Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.8, No.3, 2018
www.iiste.org

Evaluation of Resistance Reaction of Maize Inbred Lines to Major Foliar Diseases in Ethiopia

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Abstract
Maize (Zea mays L.) production in Ethiopia is constantly threatened by the potential outbreak of major foliar diseases such as Turcicum leaf blight (TLB), Gray leaf spot (GLS) and Common leaf rust (CLR). Improvement of host resistance to these diseases can provide an important component of integrated disease management, which is the most effective and practical method of managing maize diseases. This study was conducted to evaluate the reaction of maize inbred lines to TLB, GLS and CLR diseases in the rain seasons, during 2014 and 2015. 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 TLB and GLS screening nurseries under artificial inoculation at Bako Agricultural Research Center, West Shewa, Ethiopia. For rust resistance, screening was done under natural infestation at Hawassa Maize Research sub- Center, Hawassa, Ethiopia. Disease severity (1-5 scale) was assessed at ten days interval from disease onset until the maize reached the dent stage. All the inbred lines showed symptoms of the three major diseases in both seasons, but the intensity of the diseases differed significantly (P<0.001) among the inbred lines. The interaction between genotype x year was observed non-significant for the observed foliar diseases, indicating that differences in each foliar diseases; TLB, GLS & CLR severity were mainly contributed by the genotypes. Meteorological data of both seasons/years showed almost similar rain fall & temperature values, which may explains the reasons behind this. Based on combined average severity, only six inbred lines; 30G 19F2-43-1-1-1-1-1-1, CML-197 x 142-1-e(F2) 60-1-1-2-1-1, CML383, (ZM-605-C2F2-428-3-B-B-B-B-B-B-B/F7215)-2-2-2-1-1, 30G 19F2-54-1-1-1and DE-38-Z-126-3-2-2-1-1, displayed a resistance reactions to all the three diseases evaluated when compared with the multiple-resistant check (142-1-e). About, 22 and 18 inbred lines were found resistant (score 1.0-2.0 on a 1-5 scale) to TLB and GLS under artificial epiphytotic conditions, respectively. Seventeen inbred lines exhibited resistance to CLR under natural infestation in the field condition. In the resistant inbred lines, a wide range of diversity was observed for agronomic traits such as plant height (cm), ear height (cm) Plant & ear aspect (1-5), and grain yield per hectare. The results from the present study reveal a shortage of lines with multiple-resistance to these diseases in the inbred lines currently used in mid-altitude maize hybrid production in Ethiopia. It is, therefore, indispensable to look for inbred lines that have resistance to multiple foliar diseases that could be used as source of resistance for conversion of the susceptible germplasm or for direct use as parents of commercial hybrids. Focus should also be given on pyramiding genes for resistance in the breeding programmes to develop varieties with multiple resistances to these major diseases in Ethiopia.
Keywords: Foliar diseases, Grain yield**,** Inoculation, Resistance, Zea mays

1. INTRODUCTION

Maize (*Zea mays L.*) is one of the most important cereal crops in Ethiopia. It is primarily produced and consumed by the small-scale farmers that comprise about 80% of the population (Dawit et al., 2008; Mosisa et al., 2001). The mid-altitude sub-humid agro-ecology (1000 to 1800 *m.a.s.l*) is the most important maize producing environment in Ethiopia (Birhane and Bantayehu, 1989; Kebede et al., 1993; Dawit et al., 2008). Despite the importance of maize as a principal food security crop, its average yield in Ethiopia (3.4 t ha^{-1}) is still low as compared to the world average (5.7t ha^{-1})(Abate et al., 2015). A significant portion of this yield gap is attributable to biotic and a biotic stresses. Among biotic factors foliar diseases are reported to be widespread and destructive disease of maize mainly in warm and humid growing regions of Ethiopia (Tewabech et al.*,* 2001). Among these, foliar diseases; Turcicum leaf blight (TLB), Gray leaf spot (GLS) and common leaf rust (CLR) have become a serious yield limiting factors in most warm and mid-altitude maize producing regions of the country (Tewabech et al., 2012).

Earlier disease assessments in Ethiopia indicated that major foliar diseases such as TLB caused by *Exserohilum turcicum* (Pass.) *Leonard and Sugs* and CLR caused by *Puccinia sorghi* Schwein have been widely distributed causing severe epidemics every year, especially in the warm and humid areas of the country (Assefa, 1999; Tewabech et al.*,* 2001; Tewabech et al.*,* 2002). According to Assefa and Tilahun, (1992), TLB caused the highest mean grain yield loss of 50% and 1000 kernel weight loss of 16.4% on susceptible cultivars. GLS, which is caused by *Cercospora zeae-maydis* Tehon et Dan. occurs in major maize growing areas of Ethiopia, but is amajor problem for maize production in low to mid-land ofsouthern and western provinces, the largest maize production area in the country. A study conducted in Ethiopia by Dagne et al. (2004) indicated that GLS caused a yield loss of 37 percent, with estimated higher losses in years of severe epidemics. The incidence of this diseaseis severe in certain areas, but has not resulted in seriouseconomic loss except in southern and south eastern province of the country. Foliar diseases occur mainly after the tasseling stage of maize, making them difficult to control with fungicides in the field. In addition, the parental lines of the currently available commercial hybrids lack multiple resistances to all the three foliar diseases. It is, therefore, indispensable to look for inbred lines that have resistance to multiple foliar diseases that could be used as source of resistance for conversion of the susceptible germplasm or for direct use as parents of commercial hybrids.

