



RESEARCH ARTICLE

Evaluation of maize (*Zea mays* L.) germplasm for resistance to maydis leaf blight disease

K.S. Hooda, Chikkappa Gangadhar Karjagi^{1*}, Harleen Kaur², Harbinder Singh³, Aditi Ghosh¹, Shraddha Srivastava¹, Deeksha Joshi⁴, Robin Gogoi⁴, Preeti Jakhar, Sherry Jacob Rachel, Jyoti Kumari, Sushil Pandey, Ishwar Singh, Raj Kumar Gautam and Ashok Kumar

Abstract

Fungus *Cochliobolus heterostrophus* (Drech.) known as *maydis* leaf blight (MLB) or southern corn leaf blight, causes significant damage to maize crops and substantially reduces grain production. It is prevalent in warm and humid maize-growing areas throughout the world. The present study was aimed at the identification of stable sources of resistance to MLB. A set of 82 diverse inbred lines of maize were evaluated under artificial epiphytotic conditions at MLB hotspot location Delhi for two years (2020 and 2021) and in the third year at three MLB hotspot locations, namely, Delhi, Ludhiana, and Karnal during *kharif* 2022. The incidence of MLB was meticulously scored using a 1-9 rating scale. Based on two years' pooled data (2020 and 2021), eight genotypes, namely, IC0620945 (DML-1278), IC0620960 (DML-1390) (Score 1.95–2.85); IC0620992 (DML-1575) (Score 0.47–2.50), IC0620997 (DML-1634), IC0621026 (DML-1828), IC0621030 (DML-1834), IC0621040 (DML-1851) and IC0612726 (DML-212-1) were resistant to MLB with score ranging from 0.47 to 3.00. These eight lines were further subjected to multi-location evaluations, Ludhiana, Karnal, and Delhi, for validation of MLB resistance. Only four lines, viz., IC0620960 (Score 1.4–2.4), IC0620992 (Score 2.0–2.5), IC0621026 (Score 1.1–2.0) and IC0621040 (2.0–2.8) could confirm resistance to MLB at all the three locations. Thus, the identified four resistant maize inbred lines may be utilized for developing promising maize hybrids with a high degree of resistance to the devastating MLB disease. Further studies can focus on understanding the genetic basis of resistance in these resistant sources and accelerate the variety development using marker-assisted breeding.

Keywords: Maize, *maydis* leaf blight, host plant resistance, artificial epiphytotic conditions, hotspot locations.

Introduction

Maize (*Zea mays* L.) is a versatile crop with wide adaptability across different agro-climatic conditions in the world. It is the queen of cereals because of its highest genetic yield potential among cereals. Annually, it is cultivated on 205 million ha (2022) with a production of 1163 million MT with an average productivity of 5.72MT/ha in the world (FAOSTAT, 2022). The major production constraints, which lead to lower yield are biotic and abiotic stresses. Amongst biotic stresses, maydis leaf blight (MLB) also known as southern corn leaf blight (SCLB), caused by the fungus *Cochliobolus heterostrophus* (Drech.) [(*Bipolarismaydis* (Nishik. and Miyake) Shoemaker)] is one of the serious foliar diseases of maize prevalent in warm and humid maize growing areas throughout the world (Singh et al. 2016; Jeevan et al. 2020; Kumar et al. 2022). In India, the disease has acquired prominence and has spread widely in India's highlands, plains, and even to some parts of peninsular regions. In India, several MLB endemic sites (hotspots) have been identified, viz., Ludhiana (Punjab), Karnal (Haryana), Delhi (NCT), and Dholi (Bihar) for screening in hotspot locations (Kumar et al. 2022). MLB can cause yield losses of up to 40% (Fisher et al. 1976, Gregory et

ICAR-National Bureau of Plant Genetic Resources, New Delhi 110 012, India

¹ICAR-Indian Institute of Maize Research, Ludhiana 141 001, Punjab, India

²Department of Plant Breeding, Punjab Agricultural University, Ludhiana 141 001, Punjab, India

³Chaudhary Charan Singh Haryana Agricultural University, Uchani, Pin, Karnal, Haryana, India

⁴Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

***Corresponding Author:** Chikkappa Gangadhar Karjagi, ICAR-Indian Institute of Maize Research, Ludhiana 141 001, Punjab, India, E-Mail: cg.karjagi@icar.gov.in

How to cite this article: Hooda K.S., Karjagi C.G., Kaur H., Singh H., Ghosh A., Srivastava S., Joshi D., Gogoi R., Jakhar P., Rachel S.J., Kumari J., Pandey S., Singh I., Gautam R.K. and Kumar A. 2024. Evaluation of maize germplasm for resistance to maydis leaf blight of maize (*Zea mays* L.). Indian J. Genet. Plant Breed., **84**(4): 652-658.

