Chapter 4

Genetic Variability for Resistance to Leaf Blight and Diversity among Selected Maize Inbred Lines

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Abstract

Maize (*Zea mays* L.) is an important staple food crop in sub-Saharan Africa (SSA). The productivity of the crop is limited partly by the leaf blight disease caused by *Exserohilum turcicum*. In breeding for resistance to leaf blight, the germplasm needs to be well-characterized in order to design efficient breeding programs. This study evaluated the (i) genetic variability among maize inbred lines and (ii) diversity of selected medium to late maturity tropical maize inbred lines for hybrid breeding. Plants of 50 maize inbred lines were artificially inoculated in the field during 2011 and 2012. Disease severity and incidence as well as grain yield were measured. A subset of 20 elite maize inbred lines was genotyped using 20 SSR markers. The germplasm showed significant differences in reaction to leaf blight and were classified as either resistant or intermediate or susceptible. Mean disease severity varied from 2.04 to 3.25. Seven inbred lines were identified as potential sources of resistance to leaf blight for the genetic improvement of maize. The genotyping detected 108 alleles and grouped the inbred lines into five clusters consistent with their pedigrees. The genetic grouping in the source population will be useful in the exploitation of tropical maize breeding programs.

Keywords: leaf blight, inbred line, mid-altitude, maize, pedigree

1. Introduction

Maize (*Zea mays* L.) is an important staple food crop in sub-Saharan Africa (SSA). It is the third most important cereal crop after wheat and rice [1]. It is used for both livestock feeds and human consumption. In SSA, maize accounts for about 70% of the human food [2]. The demand for maize is expected to increase by >90.0% in SSA by 2020 [3]. However, the productivity of the crop is limited by several abiotic and biotic stresses. Among these abiotic factors,

insect pests, such as the stem borers and weevils, cause considerable economic damage on the crop [4, 5]. In addition, fungal diseases such as gray leaf spot (*Cercospora zeae-maydis* Tehon & Daniels), common leaf rust (*Puccinia sorghi* Schr.), and turcicum leaf blight (TLB) (*Exserohilum turcicum*) often pose a serious threat to maize production [6].

In particular, TLB, also known as the northern corn leaf blight, can devastate the crop in high rainfall, humid areas [6, 7]. TLB reduces the seed quality, resulting in diminished germination capacity, low sugar content as well as predisposition to stalk rot [8, 9]. The use of resistant varieties is an inexpensive method for combating TLB [10]. Currently, there are efforts to incorporate durable resistance into maize germplasm particularly in SSA where some commercial varieties as well as elite parental inbred lines are reportedly vulnerable to TLB [11, 12]. For example, in Ethiopia, maize productivity is low (averaging about 2.5 t/ha) in the smallholder production systems partly due to TLB and other stresses. Spurred by the need to enhance maize productivity for farmers, the national maize improvement program in Ethiopia recently embarked on a breeding project aimed at developing leaf blight resistant hybrid varieties that are adapted to the major maize-growing areas of the country which are predominantly in the mid-altitude to subhumid agroecologies [13]. However, hybrid breeding for resistance to leaf blight requires knowledge of the genetic variability of the germplasm in terms of its reaction to TLB as well as its characterization into distinct genetic groups that can be hybridized in order to exploit heterosis.

The variability in the host (maize) plant resistance to the disease occurs in either the qualitative or the quantitative form. The qualitative form of resistance is race specific and is governed by a single or few genes but the quantitative form of resistance is race nonspecific and polygenic [14, 15]. In addition, qualitative resistance can break down due to the emergence of new virulent races of the pathogen through genetic mutation and recombination events [12, 15]. The pathogen *E. turcicum* exhibits a wide range of variability [16], and new races are capable of overcoming previously resistant varieties [7]. For instance, the resistance conferred by the Htn gene(s) is characterized by chlorotic and necrotic lesions or lesions surrounded by a yellow-to-light-brown margin (without spore formation), which limits the growth and spread of the disease [12, 14]. In contrast, the resistance conferred by Htn gene is expressed as a delay in lesion formation typically showing at the pollination stage [17, 18]. Lesion size, together with area under disease progress curve (AUDPC) as well as disease severity and incidence, are commonly used in evaluating maize genotypes for resistance to TLB [19, 20]. However, phenotypic evaluations in conventional breeding approaches are unable to detect the presence of favorable alleles in the germplasm. Therefore, marker-assisted selection and DNA fingerprinting techniques have been effectively used to increase the efficiency of conventional breeding, particularly the time required for developing new improved varieties in maize [12].

The presence of discrete genetic groups among inbred lines is attributed to increased allelic diversity which is useful in optimizing hybrid vigor. Assigning inbred lines into well-differentiated genetic clusters can reduce the creation and evaluation of many undesirable crosses [21]. Molecular markers assist in characterizing inbred lines and in establishing distinct clusters of genotypes based on genetic diversity, which is useful in maize breeding programs [22, 23]. Molecular markers were applied successfully to allocate maize germplasm into heterotic

groups [24–26]. In a study which compared different markers for their effectiveness in estimating genetic grouping among maize inbred lines, SSR markers revealed the highest level of polymorphism due to their codominant nature and high number of alleles per locus [27]. Therefore, the study reported in this chapter was designed to evaluate the (i) genetic variability in reaction to TLB among maize inbred lines under field conditions and (ii) diversity of selected medium to late maturity tropical maize inbred lines for hybrid breeding using selected SSR markers.

2. Materials and methods

2.1. Field evaluation

2.1.1. Germplasm and testing location

Fifty inbred lines were used in the study. The lines were adapted to the mid-altitude agroecologies in Ethiopia and were obtained from the national maize research program and the international maize and wheat improvement center (CIMMYT). Inbred line CML-197, which was obtained from CIMMYT, served as susceptible check (**Table 1**). The field trial was conducted at Bako (37°09′ E; 09°06′ N; 1650 m above sea level). It receives approximately 1200 mm rainfall annually (**Table 2**) and is representative of the mid-altitude subhumid agroecological region in Ethiopia.

