

Screening of Maize Inbred Lines under Artificial Epiphytotic Condition for Turcicum Leaf Blight Resistance

ABSTRACT

Maize is the leading cereal crop worldwide with wide adaptability and high productivity potential. Foliar diseases are arguably the major biotic constraints for maize yield. Turcicum Leaf Blight (TLB) is considered to be the most persistent and destructive maize disease among various foliar diseases. The present study is an attempt to screen forty maize inbreds for resistance against TLB under artificially inoculated field conditions. The experiment was conducted in two different environments, viz. Varanasi (E₁) and Nagenahalli (E₂) to know the disease reaction of maize inbreds for two successive years. Screening of 40 maize inbreds revealed, 10 resistant, 13 partial resistant, 7 partial susceptible and 10 susceptible in Varanasi whereas at Nagenahalli, 3 resistant, 6 partial resistant, 14 partial susceptible, 7 susceptible and 10 highly susceptible genotypes. Based on this study, the selected resistant lines will be used in future breeding programs and could potentially be used to develop promising genotypes with suitable levels of resistance.

Keywords: Maize; resistance; screening; susceptible; turcicum leaf blight.

1. INTRODUCTION

Maize (*Zea mays* L.; 2n=20) is one of the most versatile crops grown worldwide in a wide range of environments. Biotic and abiotic stresses are the major constraints to maize productivity [1]. Maize foliar diseases are probably the main biotic constraints for maize production worldwide and the prevalence of such foliar diseases varies by region or season [2]. Among various foliar diseases, Turcicum leaf blight (TLB) is considered to be the most persistent and devastating diseases of maize [3].

Turcicum leaf blight (TLB) is caused by *Exserohilum turcicum* (Pass.), which is a ubiquitous foliar disease of corn or maize [4]. Globally, TLB causes significant losses in maize yield under optimal environmental conditions [5]. Grain yield can be reduced up to 80% when TLB becomes severe [6]. In India, TLB occurs in the states of Karnataka, Himachal Pradesh (HP), Uttarakhand, Uttar Pradesh (UP), Orissa, Andhra Pradesh (AP), and North-Eastern Hill states. Symptoms can range from small cigar shaped lesions to complete destruction of plants [3]. Resistant cultivars have been used to control TLB. There are two types of disease resistance, namely qualitative and quantitative. The qualitative disease resistance is race-specific whereas quantitative or polygenic disease resistance is normally non race-specific and more prevalent in the Indian sub continent [7]. Quantitative TLB resistance is durable in nature and characterized by fewer and typically smaller disease lesions with a prolonged incubation period [8,9,10,11]. TLB can be controlled effectively by cultivating resistant cultivars [12]. The resistant cultivars are not only environment friendly but also suitable to adopt at farmer's level. Since some new races of phytopathogens are emerging continuously, currently available resistant sources may become susceptible. Therefore, new sources of resistance need to be identified through artificial epiphytotics to cater for resistance breeding programs. Keeping this in view the experiment was conducted to identify

resistant sources against TLB disease under artificial epiphytotic condition in different environments, which would be further useful in improvement of maize populations.

2. MATERIALS AND METHODS

2.1 Plant materials

A panel of 40 maize inbreds, including 3 checks *viz.*, V-336 & CM-145 as resistant checks and CM-212 as susceptible check [13] (received from DMR, New Delhi; ICAR-VPKAS, Almora and Maize programme, Banaras Hindu University, Varanasi) were subjected to TLB disease screening for disease reactions in maize. The maize inbred lines were planted at two different locations *viz.* Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi and Agricultural Research Station, Nagenahalli under University of Agricultural Sciences, Karnataka during *Kharif* (Rainy) season 2015 to *Kharif* (Rainy) season in 2016 in a randomized complete block design (RCBD) with two replications. Each replication consisted of two-row plots of 3 meter in length and 70 cm in width with plants spaced at 25 cm from each other within a row. Recommended agronomical practices were adopted to raise a good crop [14].

