.

a). Grain yield

Significant differences were observed amongst the hybrids for grain yield. Grain yields ranged from 6.20 to 11.2 tons/ha with overall mean of 8.1 tons/ha. The highest yielding hybrids were EH11 and EH12, whilst the lowest yielding hybrid was EH7 with 6.2 tons/ha. The average yield of commercial checks was 7.5 tons per hectare. Sixty seven percent of the experimental hybrids yielded more than the mean of the commercial check varieties.

b). Gray Leaf spot scores

There were significant differences amongst the hybrids for GLS scores. The average GLS severity score for all the hybrids was 2.5. The GLS scores ranged from 1.0 to 3.5. The commercial check hybrids PhB3253, H515, H516 and WS505 were most susceptible with scores of 3.5, 3.0, 3.5 and 3.5, respectively. Whereas, the most highly resistant hybrids were EH9, EH3 and EH7.

c). Number of days to 50 percent anthesis

There were significant differences amongst the hybrids for the number of days to 50 percent anthesis. The average number of days to pollen shed was 70 whilst the pollen shedding period ranged from 69 to 71 days. The earliest hybrid to flower was WS505 at 69 days from planting. Hybrid that took longest to flower was PhB3253 at 71 days after planting. The checks on average flowered in about 70 days.

Table 3.5: **Mean values for grain yields, GLS scores and other agronomic characters measured on maize hybrids evaluated at Bungoma Long rains season of 2008**

ENTRY	HYBRID	GRAIN YIELD (tons/ha)	GLS	DAY SHD	DAY SLK	PLT HT(CM)	EAR HT(CM)	PLANT ASP	EAR ASP	PLT STD (%)
11	EH11	11.2	2.0	70.0	73.0	226.5	106.50	1.5	1.3	95.5
12	EH12	11.2	2.0	69.5	71.5	239.0	110.00	2.3	1.0	98.9
4	EH4	9.0	2.0	70.5	73.5	230.5	108.00	2.3	2.0	94.3
10	EH10	8.8	2.5	70.5	74.5	218.5	111.00	2.0	1.8	93.2
6	EH6	8.6	3.3	69.5	72.5	212.0	84.50	2.0	1.8	79.5
2	EH2	8.3	2.8	70.5	74.0	227.0	109.50	2.3	1.5	83.0
9	EH9	8.2	1.0	70.5	74.0	225.0	100.50	1.8	1.0	94.3
1	EH1	7.7	2.3	70.5	73.0	202.0	103.50	3.0	1.3	78.4
3	EH3	6.9	1.5	70.5	73.5	188.5	88.50	2.8	2.0	93.2
8	EH8	6.8	2.3	69.5	73.5	197.0	94.00	2.5	1.8	92.0
5	EH5	6.5	2.8	70.0	73.5	209.5	102.50	1.8	1.5	95.5
7	EH7	6.2	1.5	69.5	74.5	206.0	87.00	2.3	1.5	97.7
14	PHB3253	7.8	3.5	71.0	73.5	218.5	89.50	2.3	2.3	97.7
13	H515	7.7	3.0	70.0	73.5	219.5	110.00	1.5	2.0	90.9
15	H516	7.3	3.5	70.5	74.5	202.0	96.50	2.5	2.5	93.2
16	WS505	7.0	3.5	69.0	73.0	214.0	108.50	3.0	2.8	92.0
MEAN		8.1	2.5	70.1	73.5	214.7	100.63	2.2	1.7	91.8
LSD (5%)		3.0	1.4	1.7	1.7	38.7	34.70	1.2	0.9	13.8
CV (%)		17.2	26.8	1.1	1.1	8.5	16.18	25.7	24.9	7.0
SEM		1.0	0.5	0.6	0.6	12.9	11.51	0.4	0.3	4.6
P		*	**	*	*	*	NS	*	**	NS

List of abbreviations Key: ** - significant (P < 0.01), *- significant (P<0.05), NS – non-significant, SEM – standard error of mean. SHD Days to pollen shed; SLK-Days to silking; HT (cm)-Plant height in centimeters; ASP-Aspect; PLT STD-Plant stand. GLS-Gray leafspot score

d). Number of days to 50 percent silk emergence

There were significant differences amongst the hybrids for the number of days to 50 percent silk emergence. The average number of days to 50 percent silk emergence was 74 whilst the silking period ranged from 71 to 74 days. The latest silking hybrids were EH10, EH7 and H516.The earliest silking hybrid was at 72 days after planting. The checks had a mean of 74 days to 50 percent silking.