Use of host–resistance is the most practical method of managing crop diseases (Fehr, 1987; Wang, 2005; Wang et al., 2006). Planting of resistant cultivars can effectively reduce the rate of disease development, and that practice is now widely recommended (Ward et al., 1997; Dagne et al., 2004; Abera et al., 2016; Garoma et al., 2016). Development of germplasm with good agronomic characteristics and resistance to multiple foliar diseases is particularly challenging, and this goal has been identified as one of the top priorities for research and development of maize in sub-Saharan Africa (DeVries and Toenniessen, 2001). Understanding of inbred lines' disease reactions is essential for parental selection and resistant hybrid development (van et al., 2012; Technow et al., 2013; Tian et al., 2011; Barakat et al., 2009). The objective of this study was therefore, to identify sources of resistance to major maize foliar diseases; GLS, TLB, and CLR through evaluation of the locally developed and adapted maize inbred lines for using in maize improvement program in Ethiopia.

2. MATERIALS AND METHODS

2.1. Description of the study area

The present experiment was conducted consecutively for two years in the main cropping seasons of 2014 and 2015 (May to December). Both experiments were conducted in the field under artificial epiphytotic conditions for evaluation of TLB and GLS, and natural infestation was used for CLR under disease hot spot area. For accurate evaluation of disease reactions under appropriate environments, the screening nursery was located in disease epidemic areas: TLB and GLS nurseries were in Oromia administrative region at Bako Agricultural Research Center, located at (37E, 09^0 06^0 N and at an altitude of 1650 m, minimum and maximum average temperature of 15.6^0C and 30.7^0C, respectively), and CLR nursery was in SNNP regional administration, Hawassa maize research sub-center, Hawassa, located at (12^0 31'N, 39^0 33'E, Elevation of 2490m, minimum and maximum temperature of 6^0C and 30^0C, respectively).

2.2. Field Procedures

Included entries were 52 inbred lines (Table 1), arranged in alpha lattice design with three replications. Inbred line; CML 142-1-e was used as a resistant check for TLB, GLS &CLR in both cropping seasons. Each inbred line was planted in a plot consisting of two rows of 3.6m long spaced at 25 and 75cm between plants with in rows and rows respectively. Plots were hand sown with fertilization of P_2O_5 and nitrogen at the rate of 69, 92 kg h^{-1} respectively and all agronomic management practices recommended for the area were applied based on recommendation.

Table 1: List of maize inbred and their pedigree used for the study

Line code	Pedigree	origin
L1	(CML205/CML208//CML202)-X-2-1-2-B-B-B	Bako
L2	(DRBF2-60-1-2)-B-1-B-B-B/F7215)-1-1-3	Bako
L3	(TZM1102/TZM1501)-2-3-B-B-B-B	Bako
L4	(ZM-605-C2F2-428-3-B-B-B-B-B-B-B/F7215)-2-2-2-1-1	Bako
L5	30G 19F2-43-1-1-1-1-1-1	Bako
L6	30G 19F2-43-1-1-1-1-1-2	Bako
L7	30G 19F2-54-1-1-1	Bako
L8	30G 19F2-9-1-1-2-1-1-1	Bako
L9	30H83-5-1-4-2-1-1-1	Bako
L10	30H83-7-1-1-1-2-1-1	Bako
L11	30H83-7-1-2-1-1-1	Bako
L12	30V53F2-20-2-1-3-3-1-1	Bako
L13	3253F3 -9-WF- 3-1-1	Bako
L14	35B-190-O-S10-9-1-1	Bako
L15	CML-197 x 142-1-e(F2) 17-1-1-1-1-1	Bako
L16	CML-197 x 142-1-e(F2) 197-1-1-1-1-1	Bako
L17	CML-197 x 142-1-e(F2) 197-1-1-1-2-1	Bako
L18	CML-197 x 142-1-e(F2) 197-1-1-1-2-2	Bako
L19	CML-197 x 142-1-e(F2) 60-1-1-1-1-1	Bako
L20	CML-197 x 142-1-e(F2) 60-1-1-2-1-1	Bako
L21	CML383	CIMMYT
L22	CML444	CIMMYT
L23	CML445	CIMMYT
L24	DE-38-Z-126-3-2-2-1-1	Bako
L25	DE-78-Z-126-3-2-1-2-1	Bako
L26	FH625-272-1-2-1	Bako
L27	Gibe-1-198-2-2-1-1	Bako
L28	IL0'OOE-5-2-4-2-1-1	Bako
L29	ILOO'E 1-9-1-1-2-1-2	Bako
L30	KULENI 320-2-3-1-1-2-1-1	Bako
L31	Kuleni C 1-0080-2-4-1-2-1	Bako
L32	Kuleni-0017-2-1-1	Bako
L33	POO9A-134-2-3-2-1-1-1	Bako
L34	POOL9A-128-5-1-1-1-2-1	Bako
L35	TZMI719	IITA
L36	TZMI723	IITA
L37	TZMI730	IITA
L38	TZMI733	IITA
L39	TZMI745	IITA
L40	TZMI746	IITA
L41	TZMI747	IITA
L42	TZMI751	IITA
L43	TZMI753	IITA
L44	TZMI754	IITA
L45	TZMI755	IITA
L46	TZMI761	IITA
L47	TZMI763	IITA
L48	TZMI764	IITA
L49	CML 197	CIMMYT
L50	CML202	CIMMYT
L51	CML395	CIMMYT
L52	142-1-e	Bako