2.2 Disease assessment

These lines were screened against TLB to evaluate their reaction under artificially inoculated conditions. For inoculum preparation, a lesion was isolated from TLB infected maize leaves and placed it in a moist chamber for two to three days to permit sporulation. The newly formed spores were picked up from the lesions with the help of sterile fine needle under a dissecting microscope and transferred on to acidified PDA and incubated at 20°C to 25°C temperature. Colonies of *Exserohilum turcicum* were subsequently sub-cultured and used to inoculate sorghum grains and allowed to colonize the grains for ten days. After proper fungal growth, the grains were dried in shade at room temperature. A fine powder of these grains was prepared with the help of a mixer-grinder and a pinch of this powder (2 gm/plant) was put in the leaf whorl. Inoculation was made at around 4-6 leaf stages in evening hours when there was sufficient moisture in the air [5].

Disease scoring commenced three weeks after inoculation. Inbred lines were evaluated under the epiphytotic condition by using a disease rating scale of 1 to 5 [15,16,17]. Disease scoring was done at three different stages *viz.*, flowering stage, dough stage and brown husk stage. The PDI (Percent Disease Index) was determined based on the disease scores at the three stages *viz.*, 50th, 60th and 70th days after sowing following Wheeler [18].

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of ratings} \times \text{Maximum disease rating scale}} \times 100$$

The host plant reactions were classified based on the AUDPC (Area Under Disease Progress Curve). It was computed according to the formula described by Campbell and Madden [19]:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2] + t_{i+1} + X_i$$

Where X_i is the percentage of affected foliage at observation of i^{th} , t_i is the time of each observation and n is the total number of disease observations.

2.3 Data Analysis

The ANOVA (Analysis of Variance) was calculated for AUDPC data as randomized complete block design (RCBD) in the both environments using SAS (V 9.2) software package. Disease data were analyzed after checking for good fitness to ANOVA.

3. RESULTS

3.1 Screening Maize Inbred Lines in Different Environments

The 40 inbred lines were evaluated for TLB reactions under artificial epiphytotic conditions in two different environments; BHU, Varanasi and Nagenahalli, Karnataka. The ANOVA revealed a significant difference among the inbreds in both the environments (Table 2).

3.2 E₁-Varanasi, Uttar Pradesh, India

The experiment was conducted with 40 maize inbreds in RBD with two replications as mentioned in materials and methods during *Kharif* 2015-16. These inbred lines were classified on the basis of AUDPC values into Resistant (450-599.99), Partial resistant (600- 749.99), Partial susceptible (750-899.99), Susceptible (900-1049.99) and highly susceptible (1050-1199.99) [20]. In Varanasi, out of 40 inbreds, 10 inbreds (HKI-586, HUZM-53, CM-145, V-336, V-338, HKI-PC-8, HUZM-47, CM-104, CM-105 and CML-192) were classified as TLB resistant based on AUDPC values ranged from 480.00- 599.38 (Table 1) whereas, 13 partial resistant; 7 partial susceptible; 10 susceptible and no inbred was identified as highly susceptible (Table 3).

3.3 E₂-Nagenahalli, Karnataka, India

In Nagenahalli, out of 40 inbreds, 3 inbreds (CM-104, CM-145 and V-336) were classified as resistant based on AUDPC values ranged from 558.00- 596.50 (Table 1) whereas, 6 partial resistant; 14 partial susceptible; 7 susceptible and 10 inbreds were identified as highly susceptible (Table 3).

Table 1. Pedigree details, place of origin and AUDPC (Area Under Disease Progress Curve) values of 10 TLB resistant maize inbreds at BHU, Varanasi and three at Nagenahalli, Karnataka

S. No.	Inbreds	Pedigree	Place of origin	AUDPC values	
				Varanasi	Nagenahalli
1	HKI-586	CH 3	Karnal	508.75	643.50
2	HUZM-53	ISO2 X 1381 WA	Varanasi	578.13	603.00
3	V-338	B1045010	Almora	575.00	656.15
4	HKI-PC-8	LMC 8	Karnal	599.38	915.00
5	HUZM-47	P502C2-185-3-4-1-3-B-1-B-B	Varanasi	569.38	643.20
6	CM-104	A.Theo21	Amberpet	518.13	574.00
7	CM-105	Peru330	Amberpet	578.13	657.00
8	CML-192	Pool 34Q	CIMMYT	563.75	638.25
9	CM-145 (Check)	Pop 31	Almora	480.00	558.00
10	V-336 (Check)	CML 145,P63CDHC181-3-2-1-4#2-BBBB#F-BBBBB#	Almora	513.75	596.50

