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RESEARCH ARTICLE

Identification of Multiple Disease-Resistant Maize (*Zea mays*) Germplasm Genetic Resources

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Abstract

Development of maize (*Zea mays* L.) hybrids with durable resistance to multiple diseases is essential for sustaining productivity under humid subtropical conditions. The 28 Maize inbred lines, along with checks, were evaluated under artificial epiphytotic conditions over five consecutive *Kharif* seasons (2021–2025) for resistance to maydis leaf blight (MLB), banded leaf and sheath blight (BLSB), and charcoal rot (CR). Significant genotypic variation was observed for all the diseases. Pooled mean disease scores varied from 2.80–7.24 for MLB, 2.96–7.50 for CR, and 5.00–7.75 for BLSB on a 1–9 disease rating scale. Inbreds *viz;* 575, PML 920, PML 387, and LM 14 for MLB; PML 1225, CML 451, EML 104, and EML 101 for CR; and PML 387 and LM 14 for BLSB were identified as promising resistant inbred lines. MDRI analysis identified LM 14 and PML 387 as stable broad-spectrum resistant donors for climate-resilient maize breeding. These elite inbred lines can serve as valuable parental resources for breeding maize hybrids with stable, broad-spectrum resistance to multiple diseases under humid subtropical growing conditions.

Keywords: Banded leaf and sheath blight, Charcoal rot, Maize, Maydis leaf blight, Multi-disease resistance index, Resistance

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Introduction

Maize (*Zea mays* L.) is one of the world's most important cereal crops and ranks first globally in production among cereals. In Punjab, India, maize is emerging as an important alternative crop in the *Kharif* season because of the need to diversify the rice–wheat production system and reduce groundwater depletion caused by intensive paddy cultivation (Kushwah *et al*., 2024). Punjab's warm, humid monsoon season, followed by intermittent rainfall and high relative humidity during June to September, provides highly favourable conditions for the development of several fungal and bacterial diseases in maize. Punjab lies in the north-western Indo-Gangetic plains and experiences hot summers, humid monsoon weather, and moderate temperatures during crop growth. During *Kharif* maize, frequent rains, prolonged leaf wetness, high temperatures, and dense crop canopy create congenial microclimatic conditions for foliar and stalk diseases. These changing climatic patterns, combined with increasing maize area under conservation agriculture and residue retention systems, may intensify disease pressure in the coming years. Therefore, identification and deployment of resistant cultivars suited to Punjab agro-climatic conditions is becoming increasingly important (Nisa *et al*., 2024a).

Among the major diseases prevalent in north Indian maize-growing regions, maydis leaf blight (MLB) is one of the

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most destructive foliar diseases. In humid and warm conditions such as those prevailing during the *Kharif* season in Punjab, MLB can spread rapidly and cause substantial economic damage (Nisa *et al*., 2024b; Vivek *et al*., 2010). Another important disease is charcoal rot, which infects roots and basal stalk tissues, causing shredding of stalk pith, lodging, premature drying, and poor grain filling (Kaur *et al*., 2010). Banded leaf and sheath blight is another serious disease of maize under high humidity conditions. The disease generally initiates on lower leaf sheaths near the soil surface and progresses upward as characteristic banded lesions on sheaths, leaves, and cobs. Severe infection can lead to sheath rotting, ear damage, lodging, and drastic reduction in grain and fodder yield (Kumar and Kaur, 2020). High levels of stable resistance to BLSB are relatively scarce in elite maize germplasm, making breeding efforts more challenging (Hooda *et al.,* 2017).

Use of fungicides can provide temporary disease suppression, but repeated chemical application increases production cost and raises environmental concerns. Therefore, genetic resistance is considered the most economical, farmer-friendly, and environmentally sustainable strategy for disease management (Pratt *et al*., 2013). In hybrid maize breeding, resistant inbred lines are essential because they serve as parental lines for the development of superior hybrids with durable resistance (Singh *et al*., 2026). Multi-year screening of inbred lines under natural epiphytotic conditions is especially valuable because disease severity fluctuates across seasons depending on weather parameters such as rainfall, temperature, and humidity (Kaur *et al.,* 2022a). Genotypes showing consistent performance over years are therefore considered more reliable resistance donors (Yadav *et al*., 2023; Sandhu *et al.,* 2025).

