

Genetic Response of Selected Maize Genotypes to Gray Leaf Spot (*Cercospora zeina* L.) Infestation

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Received: May 19, 2023

Accepted: May 25, 2024

Online Published: June 15, 2024

doi:10.5539/jas.v16n7p140

URL: <https://doi.org/10.5539/jas.v16n7p140>

Abstract

Gray leaf spot (GLS) caused by *Cercospora zea-maydis* and *Cercospora zeina* is one of the most yield limiting diseases of maize globally. Yield losses of up to 60 percent in susceptible genotypes are not uncommon. The objectives of the study were to evaluate the response of diverse maize genotypes to Gray leafspot infestation in western Kenya; determine the genetics of GLS resistance in maize inbred lines CML312, CML389 and to evaluate the relationship between GLS assessment methods, severity and lesion length. 13 maize inbred lines, 2F₁ hybrids, and F₂ populations of crosses MSN21 and CML389 or CML312 were evaluated under artificial GLS infestation during the 2007/08 seasons at Maseno university. Among the inbred lines, MSN21 was the most susceptible to GLS and had the highest disease severity rating. The inbred line CML389 and their F₁ hybrids showed high levels of GLS resistance. CML312 and CML384 showed tolerance to GLS. Correlation between the lesion length and severity ratings was positive and highly significant ($r = 0.9$; $P < 0.001$), suggesting that both could be used in disease damage assessment. The frequency distribution of severity data for the F₂ 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 F₂ 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.

Keywords: maize, gray leafspot, genotypes, genetic response, resistance, tolerance

1. Introduction

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 & van Engelen, 1997). It is wholly produced under rainfed 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.

Cercospora zeina has been reported as the predominant species associated with Gray leaf spot (GLS) in Africa (Meise et al., 2009; Okori et al., 2015; Dave Berger, Personal Communication) and 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 worldwide 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 in Kenya, Zimbabwe, Cameroon and Zaire in the 1995/96 cropping season (Ward et al., 1999). The disease increased dramatically between 1991 and 1997 in Malawi (Ngwira et al., 1998). Since then, GLS has become an important disease of maize throughout most of eastern and southern Africa where the incidence and its severity has been increasing (Pixley, 1997; Tembo & Pixley, 1999).

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 leading to build-up of inoculum reservoirs and GLS

incidence (Gevers et al., 1994; Gevers et al., 1994; Pratt et al., 1997). 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 & Rossi, 1983; Stromberg & Donahue, 1986; McGee, 1988). Changes in the production systems and planting of susceptible varieties pose great danger to the use of newly released commercial maize hybrids in Africa. There is therefore need for maize breeders to breed hybrids with genetic resistance to GLS (Vieira et al., 2009). The effectiveness of this strategy has been demonstrated in the United States (US) and in Africa where GLS losses have been reported (Menkir & Ayodele, 2005; Derera et al., 2008). Concerted effort on breeding for gray leaf spot resistance is required for Eastern and Southern Africa region (Devries & Toeniesen, 2001). Host genetic resistance has been suggested to be the most appropriate method of reducing yield losses due to GLS in smallholder farms in Africa (Menkir, 2005).

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). Losses associated with GLS are greatest when photosynthetic tissues are blighted and prematurely killed prior to grain fill (Donahue et al., 1991). Host resistance is the most effective and cost-efficient means of managing GLS and preventing leaf blighting (Graham et al., 1993; Coates & White, 1994). However, no commercial hybrids with adequate resistance are currently available in Kenya, as they have not been improved for resistance to GLS and therefore resistant variety development should be a priority. Hybrids such as H624 and H511 usually develop high levels of GLS disease (Bigirwa et al., 2001; Ininda et al., 2007). 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.

Previous studies have suggested that resistance to GLS in maize could be due to quantitative factors or a single gene (Gevers et al., 1994). Inheritance studies have indicated that resistance to gray leaf spot is governed by a large number of genes of small effect, with the prevalence of additive effects. These QTL may be determined by using RFLP markers (Saghai Maroof et al., 1996), microsatellites (Juliatti et al., 2009; Asea et al., 2009), and by SNP (Pozar et al., 2009). Other studies have shown that host resistance to gray leaf spot is regulated by a small number of quantitative loci, with five or more genes involved, which are inherited additively (Saghai-Maroof et al., 1996). Such disease progress rate reducing polygenic resistance increases the level and stability of resistance (Dagne et al., 2008). GLS resistance is not very complex and could be evaluated effectively using inbreds *per se* (Thompson et al., 1987). Therefore, a study elucidating the genetics of GLS resistance in specific inbred lines may be necessary in order to formulate appropriate breeding strategies. There is also the need to introgress GLS resistance genes into elite breeding lines.

