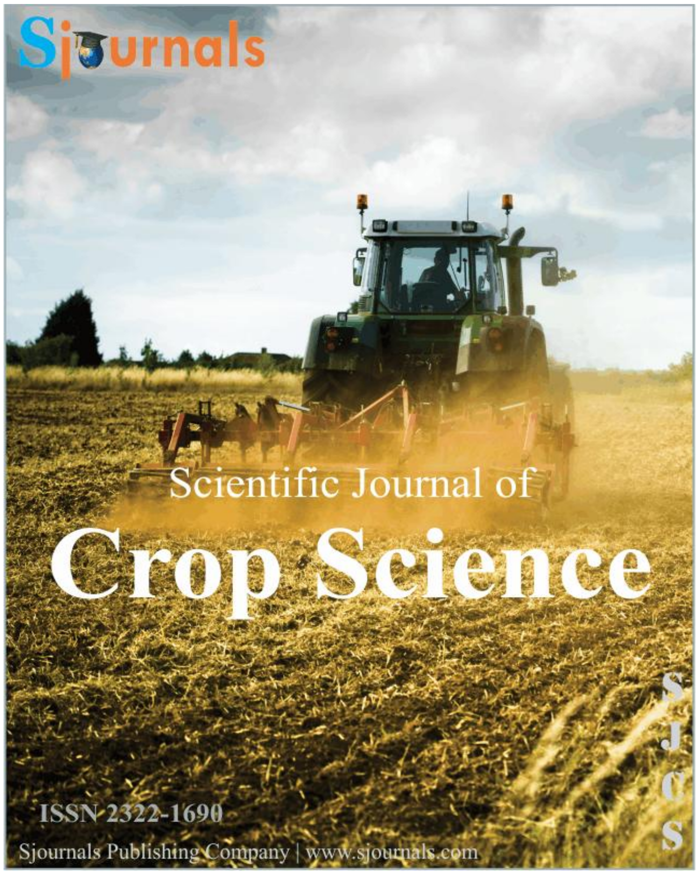
Sjournals
Scientific Journal of
Crop Science
SJCS
ISSN 2322-1690
Sjournals Publishing Company | www.sjournals.com

Sjournals
Scientific Journals
Scientific Journal of Crop Science (2016) 5(11) 137-145
ISSN 2322-1690
doi: 10.14196/sjcs.v5i11.2252

Contents lists available at Sjournals
Scientific Journal of **Crop Science**
Journal homepage: www.Sjournals.com

Original article

Evaluation of quality protein maize inbred lines for resistance to Turcicum leaf blight and grey leaf spot disease under field condition at mid altitude sub-humid agro-ecology of Ethiopia

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ARTICLE INFO

Article history,
Received 16 October 2016
Accepted 17 November 2016
Available online 21 November 2016
iThenticate screening 18 October 2016
English editing 15 November 2016
Quality control 19 November 2016

Keywords,
Inbred line
Turcicum leaf blight
Grey leaf spot
Resistance

ABSTRACT

Grey Leaf Spot and Turcicum Leaf Blight, severe foliar diseases of maize caused by the fungi Cercospora zeamaydis and Exserohilum turcicum, are characterized by relatively rapid leaf necrosis and premature death of foliage which reduces grain yield of maize crop in Ethiopia, the diseases become a major problem in all major maize growing areas. The study was carried out with the objective to evaluate and identify resistant/tolerant quality protein maize inbred lines to turcicum leaf blight and grey leaf spot diseases. A total of 25 QPM inbred lines were evaluate and necessary data on incidence, disease severity, AUDPC, plant height and grain yield were recorded. Result of combined analysis variance showed significant ($p<0.05$) difference of year x inbred lines interaction for TLB and GLS severity. The performances of the inbred lines were not consistent across years as evident from the significant year x genotypes interactions. Out of twenty five quality protein inbred lines, four of them showed resistant to turcicum leaf blight and three inbred lines resistance to grey leaf spot disease. The majority of quality protein inbred lines were showed moderate resistant to both diseases. Some quality protein maize inbred lines resistance to both diseases, indicating that it can carry genes for multiple traits. However, further study required through fine mapping and identification of co-located QTLs. Overall, the identified resistance inbred lines can be utilized in future maize breeding program like for possible use of introgression into cultivars, as source of donor and varietal resistance development.

