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Turcicum Leaf Blight Resistance in Maize: Field Screening of New Inbreds and Hybrids

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ABSTRACT

This paper presents identification of sources of Turcicum Leaf Blight (TLB) resistance in maize. Maize is affected by more than 60 diseases, of which 16 are major ones. TLB, caused by E. turcicum, is considered a serious disease where climatic conditions are cool with high relative humidity. Severe losses in grain yield ranging from 25 to 90 per cent have been reported in India. Seventy two hybrids developed by line x tester design along with 18 parents and three checks were screened against turcicum leaf blight (TLB) under artificial epiphytotics during rainy season of the years, 2015 and 2016. Disease score and percent disease indexes were recorded at tasseling and 20 days after tasseling (DAT). Nineteen hybrids with a disease score of 0, 33 hybrids with a score of 1 and 20 with a score of 2 at tasseling were categorised as resistant. The range of per cent disease index (PDI) was between 0 per cent and 28.6 per cent. At 20 days after tasseling (DAT), one hybrid with a disease score of 1, fifteen hybrids with 2 were found to be highly resistant and resistant, respectively. Twenty nine scored disease score 3 indicating moderate resistance. Twenty four hybrids registered susceptible, (score of 4) while three hybrids recorded highly susceptible reaction (disease score 5). After 20 DAT, the PDI ranged from 20.40 to 68.57 per cent. Thirty four hybrids showed less than 45 per cent disease index falling under moderately tolerant category and twenty six hybrids showed more than 50 per cent disease index falling under susceptible category. After 20 DAT, parents PDM-8, PDM-60 and PDM-254 had disease index of <10% indicating their resistance to TLB. The inbreds, PDM-4611, PDM-1, PDM-32 IDM-, PDM-36 and PDM-83 recorded disease index of more than 50% while, PDM-259, PDM-127, PDM-51 and PDM-52 recorded disease index of more than 35% but less than 50% indicating they are highly susceptible to TLB. The results suggested the possibility of improving resistance against TLB further through population improvement approach, preferably by reciprocal recurrent selection.

Keywords: Inbred, Hybrid, Maize, Turcicum Leaf Blight, Resistance

I. INTRODUCTION

Maize (*Zea mays* L.) is a cereal crop of worldwide importance. Though maize is highly versatile and offers diverse uses as food, feed and an industrial raw material, its production is plagued by a number of constraints. Maize is affected by more than 60 diseases and 16 out of 61 diseases are adversely affecting this crop and have been identified as major ones [1]. These diseases cause epidemics in favourable conditions with severe consequences on maize production. Therefore, development of disease-resistant genotypes/hybrids is one of the main objectives of maize breeding programs.

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Among the diseases of maize, foliar diseases occupy significant position and are the important factors reducing the yield and quality of maize produce. One among them is Turcicum (TLB) or northern corn leaf blight (NCLB) caused by the ascomycetes fungi *Exserohilum turcicum*. It is a ubiquitous foliar disease of maize causing significant yield loss. TLB is a serious disease, under low temperature and high humidity conditions. TLB is characterized by long elliptical, greyish green or tan leaf lesions that first appear on the lower leaves and increase in size and number until very little living tissue is left. The pathogen causes the loss of chlorophyll from the leaves, in turn leading to reduction in photosynthesis, accumulation of carbohydrates in grains and ultimately loss of grain yield [2]. Maize grain yield loss varies from 25 to 90 per cent in different parts of India depending upon the severity of TLB epiphytotics [3] [4]. Yield losses have approached 50%, when the disease is severe at 2-3 weeks after pollination [5]. Observation of near epiphytotic levels of the disease in recent years is an indication that the level of resistance in the commercial varieties/hybrids is low or the resistance has broken down.

Though, the TLB disease can be managed by chemicals and crop husbandry practices, the most appropriate and economical strategy is to exploit host plant resistance. The resistant varieties are not only environmental friendly but also convenient to adopt at farmer's level. The survey of literature indicated the possibility of breeding for resistance to TLB [6] [7] [8]. Since new races of pathogens will be emerging continuously and some resistance sources may become susceptible; there is a need to identify new sources of resistance through artificial epiphytotics year after year to cater to the resistance breeding programme. With these facts in mind the present study was carried out to screen and identify the parental lines and experimental hybrids of maize for resistance to TLB. The paper identified TLB resistant hybrids and sources of resistance against TLB which would be useful further in improvement of maize populations and inbreds through population improvement programmes.

