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# Resistance of some selected maize genotypes to gray leaf spot disease (*Cercospora zeae-maydis*) in Ethiopia

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## Abstract

Fourteen advanced maize inbred lines and locally adapted hybrid maize (BH-540) as a check were used to investigate their reaction to GLS disease. Field experiments were conducted at Bako National Maize Research Centre in 2015 and 2016 main planting seasons arranged in a randomized complete block design (RCBD) with three replications. Artificial inoculation with *Cercospora zeae-maydis* was conducted by applying dry, ground, infected maize leaves into the whorls of younger maize plants. Data on agronomic and disease parameters (latent period, disease severity, disease incidence and lesion type) were recorded from the middle two rows. From the combined analysis of variance, maize genotypes showed significant differences with reaction to GLS indicating the existence of genetic variability among the selected genotypes. Highly significant differences were also observed among entries for all agronomic parameters in both seasons. Gray leaf spot incidence and severity varied among genotypes and between years. The mean GLS incidence and severity were higher in 2016 than 2015. GLS disease incidence in two years ranged from 35% on Sc22 to 95% on CML-387 and severity ranged from 15% on A-7016 to 75% on CKL05003. Significant differences in epidemic variability were also observed among genotypes and seasons. From the analysis of disease progress curves Logistic model ($R^2$=94.55) better described the disease progress curves than the Gompertz model ($R^2$=91.50). Parents; P6 and P8 had the most desirable quality for the most of agronomic traits whereas P2, P7 and P9 were the best parents for grain yield. Among all inbred lines, P6, P7 and P14 were identified as the most desirable sources of genes for GLS disease resistance. But P6, P7, P8 and P14 were identified as the best genotypes in yield, yield related traits and GLS disease parameters. Thus, these parents were recommended to be used in breeding programs with a purpose of developing high yielder and GLS disease resistant open pollinated varieties. In conclusion this study identified potential and promising high yielding and GLS resistant open pollinated genotypes (CKL05017-B-B, CML-395, CML-387, A-7016, Gutto and Sc22). Therefore, it is recommended that these OPVs can be used by resource poor farmers for direct production where this disease is the most prevalent and/or for further breeding programs in generating novel hybrids for future use.

Keywords*: Zea mays, Cercospora zeae-maydis,* Resistance, Epidemics, Logistic, Gompertz, Models.

## Introduction

Maize (*Zea mays* L) is one of the world's most widely cultivated crops, providing food and animal feed as well as being a source of biofuel. According to the Food and Agriculture Organization of the United Nations FAOSTAT (2016) 1012 million metric tons of maize was produced in 2016 worldwide, making it the world's highest yielding grain crop. The world population is expected to reach 9.1 billion by 2050. This means that food production would have to increase by 70% to feed the world. However, the world's total arable land has reached close to its maximal usage (Holden *et al*., 2004; Masuka *et al*., 2017). Hence, increasing land productivity through the make use of highly productive crops like maize is very crucial in feeding the world population.

Maize which is a $C_4$-plant and a model organism in plant genetics is also one of the most important cereals broadly adapted worldwide (Christian *et al*., 2012). By 2020 maize production in industrialized and developing countries will surpass that of wheat and rice and it has increased since 1997 by 45% at the global level and by 72% in developing countries (FAO, 2016). Within the developing world the demand of maize for food will be the greatest in sub-Saharan Africa (40 million tons) followed by Latin America (30 million

tons), and then South and Southeast Asia (25 million tons). This can be achieved when high yielding and disease resistant maize varieties are used by farmers and commercial maize producers (FAO, 2016; Ali and Yan, 2012).

In Ethiopia it is grown in the lowlands, the mid-altitudes and the highland regions. It is an important field crop in terms of area coverage, production and utilization for food and feed purposes. However, maize varieties mostly grown in the highlands (altitude = 1,700–2,400 m.a.s.l) of Ethiopia are local cultivars. They are low yielding, vulnerable to biotic and a biotic constraints and also exhibit undesirable agronomic performances such as late maturity and susceptibility to root and stalk lodging (EARO, 2000; Legesse *et al*., 2012). Thus, enhancement of maize production and productivity can be achieved through identification of potentially superior inbred line combinations in the form of hybrids (Mosisa *et al*., 2007). Currently the average national yield of maize is very low under small scale farmers of Ethiopia 3.7 t/ha (CSA 2017). Foliar diseases of maze are the number one factors in contributing in the reduction of maize production and productivity across the world (Berger *et al*., 2014; Masuka *et al*., 2017).

Gray leaf spot (GLS) caused by *Cercospora zeae-maydis* (Tehon and Daniels, 1925) is one of the necrotrophic and polycyclic foliar diseases of maize that poses a serious problem to maize production in Sub Saharan Africa. This pathogen causes intense water loss from the plant thereby leading to severe blighting of the leaves and reduced photosynthesis. This eventually leads to undersized ears, low grain yield and premature death of maize plants. Severe blighting of the upper eight or nine leaves that contribute 75 to 90% of the photosynthates for grain fill may lead to stalk weakening or even infectious stalk rot diseases leading to premature stalk death and lodging (Lipps *et al.,* 1996: Ward *et al.*. 1999 ; Poland *et al.,* 2009).

GLS has been rigorous in recurrence and distribution and has led to economic yield losses: over 60% in western Kenya (Kinyua *et al.,* 2010), 10 to 60% in Tanzania (Sibiya *et al* , 2012), 60% in South Africa (Vivek *et al.,* 2001) and 49.5% in Ethiopia (Meseret, 2013). One approach to combat GLS would be to apply chemical fungicides. However, the application of fungicides is not economical for maize production (Ward *et al.*. 1997: Derera *et al.*. 2008) since the resource poor small scale farmers lack the financial means to apply fungicides and other management options to control the disease. Moreover, the farmers obtain low yields and most of them own small parcels of land.

