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Screening of short and medium-duration genotypes against *Turcicum* leaf blight disease of maize

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Abstract
Maize is one of the most important and widely cultivated cereal crops worldwide, known for its adaptability and high productivity potential. Among various diseases that cause significant yield losses, *Turcicum* leaf blight (TLB), a foliar disease caused by *Exserohilum turcicum*, can reduce yields by 25% to 90%. The disease affects maize from the seedling stage to maturity. Field screening was conducted under artificial inoculation conditions, and disease severity was recorded using a 1-9 scale. The present study involved screening of short and medium-duration entries against TLB. The screening trials revealed that some genotypes showed resistant and moderately resistant reactions. The resistant genotypes identified in this study could serve as valuable parental sources for resistance breeding programs aimed at developing TLB-tolerant hybrids.

Keywords: Maize, *Turcicum* leaf blight, screening, resistance

Introduction
Maize (*Zea mays* L.) is one of the most important staple food crops, originating from Central America and Mexico, and it is often referred to as the “queen of cereals”. It is cultivated in almost 170 countries worldwide, occupying an area of 193.7 million hectares and a total production of 1,147.7 million tons having average productivity of 5.75 tons per hectare (Anonymous, 2020). Globally, the United States of America ranks first in terms of production and productivity (361.09 million tons and 10.73 tonnes/ha, respectively), followed by China (215.81 million tons and 5.80 tonnes/ha, respectively). India ranks sixth in total maize production (23.67 million tons), area of 9.25 million hectares and productivity is 2.55 tonnes/ha (FAOSTAT, 2014) [8].

It faces various biotic and abiotic stresses throughout its growth period, foliar diseases being one of the most important diseases causing significant yield losses. One major foliar disease of maize is the *Turcicum* leaf blight (TLB), also called as northern corn leaf blight (NCLB) in the United States of America, caused by the ascomycete fungus, *Setosphaeria turcica* and its conidial stage *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Heliminthosporium Turcicum* Pass). It is a heterothallic facultative parasitic fungus (Luttrell, 1957) [11], and it reproduces both asexually and sexually. It was reported first by Passerine (1876) in Perma, Italy and was followed by a serious outbreak of TLB in Connecticut, New England, in 1889, which was reported by Drechsler in 1923. It can lead to severe yield losses of 25% to 90% in various regions of India, which depend on the disease severity of TLB outbreaks (Chenulu and Hora, 1962) [6]. Yield losses have reached 50% when the disease has reached severe levels at 2-3 weeks after pollination (Shurtleff, 1980) [16].

The disease is mostly favoured by high humidity and mild temperature (Ullstrup, 1970). It affects the crop from the seedling stage till maturity, and the symptom of the disease is shown by having greyish green or brown leaf lesions with a long elliptical shape, which first appear on the lower leaves and gradually extend throughout the foliage. Premature death of blighted leaves occurred if the disease started during the early stage of the crop. The application of chemical treatments and different crop management practices can manage TLB disease, but the most eco-friendly and cost-efficient strategy is to adopt host-plant resistance. Among different practices, resistance breeding is the most practical, cost-effective means to manage the disease. Hence, the present investigation was undertaken to screen short and medium-duration entries against *Turcicum* Leaf Blight under field conditions.

Materials and Methods

Collection of Diseased Samples and Pathogen Isolation

Diseased maize leaf samples exhibiting typical symptoms of *Turcicum* Leaf Blight (TLB), characterized by elongated necrotic lesions, were collected in paper poly bags from different maize-growing areas of Manipur during the survey and transported to the laboratory for pathogen isolation. The causal pathogen, *Exserohilum turcicum*, was isolated from infected leaf tissues following the standard hyphal tip isolation technique. The obtained pure cultures were maintained on Potato Dextrose Agar (PDA) slants and stored at 5 ± 1 °C in a refrigerator for subsequent laboratory and field investigations.

Isolation of the pathogen

Isolation of the fungus was done through the standard tissue isolation technique. The necrotized leaf lesions, along with the healthy portions, were surface sterilized in 1:1000 sodium hypochlorite solution for 30 sec and again washed thoroughly three times in sterile distilled water to remove the traces of sodium hypochlorite. The sterilized bits were then transferred aseptically to sterile Petri plates that contained PDA media. The inoculated petri dishes were incubated at room temperature (25 ± 1 °C) for periodic observation of fungal growth.

