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Field Screening of Maize (*Zea mays* L.) Genotypes against Turcicum Leaf Blight (TLB) under Artificial Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: There are a variety of critical problems facing tropical maize cultivation, such as disease infestation, insect/pest and weed problems. Foliar diseases in particular cause significant losses in agricultural yield. Leaf blight, known as northern leaf blight, is a disease of maize leaves caused by an overgrowth fungus. Therefore, it was necessary to develop sources of resistance to

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TLB and then a field experiment was conducted to study the performance of 64 (44 test crosses, 11 lines, 4 examiners and 5 assays) During Spring 2021-22 at the Agricultural College Farm, Babatla.
Methods: Screening was carried under field conditions by adopting an artificial disease inoculation technique Disease score was recorded at tasseling, 20 DAT and maturity stages by using 1-9 scale.
Results and Discussion: At maturity stage 12 crosses, seven lines and one check showed resistant reaction (R); 25 crosses, 4 inbreds, one check showed moderately resistant reaction (MR); 7 crosses, 3 inbreds and one check showed moderately susceptible reaction (MS) and one inbred, two checks showed susceptible reaction (S) under field screening.
Conclusion: The lines VL171488-2, VL18828, VL19705-8, VL18142, VL175869, SNL19582-22 and tester LM13 showed resistant disease reaction for TLB pathogen at phenotypic level. These lines can be used in future TLB resistance breeding programmes.

Keywords: Genotypes; Zea mays; Turcicum leaf blight; resistance breeding programmes.

1. INTRODUCTION

"Maize with a notable productive potential among the cereals, is the third most important grain crop after wheat and rice" [1]. In India, it is cultivated over an area of 9.89 m ha with a production and productivity of 31.60 million tonnes and 3199 kg ha^{-1}, respectively. In Andhra Pradesh, maize is cultivated in an area of 0.3 m ha, with a production and productivity of 1.78 million tonnes and 5917 kg ha^{-1}, respectively [2].

"A variety of biotic stresses, such as disease incidence, insects/pests, and weed problems like *Striga* spp., are common in tropical maize-growing areas. Foliar diseases, in particular, cause significant losses. The turcicum leaf blight, commonly known as northern leaf blight, is a foliar disease of maize caused by the ascomycetes fungus, *Setosphaeria turcica* and its conidial stage *Exserohilum turcicum* (Pass.) Leonard and Suggs. (syn. *Heliminthosporium turcicum* Pass) affects the photosynthesis and reduces kernel yield to an extent of 28 to 91%" [3,4]. The disease is characteried by long elliptical, greyish green or brown leaf lesions which emerge first on the lower leaves and gradually extend throughout the foliage. 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, have reduced germination capacity, vigor, grain yield and total sugar content [5,6] has restricted starch formation, chaffy kernels and infected plants are liable to infection with stalk rots [7]. "The disease is prevalent in Karnataka, Bihar, Himachal Pradesh, Andhra Pradesh and Maharashtra" [8,9]. "Several disease management options have been recommended to reduce the impact of maize foliar diseases. Among these practices, planting of resistant cultivars can effectively reduce the rate of disease development and is widely recommended. Breeding for resistance is a practical, cost-effective means to manage the disease" [10]. The development of resistance against turcicum leaf blight will have large effect on the maize crop improvement programmes.

2. MATERIALS AND METHODS

2.1 Field Layout

For the identification of resistant sources against *E. turcicum,* 64 genotypes were evaluated in an augmented design at Agricultural College Farm, Bapatla. Each entry is planted in one row of 1.6 meters length, adopting a spacing of 60 x 20 cm for screening against *Exserohilum turcicum*. The block was flanked by 3 border rows of susceptible cultivar, P3396.

For screening of germplasm against turcicum leaf blight resistance, artificial inoculation was preferred over natural infection as it ensures that the plants are properly exposed to right amount of inoculum for cause of the disease. The steps followed in screening of maize genotypes by artificial inoculation are given below.

2.2 Collection of Bacterium

Exserohilum turcicum inoculum was collected from the Department of Plant Pathology at Agricultural College, Bapatla for artificial disease inoculation.