Source of support: Consortium Research Platform on Agrobiodiversity (CRPAB)

Conflict of interest: None.

Received: Feb. 2024 **Revised:** Aug. 2024 **Accepted:** Sept. 2024

al. 1979). The effective management of MLB using chemical fungicides diminishes under significant disease prevalence coupled with the cultivation of susceptible cultivars in large areas (Poole and Arnaudin, 2014; Jeevan et al. 2020). Even though integrated disease management (IDM) practices are available to reduce the adverse impact of MLB on maize yield, the use of resistant cultivars, an integral component of IDM, often misses out due to the non-availability of resistant cultivars. Thus, the use of resistant cultivars remains a major challenge in the maximization of yield realization. Therefore, the development of MLB-resistant cultivars is considered as an economically viable and environment-friendly means of controlling the MLB disease. Extensive studies have not been conducted in India or elsewhere for the identification of genotypes resistant to MLB across locations (Sharma and Rai 2005; Chandrashekara et al. 2014; Jeevan et al. 2020). Therefore, it is important to evaluate a diverse set of maize genotypes over the years and their validation across locations for the effective use of resistant genotypes in regular breeding programme or genetic studies. The present study was undertaken to identify MLB-resistant genotypes through repeated evaluation of a selected set of maize genotypes conserved at National Genebank (NGB) at National Bureau of Plant Genetics Resources, New Delhi, under artificial inoculated epiphytotic conditions at MLB hotspot locations over the years and validation of resistance across MLB hotspot locations.

Materials and methods

Plant material

A set of 82 inbred lines comprising both indigenous and exotic collections, along with a resistant check (DML-1851) and susceptible check CM 500, were used in the present study. The lines were procured from the National Genebank (NGB) of ICAR-National Bureau of Plant Genetic Resources, New Delhi, by ICAR-Indian Institute of Maize Research, Ludhiana, India. The set represents the diverse genetic background of the active germplasm being used in the Indian maize breeding program. The sub-set from 82 genotypes comprising eight genotypes was selected based on the two-year resistant reaction at the Delhi hotspot location for evaluation under artificial epiphytotic conditions at other hotspot locations for MLB, namely, Delhi, Ludhiana, and Karnal during *Kharif* 2022.

Experimental locations and design

A genetically diverse set of inbred lines was evaluated under the artificial epiphytotic condition of MLB at Delhi for two years, *i.e.*, 2020 and 2021, in alpha design with two replications and in the third year at three MLB hotspot locations, namely Delhi, Ludhiana, and Karnal during *kharif* season of 2022 in RBD. The plot area of each genotype was 75 × 20 cm² in size, comprising a single row of three-meter

length. The genotypes were sown in single-row plots of 3 m in length with a spacing of 20 × 75 cm. The standard packages of practices were followed during the experiment to raise inbred lines under optimal growing conditions but under MLB epiphytotic conditions.

Creation of artificial MLB epiphytotic

The culture of MLB isolates was grown on sorghum grains three weeks before planting the test material. The well-grown pathogen culture on sorghum grain was stored at 6–9°C till inoculation. At the time of inoculation, the sorghum grains carrying MLB inoculum or culture were ground in a food chopper and were diluted to the desired level by adding flour of ground sorghum seeds. Subsequently, the inoculation was done when plants were 30 to 45 cm height by placing a small quantity of ground flour into the whorl during a cloudy day or evening to avoid mortality of inoculum by direct exposure to sunlight and to create artificial MLB epiphytotic conditions. The inoculation was carried out twice at weekly intervals between the first and second inoculation to ensure proper disease establishment (Hooda et al. 2018).