2.1.2. Field experiments

Inbred lines were evaluated using the lattice design with three replications. Trials were conducted for two consecutive seasons (in 2011 and 2012) during the main rainy season (May to September) in Ethiopia. The seed of each genotype was planted manually in the field in a two-row plot 5.1 m long × 0.75 m at 30.0 cm intra-row spacing. Phosphorus (in the form of diammonium phosphate) was applied once at planting at 100.0 kg/ha. Nitrogen fertilizer (in the form of urea) was applied at 100.0 kg/ha in two splits with 50% at planting and the rest at 37 days after emergence. Standard maize trial management practices were applied throughout each season at the location.

2.1.3. Leaf blight inoculum collection, preparation and inoculation

Isolates of *E. turcicum* were obtained from diseased maize leaf samples that were collected from fields where the disease is prevalent. The infected leaves were excised into small sections (approx. 1.0 cm^2 each) prior to surface sterilization using 2.5% Sodium hypochlorite for about 3 min and subsequently rinsed with sterile distilled water and blot-dried before plating on PDA in petri dishes for incubation at room temperature for 3–4 days. Pure cultures were prepared by subculturing from the isolation plates followed by incubation for 7–10 days in order to obtain sufficient growth. The inoculum was prepared by flooding the cultures with sterile distilled water and scrapping the surface with microscopic slides to dislodge the conidia and then filtered using cheese cloth after which the concentration of the conidia suspension was adjusted to approximately 105 conidia per milliliter using a hemocytometer [28].

Entry	Pedigree	Origin
1	CML 202	CIMMYT
2	CML 442	CIMMYT
3	CML 312	CIMMYT
4	CML 464	CIMMYT
5	Gibe-1-91-1-1-1-1	BAKO
6	CML 445	CIMMYT
7	CML 443	CIMMYT
8	CML 197	CIMMYT
9	A-7033	BAKO
10	CML 205/208//202-X-2-1-2-B-B-B	BAKO
11	CML 395	CIMMYT
12	F-7215	BAKO
13	DE-78-Z-126-3-5-5-1-1	BAKO
14	30H83-7-1-1-1-2-1	BAKO
15	I100E-1-9-1-1-1-1-1	BAKO
16	SZYNA99F2-81-4-3-1	BAKO
17	X1264DW-1-2-1-1-1	BAKO
18	124-b (113)	BAKO
19	SC22	BAKO
20	SC715-121-1-3	BAKO

Table 1. The pedigree and origin of maize inbred lines that were evaluated for diversity using SSR markers.

Maize plants growing in the field were inoculated at the four to six leaf growth stages during the middle of the main rainy season (mid-July) in Ethiopia. The inoculations were accomplished by spraying (manually, with the aid of an atomizer) the maize plant with the conidia suspension until runoff after which fine mist water was sprayed over the inoculated plants in order to create conducive conditions for disease development. This inoculation procedure was carried out during the evening when there was sufficient moisture in the air.

2.1.4. Data collection and analysis

In each season, the disease was visually assessed in the field 2–3 weeks after inoculation. Ten randomly selected plants were tagged and used for successive disease assessments. Plants were rated at 10-day intervals for percent incidence, lesion length, and lesion width. In order to determine the rate of lesion expansion, 2 lesions out of the 10 plants were measured (and marked for subsequent tracing) at 10-day intervals.

Month	2011			2012		
	Rainfall (mm)	Temperature (C⁰)	RH (%)	Rainfall (mm)	Temperature (C⁰)	RH (%)
January	15.90	20.20	58.00	0.00	20.40	52.70
February	2.00	20.90	50.90	4.40	21.80	47.50
March	58.80	21.90	53.90	16.20	23.00	48.90
April	68.10	20.40	52.40	30.70	24.00	62.50
May	222.20	21.30	58.50	92.8	23.00	55.60
June	295.00	19.90	67.50	153.30	20.20	66.90
July	224.10	19.30	69.30	138.20	19.50	76.00
August	294.60	19.10	75.60	263.60	19.70	64.00
September	131.30	20.00	65.90	157.50	20.10	74.40
October	53.20	20.20	59.80	6.00	21.00	50.50
November	60.10	20.00	59.80	17.10	20.30	49.70
December	0.00	19.80	54.50	6.70	21.5	45.70
Total	1425.30			886.50		

RH = relative humidity.

Table 2. Average monthly rainfall, temperature, and relative humidity at Bako during the 2011 and 2012 cropping seasons.

Disease severity was scored using a scale of 1–5 where:

1.0 = very slightly infected, one or two restricted lesions on lower leaves or trace.

2.0 = slight-to-moderate infection on lower leaves, a few scattered lesions on lower leaves.

3.0 = abundant lesions on lower leaves, a few on middle leaves.

4.0 = abundant lesions on lower and middle leaves extending to upper leaves.

5.0 = abundant lesions on all leaves, plant may be prematurely killed by blight.

The AUDPC was determined from the disease severity scores obtained in both seasons. The AUDPC parameter was calculated using Eq. (1) below as described previously [29]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})(t_{i+1} - t_i)}{2} \quad (1)$$

where n = number of observations, t_i = number of days after planting for the i^{th} disease assessment, and y_i = disease severity.

The parameter was used to quantify the epidemic from the beginning to the peak of the disease. The grain yield was calculated using the average shelling percentage of 80% adjusted to 12.5% moisture. Data sets of the quantitative measurements from individual trials were subjected to standard analysis of variance procedures using the GenStat release 14.2 computer software program [30].

2.2. Marker evaluation

2.2.1. Germplasm

Twenty maize inbred lines were used in the study. Eight of these inbred lines were originally developed for the mid-altitude and subhumid agroecologies at CIMMYT, whereas the remainder was developed by the local Ethiopian maize research program and was well adapted to mid-altitude areas. The local inbred lines were developed from three heterotic groups (that are commonly used in the country) namely Kitale synthetic II, Ecuador 573, and Pool 9A.

2.2.2. DNA sampling

DNA was collected from 3- to 4-week-old plants (tagged for identification), using Whatman FTA cards and the modified protocol of FTA paper technology [31]. Ten DNA samples from each of the 20 inbred lines were then bulked (in order to eliminate variation within each entry) and used for the diversity analysis at the INCOTEC-PROTEIOS laboratory in South Africa (Incotec, SA Pty. Ltd., South Africa) utilizing 20 SSR markers. PCR products of all of the 20 primers were fluorescently labeled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). Analysis was performed using GeneMapper 4.1. The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA). The polymorphism information content (PIC) was calculated as:

$$PIC = 1 - \sum fi.$$

where fi is the frequency of the i^{th} allele [32].

