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Research Article

Reaction of Highland Maize Inbred Lines Against Turcicum Leaf Blight (*Exserohilum turcicum*) Disease Under Artificial Inoculation

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Abstract

Maize is one of the most extensively grown crops in Ethiopia and the rest of the world. However, the maize production in Ethiopia is mainly threatened by the major foliar diseases like turcicum leaf blight (TLB), which cause up to 62.4% yield loss. To develop TLB-resistant maize varieties, it is important to look for resistant inbred lines. Therefore, the objective of this study was to evaluate the reaction of the highland maize inbred lines against TLB disease under artificial inoculation. Eighty inbred lines were obtained from the highland maize breeding program based at Ambo Agricultural Research Center (AARC). The experiment was arranged in a row-column design with two replications. The experiment was established at the AARC TLB screening maize pathology dedicated disease nursery field in the 2022 maize cropping season. The inbred lines were artificially inoculated using TLB disease inoculum. A scale of 0 to 9 was used to score, and the TLB disease severity was recorded four times at ten-day intervals starting from the disease onset. Disease data were analyzed using R-software version 4.0.5. The severity of the TLB disease was significant among the inbred lines. Accordingly, out of 80 genotypes screened for TLB Inbred lines coded as IL1-IL4 were resistant whereas IL5-IL22 were selected as moderately resistant.

Keywords

Maize, Inbred Line, TLB Disease, Inoculation, Reaction

1. Introduction

Maize (*Zea mays* L) is one of the most widely cultivated crops in the world next to wheat and rice [4]. It is a food security crop in many developing countries, including Ethiopia [1]. In Ethiopia, 2.5 million hectares of land were covered by maize with an average production of 10.5 million tons and productivity of 4.2 tons ha^{-1} [4]. Maize accounts for 35% of cereals, followed by wheat, teff, and sorghum with 19, 18, and 15 percent, respectively [2]. However, TLB is the most important maize foliar disease, which causes moderate to severe yield loss in Ethiopia [16, 6]. In Ethiopia, a yield loss due to TLB was estimated at about 62.4% [15]. High relative humidity and low night temperature jointly favor the development of the TLB disease. But the development of the disease mostly depends on weather conditions, stage of plant

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growth, and the level of resistance in maize cultivars [10].

Even though the pathogen attacks all parts of the maize plant, the most conspicuous lesions are mainly found on the foliage. The Lesions affect the leaf parts, which resulting in limited carbohydrate that used to fill the grains [15]. Physiological changes on severely infected leaf parts are like a scorched or burnt appearance, resulting in premature death of leaves [5]. The early occurrence of the disease causes leaf necrosis and premature death of foliage, which finally reduces the grain yield [12]. Therefore, the disease is ranked as the most important problem and is considered as a high research priority of maize in Ethiopia [18]. The use of host plant resistance breeding is the most effective and cost-efficient method to manage the disease than chemical methods. There are different Methods to manage the disease which include cultural practices, chemical and host plant resistance [11]. The use of host plant resistance is the most effective and cost-efficient means of managing this disease. It is, therefore, desirable to identify resistant inbred lines from diverse sources in maize pre-breeding program to improve genetic resistance to this foliar disease. Though early research efforts were made to identify maize germplasm resistant to this disease and utilize them for maize breeding program, subsequent studies for additional sources of maize germplasm need to be screened under artificial inoculation to identify the reaction of advanced new inbred lines against the target disease. This study aimed to evaluate the reaction of highland maize inbred lines against TLB disease.

2. Materials and Methods

2.1. Description of the Study Area

The field experiment was conducted at Ambo Agricultural Research Centre (AARC), a maize pathology dedicated disease screening nursery, during the 2022 main season. AARC is located at an altitude of 2175 meters above sea level, between latitude 8 ℃57' 58" N and longitude 37 °51' 33" E. Ambo ARC is located between 8 ℃57' 58" N latitude and 37 °51' 33" E longitudes and at an altitude of 2175 m.a.s.l. Its annual average temperature and rainfall were 27.5 ℃ and 1077.7 mm, respectively.