Disease scoring of genotypes for MLB

The disease reaction was measured and recorded on all the plants in each entry in the trial at 30 to 35 days after inoculation by following a 1 to 9 disease rating scale (Hooda et al. 2018), as shown in Table 1. To determine the overall disease rating of a genotype, the disease score of each plant was averaged for each replication, and the average disease rating of each genotype in each replication was subjected to statistical analysis to estimate the adjusted mean disease score and classify the genotype as given below.

Statistical analysis

The disease score data was analyzed online at the IASRI NARS portal (<https://drs.icar.gov.in/Analysis%20of%20data/Resolvable%20Block%20Design.html>). The data was subjected to analysis of variance (ANOVA) to determine significant differences among the genotypes, followed by post-hoc analyses to determine the difference between genotypes.

Results

A set of 82 maize inbred lines were evaluated along with checks during *kharif* 2020 for MLB under artificial epiphytotic conditions in Delhi, the MLB hotspot location. The disease score for MLB was recorded on individual plants in each genotype by following a uniform rating scale of 1–9 (≤ 3.0 = Resistant; 3.1–5.0 = Moderately resistant; 5.1–7.0 = Moderately susceptible; > 7.0 = Susceptible) as shown in Table 1. The reaction of genotypes to MLB inoculation was compared with CM 500, a highly susceptible check genotype, and the same was also sown after every 10th row

Table 1. Disease rating scale for evaluating the impact of MLB on maize plants

Rating scale	Degree of infection (percent DLA*)	PDI**	Disease reaction
1.0	Nil to very slight infection ($\leq 10\%$)	≤ 11.11	Resistant (R) (Score: < 3.0)
2.0	Slight infection, with a few lesions scattered on two lower leaves (10.1–20%).	22.22	(DLA: < 30%)
3.0	Light infection, a moderate number of lesions scattered on four lower leaves (20.1–30%).	33.33	(PDI: < 33.33)
4.0	Light infection, a 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)
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	(DLA: 30.1–50%) (PDI: 33.34–55.55)
6.0	Heavy infection, an 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	Moderately susceptible (MS) (Score: 5.1–7.0)
7.0	Heavy infection, an 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	(DLA: 50.1–70%) (PDI: 55.56–77.77)
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)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%).	99.99	(DLA: >70%) (PDI: >77.77)

*DLA = Diseased leaf area; PDI = Percent disease index, Hooda et al. 2018

to provide a uniform and adequate source of inoculums and also assess uniformity in disease incidence throughout the experimental area during the season. The genotypes showed significant variations in reaction to MLB and none of the genotypes were immune to MLB (Table 2). However, out of 82 lines, 25 lines were resistant, and 41 lines were moderately resistant reaction, and the remaining lines were either moderately susceptible (12) or susceptible (1). Based on the visual assessment concerning vigor and superior performance at the phenotypic level, a set of 44 visually productive lines showing resistant or moderately resistant reactions to MLB during *kharif* 2020 along with the susceptible check were further evaluated during *kharif* 2021 under similar conditions by following the same rating scale of 1-9.

The disease reaction varied from 0.47 (DML-1575) to 8.54 (DML-165) and 2.05 (DML 194-1) to 8.80 (DML-1897) during the *kharif* seasons of 2020 and 2021, respectively, signifying the presence of notable level of differences among the genotypes. The results showed that the genotypes exhibited a wide range of reactions, from resistant to susceptible, in relation to reaction to MLB in maize. During *kharif* 2021, the lines were screened for the second year by exposing to artificial created MLB epiphytotic condition through inoculation of *Bipolaris maydis* [(Nisikado and Miyake) Shoem] (*teleomorph*: *Cochliobolus heterostrophus*) grain culture in the whorl leading to expression of mean score of 7.7 on the susceptible check CM 500 ensuring high disease pressure during field screening experiment. Based on two years' pooled data (2020 and 2021), 8 genotypes