e). Plant height

There were significant differences amongst the hybrids for plant height. Plant heights ranged from 197 to 239 cm. The mean plant height for all the genotypes was 215 centimeters (cm).Hybrid EH12 had the highest plant height (239cm). The commercial checks had an average plant height of 214cm.

f). Ear height

There were no significant differences amongst the hybrids for ear height. The mean ear height for the genotypes was 101 cm. Ear heights ranged from 84.5 cm to 111.0 cm.

g). Plant Aspect

There were significant differences amongst the hybrids for plant aspect scores. The overall mean plant aspect for all the hybrids was 2.2. The plant aspect scores ranged from 1.5 to 3.0. The hybrids EH11 and H515 had the best score of 1.5.The worst hybrids were EH1 and WS505 with scores of 3.0. The commercial hybrid checks had an average score of 2.3.

h). Ear Aspect

There were significant differences amongst the hybrids for ear aspect scores. The overall mean ear aspect for all the hybrids was 1.7. The ear aspect scores ranged from 1.0 to 2.8.

Commercial hybrids H516 and WS 505 had the highest (worst) scores of 2.5 and 3.0, respectively. EH 9 and EH12 had the lowest (best) score of 1.0.

i). Plant Stand Percent

There were no significant differences amongst the hybrids for the plant stand percentage. The overall mean number of plants per plot was 91.8 percent. The plant stand percentage ranged from 79.5 to 98.9 percent.

4.7. Hybrid Evaluation at Maseno during the long rains season of 2008

Mean values for gray leaf spot of hybrids at Maseno during the long rains season of 2008 are presented in Table 4.6. Grain yield and other secondary data were not taken because of severe plant stunting and field fertility variability resulting from previous erosion of top soil and soil acidity.

4.7.1 GLS severity

There were significant differences amongst the hybrids for GLS scores. The average GLS severity score for all the hybrids was 2.3. The GLS scores ranged from 1.0 to 3.5. The susceptible check hybrids PhB3253 had a severity score of 3.0, while WH505 had the highest severity score of 3.5. The best overall score was exhibited by EH9 with a score of 1.0.

Table 4.6: Mean GLS severity scores for the maize hybrids evaluated at Maseno University Farm in long rains season of 2008

ENTRY	HYBRID	GLS
9	EH9	1.0
3	EH3	1.5
7	EH7	1.5
8	EH8	1.5
11	EH11	1.8
1	EH1	2.0
4	EH4	2.0
10	EH10	2.3
12	EH12	2.3
2	EH2	2.5
5	EH5	3.0
6	EH6	3.0
13	H515	3.0
14	PHB3253	3.0
15	H516	3.3
16	WS505	3.5
MEAN		2.3
LSD (5%)		1.3
CV (%)		27.3
SEM		0.4
P		*

Key: *- significant (P<0.05), SEM – standard error of mean.

4.8 Combined Hybrid Grain Yields and GLS Scores

Based on the overall mean grain yield of all sites, 50 percent of the experimental hybrids performed better than the commercial hybrids (Table 3.7). Hybrid EH10 had the best mean of 7.50 tons per hectare. The best performing commercial hybrid was H516 with a mean yield of 6.1 tons per hectare. The average grain yield of check varieties was 5.7 tons/ha. The lowest yielding hybrid was EH8 with a mean of 5 tons per hectare.

The combined mean GLS scores ranged from 1.1 to 2.8 (Table 3.7). The best was EH9, and the most susceptible was Phb3253 with a score of 2.8. EH10 has a score of 2.2.

Table 3.7: Combined means for grain yields and GLS scores for all sites and seasons.

Entry	Hybrid	Source	Mean Grain Yield (tons/ha)	Mean GLS scores
10	EH10	Experimental	7.50	2.2
6	EH6	Experimental	6.94	2.2
11	EH11	Experimental	6.89	1.8
2	EH2	Experimental	6.77	2.5
12	EH12	Experimental	6.53	2.0
4	EH4	Experimental	6.44	1.8
15	H516	Commercial	6.10	2.8
13	H515	Commercial	5.80	2.3
14	Phb3253	Commercial	5.65	2.8
1	EH1	Experimental	5.61	2.0
5	EH5	Experimental	5.55	2.4
16	WS505	Commercial	5.30	2.5
3	EH3	Experimental	5.06	1.6
7	EH7	Experimental	5.03	1.8
9	EH9	Experimental	5.01	1.1
8	EH8	Experimental	5.00	1.7
	Mean		**5.95**	**2.09**
	SEM		**0.20**	**0.11**