3. Results and discussion

3.1. Disease development and severity

Disease ratings were significantly different among the 50 inbred lines ($P < 0.001$), and 11 were classified as resistant, 26 as intermediate, whereas the remainder was classified as susceptible (**Tables 3** and **4**). The resistant inbred lines (e.g., 136-a and 142-1-e) attained lower disease severity scores compared to the susceptible check CML-197 (**Tables 3** and **4**). No accession was immune to the disease. In addition, there were highly significant ($P < 0.001$) differences for lesion length among inbred lines in both 2011 and 2012. The inbred lines Pool9A-4-4-1-1-1, SZSYNA-99-F2-803-4-1, and CML 197 showed comparatively larger lesion lengths, whereas the lesion length of CML 202 and CML 312 showed consistently small lesion lengths over the two seasons. Resistance to *E. turcicum* in maize germplasm was previously associated with a reduction in percent leaf area as well as small lesions [33].

The significant differences detected among genotypes in this study across the 2 years (cropping seasons) was attributable to a range of factors such as favorable climatic conditions, the inoculation method employed, and proper disease rating. In other studies, the development of NLB was attributed to pathogenic fitness and environmental conditions [34]. In Ethiopia,

No.	Inbred line	DSS	Reaction type	Incidence (%)	Lesion length (cm)	AUDPC	TSW	Yield (t ha^{-1})
1	CML 202	2.00	R	46.81	9.88	408.3	223.3	2.22
2	CML442	2.734	I	78.43	13.40	612.5	223.3	2.40
3	CML 312	2.413	I	61.52	10.35	385.0	276.7	3.03
4	CML 464	2.210	I	55.64	13.82	595.0	223.3	3.79
5	Gibe-1-91-1-1-1-1	2.534	I	71.32	14.57	408.3	321.7	2.90
6	CML 445	2.523	I	65.20	14.02	571.7	213.3	3.34
7	CML 443	2.934	S	69.61	13.48	595.0	211.7	2.07
8	Gibe-1-158-1-1-1-1	2.496	I	66.42	11.37	507.5	281.7	3.43
9	A7033	2.881	S	68.63	15.37	641.7	273.3	2.58
10	(CML 205/CML208//CML 202)-X2-1-2-B-B-B	2.696	S	83.58	15.88	571.7	300.0	5.60
11	CML395	2.388	I	71.08	14.07	420.0	338.3	4.96
12	CML 444	2.526	I	69.12	18.28	443.3	260.0	2.95
13	DE-78-Z-126-3-2-2-1-1	2.688	S	67.89	14.48	536.7	280.0	4.14
14	30H83-7-1-1-1-2-1	2.00	R	53.19	10.90	495.8	210.0	3.14
15	ILoo'E-1-9-1-1-1-1-1	2.00	R	56.62	15.62	420.0	346.7	4.83
16	SZSYNA-99-F2-814-3-1	2.00	R	42.40	10.77	466.7	315.0	2.46
17	X1264DW-1-2-1-1-1-1	2.889	S	70.59	15.00	571.7	213.3	1.94
18	124-b(113)	2.559	I	59.80	15.27	606.7	365.0	3.53
19	SC22	2.760	S	85.78	14.72	501.7	271.7	3.56
20	SC-715-1211-3	2.466	I	67.40	13.47	396.7	336.7	3.45
21	DE-105-Z-126-30-1-2-2-1	2.00	R	61.27	14.55	420.0	235.0	2.89
22	Gibe-1-20-2-2-1-1	2.663	S	69.12	18.78	501.7	301.7	2.62
23	Kuleni-0080-4-2-1-1-1-1	2.022	I	61.52	16.38	449.2	326.7	3.72
24	Pool9A-4-4-1-1-1	2.677	S	68.63	21.35	670.8	288.3	4.85
25	30H83-5-1-4-2-1-1	2.486	I	63.97	16.27	484.2	308.3	4.27
26	Iloo'E-5-5-3-1	2.639	I	74.26	13.48	560.0	328.3	4.41
27	SZSYNA-99-F2-2-7-3-1-1	2.00	R	57.35	11.77	478.3	206.7	2.77
28	SC-715-154-1-1	2.206	I	65.20	11.97	402.5	280.0	5.89
29	BH6609(F2)-10-2-1-2-1	2.333	I	61.76	11.83	402.5	300.0	3.98
30	143-5-I	2.305	I	60.29	15.48	420.0	325.0	6.84
31	144-7-b	1.90	R	58.09	12.87	385.0	330.0	4.45

No.	Inbred line	DSS	Reaction type	Incidence (%)	Lesion length (cm)	AUDPC	TSW	Yield (t ha^{-1})
32	(LZ-955459/LZ955357)-B-1-B-B	2.369	I	67.16	12.20	431.7	256.7	2.98
33	139-5-j	2.00	R	53.43	13.78	385.0	258.3	2.56
34	30H83-56-1-1-1-1-1	2.351	I	57.35	10.22	495.8	205.0	3.57
35	SZSYNA-99-F2-80-3-4-1	2.653	I	73.53	20.05	525.0	293.3	3.15
36	124-b(109)	2.901	S	81.86	15.48	536.7	310.0	5.54
37	F7215	2.417	I	63.73	14.72	455.0	393.3	3.86
38	136-a	1.80	R	51.47	13.82	238.0	396.7	4.41
39	DE-78-Z-126-3-2-1-2-1	2.631	I	70.83	14.85	595.0	286.7	3.83
40	Gibe-1-186-2-2-1	2.549	I	51.96	14.88	350.0	373.3	2.70
41	Pool9A-128-5-1-1-1	2.718	I	71.43	13.12	595.0	278.3	2.45
42	30H83-7-1-5-1-1-1-1	2.00	R	52.45	12.05	379.2	220.0	2.60
43	SZSYNA-99-F2-3-6-2-1	2.587	I	70.83	12.33	618.3	256.7	2.36
44	SC-715-13-2-1	2.434	I	61.76	12.87	420.0	248.3	2.34
45	SC-22-430(63)	3.033	S	80.15	11.57	478.3	311.7	2.48
46	Kuleni-C1-101-1-1-1	3.028	S	75.49	17.07	700.0	258.3	2.84
47	Iloo'E-1-12-4-1-1	2.355	I	51.96	10.30	443.3	276.7	2.43
48	(DRB-F2-60-1-2)-B-1-B-B-B	2.791	S	75.98	16.23	600.8	270.0	2.66
49	142-1-e	2.00	R	62.01	15.02	595.0	323.3	3.94
50	CML 197	3.028	S	88.48	18.07	525.0	271.7	50
	LSD	0.4260	—	18.513	7.504	129.93	72.64	1.465
	Pr > f	**	—	**	**	**	**	**
	CV (%)	3.3	—	17.6	10.6	16.2	15.9	25
	Overall mean	2.486	—	65.49	14.16	493.9	284.1	3.52