were found resistant to MLB *viz.*, IC0620945 (DML-1278) (Score 2.51–2.65); IC0620960 (DML-1390) (Score 1.95–2.85); IC0620992 (DML-1575) (Score 0.47–2.50), IC0620997 (DML-1634) (Score 2.66–3.00), IC0621026 (DML-1828) (Score 2.74–2.90), IC0621030 (DML-1834) (Score 2.78–2.95), IC0621040 (DML-1851) (Score 2.32–2.45) and IC0612726 (DML-212-1) (Score 3.00–2.15) (Table 2). These eight lines were further subjected to multi-location evaluation (Ludhiana, Karnal and Delhi) for validation/confirmation of MLB resistance in these lines. The disease score of the eight lines are given in Table 3. However, out of eight lines, four lines *viz.*, IC0620960 (DML-1390) (Score 1.4–2.4), IC0620992 (DML-1575) (Score 2.0–2.5); IC0621026 (DML-1828) (Score 1.1–2.0) and IC0621040 (DML-1851) (2.0–2.4) showed resistance to MLB at all the three locations (Table 3). The resistant sources identified based on two years were further confirmed in the third year of investigation which can be successfully utilized in maize improvement or breeding programmes with MLB resistance in the high yielding genetic backdrop as well as undertaking genetic studies followed by mapping genomic regions determining disease resistance.

Discussion

Globally, 115 diseases (caused by fungi, bacteria, viruses, nematodes, etc.) have been reported to infect maize crops, which adversely affects the potential yield (Khokar et al. 2014; Singh et al. 2022). On the contrary, the maize crop carries immense significance in ensuring food, feed, fodder, and energy security in the world. For example, the USA, being the largest producer of maize, uses ~45% of its

Table 2. Screening of maize inbred lines against MLB under artificial epiphytotic conditions during *kharif* 2020 and 2021