Warm, humid conditions (22 to 30°C with periods of prolonged fog or dew) favor the development of GLS. Fields planted along rivers or other low-lying areas are most likely to experience severe GLS epidemics (Ward et al., 1999; Asea *et al.,* 2002). Widespread adoption of conservation tillage practices to reduce soil erosion, with the associated higher levels of residue remaining on the soil surface, and continuous planting of predominantly susceptible maize cultivars helped the spread of *C. zeae-maydis* and increases the incidence of GLS.

In tropical environments where little conservation tillage is practiced, the use of susceptible genotypes in environments highly conducive to the disease has resulted in rapid spread of GLS where it was previously unknown (Derera *et al*., 2008). The rapidity with which GLS became endemic was probably exacerbated by production of multiple crops of maize in the same area during the same year, combined with the wide spread use of stover (dry stalks) as mulch for other adjacent crops (e.g., bananas), in complex agro ecosystems characteristic of sub-Saharan Africa ( De Nazareno et al., 1993; Gordon *et al*., 2006; Worku *et al*., 2012).

Under severe disease pressure a toxin called cercosporin is produced which causes extensive blighting of the upper leaves resulting in significant yield losses (Lipps, 1987). This disease is most severe and damaging during high relative humidity and prolonged late-season rains (Beckman and Payne, 1983). The extent of the damage has been found to be dependent on the hybrid affected and prevailing environmental conditions (Ward et al., 1999). Increased incidence of GLS in Africa has been associated with continuous cultivation of maize, and use of susceptible maize cultivars (Gevers *et al*., 1994; De Nazareno *et al*., 1993; Dagne *et al*., 2008).

GLS disease epidemics have been managed conventionally through deep tillage to burry previous maize residue, fungicide application, and field hygiene (Ward *et al*., 1997). However, these measures have not been efficient in the management of GLS (Bigirwa *et al*., 2001). A reduction in conservation tillage would have to be universally adopted to have an economic impact on GLS epidemics (Lipps *et al*., 1996). Fungicide application is costly and not practical in most operations for the resource-poor farmers. Most hybrids currently in production in Ethiopia are susceptible to GLS (Tewabech *et al*., 2012). Availability and adoption of resistant hybrids would provide a cost-effective means of controlling GLS for recourse poor farmers.

Efforts to manage GLS have focused on genetic resistance. Screening efforts have revealed diverse sources of resistance (Herman *et al*., 2011). Many

researchers examined the genetic basis of resistance by generation means and diallel analyses (Carson *et al*., 2002; Zhang *et al*., 2014) and molecular mapping (Berger et al., 2014). Resistance to *C. zeae maydis* is quantitative (partial resistance) and additive effects are predominant, but dominance is significant as well. Complete resistance to *C. zeae-maydis* has not been identified (Sibiya *et al*., 2013; Berger *et al*., 2014).

In maize, hybrid breeding remains the method of choice for attaining maximum genetic gain from the effects of heterosis. Nevertheless, identification of parental inbred lines leading to superior hybrid combinations is a crucial factor (Hallauer *et al*., 2010; Badu *et al*., 2013). Such activities using conventional breeding methods are expensive and time consuming. Furthermore, larger numbers of possible hybrid combinations to be produced from a relatively small number of inbred lines, render the evaluation of all possible combinations unfeasible (Bello *et al*., 2012 ; Badu *et al*., 2014). Use of the advanced generation grain instead of F1 (F2 in the case of OPVs) seed resulted in 32% average yield loss for hybrids, 16% yield loss for top crosses and 5% yield loss for OPVs.

Setimela *et al.* ( 2017) conducted a simple break-even yield analyses to identify scenarios where use of OPV rather than hybrid varieties might be economically advantageous and they concluded that in some farming systems, particularly where yield levels are low (e.g. below 1.5 t ha-1) and hybrid seed and fertilizer prices are high relative to price of grain, highest return to investment may result from use of improved OPV seed, which is cheaper than hybrid seed and can be recycled with little or no yield loss. Open-pollinated varieties (OPVs) still represent a significant proportion of the maize seed system in many countries of sub- Saharan Africa (Masuka *et al*., 2017). Improved OPVs are multiple line synthetics and can often be recycled for up to 3 yr without a significant loss in yield, but they yield approximately 20 to 25% less than hybrids (Pixley, 2006). Maintaining OPVs without yield loss depends on their degree of isolation from pollen contamination by seed admixture with other varieties, conditions that are often difficult for smallholder farmers to control (Assefa *et al*., 2008 ; Badu *et al.,* 2015).

The improved OPVs are particularly advantageous if the money saved by using OPV instead of hybrid seed is used to purchase additional inputs such as fertilizer, herbicide or hiring additional labor. Although use of OPV instead of hybrid seed is a backward step in terms of expected grain yield, improved OPVs represent an economical option for resource-poor maize farmers in marginal areas or when hybrid seed and fertilizer prices are high relative to price of grain. Hence, searching for locally adapted and improved OPVs remains very crucial for resource poor farmers in small scale farming systems in Ethiopia. Presently GLS is becoming one of the major constraints of maize production in maize belt areas of the country causing a yield reduction of up to 49.5% (Meseret, 2013). Genetic resistance is the most economic and effective means of reducing yield losses caused by this disease. However, breeding genotypes for disease resistance is a continuous process and plant pathologists and/or breeders need to add new effective sources to their breeding materials. In view of its expansion, seriousness, and potential destructiveness of this disease, it is necessary to develop tolerant and /or resistant genotypes for resource poor farmers. Therefore, the objectives of this study were to develop GLS resistant OPVs for production or breeding materials and evaluate effects of gray leaf spot on yield and yield related parameters of maize. This paper presents results of field experiments conducted at Bako, Ethiopia, in 2015 and 2016 main cropping seasons based on agronomic, plant breeding and genetics and epidemiological data.