2.2. Experimental Materials and Trial Management

The experiment was conducted in the field under artificial epiphytotic conditions for evaluation against TLB. A total of 80 highland maize inbred lines were used from the highland maize breeding program. The experiment was arranged using row-column design with two replications. All the inbred lines were developed by the highland maize breeding program. Each inbred line was planted in a 2 m long plot with 25 cm spacing between hills within a row and 75 cm between rows. Inbred line 142-1-e was used as a susceptible check and spreader rows, which were planted perpendicular to the main plot to enhance TLB disease distribution. Two seeds per hill were planted and later thinned to one plant per hill. For disease inspections, there was 1 m of space between two columns. A 200 kg ha^{-1} nitrogen fertilizer was applied in two splits: half at planting and half at 40 days after emergence. During planting, 150 kg ha-1 diammonium phosphate (DAP) fertilizer was applied. Hoeing, slashing and insect pest management practices were applied as per the recommendations.

2.3. Inoculum Preparation and Inoculation

The inoculum of *E. turcicum* was prepared a year before experimentation by collecting from heavily infected maize fields showing distinct TLB symptoms. TLB-infected leaf powder is effective to induce infection under field condition [3]. The infected leaves were dried under a shade and grounded in to mill about the coarseness of wheat bran and stored in paper bags at a temperature of 4 oC until inoculation date. The pulverized leaves were then dusted into the whorls of the plants according to [17] by placing a pinch of leaf mill (1 teaspoon quantity) when the plant attains 6–8 leaf stages during a moist environment to facilitate spore germination. A second inoculation was made ten days later after the first inoculation to ensure adequate infection. Inoculation was done late in the afternoon to allow a successful infection when dew and ambient temperature were optimal.

2.4. Assessment of TLB Disease Reaction

The first date of disease appearance, disease incidence, disease severity, data were collected starting from the second week of artificial inoculation. The disease severity was recorded four or five times at a ten-day interval. progress of severity of foliar diseases on each inbred lines were quantified at ten days intervals starting from onset of disease until dent stages and the highest or final severity value of each inbred lines was used for statistical analysis. The percentage of diseased plants relative to total plants in a plot was used to calculate disease incidence. Disease severity was rated using a 0 to 9 scale following the [8] procedure, where 0 represents no disease symptom and 9 represents very large disease pustules. where 0= No sign of disease or host response; 1= Chlorotic or necrotic flecks but no sign of sporulating pustules; 2= Very small pustules; strong host incompatibility; low level of disease; 3= Small pustules; obvious host incompatibility; low level of disease, 4= Small pustules, some larger; obvious host incompatibility; increased level of disease; 5= Small and medium sized pustules; some host incompatibility; moderate level of disease; 6= Medium sized pustules; little incompatibility; moderate to higher level of disease; 7= Predominantly large pustules; v. little to nil incompatibility; moderate to high level of disease; 8= Large pustules; very compatible host response; high level of disease; 9= Large pustules; very compatible host response; plants smothered

with disease. The disease reaction was categorized as follows: 0 = Resistant (R); 1 = Resistant (R); 2 = Resistant to Moderately Resistant (RMR); 3 = Moderately Resistant (MR); 4 = Moderately Resistant to Moderately Susceptible (MRMS); 5 = Moderately Susceptible (MS); 6 = Moderately Susceptible to Susceptible (MSS); 7 = Susceptible (S); 8 = Susceptible to Very Susceptible (SVS); 9 = Very Susceptible (VS) based on -Rate of Reference Set (RRS) and checks reaction procedure.

2.5. Data Analysis

Data was analyzed using R software version 4.3.6, and mean separation was performed using R studio Library (lme4).

