



RESEARCH ARTICLE

# Marker-assisted introgression of turicum leaf blight (*Ht1*) resistant gene into northeastern Himalayan region maize landrace

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## Abstract

The local people of north-eastern Himalayan region (NEHR) cultivate the maize landraces and consume them as food. These landraces possess desirable agronomic traits but are susceptible to turicum leaf blight (TLB) disease caused by the fungus *Exserohilum turicum*. Thus, we aimed to screen maize landraces under field conditions and introgress the *Ht1* gene into the susceptible landrace. Screening of four landraces against TLB was conducted under artificially inoculated field conditions during *Kharif* 2023, with two standard checks. Disease reaction rating on a scale of 1-9 was used to calculate the percent disease index (PDI) values of landraces. Among the four landraces, two - LMC-15 and LMC-16 - were identified as susceptible, with a disease rating of 8, while LMC-4 and LMC-7 received a rating of 6 and were categorized as moderately susceptible. The  $F_1$  generation was produced during the spring season of 2024 through a cross between the donor parent BML-6 and the susceptible recurrent parent LMC-15. The true  $F_1$  plants were evaluated using a SSR marker *umc1042*, which is linked to the TLB resistance gene *Ht1*. Genotyping of 120  $F_2$  plants was performed using the *umc1042* SSR marker. This marker exhibited a segregation ratio of 27:61:32 in the  $F_2$  population. The chi-square value for the genotype was 0.45, which is below  $\chi^2$  ( $p \leq 0.05$ ) and therefore considered non-significant, indicating a good fit to the expected 1:2:1 ratio. Overall, the findings confirm that MAS is an efficient and reliable approach for introgressing *Ht1* into susceptible maize lines.

**Keywords:** *Exserohilum turicum*; *Ht1* gene; maize; marker-assisted selection; NEHR; SSR marker

## Introduction

Maize is the second most important crop in the north-eastern Himalayan region (NEHR) of India, following rice and is predominantly cultivated in rainfed upland areas (1). The Indian NEHR is the centre of maize diversity, with a unique collection of landraces. These landraces have valuable agronomic traits and possess the ability to tolerate stressful conditions; therefore, they are carefully preserved and traditionally maintained by NEHR farmers (2). Over the last few decades, turicum leaf blight (TLB) has severely affected maize production, causing significant yield losses (3). TLB, caused by the fungus *Exserohilum turicum*, is a destructive foliar disease of maize (4). The disease is found worldwide, mainly in regions where the relative humidity ranges from 75 % to 90 % and temperature ranges from 22 °C to 25 °C during the growing season (5, 6). In India, the disease occurs most frequently across all major maize-growing areas during both the rainy (*Kharif*) and winter (*Rabi*) seasons (7). Symptoms initially appear as small, oval, gray-green, water-soaked spots that later develop into elongated, spindle-shaped, necrotic lesions (8).

Utilizing resistant cultivars is the most effective, economical

and environmentally friendly strategy for managing TLB. Several qualitative TLB-resistance *Ht* genes have been identified, namely *Ht1*, *Ht2*, *Ht3* and *HtN* corresponding to their locus designations (9, 10). The *Ht1* gene has been widely studied and used in maize breeding for TLB resistance because it was the first major gene identified for disease and demonstrated durable and consistent effects across environments (11). It produces a chlorotic-lesion resistance phenotype rather than typical necrotic lesions; in resistant plants, lesions remain chlorotic with limited sporulation, thereby reducing disease spread (12).

Marker-assisted selection (MAS) is considered a powerful and efficient breeding strategy because it enables rapid and accurate selection of target genes and its use has produced notable improvements in maize breeding programs aimed at developing TLB-resistant cultivars (13, 14). However, limited information is available on *Ht* genes and associated molecular markers for use in MAS in breeding maize for TLB resistance. Thus, the present study was undertaken to identify resistant (R) and susceptible (S) NEHR maize landraces through artificial inoculating with *E. turicum* and to introgress the TLB-resistant gene *Ht1* into a susceptible NEHR maize landrace.

## Materials and Methods

### Plant materials and development of mapping population

Four agronomically superior, high-yielding and popular landrace were collected from different district of Manipur. The landraces tested were LMC-4, LMC-7, LMC-15, LMC-16 and the checks used were VLB-55 (resistant check) and early composite (susceptible check). They were inoculated with *E. turcicum* to determine their resistance to TLB, using a rating score of 1-9. The seeds of NEHR landraces were obtained from Central Agricultural University, Imphal, India and used in this study. Another genotype BML-6 (TLB resistant), was used as the donor parent and was provided by ICAR-IIMR, Ludhiana. LMC-15, which exhibiting the highest PDI value (susceptible to TLB), was utilized as the recurrent parent to introduce the *Ht1* gene from the donor parent BML-6.

The study involved 120 F<sub>2</sub> plants, produced by crossing LMC-15 and BML-6 in the spring season of 2024 at the research farm of the Department of Genetics and Plant Breeding at CAU, Imphal. This farm is situated at a latitude of 24°82'N and a longitude of 93°90'E, at an elevation of 790 m MSL in the Imphal West district of Manipur. The hybrid seeds from this cross were collected separately and planted in the *Kharif* season of 2024 along with both parents. The hybridity of F<sub>1</sub>S was validated using *Ht1* gene linked *umc1042* SSR marker and the true F<sub>1</sub>S were self-pollinated to produce the F<sub>2</sub> generation.

