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Identification of reaction pattern to turcicum leaf blight among early maturing maize (*Zea mays* L.) inbred lines

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Abstract
Turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs is the most important biotic stress in temperate agro-ecologies of the valley of Kashmir. Plant genetic resistance to this disease is a safe alternative and most economic strategy. In the present study 36 early maturing inbred lines of maize were screened for resistance against *E. turcicum* under field conditions. When tested against ten isolates of *E. turcicum* under artificial conditions, twenty-seven genotypes viz., KDM-1156, KDM-362A, V-351, KDM-445B, KDM-916A, KDM-1095, KDM-439, KDM-895A, KDM-914A, KDM-342A, PS66, CML239, CML240, CML244, CML245, CML446, CML460, CML470, CML152, CML474, CML472, CML350, VL1018140, VL1034, VL109452, VL127 and VL1018527 were found resistant. Nine genotypes viz., PS45, CML165, CML459, VL1249, VL1030, SMC5, SMC3, KDM-962B and KDM-969 were found moderately resistant. The resistant inbred lines will be further used in a crossing programme to develop high yielding and disease resistance varieties for high altitude temperate agro-ecologies.

Keywords: Turcicum leaf blight, *Exserohilum turcicum*, diallel, resistant, Variability

Introduction
Maize (*Zea mays L.,* 2n=20) is a widely distributed crop grown throughout the world in tropical, sub-tropical and temperate regions under irrigated to semi-arid conditions. Being a versatile crop, it adapts to the wide range of production environments. In India, maize is the third most important cereal after rice and wheat which provides food, feed, fodder and serves as a source of basic raw material for a number of industrial products viz., starch, protein, oil, alcoholic beverages, food sweeteners, cosmetics, bio-fuel, etc. No other cereal can be used in as many ways as maize and that is why it is called "Queen of cereals". Virtually every part of the plant has an economic worth. At global level maize is cultivated over an area of 177.4 million hectares with an annual production of about 960.2 million tonnes and average productivity of 5.5 tonnes ha^{-1} (Anonymous, 2013-14) [2]. Turcicum leaf blight also called as Northern leaf blight of maize incited by the fungus
Exserohilum turcicum (Pass.) Leonard and Suggs is widely spread and economically most important disease of maize globally and occur frequently under mountain agro ecologies of Jammu and Kashmir. Major challenge to increase maize production primarily involves the predominance of cultivated land races which are more susceptible to various biotic stresses particularly Turcicum leaf blight. The disease causes enormous damage to crop in terms of grain yield particularly if the disease establishes before silking (Nwanosike *et al*, 2015) [10].
In Jammu and Kashmir, Turcicum leaf blight is the major constraint to maize production and the increased incidence often necessitates regular survey and surveillance of the disease to get comprehensive information on disease distribution and level of severity. Epidemiological studies play a vital role in developing prediction and forecasting models about disease progress 13 in relation to disease severity and environmental factors. Maize grain yield loss varies from 25 to 90 per cent in different parts of India depending upon the severity of Turcicum Leaf Blight epiphytotics (Chenula and Hora, 1962; Jha, 1993) [5, 8]. Yield losses have approached 50%, when the disease is severe at 2-3 weeks after pollination (Shurtleff, 1980) [13] Turcicum leaf blight (TLB) is considered a serious disease under mountain agro ecologies of Jammu and Kashmir. Turcicum leaf blight affects the maize crop from the seedling stage to maturity. The symptoms first appear as grayish green small elliptical spots on the leaves with water soaked lesions parallel to leaf margins, finally attaining a spindle shape with long elliptical grayish or tan lesions. If the disease starts at an early stage, it causes premature death of blighted leaves.

As a result, the crop loses their nutritive value as fodder (Payak and Renfro, 1968), have reduced germination capacity, vigor, GY and total sugar content (Ferguson *et al*., 2004) [7], has restricted starch formation, chaffy kernels and infected plants are liable to infection with stalk rots (Cuq *et al*., 1993) [6]. The fungus has a wide host range and a high pathogenic variability with several races already reported in different parts of the world (Pratt R. G., 2003; Agrios, 2005) [12, 1].

Genetic resistance of crop plants to infection by the pathogen is a safe alternative and most economical and eco friendly disease management venture. The resistant varieties are not only environmental friendly but also suitable to adopt at farmer's level. Keeping the above points in mind, the present study was carried out to screen the thirty-six maize inbred lines for identification of resistant sources against by TLB disease under artificially inoculated field conditions which would be useful further in improvement of maize populations through population improvement programmes.

Materials Andmethods

Pathogen isolation: Diseased maize leaf samples collected during survey from different maize growing areas of Kashmir Province during Kharif 2016 -2017 were attempted for the isolation of *E. Turcicum* isolates. The cultures of *E. turcicum* isolates were obtained by single spore isolation technique (Tuite, 1969) [15]. Ten single spore cultures of *E. turcicum* isolated from diseased samples of different locations representing 4 maize cultivars and 8 local landraces were maintained on potato dextrose agar slants for screening of maize genotypes.