Key: SEM-standard error of mean

CHAPTER 5

DISCUSSION

5.1 Inbred lines and F_1 hybrid response to GLS

This study enabled a comparison of inbred lines for their response to GLS at Maseno. The inbred line MSN21 had the highest GLS severity ratings of 4.0 to 4.5 and was thus the most susceptible (Tables 3.1 and 3.2). In the long rains season of 2007, inbred lines CML389 and CML388 showed high levels of GLS resistance (severity scores for both were 1). Other lines from CIMMYT breeding programme CML312 and CML384 had severity scores of 2.8 and 2.5, respectively. The ratings are in agreement with previous ratings done and reported by CIMMYT researchers (Anon, 2008). They reported that lines CML388 and CML389 are highly resistant to GLS, whereas, lines CML312 and CML384 are tolerant to GLS. Hybrid of MSN21 and CML389 had a severity rating score of 1, suggesting that the resistant genes in CML389 may be dominant.

GLS evaluation in long rains season of 2008 was based on two measures, severity and lesion length. Both gave comparable results, and again this further confirmed high levels of GLS resistance in CML389 (Severity score of 1 and lesion length of 1cm). Very highly susceptible line MSN21 had a severity score of 4.0 and the longest lesion length of 2.8 cm. It is interesting to note that a 3-way cross hybrid of resistant lines CML388, CML389 and a susceptible line M112 showed high levels of GLS resistance (Severity rating of 1 and lesion length of 0.9cm). This further suggests that resistance genes in these lines have predominantly dominant effects.

5.2 F_2 population response to GLS and genetics of resistance in CML 389

GLS response of MSN21xCML312 F_2 population showed a continuous variation from a severity score of 1.5 to 5.0 (Fig. 4.1). There was a skew in distribution towards the tolerant parental value. This suggests that GLS tolerance in the inbred line CML312 is conditioned by quantitative genes that may have some degree of dominance.

For the MSN21 x CML389 F_2 population, the GLS severity and lesion length data clearly showed a segregation pattern with two peaks (Fig. 4.2 and 4.3). This suggests at least 2 major genes condition resistance to GLS resistance in inbred line CML389. The number of plants falling within the 2 phenotypic classes fitted a 9R to 7S ratio, suggesting that two major genes with complementary epistatic interaction are involved.

These results are largely in agreement with the reports of other studies. (Coates and White, 1998; Derera *et al.,* 2008; Donahue *et al.*, 1991; Elwinger *et al.,* 1990, Huff *et al.,* 1988; Thompson *et al.,* 1987; Ulrich *et al.,* 1990). Both additive and dominance effects have been documented to play a major role in GLS resistance in the South African maize germplasm (Gevers *et al.,* 1994, Hohls *et al.,*. 1995). Maize inbred line VO613Y of South Africa has been found to have a high degree of partial resistance to GLS with 2 major quantitative trait loci (QTLs) identified (Gordon *et al.*, 2004). These QTLs accounted for up to 47% of the phenotypic variation. Whereas the source of GLS resistance QTLs in VO613Y is unknown, the probable source of resistance genes in CML389 can be guessed based on its pedigree. The likely source is Experimental Variety 7992 that is extensively used in most CIMMYT inbred lines with resistance to GLS (Table 1). These CIMMYT bred maize lines with EV7992 in their pedigree are very resistant to gray leaf spot.

5.3 Correlation between GLS severity and Lesion length

The highly significant and positive correlation ($r = 0.932$, $p<0.01$) between GLS severity and lesion length shows that these traits are highly associated in a linear way. It appears that most susceptible plants with high severity ratings also tended to have long lesion lengths. These results are not unusual as *Cercospora zeae maydis* resistance in maize is manifested in reduced growth and development of the pathogen, leading to reduced lesion size and leaf area affected (Bosque-Perez *et al.,* 1998, Gordon *et al.*, 2006). In fact, the highly positive relationship between the two measures of maize response to *C. zeae maydis* suggests that either or all of them can be used to assess GLS response in maize. However, measuring lengths of GLS lesions is laborious, and should be used only when the samples are not many.

