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Screening of Maize Gemplasm against Turcicum Leaf Blight of Maize Caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs.

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ABSTRACT

Management of the turcicum leaf blight through the continuous use of chemicals may alarm new problems in crop production like residual toxicity, environmental pollution and development of resistance in pathogens against the chemicals. Hence the host plant resistance is a cheap and environmentally reliable component to minimize the disease intensity below the threshold level. Keeping in view the above points, screening of forty eight maize germplasm along with two standard checks i.e., CI 4 (Resistance check) and CM 202 (Susceptible check) were evaluated against *E. turcicum* under artificially inoculated field conditions at MARS, Dharwad during *kharif* 2018. Using 1-9 rating scale, disease reaction and AUDPC values of germplasm were also calculated. Twelve germplasm viz., GPM 340, GPM 03, GPM 737, SBS 67, SBS 5, DQL 2299, SBS 148, CM 500, INDIMYT 100, BML 7, LM 13, BGS 24 and CI 4 (RC) recorded resistant reaction. The least AUDPC value recorded in BGS 24 (137.25) under resistant reaction.

Keywords Disease severity, *Exserohilum turcicum,* Maize, Area under disease progress curve (AUDPC), Turcicum leaf blight.

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INTRODUCTION

Turcicum leaf blight (TLB) is one of the ubiquitous foliar disease of maize. It is caused by the anamorph of the Deuteromycete, *Exserohilum turcicum* (Pass.) Leonard and Suggs. and the telomorph of the ascomycete, *Setosphaeria turcica* (Luttrell) Leonard and Suggs. First time, it was reported by Passerine (1876) in Perma, Italy, this was followed by a serious outbreak of TLB in Connecticut, New England in 1889 (Drechsler 1923). The disease appears particularly during growing season in the areas of high humidity and moderate temperature. In India TLB disease was first reported by Butler (1918) on sorghum and later by Mitra (1923) on both sorghum and maize from Punjab. Laxminarayan and Shankerlingam (1983) reported the hot spot locations in the country which includes Dharward (Karnataka), Kolahpur (Maharashtra), Karimnagar (Andhra Pradesh) and Dholi (Bihar). However, the disease is most prevalent in all the major maize growing regions of