1. Introduction

Maize (*Zea mays L.*) is one of the most important cereal crops in Ethiopia, ranking second in area coverage and first in total production. A total of 7.2 million tons of maize was produced on 2.1 million ha of land in 2014/15 (CSA, 2015). In last decade, the yield of maize increased nationally. This due to the continuous investment of maize research efforts resulted in technology development (the released of hybrids and Open Pollinated Varieties) for different agro-ecologies. However, the national average yields is still very low as compared to the world average of (5.5 t ha^{-1}) (Abate et al., 2015). An important portion of this low yielding in maize is attributable to abiotic, biotic stresses and poor management practise as well as climatic change.

Among biotic constraint; foliar diseases such as Turcicum leaf blight (TLB), Grey Leaf Spot (GLS) and common rust are the most economically important diseases on maize crop production (Tewabech et al., 2001) and Maize Lethal Necrosis Disease (MLND), the newly emerged devastating disease of maize in Eastern Africa including Ethiopia (Mahuku, 2015). Due to the presence of conducive environmental factors such as relatively higher humidity, moderate to high temperature and/ or warm areas during the maize cropping season at mid-altitude sub-humid agro-ecology of Ethiopia, turcicum leaf blight and grey leaf spot diseases are considered as the most economically important, which are identified to cause significant yield lose. However, disease severity and yield loos are varies from year to year, agro-ecology, crop management and type of variety.

Turcicum leaf blight is caused by *Exserohilum turcicum* causes leaf necrosis and premature death of foliage which reduces grain yield of crop. Raymundo and Hooker (1981) observed that yield reduction due to TLB was about 63% for early maturity susceptible hybrids. Yield losses may occur up to 60-70% if infection of blight occurs in early developmental stage of the plant. Two high yielding maize varieties were withdrawn from production in Ethiopia due to susceptible to TLB. On the other hand, grey leaf spot shows necrotic lesion tend to be long and individual lesion may merge lead to leaf senesence greatly reducing the photosynthic areas leading to poor grain filling (Darera et al., 2008). A yield loss due to GLS was estimated about 37 % in Ethiopia (Wegary et al., 2004) and 60% in South Africa (Ward et al., 1997).

Methods to manage TLB and GLS diseases include cultural practices, chemical and host plant resistance (Pratt et al., 2003). Host plant resistance is the most effective and cost-efficient means of managing GLS and preventing leaf blight because chemicals are expensive, often ineffective and inconsistence with environment. It is desirable to identify resistance inbred lines from divers resources and thus, very important in maize pre-breeding program in order to improve genetic resistance to foliar disease. Early research efforts made to identify maize germplasm resistance to turcicum leaf blight and grey leaf spot diseases and utilizing them for maize breeding program. To obtain new and stable source of resistance, subsequent study for additional source of maize germplasm should be screened under artificial inoculation and or at hotspot areas across environments and over years. The question was which inbred lines resistant to TLB and GLS severity over two years even fluctuation environment happened. Therefore, the objective of study was to evaluate and identify new source of quality protein maize inbred lines with potentially resistant/tolerant to turcicum leaf blight and grey leaf spot for use in future maize breeding program.

2. Materials and methods

2.1. Description of experimental site

The experiments were conducted at Bako National maize research centre of maize disease nursery field during main season of 2012 and 2013. Bako receives the annual rain fall about 1237 mm and situated an altitude of 1650 m above sea level, which represent mid altitude sub-humid agro-ecology zone of Ethiopia.