## Materials and Methods

Description of the Study Area: Field experiments were conducted at Bako National Maize Research Center, western Ethiopia during the main seasons (May to November) in 2015 and 2016. Bako is situated at an altitude of 1650 meters above sea level, 9°06' north latitude and 37°09' east longitude. Average annual rainfall at this location is 1246 mm. The rainy season lasts from April to October, with maximum rainfall in July and August. The soil type of the center is reddish brown Nitosols, according to FAO/UNESCO soil classification, which were developed from basalt parent materials. The soil is deep-weathered, well-drained, slightly acidic in reaction, clay to sandy clay loam at the surface, low in available P, total N, organic matter and available water holding capacity (Wakene, 2001).

Experimental Materials: Fourteen advanced maize inbred lines and a locally adapted commercial hybrid (BH-540) were obtained from the Ethiopian National Maize Research Co-ordination Center, Bako and International Maize and Wheat Improvement Centre (CIMMYT) were used for this study to develop GLS resistant OPV (open pollinated) or breeding materials. The selection of these genotypes was based on *per se* performance evaluation for reaction to GLS disease and general agronomic performance. Inbred lines 143-5-i, Gutto, A-7016 and SC-22, CML-197, CML-202, CML-387 and CML-395 were obtained from Bako

national maize research center, Ethiopia.

The Ethiopian national maize program inbred lines were locally developed from the materials introduced during 1960s and 1970s from East Africa and CIMMYT-Mexico. Based on their reaction to GLS disease, the lines were categorized as resistant, moderately resistant, moderately susceptible and susceptible. This categorization will be based on the basis of disease severity ratings using a 1-5 scale (Roane *et al.,* 1974) where 1.0–2.0=resistant; 2.1-2.5=moderately resistant; 2.6–3.0=moderately susceptible; and >3.0 susceptible.

Field Experimental Procedures: Fourteen advanced maize inbred lines and locally adopted hybrid (BH-540) replicated three times were used to investigate their resistance to GLS. These materials were evaluated in a randomized complete block design with three replications. Each plot consisted of four rows of 5.1 m length, spaced at 0.75 m apart. Plots were hand sown with high density and later thinned to a final plant density of 44,444 plants per hectare. Fertilization ($P_2O_5$ and nitrogen at the rate of 100 kg ha-1 each) and standard agronomic management practices recommended for the area will be applied.

The pathogen, *C. zeae-maydis* was collected in previous years from infected maize fields showing distinct GLS symptoms. Infected and dried maize leaves were ground into powder and stored in paper bags at a temperature of 4 $^{o}$C. The pulverized leaves were dusted into the whorls of the leaves of the plants where it remained long enough to permit spore germination at 6 weeks and 8 weeks after planting. The inoculation was done twice under dew conditions with a seven-day interval starting from the 6-leaf stage of the plant to ensure adequate infection.

Measurements of Disease Parameters:

Disease Severity: Severity was recorded on ten randomly tagged plants per plot. It was assessed using the 1-5 standard disease scoring scale recommended by Roan et al. (1974), where 1 = very slightly infected, one or two restricted lesion on lower leaves or trace. 2= slightly to moderate infection on lower leaves, a few scatter lesions on lower leaves. 3= abundant lesions on lower leaves, a few on middle leaves. 4 = abundant lesions on lower and middle leaves extending to upper leaves. 5= abundant lesions on all leaves, plant may be prematurely killed by blight. The rating was made at seven-day interval starting at about 2-3% infection on the lower leaves of the susceptible variety, BH-540. Then the severity grades were converted into percentage severity index (PSI) for analysis using the formula Wheeler (1969) as follows:

$$PSI = \frac{\text{Numerical rating} \times 100}{\text{Total no of plants observed} \times \text{maximum rating}}$$

Area under the disease progress curve (AUDPC): The disease severity scores were used to calculate infection rate and AUDPC for each treatment. AUDPC was calculated with the formula suggested by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n-1} 0.5\left(x_{i+1} + x_i\right)\left(t_{i+1} - t_i\right)$$

Where, $x_i$ is the cumulative disease severity expressed as a proportion at the $i^{th}$ observation, $t_i$ is the time (days after sowing) at the $i^{th}$ observation and n is total number of observations. Since common rust severity was expressed in percent and time (t) in days, AUDPC values were expressed in %-days (Wilcoxson *et al*., 1975). AUDPC values were then used in analysis of variance (ANOVA) to compare amounts of disease among plots with different treatments. Logistic equation, ln [(Y/1-Y)], (Van der Plank, 1963; Madden *et al*., 2007) was used for estimation of infection rate from each treatment.

Disease parameters were taken from the two middle rows. Latent period was measured as the number of days from disease inoculation to the date on which a clear grey leaf spot lesion were observed on 50% of the plants in a plot. Since the first inoculation alone may not ensure enough infection, the second inoculation was considered for calculating the latent period. To accommodate any variations and peculiarities of disease progression attributed to the stage of plant infection and prevailing weather conditions, disease severity was recorded four times at intervals of seven days using a widely-used 1-5 scale. Rating was started when obvious genotypic differences for GLS reaction become apparent and continued until the leaves start to senesce (14 DAI to 84 DAI). Disease incidence was recorded at the third week after silking as the ratio of infected leaves to the total number of leaves on a particular plant and expressed as a percentage (Ringer and Grybusks, 1995).