2.3. Inoculum preparation and Inoculation
The pathogens artificial inoculum of *E. Turcicum* and *C. zeae maydis* was prepared by collecting heavily infected maize fields showing distinct TLB and GLS symptoms respectively, which were collected in the previous year. This was done when leaves become fully mature. Infected leaves were kept in large paper bags at room temperature conditions protected from moisture. Dry leaves were then, ground into a meal about the coarseness of wheat bran and stored in paper bags at a temperature of 4^{o}C.Then,inoculationof *E. Turcicum* and *C. zeae maydis* was done according to Dagne (2008) by placing a pinch of leaf meal into whorl of each plant, when plant attains 6-8 leaf stage. A second inoculation was made seven to ten days later to ensure adequate infection, and natural infestation was used for *P.sorghi*, which is a causative agent of CLR on maize.

2.4. Evaluation of disease reactions
Data were collected on a plot basis from the two rows of the experiment. The progress of severity of foliar diseases on each inbred lines was quantified at 10 days intervals starting from onset of disease until dent stages for successive disease assessments and the highest or final severity value of each inbred lines attained was used for statistical analysis. Turcicum leaf blight and GLS was assessed using a modified 1-5 scale by CIMMYT(1985) as follows; 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.CLR disease severity rating was done using a scoring key modified from Danson et al. (2008) as follows: 1=no disease (no rust pustules seen); 2=10 to 15 % of leaf surface diseased (numerous pustules on the leaf surfaces some erumpent); 3=30 to 40% of leaf surface diseased (many erumpent pustules over the leaf surfaces); 4=45 to 65 % of leaf surface diseased (many erumpent pustules surrounded with huge blighted and sometimes rusty chlorotic zones). 5=over 75 % of leaf surface diseased (many huge dry pustules surrounded by dead rusty wilted and blighted areas on the leaves). The categorization on each disease reactions was made on the basis of disease severity ratings using a 1-5 scale (Roane *et al.,* 1974) 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. Evaluation for agronomic traits
Agronomic performance of the inbred lines was also evaluated on a per-plot basis; observations on plant height (cm), ear height (cm), plant aspect (1-5), ear aspect (1-5) and grain yield (T h^{-1}) at maturity. An average performance of the plot was used for statistical analysis.

2.6. Experimental design and statistical analysis
Alpha lattice design was employed for the study and the experiment was conducted twice. Data were subjected to analysis of variance (ANOVA) using SAS version 9.2 (SAS Institute, 2004). Single and interaction effects of factors were determined using the general linear model (GLM) procedure of SAS. Mean values among treatments were compared by the t-tests at α=0.05 level of significance. The correlation among disease parameters and some agronomic traits was analyzed by using Pearson correlation coefficient analysis following PROC CORR procedure of the SAS software (SAS Institute, 2004).

3. RESULTS
3.1 Reactions of the inbred lines to the foliar diseases
An average score of resistance of each inbred lines to; TLB, GLS and CLR were calculated. For each disease the average score between the genotypes x environment/year was insignificantly different thus, data from both years were pooled for statistical analysis (Table 2).
3.1.1 Grey leaf spot resistance: The mean disease severity result indicated significant (P < 0.001) variation among the inbred lines for GLS resistance in the pooled data (Table 2). The mean analysis indicated a non significant interaction between genotypes x planting years, indicating that differences in the GLS scores were mainly contributed by the inbred lines. Based on mean GLS severity of the two years, 17 inbred lines were found resistant (score 1.0 to 2.0 on a 1-5 scale), 9 moderately resistant (score 2.1 to 2.5), 14 susceptible (score 2.5 to 3.0), and 11highly susceptible (score>3.0) (Fig 1).The resistant check 142-1-e had a severity score of 1.50. The resistant inbred lines, which have showed very comparable resistance with the resistant check (142-1-e), were selected as GLS resistance sources for use in resistance breeding programs. These 17 resistant lines also exhibited a wide range of diversity for yield and other agronomic traits (Table 2).
3.1.2 Leaf blight resistance: The study result of 52 inbred lines for TLB indicated significant (P<0.001) variation in the pooled analysis (Table 2). The interaction between genotype x year was observed non-significant, indicating that differences in leaf blight severity were mainly contributed by the genotypes. Based on mean leaf blight severity for the two years, 21 inbred lines were found resistant (score 1.0 to 2.0 on a 1-5 scale), 6