Punjab Agricultural University has a vibrant crop improvement programme and has released several hybrids developed from locally adapted germplasm. However, continued emergence of disease pressure necessitates screening of parental lines of released hybrids and newly developed inbreds against prevalent pathogens. Identification of inbreds possessing resistance to multiple diseases such as MLB, BLSB and charcoal rot can substantially strengthen breeding programmes and reduce vulnerability of future hybrids (Kaur *et al*., 2021). Therefore, the present investigation was undertaken to evaluate a diverse set of maize inbred lines over multiple years under Punjab agro-climatic conditions for resistance to Maydis leaf blight (MLB), banded leaf and sheath blight (BLSB) and charcoal rot (CR), and to identify stable donor lines for their effective utilization in maize disease resistance breeding programmes.

Materials and Methods

Experimental site

The present investigation was conducted at the experimental fields of Punjab Agricultural University (PAU), Ludhiana, during the *Kharif* seasons of 2021 to 2025. Ludhiana is situated in the central plain zone of Punjab at approximately 30.9° N latitude and 75.8° E longitude, with an altitude of about 247 m above mean sea level. The region has a semi-arid to sub-humid climate characterized by hot summers, monsoon rainfall during July-September, and mild winters. During the *Kharif* seasons of 2021–2025, the experimental site experienced monsoon-associated rainfall, warm temperatures and high relative humidity, with year-to-year variation. The average seasonal rainfall, mean temperature and relative humidity during the crop period were 633.3 mm, 34.9°C maximum to 23.4 °C minimum, and 78%, respectively, with variation among years. These environmental conditions provided favourable conditions for the development of foliar and stalk diseases and may have contributed to differences in disease severity observed among seasons.

Plant material and experimental design

A total of 28 maize inbred lines along with resistant and susceptible checks were evaluated for resistance against major diseases of maize. The test material comprised advanced breeding lines developed under the maize improvement programme of PAU. These lines are being maintained through continuous selfing/sibing at PAU. The resistant check, used in the study, was LM 13, while CM 600 served as the susceptible check. The experiment was laid out in a randomised block design with three replications during each year. Each genotype was planted in two-row plots of 3.0 m length with row-to-row spacing of 60 cm and plant-to-plant spacing of 20 cm. Recommended agronomic practices for maize cultivation in Punjab were followed to raise a healthy crop, except for fungicidal sprays, which can restrict disease development. Standard fertilizer application, irrigation, interculture and weed management practices as recommended by PAU were followed uniformly across all replications and years.

Disease screening

The test entries were screened under artificial

epiphytotic field conditions against maydis leaf blight caused by *Drechslera maydis*, banded leaf and sheath blight caused by *Rhizoctonia solani* and charcoal rot caused by *Macrophomina phaseolina*.

Maydis leaf blight: The most virulent isolate of *Drechslera maydis* (*Dm1*) was mass-multiplied on sterile sorghum grains (Kaur *et al.,* 2022b). The whorl inoculation procedure was performed on 35-40-day-old plants in the late evening by placing dried powder from ground sterile grains in whorls. Moisture was provided by spraying water with a knapsack sprayer for spore germination (Hooda *et al.,* 2018).

Banded leaf and sheath blight: The most virulent isolate of *Rhizoctonia solani* (*Rs12)* was mass-multiplied on maize-sand media in flasks (Kumar *et al.,* 2021). Inoculation was done after 30–40 days of sowing by the artificial sheath inoculation method (Pascual and Raymundo, 1989).

Charcoal rot: Mass culture of *Macrophomina phaseolina* was prepared on toothpicks using potato dextrose broth media. The toothpicks fully covered with fungal growth were used to inoculate plants at the flowering stage (75-80 days old). The second lower internode of the plant just above the soil level was opened with a jabber, and a toothpick was inserted as explained by Payak and Sharma (1983).