II. EXPERIMENTAL MATERIAL AND METHODS

The base material for the present study was selected from 100 inbred lines previously screened against TLB (in two consecutive years, 2013-2014). The previous screening categorised inbreds into resistant, moderately resistant, moderately susceptible and susceptible groups. From this, 18 inbreds were selected in medium maturity category possessing various degree of TLB resistance (Table 1). These were crossed to generate 72 experimental hybrids in a 12 x 6, Line x Tester design during *rabi* 2014. This produced hybrids in all possible combination of Resistant (R), moderately resistant (MR), and susceptible (S) inbreds such as R x R, R x MR, R x S, S x S and their reciprocal like crosses. The screening experiment was conducted during two consecutive *kharif* seasons (2015 and 2016).

The experimental hybrids along with parents and checks were sown in the field in RCBD with two replications during two consecutive *kharif* seasons (2015 and 2016) at ICAR-IARI's Regional Research Centre, Dharwad. Hybrids and parents were sown in two rows each. The artificial epiphytotics of TLB were created by following the standard artificial inoculation technique.

III.CREATION OF ARTIFICIAL EPIPHYTOTICS

The TLB pathogen (*E. turcicum*) was isolated from maize leaves showing the typical *turcicum* leaf blight (TLB) symptoms from two different maize fields at Dharwad. The blighted leaf tissue was cut into small pieces and was

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surface sterilized by treating with 0.1% mercuric chloride ($HgCl_2$). The sterilized leaf pieces were washed three times in sterile double distilled water and dried on sterile filter paper. These tissue pieces were inoculated onto petri-plates containing sterilized agar media. The plates were incubated at 24± 2°C for 7 days. The well developed fungal colonies inoculated on PDA slants and incubated. Colonies showing typical features of *E. Turcicum* were maintained on PDA slants were mass multiplied on sterile (autoclaved twice) and sucrose treated sorghum kernels in flasks. The flasks were maintained in the lab carefully to avoid any contamination for two weeks and shaken alternate day to distribute the fungus evenly over sorghum kernels. The infested sorghum kernels were then air dried and made into a fine powder. A pinch of this powder was inoculated in to the leaf whorl. In each genotype/hybrid, the first inoculation was done at 4 to 5 leaf stage. The treatment was repeated thrice in an interval of five days to ensure high pathogen load. The inoculum was applied just before sunset to allow the dew to initiate spore germination of *E. turcicum* during the night. Additionally, the water sprays were given to the inoculated rows to provide sufficient humidity for facilitating spore germination and disease development. Spreader rows of susceptible inbred (CM-202) were planted after every five rows to ensure sufficient inoculum load of TLB.

Table 1: Characteristic features of the genotypes used in the study (based on pooled data from 2013-2014 at Dharwad)

Sl. No.	Inbred	Pedigree	TLB Score	Level of resistance
I	**Lines (female)**			
1	PDM-259	Comp 8527-8-1-2-3-1-1-1	3.0	Moderately Resistant
2	PDM-127	Comp 85164-10-1-2-5-1-1-1	3.0	Moderately Resistant
3	PDM-136	MDR-10-5-1-2-3-5-1-1-1	2.0	Resistant
4	PDM-24	Ageti-9-8-1-2-3-1-1-1-1	2.0	Resistant
5	PDM-423	(Comp 8551 X 8527) X 6-2-1-7-13-2-3	3.0	Moderately Resistant
6	PDM-8	MDR pool-12-8-7-2-1-1-1	1.0	Resistant
7	PDM-60	MDR pool-5-2-4-1-1-1-1	1.0	Resistant
8	PDM-51	Comp85164 X 8527) -8 4-2-8-7-1-1-1	3.0	Resistant
9	PDM-52	(Comp 8551 X 8527) X 6-2-1-7-13-2-2	3.0	Moderately Resistant
10	PDM-254	Ageti-68-9-8-1-2-3-1-1-1	2.0	Resistant
11	PDM-36	Comp 8551-11-3-2-3-1-1-1	3.0	Moderately Resistant
12	PDM-83	Comp 8551 x Ageti-76-11-3-2-3-1-1-1	5.0	Susceptible
II	**Testers (male)**			
1	PDM-4611	PC3x PC4-7-2-3-1-1-1-1	4.0	Susceptible
2	PDM-59	MDR-15-58-1-2-3-5-1-1-1	2.0	Resistant
3	PDM-258	MDR-7-4-2-2-3-5-1-1-1Ä	2.0	Resistant
4	PDM-32	(Comp 8551 X 8527) X 6-2-1-7-13-2-1	3.0	Susceptible (moderate)
5	PDM-1	Ageti-76-9-8-1-2-3-1-1-1	4.0	Susceptible
6	PDM-4711	PC3 x Comp 8551-5-3-2-1-1-1	5.0	Susceptible