Table 2. Analysis of variance for AUDPC of TLB in 40 maize inbred lines evaluated at BHU, Varanasi (E₁) and Nagenahalli, Karnataka (E₂)

Source of variation	df	Mean Sum of Square	
		BHU, Varanasi	Nagenahalli, Karnataka
Replication	1	16953.66	72363.46
Treatment	39	50303.71**	64722.32**
Error	39	721.77	1660.44
CV %		3.64	4.63

**Significant at 0.01 probability level.

Table 3. Classification of maize inbred lines based on AUDPC values into Resistant (450-599.99), Partial resistant (600-749.99), Partial susceptible (750-899.99), Susceptible (900-1049.99) and Highly susceptible (1050-1199.99)

	E ₁ (BHU, Varanasi)	E ₂ (Nagenahalli, Karnataka)
Resistant	HKI-586, HUZM-53, CM-145, V-336, V-338, HKI-PC-8, HUZM-47, CM-104, CM-105, CML-192	CM-104, V-336, CM-145
Partial resistant	V-341, CM-141, CML-118, HKI-536, HKI-287, HUZM-478, HKI-193, V-348, V-388, HUZM-509, HUZM-81-1, HUZM-356, HUZM-457	CML-192, CM-105, HUZM-47, V-338, HUZM-53, HKI-586
Partial susceptible	HUZM-185, HKI-1105, HKI-335, CML-152, V-342, V-346, V-273	V-342, CML-152, HUZM-356, HUZM-81-1, HUZM-509, V-388, V-348, HKI-193, HUZM-478, HKI-287, HKI-536, CML-118, CM-141, V-341
Susceptible	Dhiari Local, V-335, HUZM-121, HUZM-36, CM-212, V-25, HKI-162, HUZM-88, CML-395, CM-126	V-273, V-346, CML-395, HKI-335, HUZM-457, HKI-PC-8, HUZM-185
Highly susceptible	-	CM-126, HUZM-88, HKI-162, V-25, CM-212, HUZM-36, HKI-1105, HUZM-121, V-335, Dhiari Local

4. DISCUSSION

TLB is the major foliar disease, affecting the maize production in India and has a worldwide distribution. The grain yield loss was reported upto 20% by Vestal and Semeniuk [21], 40% to 68% by Ullstrup [22] and 27.6% to 90.7% by Chenulu and Hora [23] while Rai [24] reported yield losses ranging from 26.6% to 97.5% and this was directly correlated to the disease severity. Singh et al. [16] reported heavy losses by TLB from a study conducted for several years at Almora. The most effective way to control losses due to TLB is to identify additional sources of disease resistance. Keeping this in view the experiment was carried out to understand the disease reactions and to identify additional sources of resistance in the two environments.

In the present study, inbreds were screened in Varanasi as well as in Nagenahalli under artificial epiphytotic conditions during *Kharif*, 2015 to *Kharif*, 2016. ANOVA revealed significant differences among the inbreds in both the environments. The study indicated that disease severity was always higher in Nagenahalli which appears to have a more conducive environment for disease development compared to Varanasi. Over-all more resistant inbreds (HKI-586, HUZM-53, CM-145, V-336, V-338, HKI-PC-8, HUZM-47, CM-104, CM-105, and CML-192) were

identified in Varanasi than Nagenahalli (CM-104, V-336 and CM-145). One of the major reasons for getting more resistant inbreds in Varanasi is due to relatively less disease pressure in Varanasi compared to Nagenahalli. The 40 maize inbreds were critically evaluated in two environments viz., E₁ (BHU, Varanasi), and E₂ (Nagenahalli, Karnataka). Similarly, Kumar et al. [25] conducted an experiment with 60 indigenous and exotic inbred lines during 2005-2006 at Almora (Uttarakhand) and Nagenahalli (Karnataka).

