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Disease Reaction Studies for Turcicum Leaf Blight Exserohilum Turcicum Disease in Maize Zea Mays L. Under Artificial Epiphytotic Conditions

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Abstract
Maize is widely cultivated around the globe under varied climatic conditions. India is one of the major maize producing countries and stands fourth in terms of production. Turcicum leaf blight (TLB) disease caused by the pathogen, *Exserohilum turcicum* is one of the major diseases of maize causing varying extent of grain yield loss from 15 to 30%. To overcome the losses due to the disease, resistant cultivars needs to be developed. Hence, the identification of resistant sources is important in order to develop TLB resistant hybrids. Present investigation was conducted to identify TLB resistant inbreds and hybrids. Simplified Triple Test Cross (STTC) design was employed to develop 96 hybrids using twenty inbred lines. Further the hybrids and inbred lines along with two checks (Resistant –NAI 137; Susceptible – CM 202) were evaluated for TLB disease reaction under artificial epiphytotic condition during *rabi* 2019 in simple lattice design with two replications. Scoring was done from 60 to 80 days after sowing based on 1-9 TLB disease scale. Among the inbred lines V-86, MAI-295, V-70, MAI-214, V-85, 40375, V-72, V-39, 40061 were resistant and MAI-288, V-24 were susceptible. Among hybrids, 36 were resistant, 44 were moderately resistant. 14 were moderately susceptible and two hybrids were susceptible to TLB disease. Identified TLB resistant hybrids needs to be further evaluated to test their performance and stability across locations. These resistant sources can be used to transfer TLB disease resistance.

Keywords: Maize, Exserohilum turcicum, Resistant, Susceptible

I. Introduction

Maize is widely cultivated around the globe under varied climatic conditions. India is among the major maize producing countries and stands fourth in terms of production. World maize production is 28.76 Mt with an average productivity of 3.06 t ha^{-1} covers an area of 9.57 Mha [4]. Its multifaceted utilization for human needs and animal feed makes it one of the important crops. Many biotic and abiotic factors limit maize production [6]. In general, 9% grain yield loss occurs due to diseases [13]. Globally, diseases reported to occur in maize are 112. In India, 35 diseases are reported to affect grain yield [1].

Based on the intensive research conducted in maize, 16 diseases have been identified which adversely affects productivity [15]. Among them, *Exserohilum turcicum* causing Turcicum leaf blight (TLB)

disease caused by the pathogen, is one of the major diseases of maize resulting in varying extent of grain yield loss from 15 to 30%, which may shoot upto 70% under adverse conditions [9]. It is a fungal disease with serious effect on the maize foliage owing to loss in leaf area and reduces photosynthetic activity leading to severe grain yield loss [8]. Characteristic symptoms of TLB disease occurs on the lower leaves as long elliptical necrotic lesion and then spreads althrough the foliage. These lesions tend to reduce the green photosynthetic area thereby, resulting in deformed cobs with shriveled grains [14]. Secondary spread of the disease occurs through air borne conidia as pathogen survives in the plant debris. The disease is known to occur widely in both tropical and temperate regions. High temperature and humidity prevailing during early growth stages to grain filling stages triggers the development and spread of the disease [19].

To effectively combat the loss, effective management of TLB disease is necessary. Resistant hybrids and cultivars are of prime importance to manage the disease efficiently. Breeding for disease resistance is the most practical, cost-effective and eco-friendly method of managing the disease [5]. Prior to the development of resistant hybrids, there is a need to identify resistant sources. Further this can be made use in the breeding programme for population improvement, development of hybrids or transfer of the resistant genes to a widely adapted hybrid by backcross method. Hence, the present investigation was conducted with an objective to identify inbred lines and hybrids resistant to TLB disease.

II. Material and methods

Experimental material comprised of 96 hybrids and their parents. Inbred lines used in the study as parents were selected based on grain yield and TLB disease reaction [2]. Two checks were used, NAI 137 as resistant check and CM-202 as a susceptible check for TLB disease. Material was sown with the recommended spacing of 0.6 × 0.3 m under artificial epiphytotic condition during *rabi* 2019. The experimental material was evaluated for TLB disease reaction under artificial epiphytotic condition during *rabi* 2019.