Lesion type was also scored on ten randomly selected plants at the third week after silking on a scale of 1-4, where 1=flecks to chlorotic lesion, 2=chlorotic lesion with some necrosis, 3=chlorotic lesion with considerable necrosis and 4=susceptible or wilted lesion (Pratt *et al*., 1997). Plant height was measured in centimeters two weeks after pollen shed had ceased, as the distance from the soil surface to the base of lowest tassel branch. Days to maturity were recorded as the number of days from emergence to when 50% of the plants in a plot formed a black layer at the

tip of each kernel on the ears. Ears harvested from each plot were shelled and weighed to determine the percentage moisture content.

Statistical Analysis: Percent disease incidence data covering a wide range of values; were transformed using arcsine transformation to satisfy the assumption of analysis of variance (Steel and Torrie, 1980). Analyses of variances was performed using SAS software (SAS Software Inc., 2000) for each year separately and combined over years after testing homogeneity of error variances using Bartlett's test. Pearson correlation coefficient was calculated between pairs of traits using over years means to determine

## Results and Discussion

Reaction of maize genotypes to GLS disease under field condition: From the combined analysis of variance, maize genotypes showed significant differences with reaction to GLS indicating the existence of genetic variability among the selected genotypes (Table 1). Highly significant differences were also observed among entries for all agronomic parameters in both seasons (Appendix 1). Severe epidemics of gray leaf spot were noted on susceptible genotypes. There were also significant ($P < 0.05$) differences in the overall mean of gray leaf spot incidence, severity, AUDPC, yield and yield components among different genotypes.

The inbred line P14 was the highest yielding parent with 2.9 t ha-1 followed by P6 with 2.5 t ha-1 while P1 and P2 had the lowest grain yields of 1.6 and 1.7 t ha-1, respectively (Table 3). This implies that P6 and P14 had the highest frequencies of yield favoring alleles as opposed to the other inbred lines. The inbred line P14 (GLS score of 1.65) was immune to GLS while P10 was highly resistant to GLS with score of 2.3. Thus, P6 could be an excellent source of both grain yield and GLS resistance genes while P8 could be an excellent source of grain yield genes. The parental means of these inbred lines also revealed the breeding potential for the characters involved and which could easily be used to discriminate poor lines in future breeding efforts.

Table 2. Mean values of Gray leaf spot disease parameters of 14 genotypes and F1 hybrid evaluated at Bako in 2015 and 2016 main cropping seasons.

| Entry | DFDA | INC | Severity | AUDPC | | NL | LL | LW |
|---|---|---|---|---|---|---|---|---|
| | days | % | 1-5 scale | IPP | Severity | count | cm | cm |
| P1 | 40.00 | 50.00 | 2.40 | 1955.00 | 1275.00 | 1.85 | 4.80 | 0.75 |
| P2 | 45.00 | 55.00 | 2.55 | 1685.60 | 1480.00 | 1.50 | 5.53 | 0.85 |
| P3 | 37.50 | 65.50 | 2.50 | 2425.50 | 1385.00 | 2.70 | 13.75 | 1.50 |
| P4 | 40.50 | 60.50 | 1.50 | 1210.50 | 1425.50 | 1.53 | 7.80 | 0.85 |
| P5 | 35.00 | 60.28 | 2.60 | 2800.00 | 16870.00 | 2.56 | 14.65 | 2.50 |
| P6 | 60.60 | 45.78 | 1.50 | 1264.50 | 1000.50 | 0.25 | 2.70 | 0.58 |
| P7 | 62.00 | 40.00 | 1.55 | 1155.00 | 1250.50 | 0.70 | 6.45 | 0.77 |
| P8 | 50.33 | 39.80 | 1.45 | 1125.00 | 1575.40 | 0.85 | 1.65 | 1.05 |
| P9 | 45.00 | 60.00 | 2.52 | 2460.50 | 1265.50 | 0.75 | 5.70 | 1.85 |
| P10 | 38.00 | 70.60 | 2.60 | 2753.00 | 1750.00 | 1.50 | 13.75 | 1.25 |
| P11 | 30.00 | 75.75 | 3.85 | 5150.50 | 2705.00 | 4.80 | 20.00 | 3.50 |
| P12 | 30.50 | 71.86 | 2.75 | 2280.00 | 1490.00 | 2.00 | 7.30 | 1.80 |
| P13 | 49.00 | 35.00 | 1.20 | 1220.50 | 1858.00 | 3.75 | 13.00 | 0.88 |
| P14 | 65.00 | 34.75 | 1.65 | 900.00 | 1140.50 | 0.50 | 1.58 | 0.56 |
| BH 540 | 27.00 | 72.70 | 3.85 | 2450.00 | 1682.00 | 4.90 | 22.75 | 5.83 |
| Mean | 48.55 | 61.42 | 2.83 | 2103.00 | 1436.00 | 1.82 | 10.50 | 2.00 |
| CV | 18.55 | 20.45 | 14.03 | 22.48 | 9.65 | 18.50 | 20.06 | 13.00 |
| SE(m) | 4.45 | 5.70 | 0.58 | 195.40 | 69.75 | 0.43 | 1.80 | 0.35 |
| F. test | ** | ** | ** | ** | ** | ** | ** | ** |

** = 0.05 and **= 0.01 significant probability level*

DFDA =days of first disease appearance, AUDPC= areas of disease progresses curve, IPP = percent of infected plants per plot,LL lesion length

Gordon *et al*. (2006) also reported GLS resistant maize inbred lines have lower severity scores under field condition followed by higher yield which is in agreement with the present finding a yield reduction of up to 45% was noted from the field experiments in both seasons indicating the potential of this pathogen in limiting maize productivity.