Maize inbreds CM-145, V-336 and CM-104 were classified as resistant in both the environments while, CM-212, V-25 and HKI-162 were classified as stable for susceptibility over the environments. This indicated consistency for resistance as well as susceptibility in these inbred lines. Several workers have previously reported genotypes showing their stability for resistance and susceptibility over environments and over the years. Sharma and Payak [26] reported durable resistance in CM-104 and CM-105 against *E. turcicum*. Chandrashekhara et al. [27] have also reported about the resistance of NCLB in lines CM-145, V-338, V-336, CM-138, CML-235, and NAI-135 in Almora as well as Nagenahalli in two separate experiments. Most of lines in the present study were classified as partially resistant and partially susceptibility such as V-341, CM-141, CML-118, HKI-536, HKI-287, HUZM-478, HKI-193, V-348, V-388, HUZM-509, HUZM-81-1, HUZM-356, HUZM-457, CML-192, CM-105, HUZM-47, V-338, HUZM-53, HKI-586, HUZM-185, HKI-1105, HKI-335, CML-152, V-342, V-346, V-273, and HKI-PC-8. Some of these lines which expressed resistance/partial resistance in E₁ exhibited susceptible/partial susceptible reaction in E₂, thus indicated strong influence of the environment on disease reactions. Majority of lines expressed resistance/partial resistance reaction in Varanasi, while susceptible/partial susceptible reaction in Nagenahalli. Kumar et al. [25] reported that virulence pattern of the TLB Pathogen from Almora changed, as inbreds CM 138, CML 235 and NAI 135, which showed a different resistant reaction over the two different locations. These results indicated changes in the virulence pattern of the pathogen as well as the impact of the environment. The climatic factors over the locations do have its influence on the number of maize inbreds as far as TLB resistant -susceptible reactions is concerned.

5. CONCLUSION

In the present study, an attempt has been made to identify the TLB resistance sources in Varanasi as well as in Nagenahalli under artificially inoculated field conditions. Out of 40 maize inbreds, 10 inbreds viz., HKI-586, HUZM-53, CM-145, V-336, V-338, HKI-PC-8, HUZM-47, CM-104, CM-105 and CML-192 were identified as resistant in Varanasi whereas only 3 inbreds viz., CM-104, V-336 and CM-145 were identified as resistant in Nagenahalli. Comparative study in two environments indicated that average disease incidence was higher in Nagenahalli than Varanasi, so as a hot spot for TLB, Nagenahalli is an ideal location to study the disease.

REFERENCES

1. Malik VK, Singh M, Hooda KS, Yadav NK, Chauhan PK. Efficacy of newer molecules, bioagents and botanicals against maydis leaf blight and banded leaf and sheath blight of maize. Plant Pathol J. 2018;34(2):121-125.
2. Smith DR. Global disease assessment of corn. In: Proceeding 54th Annual Corn and Sorghum Research Conference, Chicago. 1999;p.54.
3. Jakhar DS, Singh R, Kumar S, Singh P, Ojha V. Turcicum leaf blight: A ubiquitous foliar disease of maize (*Zea mays* L.). Int J Curr Microbiol Appl Sci. 2017;6:825-831.
4. Leonard KJ, Suggs EG. *Setosphaeria prolata* is the ascigenous state of *Exserohilum prolata*. Mycologia. 1974;66:181-297.