2.3 Mass Multiplication

The mass multiplication of the pathogen *E. turcicum* was done on sterilized sorghum grain culture [11] and was presented in Fig. 1.

About an inch layer of sorghum grains (nearly 40 to 45 g) wass dispensed in a conical flask (500 ml) and soaked in water for about 3-4 hours and excess water was drained off after soaking. The flask containing sorghum grains was autoclaved twice, seeded with fungus under aseptic condition and kept for incubation at 25- 27 ^{0}C. The flasks were shaken once in 2-3 days to facilitate uniform growth of the pathogen on grains. After incubation of about a fortnight the material was ready for inoculation (Fig. 1). The above impregnated sorghum grains were allowed for drying by spreading them on a clean paper sheet in shade at room temp. After drying, prepared a fine powder of these grains with the help of mixer- grinder and put a pinch of this powder in the leaf whorl.

2.4 Artificial Disease Inoculation

Three weeks old culture of *E. turcicum* multiplied on sorghum grains was powdered and inoculated into the whorls of test plants at 32 DAS following whorl drop method of inoculation [12] and was followed by water spray so as to maintain humidity for infection. The inoculation was done in the evening time between 5 and 6 pm (Fig. 1).

2.5 Disease Score

Turcicum leaf blight severity was recorded on five plants in each entry at the time of tasseling, twenty days after tasseling and at maturity using 1-9 disease rating scale [13,14] presented in the Table 1.

2.6 Per cent Disease Index (PDI)

Based on disease severity data, per cent disease index (PDI) was calculated from the formula given by Wheeler [15].

$$\text{PDI (\%)} = \frac{\text{Sum of individual disease ratings}}{\text{No. of observations assessed x Maximum disease rating}} \times 100$$

Table 1. Disease scoring scale (1-9) for turcicum leaf blight [13,14]

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 DLA : ≤ 30% 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 ear (30.1-40%).	44.44	Moderately resistant (MR) Score: 3.1–5.0 DLA : 30 –50% 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 ear (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 ear (50.1-60%)	66.66	Moderately susceptible (MS) Score: 5.1-7.0 DLA : 50.1 – 70% 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 ear (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 DLA : >70% 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- Disease leaf area; PDI**- Per cent disease index*

3. RESULTS AND DISCUSSION

The plants were inoculated at the stage of tasseling (32 – 45 DAS). There was clear cut differential responses of both inbreds and hybrids against TLB under artificial epiphytotics. The disease score, per cent disease index values and disease reaction of maize genotypes (inbreds and hybrids) are presented in Table 2. The genotypes were scored on 1-9 scale [13,14].

The disease score at the time of tasseling ranged from 1.0 to 3.0 for hybrids and 1.0 to 4.0 for inbreds. The PDI values ranged from 2.2 - 6.7% for hybrids and 2.2 - 11.1% for inbreds. Thus, at the stage of tasseling all the genotypes showed apparently resistant to moderatley resistant (MR) reaction indicating the requirement of sufficient time period for the screening. The susceptible check scored the reaction value of 3.0. The disease score at the time of 20 days after tasseling was more compared to before tasseling *i.e*., 1.0 to 6.0 in hybrids an 2.0 to 6.0 in inbreds indicating the rapid progress in disease spread and most of the genotypes started showing the symptoms of susceptibility reaction. The PDI values of the hybrids were 2.2 to 13.3%, while the inbreds were 4.4 to 13.3 %.