moderately resistant (score 2.1 to 2.5), 19susceptible (score 2.6 to 3.0), and 5highly susceptible (score >3.0) compared with 1.5 score for the resistant check 142-1-e (Fig. 1). Twenty-eight morphologically diverse lines with mean ≤ 2.50 leaf blight score were selected as sources of TLB resistance for maize breeding programs (Table 2).

3.1.3 Common leaf rust resistance: Significant differences (*P* < 0.001) were observed in the evaluated 52 inbred lines for common leaf rust resistance in the field (hot-spot area), under natural infestation (Table 2). Only 16 inbred lines were resistant (score 1-2 severity), which is very comparable with resistant check (severity score 1.5), and 19had moderate resistance (score 2.1- 2.5 severity), 11were susceptible and only 5 highly susceptible (Fig. 1). These inbred lines showed resistance in the field screening at least as compared as the rust resistant check, 142-1-e and good agronomic performance were selected for maize resistance breeding at epidemics of the CLR disease.

Table 2: Combined mean of TLB, GLS and CLR disease reaction of inbred lines at Bako and Hawassa, over two years (2014 and 2015 main seasons)

Line code	TLB		GLS		CLR	
	Severity	Response	Severity	Response	Severity	Response
L1	2.75	S	2.61	S	2.00	R
L2	2.00	R	2.50	S	2.25	MR
L3	2.25	MR	2.75	S	2.22	MR
L4	1.25	R	1.50	R	2.00	R
L5	1.50	R	1.25	R	1.50	R
L6	2.25	MR	1.75	R	2.25	MR
L7	2.00	R	2.00	R	1.75	R
L8	2.75	S	2.70	S	2.25	MR
L9	2.00	R	1.50	R	2.25	MR
L10	2.00	R	2.70	S	2.25	MR
L11	1.75	R	2.25	MR	2.00	R
L12	1.75	R	1.00	R	2.25	MR
L13	2.25	MR	2.75	S	2.00	R
L14	1.75	R	2.60	S	2.00	R
L15	2.60	S	2.21	MR	1.75	R
L16	3.20	HS	1.75	R	2.25	MR
L17	2.75	S	1.75	R	2.70	S
L18	2.60	S	2.50	MR	2.70	S
L19	2.60	S	1.25	R	2.25	MR
L20	1.50	R	1.50	R	2.00	R
L21	1.75	R	1.75	R	1.75	R
L22	2.00	R	3.10	HS	2.25	MR
L23	1.75	R	2.60	S	2.61	S
L24	1.75	R	1.50	R	2.00	R
L25	2.00	R	2.25	MR	2.50	MR
L26	2.00	R	4.50	HS	2.00	R
L27	2.10	MR	3.20	HS	2.00	R
L28	3.20	HS	2.25	MR	2.25	MR
L29	2.75	S	2.00	R	2.25	MR
L30	2.70	S	2.75	S	2.00	R
L31	3.10	HS	3.10	HS	2.25	MR
L32	2.72	S	2.75	S	2.70	S
L33	2.75	S	2.75	S	2.25	MR
L34	2.75	S	3.30	HS	2.25	MR
L35	2.00	R	1.75	R	2.60	S
L36	1.75	R	3.25	HS	2.60	S
L37	2.76	S	1.75	R	2.00	R
L38	3.10	HS	2.60	S	2.25	MR
L39	1.5	R	2.50	MR	2.25	MR
L40	1.75	R	1.50	R	2.25	MR
L41	2.75	S	4.25	HS	2.25	MR

L42	4.10	HS	4.25	HS	2.75	S
L43	1.75	R	1.75	R	2.75	S
L44	2.75	S	4.00	HS	2.20	MR
L45	2.75	S	4.00	HS	3.10	HS
L46	2.85	S	2.50	MR	3.30	HS
L47	2.75	S	2.25	MR	3.20	HS
L48	2.5	S	3.25	HS	3.10	HS
L49	5.00	HS	1.75	R	2.00	R
L50	1.75	R	2.50	MR	2.00	R
L51	2.10	MR	3.00	S	2.00	R
L52	1.50	R	1.75	R	1.75	R
Mean	2.34		2.44		2.34	
CV (%)	11.69		10.57		4.01	

Note: Means followed by a different letter across column are significantly different at α=0.05 (P <0.05).
1.0-2.0=resistant (R); 2.1-2.5=moderately resistant (MR); 2.6-3.0=susceptible (S); >3=highly susceptible (HS).