5. Carson ML. Inheritance of latent period length in maize infected with *Exserohilum turcicum*. Plant Dis. 1995;79:581-585.
6. Tefferi A, Hulluka M, Welz HG. Assessment of damage and grain yield loss in maize caused by northern corn leaf blight in western Ethiopia. J Plant Dis Protect. 1996;103:353-363.
7. St Clair DA. Quantitative disease resistance and quantitative resistance loci in breeding. Annu Rev Phytopathol. 2010;48:247-268.
8. Brewster VA, Carson ML, Wicks ZWIII. Mapping components of partial resistance to northern leaf blight of maize using reciprocal translocation. Phytopathology. 1992;82:225-229.
9. Smith DR, Kinsey JG. Latent Period- A possible selection tool for *Exserohilum turcicum* resistance in corn. Maydica. 1993;38:205-208.
10. Abebe D, Singburadom N, Sangchote S, Sarobol E. Evaluation of maize varieties for resistance to northern leaf blight under field conditions in Ethiopia. Kasetsart J Natural Sci. 2008;42:1-10.
11. Gulzar S, Dar ZA, Ahangar MA, Lone AA, Bhat MA, Kamal-ud-din, Khan MA, Sofi PA, Yousuf V, Yousuf N, Majid A. Identification of reaction pattern to turcicum leaf blight among early maturing maize (*Zea mays* L.) inbred lines. J Pharmacogn Phytochem. 2018;7:657-1660.
12. Dingerdissen AL, Geiger HH, Lee M, Schechert A, Welz HG. Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment. Mol Breed. 1996;2:143-156.
13. Singh R, Srivastava RP, Mani VP, Khandelwal RS, Ram L. Screening of maize genotypes against northern corn leaf blight. The Bioscan. 2014;9:1689-1693.
14. Mallikarjuna N, Puttaramanai NK, Kumar HR, Raveendra, Kumar VBS. Evaluation of maize germplasm for resistance to turcicum leaf blight. Int J Pure Appl Biosci. 2018;6:1601-1605.
15. Payak MM, Sharma RC. Disease rating scales in maize in India, In techniques of scoring for resistance to important diseases of maize, All India Coordinated Maize Improvement Project, Indian Agricultural Research Institute, New Delhi. 1983;p.1-4.
16. Singh R, Mani VP, Koranga KS, Bisht GS, Khandelwal RS, Bhandari P, Pant SK. Identification of additional sources of resistance to *Exserohilum turcicum* in maize (*Zea mays* L.). SABRAO J Breed Genet. 2004;36: 45-47.
17. Manu TG, Naik GB, Murali R, Nagaraja H. Identification of sources of resistance against turcicum leaf blight of maize. Int J Chem Stud. 2017;5:1664-1668.
18. Wheeler BEJ. An introduction to plant diseases. John Wiley and Sons. Ltd. London. 1969.
19. Campbell CL, Madden LV. Introduction to Plant Disease Epidemiology. John Wiley and Sons, Inc. New York. 1991.
20. Srivastava RP. Mapping of QTLs for northern corn leaf blight in maize (*Zea mays* L.). Doctoral dissertation, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India. 2015.
21. Vestal EP, Semeniuk S. *Puccinia sorghi* and *H. turcicum* on corn in Iowa during 1950. Plant Dis Rep. 1951;35:212-214.
22. Ullstrup AJ. The effect of some leaf diseases on grain yield in corn. Phytopathology. 1951;41:34-36.
23. Chenulu VV, Hora TS. Studies on losses due to *Helminthosporium* blight of maize. Indian Phytopathol. 1962;15:235-237.

24. Rai GS. Status of breeding and management of turcicum leaf blight and premature drying, Proc. 30th Annual Maize workshop. New Delhi: AICRP, IARI. 1987;p.21-27.
25. Kumar S, Pardurange Gowda KT, Pant SK, Shekhar M, Kumar B, Kaur B, Hettiara Chchi K, Singh ON, Prasanna BH. Sources of resistance to *Exserohilum turcicum* (Pass.) and *Puccinia polysora* (Underw.) incitant of turcicum leaf blight and polysora rust of maize. Arch. Phytopathol. Plant Protect. 2011;44:528-536.
26. Sharma RC, Payak MM. Durable resistance in the maize inbred lines. Theor Appl Genet. 1990;80:542-544.
27. Chandrashekara C, Jha SK, Agrawal PK, Singh NK, Bhatt JC. Screening of extra early maize inbred under artificial epiphytotic condition for North-Western Himalayan region Vivekananda Parvatiya Krishi Anusandhan Sansthan, (ICAR), Almora (Uttarakhand), India. Maize Genet Coop News Lett. 2012;p.86.