2.2. Source of inbred lines

A total of twenty five quality proteins maize inbred lines were used in the study. Among 25 inbred lines, 17 were developed by Bako National maize research centre; Ethiopia Institute of Agriculture Research, and 8 inbred lines acquired from CIMMYT and adapted to local condition. The non-quality protein maize inbred lines of CML-197 and SC-22 were used as susceptible check for TLB and GLS respectively.

2.3. Experimental design

The trials were arranged as 5 x 5 alpha lattice design with two replications and conducted for two years (2012 and 2013) during the main cropping season of Ethiopia. Each inbred line planted on two rows of 3.6 m length, 75 cm x 30 cm between rows and plant respectively. Two seeds were planted per hill in a row and later thinned to one. Agronomic practises including fertilizer application, weed management and others were applied as per research recommendation.

2.4. Inoculum preparation and inoculation

Evaluation of GLS and TLB resistance under natural condition is not reliable. Application of artificial inoculation would be ensured disease pressure for evaluation. The inoculum was collected from natural infected maize leaves a year before experimentation. The infected leaves were dried under shade and crushed/grounded in to powder and preserved at $4^{o}c$ in refrigerator until inoculation date. The pulverized leaves then dusted in the whorls of the plants during moist environments in order to retain long enough to permit spore germination. The inoculation was applied twice during both seasons at ten days interval starting from six leaves stage of the plant.

2.5. Disease assessment

TLB and GLS were visually assessed in the field 1-2 weeks after artificial inoculation. Data collected included first disease appearance date, disease incidence, disease severity, and other agronomic traits including plant height (cm) and grain yield (t/ha). Each plot was assessed at 10 days interval for incidence and severity scale for four times. 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 = Slight infection- very few lesion on leave, usually on the lower leaves of the plant only
1.5 - 2.0 = Light infection- few to moderate lesion on leaves below top ear, no lesion on leave above the top ear
2.5 - 3.0 = Moderate infection- moderate to large number of lesion on leave below top ear, few lesion on leaves above the top ear
3.5 - 4.0 = Heavy infection- large number of lesion on leaves below top ear, moderate to large number of lesion on leaves above the top ear
4.5 - 5.0 = Very heavy infection- all leaves with large number of lesions leading to premature death of the plant and light ears

2.6. Area under disease pressure curve (AUDPC)

Severity data for the observed TLB and GLS diseases were recorded at ten days interval starting from the initial disease appearance date up to the disease reached pick. Disease progress curve was developed from ten days interval reading and its values were computed to integrate TLB and GLS diseases assessment of severity. The area under disease progress curve was used to quantify the beginning of epidemic and the time until disease reached maximum stage. In order to draw AUDPC, the disease severity was taken five times including per se evaluation. AUDPC was calculated by the following formula (Campbell and Madden, 1991):

$$AUDPC = \sum_{i=1}^{n-1} \frac{[(\mathrm{yi}+1+yi)]}{2} * (\mathrm{ti}+1^{-\mathrm{t}}\mathrm{i})$$

Where, n = total number of observation, t_i = days after second inoculation date for the i^{th} disease assessment and yi disease severity.

2.7. Data analysis

Disease incidence, disease severity, plant height, grain yield and area under disease progress curve were analysed by using PROC GLM of SAS software (SAS, 2004). Mean separation was performed to compare treatment means using LSD-test at 5% 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 and discussion

A total of twenty five quality protein maize inbred lines were screened over two years for resistance to TLB and GLS. Mean values of disease assessment varied due to year and inbred lines. The combined analysis variance result showed significant ($p<0.05$) difference of year x inbred lines for TLB and GLS severity as well as for yield and plant height (Table 1 and 2). Some inbred lines scored different mean values of severity for TLB and GLS for tested years. This might be due to varied rainfall distribution and relative humidity during tested years (Table 3). Disease severity ranged from 1.61 to 4.37 and 1.75 to 4.10 for TLB and GLS respectively. Inbred lines with mean values of severity greater than three were categorized under susceptible to TLB and GLS. Whereas inbred lines with mean values of severity less than two were considered as tolerance/resistance to TLB and GLS diseases (Table 1 and 2).