A. Artificial inoculation of pathogen by leaf whorl technique

Artificial epiphytotic condition was created by following leaf whorl drop method [20]. Fungal pathogen *Exserohilum turcicum* was isolated from TLB infected leaves by placing them into PDA (Potato Dextrose Agar) petri dishes. After 24 hours, *E. turcicum* colonies were identified and transferred on to PDA slants and incubated for 15 days at room temperature. Further hyphal tip isolation protocol [17] was used to obtain pure culture. Sterilized sorghum grains were aseptically inoculated and incubated for mass multiplication of the pathogen [10]. After 20 days, fully sporulated sorghum grains were ground into fine powder. 1 to 1.5 gram of fine powder along with jaggery was mixed with water to form a solution. Test plants were sprayed with this solution on their leaf whorls from 45th days after sowing and continued for 9–10 days in order to create optimum inoculums load.

B. Scoring of the disease based on disease severity

Disease scoring was done based on the necrotic lesions on leaf. By visualizing the prominent symptoms of TLB such as necrotic areas, *per cent* disease severity was noted from 60 to 90 days after sowing [7]. Further, obtained TLB disease severity percentage was converted to disease score based 1-9 scale given by [11] (Table I). *Per cent* index (PDI) was calculated from the obtained disease scores from the formula

given by [21]. Based on disease scores, experimental material was categorized into resistant and susceptible groups.

$$\text{PDI (\%)} = \frac{\text{Sum of Numerical grading}}{\text{Total number of plants observed} \times \text{Maximum disease grade}} \times 100$$

TABLE I: Severity of turcicum leaf blight disease in 1-9 scale

Rating scale	Degree of infection (% Diseased leaf area)	Disease severity	Reaction
1.0	Zero to very slight infection (≤10%).	≤11.11	Resistant Score: ≤3.0) PDI: <33.33
2.0	Little infection, 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, 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 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, 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 Score:>7.0 PDI: >77.77
9.0	Very heavy infection, lesions abundant scattered on almost all leaves, plants prematurely dried or killed (>80%).	99.99	

III. RESULTS AND DISCUSSION

A. Analysis of variance for TLB disease percentage in maize

Analysis of variance (ANOVA) for TLB disease percentage in maize (Table II). Significance of mean sum of squares due to genotypes (parents, hybrids and checks) indicated significant difference among the genotypes for all productivity per se traits and disease percentage investigated in the study

TABLE II: Analysis of variance of simple lattice design for disease percentage

Source of variation	Degrees of freedom	Sum of Squares	Mean sum of squares
Replications	01	53.44	53.44
Genotypes (unadjusted)	115	53348.76	1463.90**
Error	19	14780.58	777.92
Blocks within replicated	11	2961.15	269.19

(adjusted)			
Intra block error	07	16329.71	378.42

Note: * Significant @ P = 0.05 ; **Significant @ P = 0.01

B. Analysis of variance for disease response groups for genotypes (parents and crosses)

The values of TLB disease percentage of different genotypes falling under different disease response groups were subjected to analysis of variance (Table III). Significance of mean sum of squares between response groups justifies the classification of parents and hybrids into four different disease response groups. It emphasizes that there existed significant difference interms of disease reaction among the parents and hybrids that were screened for TLB resistance

There was significant difference among hybrids and parents of each of the disease response group. For hybridization programme, the parents can be selected between the disease response groups to have contrasting disease reaction. The results were similar to that of [18] in Blackgram.

TABLE III: Analysis of variance for disease response groups for genotypes (parents and hybrids)

Source of variation	Degrees of freedom	Mean sum of squares	'F' statistics	P - value	F critical
Between response group	03	90.94**	294.99	5.04×10^{-25}	2.68
Within response group	117	0.30	-	-	-
Total	120	-	-	-	-

Note: * Significant @ P = 0.05 **Significant @ P = 0.01

C. Disease reaction of parents TLB disease under artificial epiphytotic condition

Identification of resistant sources for TLB disease is an important arena since it is the pre-requisite for the development of TLB disease resistant hybrids. Host plant resistance is the most cost effective, eco-friendly and durable method of disease management. Hence, parents and hybrids were screened for TLB disease reaction under artificial epiphytotic conditions to create increased disease pressure.

Screening results revealed that among parents, nine inbred lines had the disease score less than 3 and were categorized as resistant (Table IV). Varying frequency of parents for different disease groups are showed in Figure 1. These inbred lines can be used resistant breeding programme to develop hybrids with TLB disease resistance. Five inbreds were moderately resistant to TLB disease with the disease score between 3.1-5. Moderately susceptible inbreds were four in number with disease score of 5.1-7.1. Two inbred lines were susceptible to TLB disease with disease score of 7.1-9.0. These inbred lines falling under different disease groups can be employed to study the genetic acrhitecture of TLB disease resistance by crossing in definite fashion.