This study therefore aimed at evaluating the response of diverse maize inbred lines under Gray leaf spot (GLS) infestation in western Kenya; to determine the genetics of GLS resistance in two inbred lines (CML312 and CML389); and to study the inheritance of GLS resistance/tolerance in CML389 and CML312 as well as to evaluate the relationship between different GLS assessment methods (severity and lesion length).

2. Materials and Methods

2.1 Site Description

Field experiments were conducted at Maseno University, 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, texture and classified as dystric nitisols (Jaetzold & Schmidt, 1982). It experiences mean minimum and maximum temperatures of 15.4 and 29.9 °C respectively with an average annual rainfall of 1250 mm. This site has a bimodal type of rainfall where the first peak falls between April and August (Long rains) and the second peak between September and December (Short rains). The short rains season (September to December), however, are sometimes unreliable.

2.1.1 Experiment I: Inbred Lines and F₁ Hybrid Screening for GLS

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

2.1.2 Plant Materials

The maize inbred lines screened were obtained from either CIMMYT or Maseno University. The list of materials evaluated is in (Table 1). In 2007, five inbred lines and two F₁ hybrids were evaluated while in 2008, eleven inbred lines and one three-way cross hybrid were evaluated. The experiments were conducted in a randomized

block design with three replications. Inbred lines and F₁ 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 F₁ hybrids were obtained by crossing MSN21 and CML 389 or CML 312.

Table 1. List of inbred lines and F₁ hybrids screened for GLS at Maseno in 2007 and 2008 long rains seasons

Entry	Material	Source/Status	GLS response status
1	CML389*¶	CIMMYT	Resistant
2	CML312*¶	CIMMYT	Tolerant
3	CML384*	CIMMYT	Tolerant
4	CML388*	CIMMYT	Resistant
5	MSN21*¶	MASENO	Susceptible
6	MSN21 × CML389F ₁ *	F ₁ HYBRID	Unknown
7	MSN21 × CML312F 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

Note. * Lines/Hybrids evaluated 2007; ¶ Lines/Hybrids evaluated 2008.

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

2.1.3 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 four 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 P₂O₅ 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.

2.1.4 Inoculation

The pathogen, *C. zea-maydis*, was artificially inoculated using infected leaves collected in previous years from infected maize fields showing distinct GLS symptoms. The infected dried leaves were ground into powder with a hammer mill and stored in paper bags at a temperature of 4 °C. The pulverized leaf was dusted into the whorls of the leaves of the plants where it remained long enough to permit spore germination. The inoculation was done twice under dew conditions with a ten-day interval starting from the 8-leaf stage of the plant to ensure adequate infection. Approximately 3 g of GLS inoculum was placed in the whorl of each plant to augment natural infestation.

2.1.5 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 six mid leaves with fully developed lesions of at least 5 randomly selected plants per plot (Plate 1).

2.2 Experiment II: F₂ Hybrid Evaluation for GLS Response

2.2.1 Plant Materials

The F₁ hybrids of MSN21 × CML389 and MSN21 × CML312 were advanced to F₂ generation by self-pollination of individual plants. This was done at Maseno during the long rains season of 2008. A randomized block design experiment in three replications was conducted. The F₂ seeds of (MSN21 × CML312) were planted on 4 rows, 5 meters long, and MSN21W × CML 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.

2.2.2 Agronomic Practices

All agronomic practices were done in similar way as described in section 2.1.3. GLS inoculation was done in a similar way as previously described in section 2.1.4.

2.2.3 GLS Severity Assessments

A total of 66 F₂ surviving plants of the MSN21 × CML312 were evaluated for GLS severity towards physiological maturity on a scale of 1-5, as outlined in Section 2.1.6. Ratings were on individual plant basis. A total of 184 surviving plants of the MSN21 × CML389 F₂ populations were evaluated for GLS severity on a scale of 1 to 5. In addition, fully developed GLS lesion lengths were measured, on at least 5 middle leaves per plant. Lesion length measurement was done only on the MSN21 × CML389 F₂ population which appeared interesting based on segregation pattern of severity ratings.