This investigation identified the most promising OPVs. Since the most adopted hybrid maize varieties are becoming susceptible to this foliar disease in western, southern, southwestern and north western parts of the country, this information can be used by plant breeders and pathologists to develop GLS resistant maize varieties which can be used to abate food shortage in the maize belt areas of the country.

Significant differences in epidemic variability were also observed among genotypes and seasons. From the analysis of disease progress curves Logistic model ($R^2$=91.5) better described the disease progress curves than the Gompertz model ($R^2$=90.5) (Table 3). Parents; P6 and P8 had the most desirable quality for most agronomic traits whereas P7 and P8 were the best genotypes for grain yield. Among all inbred lines, P6, P7 and P8 were identified as the most desirable sources of genes for GLS disease resistance. But P6, P7, P8, P10 and P14 were identified as the best genotypes in yield, yield related traits and GLS disease parameters (Table 2).

GLS Disease Incidence: Incidence data indicated significant ($p \leq 0.01$) difference among genotypes at all dates of assessments. Variation in GLS incidence also varied among genotypes and between years. The mean GLS disease incidence in two years ranged from 35% on P13 to 75% on P11. At the first date of disease assessment the mean percentage incidence on susceptible genotypes P11 was 52% and that of the moderately resistant genotypes P6 and P8 were 15 and 25%, respectively (Table 2).

Herman *et al*. (2011) and Derera *et al*. (2008) also indicated the presence of significant differences between early maturing and late maturing maize genotypes on their level of GLS severity scores of maize genotypes. In the case of the resistant inbreed lines fewer numbers of lesions were observed while the severity of the disease is slightly increasing with time, as opposed to the susceptible ones, where the disease severity increases remarkably as time elapses.

Lesion Size: There was a highly significant ($p \leq 0.01$) difference for lesion length among tested genotypes. Similarly Lesion width showed significant differences in both years. The genotypes P1, P2, P10 and P12 had the highest (22.50 cm) lesion length and width (5.20 cm), where the lesion length showed highly ($p \leq 0.01$) significant difference from other maize genotypes. Lesion length of the genotype P8 was the smallest (1.65 cm) and showed significant difference from the genotypes P3, P5, P10, P11 and P13. Similar result was indicated by Dagne *et al.* (2008) where lesion length of genotype CML-395 was 1.50cm. However the result indicated by this author for this inbred line was smaller than the figure observed from the current study. Even if the lesion length of genotypes P6 was the shortest, the lesion width of this genotype was the second widest among all other maize genotypes. Menkir and Ayodele (2005) and Gordon *et al*. (2006) indicated components associated with partial resistance to gray leaf spot of maize include prolonged latent and incubation periods, reduced infection rates, low sporulation and fewer and smaller lesions.

Understanding the genetics and heritability behaviors of components expressing partial resistance has enabled selection of this type of resistance possible in several host–pathogen systems based on component measurements (Gordon *et al*., 2006). However, little has been reported on the components expressing partial resistance to gray leaf spot of maize and possible applications in the selection and breeding of resistant genotypes (Gordon *et al*., 2006). Therefore, the information obtained from this study can be used by plant pathologists and breeders to develop GLS resistant OPVs for resource poor farmers of the country.

Disease progress rates: Disease progress rates calculated from the data taken seven days after the GLS disease symptom development showed significant ($p \leq 0.01$) difference on the maize genotypes. Disease progress rate of genotypes P1, P2, P3, P4, P10, and P11 were 0.04, 0.01, 0.04, 0.02, 0.01 and 0.04 units-days, respectively (Table 4). These results indicated that the disease progressed faster on BH-540(susceptible) and P11, which were 4.5 times faster than the genotype P14. Disease progress rates of the resistant genotypes, namely P6, P7 and P14 showed little increase in rate starting from the time of disease onset onwards, while the susceptible genotypes P1, P2 and P11 showed variability in disease progress rates from time to time, i.e. progress rate increased over time.

The overall data calculated for disease progress rates also showed significant ($p \leq 0.01$) difference among maize genotypes in the final assessment. During the last progress disease assessment, the fastest disease (0.032 units-day$^{-1}$) progress rate was for the genotype P11, which had a significant difference from the other maize genotypes and the slowest (0.0195 units-day$^{-1}$) was for the maize

genotype P14 (Table 6). This result further confirmed the reaction of BH-540 and P14 as susceptible and resistant, respectively, in line with earlier findings. Sibiya *et al*. (2012) indicated the use of genetically resistant maize hybrids is the preferred means of controlling gray leaf spot, caused by *Cercospora zeae-maydis*. One problem faced by maize breeders attempting to breed for resistance to gray leaf spot is the high degree of genotype–environment interactions observed in disease trials causing variability in yielding ability of maize genotypes (Herman et al., 2013).

The relationship between GLS and maize yield is most often summarized as a simple empirical model that describes average crop performances in the presence of this pathogen. Such models may be robust and useful for surveys but their use is usually constrained to the specific conditions under which the model was developed. Changes in production system usually invalidate the relationship. The alternative is to base the relationship on an epidemiological analysis of the pathogen population and a physiological concept of host growth and development. This study provides the knowledge and conceptual basis and discusses the limitations to progress in the development of such models in which the Logistic model ($R^2$ =91.5) described the disease progress curves than the Gompertz ($R^2$=90.5). Gordon et al. (2006) also showed that a host-based assessment of GLS disease is well suited to yield investigations and to multiple pest constraints and that disease is logically related to yield via radiation interceptions and radiation use efficiency.