3.2 Agronomic performance of the selected inbred lines: Significant differences ($P < 0.001$) among the evaluated inbred lines were observed for plant height (PH), Plant aspect (PA), ear height (EH), ear aspect (EA) and grain yield per hectare (Table 3). Ear height of the selected inbred lines resistant or moderately resistant to at least two diseases evaluated are ranged from 78cm (TZMI753) to 121.5cm (35B-190-O-S10-9-1-1). In total, almost all inbred lines evaluated were in the medium to late maturity group (data not shown). Plant height of the selected multiple disease resistant inbred lines are medium, ranged between162 cm (DE-38-Z-126-3-2-2-1-1) to 228 cm (CML383) and only one (30H83-5-1-4-2-1-1-1) lines was short (148cm). Among the selected inbred lines with multiple disease resistance, the highest grain yield (5.43t h^1) was observed in the inbred line CML383. Nine inbred lines, which are resistant or moderately resistant to the three diseases, recorded significantly higher grain yield compared with the trial, mean (Table 3).

Table 3: Combined mean of inbred lines agronomic performances at Bako and Hawassa, over two years (2014 and 2015 main seasons)

Line code	PH (cm)	PA (1-5)	EH (cm)	EA (1-5)	GY (t/h)
L1	218.50cd	3.00cde	114.00def	2.75abc	4.84a
L2	219.00cd	3.00cde	91.00ouv	2.50bcd	4.26ab
L3	173.00no	3.00cde	96.50lmo	3.25a	2.91abc
L4	171.50no	2.25fgh	84.50uvx	3.25a	2.50cd
L5	203.00hi	2.00gh	88.50ouv	2.25cd	3.11abc
L6	204.00hi	2.00gh	85.50uvx	2.00d	1.94fg
L7	194.00jk	2.25fgh	86.00uvx	3.00ab	4.32ab
L8	137.50tu	2.75def	63.00z	3.00ab	1.37hi
L9	148.00rs	2.75def	67.00za	2.75abc	2.09efg
L10	229.50bc	2.50efg	84.50uvx	3.00ab	4.37ab
L11	230.00bc	2.75def	84.50uvx	3.25a	5.44a
L12	189.00k	2.25fgh	82.00uvx	3.00ab	3.41abc
L13	166.00op	3.50abc	76.50za	2.75abc	4.61ab
L14	192.00jk	3.00cde	121.50cde	3.00ab	1.90ghi
L15	213.00ef	3.50abc	122.50bcd	2.50bcd	3.36abc
L16	229.00bc	2.25fgh	115.00def	3.00ab	4.32ab
L17	244.00b	2.50efg	132.50bc	2.00d	3.67abc
L18	238.50bc	3.25bcd	136.00b	2.50bcd	3.34abc
L19	199.00hi	2.00gh	105.00flm	2.50bcd	2.87abc
L20	206.00fg	1.75h	103.50lm	2.75abc	2.73bc
L21	228.50bc	3.00cde	98.50lmo	2.25cd	5.43a
L22	173.00op	3.75ab	102.00lmo	3.00ab	2.48cd
L23	204.50hi	3.00cde	72.00za	3.25a	2.98abc
L24	162.00qr	2.00gh	86.00uvx	2.50bcd	3.97ab
L25	184.50op	3.50abc	90.50uvo	2.00d	4.27ab

L26	188.50km	4.00a	100.50lmo	3.00ab	2.33cd
L27	217.50de	3.75ab	108.00lef	3.25a	4.54ab
L28	237.50bc	2.50efg	109.50def	3.00ab	5.27a
L29	242.00bc	2.75def	99.50lmo	2.00d	4.74ab
L30	208.50fg	3.50abc	109.50def	2.75abc	2.42cde
L31	203.00hi	3.25bcd	117.00def	3.00ab	3.28ab
L32	192.50jk	2.75def	106.50flm	2.75abc	3.72ab
L33	158.50st	3.50abc	83.00uvx	2.75abc	1.03ij
L34	207.50ef	3.00cde	95.00imo	3.00ab	3.28ab
L35	205.00fg	2.25fgh	102.50lm	3.00ab	3.03ab
L36	190.00kl	2.75def	81.00uvx	2.75abc	1.81gh
L37	202.00hi	2.00gh	116.50def	2.50bcd	4.64a
L38	156.50rst	2.75def	79.00vx	3.00ab	2.49cde
L39	215.00ghi	2.00gh	117.50def	2.50bcd	4.72a
L40	219.50cde	2.25fgh	108.50ef	2.50bcd	4.17ab
L41	160.50rst	3.25bcd	90.50uv	2.75abc	2.15de
L42	131.00u	3.25bcd	93.50mo	2.75abc	0.25j
L43	162.5qr	2.25fgh	78.00zvx	2.75abc	2.73bcd
L44	155.50st	3.25bcd	90.50uv	2.50bcd	2.22def
L45	143.00st	3.50abc	82.50uv	3.25a	1.91gh
L46	156.50st	2.75def	72.50za	2.75abc	2.56cde
L47	194.00jk	2.50efg	104.00flm	2.75abc	1.84ghi
L48	165.00pq	3.00cde	76.00z	3.00ab	2.82bcd
L49	189.50kl	4.00a	113.00def	2.75abc	3.34abc
L50	187.50lm	3.25bcd	90.50ou	3.00ab	2.38cde
L51	177.00pq	3.00cde	98.00lmo	2.25cd	4.24ab
L52	269.00a	2.50efg	153.00a	2.50bcd	4.61ab
Mean	195.99	2.82	97.38	2.74	3.28
CV (%)	5.20	9.71	7.11	11.19	9.69