Data scoring: Disease data were recorded using a 1-9 scale (Hooda *et al.,* 2018) on all plants in a plot and averaged plot-wise for statistical analysis. Disease severity was assessed using the standard 1-9 rating scale, where 1.0 -3.0: resistant, 3.1-5.0: moderately resistant, 5.1-7.0: moderately susceptible and 7.1-9.0: susceptible. For foliar blights (MLB and BLSB), scoring was done from tasseling to grain filling stages when symptoms were fully expressed. Charcoal rot observations were recorded at the time of harvesting. The inoculated plants were split open longitudinally to see the extent of pith damage in the form of blackish discolouration. The disease intensity and severity were recorded using a 1-9 rating scale given by Payak and Sharma (1983). Year-wise disease scores were recorded separately from 2021 to 2025 for MLB and CR, and from 2021 to 2024 for BLSB. Some entries marked as NT (not tested) in particular years were excluded from annual mean calculations for that season. The pooled mean disease score of each genotype across years was calculated as:

$$\text{Mean score} = \sum X_i / n$$

where X_i is the disease score in the ith year, and n is the number of years tested.

Multi-disease resistance index (MDRI) and Selection criteria for Punjab conditions: To identify genotypes with combined resistance against multiple diseases, a multi-disease resistance index was computed as:

$$MDRI = (MLB + CR + BLSB)/3$$

Lower MDRI values indicated superior overall disease resistance. Genotypes showing consistently low disease scores across years, stable performance under natural epiphytotic conditions, and low MDRI values were identified as promising donors for maize breeding programmes targeting Punjab agro-climatic conditions.

Statistical analysis

Descriptive statistics including mean, standard error (SE), standard deviation (SD), coefficient of variation (CV), minimum, maximum, skewness and kurtosis were computed separately for each disease and year. Two-way analysis of variance (ANOVA) was performed considering genotypes and years as fixed effects to determine the significance of variation due to genotype, environment (year), and their interaction. Mean comparisons and ranking of genotypes were based on pooled means across years. All statistical analyses were performed using standard procedures described by Gomez and Gomez (1984) and Panse and Sukhatme (1985).

Results

Disease reaction of maize inbred lines

Maydis leaf blight (MLB): Considerable variability in MLB severity was observed among maize inbred lines across five years (2021–2025), indicating the presence of exploitable resistance sources. Pooled mean disease scores ranged from 2.80 to 7.24 on a 1–9 scale, where lower values indicate higher resistance. Among the test

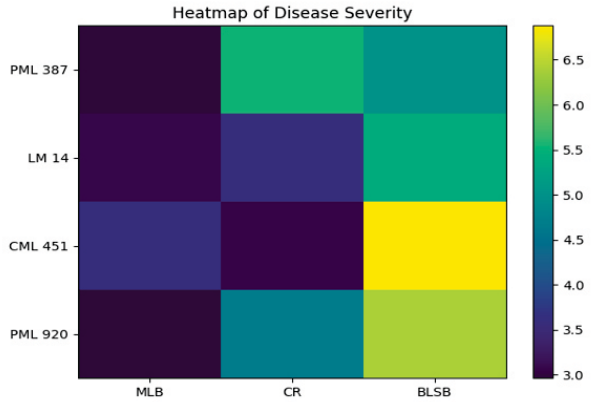

Fig. 1: Disease severity profiling of maize genotypes against important diseases using heatmap analysis.

entries, CML 575 (2.80) recorded the lowest mean score, followed by PML 920 (2.96), PML 387 (2.98) and LM 14 (3.08), demonstrating consistent resistance across years (Table 1). These genotypes performed comparably to or better than the resistant check LM 13 (2.84), whereas the susceptible check CM 600 (7.24) consistently exhibited high disease severity. In contrast, PML 1224 (6.49), PML 333 (6.26), PML 1231 (6.10) and PML 1012 (5.74) showed moderately susceptible reactions. The distribution of disease responses across genotypes is illustrated in the heat map (Fig. 1), highlighting clear variability in resistance levels. Year-wise variation in disease severity (Table 2) showed relatively higher disease levels during 2024.

Table 1: Pooled disease severity and multi-disease resistance of maize inbred lines evaluated under artificial inoculation conditions at Ludhiana centre during *kharif* seasons (*Kharif* 2021-*Kharif* 2025)