DSS = disease severity score (0.00–5.00); R = resistant (1.0–2.00); I = intermediate (2.10–2.50); susceptible (2.51–5.00); and TSW = thousand seed weight.

** = Significant at 0.05 and 0.01 probability levels, respectively.

Table 3. Maize leaf blight reactions, grain yield, and thousand seed weight of 50 inbred lines tested during 2011 at Bako research Center in Ethiopia.

the disease infection and epidemics in maize occur largely during the main production season particularly in the wet and humid areas. Therefore, breeding for resistance to the disease in such areas is critical.

Disease severity scores in both cropping seasons were significantly different ($P < 0.01$) (**Tables 3** and **4**). During the two seasons, the lowest severity scores were observed for the inbred lines CML 202, 144-7-b, and 142-1-e. In contrast, relatively high severity scores were

No.	Inbred line	DSS	Reaction type	Incidence (%)	Lesion length (cm)	Lesion width (cm)	TSW	Yield (t/ha)
1	CML 202	2.39	R	40.69	12.00	1.33	173	2.15
2	CML442	2.69	S	72.55	13.67	1.67	210	2.67
3	CML 312	2.47	I	64.22	12.33	0.83	220	3.25
4	CML 464	1.92	R	52.45	13.00	1.03	207	3.01
5	Gibe-1-91-1-1-1-1	2.53	S	74.02	20.33	1.50	260	3.76
6	CML 445	2.42	I	65.69	14.33	1.17	207	3.36
7	CML 443	2.97	S	64.71	13.00	1.00	183	1.93
8	Gibe-1-158-1-1-1-1	2.39	I	58.82	12.00	1.57	270	2.93
9	A7033	2.81	S	58.82	13.33	1.33	240	2.41
10	(CML 205/CML208//CML 202) -X2-1-2-B-B-B	2.64	S	86.76	22.33	1.83	237	5.83
11	CML395	2.33	I	70.59	21.67	2.00	313	5.04
12	CML 444	2.61	S	65.69	23.33	2.00	230	2.67
13	DE-78-Z-126-3-2-2-1-1	2.67	S	65.20	18.33	1.50	250	2.6
14	30H83-71-1-1-2-1	1.89	R	39.71	13.33	1.67	187	2.91
15	ILoo'E-1-9-1-1-1-1-1	2.14	I	54.41	23.67	1.33	293	4.62
16	SZSYNA-99-F2-814-3-1	1.69	R	27.94	14.00	1.00	257	2.01
17	X1264DW-1-2-1-1-1-1	2.36	I	72.55	19.33	1.33	183	1.92
18	124-b(113)	2.34	I	45.10	16.33	1.67	303	3.13
19	SC22	2.05	I	91.18	16.67	2.00	230	3.14
20	SC-715-121-1-3	3.07	S	70.10	16.00	2.17	270	2.64
21	DE-105-Z-126-30-1-2-2-1	1.57	R	69.61	20.67	1.83	230	3.42
22	Gibe-1-20-2-2-1-1	2.48	I	77.45	25.00	1.33	287	3.08
23	Kuleni-0080-4-2-1-1-1-1	4.29	I	58.33	20.33	1.33	283	3.8
24	Pool9A-4-4-1-1-1	2.42	I	62.25	25.67	1.67	270	5.35
25	30H83-51-4-2-1-1	2.47	I	67.16	22.00	2.00	260	4.39
26	Iloo'E-5-5-3-1	2.61	S	77.94	14.33	1.00	260	3.5
27	SZSYNA-99-F2-2-7-3-1-1	2.22	I	55.88	15.00	1.50	170	2.88
28	SC-715-154-1-1	2.14	I	73.53	15.67	1.83	217	5.01
29	BH6609(F2)-10-2-1-2-1	2.33	I	54.90	15.33	1.53	243	1.74
30	143-5-I	2.08	I	51.96	18.00	2.17	273	5.95
31	144-7-b	1.89	R	59.31	18.00	1.00	333	3.47
32	(LZ-955459/LZ955357)-B-1-B-B	2.28	I	67.65	16.67	1.33	200	2.72