Note: Means followed by a different letter across column are significantly different at α=0.05 (P <0.05).

3.3 Multiple resistances of the inbred lines to the three foliar diseases

A wide range of reactions to TLB, GLS and CLR was observed in the 52 inbred lines tested. The proportions of lines that showed resistance (R) and moderately resistance (MR) reactions to inoculation of different pathogens varied (Fig. 1). The percentage of inbred lines resistant and moderately resistant to TLB was 53.8, but the rest of them exhibited susceptible and highly susceptible reactions. Most lines that were resistant to CLR showed a moderately resistance (36.5%) and 32.7% were shown resistance reaction but the rest showed susceptible and highly susceptible reactions. The percentage of lines that exhibited resistance or moderately resistance reactions to GLS was 34.6 and 17.3%, respectively. A small percentage of lines were resistant to the three diseases; TLB, GLS and CLR simultaneously. Only six inbred lines; 30G 19F2-43-1-1-1-1-1-1, CML-197 x 142-1-e(F2) 60-1-1-2-1-1, CML383, (ZM-605-C2F2-428-3-B-B-B-B-B-B/F7215)-2-2-2-1-1, 30G 19F2-54-1-1-1 and DE-38-Z-126-3-2-2-1-1, displayed a resistance reactions to all the three diseases evaluated when compared with the multiple-resistant check (CML 142-1-e). Inbred lines; 30H83-5-1-4-2-1-1-1, 30H83-7-1-2-1-1-1, 30V53F2-20-2-1-3-3-1-1, 35B-190-O-S10-9-1-1, FH625-272-1-2-1, TZMI719, TZMI730, TZMI746, TZMI753 and CML202 were at least resistant against 2 diseases evaluated (Table1). The percentage of lines that exhibited MR reactions to the three diseases was 23%. Generally, about 30.7% of the lines tested were at least resistant to 2 diseases tested.

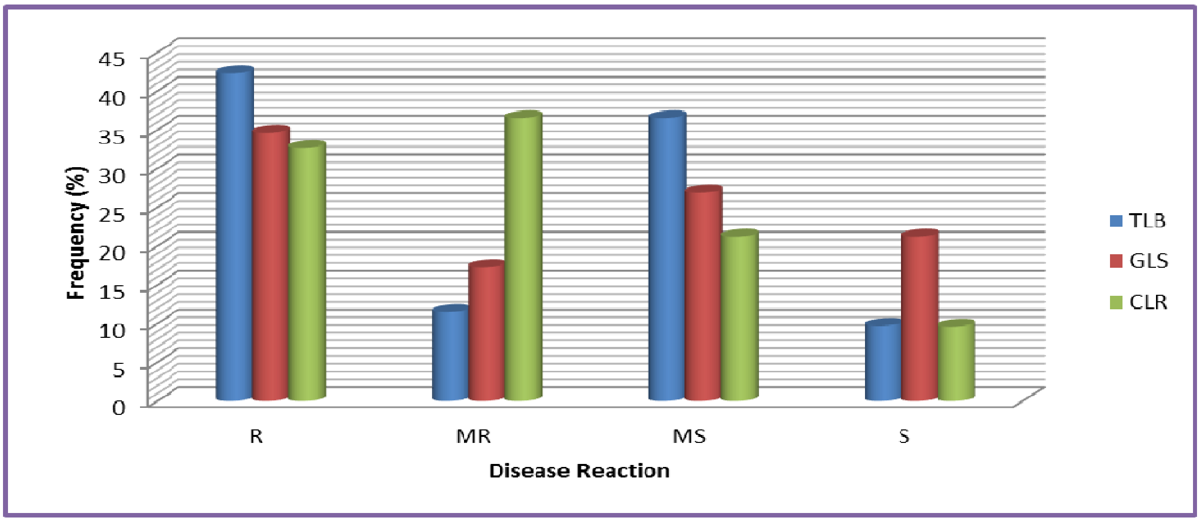

Figure 1. Frequencies of the inbred lines with resistant (R), moderately resistant (MR), moderately susceptible(MS), and susceptible (S) reactions to Turcicum leaf blight (TLB), gray leaf spot (GLS) and common leaf rust (CLR).