3. Results and Discussions

The genotype (G), recording time (T), genotype by recording time interaction (GxT), and error variances were significant for the TLB disease severity at each scoring interval (Table 1). shows the amount of residual, or unexplained variance at each recording time. The %variance GxT column summarises the percentage of genetic variance explained at each recording time in the model fitted to the Genotype by Time interaction term in the model.

Table 1. *Analysis of variances for TLB disease severity at different recording time.*

Time	Genotype	Mean TLB	Genetic Variance	Error Variance	%variance GxT
TLB_1	80	1.88	0.188	0.379	67.66
TLB_2	80	3.11	0.669	0.642	100
TLB_3	80	3.89	1.023	0.671	98.38
TLB_4	80	4.15	1.543	0.477	100
TLB_5	80	4.36	1.787	0.462	100

Genetic correlation between assessment times and the residual error correlation between assessment times were generated to indicate the trend of TLB disease severity (Table 2). This table contained two correlation matrices that have been produced in the analysis output; Genetic correlations between assessment times and the other for Residual error correlations between assessment times, pooled across all plots in the experiment. The genetic correlations show the similarity in the genotypes response to TLB disease between recording times, and there is a very strong correlation between each recording time. The residual correlations are weaker than the genetic correlations, and these terms are important in the statistical model as we are making repeated measurements of disease on each plot over time.

Table 2. *Correlation over time for genetic and residual variances.*

Genetic	TLB_1	TLB_2	TLB_3	TLB_4	TLB_5
TLB_1	1	0.82	0.82	0.82	0.82
TLB_2	0.82	1	0.99	1.00	1.00
TLB_3	0.82	0.99	1	0.99	0.99
TLB_4	0.82	0.99	0.99	1	1.00
TLB_5	0.82	0.99	0.99	0.99	1

Residual	TLB_1	TLB_2	TLB_3	TLB_4	TLB_5
TLB_1	1	0.69	0.47	0.38	0.31
TLB_2	0.69	1	0.68	0.55	0.46
TLB_3	0.47	0.68	1	0.81	0.67
TLB_4	0.38	0.55	0.81	1	0.83
TLB_5	0.31	0.46	0.67	0.83	1

The TLB values for each Genotype (G) at each Time (T) assessment are given in (Table 2), and these are predictions of random effects from the statistical model, so they form Best Linear Unbiassed Predictors, or BLUPs. Statistical and quantitative genetics theory show that BLUPs are the best values for selection within a breeding program as they given the highest level of accuracy for genotype selections. Due to the very high correlations between genotypes over time, an overall prediction of TLB for each Genotype was formed, as these values simplify the selection process due to the similarity in Genotype rank position at each assessment time. The times chosen for this overall average are assessment times 4. But assessments 1 and 2 were excluded recording times, due to the low mean TLB value at time 1 and the lower genetic variance for assessment times 1 and 2, as there is less discrimination between genotypes in these assessments.

Table 3. *Inbred lines reaction against TLB under artificial inoculation at Ambo during 2022.*

Entry	Inbred lines Code number	Disease Severity (Scale 0-9)	Resistance Category
1	IL1	1.87	RMR
2	IL2	2.20	RMR
3	IL3	2.26	RMR
4	IL4	2.40	RMR
5	IL5	2.52	MR
6	IL6	2.56	MR
7	IL7	2.65	MR
8	IL8	2.66	MR
9	IL9	2.67	MR
10	IL10	2.68	MR
11	IL11	2.70	MR
12	IL12	2.96	MR
13	IL13	3.00	MR
14	IL14	3.02	MR
15	IL15	3.06	MR
16	IL16	3.10	MR
17	IL17	3.17	MR
18	IL18	3.23	MR
19	IL19	3.33	MR
20	IL20	3.33	MR
21	IL21	3.46	MR
22	IL22	3.49	MR
23	IL23	3.54	MRMS
24	IL24	3.65	MRMS
25	IL25	3.75	MRMS
26	IL26	3.76	MRMS
27	IL27	3.79	MRMS
28	IL28	3.80	MRMS
29	IL29	3.80	MRMS
30	IL30	3.82	MRMS
31	IL31	3.82	MRMS
32	IL32	3.84	MRMS
33	IL33	3.85	MRMS
34	IL34	3.87	MRMS
35	IL35	3.87	MRMS
36	IL36	3.89	MRMS
37	IL37	3.90	MRMS