The disease incidence at the time of maturity was severe and most of the genotypes scored the susceptibility reaction to the pathogen and the values ranged from 1.0 to 7.0 in hybrids to 2.0 to 7.5 in inbreds indicating the presence of resistance genes in some of the lines. The disease reaction of the susceptible check during this stage was 8.0 indicating the spread of the disease not very fast among the genotypes and also from spreader rows to the tester genotypes this is mainly because of the limited favourable conditions *i.e*., temperature, humidity and moisture. Percent disease index (PDI) for hybrids and inbred lines were ranged between 8.9 – 16.7% and 4.4 - 16.7% respectively. One hybrid (SNL19564-20 x LM13) possessed a disease score of 1.0 and lowest per cent disease index (PDI) of 2.2%, indicating its highly resistant nature against TLB. Among the checks P3396 is classified as susceptible check with disease score 8.0 and PDI 17.7% in P3396 and DKC8171 the disease score was 8.0 and PDI was 17.8 %. Twelve hybrids, seven inbred lines, one check with disease score ≤ 3.0 was observed in the present investigation indicating their resistant reaction against TLB. 25 hybrids, four inbred lines and one check with disease score 3.1 – 5.0 were noted and categorized as moderately resistant to TLB. Seven hybrids and three inbred lines and one check with disease score of 5.1 **-** 7.0 were observed and fall under the category of moderately susceptible reaction to disease. one inbred line (SNL19564-20) and two checks (P3396 and DKC8171) with disease score 8.0 and 7.5 respectively., were categorized as TLB susceptible.

Among the inbreds, six lines *viz*., VL171488-2, VL18828, VL19705-8, VL18142, VL175869-14, SNL19582-22 and one tester, LM13 were categorized as resistant with disease score ≤ 3.0. Three lines *viz*., VL19978-6, VL19255, SNL19588-23 and one tester, LM14 were categorized as moderately resistant lines. One line, CAL1733-13, and two male testers, BML6 and BML7 were categorized as moderately susceptible lines. Only one line, SNL19564-20 with disease score 7.5 was categorized as susceptible to disease TLB (Fig. 3).

Thus, the present study revealed that there was a gradual increased in the disease incidence from tasseling to maturity stage as earlier reports by Bhat et al. [16]. Eighteen genotypes having resistant reaction during tasseling stage turned as moderately resistant at 20 DAT, six of the moderately resistant genotypes at 20 DAT showed moderately susceptible at maturity stage. Frequency distribution of genotypes against TLB reaction were presented in Fig. 2. Mir et al. [17] confirmed that among 10 inbred lines evaluated, three were found moderately resistant, five lines moderately susceptible and the rest two, were severely affected by TLB and rated as susceptible. Mallikarjuna et al. [18] out of 135 genotypes evaluated, none of the genotypes showed resistant, 34 genotypes expressed moderately resistant reaction, 73 showed moderately susceptible reaction and 29 genotypes exhibited susceptibility reaction to TLB disease. Yousuf et al. [1] confirmed that among seventy landraces, forty-three lines were categorised as resistant, eighteen moderately resistant, five moderately susceptible and landrace Tral 3 recorded highest percent disease index (PDI) as 78.91 per cent and rated as susceptible. Bantu et al. [19] screened 70 medium maturing maize inbreds against TLB under artificial epiphytotic conditions. The per cent disease index ranged from 13.3 - 80.0 and area under disease progressive curve was 300.0 - 1591.7.

All the 64 genotypes (44 test crosses, 11 lines, 4 testers and 5 checks) were screened against

1. Autoclaving of sorghum grains

2. TLB Pure culture

3. Seeded with fungus

4.

5. Incubation at 25-27 ^{0}C

6. Fine Powder of sorghum grains

7. Whorl drop inoculation

Fig. 1. Mass multiplication of the pathogen *E. turcicum* on sterilized sorghum grain culture (Joshi et al., 1969)

Table 2. Screening against TLB caused by *Exserohilum turcicum* in maize (*Zea mays* L.) during *rabi* 2021-22 at Bapatla

