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Research Article

Screening of Maize Inbred Lines Under Artificial Epiphytotic for Their Reaction to Grey Leaf Spot (*Cercospora zeae-maydis*)

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Abstract

Maize (Zea mays L) is one of the most widely cultivated crops and it is the basis for food security in many developing countries in Africa, and is an important food crop in Ethiopia. Despite of, its importance the crop it is affected by many biotic stresses such as pest and diseases attack. Grey leaf spot, caused by Cercospora Zeae maydis is the most important foliar disease of maize. The disease is characterized by relatively rapid leaf necrosis and premature death of foliage which eventually reduces grain yield. Development of host resistance to this disease can provide an important component of integrated disease management; which is the most effective and practical method of managing maize disease. The study was conducted to evaluate the reaction of maize inbred lines to GLS in the main cropping season during 2020. The inbred lines were obtained from Bako National Maize Research Center, breeding program, and it was arranged using alpha-lattice design with two replications. The inbred lines were evaluated in GLS screening field under artificial inoculation at Bako West Shewa, Ethiopia. Plot based Disease severity scores (1-5 scale) was used to assess at ten days intervals from disease onset to maturity. All the inbred lines showed disease symptom during the season, but the intensity of the diseases differed significantly at (P<0.05) among the inbred lines. Out of 72 genotypes screened for GLS, 20 inbred lines viz. BKL002, BKLOO4, CML 165, MBRC5BCF108-2-3-1, TZMI746, TZMI719, TZMI733, CML547, CML543, CML536, CZLQ1, CZLQ2, CML511, ILO'00E-5-5-3-1-1, 30H83-7-1-3-1-1-1-1, TZMI750, TZMI763, 30H83-7-1-2-1-1-1-#-#, DE-38-Z-126-3-2-2-2-1-1-#, TZMI407-short-#-#-# were identified to be resistant whereas TZMI746 and CML536 inbred lines are suitable candidates for utilization in both grain yield and GLS resistance, thus recommended for inclusion in hybrid development programs.

Keywords

Maize, Resistant, GLS, Inbred Lines

1. Introduction

Maize (Zea mays L) is one of the most widely cultivated crops in the world. It is one of the three most popular cereal crops next to wheat, and rice in the world. Maize is an important cereal crop in Sub-Saharan Africa (SSA) critical for food security as well as a source of income for millions of small-holder farmers [10]. It is the basis for food security in many developing countries in Africa and is an important food crop in Ethiopia [1].

In Ethiopia currently, about 2.5 million ha of land is cov-

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ered by maize with an average production of 10.5 million tons [7]. Maize accounting for 35% of cereals production followed by Wheat, Teff and Sorghum with 19, 18 and 15 percent respectively [3]. The average national yield of maize is very low under small-scale farmers, which is 4.2 t/ha in the country whereas, the world average productivity 6.1 t/ha [6].

Despite of, this global importance the crop it is affected by many biotic stresses such as pest and diseases attack. The major foliar diseases include Turcicum leaf blight (*Exserohilum turcicum* (Pass) Leonard & Suggs), grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels), leaf rust (*Puccinia sorghi* Schr.), maize streak virus disease [4, 14, 2, 9]. Of these diseases affecting maize, particularly grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) is the most important foliar disease causing moderate to severe losses in yield [14]. The disease takes heavy toll during the main season when conditions of relative humidity coupled with low night temperature. 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 [5]. Methods to manage GLS disease include cultural practices, chemical and host plant resistance [11]. Due to its inconsistency with environment and expensiveness, uses of chemical fungicides are not effective.

The most effective and cost-efficient means of managing this disease is the use of host plant resistance. It is, therefore, desirable to identify resistant inbred lines from diverse sources in maize pre-breeding program in order to improve genetic resistance to this foliar disease. Though early research efforts made to identify maize germplasm resistant to the disease and utilizing them for maize breeding program, subsequent study for additional source of maize germplasm should be screened under artificial inoculation to obtain new and stable resistance.

The objective of this study was to select maize inbred lines that are resistant/tolerant to GLS through evaluation from locally developed and adapted maize inbred lines for use in maize improvement program.

2. Materials and Methods

2.1. Description of the Study Area

The experiment was conducted at Bako national maize research center of maize disease nursery field during main season of 2020. The site is located at 9°06' N and 37°09' E and receives the annual rain fall of about 1237 mm and situated at an altitude of 1650 m above sea level, which represent mid altitude sub-humid agro-ecology zone of Ethiopia. It has minimum and maximum average temperature of 15. 6℃ and 30.7℃, respectively. The experiment was conducted in the field under artificial inoculation conditions for evaluation against GLS.