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2.2 ESTIMATE OF DISEASE SEVERITY

The TLB disease scoring was done using 1- 5 scale suggested by Payak and Sharma [9] on five random plants in each entry at the time of tasseling and at 20 days after tasseling. The scoring was done as follows.

Rating	Description
1.0 :	Very slight to slight infection, one or two to few scattered lesions on lower leaves.
2.0:	Light infection, moderate number of lesions on lower leaves only
3.0:	Moderate infection, abundant lesions are on lower leaves, few on middle leaves.
4.0:	Heavy infection, lesions are abundant on lower and middle leaves, extending to upper leaves.
5.0:	Very heavy infection, lesions abundant on almost all leaves, plants prematurely dry or killed by the disease

A disease score of 1.0 was considered as highly resistant, 2.0- resistant, 3.0-moderately resistant and the scores 4.0 and 5.0 are considered as susceptible and highly susceptible, respectively. Per cent disease index was calculated as per Wheeler [10]

$$PDI = \frac{\text{Sum of individual disease ratings}}{\text{Total No. of plants / leaves observed}} \times \frac{100}{\text{Maximum disease rating value}}$$

III. EXPERIMENTAL RESULTS AND DISCUSSION

The spreader rows of susceptible inbreds showed highly susceptible reaction to TLB in both the years indicating disease development. The spreader rows were repeated after every five test entries and hence ensured sufficient inoculum load in the screening against TLB under field conditions.

There was clear cut differential responses of both inbreds and hybrids against TLB under artificial epiphytotics. The disease reaction and per cent disease index values of inbreds and hybrids are presented in Table 2 and Table 3, respectively. Disease ratings at the time of tasseling were lower compared to that at 20 DAT. This was expected as disease development started only after 5-6 leaf stage and progressed. However, susceptible genotypes sustained more disease than intermediate ones. The resistant inbreds and hybrids showed only traces of leaf blight.

3.1 Screening at the Time of Tasseling

The disease score at the time of tasseling ranged from 0 to 2 among both inbreds and hybrids. The disease severity ranged between 0 per cent and 28.6 per cent among both inbred parents and hybrids. Thus, at the stage of tasseling all the test entries were apparently resistant. However, there was differential response ranging from resistant to highly susceptible reaction at 20 DAT.

3.2 Screening at 20 Days After Tasseling

Twenty days after tasseling (20 DAT), there was heavy incidence of TLB disease due to artificial epiphytotics. TLB disease ratings were significantly different among both inbred parents and the hybrids. The disease score ranged from 1.0 to 5.0 among both parents and hybrids. One hybrid (PDM-8 x PDM-32) possessed a disease score of 1.0 and lowest per cent disease index (PDI) of 20.4%, indicating its highly resistant nature against TLB.

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Thirteen hybrids scored 2.0 indicating their resistance against TLB. Thirty one hybrids had a rating of 3.0 indicating that they were moderately resistant. Twenty four hybrids registered susceptible reaction (rating of 4.0). Three hybrids recorded disease score of 5.0 indicating of highly susceptible reaction to the disease. The PDI values for hybrids ranged from 20.4% to 68.57%. Thirteen hybrids registered less than 25% PDI and were categorised as resistant. These hybrids have also recorded disease rating of 2.0 or less. Thirty two hybrids had less than 40% PDI and were falling under moderately resistant category. Twenty seven hybrids showed more than 50% PDI and hence classified as susceptible. Three hybrids recorded the disease score 5 and recorded PDI of more than 65% and hence were highly susceptible. TLB appears in sizeable form in Karnataka resulting in grain yield reduction ranging between 28 to 91 per cent in case of TLB [11] [12] [13].