S. No.	Genotypes	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction
	HYBRIDS	Tasseling stage			20 Days after Tasseling			Maturity stage		
1	VL171488-2 x BML6	1	2.2	R	4	8.9	MR	4	8.9	MR
2	VL171488-2 x BML7	1	2.2	R	1	2.2	R	3	6.7	R
3	VL171488-2 x LM13	1	2.2	R	4	8.9	MR	4	8.9	MR
4	VL171488-2 x LM14	2	4.4	R	4	8.9	MR	4	8.9	MR
5	VL18828 x BML6	1	2.2	R	2	4.4	R	3	6.7	R
6	VL18828 x BML7	1	2.2	R	3	6.7	R	5	11.1	MR
7	VL18828 x LM13	1	2.2	R	2	4.4	R	6	13.3	MS
8	VL18828 x LM14	1	2.2	R	2	4.4	R	5	11.1	MR
9	VL19978-6 x BML6	1	2.2	R	1	2.2	R	2	4.4	R
10	VL19978-6 x BML7	2	4.4	R	2	4.4	R	5	11.1	MR
11	VL19978-6 x LM13	1	2.2	R	3	6.7	R	5.5	12.2	MS
12	VL19978-6 x LM14	3	6.7	R	4	8.9	MR	4	8.9	MR
13	VL19705-8 x BML6	3	6.7	R	5	11.1	MR	7	15.6	MS
14	VL19705-8 x BML7	3	6.7	R	4	8.9	MR	6	13.3	MS
15	VL19705-8 x LM13	1	2.2	R	3	6.7	R	5.5	12.2	MS
16	VL19705-8 x LM14	3	6.7	R	3	6.7	R	4.5	10.0	MR
17	VL19255 x BML6	3	6.7	R	3	6.7	R	3.5	7.8	MR
18	VL19255 x BML7	3	6.7	R	4	8.9	MR	4.5	10.0	MR
19	VL19255 x LM13	3	6.7	R	4	8.9	MR	4.5	10.0	MR
20	VL19255 x LM14	2	4.4	R	3	6.7	R	4.5	10.0	MR
21	VL18142 x BML6	2	4.4	R	3	6.7	R	3.5	7.8	MR
22	VL18142 x BML7	1	2.2	R	3	6.7	R	3.5	7.8	MR
23	VL18142 x LM13	1	2.2	R	1	2.2	R	1.5	3.3	R
24	VL18142 x LM14	1	2.2	R	2	4.4	R	3.5	7.8	MR
25	CAL1733-13 x BML6	2	4.4	R	2	4.4	R	2	4.4	R
26	CAL1733-13 x BML7	1	2.2	R	1.5	3.3	R	1.5	3.3	R
27	CAL1733-13 x LM13	1	2.2	R	1	2.2	R	1.5	3.3	R
28	CAL1733-13 x LM14	2	4.4	R	4	8.9	MR	4	8.9	MR
29	VL175869-14 x BML6	2.5	5.6	R	3	6.7	R	3.5	7.8	MR
30	VL175869-14 x BML7	1	2.2	R	3	6.7	R	5	11.1	MR

S. No.	Genotypes	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction
	HYBRIDS	**Tasseling stage**			**20 Days after Tasseling**			**Maturity stage**		
31	VL175869-14 x LM13	3	6.7	R	3	6.7	R	3	6.7	R
32	VL175869-14 x LM14	3	6.7	R	3	6.7	R	3.5	7.8	MR
33	SNL19564-20 x BML6	3	6.7	R	4	8.9	MR	4	8.9	MR
34	SNL19564-20 x BML7	3	6.7	R	3	6.7	R	3.5	7.8	MR
35	SNL19564-20 x LM13	1	2.2	R	1	2.2	R	1	2.2	R
36	SNL19564-20 x LM14	2	4.4	R	3	6.7	R	3	6.7	R
37	SNL19582-22 x BML6	3	6.7	R	3	6.7	R	4.5	10.0	MR
38	SNL19582-22 x BML7	3	6.7	R	4	8.9	MR	3.5	7.8	MR
39	SNL19582-22 x LM13	2	4.4	R	4	8.9	MR	4.5	10.0	MR
40	SNL19582-22 x LM14	3	6.7	R	4	8.9	MR	4	8.9	MR
41	SNL19588-23 x BML6	3	6.7	R	6	13.3	MS	7	15.6	MS
42	SNL19588-23 x BML7	3	6.7	R	5	11.1	MR	6.5	14.4	MS
43	SNL19588-23 x LM13	2	4.4	R	2	4.4	R	2.5	5.6	R
44	SNL19588-23 x LM14	1	2.2	R	2	4.4	R	2.5	5.6	R
LINES										
45	**VL171488-2**	**3**	**6.7**	**R**	**3**	**6.7**	**R**	**3**	**6.7**	**R**
46	**VL18828**	**2**	**4.4**	**R**	**3**	**6.7**	**R**	**3**	**6.7**	**R**
47	VL19978-6	3	6.7	R	4	8.9	MR	4.5	10.0	MR
48	**VL19705-8**	**3**	**6.7**	**R**	**3**	**6.7**	**R**	**3**	**6.7**	**R**
49	VL19255	3	6.7	R	3.5	7.8	MR	4	8.9	MR
50	**VL18142**	**1**	**2.2**	**R**	**2**	**4.4**	**R**	**2.5**	**5.6**	**R**
51	CAL1733-13	5	11.1	MR	6	13.3	MS	7	15.6	MS
52	**VL175869-14**	**1.5**	**3.3**	**R**	**2**	**4.4**	**R**	**2**	**4.4**	**R**
53	SNL19564-20	5	11.1	MR	5	11.1	MR	7.5	16.7	S
54	**SNL19582-22**	**2**	**4.4**	**R**	**2.5**	**5.6**	**R**	**3**	**6.7**	**R**
55	SNL19588-23	3	6.7	R	4	8.9	MR	4.5	10.0	MR
TESTERS										
56	BML6	4	8.9	MR	5	11.1	MR	6	13.3	MS
57	BML7	2	4.4	R	4	8.9	MR	6.5	14.4	MS
58	**LM13**	**1**	**2.2**	**R**	**3**	**6.7**	**R**	**3**	**6.7**	**R**
59	LM14	3	6.7	R	4	8.9	MR	4	8.9	MR
CHECKS										