Hyphal tip isolation

For the maintenance of a pure culture of the pathogen, the hyphal tip isolation method was used. From 15 days 15-day-old culture, the spore suspension was diluted in sterilised distilled water to get eight to ten spores per ml. From this, one ml of suspension was uniformly spread on two per cent solidified water agar plates and incubated at 27 ± 1 °C for 12 hr. With a marker pen, a single spore was marked on the backside of the Petri plate with the help of a microscope. After periodically observing under the microscope, the hyphae coming from each cell of the single spore were then traced and marked. With the help of a cork borer, the tip of the hyphae was cut carefully and then transferred to PDA plates and incubated at 27 ± 1 °C for 10 days. Again, the mycelial bits of the fungus from incubated plates were transferred to the petri plates containing PDA and further incubated at 27 ± 1 °C for 10 days. Thus, the pure culture obtained was free from sectoring or saltation. For the identification of *E. turcicum*, spore morphology and colony characteristic studies were done on PDA.

Mass multiplication of the inoculum

Sterilized sorghum grains were used for the mass multiplication of *E. turcicum* (Joshi *et al.*, 1969) [10]. About 40 to 45 g of sorghum grains were dispensed in a 500 ml conical flask, soaked in water for about 3-4 hrs and excess water was drained off. The sorghum grains were autoclaved twice at 15 psi for 1 hour, inoculated with the fungus under aseptic conditions, and incubated at 25-27 °C. Once in 2-3 days, the flasks were shaken for uniform growth of *E. turcicum* on grains. The above impregnated sorghum grains were dried by spreading uniformly on a clean paper sheet in the shade.

Field Screening of Maize Genotypes for TLB

A field experiment was conducted during *kharif* 2024 at the Andro Research Farm, College of Agriculture, Central Agricultural University, Imphal, Manipur (24.763793° N, 94.052882° E; 755.02 ± 2.5 m above mean sea level) to evaluate maize genotypes for resistance to *Exserohilum turcicum*, the causal organism of *Turcicum* Leaf Blight (TLB). The experiment was laid out in an alpha lattice design with a plot length of 2 m. Each genotype was sown on 28 June 2024, maintaining a spacing of 20 × 60 cm and following the recommended agronomic practices, except for plant protection measures.

Inoculum Preparation and Inoculation Procedure

Pure cultures of *E. turcicum* were maintained on potato dextrose agar (PDA) medium and incubated at 25 ± 1 °C for 20 days to promote sporulation. A spore suspension was prepared by flooding the culture plates with sterile distilled water and gently scraping the surface to dislodge the conidia. The resulting suspension was filtered through double-layered muslin cloth to remove mycelial fragments.

The spore concentration was adjusted to 3×10^5 spores ml^{-1} using a haemocytometer. The suspension was sprayed uniformly on the maize plants at the three-to four-leaf stage twice at 30 and 40 DAS using a hand-held atomizer during the evening hours to facilitate infection. Immediately after inoculation, a fine water mist was applied to maintain adequate humidity and promote pathogen establishment.

Disease Development and Assessment

Following inoculation, the field was periodically irrigated to maintain moderate humidity conducive to disease development. Disease observations were recorded at regular intervals, and disease severity was assessed using the standard 1-9 rating scale (Table 1) recommended by the Indian Institute of Maize Research (IIMR), Ludhiana (Anonymous, 2014) [1]. Observations began 45 days after sowing when typical *Turcicum* Leaf Blight (TLB) symptoms were well expressed, and assessments were continued at weekly intervals for six consecutive weeks. Based on the mean disease scores, the genotypes were categorized as follows: The genotypes showing disease scores between 1.0-3.0 were considered as resistant (R), 3.1-5.0 as moderately resistant (MR), 5.1-7.0 as moderately susceptible (MS), 7.1-9.0 as susceptible (S).

Results and Discussion

Screening of Medium-and Short-Duration Maize Hybrids for Resistance to *Turcicum* Leaf Blight

Medium-Duration Hybrids

A total of thirty-three medium-duration maize hybrids were evaluated for their reaction to *Turcicum* Leaf Blight (TLB) under field conditions. Among them, fourteen genotypes, AMH 2022-161, BMH C-57-18, DH 383, IMH 10-24K-4, IMH 10-24K-9, IMH 2-24K-107, IMH 2-24K-112, IMH 2-24K-3, IY 8323 (DKC 9273), KDMH 140, LMH 1324, MAH 19-2, MAH 22-55, and MAH 22-79 exhibited a resistant reaction to TLB.