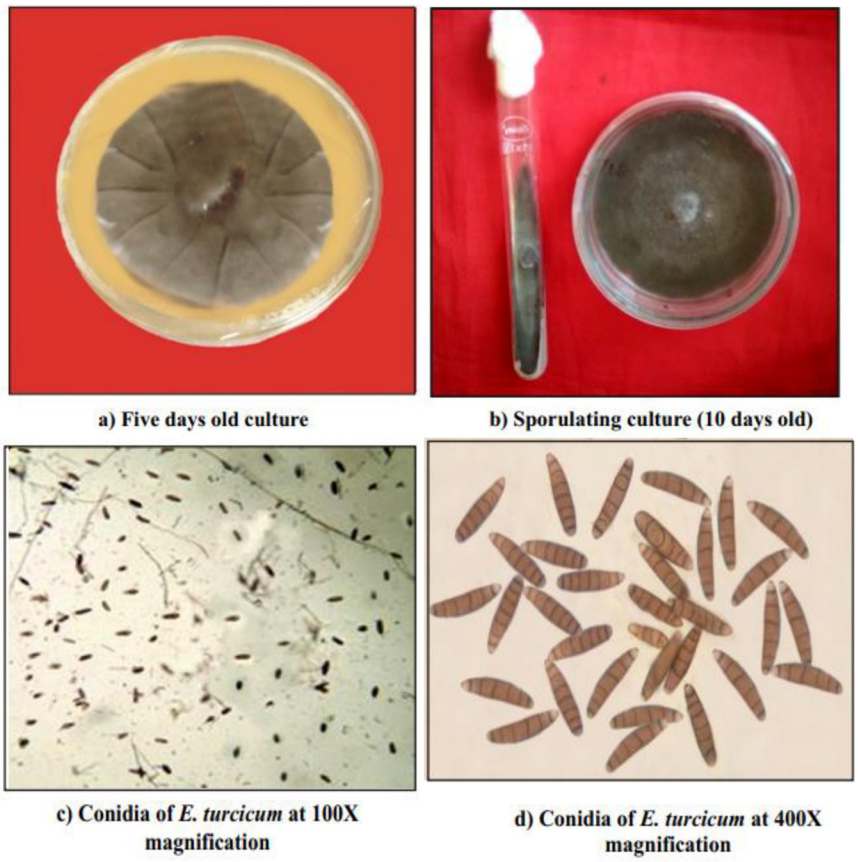

Fig. 1. Cultural and morphological characters of *E. turcicum.*

India during rainy (*kharif*) as well as in winter (*rabi*) season since last two decades.

Hence for the management of TLB, the control measures like seed treatment, application of fungicides, use of resistant and tolerant genotypes have been recommended (Anonymous 2004). Although breeding for TLB resistance started much earlier, more efforts are still needed as new challenges arise. There is possibility of emergence of new races of pathogens and some available resistance sources may become susceptible. Therefore, there is a need to identify new sources of resistance under artificial epiphytotics to cater to the resistance breeding programs. Following the difficulty in controlling TLB due to high input prices and arising of new races is unreliable. Therefore, breeding for maize resistance to TLB was more demanded as it is cheap and reliable approach for combating losses due to the disease.

MATERIALS AND METHODS

Collection of diseased samples

The leaves of maize plants severely infected by *E. turcicum* showing typical leaf blight necrotic lesion type symptoms were collected from experimental fields of All India Coordinated Maize Improvement Project, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad and further used for isolation of the pathogen. The pathogen *E. turcicum* was isolated by standard hyphal tip isolation procedures and then nucleus culture was maintained on potato dextrose agar slants, kept in refrigerator at 5°C which was further used in all the laboratory and field studies.

a) Inoculation of fungus on sorghum grains

b) Complete mycelial growth on sorghum grains

Fig. 2. Mass multiplication of *E. turcicum* on sorghum grains.

Isolation of the pathogen

The fungus was isolated following standard tissue isolation technique. The necrotized leaf bits along with healthy portions were surface sterilized in 1:1000 sodium hypochlorite solution for 30 sec and washed thoroughly thrice in sterile distilled water to remove the traces of sodium hypochlorite. Then sterilized bits were aseptically transferred to sterile Petri plates containing PDA media. The inoculated Petri dishes were incubated at room temperature (25 ± 1°C) and observed periodically for fungal growth. The growth of the fungus was conspicuous after 24 hr of incubation. The pure colonies which developed from the bits were transferred to PDA slants and incubated at room temperature for 15 days. After the incubation period, abundant sporulation was observed and the pathogen was purified following hyphal tip isolation technique as described below.

Hyphal tip isolation

The pure culture of the pathogen was obtained by hyphal tip isolation method. The spore suspension was diluted in sterilized distilled water to get eight to ten spores per ml from 15 days old culture. One ml of such suspension was spread uniformly on two per cent solidified water agar plates and incubated at 27 ± 1°C for 12 hr. Single spore was marked with a marker pen on back side of the Petri plate with the aid of microscope and it was allowed to germinate. Such plates were periodically observed for spore germination under microscope. The hyphae coming from each cell of the single spore was traced and marked. The tip of the hyphae was cut carefully with cork borer and transferred to PDA plates and incubated at 27 ± 1°C for 10 days. Later, mycelial bits of the fungus from incubated plates were transferred to the Petri plates containing PDA and incubated at 27 ± 1°C for 10 days. The pure culture thus obtained was free from saltation or sectoring. In order to confirm the identity of *E. turcicum*, spore morphology and colony characteristic studies were done on PDA. Further, the conidia of *E. turcicum* was observed under microscope (Fig. 1).

Maintenance of the culture

The hyphal tip cultures of *E. turcicum* were sub-cultured on PDA slants and kept in laboratory at 28 ± 1°C for 15 days. Such mother culture slants were preserved at 5°C in refrigerator. Further, these cultures were sub-cultured once in a month to maintain viability and used for future studies.