S. No.	Genotypes	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction	Disease score	PDI	Disease Reaction
	HYBRIDS	**Tasseling stage**			**20 Days after Tasseling**			**Maturity stage**		
60	P3396	5	11.1	MR	6	13.3	MS	8	17.7	S
61	DKC8171	5	11.1	MR	6	13.3	MR	5	11.1	MR
62	P3546	2	4.4	R	3	6.7	R	3	6.7	R
63	DKC9120	3	6.7	R	4	8.9	MR	5	11.1	MR
64	PAC751	4	8.9	MR	4	8.9	MR	4	8.9	MR

Table 3. Categorization of maize genotypes based on their response to *E. turcicum* under artificial epiphytotic conditions

Group	Score	Type	Number	Name of Genotypes
Resistant (R)	≤ 3	Hybrids	12	VL171488-2 x BML7, VL18828 x BML6, VL19978-6 x BML6, VL18142 x LM13, CAL1733-13 x BML6, CAL1733-13 x BML7, CAL1733-13 x LM13, VL175869-14 x LM13, SNL19564-20 x LM13, SNL19564-20 x LM14, SNL19588-23 x LM13, SNL19588-23 x LM14
		Inbred lines	7	VL171488-2, VL18828, VL19705-8, VL18142, VL175869, SNL19582-22, LM13
		Checks	1	P3546
Moderately Resistant (MR)	3.1 - 5	Hybrids	25	VL171488-2 x BML6, VL171488-2 x LM13, VL171488-2 x LM14, VL18828 x BML7, VL18828 x LM14, VL19978-6 x BML7, VL19978-6 x LM14, VL19705-8 x LM14, VL19255 x BML6, VL19255 x BML7, VL19255 x LM13, VL19255 x LM14, VL18142 x BML6, VL18142 x BML7, VL18142 x LM14, CAL1733-13 x LM14, VL175869-14 x BML6, VL175869-14 x BML7, VL175869-14 x LM14, SNL19564-20 x BML6, SNL19564-20 x BML7, SNL19582-22 x BML6, SNL19582-22/BML7, SNL19582-22 x LM13, SNL19582-22 x LM14
		Inbred lines	4	VL19978-6, VL19255, SNL19588-23, LM14
		Checks	3	DKC9120
Moderately Susceptible (MS)	5.1 - 7	Hybrids	7	VL18828 x LM13, VL19978-6 x LM13, VL19705-8 x BML6, VL19705-8 x BML7, VL19705-8 x LM13, SNL19588-23 x BML6, SNL19588-23 x BML7
		Inbred lines	3	CAL1733, BML6, BML7
		Checks	1	PAC751
Susceptible (S)	> 7	Hybrids	0	--
		Inbred lines	1	SNL19564-20
		Checks	1	P3396, DKC8171