Nineteen genotypes—AH 4142, AH 4152, BMH I-82-42, DH 382, HKH 417, IMH 10-24K-1, IMH 10-24K-2, IMH 10-24K-3, IMH 10-24K-5, IMH 10-24K-8, IMH 5-24K-398, IY 8291 (DKC 9274), IY 8384, JH 17026, JH 21021, LMH 6524, IX 7841 (DKC 7250), IX 8455 (DKC 9254), and IX 8466 (DKC 9256) were found to be moderately resistant to TLB (Table 2).

Table 1: Disease scoring according to Chung *et al.* (2010); Mitiku *et al.* (2014)

Rating scale	Degree of infection (% DLA*)	PDI**	Disease Reaction
1.0	Nil to very slight infection (≤10%).	≤ 11.11	Resistant (Score: ≤ 3.0) (PDI: ≤ 33.33)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20%).	22.22	
3.0	Light infection, moderate number of lesions on four lower leaves (20.1-30%).	33.33	
4.0	Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%).	44.44	Moderately Resistant (Score: 3.1-5.0) (PDI: 33.34-55.55)
5.0	Moderate infection, an abundant number of lesions scattered on lower leaves, a moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	55.55	
6.0	Heavy infection, an abundant number of lesions scattered on lower leaves, moderate infection on middle leaves, and a few lesions on two leaves above the cob (50.1-60%).	66.66	Moderately susceptible (Score: 5.1-7.0) (PDI: 55.56-77.77)
7.0	Heavy infection, an abundant number of lesions scattered on lower and middle leaves, and a moderate number of lesions on two to four leaves above the cob (60.1-70%).	77.77	
8.0	Very heavy infection, lesions are abundantly scattered on the lower and middle leaves and spreading up to the flag leaf (70.1-80%).	88.88	Susceptible (Score: > 7.0) (PDI: > 77.77)
9.0	Very heavy infection, lesions abundantly scattered on almost all leaves, plants prematurely dried or killed (>80%).	99.99	

*DLA-Diseased leaf area; **Percent disease index (PDI)

Short-Duration Hybrids

In the case of short-duration maize hybrids, twenty-three genotypes were evaluated. Four genotypes, DH 380, FDH 93, FMH 66, and AHD 1639, showed a resistant reaction to TLB.

Fifteen genotypes, AMH 2022-141, DH 381, FH 4206, FH 4209, FH 4236, IMH 10-24K-6-E, IMH 5-24K-663, JH 32982, JH 32984, KGH 23-17, KGH 23-41, LMH 2438, LMH 2447, LMH 2342, and IX 7851, exhibited moderately resistant reactions, while four genotypes, AH 4681, IMH 10-24K-7-E, IMH 5-24K-197, and IMH 5-24K-303 were found to be moderately susceptible (Table 3).

The present findings are in agreement with the earlier studies of Harlapur *et al.* (1999) [9], who screened thirty-seven maize inbreds under artificially inoculated conditions and reported that CI-4, CM-104, and NAI-147 were resistant, CM-111, CM-501, CM-121, KDMI-12, and CM-118 exhibited intermediate reactions, and CM-202, CM-115, CM-117, CM-128, CM-600, and KDMI-10 were highly susceptible to *E. turcicum*.

Similarly, Mallikarjuna *et al.* (2018) [12] screened 135 genotypes and reported that none were completely resistant, while 34 genotypes were moderately resistant, 73 moderately susceptible, and 29 susceptible to TLB.

Bantu *et al.* (2021) [4] evaluated seventy medium-maturing maize inbreds under artificial epiphytotic conditions and observed that the per cent disease index (PDI) ranged from 13.3 to 80.0, and the area under the disease progress curve (AUDPC) varied between 300.0 and 1591.7.

Babita and Mani (2011) [3] screened temperate maize lines against northern corn leaf blight and identified five inbreds showing resistant reactions.

Singh *et al.* (2014) [15] evaluated 118 maize genotypes under artificial epiphytotic conditions and reported 26 resistant, 56 moderately resistant, 26 susceptible, and 10 highly susceptible genotypes.

Likewise, Manu *et al.* (2017) [13] screened 115 maize inbred lines against TLB and found 21 resistant (disease score 1), 51 moderately resistant, and the remaining lines ranged from moderately susceptible to highly susceptible.

Chandrashekara *et al.* (2012) [5] evaluated thirty-five inbred lines under artificial epiphytotic conditions at Hawalbagh, Almora, India, and reported 10 resistant, 18 moderately resistant, 3 susceptible, and 4 highly susceptible entries. The inbred lines V373, V398, V407, V418, VQL2, and CM 145 exhibited a high degree of resistance to TLB.