2.2. Description of Experimental Materials and Design

A total of Seventy-two white maize inbred lines were used and arranged in 9x8 alpha lattice design with two replications. Some of the genotypes were developed by BNMRC and the rest are obtained from CIMMYT. Each inbred line was planted in a plot consisting of two rows of 3.6m long spaced at 25 and 75cm between plants and rows, respectively. Maize inbred lines SC-22 was used as susceptible check. Nitrogen (N2) and diammonium phosphate (P_2O_5) fertilizers were applied at the recommended rates of 92 kg/ha and 69 kg/ha, respectively. All agronomic management practices for the area were applied as per the recommendations.

2.3. Inoculum Preparation and Inoculation

Inoculum of *C. zeae maydis* was prepared a year before experimentation by collecting from heavily infected maize fields showing distinct GLS symptoms. The infected leaves were dried under shade and crushed/grounded in to mill about the coarseness of wheat bran and stored in paper bags at a temperature of 4℃ until inoculation date. The pulverized leaves then dusted in the whorls of the plants according to Dagne [4] by placing a pinch of leaf mill when plant attains 6-8 leaf stage during moisten environments in order to retain long enough to permit spore germination. Some of the genotypes were developed by BNMRC and the rest are adopted from CIMMYT. A second inoculation was made ten days later after the first inoculation to ensure adequate infection.

2.4. Assessment of Disease Reaction

The GLS disease symptom was visually assessed in the field two weeks after artificial inoculation on a plot basis from the two rows. Data collected included date first disease appeared, disease incidence, disease severity, and other agronomic traits including plant height (cm) and grain yield (t/ha). The progress of severity of the disease on each inbred lines was quantified at ten days intervals starting from onset of disease until dent stages and the highest or final severity value of each inbred lines were used for statistical analysis. Disease incidence was measured as percent of infected plants per total plant per plot. Disease severity was rated based on 1-5 scoring scale (CIMMYT, 1985); where 1=no disease symptoms, 2=moderate lesion below the leaf subtending the ear, 3=heavy infestation on and below the leaf subtending the ear with few lesions above it, 4=severe lesion on all but the uppermost leaves which may have a few lesions, 5=all leaves dead. The categorization of the disease reactions was made on the basis of disease severity ratings using a 1-5 scale [12] with some modifications, where; 1.0–2.0=Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6-3.0= Susceptible (S), and >3.0 Highly susceptible (HS).

2.5. Statistical Data Analysis

Data were analyzed using PROC GLM of SAS version 9.2 [13]. Mean separation was performed to compare treatment means using LSD-test at 5% level of significance.

3. Results and Discussion

A total of seventy-two Maize inbred lines were screened for resistance to GLS. The mean disease severity and yield results indicated significant ($P < 0.05$) variation among the inbred lines for GLS resistance (Table 1).

Table 1. *Mean GLS severity, yield and other agronomic traits of 72 Maize inbred lines evaluated under artificial inoculation during 2020 main cropping season at Bako.*

Entry No	Pedigree	Plant Height (Cm)	Ear Height (Cm)	Anthesis date (days)	Yield t/ha	Disease Severity Scale (1-5)	Resistance Category
1	142-1-e	229.8	141.0	90.7	2.95	2.3	MR
2	F 7215	163.1	77.5	92.9	1.20	3.1	SS
3	BKLOO1	188.2	89.0	86.2	2.36	2.6	SS
4	BKL002	109.4	43.7	80.6	2.01	1.4	RR
5	BKLOO3	136.6	79.0	89.3	0.91	2.2	MR
6	BKLOO4	157.4	69.2	89.6	1.39	1.8	RR
7	CML 161	113.7	59.1	84.8	1.10	2.2	MR
8	CML 165	92.9	37.8	89.1	1.08	1.5	RR
9	CML 312BK	181.3	87.7	86.7	2.25	2.2	MR
10	CML 144	141.4	64.2	88.6	1.11	4.1	HS
11	CML 202	140.2	66.1	89.2	0.45	3.0	SS
12	CML 159	133.2	57.1	87.3	1.27	4.2	HS
13	A7033	195.1	110.5	81.3	3.21	3.9	HS
14	SC 22	152.3	84.8	85.2	1.81	3.7	HS
15	CML 395	109.4	60.2	83.6	0.71	4.8	HS
16	CML 204	158.3	74.1	84.7	0.93	3.8	HS
17	KUBA/GUDAC1…	137.9	61.6	80.6	0.96	2.1	MR
18	124b(113)	191.7	84.0	81.3	4.03	4.4	HS
19	CML 176/KULEN...						
20	CML 334	145.5	77.9	89.4	0.92	3.4	SS
21	MBRC5BCF108-2-3-1	130.6	53.9	83.9	1.30	1.7	RR
22	Zim line/kat#24						
23	CML 445	109.3	38.6	88.2	0.81	3.1	SS
24	TZMI723	152.6	61.4	91.9	1.09	2.7	SS
25	TZMI730	167.4	99.2	85.4	3.69	2.4	MR
26	TZMI746	148.1	84.8	90.3	2.30	1.3	RR
27	TZMI719	138.1	70.0	90.4	0.78	1.7	RR
28	TZMI733	135.0	66.8	92.5	1.27	1.6	RR
29	CML547	163.2	62.4	82.3	1.18	1.8	RR
30	CML444						