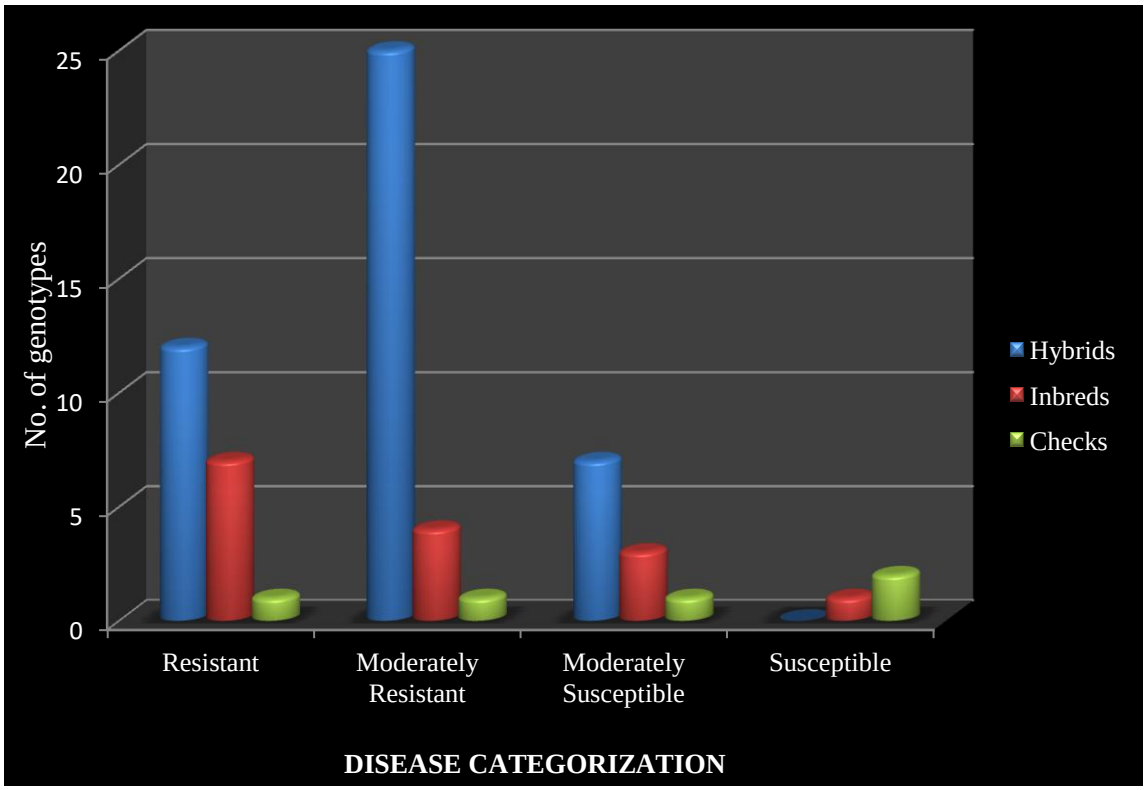

Fig. 2. Frequency distribution of genotypes for turcicum leaf blight (TLB) disease response

Fig. 3. Inbred lines showing moderately susceptible (BML7) and resistant (VL18828) reaction towards TLB incidence

turcicum leaf blight to identify tolerant/susceptible genotypes for leaf blight. 12 crosses, seven lines and one check showed resistant reaction (R); 25 crosses, four inbreds, one check showed moderately resistant reaction (MR); 7 crosses, 3 inbreds and one check showed moderately susceptible reaction (MS) and one inbred and two checks showed susceptible reaction(S). The classification of inbreds, hybrids and checks were presented in Table 2.

4. CONCLUSION

The development of resistance against turcicum leaf blight will have large effect on the maize crop improvement programmes. The lines VL171488-2, VL18828, VL19705-8, VL18142, VL175869, SNL19582-22 and tester LM13 showed resistant disease reaction for TLB pathogen at phenotypic level. These lines can be used in future TLB resistance breeding programmes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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