S. No.	Inbred line	Mean disease score (1-9)*					
		Maydis leaf blight	Disease Reaction	Charcoal rot	Disease Reaction	Banded leaf and sheath blight	Disease Reaction
1	LM 6	4.08	MR	4.72	MR	6.63	MS
2	LM 21	4.18	MR	4.62	MR	7.03	MS
3	LM 25	3.80	MR	4.08	MR	6.84	MS
4	PML 1224	6.49	MS	4.15	MR	7.08	MS
5	PML 368	3.98	MR	4.90	MR	6.44	MS
6	PML 387	2.98	R	5.53	MS	5.00	MR
7	PML 503	3.72	MR	4.89	MR	5.63	MS
8	LM 14	3.08	MR	3.59	MR	5.40	MS
9	PML 1230	3.78	MR	4.10	MR	6.78	MS
10	PML 1231	6.10	MS	4.62	MR	6.98	MS
11	PML 1012	5.74	MS	4.20	MR	6.75	MS
12	PML 243	4.54	MR	4.27	MR	6.13	MS
13	PML 1225	4.66	MR	2.96	R	7.20	S
14	PML 333	6.26	MS	4.44	MR	6.28	MS
15	PML 920	2.96	R	4.66	MR	6.39	MS
16	CML 451	3.60	MR	3.06	MR	6.88	MS
17	PML 1229	4.23	MR	5.15	MS	6.71	MS
18	PML 81	3.90	MR	4.19	MR	6.18	MS
19	CML 575	2.80	R	4.58	MR	7.43	S
20	PML 1228	4.30	MR	4.58	MR	6.75	MS

*Reaction scale: Resistant (⩽3.0); Moderately resistant (3.1–5); Moderately susceptible (5.1-7.0); Susceptible (>7.0).

Table 2: Year-wise descriptive statistics of disease severity in maize inbred lines

Disease	Year	Mean	SD	CV (%)	Minimum	Maximum	Skewness
MLB	2021	4.46	1.31	29.43	2.5	7.0	0.74
MLB	2022	4.09	1.37	33.53	2.5	7.2	1.17
MLB	2023	4.33	1.28	29.59	3.0	7.5	0.93
MLB	2024	4.67	1.35	28.99	2.5	7.0	0.14
MLB	2025	4.58	1.23	26.78	2.8	7.5	0.38
CR	2021	4.48	0.96	21.38	3.0	7.2	0.97
CR	2022	4.24	1.03	24.26	2.38	7.5	1.00
CR	2023	4.58	1.05	22.83	3.0	8.0	1.01
CR	2024	4.36	1.05	24.10	3.0	7.8	1.30
CR	2025	4.31	0.88	20.45	3.0	7.0	0.78
BLSB	2021	5.96	0.54	9.06	4.8	7.0	-0.13
BLSB	2022	6.49	0.90	13.87	5.0	8.5	0.51
BLSB	2023	6.83	0.96	14.05	5.0	8.4	-0.33
BLSB	2024	6.79	0.90	13.23	5.0	8.5	-0.51

Charcoal rot (CR): Disease severity of CR varied significantly among genotypes across years, with pooled mean scores ranging from 2.96 to 7.50 (Table S2). The most resistant genotype was PML 1225 (2.96), followed by CML 451 (3.06), EML 104 (3.08), EML 101 (3.13) and LM 14 (3.59), indicating stable performance under diverse environmental conditions (Table 1). The susceptible check CM 600 (7.50) showed consistently high disease intensity, whereas the resistant check LM 13 (3.76) maintained comparatively lower scores. Some genotypes, such as PML 387 (5.53) and PML 1229 (5.15), exhibited moderate susceptibility to charcoal rot despite favourable performance against other diseases. The heat map (Fig. 1) further illustrated the disease-specific variation in resistance responses. Year-wise variation (Table 2) indicated slightly higher disease severity during 2023.

Banded leaf and sheath blight (BLSB): BLSB exhibited comparatively higher disease severity than the other diseases, with pooled mean scores ranging from 5.00 to 7.75. The lowest disease severity was recorded for PML 387 (5.00), followed by LM 14 (5.40), PML 503 (5.63) and PML 81 (6.18), indicating relatively better tolerance (Table 1). Tolerant check LM 13 (5.08) performed similarly to PML 387, whereas the susceptible check CM 600 (7.75) showed consistently high disease intensity. Highly susceptible genotypes included CML 575 (7.43), PML 1225 (7.20) and PML 1224 (7.08). The heat map (Fig. 1) showed generally higher BLSB scores compared to MLB and CR. Year-wise trends (Table 2) indicated a gradual increase in disease severity from 2021 to 2024.