Field screening of maize germplasm against turcicum leaf blight: For the identification of sources of resistance to *E.turcicum*, a set of 36 maize germplasm lines consisting of materials belonging to CIMMYT, VPKAS Almora and SKUAST-K Srinagar were initially screened under artificially inoculated field conditions. The experiment was carried out at Mountain Crop Research Station, Larnoo located at latitude 33°37'N, longitude 75°22'E and an altitude of 2286 metres above mean sea level. The experiment was established during *Kharif* 2017 following a randomised complete block design with two replications. Test lines were planted in 2 row plots of 3 m length with plant spacing of 60×20cm.The plot was bordered by susceptible disease spreader rows on each side of the inbred lines.

Evaluation of selected maize genotypes against *E. turcicum* isolates under controlled conditions: In order to validate the resistance, a selected set of 20 genotypes which showed moderately resistant to resistant reaction against Turcicum leaf blight under field conditions, was further screened under controlled conditions against all the collected isolates separately, to investigate genotype isolate interactions. Five seeds of each genotype were sown in pots, filled with sterilized potting medium prepared by mixing soil, FYM and sand at the ratio of 6:2:1, respectively. Fertilizers were applied as per recommendation and watering was done as per the moisture status of the potting medium. After germination one plant was maintained in each pot. The treatments were arranged in a completely randomized block design with three replications per treatment. Spore suspension of each isolate was prepared separately and spraying of spore suspension of each isolate was done separately in evening by using a glass atomizer at three to four leaf stages of plants, grown in glasshouse.

Disease assessment: Development of disease was assessed by using 1-9 scale of Indian Institute of Maize Research, Ludhiana (Anonymous a, 2014) [3]. The genotypes showing disease score between 0.1-3.0 were considered as resistant (R), 4-5 as moderately resistant (MR), 6-7 as moderately susceptible (MS), 8-9 as susceptible (S). The observations were recorded on weekly basis for 6 weeks**,** commenced from 45 days after sowing.

Table 1

Rating scale	Degree of infection (per cent DLA*)	PDI**	Disease reaction
1.0	Nil to very slight infection (≤10%).	≤11.11	Resistant (R) (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 scattered 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 (MR) (Score: 3.1–5.0) (PDI: 33.34-55.55)
5.0	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	55.55	
6.0	Heavy infection, 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	Mod. susceptible (MS) (Score: 5.1-7.0) (PDI: 55.56-77.77)
7.0	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%).	77.77	
8.0	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%).	88.88	Susceptible (S) (Score: >7.0) (PDI: >77.77)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%).	99.99	

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

Results and Discussion

The turcicum leaf blight disease of maize was prevalent in all the surveyed areas of Kashmir valley. Ten single spore cultures of *E. turcicum* isolates of diverse locations were maintained on potato dextrose agar medium. The isolates were designated as Kti-1to Kti-10(Table 3).

Germplasm screening under field conditions: Thirty-six maize germplasm lines were initially screened for resistance against *E. turcicum* under artificially inoculated field conditions (Table 1). The lines *viz*., KDM-1156, KDM-362A, V-351, KDM-445B, KDM-916A, KDM-1095, KDM-439, KDM-895A, KDM-914A, KDM-342A, PS66, CML239, CML240, CML244, CML245, CML446, CML460, CML470, CML152, CML474, CML472, CML350, VL1018140,

VL1034, VL109452, VL127 and VL1018527 showed resistant reaction with disease grade ≤ 3 against *E.turcicum*, while as PS45, CML165, CML459, VL1249, VL1030, SMC5, SMC3, KDM-962B and KDM-969 were found moderately resistant with disease score of 4.0-5.0. The genotypes CML474 and CML472 showed maximum resistance with disease intensity percent of 3.9 & 3.5 respectively. From the thirty-six genotypes, 27 genotypes which showed resistant reaction against *E.turcicum* were selected along with highly susceptible check genotypes for further evaluation under Controlled conditions.