3.4 Correlation Analysis

Pearson correlation analysis revealed grain yield (GY) is not significant and negatively correlated with TLB, CLR and PA, but significant and negatively correlated with GLS (r=-0.382)(Table 3).This indicated that GLS disease progress had a negative effect on yield. However, positive associations were observed among morphological traits. Plant height showed significant and positive association with EH (r=0.679) and GY (r=0.667) indicating, increments in plant height resulted in the exertion of ear height and this might be the reason for grain yield increments.

Table 4: Phenotype correlation analysis result among TLB, GLS, CLR disease reactions, grain yield and some other maize agronomic traits.

	TLB	GLS	CLR	PH	EH	GY
TLB	1	0.193	0.153	-0.092	0.243	-0.195
GLS		1	0.136	-0.439**	-0.16	-0.382**
CLR			1	-0.157	-0.081	-0.224
PH				1	0.679**	0.667**
EH					1	0.355**
GY						1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

3.5 Cluster Analysis

Based on the combined mean data of the 52 inbred lines disease resistance; TLB, GLS, CLR and some agronomic traits, clustering analysis result fall into four major clusters (branches) (Figure 2 and Table 4). Accordingly, some of the inbred lines with multiple disease-resistant types were clustered on different branches (Figure 2 & Table 4), indicating these inbred lines might constitute different resistance genes governing resistance for the three major diseases evaluated. The modern advanced molecular methods can be used, to locate the genes and incorporating mechanism of resistances of such inbred lines.

Table 5: Cluster analysis of 52 maize inbred lines based on GLS, TLB, CLR disease resistance, grain yield and some agronomic traits

No of Cluster	Number of inbred lines	Name of inbred lines
I	6	L10, L11, L28, L29, L2 and L21
II	17	L13, L48, L3, L51, L22, L38, L46, L24, L33, L43, L44, L4, L41, L8, L9, L42 and L45
III	26	L5, L6, L12, L36, L27, L40, L31, L37, L19, L47, L20, L35, L32 , L7, L25, L50, L1, L39, L30, L15, L26, L14, L49, L34, L16 and L23
IV	3	L17, L18 and L52

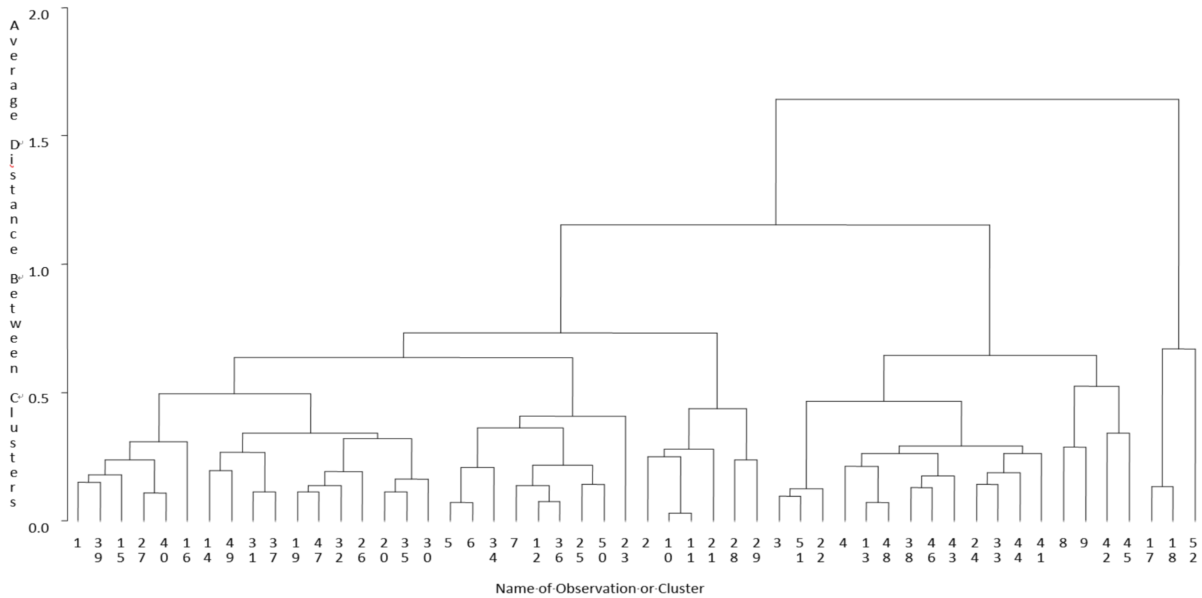

Figure 2. Tree diagram of 52 maize inbred lines based on TLB, GLS, CLR, PH, EH, PA, EA and grain yields traits.