Entry	Inbred lines Code number	Disease Severity (Scale 0-9)	Resistance Category
38	IL38	3.90	MRMS
39	IL40	3.90	MRMS
40	IL41	3.91	MRMS
41	IL41	4.16	MRMS
42	IL42	4.17	MRMS
43	IL43	4.19	MRMS
44	IL44	4.20	MRMS
45	IL45	4.22	MRMS
46	IL46	4.22	MRMS
47	IL47	4.25	MRMS
48	IL48	4.26	MRMS
49	IL49	4.28	MRMS
50	IL50	4.29	MRMS
51	IL51	4.29	MRMS
52	IL52	4.33	MRMS
53	IL53	4.35	MRMS
54	IL54	4.41	MRMS
55	IL55	4.45	MRMS
56	IL56	4.55	MS
57	IL57	4.56	MS
58	IL58	4.59	MS
59	IL59	4.60	MS
60	IL60	4.63	MS
61	IL61	4.65	MS
62	IL62	4.67	MS
63	IL63	4.80	MS
64	IL64	4.80	MS
65	IL65	4.92	MS
66	IL66	5.02	MS
67	IL67	5.10	MS
68	IL68	5.10	MS
69	IL69	5.45	MS
70	IL70	5.45	MS
71	IL71	5.46	MS
72	IL72	5.50	MS
73	IL73	5.87	MSS
74	IL74	6.00	MSS
75	IL75	6.23	MSS
76	IL76	6.70	S

Entry	Inbred lines Code number	Disease Severity (Scale 0-9)	Resistance Category
77	IL77	6.82	S
78	IL78	6.93	S
79	IL79	7.28	S
80	IL80	7.29	S

IL= Inbred Lines

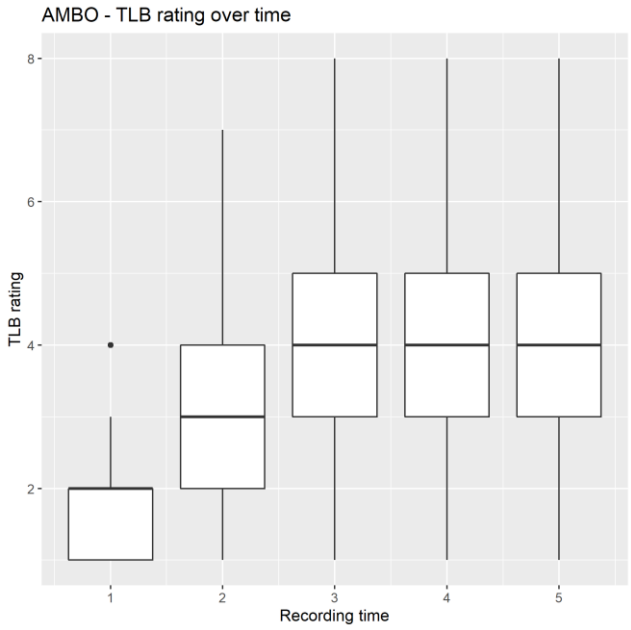

Figure 1. *TLB rating distribution.*

Six distinct disease reaction types were identified from the responses of inbred lines against TLB disease (Table 3). The more susceptible inbred lines most likely lost their active leaf tissues, which led to a reduction in photosynthetic leaf area. As a result, the plant finally produced few kernels and/or might have contributed to the overall yield loss, demonstrating a negative link with the severity of the disease. Similar research results have been reported by [13], who found significant variance between 27 populations of maize and 38 inbred lines that were evaluated for resistance to turcicum leaf blight. Further evaluation for stability to TLB across location and years at hotspot locations and/or under controlled conditions should be conducted on the moderately resistant and resistant inbred lines. In the future, the inbred lines that were selected can also be utilized to create hybrids and composites for TLB resistance breeding projects. These results are consistent with research by [9, 14], which found that different maize germplasms responded differently to diseases. Out of 80 genotypes screened for TLB Inbred lines coded as IL1to IL4 were resistant (RMR) whereas IL5-IL22 were selected as moderately resistant (MR). Moreover, [7], figured out promising sources of resistance to maize GLS disease 10 inbred lines as resistant, some as moderately resistant and as susceptible.