No.	Inbred line	DSS	Reaction type	Incidence (%)	Lesion length (cm)	Lesion width (cm)	TSW	Yield (t/ha)
33	139-5-j	2.03	I	44.12	19.33	1.07	237	1.8
34	30H83-561-1-1-1-1	2.22	I	50.98	13.00	0.83	203	2.93
35	SZSYNA-99-F2-80-3-4-1	2.81	S	76.47	27.67	1.83	237	3.38
36	124-b(109)	3.03	S	82.84	19.33	1.33	270	5.59
37	F7215	2.5	I	62.75	21.33	1.07	273	2.92
38	136-a	1.75	R	42.16	17.33	1.33	363	3.62
39	DE-78-Z-126-3-2-1-2-1	2.56	S	65.20	19.00	1.00	237	3.54
40	Gibe-1-186-2-2-1	2.81	S	49.02	19.33	1.33	360	2.3
41	Pool9A-128-5-1-1-1	2.67	S	68.36	15.33	1.67	223	2.87
42	30H83-71-5-1-1-1-1	1.94	R	50.00	16.67	2.00	193	2.84
43	SZSYNA-99-F2-3-6-2-1	2.28	I	63.24	15.33	2.00	233	2.36
44	SC-715-13-2-1	2.47	I	66.67	16.33	1.17	210	2.34
45	SC-22-430(63)	3.08	S	89.71	14.33	1.67	227	2.05
46	Kuleni-C1-101-1-1-1	2.81	S	68.63	7.67	1.17	237	3.07
47	Iloo'E-1-12-4-1-1	2.17	I	33.33	10.67	1.33	243	1.57
48	(DRB-F2-60-1-2)-B-1-B-B-B	2.72	S	67.65	21.00	2.00	230	2.48
49	142-1-e	1.81	R	49.51	16.33	1.50	287	4.29
50	CML 197	3.39	S	98.53	19.67	2.67	213	4.41
	LSD	0.396	—	19.159	9.013	0.902	47	1.3
	Pr > f	**	—	**	**	*	**	**
	CV (%)	10.1	—	18.8	32.1	36.9	11.9	24.7
	Overall mean	2.43	—	62.93	17.31	1.51	245	3.23

DSS = disease severity score (0.00–5.00); R = resistant (1.0–2.00); I = intermediate (2.10–2.50); susceptible (2.51–5.00); and TSW = thousand seed weight.

*; ** = Significant at 0.05 and 0.01 probability levels, respectively.

Table 4. Maize leaf blight reactions, grain yield, and thousand seed weight of 50 inbred lines tested during 2012 at Bako research Center in Ethiopia.

observed for CML 197, Kuleni-C1-101-1-1-1, and SC-22-430(63), suggesting that they were susceptible to the disease. The final severity score and AUDPC values provided sufficient estimation of the reaction of the inbred lines to *E. turcicum*. The inbred lines that were classified as resistant showed significantly lower AUDPC values than the susceptible ones (**Figure 1**). Furthermore, susceptible inbred lines tended to show a rapid increase in severity of the disease compared with the resistant lines culminating in higher severity scores toward maturity unlike the resistant ones. The severity of the disease was slightly higher in 2011 than 2012 (**Tables 3** and **4**). This was likely due to the low rainfall that was received at flowering in 2012,

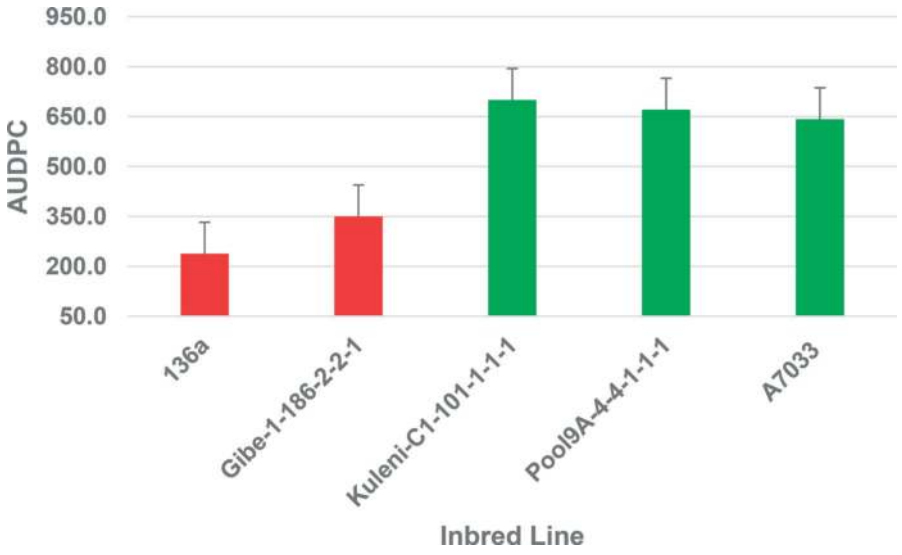

Figure 1. Area under disease progress curve for resistant (red) and susceptible (green) maize inbred lines inoculated with isolates of *E. turcicum* in the field.

which was not conducive for the development of the disease. Nonetheless, the environmental conditions were generally favorable for leaf blight development during the two testing seasons. Previous studies involving leaf blight showed that the dropper inoculation was efficient and minimized the chances of disease escape from evaluation [9]. In this study, the inoculation technique was easy to employ and reliable. There were clear differences between resistant and susceptible genotypes, and at the flowering stage, the later genotypes exhibited a moderate increase in diseased leaf tissue. In some cases, relatively less susceptible individual genotypes were identifiable. The selection of such less susceptible genotypes can result in the accumulations of minor genes that can elevate the level of field resistance [35–37].

3.2. Genetic polymorphism

The twenty SSR primers identified 108 alleles among the 20 maize inbred lines. Between 1 to 11 alleles were scored across the SSR loci (**Table 5**). Two loci (Phi 037, Umc1296) each revealed only a single allele. The maximum number of alleles (11) was detected at the Bnlg 2190 locus. The maximum PIC estimated for all loci was 0.8028 with a mean of 0.54 (**Table 5**). The expected heterozygosity (He) values, as a measure of allelic diversity at a locus, varied from 0.0000 to 0.8395 with an average of 0.5774. These values were well correlated with the number of alleles. Ten SSR loci (Umc1568, Nc003, Umc2214, Umc2038, Phi085, Umc1153, Bnlg238, Phi054, Bnlg2190, and Bnlg240) attained a PIC value >0.6, which indicated their potential to detect differences between the inbred lines.