Table 3. Disease progress rates, intercepts and adjusted coefficients of determination ($r^2$) for GLS of maize.

| | | Logistic [a] | | | Gompertz [a] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| year | Inoculation date/ days after planting | Rate | Intercept | AdjR2 (%) | Rate | Intercept | Adj$R^2$ (%) | $AUDPC_c$ | Final severity (%) |
| 2015 | 10 August 75 | 0.13 | -5.45 | 88.00 | 0.12 | -6.70 | 89.80 | 1250.00 | 15.00 |
| | 17 August 82 | 0.12 | -6.53 | 85.75 | 0.10 | -7.75 | 87.20 | 1375.00 | 20.00 |
| | 25 August 90 | 0.14 | -4.40 | 90.20 | 0.12 | -8.40 | 91.50 | 1665.00 | 35.00 |
| | 2 September 97 | 0.13 | -5.55 | 92.50 | 0.11 | -5.55 | 85.03 | 1700.00 | 45.00 |
| | BH-540 | 0.15 | -6.20 | 90.25 | 0.20 | -5.55 | 88.45 | 2450.00 | 55.00 |
| | LSD[b] | * | * | ** | ns | * | ** | ** | ** |
| 2016 | 20 July 65 | 0.13 | -7.25 | 90.00 | 0.14 | -6.55 | 88.65 | 1480.00 | 10.00 |
| | 27 July 72 | 0.15 | -7.00 | 91.55 | 0.13 | -5.75 | 90.70 | 1575.00 | 25.00 |
| | 4 September 79 | 0.14 | -6.75 | 91.00 | 0.11 | -6.31 | 89.65 | 1870.00 | 40.50 |
| | 11September 86 | 0.15 | -5.58 | 90.50 | 0.12 | -5.50 | 89.50 | 1900.00 | 60.55 |
| | BH-540 | 0.30 | -6.55 | 94.55* | 0.25 | -9.00 | 90.25 | 2860.00 | 72.50 |
| | LSD | * | ** | ** | * | ** | ** | ** | ** |

*ns = not significant,*

*A Model equations were y = 1/(1 + exp (–[a + rt]) for the logistic model and y = exp (–B exp (–kt)) for the Gompertz model, in which r and k represent the rate parameters for logistic and Gompertz models, respectively. All rate and adjusted coefficient of determination values are significant at P ≤ 0.0001.*

*b Cumulative foliar disease severity.*

*C Fisher's least significant difference (P ≤ 0.05) ,BH-540 standard check*

Values were derived by fitting data obtained using the Horsfall–Barratt scale to two growth models, and by calculating mean final percent foliar disease severity, area under the disease progress curve (AUDPC) of 14 genotypes and F1 hybrid due to ten epidemics from field experiments at Bako in 2015 and 2016 main cropping seasons. Carson *et al*., (2002) and Berger *et al.* (2014) also investigated infection by plant pathogens and yield is linked by epidemiological and physiological processes that may be considered as three major functional relationships. Disease severity is determined by a function of the degree of infection, colonization, and damage of host tissues. The amount of host development and growth is a function of disease severity, and yield realization is a function of host development and growth (Kranz *et al*., 1978; Madden *et al*., 2007; Bergua *et al*., 2008).

Area under Disease Progress Curve (AUDPC): Area

under the disease progress curve (AUDPC) showed significant ($p \leq 0.01$) difference among maize genotypes. The analysis of variance (ANOVA) indicated that the highest AUDPC (1650.17%-days) was recorded on the susceptible genotype and the lowest AUDPC (1369.16%-days) was noted on the resistant genotype (Table 4). Higher areas under disease progress curves were recorded on the susceptible than the resistant maize genotypes (Table 3). Previous works at Bako by Dagne *et al.* (2008) indicated genotypes considered as susceptible such as Abobako, BH-540 and Local-M had AUDPC values more than resistant genotypes Kuleni and BH-660 genotypes.

Table 4. E**ff**ect of di**ff**erent maize genotypes on GLS disease severity (%) and AUDPC (%-days) evaluated at Bako in 2015 and 2016 main cropping seasons

| Genotypes | AUDPC (%-days) | Last Severity (%) |
|---|---|---|
| 1.CKL 05019 | 1640.00 | 55.80 |
| 2.CKL05003 | 1950.30 | 75.85* |
| 3.CKL05002-B | 1640.80 | 65.00 |
| 4.POOE-3-2-1-2-1 | 1581.75 | 65.00 |
| 5.POOL-9A-4-4-1-1-1 | 1458.50 | 45.06 |
| 6.CKL05017-B-B | 865.60 | 25.50** |
| 7.CML-197 | 548.00 | 15.25** |
| 8.CML-395 | 847.50 | 20.10** |
| 9.CML-202 | 1598.00 | 42.30 |
| 10.CML-387 | 1412.56 | 36.95 |
| 11.143-5-i | 1456.40 | 45.60 |
| 12.Gutto | 1827.20 | 68.07 |
| 13. A-7016 | 517.10 | 25.00** |
| 14. SC-22 | 340.00 | 15.30** |
| BH-540 | 1862.10 | 72.75* |
| CV% | 8.6 | |
| LSD (5%) | 5.873 | |

** = 0.05 and **= 0.01 significant probability level, AUDPC= areas of disease progresses curve,*

This study also indicated similar result that susceptible genotypes had highest area under disease progress curves. No significant difference was observed between the average scores of the susceptible genotypes: having the highest (1735.22%-days) AUDPC for P6, followed by the AUDPC (1684.31%-days.) for the maize genotypes P8. The AUDPC values for BH-540 and P14 were significantly ($p \leq 0.01$) different from the other genotypes. The AUDPC values for the genotypes P14 were lower by 368.44% - and 317.53% -days than the values for BH-540 and P6, respectively.