Table 1
Mean of grey leaf spot severity, grain yield and plant height of 25 QPM inbred lines tested during 2012 and 2013.

Entry	QPM inbred lines	GLS severity (1-5 scale)	Reaction to GLS	Plant height (cm)	Yield t ha^{-1}
1	CML-144	3.11	S	159.75	3.04
2	BQ00RC3- 356-1-1-2-1-1-1	1.94	R	143.25	3.34
3	BQ00RC3#32-1-2-2-1-1-1	2.60	MR	188.25	3.03
4	CML-149	3.81	S	188.00	2.93
5	CML-140	2.81	MR	177.50	2.94
6	Obtanpa 180-1-1-2-1-1	2.47	MR	165.00	2.64
7	Pool15QMES-693-B-2-B#-B-B-B	3.10	S	137.50	2.18
8	(GH-132-28)-22-1-6-1-1	2.63	MR	154.75	1.98
9	CML-144/Kulen(F2)-11-2-1-2-1-1	2.19	MR	181.75	2.58
10	CML-176/Kulen(F2)-3-1-1-2-1-1	2.60	MR	139.75	2.26
11	BQ00RC3 #-331-2-1-1-1-1	2.01	R	173.25	3.07
12	CML-142/144-7-b(F2)-9-2-1-2-2-1	2.19	MR	153.00	2.69
13	CML-193	3.36	S	124.25	2.59
14	CML-142	2.53	MR	199.50	2.78
15	Pool15QMES-212-B-2-B-B-B-B-B	4.10	S	118.25	1.86
16	CML-142/144-7-b(F2)-9-2-2-1-1-1-1	2.31	MR	156.50	2.15
17	CML-144/144-7-b-(F2)-4-2-1-1-1-1-1	2.35	MR	174.25	3.23
18	Obtanpa 244-2-1-1-1	3.29	S	130.25	2.08
19	Obtanpa 14-6-1-1	3.41	S	179.00	1.83
20	Obtanpa 118-1-2-2-2-1	3.00	S	178.00	2.06
21	Obtanpa 204-3-2-2-1	2.22	MR	149.25	3.05
22	BQ00RC3# -354-2-1-2-1-1-1	2.72	MR	136.25	2.08
23	CML-176/Kulen(F2)-4-3-1-1-1	1.75	R	162.50	3.18
24	CML-197	2.34	MR	153.75	2.03
25	SC-22	3.25	S	149.50	2.61
	LSD(0.05)	0.42		6.59	ns
	CV (%)	10.91		14.93	23.28

Resistance(R) = ≤2, moderate resistance (MR) = >2 MR ≤3 and Susceptible (S)= >3 or (3-5).

Table 2
Mean of leaf blight reaction, grain yield and plant height of 25 QPM inbred lines tested during 2012 and 2013.