Table 2: Disease screening of medium-duration maturity maize genotypes

Sl. No.	Genotype	TLB (1-9)	Reaction
1	AH 4142	3.1	MR
2	AH 4152	4.0	MR
3	AMH 2022-161	2.2	R
4	BMH C-57-18	1.9	R
5	BMH I-82-42	3.2	MR
6	DH 382	3.5	MR
7	DH 383	2.8	R
8	HKH 417	3.4	MR
9	IMH 10-24K-1	3.2	MR
10	IMH 10-24K-2	3.8	MR
11	IMH 10-24K-3	3.4	MR
12	IMH 10-24K-4	2.6	R
13	IMH 10-24K-5	4.2	MR
14	IMH 10-24K-8	3.1	MR
15	IMH 10-24K-9	3.0	R
16	IMH 2-24K-107	3.0	R
17	IMH 2-24K-112	2.6	R
18	IMH 2-24K-3	3.0	R
19	IMH 5-24K-398	4.1	MR
20	IY 8291 (DKC 9274)	3.1	MR
21	IY 8323 (DKC 9273)	3.0	R
22	IY 8384	3.7	MR
23	JH 17026	4.1	MR
24	JH 21021	3.8	MR
25	KDMH 140	2.1	R
26	LMH 1324	3.0	R
27	LMH 6524	3.7	MR
28	MAH 19-2	2.9	R
29	MAH 22-55	2.5	R
30	MAH 22-79	2.9	R
31	IX 7841 (DKC 7250)	4.7	MR
32	IX 8455 (DKC 9254)	3.6	MR
33	IX 8466 (DKC 9256)	3.4	MR
Checks			
C1	Bio 9544	2.2	R
C2	CMH 08 292	2.6	R
C3	Dhari Local	8.0	S
C4	LG 34.05	2.3	R
C5	RCRMH4-1	3.5	MR
C6	VAMH 12014	2.9	R
	CV (%)	19.5	
	F (Prob)	0.0	
	CD (5%)	1.4	
	CD (1%)	1.9	

Table 4: Disease screening of short-duration maturity maize genotypes

Sl. No.	Genotype	TLB (1-9)	Reaction
1	AH 4681	5.6	MS
2	AMH 2022-141	3.2	MR
3	DH 380	3.0	R
4	DH 381	3.7	MR
5	FDH 93	2.6	R
6	FH 4206	3.2	MR
7	FH 4209	4.6	MR
8	FH 4236	3.1	MR
9	FMH 66	2.9	R
10	IMH 10-24K-6-E	3.3	MR
11	IMH 10-24K-7-E	5.3	MS
12	IMH 5-24K-197	5.3	MS
13	IMH 5-24K-303	5.2	MS
14	IMH 5-24K-663	4.7	MR
15	JH 32982	5.0	MR
16	JH 32984	4.1	MR
17	KGH 23-17	3.5	MR
18	KGH 23-41	4.5	MR
19	LMH 2438	3.5	MR
20	LMH 2447	3.7	MR
21	AHD 1639	2.7	R
22	LMH 2342	4.3	MR
23	IX 7851	3.2	MR
Checks			
C1	BIO 605	2.9	R
C2	Dhari Local	6.4	MS
C3	DKC 7074	3.0	R
C4	LG 34.05	4.3	MR
C5	LQMH 1	4.1	MR
C6	RCRMH4-1	4.2	MR
C7	VAMH 12014	3.1	MR
	CV (%)	14.2	
	F (Prob)	0.0	
	CD (5%)	1.2	
	CD (1%)	1.6	

Conclusion

The present investigation demonstrated considerable variability among maize genotypes in their response to *Exserohilum turcicum*, the causal agent of *Turcicum* Leaf Blight (TLB). Several short-and medium-duration hybrids exhibited resistant or moderately resistant reactions, highlighting their potential as valuable genetic resources for resistance breeding. Identification of such resistant sources is crucial for developing durable and sustainable TLB resistance, particularly in regions prone to frequent disease outbreaks.

The incorporation of resistance genes from these sources into susceptible but agronomically superior cultivars can greatly enhance yield stability and resilience. Furthermore, the utilization of these resistant genotypes in population improvement and hybrid development programs will contribute significantly to the improvement of maize germplasm and the development of high-yielding, TLB-resistant maize cultivars for sustainable maize production.

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