Mass multiplication of inoculum

The mass multiplication of *E. turcicum* was prepared on sterilized sorghum grains (Joshi *et al.* 1969). About an inch layer of sorghum grains (nearly 40 to 45 g) was dispensed in a 500 ml conical flask, soaked in water for about 3-4 hs and excess water was drained off. The flasks containing sorghum grains were auto-

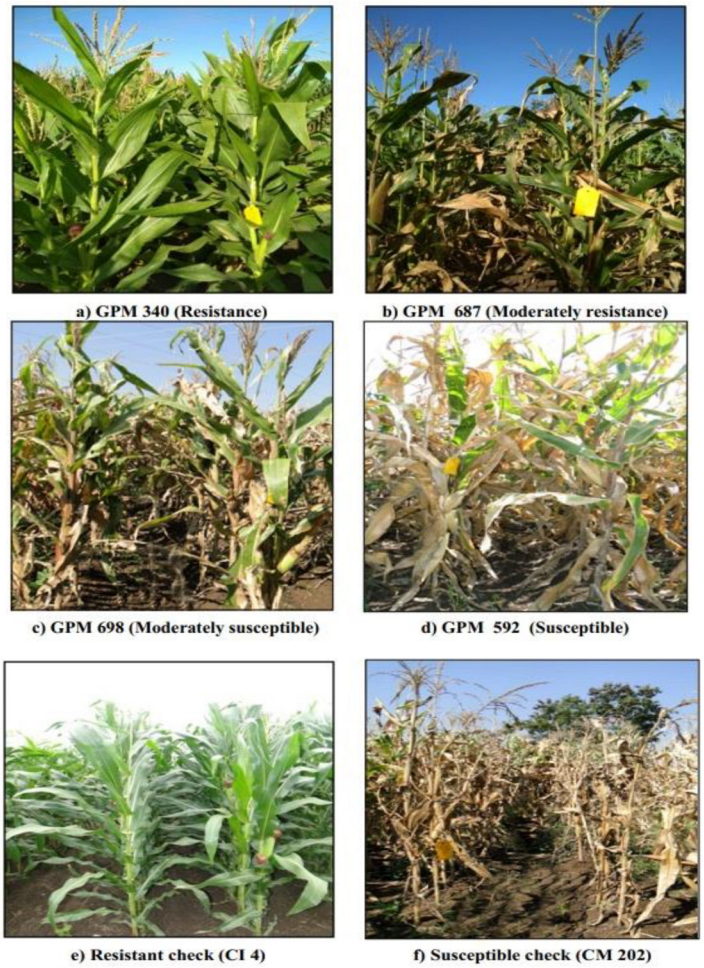

Fig. 3. Screening of germplasm against turcicum leaf blight diseas.

claved twice at 15 pounds per inch square pressure for one hour, seeded with fungus under aseptic condition and kept for incubation at 25- 27°C. The flasks were shaken once in 2-3 days to facilitate uniform growth of *E. turcicum* on grains. After incubation of about a fortnight, the material was ready for inoculation. The above impregnated sorghum grains were allowed for drying by spreading them on a clean paper sheet in shade at room temperature. After drying, fine powder of these grains was prepared with the help of mixer - grinder and put a pinch of this powder in the leaf whorl. The inoculum was directed into the whorl of the plant @ 2g/plant followed by water spray in the whorls so as to maintain adequate moisture for longer period to permit spore germination. Artificial inoculation was done twice i.e., at 30 and 40 days after sowing (Fig. 2).

Screening of germplasm

A field experiment was conducted to screen maize genotypes for turcicum leaf blight at MARS, Dharwad during *kharif* 2018. Forty eight maize germplasm and fifty five hybrids were screened against turcicum leaf blight under artificial inoculated field conditions. The resistant check CI-4 and susceptible check CM-202 were planted along with test entries in plot size of 4.8 sq m and replicated twice. The crop was raised by following the recommended agronomic practices except disease management. The test

Table 1. Screening of maize germplasm against turcicum leaf blight of maize. AUDPC - Area under disease progress curve.