Table 2: Reaction of maize experimental parents against TLB during 2015 and 2016

Sl. No.	Entry	TLB Score (1-5) at		PDI at		
		Tasseling	20 DAT	Tasseling	20 DAT	Reaction type
Lines						
1	PDM-259	0.0	3.0	0.00	37.14	MR
2	PDM-127	1.0	3.0	14.30	37.14	MR
3	PDM-136	0.0	2.0	0.00	22.86	R
4	PDM-24	0.0	2.0	0.00	22.86	R
5	PDM-423	0.0	3.0	14.30	37.14	MR
6	PDM-8	0.0	1.0	0.00	8.57	R*
7	PDM-60	0.0	1.0	0.00	8.57	R*
8	PDM-51	1.0	3.0	14.30	37.14	MR
9	PDM-52	1.0	3.0	14.30	37.14	MR
10	PDM-254	0.0	1.0	0.00	8.57	R*
11	PDM-36	1.0	4.0	14.30	51.43	S
12	PDM-83	2.0	5.0	28.86	51.43	S
Testers						
1	PDM-4611	1.0	4.0	14.30	51.43	S
2	PDM-59	0.0	2.0	0.00	22.86	R
3	PDM-258	0.0	2.0	0.00	22.86	R
4	PDM-32	1.0	4.0	14.30	51.43	S
5	PDM-1	2.0	5.0	28.86	66.11	S
6	PDM-4711	0.00	4.0	14.30	51.43	S
Range	**Min.**	**0.00**	**1.00**	**0.00**	**8.57**	
	Max.	**2.00**	**5.00**	**28.86**	**66.11**	
CV		**10.50**	**12.25**	**11.55**	**15.32**	

R*-Highly resistant, R- Resistant, DAT-Days after tasseling
MR- Moderately resistant S-Susceptible CV-Coefficient of variation

Table 3. Reaction of maize experimental hybrids against TLB during 2015 and 2016

Sl. No.	Experimental hybrids	TLB Score (1-5) at		PDI (%) at		R type	Sl. No.	Experimental hybrids	TLB Score (1-5) at		PDI (%) at		R type
		Tasseling	20 DAT	Tasseling	20 DAT				Tasseling	20 DAT	Tasseling	20 DAT	
1	PDM-259 x PDM-	1.0	3.0	14.30	37.14	MR	37	PDM-60 x PDM-4611	0.0	3.0	0.00	37.14	MR
2	PDM-259 x PDM-59	0.0	3.0	14.30	37.14	MR	38	PDM-60 x PDM-59	0.0	2.0	0.00	22.86	R
3	PDM-259 x PDM-258	0.0	3.0	14.30	37.14	MR	39	PDM-60 x PDM-258	1.0	2.0	0.00	22.86	R
4	PDM-259 x PDM-32	1.0	3.0	7.10	37.10	MR	40	PDM-60 x PDM-32	0.0	3.0	0.00	37.14	MR
5	PDM-259 x PDM-1	1.0	4.0	14.30	51.43	S	41	PDM-60 x PDM-1	2.0	4.0	28.60	51.43	S
6	PDM-259 x PDM-	1.0	3.0	7.10	37.10	MR	42	PDM-60 x PDM-4711	2.0	4.0	28.60	51.43	S
7	PDM-127 x PDM-	2.0	5.0	28.60	66.11	S	43	PDM-51 x PDM-4611	1.0	4.0	14.30	48.57	S
8	PDM-127 x PDM-59	1.0	2.0	7.10	22.86	R	44	PDM-51 x PDM-59	0.0	3.0	0.00	37.14	MR
9	PDM-127 x PDM-258	1.0	2.0	7.10	22.86	R	45	PDM-51 x PDM-258	1.0	3.0	0.00	37.14	MR