S. No.	Genotypes	IC Number	Pedigree	Disease Scores (Mean \pm SEM), Reaction	
				2020	2021
1	DML-1109	IC0620926	SUWAN-Ä-Ä-Ä-Ä	3.40 (\pm 0.62), MR	8.75 (\pm 0.21), S
2	DML-1117	IC0620929	MRCHY4852-1	2.67 (\pm 0.88), R	5.85 (\pm 1.63), MS
3	DML-1132	IC0620930	MRCHY4895-1	3.43 (\pm 0.62), MR	4.00 (\pm 1.41), MR
4	DML-119	IC0612743	HY10RN-8-235-118-1-3-1-4	3.56 (\pm 0.88), MR	6.65 (\pm 0.49), MS
5	DML-1257	IC0620944	7025/7026-Ä-Ä-Ä-Ä-Ä	3.40 (\pm 0.91), MR	2.28 (\pm 0.00), R
6	DML-1278	IC0620945	BIO 9637-Ä-Ä-Ä-Ä-Ä	2.51 (\pm 0.88), R	2.65 (\pm 0.49), R
7	DML-1288	IC0620947	BIO 9681-Ä-Ä-Ä-Ä-Ä	1.68 (\pm 0.89), R	6.18 (\pm 0.00), MS
8	DML-1352	IC0620951	MRCHY4780-4-Ä-Ä-Ä-Ä	2.24 (\pm 0.90), R	6.40 (\pm 2.55), MS
9	DML-1836	IC621032	JAY 2-1-2-1	3.20 (\pm 0.89), MR	5.15 (\pm 1.63), MS
10	DML-1390	IC0620960	MH 102-Ä-Ä-Ä-Ä-Ä	1.95 (\pm 0.89), R	2.85 (\pm 0.35), R
11	DML-1413	IC0620963	MRCHY4839-1	1.68 (\pm 0.89), R	7.48 (\pm 0.00), S
12	DML-1414	IC0620964	MRCHY4809-2	2.07 (\pm 0.63), R	4.45 (\pm 2.33), MR
13	DML-1451	IC0620973	MRCHY5222-2	3.48 (\pm 0.87), MR	5.00 (\pm 0.00), MR
14	DML-1451-1	IC0620974	MRCHY5222-2	3.15 (\pm 0.88), MR	4.00 (\pm 1.41), MR
15	DML-1473-1	IC0620977	MRCHY4956-5	2.09 (\pm 0.62), R	7.08 (\pm 0.00), S
16	DML-1479	IC0620981	MRCHY4961-4	1.95 (\pm 0.89), R	3.90 (\pm 0.42), MR
17	DML-1496-1	IC0620983	MRCHY5035	3.23 (\pm 0.89), MR	3.30 (\pm 0.28), MR
18	DML-1498	IC0620985	MRCHY5035	3.43 (\pm 0.88), MR	5.50 (\pm 0.71), MS
19	DML-1545	IC0620988	HQPM -1-Ä-Ä-Ä-Ä-Ä	2.28 (\pm 0.88), R	3.50 (\pm 2.12), MR
20	DML-1575	IC0620992	HQPM -5-Ä-Ä-Ä-Ä-Ä	0.47 (\pm 0.89), R	2.50 (\pm 0.71), R
21	DML-1611	IC0620995	PEHM-2-Ä-Ä-Ä-Ä-Ä	1.80 (\pm 0.62), R	4.20 (\pm 1.13), MR
22	DML-1634	IC0620997	PRAKASH-Ä-Ä-Ä-Ä-Ä	2.66 (\pm 0.62), R	3.00 (\pm 0.00), R
23	DML-1650	IC0621001	Seed Tech 2324-Ä-Ä-Ä-Ä-Ä	3.07 (\pm 0.89), MR	3.08 (\pm 0.00), MR
24	DML-1788-1	IC0621009	JKC-5-2-1-1-1	3.39 (\pm 0.89), MR	4.40 (\pm 1.41), MR
25	DML-1811	IC0621016	CML 408-2-1-1-1	3.12 (\pm 0.62), MR	5.15 (\pm 2.33), MS
26	DML-1815	IC0621018	WNCDMR11R 1611	1.57 (\pm 1.25), R	4.35 (\pm 0.07), MR
27	DML-1822	IC0621023	HKI 164-D-3-3-2	3.34 (\pm 0.89), MR	7.75 (\pm 1.77), S
28	DML-1828	IC0621026	BML 15-4-1-1-1	2.74 (\pm 0.88), R	2.90 (\pm 0.14), R
29	DML-1831	IC0621028	PFSR5106/1	3.42 (\pm 0.88), MR	2.35 (\pm 0.92), R
30	DML-1832	IC0621029	CM117-3-4-1-1-1	3.14 (\pm 0.88), MR	5.30 (\pm 1.56), MS
31	DML-1834	IC0621030	HKI 326-3-1	2.78 (\pm 0.87), R	2.95 (\pm 0.78), R
32	DML-1835	IC0621031	HKI 586-1WG33	2.94 (\pm 0.88), R	6.50 (\pm 0.71), MS
33	DML-1837	IC0621033	JAY 3-7-1	2.20 (\pm 0.89), R	3.15 (\pm 0.49), MR
34	DML-1842	IC0621035	DT/LN/EM-46-3-1 \times CML 311-2-1-3)-B-1-1	3.57 (\pm 0.90), MR	3.15 (\pm 1.63), MR
35	DML-1851	IC0621040	(CLQ-6601 \times CL-02843)-B-26-3-1-BB-2-1-1	2.32 (\pm 0.90), R	2.45 (\pm 0.49), R
36	DML-1857	IC0621042	(CA14502/CA14509)-F2-8-1-B*8-1-1	3.06 (\pm 0.88), MR	3.65 (\pm 0.92), MR
37	DML-1879	IC0621050	EY-DMR-C5-S2-BB-3-2-*6-1-BBB-1-1-1	1.95 (\pm 0.89), R	6.98 (\pm 0.00), MS
38	DML-1895	IC0621053	CA14514-B-2-B-2-BBB-1-1-1	3.59 (\pm 0.65), MR	4.45 (\pm 1.91), MR
39	DML-1897	IC0621054	(87036/87923) \times 800-3-1-BB-1-1-1	2.59 (\pm 0.89), R	8.80 (\pm 0.28), S

Cont...

S. No.	Genotypes	IC Number	Pedigree	Disease Scores (Mean \pm SEM), Reaction	
				2020	2021
40	DML-1909-1	IC0621061	DTPYC9-F134-2-1-2-1-2-1-1-BB	2.52 (\pm 0.89), R	7.40 (\pm 0.57), S
41	DML-194-1	IC0612741	EC645987-2-3-2-1-1	3.57 (\pm 0.89), MR	2.05 (\pm 0.92), R
42	DML-196	IC0612730	EC 44612-4-6-2-1-1-7	2.13 (\pm 0.88), R	4.5 (\pm 2.12), MR
43	DML-212-1	IC0612726	EC 618226-2-3-9-12-1	3.00 (\pm 0.90), R	2.15 (\pm 1.20), R
44	DML-416	IC0612733	EC646076-7-2-2-1-5	2.96 (\pm 0.87), R	6.00 (\pm 1.41), MS
	RC [DML-1851]			-	2.94 (\pm 0.88), R
	SC [CM 500]			5.23 (\pm 0.88), MS	7.70 (\pm 0.88) (S)
	Overall mean			3.76	4.96
	CV (%)				23.10