Entry No	Pedigree	Plant Height (Cm)	Ear Height (Cm)	Anthesis date (days)	Yield t/ha	Disease Severity Scale (1-5)	Resistance Category
31	CML543	134.2	64.1	87.7	0.37	1.7	RR
32	CML536	164.7	74.6	89.2	2.65	1.5	RR
33	124-b(109)	184.9	88.6	81.6	2.62	4.1	HS
34	CZLQ1	146.9	70.8	81.6	1.42	2.0	RR
35	CZLQ2	157.2	85.1	89.7	1.05	1.5	RR
36	CZLQ3	97.6	39.3	87.3	0.99	2.1	MR
37	CZLQ5	113.7	46.4	83.8	1.91	4.6	HS
38	CML511	126.9	54.4	87.1	1.49	2.0	RR
39	TZMI745	166.2	91.0	85.2	2.41	3.3	SS
40	ILO'00E-5-5-3-1-1	145.3	63.6	86.6	1.75	1.9	RR
41	35B-190-O-S-10-2-1-2-2	184.4	96.7	88.0	1.63	3.6	HS
42	30H83-7-1-3-1-1-1-1	153.7	67.0	82.3	2.21	1.5	RR
43	30H83-7-1-5-1-1-1-1	180.7	85.4	88.8	1.72	3.1	SS
44	30H83-7-3-4-1-1-1	205.8	85.7	79.7	5.47	1.5	RR
45	TZMI750	210.5	82.2	88.9	1.42	1.5	RR
46	TZMI751	117.2	65.4	86.7	1.15	3.8	HS
47	TZMI753						
48	TZMI754	121.1	70.0	93.8	1.19	3.0	SS
49	TZMI755	125.7	76.4	93.3	1.33	4.0	HS
50	TZMI759	104.5	58.9	83.9	1.25	3.2	SS
51	TZMI760	160.8	97.7	88.2	1.83	2.5	MR
52	TZMI761						
53	TZMI763	162.8	63.0	93.1	0.18	1.4	RR
54	TZMI764	120.7	57.1	89.3	1.70	4.7	HS
55	TZMI766	149.5	86.3	85.0	3.45	3.6	HS
56	CML498	88.0	28.7	90.9	0.10	2.3	MR
57	CML539	103.1	42.9	85.3	0.42	4.6	HS
58	CML488						
59	TZMI717	162.8	75.2	94.2	0.68	3.2	SS
60	GIBE-1-178-2-1-2-1-#-#	143.3	69.6	89.2	0.17	3.3	SS
61	DE-38-Z-126-3-2-2-2-2-#	139.7	63.6	79.7	1.99	2.2	MR
62	30H83-7-1-5-1-1-1-1-#	216.4	101.7	87.0	1.34	3.6	HS
63	CKL05019-#	176.3	88.5	86.2	3.22	2.8	SS
64	CML 197	175.0	103.8	86.4	3.92	4.4	HS
65	30H83-7-1-2-1-1-1-#-#	170.6	63.3	81.8	3.39	1.8	RR
66	DE-38-Z-126-3-2-2-2-1-1-#	153.3	77.6	83.1	1.95	1.6	RR
67	TZMI407-short-#-#-#	119.3	51.3	88.1	0.82	1.5	RR