5.4 Hybrid response to GLS

Developing maize varieties with multiple disease resistance is a high priority in many breeding programs, especially in sub-Saharan Africa, where increasing intensity of maize production has resulted in maize being produced essentially year-round in many areas with environments that are favorable to disease development (Gordon *et al.,*2004). Because of the nature of these farming systems, and the potential catastrophic consequences of crop failure due to disease epidemics the development of durable resistance (Simmonds, 1985; Allard, 1999), should be the top priority.

Resistance to GLS is an essential trait in most maize improvement programs (Schechert *et al.,* 1999). Currently, only a few high-yielding maize hybrids resistant to GLS are available in Africa, particularly in Kenya. Nearly all the commercial hybrids evaluated were susceptible to GLS. The only hybrid that showed very good GLS resistance was the experimental hybrid EH 9. However, this hybrid had lower grain yields compared to others. The commercial hybrids PhB3253 and Kenya Seed H516 were the most susceptible to GLS (both with severity ratings of 2.8). A recent GLS evaluation of diverse maize varieties and lines at Kakamega (1585 m above sea level) in western Kenya also found PhB3253 to be most susceptible with a severity score of 3.5 (Ininda *et al.*, 2007).

It has been reported that variation in GLS severity among locations is a common phenomenon (Carson *et al.,* 1997) and is most frequently attributed to environmental conditions and tillage practices (Ward *et al.*, 1997). Results from this study showed that some hybrids exhibited mild GLS infections at Maseno but the same hybrids exhibited higher infections at Bungoma and vice versa. This observation is similar to that of Bubeck *et al.*, (1993) who reported that variation in GLS severity may be due to differential sensitivities of maize genotypes to environmental conditions since the quantitative trait loci (QTL) effects associated with GLS resistance may be inconsistent over environments.

For both Maseno and Bungoma, the mean GLS severity scores were higher during long rains seasons (Bungoma 2.5, Maseno 2.3) compared to the short rains seasons (Bungoma 1.7, Maseno 1.8). The differences in severity levels can be attributed to differential seasonal rainfall amounts and relative humidity in these regions (Appendix 6, and 7). In western Kenya, where Maseno and Bungoma are located, the long rains season is usually associated with higher amounts of rainfall and relative humidity compared to short rains season. It has been observed that Warm (22 to 30° C) and humid environmental conditions favor GLS disease development (Bhatia and Munkvold, 2002; Lipps, 1987; Rupe *et al.,* 1982).

5.5 Combined hybrid yield performance under GLS infestation

In terms of grain yield, the overall best performing hybrid was the experimental EH10 (mean 7.5 tons/ha, check means 5.7 tons/ha). In this study, 50 percent of the experimental hybrids performed better than the commercial check hybrids for grain yield under artificial GLS infestation. The superiority of some of the experimental hybrids over commercial checks can be explained by the breeding approach, the ingenious choice of germplasm with emphasis on isolation and renewed contact (Dogget, 1988, Eberhart *et al.,* 1967). Most of these hybrids have at least one or all parental inbred lines developed from local maize landraces from western Kenya (MM Dida, Personal communication). The landraces are likely well adapted to prevailing local conditions in this region. Moreover, the inbred lines used in making the hybrids have also been selected for resistance to prevailing biotic and abiotic constraints such as Maize streak virus, Northern leaf blight, *Pysoderma maydis* and low pH acid soils (MM Dida Personal communication).

The combined use of improved, previously isolated and locally adapted germplasm for maize improvement appears to be very promising. This breeding concept is not new, M. N. Harrison used it to develop the first successful Kenyan maize hybrid 611 (Darrah *et al.*, 1972) which was the predecessor of the legendary hybrid 614D, commonly grown in the highlands of Eastern Africa.

5.6 Other Secondary Traits

With exception of Bungoma Long Rains 2008 data, there were significant differences in plant stand count (Tables 3.3, 3.4 and 3.5). Generally, commercial check hybrids had higher stand counts compared to experimental hybrids. These checks seed were dressed with Raxil® (Bayer CropScience) that may have protected these from soil borne pathogens and insects pests.

There were no significant differences for both days to flowering and silking for Bungoma SR2007 data (Table 3.4), However, there were significant differences in these traits for Maseno SR 2007 and Bungoma LR 2008 (Tables 3.3 and 3.5). The non significant difference of these traits in Bungoma SR season may be attributed to drought stress that was experienced during this season (Appendix 6). The genetic differences between maize genotypes are often not easily detected under drought stress (Banzinger *et al*., 2000).