The genetic diversity of the germplasm is one of the most important factors limiting the number of alleles identified per microsatellite locus during screening. However, other factors

SSR locus	Repeat types	Bin number	Number alleles	PIC value	He
Umc1568	TCG	1.02	6	0.6833	0.7250
Bnlg176	___	1.03	4	0.3092	0.3378
Bnlg182	___	1.03	6	0.5510	0.5888
Phi 037	AG	1.08	1	0.0000	0.0000
Bnlg 108	___	2.04	4	0.4253	0.4637
Nc003	AG	2.06	6	0.7429	0.7778
Umc2214	CTT	2.1	8	0.7075	0.7350
Bnlg602	___	3.04	6	0.4701	0.4900
Umc2038	GAC	4.06	4	0.6311	0.6925
Phi085	AACGC	5.06	4	0.6695	0.7222
Umc1153	TCA	5.09	8	0.6683	0.7036
Bnlg238	___	6	8	0.7689	0.7922
Umc1296	GGT	6.07	1	0.0000	0.0000
PhiI015	AAAC	8.08	7	0.5112	0.5938
Umc1367	CTG	9.05	2	0.4949	0.5850
Phi054	AG	10.03	6	0.8028	0.8255
Umc1677	GGC	10.05	7	0.3047	0.3750
Bnlg2190	AG	10.06	11	0.8224	0.8395
Bnlg240		8.06	7	0.7777	0.8025
umc2361	CCT	8.06	2	0.3743	0.4986

PIC = polymorphic information content and He = heterozygosity.

Table 5. Information about the 20 SSR loci used in this study.

such as the number of SSR loci and repeat types as well as the methodologies employed for the detection of polymorphic markers have been reported to influence allelic differences. In this work, the mean number of alleles (5.4) was in agreement with those reported in maize [38]. Similarly, values of number of SSR loci used in this study closely agreed with the findings reported previously [13, 39]. In addition, the mean PIC value determined in the present investigation was in agreement with the findings that were obtained in earlier studies that involving the use of SSR markers on maize inbred lines [40, 41]. The PIC value demonstrates the usefulness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships. The dinucleotide SSR loci (phi054, nc003, bnlg2190) identified the largest mean number of alleles (7.67) and mean PIC (0.79), as compared to tri-, tetra-, and penta-nucleotide repeats in the study, which was in close agreement with previous observations in maize [40, 42].

In this study, automated analysis was used for screening the microsatellites, resolving allelic variation better than using gel electrophoretic analysis for instance. This may be particularly

important for SSR loci containing dinucleotide repeats whose amplification products are between 130 and 200 bp, because PCR products differing by two base pairs cannot be resolved with agarose gel electrophoresis [40, 43].

The ability to measure genetic distances between the inbred lines that reflect pedigree relationship ensures a more stringent evaluation of the adequacy of marker profile data; hence, the minimum genetic distance which was revealed between CML-202 and I100E-1-9-1-1-1-1-1-1 (0.28) was a good indication, confirming the power of SSR markers to distinguish closely related inbred lines. Similar findings were reported for maize inbred lines using SSR markers [44–46].

3.3. Cluster analysis

The dendrogram obtained using the UPGMA clustering algorithm based on SSR data matrices grouped the inbred lines into five categories (**Figure 2**). This information, in combination with the pedigree records and combining ability tests, will be valuable for selecting (or identifying) optimal crosses and assigning inbred lines into heterotic groups. The greatest distance was found between the cluster containing the inbred line CML-202 line and the cluster of the inbred line Gibe-1-91-1-1-1-1. Cluster I consisted of inbred lines that are adapted to mid-altitude as well as some originating from CIMMYT. Most of the mid-altitude inbred lines in this group originated from the heterotic group Kitale Synthetic II and constitute the largest group in the cluster. In Cluster II, CIMMYT inbred lines CML312

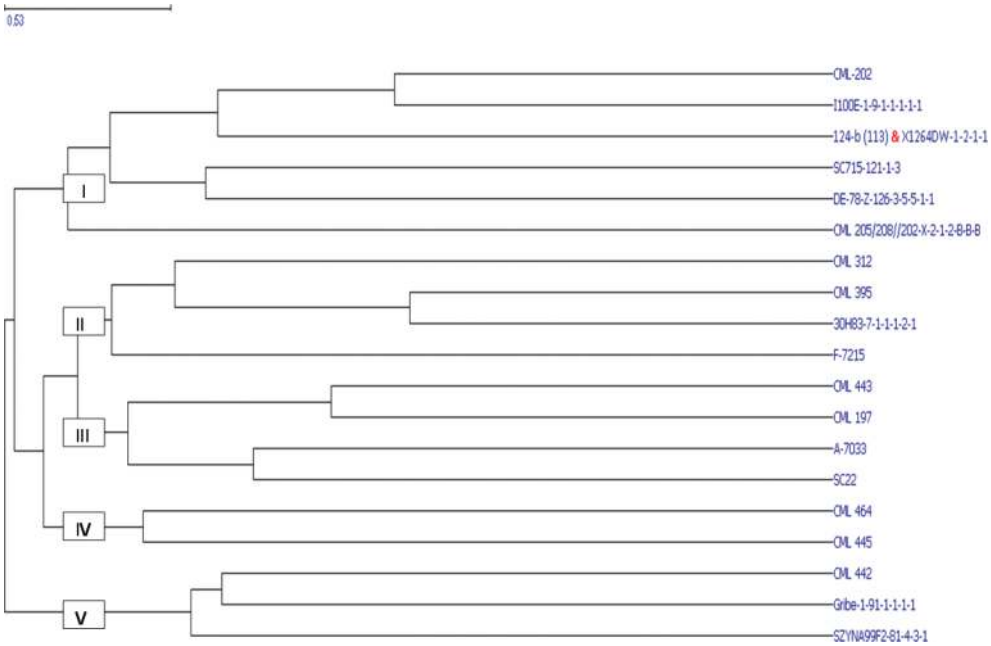

Figure 2. Dendrogram showing genetic relationship among 20 maize inbred lines tested using 20 SSR markers. The five clusters among the inbred lines are denoted from I to V.

and CML395 were grouped along with two local inbred lines, with two subdivisions in the main group. Cluster III contained two major subgroups, one containing CIMMYT inbred lines and the other containing local inbred lines. In terms of pedigree, these inbred lines are closely related and belong to the heterotic group AB, thus supporting the observation of a positive relationship between the pedigree and the SSR marker groupings in this study. In another cluster, two CIMMYT inbred lines (CML-443 and CML-197) were grouped closely, as revealed on the UPGMA dendrogram (**Figure 2**). These two inbred lines were also grouped in the same heterotic groups A and AB, based on their heterosis. Cluster V consisted of one CIMMYT inbred line and two locally adapted mid-altitude inbred lines. The separation of these elite mid-altitude maize inbred lines into genetically distinct groups may be associated with high heterotic response and increased combining ability useful for hybrid development.