*RC=Resistant Check, SC=Susceptible Check, CV = Coefficient of Variation, SEM (\pm) = Standard error of mean

Table 3. Screening of maize inbred lines against MLB under field conditions at three hotspot locations during *kharif* 2022

S. No.	Genotype	Delhi	Karnal		Mean
1	DML1278	6.7, MS	6.9, MS	7.0, MS	6.9, MS
2	DML1390	1.4, R	1.9, R	2.4, R	1.9, R
3	DML1575	2.0, R	2.1, R	2.5, R	2.2, R
5	DML1634	6.8, MS	6.8, MS	6.9, MS	6.8, MS
6	DML1828	1.1, R	1.5, R	2.0, R	1.5, R
7	DML1834	6.4, MS	6.9, MS	6.9, MS	6.7, MS
9	DML1851	2.0, R	2.1, R	2.4, R	2.2, R
10	DML212-1	4.6, MR	4.6, MR	4.5, MR	4.6, MR
	CM500	7.1, S	7.1, S	7.4, S	
	SEm (\pm)	0.2			

SEm (\pm) = Standard error of mean

total produce for bioethanol production. India uses ~60% of the total maize produce as livestock feed, while in Africa, Latin America, and some Asian countries, maize serves as a staple food crop for millions of poor masses (Mallikarjuna et al. 2014; Agrawal et al. 2018). In this context, ensuring sufficient quantity of maize production in the world through continuous genetic improvement along with employing appropriate production and protection technologies is significant. Among several stress factors, MLB is one of the key factors that affect the maize yield with varying degrees across the world including India (Gogoi et al. 2020).

The previous reports across different crops, including maize, indicate that the development and deployment of resistant cultivars is the most sustainable and cost-effective strategy for disease and insect pest management in crops (Stout and Davis, 2009; Lefebvre et al. 2020; Pathania et al. 2021). The first step in the employment of host plant resistance in the management of diseases is the identification

of a stable resistance source against the particular disease. In the present study, diverse maize germplasms comprising inbred lines were taken from NGB to identify the sources of resistance against MLB. The present study evaluated 82 maize inbred lines, including two checks, for their reaction to MLB under artificial epiphytotic conditions at a hotspot location in Delhi during the *kharif* season 2020. Based on the initial screening, 44 genotypes were re-evaluated in the second year during *kharif* 2021.

As compared to previous studies (Sharma and Rai 2005; Chandrashekara et al. 2014), the present study is unique with respect to number of genotypes chosen as well as subsequent short-listing of genotypes for repeated evaluation in the second year followed by validation/confirmation at multiple hotspot locations in the third year. The information on the racial pattern of *Bipolaris maydis* [(Nisikado and Miyake) Shoem] (teleomorph: *Cochliobolus heterostrophus*) is well documented in India (Meshram et al. 2022). However, the virulence level may differ between different isolates. In this regard, the evaluation of resistant sources across all the hotspot locations is vital for the identification of stable resistant sources. The virulence level of Ludhiana isolate is relatively higher as compared to Delhi. The identification of stable resistant genotypes across locations is also vital for their deployment across geographical regions. Further, the stable resistant sources can also be used for the development of genetic resources in the form of mapping populations, which may further be used for the identification of genomic regions determining the resistance trait.

The studies focussing on the identification of resistant sources for MLB in India are limited. Further, they have not attempted to identify the stable resistant sources based on multi-location evaluation under replicated trials (Sharma and Rai 2005; Chandrashekara et al. 2014; Kumar et al. 2016), because the evaluation over years across locations with replications is vital due to significant influence of

environment on the disease development as well as host-pathogen interaction. The disease development requires fulfillment of the disease triangle, like the existence of optimum epiphytotic conditions for full expression of the disease, a sufficient load of virulent pathogens and host-plant. The time factor also adds the fourth dynamic to the disease development. The micro-climatic conditions are vital for the precise evaluation of test entries for their consistent reaction to the disease. In the present study, the results of the susceptible and resistant entries over the years at the Delhi location, as well as across multiple hotspot locations under artificial epiphytotic conditions, are consistent with acceptable coefficient variation, which indicates the precise evaluation of the test entries for their reaction to MLB.