TLB2heatmap: This graph is a heatmap of the TLB rating scores at recording time 3 for each plot in the trial, labelled by Rows and Columns in the field trial. This image is used to explore whether some areas of higher and lower TLB disease occurs spatially across the field, and our statistical analysis methods adjustment for this field trend.

This graph shows the distribution of the rating scores for all plots at each assessment time, and we can use this graph to explore the mean and variance difference between assessment times. The inbred line response against TLB disease was consistently increased over time until the 3rd or 4th disease assessment (Figure 2).

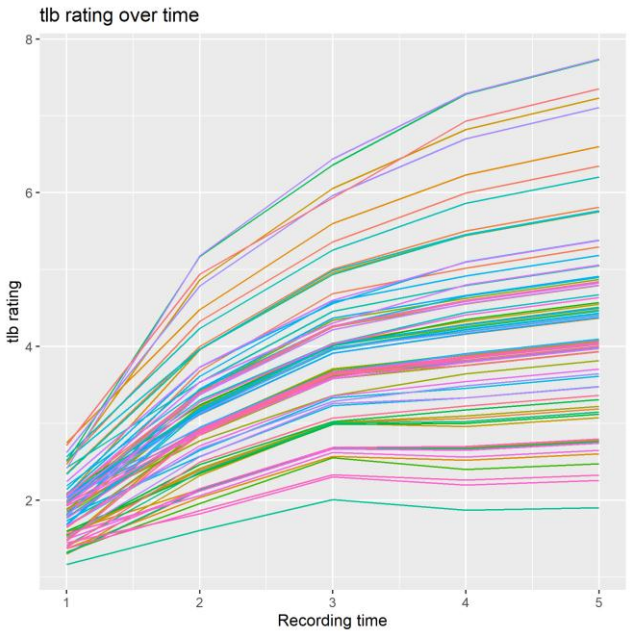

Figure 2. *TLB disease response trend overtime under artificial inoculation.*

This graph shows the genotype response of TLB rating scores over assessment times, and we can use this graph to visualise the increase in disease over time, and the consistency

of genotype ranking over assessment times.

4. Conclusion and Recommendation

The identified TLB resistant inbred lines could be used as a source of resistance in maize breeding programs, and the associated quantitative trait loci to the target resistant gene should be identified. The TLB disease resistant and susceptible inbred lines should be utilized to develop maize hybrid rating reference sets for the breeding programs, which could be used as resistant and susceptible checks. Those inbred lines that are categorized into different resistant category levels would better be supported by molecular techniques to identify the location of loci.

Abbreviations

AARC	Ambo Agricultural Research Center
FAO	Food and Agriculture Organization
CSA	Central Statistical Agency
SSA	Sub-Saharan Africa
TLB	Turcicum Leaf Blight
CIMMYT	Centro Internacional de Mejoramiento de Ma ź y Trigo
m.a.s.l	Meter Above Sea Level
EIAR	Ethiopian Institute of Agricultural Research

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Author Contributions

Midekssa Dida: Conceptualization, Data curation,Formal Analysis, Funding acquisition,Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

Kassahun Sadessa: Data curation

Demissew AbbaKemal: Data curation, Funding acquisition

Misgana Merga: Data curation,Visualization

Gudeta Biratu: Data curation,Visualization

Dufera Tullu: Data curation,Visualization

Conflicts of Interest

The Authors declare no conflicts of interest.

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