TABLE IV: Responses hybrids for turcicum leaf blight disease under artificial epiphytotic condition

Scale	Disease reaction	Parents
<3.0	Resistant	V-86, MAI-295, V-70, MAI-214, V-85, 40375, V-72, V-39, 40061
3.1-5.0	Moderately resistant	MAI-212, MAI-1, MAI-135, MAI-8, MAI-16,

5.1-7.0	Moderately Susceptible	MAI-746, MAI-194, MAI-308, MAI-202
7.1-9.0	Susceptible	MAI-288, V-24

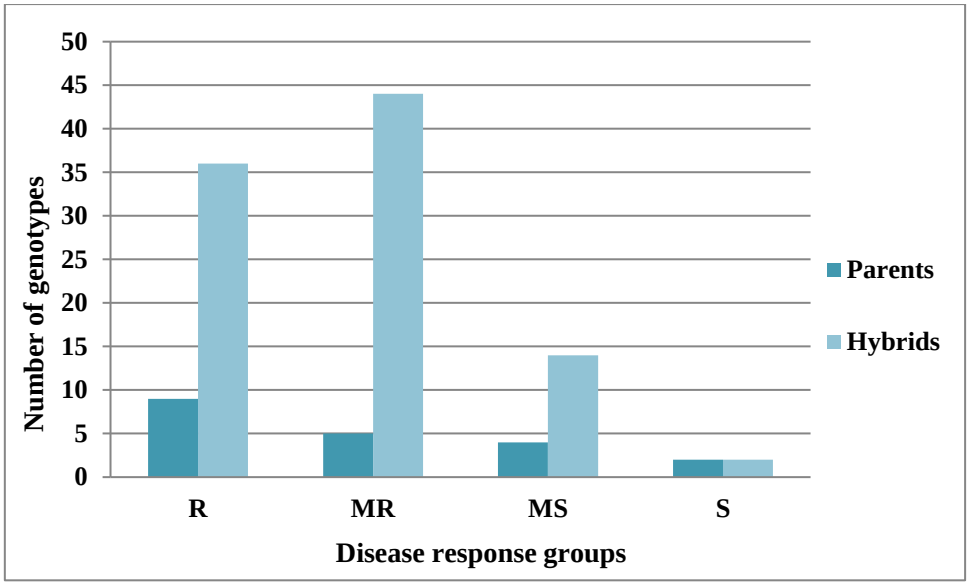

Fig 1: Distribution of genotypes (hybrids and parents) across TLB disease response groups.

D. Disease reaction of hybrids for TLB disease under artificial epiphytotic condition

Screening of single cross hybrids against TLB disease enabled grouping of hybrids into various TLB disease reaction groups and identification of TLB disease resistant hybrids. Among hybrids, 36 were resistant and 44 were moderately resistant with less than 3 and 3.1-5.0 disease score, respectively. Variation in frequency of different disease response groups of hybrids are depicted in Figure 1. Fourteen SCHs had 5.1-7.0 and two SCHs had 7.1-9.0 disease score and were categorized as moderately susceptible and susceptible disease group, respectively (Table V). So obtained can be further evaluated for TLB disease response across locations to identify stable TLB resistant hybrids. Further identification of stable TLB resistant hybrids with higher yield can be carried out and same can be further released.

TABLE V: Response of hybrids for turcicum leaf blight disease under artificial epiphytotic condition

Scale	Disease reaction	Hybrids
<3.0	Resistant	V-86 × 40375, V-86 × V-24, V-86 × 40061, MAI-212 × 40375, MAI-212 ×MAI-308, MAI-212 × V-72, MAI-212 × V-39, MAI-212 × 40061, MAI-295 × 40375, MAI-295 × V-72, MAI-295 × 40061, V-70 × MAI-308, V-70 × V-72, V-70 × V-24, V-70 × 40061, MAI-214 × 40375, MAI-214 × V-72, MAI-214 × V-39, MAI-214 × V-24, MAI-1 × 40375, MAI-1 × V-39, MAI-135 × V-72, MAI-135 × V-39, MAI-135 × 40061, MAI-746 × V-39, MAI-746 × 40061, MAI-194 × 40375, MAI-194 × V-72, MAI-194 × 40061, MAI-16 × 40375, MAI-16 × V-72, MAI-16 × MAI-288, MAI-16 ×40061, V-85 × MAI-308, V-85 × V-39, V-85 × 40061,