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10	PDM-127 x PDM-32	2.0	4.0	28.60	51.43	S	46	PDM-51 x PDM-32	0.0	3.0	0.00	37.14	MR
11	PDM-127 x PDM-1	1.0	3.0	14.30	37.14	MR	47	PDM-51 x PDM-1	1.0	4.0	14.30	51.40	S
12	PDM-127 x PDM-	1.0	3.0	14.30	37.14	MR	48	PDM-51 x PDM-4711	1.0	4.0	14.30	51.40	S
13	PDM-136 x PDM-	2.0	4.0	21.40	51.43	S	49	PDM-52 x PDM-4611	0.0	4.0	0.00	62.86	S
14	PDM-136 x PDM-59	1.0	3.0	14.30	37.14	MR	50	PDM-52 x PDM-59	1.0	3.0	0.00	37.14	MR
15	PDM-136 x PDM-258	1.0	3.0	14.30	37.14	MR	51	PDM-52 x PDM-258	0.0	3.0	0.00	37.14	MR
16	PDM-136 x PDM-32	1.0	3.0	14.30	37.14	MR	52	PDM-52 x PDM-32	2.0	4.0	28.60	62.86	S
17	PDM-136 x PDM-1	1.0	4.0	14.30	51.43	S	53	PDM-52 x PDM-1	2.0	5.0	28.60	68.57	S
18	PDM-136 x PDM-	1.0	3.0	14.30	37.14	MR	54	PDM-52 x PDM-4711	2.0	4.0	28.60	62.86	S
19	PDM-24 x PDM-4611	1.0	3.0	14.30	37.14	MR	55	PDM-254 x PDM-	1.0	4.0	28.60	62.86	S
20	PDM-24 x PDM-59	1.0	2.0	7.10	22.86	R	56	PDM-254 x PDM-59	1.0	2.0	0.00	22.86	R
21	PDM-24 x PDM-258	1.0	2.0	7.10	22.86	R	57	PDM-254 x PDM-258	0.0	3.0	0.00	37.14	MR
22	PDM-24 x PDM-32	0.0	3.0	14.30	37.14	MR	58	PDM-254 x PDM-32	0.0	3.0	0.00	37.14	MR
23	PDM-24 x PDM-1	1.0	3.0	14.30	37.14	MR	59	PDM-254 x PDM-1	1.0	4.0	14.30	62.86	S
24	PDM-24 x PDM-4711	1.0	3.0	14.30	37.14	MR	60	PDM-254 x PDM-	0.0	3.0	0.00	37.14	MR
25	PDM-423 x PDM-	0.0	2.0	0.00	22.86	R	61	PDM-36 x PDM-4611	2.0	4.0	28.60	62.86	S
26	PDM-423 x PDM-59	0.0	2.0	0.00	22.86	R	62	PDM-36 x PDM-59	1.0	4.0	14.30	62.86	S
27	PDM-423 x PDM-258	0.0	2.0	0.00	22.86	R	63	PDM-36 x PDM-258	1.0	4.0	14.30	62.86	S
28	PDM-423 x PDM-32	1.0	3.0	14.30	37.14	MR	64	PDM-36 x PDM-32	1.0	4.0	14.30	62.86	S
29	PDM-423 x PDM-1	1.0	3.0	14.30	37.14	MR	65	PDM-36 x PDM-1	2.0	4.0	28.60	62.86	S
30	PDM-423 x PDM-	1.0	3.0	14.30	37.14	MR	66	PDM-36 x PDM-4711	2.0	4.0	21.43	62.86	S
31	PDM-8 x PDM-4611	0.0	3.0	0.00	37.14	MR	67	PDM-83 x PDM-4611	2.0	4.0	28.60	51.43	S
32	PDM-8 x PDM-59	1.0	2.0	7.10	22.86	R	68	PDM-83 x PDM-59	1.0	3.0	0.00	37.10	MR
33	PDM-8 x PDM-258	1.0	3.0	7.10	37.14	MR	69	PDM-83 x PDM-258	2.0	4.0	21.40	51.43	S
34	PDM-8 x PDM-32	0.0	1.0	0.00	20.40	R*	70	PDM-83 x PDM-32	2.0	4.0	28.60	51.43	S
35	PDM-8 x PDM-1	0.0	2.0	0.00	22.86	R	71	PDM-83 x PDM-1	2.0	5.0	21.43	65.71	S
36	PDM-8 x PDM-4711	0.0	3.0	0.00	37.14	MR	72	PDM-83 x PDM-4711	1.0	4.0	14.30	51.43	S
	Range : Min	**0.00**	**1.00**	**0.00**	**20.40**			**LSD**	**0.18**	**0.22**	**0.49**	**3.26**	
	Max	**2.00**	**5.00**	**28.60**	**68.57**			**CV**	**8.50**	**9.70**			