The majority of the inbred lines (60%) that were evaluated in this study were previously developed by the national maize breeding program in Ethiopia. Because of the potential of encountering genetic admixtures or incomplete pedigree records in breeding programs, discrepancies in classification of germplasm may occur when comparing molecular results with classification based on pedigree relatedness. The effects of selection, genetic drift, and mutation may contribute to these discrepancies. The technique of clustering inbred lines can create apparent discrepancies, when one inbred line that is related to two inbred lines from separate clusters is then grouped with the inbred to which it is more closely related [40, 47]. Nonetheless, the SSR markers separated most of the inbred lines into distinguishable clusters, which generally agreed with the existing pedigree records and the findings that were reported previously [27, 42].

4. Conclusions

The inbred lines showed significant differences in reaction to the leaf blight disease and were classified into three categories namely resistant, intermediate, or susceptible. The mean disease severity and upper leaf area infection varied from 2.04 to 3.25 and 3.3% to 100% respectively. Seven inbred lines were identified as potential sources of resistance to leaf blight for the genetic improvement of maize under the mid-altitude agroecology in Ethiopia. The genotyping detected 108 alleles and grouped the inbred lines into five clusters consistent with their pedigrees. The genetic grouping present in the population as determined in this study will be useful in the exploitation of tropical germplasm for hybrid maize breeding programs. The inbred lines that were identified as resistant to leaf blight can be considered as source material for disease resistance under the mid-altitude agroecological conditions in Ethiopia. The genetic grouping of the inbred lines was valuable information for future maize breeding programs. The use of SSR markers was able to provide complimentary information regarding the relatedness of the elite inbred lines that were evaluated. The high PIC value across all loci was strong evidence confirming the potential for SSR markers to discriminate between inbred lines of diverse sources and even between closely related genotypes. A number of loci that were identified with high PIC values indicated their usefulness for diversity analysis of maize

inbred lines. The approach used in the study enables clear differentiation between inbred lines and their classification into distinct groups based on genetic distance estimates generated through selected polymorphic SSR primers.

There will be merit in establishing resistance breeding program aimed at developing varieties with increased adult plant resistance to TLB in Ethiopia. Such varieties offer one of the most effective and affordable ways to overcome the problem of leaf diseases of maize in the mid-altitude agroecology in Ethiopia and similar environments in SSA. Therefore, further testing of the resistant germplasm identified in this study across more locations and seasons will also be merited.

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References

[1] FAO. World Agricultural Production [Internet]. 2011. Available from: http://faostat.fao.org/default.aspx [Accessed: 14 June 2013]

[2] FAO. World Agricultural Production [Internet]. 2007. Available from: http://faostat.fao.org/default.aspx [Accessed: 30 November 2011]

[3] Rosegrant MW, Paisner MS, Meijer S, Witcover J. Strategies for selection of host resistance. The African Crop Science Journal. 2001;**1**:189-198

[4] Mosisa W, Legesse W, Berhanu T, Girma D, Girum A, Wende A, Tolera K, Gezahegn B, Dagne W, Solomon A, Habtamu Z, Kasa Y, Temesgen C, Habte J, Demoz N, Getachew B. Status and future direction of maize research and production in Ethiopia. In: Proceedings of the 3rd National Maize Workshop of Ethiopia; 18-20 April 2011; Addis Ababa, Ethiopia. 2012. pp. 17-23

[5] Girma D, Solomon A, Emana G, Ferdu A. Review of the past decade's (2001-2011) research on pre-harvest insect pests of maize in Ethiopia. In: Proceedings of the 3rd National Maize Workshop of Ethiopia; 18-20 April 2011; Addis Ababa, Ethiopia. 2012. pp. 166-173

[6] Tewabech T, Dagne W, Girma D, Meseret N, Solomon A, Habte J. Maize pathology research in Ethiopia in the 2000s: A review. In: Proceedings of the 3rd National Maize Workshop of Ethiopia; 18-20 April 2011; Addis Ababa, Ethiopia. 2012. pp. 193-201

[7] Juliana BO, Marco AG, Isaias OG, Luis EAC. New resistance genes in *Zea mays Exserohilum turcicum* pathosystem. Genetics and Molecular Biology. 2005;**28**:435-439

[8] Cardwell KF, Schulthess F, Ndehma R, Ngoko Z. A systems approach to assess crop health and maize yield loss due to pests and diseases in Cameroon. Agriculture, Ecosystems & Environment. 1997;**65**:33-47

[9] Muiru WM, Mutitu WE, Kemenju JW. Reaction of some Kenyan maize genotypes to Turcicum leaf blight under green house and field conditions. Asian Journal of Plant Sciences. 2007;**6**:1190-1196

[10] Dagne W, Habtamu Z, Demissew A, Temam H, Harjit S. Combining ability of maize inbred lines for grain yield and reaction to grey leaf spot disease. The East African Journal of Sciences. 2008;**2**:135-145

[11] Njuguna JAM, Kendera JG, Muriithi LMM, Songa S, Othiambo RB. Overview of maize diseases in Kenya. Maize Review Workshop in Kenya. Kakamega, Kenya; 1990. pp. 45-51

[12] Welz HG, Geiger HH. Genes for resistance to northern corn leaf blight in diverse maize populations. Plant Breeding. 2000;**119**:1-14

[13] Legesse WB, Myburg AA, Pixley KV, Botha AM. Genetic diversity of maize inbred lines revealed by SSR markers. Hereditas. 2007;**144**:10-17

[14] Singh R, Mam VP, Koranga KS, Bisht GS, Khandelwal RS, Bhandar P, Pan SK. Identification of additional sources of resistance to *Exserohilum turcicum* in maize. SABRAO Journal of Breeding and Genetics. 2004;**36**:45-47

[15] Ogiliari JB, Guimaraes MA, IO GI. New resistance genes 400 in the *Zea mays – Exserohilum turcicum* pathosystem. Genetics and Molecular Biology. 2005;**28**:435-439