There also significant differences in plant and ear aspects for all the seasons and sites (Tables 3.3, 3.4 and 3.5). These are secondary traits that are highly correlated to seed yield in maize. Therefore, as expected the hybrids with lower aspect scores had higher seed yields and those with higher aspect scores had lower yields.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

(i) Experimental hybrid EH10 showed the most consistent high yields. On average, it yielded 32% higher than commercial checks.

(ii) This study confirmed that CIMMYT maize inbred lines CML388 and CML389 are very resistant to gray leaf spot. Whereas, CML 384 and CML312 are tolerant.

(iii) A three-way cross experimental hybrid EH9 comprising of CML389 and CML388 was the most resistant to *Cercospora zeae-maydis.*

(iv) There may be at least 2 major genes that condition resistance to GLS in CIMMYT maize inbred line, CML389. These resistance genes exhibit dominance effects and show complementary epistatic interactions.

(iv) GLS tolerance in CML312 appears to be under control of quantitative genes.

(v) There is a high positive correlation between GLS lesion length and severity ratings. Therefore, any of these can be reliably used in assessing maize response to *Cercospora zeae-maydis.*

6.2 Recommendations

1. The inbred lines CML389 and CML388 are highly resistant to gray leaf spot and may be good sources of GLS resistance genes. These inbred lines were developed by CIMMYT Zimbabwe maize breeding programme and are adapted to mid altitudes. The fact that these lines were also selected for maize streak virus and highland rust resistance (*Puccinia sorghi* L.); they are highly recommended for use in breeding varieties adapted to mid altitudes and highlands of western Kenya.

2. If the higher yield performance of some experimental hybrids such as EH10 can be replicated over several sites and seasons, they can be recommended for submission for National Performance Trials (NPT) conducted by Kenya Plant Health Inspectorate Services (KEPHIS).

6.3 Recommendations for further Research

1. In this study, the F_2 segregation pattern suggested that inbred line CML389 may have at least 2 major dominant genes conferring resistance to *Cercospora zeae-maydis*. It has also been postulated that other CIMMYT lines may have the same resistance genes. Therefore, there is a need for further research to verify this postulation.

2. The dominant GLS resistance genes in CML389 appear to be very stable and confer high levels of resistance to F_1 hybrids. Therefore, there is a need to map these genes and tag them with DNA makers, to enable molecular marker assisted breeding.

REFERENCES

Aastveit, A.H., and Aastveit K. (1993). Effects of genotype-environment interactions on genetic correlations. *Theor. Appl. Genet.* **86:**1007–1013.

Adipala, E., Lipps P.E., and Madden L. V. (1993). Reaction of maize cultivars from Uganda to *Exserohilum turcicum. Phytopath* **83:**217-223.

Allard, R.W. (1999). Principles of plant breeding 2nd ed. John Wiley and Sons, New York.

Anonymous. (2008). CIMMYT (online).June 2009. Available ***HTTP:/www.cimmyt.org/english/wps/obtain_seed/germplas.htm.***

Ayers, J.E., Johnson M.W., and Hill R.R. (1984). Identifying resistance to grey leaf spot In: Proc.Maize Sorghum Res.Conf., 39th Am. Seed Trade Assoc. Washington, DC. pp157-175.

Banziger, M., Edmeades G.O., Beck D., and Bellon M. (2000). Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice. Mexico,D.F.:CIMMYT.

Banziger, M., and Vivek, B.S. (1997). *Fieldbook*: Software for managing a maize breeding programme. **CIMMYT, Mexico**

Beckman, P. M., and Payne, G.A. (1982). External growth, penetration, and development of *Cercospora zeae-maydis* in corn leaves. *Phytopath.* **72:**810-815.

Beckman, P. M., and Payne, G.A. (1983). Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. *Phytopath.* **73:**286-289.

Bernardo, R., (2002). Breeding for Quantitative Traits in Plants. *Stemma Press*, Woodbury, MN. USA. pp 212, 259.

Bhatia, A., Munkvold G.P. (2002). Relationships of environmental and cultural factors with severity of gray leaf spot in maize. *Plant Dis.* **86**:1127-1133

Bigirwa, G., Pratt, R.C., Adipala, E., and Lipps, P.E. (2001a). Assessment of gray leaf spot and stem borer incidence and severity on maize in Uganda. In *African Crop Science Conference Proceedings,* Casablanca, Morocco.11-14 Oct.1999.*Vol.*469-474.