Descriptive statistics and variability analysis

Descriptive statistics (Table 2) revealed substantial variability among the three diseases. MLB exhibited a coefficient of variation (CV) ranging from 26.78 to 33.53%, indicating wide diversity among genotypes. CR showed moderate variability (CV: 20.45–24.26%), whereas BLSB exhibited comparatively lower variability (CV: 9.06–14.05%). Positive skewness was observed for MLB and CR, indicating a greater proportion of moderately resistant genotypes, whereas occasional negative skewness in BLSB suggested clustering towards higher disease severity.

Multi-disease resistance and identification of elite donors

Based on pooled mean performance (Table 1), several genotypes exhibited superior resistance against individual diseases. PML 387 and LM 14 consistently appeared among the better-performing lines across all three diseases. This trend was further supported by the heat map (Fig. 1), which highlighted their relatively lower disease scores across diseases. The multi-disease resistance index (MDRI) provided an integrated assessment of resistance across diseases (Table 3). Lower MDRI values indicated superior combined resistance. The top-ranking genotypes were LM 14 (4.02), PML 387 (4.50), CML 451 (4.51) and PML 920 (4.67). Their comparative performance across diseases is illustrated in the radar plot (Fig. 2). Based on multi-year pooled performance, PML 387 and LM 14 were identified as promising multi-disease-resistant inbred lines, whereas CML 575 and PML 920 were superior donors for MLB, PML 1225 and CML 451 for charcoal rot, and PML 387 for BLSB (Table 4).

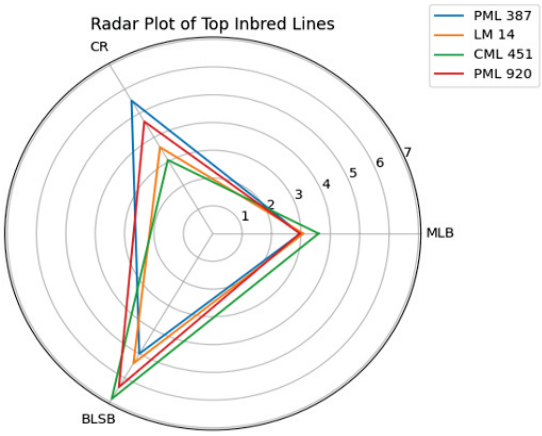

Fig. 2: Radar analysis of elite inbreds for resistance against maydis leaf blight, banded leaf and sheath blight and charcoal rot of maize.

Table 3: Genotype × year effects and multi-disease resistance ranking

Inbred line	MLB	CR	BLSB	MDRI	Rank	Interpretation
PML 387	2.98	5.53	5.00	4.50	1	Stable multi-disease resistance
LM 14	3.08	3.59	5.40	4.02	2	Balanced resistance
CML 451	3.60	3.06	6.88	4.51	3	Strong CR donor
PML 920	2.96	4.66	6.39	4.67	4	MLB donor

Table 4: Identification of promising maize inbred lines for resistance breeding against different diseases

Category	Inbred lines	Target disease(s)	Remarks
Multi-disease resistant	PML 387, LM 14	MLB, CR, BLSB	Consistent low severity
MLB donors	CML 575, PML 920	MLB	Lowest disease scores
CR donors	PML 1225, CML 451	CR	Stable resistance
BLSB donor	PML 387	BLSB	Best performer

Discussion

The substantial variation observed for MLB, charcoal rot and BLSB among the evaluated maize inbred lines demonstrated the availability of diverse resistance sources that can be effectively utilized in resistance breeding programmes. The consistent performance of resistant and susceptible checks across years further confirmed the reliability of the disease screening methodology and adequacy of disease pressure under field conditions. For MLB, the comparatively higher disease severity observed during 2024 suggested a strong environmental influence on disease development. Warm temperatures and high humidity prevailing during the *K*harif season are known to favour southern corn leaf blight development. Similar environmental effects on MLB severity have been reported in previous research (Carson and Stuber 2004; Balint-Kurti *et al*., 2010; Kaur *et al*., 2010). The stable resistance shown by CML 575, PML 920 and PML 387 highlighted their value as donor parents for breeding programmes targeting humid maize-growing regions of Punjab and north-western India.