2.3 Data Analysis

For each F₂ population, individual plant disease severity scores were plotted into frequency distribution diagrams. Mean lesion length for each individual F₂ plant of MSN21 × CML389 population were also plotted. Pearson's correlation analysis was done for the GLS severity scores and lesion length for the MSN21 × CML389 F₂ population. Based on the shape of frequency distribution of the MSN21 × CML389 F₂ 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. Results

3.1 Inbred Lines and F₁ Hybrid Screening for GLS at Maseno During the Long Rains Season of 2007

The overall mean GLS score for inbreds and F₁ hybrids evaluated at Maseno was 2.0 (Table 2). GLS severity scores ranged from 1.0 to 4.5. Inbred lines CML389 and CML388 did not show any signs of GLS attack (Plate 2) and were both rated with severity score of 1.0. The lines CML312 and CML384 had GLS severity scores of 2.8 and 2.5, respectively (Table 2). 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 2, Plate 4). The F₁ hybrid of MSN21 and CML389 had a severity score of 1.0 and was clean just like the latter parental line (CML389). The F₁ hybrid of MSN21 and CML312 had a severity rating of 1.5.

Table 2. GLS severity scores and flowering data for inbred lines and F₁ hybrids and at Maseno long rains season, 2007

Entry	Inbred Line/F ₁ Hybrid	GLS Severity Rating	DPS	DS
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	MSN21 × CML389 F ₁	1.0	72	75
7	MSN21 × CML312 F ₁	1.5	65	66
Mean		2.0	70.5	73.2
SEM		0.4	2.50	2.50
SD		1.3	66.5	73.3
CV		0.6	8.70	8.40

Note. DPS: Days to pollen shed; DS: Days to mid-silking; SEM: Standard error of mean; SD: Standard deviation, CV: Co-efficient of variation.



Plate 1. Measurement of fully expanded 6th leaf lesions using a ruler



Plate 2. 6th leaf of a resistant maize inbred line CML389 at R3 stage (Grain filling)



Plate 3. 6th leaf of a tolerant maize inbred line CML384 at R3 development stage



Plate 4. 6th leaf of a highly susceptible maize inbred line MSN21 at R3 development stage

3.2 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) 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 0.1 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 and 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. GLS Severity scores for inbred lines and a hybrid at Maseno in the long rains season, 2008

Entry	Inbred Line/Hybrid	Source	GLS Rating	GLS lesion length (cm)
1	MSN21	MASENO	4.0	2.8
2	CML389	CIMMYT	1.0	0.1
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	1.8
SEM			0.3	0.3
SD			1.0	0.9
CV			0.4	0.5

Note. SEM: Standard error of mean; SD: Standard deviation; CV: Coefficient of variation.

3.3 Evaluation of the F₂ Population Response to GLS at Maseno During the Long Rains Season of 2008

3.3.1 Variation in GLS Scores for MSN21 × CML312 F₂ Population

The GLS severity scores for the MSN21 × CML312 F₂ population showed a continuous distribution (Figure 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).

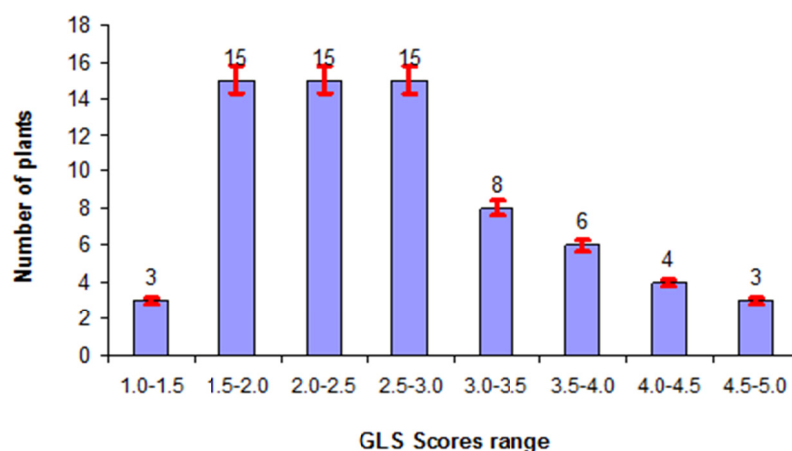


Figure 1. Frequency distribution of GLS severity rating of MSN21 × CML312 F₂ population

3.3.2 Variation in GLS Severity Rating for MSN21 × CML389 F₂ Populations

The MSN21 × CML389 F₂ population GLS severity ranged from 1 to 4.5. The frequency distribution figure showed two peaks (Figure 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 F₂ population, the distribution in lesion length data also showed a similar topology, with two major peaks (Figure 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 ($P > 0.05$).