In the second year, the test entries with moderately resistant as well as resistant entries were selected to evaluate in the second year to confirm the repeatability of the disease expression and consistency of the expression of the genotype. The results also indicated that there is scope for continuous evaluation of new and more diverse genotypes for the disease. Because, none of the genotypes tested were completely immune to MLB. Further, significant variation in disease response was observed among the genotypes indicating the presence of genetic diversity for MLB resistance.

Previous studies have been conducted to understand the genetics of MLB resistance in maize (Kumar et al. 2016; Karimishahriand Sharma 2017; Jeevan et al. 2020), which have reported that oligo genes and the predominance of non-additive gene action over additive gene action govern resistance. Further, the studies indicated the epistatic interaction between genes but none of the studies are based on multiple locations. In the present study, the resistant sources identified are based on multiple hotspot locations. Based on the results of the present study, the follow-up study would be continued, involving the development of mapping populations followed by mapping resistant genes against MLB. The knowledge of genomic regions, along with linked markers to, can aid in the development of efficient molecular marker-based selection for MLB resistance, which can accelerate the process of hybrid development.

In short, the resistant sources identified in the present study can also be integrated into the active breeding material for making experimental cross combinations to develop improved MLB-resistant hybrids because some of the earlier genetic studies have indicated partial/complete dominance of resistant reactions in some of the F_1 hybrid combinations (Kumar et al. 2016; Jeevan et al. 2020). The above strategy can contribute to sustainable maize production by reducing the impact of MLB and enhancing the overall productivity and resilience of maize crops. Therefore, the resistant sources provide valuable genetic material for maize improvement programs. The findings of the present study emphasize the existence of

genetic variation for MLB resistance among maize inbred lines. Further, the four maize inbred lines showing stable resistance to MLB would hold great potential for use as a source of MLB resistance in future breeding programs, as well as to develop genetic resources in the form of mapping populations for further use in the identification of genomic regions determining MLB resistance.

Author's contribution

Conceptualization of research (KSH, CGK); Designing of the experiments (CGK); Multiplication and maintenance of experimental material (CGK, KSH); Source of experimental materials (SJR, JK, SP); Execution of field experiments and data collection (KSH, HK, HS, RG, DJ, CGK); Analysis of data and interpretation (CGK, KSH); Preparation of the manuscript (KSH, AG, PJ, SS); Review and editing of manuscript (CGK, KSH, IS, RKG, AK).

Acknowledgment

The authors acknowledge the funding support and resources provided by the Director, ICAR-National Bureau of Plant Genetic Resources, New Delhi for carrying out this research work under Consortium Research Platform on Agrobiodiversity (CRPAB) and also Director, ICAR-Indian Institute of Maize Research, Ludhiana for institutional support.

References

- Agrawal P. K., Mallikarjuna M. G. and Gupta H. S. 2018. Genetics and applied genomics of quality protein maize for food and nutritional security. *Biotech. Crop Improv.*, **3**: Genomic Approaches, 151-178. https://doi.org/10.1007/978-3-319-94746-4_7
- Chandrashekhara C., Jha S. K., Arunkumar R. and Agrawal P. K. 2014. Identification of new sources of resistance to turicum leaf blight and maydis leaf blight in maize (*Zea mays* L.). *SABRAO J. Breed. Genet.*, **46**(1): 44-55.
- Gogoi R., Hooda K. S., Kaur H., Khokhar M. K., Basandrai A.K., Rathee V.K., Gupta P.K., Singh R.P. and Mehra R. 2020. Relooking yield losses caused by maydis leaf blight, and branded leaf and sheath blight in maize. *J. Mycol. Pl. Pathol.*, **50**(3): 225-235.
- Hooda K. S., Bagaria P. K., Khokhar M., Kaur H. and Rakshit S. 2018. Mass screening techniques for resistance to maize diseases. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana- 141004, 93pp.
- Hooda K. S., Khokhar M. K., Parmar H., Gogoi R., Joshi D., Sharma S. S. and Yadav O. P. 2017. Banded leaf and sheath blight of maize: historical perspectives, current status and future directions. *Proceedings of the National Academy of Sciences, India Section B: Biol. Sci.*, **87**: 1041-1052. <https://doi.org/10.1007/s40011-015-0688-5>
- Jeevan B., Gogoi R., Sharma D., Manjunatha C., Rajashekara H., Ram D. and Mallikarjuna M. G. 2020. Genetic analysis of maydis leaf blight resistance in subtropical maize (*Zea mays* L.) germplasm. *J. Genet.*, **99**: 1-9. <https://doi.org/10.1007/s12041-020-01245-3>
- Khokhar M. K., Hooda K. S., Sharma S. S. and Singh V. 2014. Post