The moderate year-wise fluctuation observed for charcoal rot indicated the influence of environmental stress conditions on disease expression. Charcoal rot is generally aggravated under high temperature and moisture stress conditions, particularly during the late *kharif* season. Similar observations have been reported by Muthu Kumar *et al*., (2011); Ahmadi and Kaur (2019) and Rashid *et al*., (2021). The superior performance of PML 1225 and CML 451 suggested their potential utility in developing charcoal rot-resistant, drought-resilient maize hybrids. Among the three diseases, BLSB exhibited comparatively higher overall disease severity, indicating its aggressive nature under field conditions. The gradual increase in BLSB severity from 2021 to 2024 may be attributed to prolonged leaf wetness, dense crop canopy and high relative humidity during the monsoon season, which is favourable for disease establishment and spread. Similar findings have been documented by Ahuja and Payak (1983), Kumar and Singh (2004) and Kumar *et al*., (2023). The comparatively better performance of PML 387 against BLSB highlighted its importance as a useful source of resistance for future breeding efforts.

The descriptive statistics further confirmed the presence of substantial genetic variability among the evaluated germplasm. Higher CV values for MLB and CR suggested greater diversity in disease response, whereas the relatively lower CV for BLSB reflected more uniform disease pressure across genotypes. The observed skewness patterns indicated the predominance of moderately resistant genotypes within the tested material, supporting the suitability of the germplasm for resistance breeding and donor identification. The identification of genotypes exhibiting resistance across multiple diseases is particularly important for Punjab's maize-growing environments, where humid monsoon conditions followed by intermittent moisture stress can favour the occurrence of both foliar and soil- or stalk-associated diseases. During the *Kharif* season, frequent rainfall, high relative humidity, prolonged leaf wetness and warm temperatures provide favourable conditions for the establishment and spread of foliar diseases such as maydis leaf blight and banded leaf and sheath blight. In contrast, charcoal rot, although influenced by several environmental and host-related factors, is often aggravated when high temperature and moisture stress occur during the later stages of crop growth. Thus, the transition from humid monsoon conditions to periods of intermittent moisture stress can create a disease-conducive environment in which different pathogens may affect the crop at different growth stages. This temporal and environmental overlap increases the importance of host resistance that remains effective across multiple diseases rather than resistance directed against a single pathogen. In the present study, PML 387 and LM 14 consistently exhibited comparatively lower disease scores across MLB, CR and BLSB and consequently attained the lowest MDRI values, suggesting their potential utility as broad-spectrum resistance donors. The stable performance of these lines across years is particularly valuable because environmental fluctuations can alter disease pressure and may otherwise reduce the reliability of resistance expressed in a single season.

Incorporation of such multi-disease resistant germplasm into breeding programmes could therefore provide a more robust approach for developing maize hybrids adapted to the variable disease environments of north-western India. Similarly, CML 575 and PML 920 were identified as valuable donors for MLB resistance, PML 1225 and CML 451 for charcoal rot resistance, and PML 387 for BLSB resistance. The stable broad-spectrum resistance observed in PML 387 and LM 14 may reflect the presence of multiple favourable resistance factors that restrict pathogen establishment and disease progression. Since the present study was based on phenotypic evaluation and did not include molecular or physiological characterization, the specific mechanisms underlying their resistance cannot be established. Nevertheless, the consistency of their disease response across years and across pathogens suggests that these lines may possess quantitatively expressed or combined resistance factors that contribute to greater stability under variable environmental conditions. Further molecular and physiological investigations would be useful to elucidate the genetic basis of their broad-spectrum resistance. These elite inbred lines can be effectively incorporated into breeding programmes aimed at developing disease-resistant and climate-resilient maize hybrids adapted to north-western India.

Conclusion

The multi-year evaluation of maize inbred lines revealed substantial genetic variability for resistance to Maydis leaf blight, charcoal rot and banded leaf and sheath blight. Significant variation among genotypes across years highlights the importance of both host genetics and environmental factors in disease expression. Among the evaluated lines, PML 387 and LM 14 exhibited consistent and broad-spectrum resistance, while CML 575 and PML 920 were superior against Maydis leaf blight, PML 1225 and CML 451 against charcoal rot, and PML 387 against banded leaf and sheath blight. The MDRI-based ranking further facilitated identification of elite multi-disease resistant donors. These inbred lines represent valuable genetic resources for developing high-yielding, disease-resistant and climate-resilient maize hybrids. These lines may be used for genetic studies and development of new resistant germplasm lines.

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