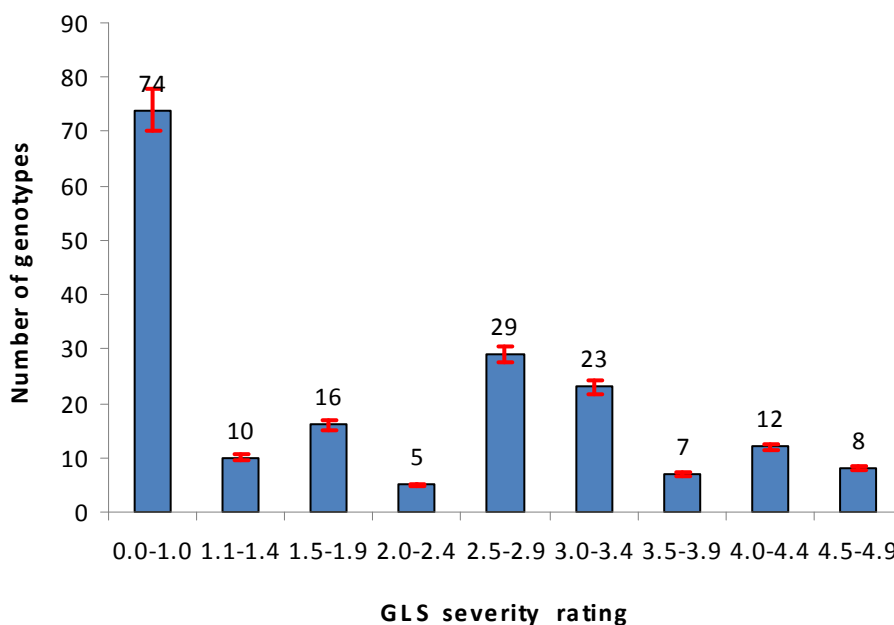


Figure 2. Frequency distribution of GLS severity rating of 184 MSN21 × CML389 F₂ population

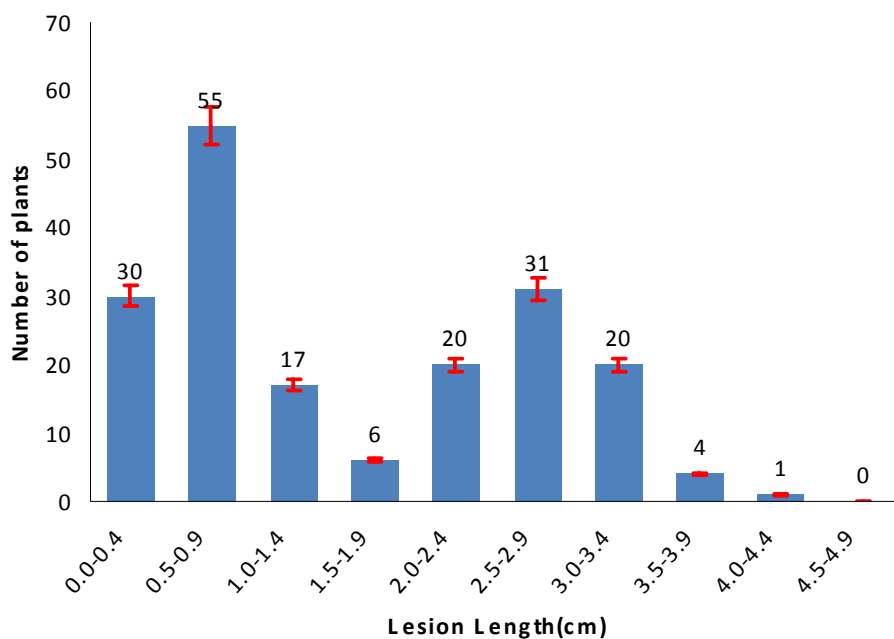


Figure 3. Frequency distribution of lesion length for the MSN21W × CML389 F₂ population

3.3.3 Correlation Between GLS severity and Lesion Length for the MSN21 × CML 389 F₂ Genotypes

Figure 4 is a correlation plot of the GLS lesion length and GLS severity data of an F₂ 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.