4. DISCUSSION

Field screening studies over two years indicated that there was clear cut differential disease response of inbred lines to *E. Turcicum* and *C. zeae maydis* due to artificial inoculation and natural infestation of *P.sorghi*. Although, the inbred lines showing moderately resistance reaction of less than 2.5 disease score remained green till maturity, the susceptible and highly susceptible lines (>2.50 disease score) failed to produce normal foliage as well as ears as disease covered the entire plant before silking and tasseling stage. The present study revealed that out of 51 inbred lines tested, only 5 lines registered multiple-disease resistance which have recorded least disease rating of less than 2.0 against the three major diseases which have showed very comparable resistance with the multiple disease resistant check 142-1-e, while more than half of the lines had exhibited maximum rating scale of >3.0 (highly susceptible reaction) against each evaluated diseases. Within the resistant inbred lines, a wide range of diversity was observed for agronomic traits such as PH, PA, EH, EA and grain yield/hectare. The investigation revealed that 6 inbred lines, namely 30G 19F2-43-1-1-1-1-1-1, CML-197 x 142-1-e(F2) 60-1-1-2-1-1, CML383, (ZM-605-C2F2-428-3-B-B-B-B-B-B-B/F7215)-2-2-2-1-1, 30G 19F2-54-1-1-1 and DE-38-Z-126-3-2-2-1-1, had registered multiple-disease resistance reaction that possessed a disease score of 1.0-2.0, which is very comparable resistance with the multiple-resistant check (142-1-e).

Multiple disease resistance of the lines against major foliar diseases occurred in 11.5% of the lines tested. Generally, 21 inbred lines were found resistant (score 1.0-2.0 on a 1-5 scale) to TLB and 17 to GLS under artificial epiphytotic condition and 16 inbred lines exhibited resistance to rust under natural infestation. The findings also revealed CLR was well controlled with most lines (36.5%) displaying moderate resistance. However, highly resistant lines are also needed in south eastern provinces of Ethiopia, owing to severe epidemics of the disease. Thus, it can be emphasized from the results that the identified resistant lines hold excellent promise for resistance against *E. turcicum, C. zeae maydis* and *P. sorghi* causing TLB, GLS and CLR of maize respectively, and can be used for developing hybrids and composites in future program of breeding for multiple-disease resistance. These findings are in agreement with the work of Patil et al. (2000), Muiru et al., 2007 and Pandurangegowda et al. (1994), who reported differential reaction to diseases among the various maize germplasm. Sharma and Payak

(1990) reported durable resistance in CM-104 and CM-105 maize inbred lines against *E. turcicum*. Promising maize disease-resistance sources were also reported by Dagne et al. (2008), who identified (143-5-I and CML-387) as sources of resistance, (Gotto LMS5, SC-22 and CML-395) moderately resistance and A-7016 and CML-197 susceptible to GLS of maize. Wende et al. (2013) identified two inbreds, 136-a and Gibe-1-186-2-2-1 as TLB resistant lines. Moderately resistant reaction was noticed in CM-111, CM501, CM-121, KDMI-12 and CM-118, where as several lines including CM-203, CM-115, CM117, CM-128, CM-600 and KDMI-10 were found highly susceptible.

The study also agrees with reports on differential reaction of various maize germiplasm to the diseases; TLB (Singh et al., 2004; Adpala et al., 1993; Chandrashekara et al., 2012; Abera et al., 2016), and GLS disease (Saghai Maroof et al., 1993). The resistance reaction of various maize germiplasm was found different for the evaluated diseases of maize. The reaction of inbred lines to the various pathogens is governed by the resistance genes incorporated in the genotypes in the breeding programmes. Thus the promising lines with good yield and other agronomic performance identified through this investigation can be deployed in disease endemic areas for sustainable maize productivity. Moreover, the proportion of lines showed multiple-resistance to the three diseases was very small in number, and a broad range of lines from various sources should be screened to identify enough number of lines that are resistant to these diseases.

5. CONCLUSION AND RECOMMENDATION

Most of the inbred lines evaluated were found to be susceptible to one or more the major leaf diseases of maize but the degree of susceptibility varied among the inbred lines. So there is a need to pyramid genes for multiple-disease resistance in the inbred lines to enable maize producers increase their productivity by decreasing losses incurred by these diseases. Maize genotypes identified as multiple disease resistant in the study should also be screened under controlled environments to correctly identify the level of resistance for each of the major diseases. The molecular methods can also be used to locate the genes and incorporating the mechanism of resistance of these screened inbred lines with desired agronomic characteristics and have potential to be used as source material in the breeding of disease resistance to overcome the problem of leaf diseases of maize in Ethiopia.

6. ACKNOWLEDGMENT

The authors would like to thank EIAR for financial support provided to conduct the experiment. We are also grateful to Bako National Maize Research Co-ordination Center breeding program for delivering maize genotypes. It is also our pleasure to thank, the National Maize Research plant protection division technical assistance staff; Geta Gelana, Mekides Kebede, Abebech yilma & Diriba Oljira for assistance they provided to us in field management and data collection.

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