Bigirwa, G., Pratt, R.C., Adipala, E., and Lipps P.E. (2001b). Farming components responsible for gray leaf spot disease severity in Districts of contrasting Incidence. Pages 85-87. In: *Proc.Seventh Eastern and Southern Africa Maize Conf.* Nairobi, Kenya, 11-15 February 2001.

Bigirwa, G., Kyetere D.T, Imanywoha, J.B., Okanya, S. (1999). Responses of maize genotypes to gray leaf spot in Uganda. Pages 35-36.In:*Proc.Sixth Eastern and Southern Africa Maize Conf.Maize Production Technology for the Future: Challenges and Opportunities.* Addis Ababa Ethiopia, 21-25 September 1998.

Bosque-Perez, N.A., Buddenhagen, I.W., Olojede, S.O. (1998). Effect of Maize Streak Virus disease on the growth and yield of maize as influenced by varietal resistance level and plant stage at time of challenge. *Euphytica* **101:**307-317.

Bubeck, D. M., Goodman, M.M., Beavis, W.D., and Grant, D. (1993). Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Sci.* **33:**838-847.

Carson, M.L., Goodman, M.M., Williamson, M.S., Haralambus, V., and Nyanapah, J.O. (1997). Pathogen variability as a potential cause of genotype x environment interaction in maize grey leaf spot trials (abstr.). *Phytopath.* **87**:15

Coates, S.T. and White, D.G. (1998). Inheritance of resistance to gray leaf spot in crosses involving selected resistant inbred lines of corn. *Phytopath* **88**:872-982

Darrah, L.L., Eberhart, S.A., and Penny, L.H. (1972). A maize breeding methods study in Kenya. *Crop Sci* **12**:605-608

Derera, J., Tongoona, P., Pixley, K.V., Vivek, B., Laing, M.D,, Rij, N.C. (2008). Gene Action Controlling Gray Leaf spot Resistance in South African Maize Germplasm. *Crop Sci.,* **48:**93-98.

Devries, J. and Toenniessen, G. (2001). Securing the Harvest. *Biotechnology, Breeding and Seed systems for African crops*, 109 pp.

Doggett, H. (1988). Sorghum. *Tropical Agriculture Series, ed.* 2nd. Longman.

Donahue, P. J., Stromberg, E.L., and Meyers, S.L. (1991). Inheritance of reaction to gray leaf spot in maize inbreds. *Crop Sci.* **31**:926-931

Eberhart, S. A., Harrison, M.N., and Ogada, F. (1967). A comprehensive breeding system. *Theor. Appl. Genet*. **37**: 169-174

Elwinger, G. F, Johnson, M.W., Hill R.R., and Ayers, J.E. (1990). Inheritance of resistance to gray leaf spot of corn. *Crop Sci.* **30**:350-358

Falconer, D.S. (1964). Introduction to Quantitative Genetics. *2. Ed. New York*: Ronald Press. 365p.

FAO (1988). Information Network on Post-Harvest Operations (INPhO), Maize in human nutrition. FAO, Rome.

Geraldi, I.O., J.B.Miranda Filho, Vencovsky, R. (1985). Estimates of Genetic Parameters for Tassel Characters. In Maize (*Zea Mays* L.) and Breeding Perspectives. *Maydica* **30**:1- 14.

Gevers, H.O., and Lake, J.K., and Hohls, T. (1994). Diallel cross analysis of resistance to gray leaf spot in maize. *Plant Dis.* **78**:379-383

Gordon, S.G., Michael, B., Inge, M., Hans, O.G., Patrick, E.L., and Richard, C.P. (2004). Linkage of Molecular Markers to Cercospora zea-maydis Resistance in Maize. *Crop Sci.* **44**:628-636

Gordon, S.G., Lipps, P.E., and Pratt, R.C. (2006). Heritability and components of resistance to *Cercospora zeae-maydis* derived from maize inbred VO613Y. *Phytopath* **96:**593-598

Hallauer, A.R., Miranda Filho, J.B. (1995). Quantitative Genetics, *In* Maize Breeding. *2nd. Ed. Ames: Iowa State University Press*, 468p.

Hallauer, A.R., and Miranda Filho, J.B. (1988). Quantitative Genetics in Maize Breeding, *2nd Edition. Iowa State University Press. Ames USA*. pp 64-67, 102-107, 150, 281-284, 292-294.

Hilty, J. W., Hadden, C.H., and Garden, F.T. (1979). Response of maize hybrids and inbred lines to gray leaf spot disease and the effects on yield in Tennessee. *Plant Dis. Rep.* **63**:515-518.