Sl. No.	Germplasm	Disease score (1-9 scale)	AUDPC value	Sl. No.	Germplasm	Disease score (1-9 scale)	AUDPC value
1	GPM 549-1	5	320.25	26	CM 119	5	343.75
2	GPM 627	5	320.25	27	CM 500	3	279.08
3	GPM 31	8	503.25	28	CM 501	5	343.75
4	GPM 340	3	251.63	29	INDIMYT 100	3	278.53
5	GPM 773	4	297.38	30	BML 7	3	223.00
6	GPM 33	6	411.75	31	CM 152	8	529.00
7	GPM 383	4	297.38	32	BML 6	4	297.50
8	GPM 744	6	411.75	33	CM 400	7	428.75
9	GPM 684	4	297.38	34	CM 600	9	541.75
10	GPM 582	7	434.63	35	LM 13	3	203.25
11	GPM 704	4	297.38	36	CM 111	4	301.30
12	GPM 03	3	251.63	37	CM 115	8	488.75
13	GPM 687	5	366.00	38	CM 123	8	457.50
14	GPM 38	6	411.75	39	CM 128	5	343.25
15	GPM 28	5	343.13	40	BGS 5	9	532.25
16	GPM 649	6	388.88	41	BGS 24	2	137.25
17	GPM 691	5	320.25	42	BGS 27	5	327.58
18	GPM 57	5	343.13	43	BGS 28	4	283.65
19	GPM 592	9	549.00	44	BGS 23	3	228.75
20	GPM 766	8	503.25	45	SBS 67	3	187.58
21	GPM 737	3	205.88	46	SBS 5	3	187.58
22	GPM 698	6	388.88	47	DQL2299	3	183.00
23	GPM 679	5	343.13	48	SBS 148	3	192.15
24	GPM 49	5	366.00	49	Resistance check CI 4	2	137.25
25	GPM 669	5	366.00	50	Susceptible check CM 202	9	549.00

genotypes were inoculated by *E. turcicum* inoculum multiplied on sorghum grains in the leaf whorls at 30 and 40 days after sowing at the rate of 2 g per plant during evening hours. A light water spray was given immediately after the inoculation to create optimum humidity for infection.

The observations on the disease severity of turcicum leaf blight was recorded on the basis of 1–9 modified disease rating scale (Anonymous 2016). Further the genotypes were categorized into resistant, moderately resistant, moderately susceptible and susceptible. Further, disease scores were used to calculate the area under disease progress curve (AUDPC) using the following formula given by Wilcoxson *et al.* (1975).

$$AUDPC = \sum_{i=1}^{k} \left(\frac{1}{2} (si + si_{-1}) d \right)$$

Where,
Si = Disease severity at the end of time
S_i-1 = Number of successive evaluations of blight
d = Interval between two evaluations.

RESULTS AND DISCUSSION

The results of conducted experiment revealed the clear cut differential reactions for different germplasm against the TLB pathogen under artificially inoculated conditions. Germplasm with disease score less than 3.0 and AUDPC values less than 280.00 showed the resistance reaction as they remained green till the maturity without any initiation of the symptoms. While germplasm with disease score above 7.0 and AUDPC values above 450.00 showed the susceptible reaction as they showed grayish elliptical to spindle shaped lesions on the foliage and resulted lesser no. of cobs (Fig. 3).

Table 2. Reaction of maize germplasm against turcicum leaf blight of maize.