Entry	QPM inbred lines	TLB severity (1-5 scale)	Reaction to TLB	Plant height (cm)	Yield t ha^{-1}
1	CML-144	2.44	MR	165.75	3.82
2	BQ00RC3- 356-1-1-2-1-1-1	1.65	R	139.25	2.74
3	BQ00RC3#32-1-2-2-1-1-1	1.94	R	181.50	2.76
4	CML-149	3.53	S	202.00	2.67
5	CML-140	2.68	MR	186.75	3.77
6	Obtanpa 180-1-1-2-1-1	2.97	MR	174.50	3.15
7	Pool15QMES-693-B-2-B#-B-B-B	4.37	S	127.25	2.21
8	(GH-132-28)-22-1-6-1-1	2.69	MR	145.00	1.67
9	CML-144/Kulen(F2)-11-2-1-2-1-1	2.18	MR	190.00	3.34
10	CML-176/Kulen(F2)-3-1-1-2-1-1	2.47	MR	155.00	2.08
11	BQ00RC3 #-331-2-1-1-1-1	2.81	MR	182.75	3.51
12	CML-142/144-7-b(F2)-9-2-1-2-2-1	2.75	MR	142.25	3.05
13	CML-193	2.87	MR	121.00	2.27
14	CML-142	2.25	MR	193.50	3.64
15	Pool15QMES-212-B-2-B-B-B-B-B	3.78	S	121.75	2.11
16	CML-142/144-7-b(F2)-9-2-2-1-1-1-1	1.68	R	161.75	2.50
17	CML-144/144-7-b-(F2)-4-2-1-1-1-1-1	2.59	MR	176.00	3.12
18	Obtanpa 244-2-1-1-1	2.41	MR	142.25	2.72
19	Obtanpa 14-6-1-1	2.84	MR	183.75	2.82
20	Obtanpa 118-1-2-2-2-1	2.45	MR	183.25	3.17
21	Obtanpa 204-3-2-2-1	1.61	R	146.50	3.38
22	BQ00RC3# -354-2-1-2-1-1-1	2.08	MR	149.25	2.93
23	CML-176/Kulen(F2)-4-3-1-1-1	2.07	MR	160.25	2.28
24	CML-197	3.28	S	158.75	2.01
25	SC-22	2.35		150.25	2.54
	LSD(0.05)	0.5		12.12	0.66
	CV (%)	13.51		5.26	16.72

Resistance(R) = ≤2, moderate resistance (MR) = >2 MR ≤3 and Susceptible (S)= >3 or (3-5).

Table 3
Average rainfall, temperature and relative humidity at Bako during year of 2012 and 2013.

Month	2012 Rainfall (mm)	2012 Temperature (^{0}c)	2012 RH (%)	2013 Rainfall (mm)	2013 Temperature (^{0}c)	2013 RH (%)
January	0.0	20.3	52.2	13.0	21.25	45.0
February	4.4	21.75	47.5	0.1	22	40.0
March	16.2	23	48.9	38.0	21.85	43.0
April	30.7	23.95	62.5	4.0	22	44.0
May	92.8	23	55.6	149.3	21.9	50.0
June	153.3	20.3	66.9	287.8	20.3	54.0
July	138.2	19.5	76.0	340.9	19.4	51.0
August	263.6	19.7	64.0	300.9	19.5	57.0
September	157.5	20.2	74.4	139.8	19.8	53.0
October	6.0	21	50.5	112.7	20.4	57.0
November	17.1	20.5	49.7	44.6	21.4	56.0
December	6.7	21.3	45.7	0.0	19.8	48.0

RH=relative humidity.

3.1. Frequency of inbred lines reaction to TLB and GLS

The proportion of quality protein maize inbred lines that showed resistance, moderate resistance and susceptible reaction to TLB and GLS were varied. No inbred lines were showed immune to TLB and GLS, but the percentage of lines that exhibited resistance to TLB and GLS were 25 and 8.3 % respectively (Figure 1). About 60 % of inbred lines showed moderate resistance to both diseases, indicating that majority of inbred lines are categorized under moderate resistant to leaf blight and grey leaf spot.

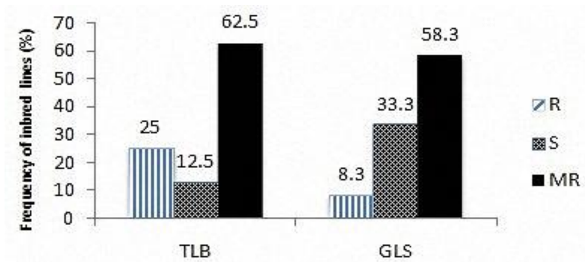

Fig. 1. Frequency of quality protein maize inbred lines with resistance(R), moderate resistance (MR) and susceptible (S) reaction to turcicum leaf blight and grey leaf spot.