Hohls. T., Shanahan, P.E., Clarke, G.P.Y., and Gevers, H.O. (1995). Genetic analyses of grey leaf spot infection of maize of maize in a single location 12 x 12 diallel. *S. Afr. J. Plant Soil* **12**: 133-139.

Horsfall, J. G., and Barratt, R.W. (1945). An improved grading system for measuring plant disease (abs) *Phytopath* **35**:655.

Huff, C.A., Ayers, J.E., and Hill, R.R. (1988). Inheritance of resistance in corn (*Zea mays*) to gray leaf spot. *Phytopath* **78:**790-794

Ininda, J., Danson, J., Lagat, M., Wei, Ying., Ajanga and Odongo, O.M. (2007). The Use of simple sequence repeats markers to study genetic diversity in maize genotypes resistant to gray leaf spot disease. *African J. Biotech* **l6**:1623-1628.

James, C. W. (1968). Crop Assessment. In *Plant Pathologists Pocket book.* 130-143 CMI SEC ED. Cambrian News Ltd. Queen Street, Aberystwyth Wales.

Jaetzold, R., and Schmidt, H. (1982). Farm management handbook of Kenya. **Vol. II /A. and II / B.** Ministry of Agriculture, Nairobi.

Jenco, J.H. (1995). Epidemiology of *Cercospora Zea maydis* on *Zea mays* in Iowa. Msc Thesis. Iowa State University, Ames.

Latterell, F. M., and Rossi, A.E. (1983). Gray leaf spot of maize: A disease on the move. *Plant Dis.* 67: 842-847.

Levy, Y. and Pataky, J.K. (1992). Epidemiology of northern leaf blight on sweet corn. *Parasitica* **20**:53-66.

Lipps, P.E. (1987). Gray leaf spot epiphytotic in Ohio corn. *Plant Dis.* **71**:281

Manh, N. Q. (1977). Inheritance of resistance to gray leaf spot in maize. *M.S. thesis*. Virginia Polytechnic Institute, Blacksburg, VA.

Mantel, S., V. Engelen, V.W.P. (1997). The impact of land degradation on food productivity. Case studies of Uruguay, Argentina and Kenya, Volume 1, **Volume 1**: main report.

McGee, D.C. (1988). Maize diseases: A reference source for seed technologists. *American Phytopathological Society*, St. Paul, MN.

Menkir, A., and Ayodele, M. (2005). Genetic Analysis of Resistance to Gray Leaf Spot of Midaltitude Maize Inbred Lines. *Crop sci.* **45**:163-170

Nelson P.T., Jines, M.P., Goodman, M.M. (2006). Selecting among available, elite tropical maize inbreds for use in long-term temperate breeding. *Maydica* **51**:255-262.

Ngwira, P.N.,Pixley, K.P., Devries,J.D.,and Kanaventi, C.C(1998). Major maize disease problems and farmer's varietal preferences in Malawi. In *Proceedings of the sixth Eastern and Southern Africa Regional Maize Conference,* held in Addis Ababa, 21-25 Sept., 1998.

Nutter, F.W., Teng, P.S., and Royer, M.H. (1993). Terms and concepts for yield, crop loss and disease thresholds. *Plant Dis.*77:211-215.

Paterniani, E. (1981). Influence of tassel size on ear placement in maize (*Zea mays* L.). *Maydica,* **26:** 85-91.

Patterson, H.D., and William, E.R. (1976). A new class of resolvable incomplete block designs. *Biometrica* **63**:83-89

Perkins, J.M., Smith, D.R., Kinsey, J.G., and Dowden, D.C. (1995). Prevalence and control of gray leaf spot. Pages 177-185.In *Proc.Ann.Conf.III.Maize Breeders School 31*, Illinois, Urbana.

Pixley, K.V. (1997). CIMMYT mid-altitude maize breeding program. In: *Jewell, D.C., Pixley, K.V., Banzinger, M., Waddington, S.R., Varughese, G., Zambezi, B.T.and Mekuria.1996.M (eds)* CIMMYT-Harare. *Annual Report,* 1996-97.CIMMYT, Harare, Zimbabwe.