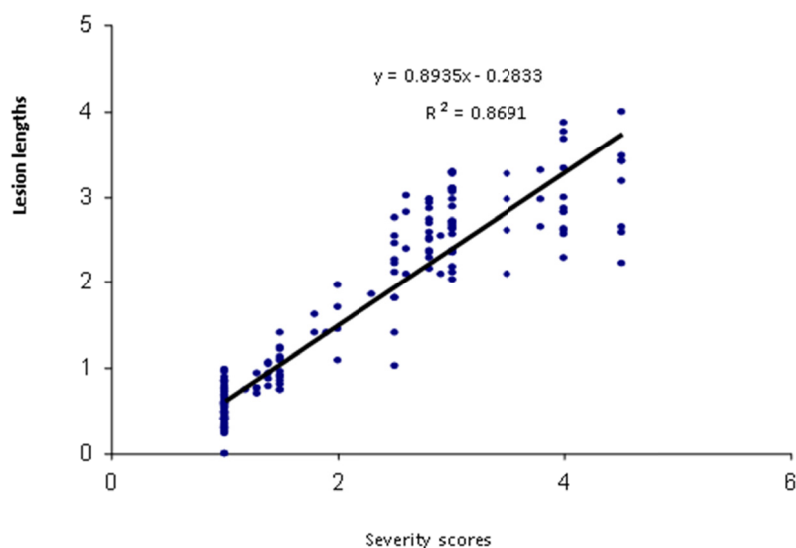


Figure 4. Correlation plot of GLS lesion length and GLS severity for the MSN21 × CML389 F₂ population

4. Discussion

4.1 Inbred Lines and F₁ 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 2 and 3). 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 program 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. This study suggests that these inbred lines may be better utilized in hybrid development for GLS resistance. The possibility of using inbred line information to indicate hybrid performance has been reported (Dagne et al., 2008). Thompson et al. (1987) and Saghai Maroof et al. (1996) reported that GLS resistance could be evaluated effectively using inbreds *per se*. Menkir and Ayodele (2005) reported that inbred lines with high levels of GLS resistance produce hybrids with high levels of GLS resistance.

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 1.0 and lesion length of 0.1 cm). Very highly susceptible line MSN21 (Plate 4) 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. Recent studies addressing the type of gene action in resistance to GLS indicate the predominance of additive gene action (Menkir & Ayodele, 2005; Derera et al., 2008). Nonetheless, they also reported significance of the non-additive effects and suggested that to fully explain the inheritance mode of GLS resistance, the model would have to include the dominance effects (de Brito et al., 2012).

4.2 F₂ Population Response to GLS and Genetics of Resistance in CML 389

GLS response of MSN21 × CML312 F₂ population showed a continuous variation from a severity score of 1.5 to 5.0 (Figure 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 × CML389 F₂ population, the GLS severity and lesion length data clearly showed a segregation pattern with two peaks (Figures 2 and 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 & 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 4). These CIMMYT bred maize lines with EV7992 in their pedigree are very resistant to gray leaf spot.

Table 4. CIMMYT Maize Inbred lines that are resistant to GLS and have population EV7992 in pedigree.

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

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

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

4.3 Correlation Between GLS Severity and Lesion Length

The highly significant and positive correlation ($r = 0.932$, $p < 0.001$) 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* species 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. zeina* 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. Conclusions

This study confirmed that CIMMYT maize inbred lines CML388 and CML389 are resistant to gray leaf spot whereas; CML 384 and CML312 are tolerant. There may be at least 2 major genes that condition resistance to GLS in CIMMYT maize inbred line, CML389. These resistance genes appear to exhibit dominance effects and show complementary epistatic interactions. GLS tolerance in CML312 appears to be under control of quantitative genes.

There is a high positive correlation between GLS lesion length and severity ratings. Therefore, any of these methods can be reliably used in assessing maize response to *Cercospora zeina*.

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 program 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.) suggests that they can be recommended for use in breeding varieties adapted to mid altitudes and highlands of Kenya.

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Acknowledgments

We appreciate the management of Maseno University, Kenya who provided land for the trials for this study.

Authors Contributions

Peter Okoth Mbogo and Prof. Mathews Dida were responsible for designing the study. The former was responsible for drafting the manuscript, collected and analyzed the data. All authors read and approved the final manuscript.

Funding

Not applicable.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Informed Consent

Obtained.

Ethics Approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

Provenance and Peer Review

Not commissioned; externally double-blind peer reviewed.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.

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