- flowering stalk rot complex of maize-present status and future prospects. *Maydica*, **59**(3): 226-242.
- Karimishahri M.R. and Sharma R.C. 2017. Inheritance of resistance to maydis leaf blight in maize. *Agriculture & Forestry/Poljoprivreda i šumarstvo*, **63**(1): 261-275.
- Kumar B., Choudhary M., Kumar K., Kumar P., Kumar S., Bagaria P. K. and Rakshit S. 2022. Maydis leaf blight of maize: Update on status, sustainable management and genetic architecture of its resistance. *Physiol. Mol. Plant Pathol.*, 101889. <https://doi.org/10.1016/j.pmpp.2022.101889>
- Kumar B., Hooda K. S., Gogoi R., Kumar V., Kumar S., Abhishek A. and Yadav O. P. 2016. Inheritance study and stable sources of maydis leaf blight (*Cochliobolus heterostrophus*) resistance in tropical maize germplasm. *Cereal Res. Commun.*, **44**(3): 424-434. <https://doi.org/10.1556/0806.44.2016.004>
- Lefebvre V., Boissot N. and Gallois J. L. 2020. Host Plant Resistance to Pests and Pathogens, the Genetic Leverage in Integrated Pest and Disease Management. In: Gullino, M., Albajes, R., Nicot, P. (eds) *Integrated Pest and Disease Management in Greenhouse Crops. Plant Pathology in the 21st Century*, vol 9. Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_9
- Mallikarjuna M. G., Nepolean T., Hossain F., Manjaiah K. M., Singh A. M. and Gupta H. S. 2014. Genetic variability and correlation of kernel micronutrients among exotic quality protein maize inbreds and their utility in breeding programme. *Indian J. Genet. Plant Breed.*, **74**(2): 166-173. <https://doi.org/10.5958/0975-6906.2014.00152.7>
- Meshram S., Gogoi R., Bashyal B. M., Kumar A., Mandal P. K. and Hossain F. 2022. Comparative Transcriptome Analysis of Fungal Pathogen Bipolarismaydis to Understand Pathogenicity Behavior on Resistant and Susceptible Non-CMS Maize Genotypes. *Front. Microbiol.*, **13**: 837056. <https://doi.org/10.3389/fmicb.2022.837056>
- Pathania A., Singh L. and Sharma P. N. 2021. Host Plant Resistance: An Eco-Friendly Approach for Crop Disease Management. In: Kaushal, M., Prasad, R. (eds) *Microbial Biotechnology in Crop Protection*. Springer, Singapore. https://doi.org/10.1007/978-981-16-0049-4_16
- Poole N. F. and Arnaudin M. E. 2014. The role of fungicides for effective disease management in cereal crops. *Canadian J. Pl. Pathol.*, **36**(1): 1-11. <https://doi.org/10.1080/07060661.2013.870230>
- Sharma R. C. and Rai S.N. 2005. Evaluation of maize inbred lines for resistance to maydis leaf blight. *Indian Phytopath.*, **58**(3): 339-340.
- Sharma R. C., Rai S. N., Mukherjee B. K. and Gupta N. P. 2001. Assessing potential of resistance source for the enhancement of resistance to Maydis leaf blight (*Bipolarismaydis*) in maize (*Zea mays* L.). *Indian J. Genet. Plant Breed.*, **63**(1): 33-36. <https://www.isgpb.org/journal/index.php/IJGPB/article/view/2086>
- Singh R. and Srivastava R. P. 2016. Southern corn leaf blight-an important disease of maize: An extension fact sheet. *Indian Res. J. Extn. Edu.*, **12**(2): 324-327.
- Stout M. and Davis J. 2009. Keys to the Increased Use of Host Plant Resistance in Integrated Pest Management. In: Peshin R., Dhawan A.K. (eds) *Integrated Pest Management: Innovation-Development Process*. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-8992-3_7