Disease rating	Reaction	No. of germplasm	Germplasm
≤ 3.0	Resistant	12	GPM 340, GPM 03, GPM 737, SBS 67, SBS 5, DQL 2299, SBS 148, CM 500, INDIMYT 100, BML 7, LM 13, BGS 24, CI 4 (RC)
3.1- 5.0	Moderately resistant	21	GPM 549-1, GPM 627, GPM 773, GPM 383, GPM 684, GPM 687, GPM 28, GPM 49, GPM 691, GPM 57, GPM 669, GPM 704, GPM 679, CM 119, CM 501, BML 6, CM 111, CM 128, BGS 27, BGS 28, BGS 23
5.1-7.0	Moderately susceptible	7	GPM 33, GPM 38, GPM 649, GPM 582, GPM 744, GPM 698, CM 400
≥7.0	Susceptible	8	GPM 31, GPM 592, GPM 766, CM 152, CM 600, CM 115, CM 123, BGS 5, CM 202 (SC)

Of the forty eight germplasm evaluated, twelve germplasm viz., GPM 340, GPM 03, GPM 737, SBS 67, SBS 5, DQL 2299, SBS 148, CM 500, INDIMYT 100, BML 7, LM 13, BGS 24 and CI 4 (RC) showed resistant reaction. Twenty one germplasm viz., GPM 549 -1, GPM 627, GPM 773, GPM 383, GPM 684, GPM 687, GPM 28, GPM 49, GPM 691, GPM 57, GPM 669, GPM 704, GPM 679, CM 119, CM 501, BML 6, CM 111, CM 128, BGS 27, BGS 28 and BGS 23 were found to be moderately resistant. Whereas seven germplasm viz., GPM 33, GPM 38, GPM 649, GPM 582, GPM 744, GPM 698 and CM 400 were moderately resistant and remaining eight germplasm viz., GPM 31, GPM 592, GPM 766, CM 152, CM 600, CM 115, CM 123, BGS 5 and CM 202 were recorded as susceptible reaction for turcicum leaf blight (Tables 1, 2).

The AUDPC values differed considerably for different maize germplasm ranging from 137.25 to 549.00. The least AUDPC value was recorded in BGS 24 (137. 25) showing resistant reaction and highest AUDPC value was recorded in GPM 592 (549.00) which was depicted as susceptible reaction. Hence, lower AUDPC values recorded by the germplasm viz., BGS 24, SBS 67, SBS 5, SBS 148 and DQL 2299 could be used for rating them as slow blighters (Table 1).

The present findings are in accordance with studies of Harlapur *et al*. (1999) reported the results of field screening of thirty seven maize inbreds under artificially inoculated conditions. The results revealed that CI-4, CM-104 and NAI-147 showed resistant reaction for *E. turcicum* and inbreds viz., CM-111, CM-501, CM-121, KDMI-12 and CM-118 were recorded intermediatery reactions. And CM-202, CM-115, CM-117, CM-128, CM-600, KDMI-10 were found to be highly susceptible.

Similarly, Kachapur *et al.* (2014) screened fifty new germplasm lines against TLB. Among them they found that GPM-378, GPM-408, GPM-496, GPM-524 and GPM-537 were showed the resistant reaction to TLB and GPM-375, GPM-440, GPM-540 and GPM-569 were shown susceptible reaction.

Thus from the present investigation, new sources of resistance were identified through artificial epiphytotics. This can cater to the resistance breeding program by combating with the new races of pathogens that would be emerging continuously and susceptibility of some resistance sources. This results would also be useful in improvement of maize germplasm and hybrids through population improvement programs for sustainable productivity.

CONCLUSION

Screening of forty eight germplasm and fifty five hybrids were carried out at Main Agricultural Research Station, UAS, Dharwad. Among forty eight germplasm evaluated against TLB, twelve germplasm viz., GPM 340, GPM 03, GPM 737, SBS 67, SBS 5, DQL

2299, SBS 148, CM 500, INDIMYT 100, BML 7, LM 13, BGS 24 and CI 4 (RC) were recorded resistant reaction. The AUDPC values differed considerably for different maize germplasm ranging from 137.25 to 549.00. The least AUDPC value was recorded in BGS 24 (137.25) under resistant reaction and highest AUDPC value was recorded in GPM 592 (549.00) which is depicted as susceptible reaction. Hence, lower AUDPC values recorded by the germplasm viz., BGS 24, SBS 67, SBS 5, SBS 148 and DQL 2299 could be used for rating them as slow blighters.

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