3.2. AUDPC analysis

AUDPC was studied to estimate the areas under the actual infection curve. It is expressed in % (scale) per days; accumulation of daily percent infection value. The disease severity was taken four times and once including per se evaluation. Then, the disease progress curve was developed from ten days interval reading and its values were computed to integrate TLB and GLS severity. The higher value of AUDPC showed more susceptible inbred lines than resistant (Figure 2 and 3). Inbred lines of Pool15QMES-612-B-2-B-B-B and CML-149 showed the highest AUDPC value and fast onset disease for TLB and GLS whereas, inbred lines such as BQ00RC3- 356-1-1-2-1-1-1, CML-176/Kulen(F2)-4-3-1-1-1 and Obtanpa 204-3-2-2-1 exhibited low AUPDC value, indicating that the lines could be an excellent source of favorable TLB and GLS resistant inbred line.

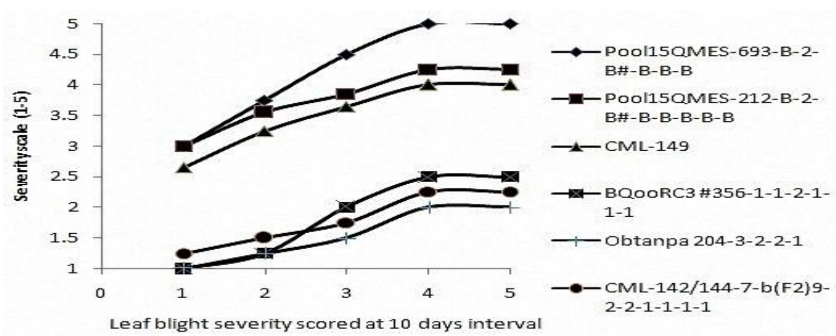

Fig. 2. Average area under disease progress curve for turcicum leaf blight severity on very susceptible vs resistance inbred lines.

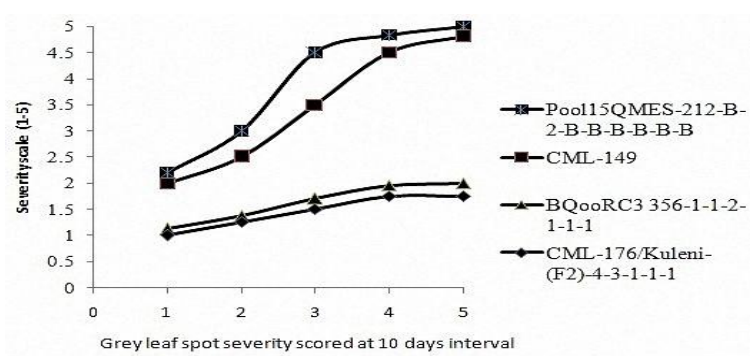

Fig. 3. Average area under disease progress curve for grey leaf spot severity on very susceptible vs resistant inbred lines.

3.3. Correlation analysis

The result of Pearson correlation analysis showed significant and positive similar trend association ($p<0.001$) among disease assessment for TLB and GLS (Table 3 and 4). But severity revealed a highly significant strong positive correlation with AUDPC ($r=0.98$) for TLB than GLS. This might be due to the faster progress of TLB than GLS within conducive environment. However, negative associations were observed between disease parameters and grain yield as well as plant height. For TLB, severity and AUDPC showed significant negative associations with grain yield and plant height. This showed that disease pressure had effect on plant height and grain yield.

Table 4
Pearson correlation among disease parameters and selected agronomic traits in 25 QPM inbred lines tested for TLB reaction.

	Incidence	Severity	AUDPC	Plant height	Yield
Incidence	1				
Severity	0.37**	1			
AUDPC	0.33**	0.98**	1		
Plant height	-0.13ns	-0.22ns	-0.23ns	1	
Yield	-0.15ns	-0.45ns	-0.40ns	0.58**	1

Table 5
Pearson correlation among disease parameters and selected agronomic traits in 25 QPM inbred lines tested for GLS reaction.