The performance of all the maize genotypes were classified into four groups. Nine lines with a disease score 1, thirteen lines with a score 2 and five lines with a score 3 were categorized as resistant. The range of per cent disease intensity (PDI) ranged from 3.5 per cent for CML472 to 31.3 per cent for CML446 for resistant lines. Nine lines with disease score 4 indicated moderate resistances and showed PDI between 34.4 per cent for KDM-969 to 38.5 per cent for CML459. Singh *et al.*(2014) [14] evaluated 118 maize genotypes out of which 26 were found resistant, 56 moderately resistant, 26 susceptible and 10 highly susceptible against turcicum leaf blight. Babita and Mani (2011) [14] screened the temperate maize lines against northern corn leaf blight and found five inbreds, viz., V 335, V 13, V 336, V 53 and V 27 resistant to disease. Inherent resistance or tolerance of crop plants to infection by the pathogen is a safe alternative and most economical and eco friendly disease management venture. Varied response of maize germplasm against TLB was observed by Muiru *et al*. (2015) [9] and suggested that there is a need to pyramid genes for resistance in the elite varieties to enable farmers increase their productivity. The resistant sources with varied levels of resistance do exist against the Turcicum leaf blight disease of maize. The determination of genetic basis of the resources and incorporation of their Significant difference was observed in disease ratings among the genotypes under field and controlled conditions. The effect of the disease was more severe in the green house plants. The differences are attributed to several factors including controlled environmental conditions, host genotype, inoculation methods and resistance variation among the genotypes.

The maize lines identified to possess resistance to Turcicum leaf blight in the present study, can be used successfully in developing high yielding early maturing hybrids/composites for the temperate mountain ecology, having resilience to Turcicum leaf blight.

Conclusion

The inbred lines identified to possess resistance to Turcicum leaf blight in the present study, can be used successfully in developing high yielding early maturing varieties having high level of resistance to Turcicum leaf blight suitable for temperate mountain ecologies. The occurrence and distribution of different isolates of *E.turcicum* with wide pathogenic variability in the field provides important information to devise a suitable disease management programme of TLB.

Resistant lines

Table 2: Reaction of maize genotypes to E. Turcicum under artificially inoculated field conditions.

S. No.	Entry	Source	% Disease intensity	Score	Response
1	KDM-962B	SKUAST-K	36.5	4	MR
2	KDM-1156	SKUAST	25.2	3	R
3	KDM-362A	SKUAST	26.3	3	R
4	KDM-342A	SKUAST	12.8	2	R
5	KDM-969	SKUAST	34.4	4	MR
6	KDM-445B	SKUAST	9.2	1	R
7	KDM-916A	SKUAST	14.2	2	R
8	KDM-1095	SKUAST	13.0	2	R
9	KDM-439	SKUAST	8.3	1	R
10	KDM-895A	SKUAST	15.7	2	R
11	KDM-914A	SKUAST	16.5	2	R

12	V-351	VPKAS Almora	12.7	2	R
13	CML 474	CIMMYT	3.9	1	R
14	CML 472	CIMMYT	3.5	1	R
15	CML 350	CIMMYT	15.8	2	R
16	SMC-3	Commercial Cultivar	34.5	4	MR
17	SMC-5	Commercial Cultivar	38.7	4	MR
18	VL1018140	CIMMYT	4.9	1	R
19	VL 1249	CIMMYT	36.9	4	MR
20	VL 1034	CIMMYT	4.2	1	R
21	VL109452	CIMMYT	4.3	1	R
22	VL1030	CIMMYT	34.6	4	MR
23	VL127	CIMMYT	10	2	R
24	VL1018527	CIMMYT	13.9	2	R
25	PS 45	MCRS Sagam	34.6	4	MR
26	PS 66	MCRS Sagam	29.5	3	R
27	CML 239	CIMMYT	16.3	2	R
28	CML 240	CIMMYT	27.9	3	R
29	CML 165	CIMMYT	34.7	4	MR
30	CML 244	CIMMYT	19.5	2	R
31	CML 245	CIMMYT	17.2	2	R
32	CML 446	CIMMYT	31.3	3	R
33	CML 459	CIMMYT	38.5	4	MR
34	CML 460	CIMMYT	9.9	2	R
35	CML 470	CIMMYT	3.8	1	R
36	CML 152	CIMMYT	12.7	2	R
1-9 scale was used:- **1,2,3**= Resistant (R), Score: ≤ 3.0, PDI: ≤ 33.33**; 4,5**= Moderately resistant (MR), Score: 3.1–5.0, PDI: 33.34-55.55**; 6,7**=Moderately susceptible (MS), Score: 5.1-7.0, PDI: 55.56-77.77; **8,9**=Susceptible (S); Score: >7.0, PDI: >77.77).					

Table 3: E. turcicum isolates collected from different locations of Kashmir valley

S.NO.	Isolate	Maize Cultivar	Place of Origin
1	Kti-1	Local	Pahalgam Anantnag
2	Kti-2	Local	Gandarbal
3	Kti-3	SMC-3	Khudwani Kulgam
4	Kti-4	Local	Tahab Pulwama
5	Kti-5	SMC-5	Pombay Kulgam
6	Kti-6	Local	Kupwara
7	Kti-7	C6	Shalimar Srinagar
8	Kti-8	Local	Shopian
9	Kti-9	Local	Bandipora
10	Kti-10	Local	Larnoo Anantnag

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