Pratt R.C., Lipps, P.E, Bigirwa, G., Kyetere, D.T. (2000). Germplasm enhancement through cooperative research and breeding using elite tropical and US corn belt maize germplasm. *African Crop Sc.* **8**:345-353

Rupe, J. C., Siegel, M.R., and Hartman, J.R. (1982). Influence of environment and plant maturity of gray leaf spot of corn caused by *Cercospora zeae-maydis*. *Phytopath* **72**:1587-1591

Saghai Maroof,M.A., Van Scoyoc, S.W., and Yu,Y.G.(1993). Gray leaf spot disease of maize: Rating Methodology and inbred line evaluation. *Plant Dis.*77:583-587

Schechert, A.W., Welz, H.G., and Geiger, H.H. (1999). QTL for resistance to *Setosphaeria turcica* in tropical African maize. *Crop sci.* **39:** 514-523.

Saleh, G.B., Abdullah., and Anuar, R. (2002). Performance, heterosis and heritability in selected tropical maize single, double and three-way cross hybrids. J. *Agric Sci,* **138:** 21-28.

Simmonds, N.W. (1985). A plant breeder's perspective of durable resistance. *FAO Plant Prot.* Bull. **33(1):**13–17.

Slopeck, S. W. (1989). An improved method of estimating percent leaf area diseased using a 1 to 5 disease scale. *Can. J. Plant Pathol.* **11**:381-387.

Smith, D.R., and White, D.G. (1987). Diseases of corn. In: Corn and corn improvement monograph no.18, 3rd edition in the series AGRONOMY. *American Society of Agronomy, Crop sci. Society of America* and *Soil Science Society of America.* Madison, Wisconsin, USA. pp 687-749.

Solomonovitz, S., Levy, Y., and Pataky, J.K. (1992). Yield loss of sweet corn cultivars in response to defoliation and to infections by Exserohilum turcicum. *Phytoparasitica.* **20:**113-121.

Stromberg, E.L., and Donahue, P.J. (1986). Hybrid performance of yield losses associated with gray leaf spot disease.p.92-104.In *Proc.41st corn sorghum Res.Conf.American Seed Trade Assoc.,* Washington, DC.

Tehon, L.R., and Daniels, E. (1925). Notes on the parasitic fungi of Illinois. *Mycologia* **17**:240 249.

Thompson, D.L., Berquist, R.R., Payne, G.A., Bowma, D.T., and Goodman, M.M. (1987). Inheritance of resistance to gray leaf spot in maize. *Crop Sci.* **27**:243-246

Thorson, P. R. (1993). Relative humidity and the development of *Cercospora zeae-maydis.* M.S.thesis.Iowa State Univ, Ames.

Thornson, P. R., and Martison, C.A. (1993). Development and survival of *Cercospora zeae-maydis* germlings in different relative humidity environments. *Phytopath.* **83:**153-157.

Twumasi-Afriyie, S., Wolde, L., Mduruma, Z., Ombhakho, G., Kyetere, D., Manirakiza, A., and Ngaboyisonga, C. (2001). Infusion, development and improvement of highland maize germplasm in Eastern Africa. *Seventh Eastern and Southern Africa Regional Maize Conference,* 80-84.

Ulrich, J.F., Hawk, J.A., and Caroll, R.B. (1990). Diallel analysis of maize inbreds for resistance to gray leaf spot. *Crop Sci.* **30**: 1198-1200.

Vencovsky, R. (1978). Herança Quantitativa. In: *Paterniani, E.; Viégas, P. (Ed.)* Melhoramento e produção do milho. Campinas: Fundação Cargill.

Vencovsky, R., Barriga, P. (1992). Genética Biométrica No Fitomelhoramento. *Ribeirão Preto*: Sbg, 496p.

Ward, J.M.J. and Nowell, D.C (1994). Epidemiology and management of grey leafspot disease, a new disease of maize in South Africa. Tenth South African maize Breeding Symposium.1994.In: press Vanderplank J.E (1993).*Plant Disease*; Epidemics and control. Academic Press New York. pp 349.

Ward, J.M.J. (1996). Epidemiology of grey leafspot disease. A new disease of maize in South Africa. University of Natal, Pietermaritzburg, 3200, South Africa.

Ward, J.M.J., Laing, M.D., and Cairns, A.L.P. (1997). Management practices to reduce gray leaf spot of maize. *Crop sci.* **37**:1257-1262.

Ward, J.M. and Nowell, D.C, (1998). Integrated management for the control of maize gray leaf spot. *Integrated Management Review* **3**:1-12

Ward, J. M. J., Stromberg, E.L., Nowell, D.C., and Nutter, F.W.Jr. (1999). Gray leaf spot: A disease of global importance in maize production. *Plant Dis.* **83**: 884-895.