	Incidence	Severity	AUDPC	Plant height	Yield
Incidence	1				
Severity	0.58**	1			
AUDPC	0.41**	0.86**	1		
Plant height	-0.04ns	-0.02ns	0.03ns	1	
Yield	-0.12ns	-0.12ns	-0.07ns	0.51**	1

Significant variation was observed in inbred lines by year for TLB and GLS disease severity, indicating the difference in severity of inbred lines in both tested years. Chandrashekara et al. (2014) evaluated thirty five maize inbred lines against Turcicum leaf blight and Mydis leaf blight showed significant difference among inbred lines, inbred lines x year interaction for severity. Similarly, 27 maize population and 38 inbred lines screened for resistance to Turcicum leaf blight and showed significant difference for tested two years (Singh et al. 2014). In present study, highly significant difference observed among inbred lines than inbred lines x year interaction. i.e, Weak significant difference observed among inbred lines x year interaction particularly for TLB. Some inbred lines showed resistant in one year whereas they acted as moderate resistant to respective disease severity on second year. Difference ranking of inbred lines in two years might be due to changing of environment, such as amount of rainfall distribution and relative humidly contributed for severity variation from year to year. Levy (1991) reported that pathogenic fitness and environmental conditions were important factors for disease development.

The study showed that the evaluated quality protein maize inbred lines categorized into three classes; susceptible, moderate and resistance according to their disease reaction based on disease severity rating scale. Majority of quality protein maize inbred lines showed moderate resistance to TLB and GLS. Four inbred lines revealed susceptible to TLB and nine inbred lines showed susceptible to GLS. Whereas four inbred lines revealed resistance to TLB and three inbred lines showed resistance to GLS, indicating that low rate of severity and AUDPC values. The more susceptible inbred lines scored higher severity as well as AUDPC values. It is likely that the more susceptible inbred lines loss their active leaf tissues and resulted in less photosynthetic leaf area, then the plant eventually produce few kernels and/or may contribute to the overall yield loss that negative correlation with disease severity. Similar finding research reported (Singh et al., 2014). These moderate and resistant inbred lines should be screened further for resistance to TLB and GLS across location and years at hotspot areas and or under controlled condition.

As high genetic variation observed among inbred lines for resistance to two diseases, some QPM inbred lines can carry genes for resistance to TLB and GLS disease. Genetic resistance to TLB can be qualitative and/or quantitative nature (Ogiliari et al., 2005), whereas resistance to grey leaf spot is inherited quantitatively (Cromley et al., 2002). In this study, some inbred lines showed low lesion number, with low mean values of severity and reduced area under disease progress curve (AUDPC) values consider to quantitatively resistant. In addition, these inbred lines also carry quality protein that controlled by *opaque 2* genes. To ensure the responsible genes either found on the same or different chromosomes for controlling multiple traits, further study on fine mapping and co-located QTLs should be required.

4. Conclusion

From this study it can be suggested that source of resistant inbred lines such as BQ00RC3- 356-1-1-2-1-1-1, CML-176/Kulen(F2)-4-3-1-1-1 and Obtanpa 204-3-2-2-1 were identified and can be utilized in future maize breeding program for possibility use of incorporating it into cultivars, as source of donor, mapping for resistant genes and varietal resistance development.

Acknowledgments

This research was supported by CIMMYT coordinated Nutrition maize for Ethiopia (NuME) project. The authors greatly acknowledged for their financial supporting. We would like to thank Geta Gelana, Mekides Kebede and Abebech Yilma for their assistance during field trial management and data collection. The authors declare that they have no conflict of interest.

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How to cite this article: Garoma, B., Tilahun, B., Dida, M., Deresa, T., Demissie, G., T/wold, A., Wegary, D., 2016. Evaluation of quality protein maize inbred lines for resistance to Turcicum leaf blight and grey leaf spot disease under field condition at mid altitude sub-humid agro-ecology of Ethiopia. Scientific Journal of Crop Science, 5(11), 137-145.