Entry No	Pedigree	Plant Height (Cm)	Ear Height (Cm)	Anthesis date (days)	Yield t/ha	Disease Severity Scale (1-5)	Resistance Category
68	Kuleni C 1-0080-2-4-1-2-1-#-#	160.5	76.8	88.0	0.79	2.4	MR
69	30G 19F2-54-1-1-1-#-#	147.1	70.9	87.9	0.80	2.4	MR
70	KULENI 320-2-3-1-1-2-1-1-#-#	179.5	93.3	83.0	2.28	2.9	SS
71	[CML444/DRB-F2-60-1-1-1-BBB//[LZ956441/LZ966205]-B-3-4-4-B-5-B*7-#-#-#	174.8	104.0	90.8	1.55	2.5	MR
72	30H83-56-1-1-3-1-1-#-#						
	Mean	149.8	72.6	87.2	1.60	2.7	
	LSD_0.05	47.7	23.9	4.2	1.18	1.1	
	CV	16.0	16.5	2.3	36.66	20.7	
	pValue	0.001	0.001	0.001	0.001	0.001	
	Min	88.7	28.3	79.9	0.10	1.3	
	Max	230.3	140.7	94.3	4.03	4.8	

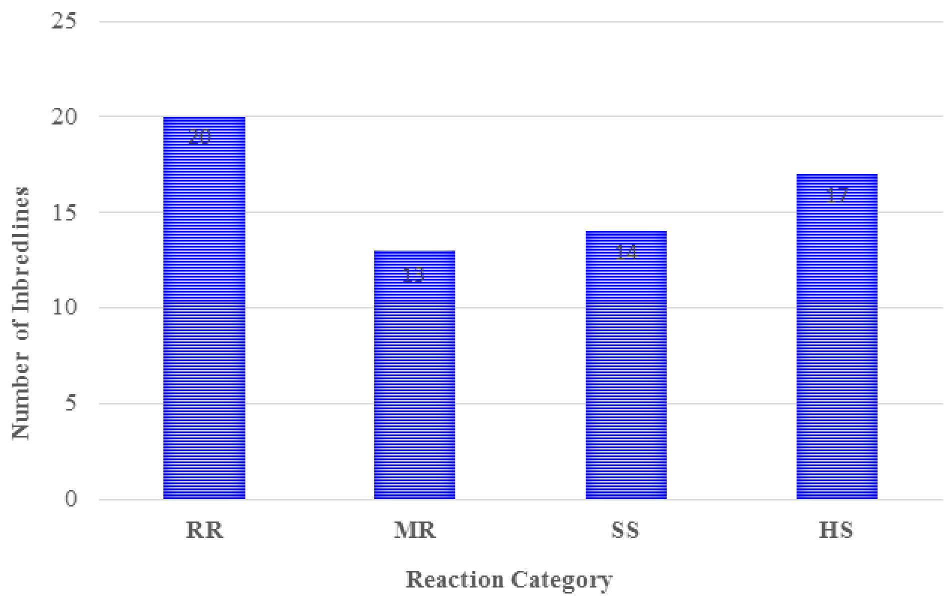

Figure 1. *Number of normal maize inbred lines with Resistant (RR), Moderately Resistant (RM), Susceptible (SS) & highly susceptible (HS).*

Disease severity ranged from 1.5 to 4.8 for GLS disease were recorded. Inbred lines with mean severity values of < 2 were categorized under resistant (RR) /tolerant to GLS. Whereas inbred lines with mean severity values ranging from 2.1- 2.5 categorized as moderately resistant (RM), from 2.6-3.5 as susceptible (SS), and those with severity value > 3.5 were considered as highly susceptible (HS) to GLS. Accordingly, 20 inbred lines were resistant/tolerant, 13 inbred lines moderately resistant and 14 were Susceptible and 17 were highly susceptible to GLS disease. Those inbred lines, which have showed resistance/tolerance were compared to the susceptible and resistant checks SC 22 and 142-1-e respectively. Best selected inbred lines could be used as source material of GLS resistance for use in maize resistance breeding programs.

Out of seventy-two genotypes screened for GLS, 20 inbred lines viz. BKL002, BKLOO4, CML 165, MBRC5BCF108-2-3-1, TZMI746, TZMI719, TZMI733, CML547, CML543, CML536, CZLQ1, CZLQ2, CML511, ILO'00E-5-5-3-1-1, 30H83-7-1-3-1-1-1-1, TZMI750, TZMI763, 30H83-7-1-2-1-1-1-#-#, DE-38-Z-126-3-2-2-2-1-1-#, TZMI407-short-#-#-# were identified to be resistant whereas (CML 144, CML 159, A7033, SC 22, CML 395, CML 204, 124b(113), 124-b(109), CZLQ5, 35B-190-O-S-10-2-1-2-2, TZMI751, TZMI755, TZMI764, TZMI766, CML539, 30H83-7-1-5-1-1-1-1-#, CML 197 were identified to be susceptible. From maize inbred lines that are categorized to be resistance 30H83-7-1-2-1-1-1-#-#, TZMI746 and CML536 inbred lines are suitable candidates for utilization in both grain yield and GLS resistance, thus recommended for inclusion in hybrid development programs. This result is similar with research conducted at Kenya [8].