PDI-Percent disease index DAT-Days after tasseling R Type-Resistance reaction type

Five female parents (lines) were found in resistant category. Among them three inbreds, *viz.*, PDM-8, PDM-60 and PDM-254 recorded a disease rating of 1.0. These lines had also registered lowest PDI of 8.57% and hence were categorized as highly resistant. Two inbreds? *viz.*, PDM-136 and PDM-24 registered a rating 2.0 and a PDI of 22.86% and hence were resistant to TLB. Five lines were moderately resistant with a rating of 3.0 and PDI of 37.14% while remaining two lines PDM-36 and PDM-83 with the rating of 4.0 and 5.0, respectively and a PDI of 51.4% were categorised as susceptible.

Among the males (testers), two inbreds *viz.*, PDM-59 and PDM-258 with a disease score of 2.0 and a PDI of 22.86% showed resistance reaction against TLB. Three testers recorded a disease score of 4.0 and PDI of 51.4% and one (PDM-1) with a disease score of 5.0 and PDI of 66.11% showed susceptible reaction.

The present study revealed that the disease rating at tasseling was not reliable as disease rating increased at 20 DAT. Out of 18 inbreds, 7 inbreds *viz.*, PDM-8, PDM-60, PDM-254, PDM-136, PDM-24, PDM-59 and PDM-258 were resistant (3 were highly resistant) to TLB at 20 DAT. This indicated that the TLB resistance is not

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uncommon in maize genotypes. Similar results with TLB resistance in maize has been reported earlier [7] [14] [15] [16]. The study has enabled us to classify inbreds and hybrids into resistant (R), moderately resistant (MR) and susceptible (S) categories. When female parent was resistant, most of the hybrids were either resistant or moderately resistant. However, when female parent was susceptible most of the hybrids were susceptible. For instance, the crosses from 61 to 72 involved susceptible female parent. Except cross number 68 (PDM-83 x PDM-59) with MR reaction, other 11 hybrids registered susceptible reaction (Table 3). This might be due to genotype x cytoplasm interaction and/or maternal effects. Such results have been earlier reported [17] in leaf blight (*Exserohilum turcicum* (Pass.)) of sorghum. However, a separate detailed study is required to understand this aspect. The unusual susceptibility of T cytoplasm (male sterile) of maize to *Helminthosprium maydis* has been reported as early as 1961 by Mercado and Lantican [18] and later confirmed by Villareal and Lantican [19].

IV. CONCLUSIONS

Field screening of maize genotypes leading to the identification of TLB resistant inbreds is useful in maize breeding programmes, particularly in areas prone to TLB. The promising lines identified in the study holds a great promise in resistance breeding against *E. Turcicum.* Moreover, these lines could be hybridized with the previously identified high yielding, MLB and TLB resistant lines such as DM-188, DM-91, DM-196, DM-111, DM-127, DM-134, DM-193, DM-136, DM-135, DM-189, DM-51, DM-137, for developing high yielding hybrids and composites with multiple disease resistance (Bhat *et al*., 2012). The results also indicated that none of the lines were immune to TLB. Though both qualitative and quantitative resistance operate against TLB, in most of maize genotypes the resistance is governed by minor genes [20]or resistance was partially dominant and controlled by many genes ([21] [22]. Hence, there is a scope for improvement in resistance through population improvement approach, preferably by reciprocal recurrent selection. Progeny testing and selfing of the individual plants, derived from single ears and selection of less-susceptible individual progenies against *E., turcicum* can result in an accumulation of minor genes and increasing the level of resistance [6] [23]. The type of resistance, mechanisms of resistance, and the locations of the gene(s) for resistance of these grain maize genotypes need to be studied. The biotechnology approaches can be used to locate the gene(s) and in incorporating it in the cultivars with desirable agronomic characteristics.

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