3.1-5.0	Moderately resistant	V-86 × MAI-308, V-86 × V-72, V-86 × MAI-288, V-86 × V-39, V-86 ×MAI-202, MAI-212 × MAI-288, MAI-212 × V-24, MAI-212 × MAI-202, MAI-295 × MAI-308, MAI-295 × MAI-288, MAI-295 × V-39, MAI-295 × V-24, MAI-295 × MAI-202, V-70 × 40375, V-70 × V-39, V-70 × MAI-202, MAI-214 × MAI-308, MAI-214 × MAI-288, MAI-214 × 40061, MAI-214 × MAI-202, MAI-1 × MAI-308, MAI-1 × V-72, MAI-1 × MAI-288, MAI-1 × 40061, MAI-135 × 40375, MAI-135 × MAI-288, MAI-135 × V-24, MAI-8 × 40375, MAI-8 × V-72, MAI-8 × V-39, MAI-8 × 40061, MAI-8 × MAI-202, MAI-746 × 40375, MAI-746 × V-72, MAI-194 × MAI-288, MAI-194 × V-39, MAI-194 × V-24, MAI-194 × MAI-202, MAI-16 × MAI-308, MAI-16 × V-39, MAI-16 × V-24, MAI-16 × MAI-202, V-85 × 40375, V-85 × V-72,
5.1-7.0	Moderately Susceptible	V-70 × MAI-288, MAI-1 × V-24, MAI-1 × MAI-202, MAI-135 × MAI-308, MAI-135 × MAI-202, MAI-8 × MAI-308, MAI-8 × MAI-288, MAI-8 × V-24, MAI-746 × MAI-308, MAI-746 × V-24, MAI-194 × MAI-308, V-85 × MAI-288, V-85 × V-24, V-85 × MAI-202,
7.1-9.0	Susceptible	MAI-746 × MAI-288, MAI-746 × MAI-202,

E. Distribution of resistant crosses in relation to TLB disease reaction of parents

Resistant hybrids were categorized based on the TLB disease reaction of parents. No hybrids resistant for TLB disease was obtained when both the parents and female and male parent were susceptible (Table VI). The proportion of resistant hybrids obtained were high when both the parents were resistant to TLB disease, reassuring that the inheritance of TLB disease resistance is predominantly controlled by additive gene effects [3] [16]. Preponderance of additive gene effects, emphasizes the utility of recurrent selection to improve the frequency of resistant alleles and obtain TLB disease resistant cultivars [12].

Table VI: Distribution of resistant crosses in relation to TLB disease reaction of parents

Sl. No.	Disease reaction of parents	Number of crosses under the category	Number of crosses resistant to TLB disease	Conditional probability of resistant crosses
1	R × R	40	38	0.57
2	R × S	40	24	0.36
3	S × R	8	4	0.06
4	S × S	8	-	-

IV. CONCLUSION AND FUTURE PROSPECTS

Ubiquitous nature of maize has made it one of the important food crop across world. Grain yield potential of the released hybrids are not fully realized due to the various diseases. Turcicum leaf blight one of the prominent diseases causes yield loss from 15 to 30%. Management of the disease requires development of TLB disease resistant cultivars/hybrids, which needs prior identification of resistant sources. In this regard, 20 parents and 96 hybrids were screened against TLB disease. Among the inbred

lines V-86, MAI-295, V-70, MAI-214, V-85, 40375, V-72, V-39, 40061 were resistant and MAI-288, V-24 were susceptible. Among hybrids, 36 were resistant, 44 were moderately resistant. 14 were moderately susceptible and two hybrids were susceptible. TLB resistant parents form a pre-requisite for the transfer and development of TLB disease resistant cultivars. Whereas, resistant hybrids can be further evaluated for its performance for yield and its attributing traits and can be released cultivation. Distribution of resistant crosses in relation to parental disease reaction revealed that proportion of resistant hybrids was high when both parents were resistant. This inferred predominance of additive gene effects for inheritance of TLB disease resistance. Consequently, population improvement approaches such as recurrent selection will be helpful to develop TLB resistant cultivars.

V. REFERENCES

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