Association of GLS Disease Parameters with Yield and Yield Components: Correlation of disease parameters with yield and seed weight was determined for the year 2015 and 2016 (Table 5). There were significantly negative and non-significant correlations between the disease parameters and thousand seed weight in both years. Yield, had weak and non-significant positive correlations with the disease parameters (Table 4) during both years. This indicated that all the parameters effectively measured the disease progress and had a negative effect on yield and seed weight. Gordon *et al*. (2006) also investigated the association of yield and yield components with disease parameters, which included severity, AUDPC, incidence and disease progress rate, using correlation analysis in which incidence, severity, AUDPC and disease progress rate were negatively correlated with yield components regardless of relative yield loss. However, the significant association depended on the maize genotypes correlated with the respective disease parameters.

Mostly non-significant negative associations were observed on the maize genotype, CML-197. The only significant associations were observed on AUDPC ($r = -0.73$) and disease progress rate ($r = -0.75$). On the susceptible variety BH-540, most of the disease parameters were strongly (negatively

or positively) associated with grain yield, relative yield loss, TKW. Severity assessed at 90 DAP had the strongest negative association with maize grain yield (r = -0.81), Relative yield loss (r = 0.80), TKW (r = -0.81) above all other disease parameters (Table 6).

On other susceptible maize genotypes P1, P2, P11, most of the disease parameters were strongly associated with grain yield, relative yield loss. Grain yields of these variety were significantly associated with GLS severity assessed at 90 DAP and AUDPC. The strongest associations were observed between disease progress rates with lesion size (r = -0.83), and followed by grain yield (r = 0.80). On the maize genotypes BH-540, P6 and P14, even if all disease parameters were negatively correlated with yield parameters, associations were mostly non-significant. For the maize variety BH-540, there were strong associations of the maize lesion size in line with GLS severity (r = -0.87), AUDPC (r = -0.88) and disease progress rates (r = -0.87). On the maize variety P14, the only significant association was that of the disease progress rate correlated with the ear size (Table 5). To evaluate the association of maize grain yield with disease parameters, generally, the better estimator of the degree of association was different among the susceptible and moderately resistant genotypes. For instance, disease severity assessed at 90 DAP was strongly associated with yield on susceptible maize genotypes; similarly, the disease progress rate was strongly associated with yield on moderately resistant genotypes (Table 6).

Table 5. Phenotypic correlation coefficients among disease parameter, agronomic traits and grain yield for 14 maize inbred lines evaluated at Bako, Ethiopia in 2015 and 2016 main cropping seasons.

| Traits | Lesion size(cm) | AUDPC (%days) | Disease progress rate | Disease Severity (%) | TKW | Grain yield(kg/ha) |
|---|---|---|---|---|---|---|
| Lesion size(cm) | 0.875 | | | | | |
| AUDPC(%-days) | -0.830 | 0.825 | | | | |
| Disease progress rate | -0.752 | 0.875 | 0.945 | | | |
| Disease severity (%) | -0.750 | -0.540 | -0.645 | 0.875 | | |
| TKW | -0.455 | -0.725 | -0.540 | -0.750 | 0.650 | |
| Grain yield(kg/ha) | -0.680 | -0.760 | -0.565 | -0.850 | 0.975 | 0.850 |

** = 0.05 and **= 0.01 significant probability level*

Modeling the Relationships between Severity and AUDPC with Grain Yield: Regression of GLS severity and AUDPC on grain yield data revealed significant difference as compared to regressions of other disease parameters on yield for all maize genotypes. Therefore, these two parameters (i.e. severity and AUDPC) were used as predictors and grain yield as dependent variable to estimate maize grain yield losses. The severity assessed at the last day revealed better coefficient of determination and showed significant relationship with yield for the maize genotypes BH-540 ($R^2$ = 76.00), P14 ($R^2$ = 68.00) and P6 ($R^2$ = 61.75). However, for the maize genotypes P7 ($R^2$ = 85), P8 ($R^2$ = 80.6) and P6 ($R^2$ = 88.9), AUDPC predicted grain yield losses better than GLS severity because of $R^2$ of AUDPC was higher than that of the $R^2$ for severity.

The regression equations illustrated that for every 1% increase in disease severity assessed on the genotypes at the final day of assessment, there were grain yield losses of 160.0, 120.50, and 45.50 kg $ha^{-1}$ for the maize genotypes P6, P7 and P14, respectively (Figure A, B and C). Similarly, based on the regression equations, for every 1% increase in AUDPC there were 47.0, 35.5 and 10.0 kg $ha^{-1}$ yield losses that were calculated for the genotypes CML-197(susceptible), CML-387(moderately resistant) and CML-395(resistant), respectively (Figure A, B and C).