[16] Yeshitela D. Cloning and Characterization of Xylanase Genes from Phytophathogenic Fungi with a Special Reference to *Helminthosporium turcicum* the Cause of Northern Leaf Blight of Maize [Thesis]. Finland: University of Helsinki; 2003

[17] Gevers HO. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize. Plant Disease. 1997;**59**:296-299

[18] Leonard KJ. Proposed nomenclature for pathogenic races of *Exserohilum turcicum* on corn. Plant Disease. 1989;**73**:776-777

[19] Adipala E. Reaction of 13 maize genotypes to *E. turcicum* in different agro-ecological zones of Uganda. East African Agricultural and Forestry Journal. 1994;**59**:213-218

[20] Pratt RC, Gordon K, Lipps P, Asea G, Bigrawa G, Pixley K. Use of IPM in the control of multiple diseases of maize. The African Crop Science Journal. 2003;**11**:189-198

[21] Terron A, Preciado E, Cordova H, Mickelson H, Lopez R. Determinacion del patron heterotico de 30 lineas de maiz derivadas del la poblacion 43 SR del CIMMYT. Mesoamerican Agronomy. 1997;**8**:26-34

[22] Melchinger AE, Gumber RK. Overview of heterosis and heterotic groups in agronomic crops. In: Lamkey KR, Staub JE, editors. Concepts and Breeding of Heterosis in Crop Plants. Madison, USA: CSSA; 1998

[23] Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK, Beck D, Bohn M, Frisch M. Use of SSRs for establishing heterotic groups in subtropical maize. Theoretical and Applied Genetics. 2003;**107**:947-957

[24] Lee M, Godshalk EB, Lamkey KR, Woodman WW. Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. Crop Science. 1989;**29**:1067-1071

[25] Livini C, Anjmone-Marsan P, Melchinger AE, Messmer MM, Motto M. Genetic diversity of maize inbred lines within and among heterotic groups revealed by RFLPs. Theoretical and Applied Genetics. 1992;**84**:17-25

[26] Dubreuil P, Dufour P, Krejci P, Causse M, Vienne D, Gallais A, Charcosset S. Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Science. 1996;**36**:790-799

[27] Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick P, Castiglioni P, Taramino G, Motto M. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theoretical and Applied Genetics. 1998;**97**:1248-1255

[28] Wende A, Shimelis H, Derera J, Worku M, Laing M. Heterosis and combining ability of elite maize inbred lines under northern corn leaf blight disease prone environments of the mid-altitude tropics. Euphytica. 2016;**208**:391-400

[29] Campbell CL, Madden LV. Introduction of Plant Disease Epidemiology. New York, USA: Wiley; 1991

[30] Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. GenStat for Windows (11th Edition) Introduction. VSN International: Hemel Hempstead; 2008

[31] Mbogori MN, Kimani M, Kuria A, Lagat M, Danson JW. Optimization of FTA technology for large scale plant DNA isolation for use in marker-assisted selection. The African Journal of Biotechnology. 2006;**5**:693-696

[32] Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovitch S, Ziegle J. An evaluation of the utility of SSR loci as molecular markers in maize: Comparison with data from RFLP and pedigree. Theoretical and Applied Genetics. 1997;**95**:163-173

[33] Muriithi LMM. Response of maize genotypes to *Turcicum* blight and other economically important diseases. In: Proceedings of the 3rd KARI Scientific Conference; 7-9 October 1992; Nairobi, Kenya

[34] Levy Y. Variation in fitness among field isolates of *Exserohilum turcicum* in Israel. Plant Disease. 1991;**75**:163-166

[35] Bowen KL, Pedersen WL. Effects of northern leaf blight components on corn inbreds. Plant Disease. 1988;**72**:952-956

[36] Ceballos H, Deutsch JA, Gutiérrez H. Recurrent selection for resistance to *Exserohilum turcicum* in eight subtropical maize populations. Crop Science. 1991;**31**:964-971

[37] Ojulong HF, Adipala E, Rubaihayo PR. Diallel analysis for reaction to *Exserohilum turcicum* of maize cultivars and crosses. The African Crop Science Journal. 1996;**4**:19-27

[38] Vaz Patto MC, Satovic Z, Pêgo S, Fevereiro P. Assessing the genetic diversity of Portuguese maize germplasm using microsatellite markers. Euphytica. 2004;**137**:63-72

[39] Pinto LR, Vieira MLC, de Souza CL, de Souza AP. Genetic diversity assessed by microsatellites in tropical maize population submitted to high-density reciprocal recurrent selection. Euphytica 2003;**134**:277-286

[40] Senior ML, Murphy JP, Goodman MM, Stuber CW. Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Science. 1998;**38**:1088-1098

[41] Heckenberger M, Bohn M, Zeigle JS, Joe LK, Hauser JD, Hutten M, Melchinger AE. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. Genetic and technical sources of variation in SSR data. Molecular Breeding. 2002;**10**:181-191

[42] Enoki H, Sato H, Koinuma K. SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theoretical and Applied Genetics. 2002;**104**:1270-1278

[43] Sibov ST, de Souz CL, Garica AF, Silva AR, Mongolin CA, Benchimol LL, de Souza AP. Molecular mapping in tropical maize (*Zea mays L.*) using microsatellite markers. Map construction and localization of loci showing distorted segregation. Hereditas. 2003;**139**:96-106

[44] Boppenmeier J, Melchinger AE, Brunklaus-Jung E, Geiger HH, Herrmann RG. Genetic diversity for RFLP in European maize inbreds: In relation to performance of Flint x dent crosses for forage traits. Crop Science. 1992;**32**:895-902

[45] Lanza LLB, de Souza Jr CL, Ottoboni LM, Vieira MLC, de Souza AP. Genetic distance of inbred lines and prediction of maize single cross performance using RAPD markers. Theoretical and Applied Genetics. 1997;**94**:1023-1030

[46] Li Y, Du J, Wang T, Shi Y, Song Y, Jia J. Genetic diversity and relationships among Chinese maize inbred lines revealed by SSR markers. Maydica. 2002;**47**:93-101

[47] Ajmone-Marsan P, Castiglioni P, Fusari F, Kuiper M, Motto M. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theoretical and Applied Genetics. 1998;**96**:219-227