4. Conclusion

From the above result twenty inbred lines were showed resistant to GLS (*C. Zeae maydis*) under artificial inoculation. It is recommended that maize inbred line that showed to be resistant in the study would better be repeated under controlled environment in order to accurately confirm the extent of their resistant to GLS disease. Additionally, to identify the gene or genes causing the resistance and add them to cultivars with desirable agronomic traits, it would be preferable to employ molecular techniques. In addition, the investigation's promising lines with high yield and other agronomic traits can be used to sustainably increase the yield of maize in disease-endemic areas. As an alternative, the aforementioned promising genotypes could be employed as parents in hybridization to give current high yielding cultivars that have been adapted the gene for resistance Grey leaf spot.

Abbreviations

GLS: Grey Leaf Spot
BNMRC: Bako National Maize Research Center
FAO: Food and Agriculture Organization
CSA: Central Statistical Agency
SSA: Sub-Saharan Africa
LSD: Least Significance Difference

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Author Contributions

Mideksa Dida is the sole author. The author read and approved the final manuscript.

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Abate, T. S., S. H. Bekele, M. Abebe, W. Dagne, K. Yilma, et al., 2015. Factors that transformed maize productivity in Ethiopia. Food science, 7: 965-981.

[2] Berger, K, Maryke C, Jeanne N, Felix M, Frederik J, Pangirayi T, and Alexander A. 2014. Mapping QTL conferring resistance in maize to grey leaf spot disease caused by Cercospora Zeina. BMC Genetics, 15: 60.

[3] Central Statistical Agency (CSA). (2020). Annual Agricultural Sample Survey Area and production of major crops. The Federal Democratic Republic of Ethiopia, CSA, Addis Ababa, Ethiopia.

[4] Dagne W, Habtamu Z, Demissew A, and Harjit S. 2008. The Combining Ability of Maize Inbred Lines for Grain Yield and Reaction to Grey Leaf Spot Disease. East Afr. J. of Sci. 2(2): 135- 145. http://dx.doi.org/10.4314/eajsci.v2i2.40373

[5] Dhami, N. B., Kim, S., Paudel, A., Shrestha, J., & Rijal, T. R. (2015). A review on threat of gray leaf spot disease of maize in Asia. *Journal of Maize Research and Development*, *1*(1), 71–85. https://doi.org/10.3126/jmrd.v1i1.14245

[6] FAOSTAT. (Food and Agriculture Organization of United Nations) (2020). FAOSTAT [Online]. Available at http://dx.doi.org/10.5194/essd-2020-202-rc3 Accessed August.

[7] FAOSTAT. (Food and Agriculture Organization of United Nations), 2021. FAOSTAT [Online]. Available at http://faostat3.fao.org (Accessed March, 2022).

[8] LAGAT, N., 2022, April. Evaluation of maize (Zea mays) inbred lines for grey leaf spot (Cercospora Zeae-maydis) resistance under artificial inoculation in Kenya. In *Egerton University International Conference*.

[9] Masuka, B., G. N. Atlin, M. Olsen, C. Magorokosho, M. Labuschagne, J. Crossa et al. 2017. Gains in maize genetic improvement in eastern and southern Africa: I. CIMMYT hybrid breeding pipeline. Crop Science. 57: 1–12.

[10] Prasanna *et al*. (2020) Prasanna B, Suresh LM, Mwatuni F, Beyene Y, Makumbi D, Gowda M, Molnar T. Maize lethal necrosis (MLN): efforts toward containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa. *Virus Research*. 2020; 282: 197943. http://doi.org/10.1016/j.virusres.2020.197943

[11] Pratt RC, Gordon K, Lipps P, Asea G, Bigrawa G, Pixley K. 2003. Use of IPM in the control of multiple disease of mazie. Afr. Crop. Sci. J., 11, 189-198. http://dx.doi.org/10.4314/acsj.v11i3.27570

[12] Roane CW, Harison RL and Genter CF. 1974. Observations on grey leaf spot of maize in Virginia. Plant Disease Reporter 58: 456-459.

[13] SAS Institute Inc. 2004. SAS/STATA guide for personal com-puters. Version 9.2 edition. Carry (NC): SAS Institute

[14] Tilahun, T., D. Wagary, G. Demissie, M. Negash, S. Admassu and H. Jifar, 2012. Maize pathology research in Ethiopia in the 2000s: A review. In Meeting the Challenges of Global Climate Change and Food Security through Innovative Maize Research (193).

Biography

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