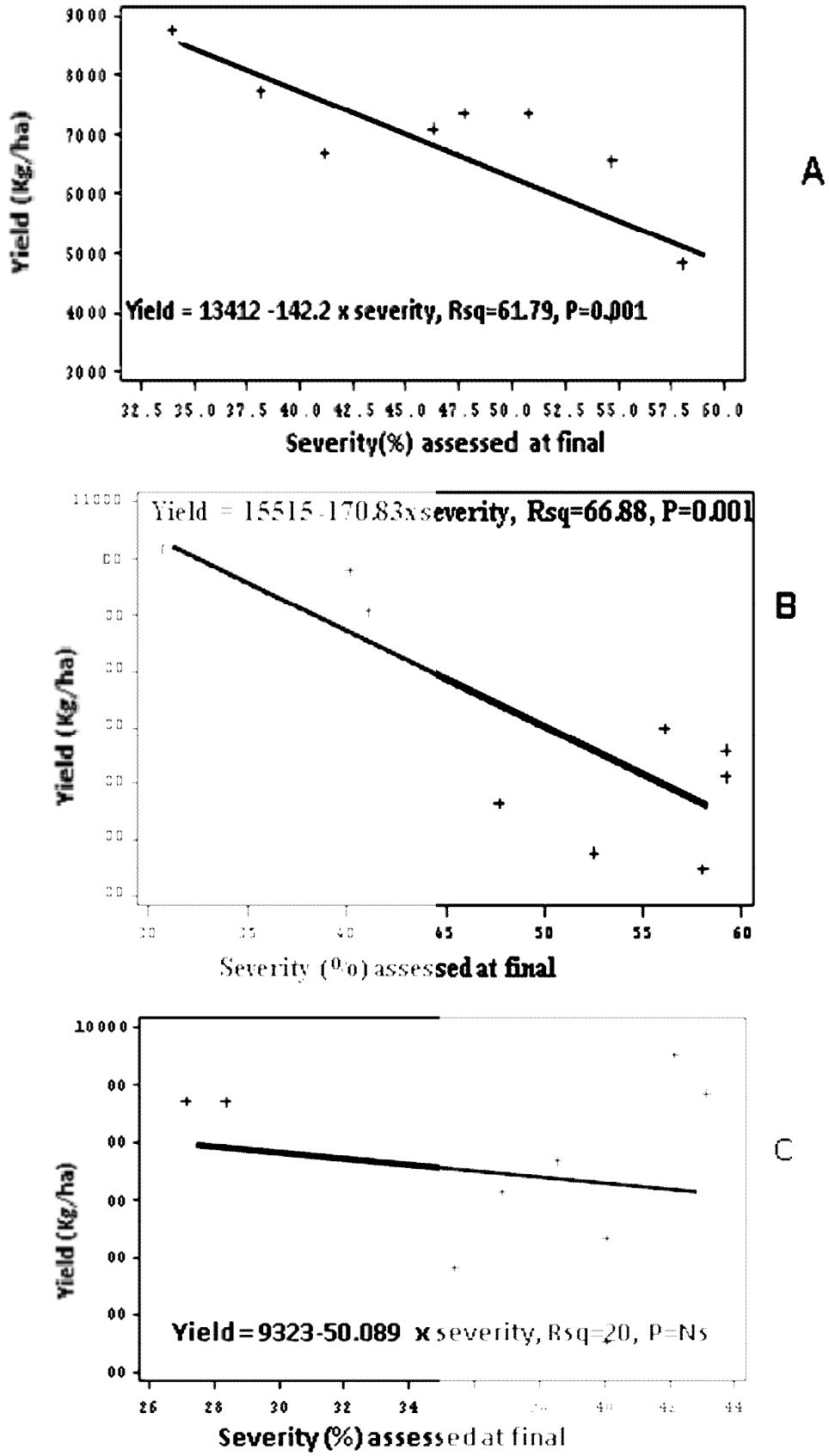


Figure 1. Estimated relationships between maize GLS severities and grain yields at Bako in 2015 and 2016 main cropping seasons (mean values). A, B and C: Severity vs Yield of the maize genotypes CML-197, CML-387 and CML-395, respectively.

## Conclusion

Combined analysis of variance, showed the presence of significant differences with reaction to GLS among the genotypes indicating the existence of genetic variability among the selected maize genotypes. Highly significant differences were also observed among entries for all agronomic parameters in both seasons. Severe epidemics of gray leaf spot were noted on susceptible genotypes. There were also significant differences in the overall mean of gray leaf spot incidence, severity, AUDPC, yield and yield components among different genotypes and components of partial resistance. Inbred lines P6, P7, P8, and P14 showed better GLS disease resistance. Therefore, these parents can be used by resource poor maize growers where this disease is the most prevalent or the information can be utilized by plant pathologist and/or breeders in generating novel GLS resistant hybrids for resource rich and commercial farmers in Ethiopia where this disease is dominantly prevailing.

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Appendix 1. Effect of different maize genotypes on GLS disease progress rate (unit-day$^{-1}$) at Bako in 2015 and 2016 main cropping season.

| Genotype | GLS progress rate | SE of (r) [a] | ($R^2$%) [b] | P |
|---|---|---|---|---|
| 1.CKL 05019 | 0.041 | 0.0025 | 78.20 | .0001 |
| 2.CKL05003 | 0.043 | 0.0025 | 66.70 | .0001 |
| 3.CKL05002-B | 0.010 | 0.0025 | 85.15 | .0001 |
| 4.POOE-3-2-1-2-1 | 0.021 | 0.0025 | 75.10 | .0001 |
| 5.POOL-9A-4-4-1-1-1 | 0.029 | 0.0025 | 64.80 | .0001 |
| 6.CKL05017-B-B | 0.038 | 0.0025 | 88.65 | .0001 |
| 7.CML-197 | 0.030 | 0.0025 | 82.75 | .0001 |
| 8.CML-395 | 0.034 | 0.0025 | 89.25 | .0001 |
| 9.CML-202 | 0.029 | 0.0025 | 88.77 | .0001 |
| 10.CML-387 | 0.016 | 0.0025 | 88.60 | .0001 |
| 11.143-5-i | 0.025 | 0.0025 | 82.89 | .0001 |
| 12.Gutto | 0.026 | 0.0025 | 71.50 | .0001 |
| 13. A-7016 | 0.093 | 0.0025 | 75.01 | .0001 |
| 14. SC-22 | 0.015 | 0.0025 | 77.69 | .0001 |
| BH-540 | 0.018 | 0.0025 | 92.57 | .0001 |
| CV% | | | | |
| LSD (5%) | | | | |

** = 0.05 and **= 0.01 significant probability level*

*CV = coefficient of variation [a]= standard error of main factor [b] = Coefficient of determination or proportion explained by the model, P = Significance probability level of rates when regressed over time*.

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