### Inoculation and disease evaluation

#### Mass multiplication of inoculums

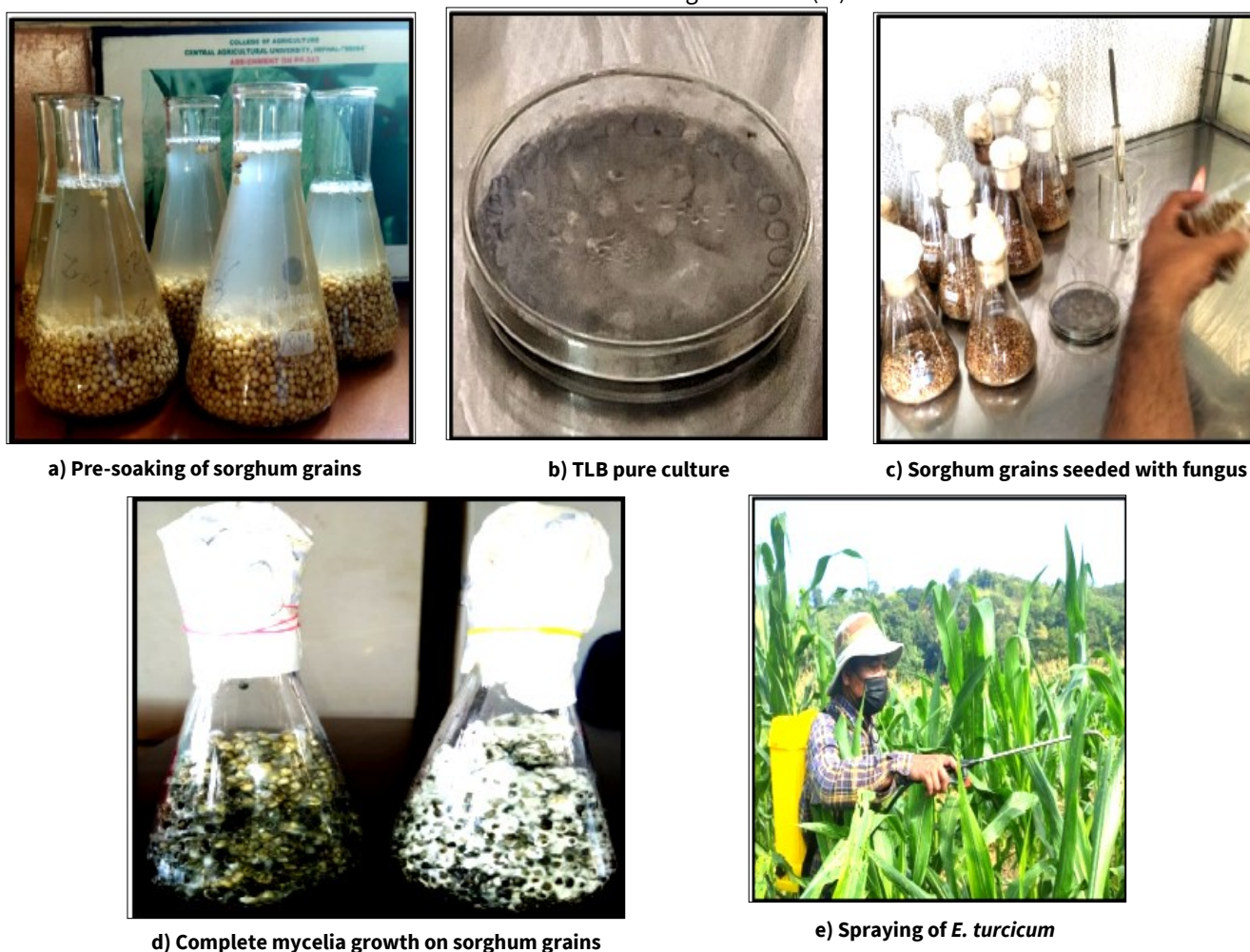
Pure culture media of *E. turcicum* obtained from the Department of Plant Pathology, Central Agricultural University, Imphal. Mass multiplication of *E. turcicum* was carried out on sterilized whole sorghum grains (15). A layer of sorghum grain, approximately one inch in depth, is placed in a conical flask and soaked in water for duration of 4-6 hr. After soaking, excess water was removed. The flasks containing sorghum grains were autoclaved twice on alternate days, seeded with fungus under aseptic conditions and incubated at 25-27 °C. Every 2-3 days, the flasks were shaken to promote uniform growth of *E. turcicum* on sorghum grains. After an incubation period of approximately two weeks, the material was ready for inoculation (Fig. 1).

#### Artificial inoculation

Suspensions of *E. turcicum* spores were quantified using a haemocytometer and maintained a concentration of  $3 \times 10^5$  spores mL<sup>-1</sup>. The diluted suspension was used for inoculating plants as a spray applied using a knapsack sprayer directed toward the whorl. Inoculations were preferably done late in the afternoon. Artificial inoculation was performed twice, at 30 and 40 days after sowing (Fig. 1).

#### Disease score

The percent disease index (PDI) of TLB was calculated at four stages, 40, 50, 60 and 70 days after sowing and the disease reaction was measured using a 1-9 scale (4) (Table 1). The PDI was calculated using the formula (16).



**Fig. 1.** Mass multiplication of *E. turcicum* on sterilized sorghum grains and artificial inoculation of pathogen.

**Table 1.** Rating scale for TLB disease in maize

Rating scale	Degree of infection (percent DLA*)	PDI**	Disease reaction
1.0	Nil to very slight infection ( $\leq 10\%$ )	$\leq 11.11$	Resistant (R)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20 %)	22.22	(score: $\leq 3.0$ )
3.0	Light infection, moderate number of lesions scattered on four lower leaves (20.1-30 %)	33.33	(PDI: $\leq 33.33$ )
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 (MR)
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	(Score: 3.1-5.0)
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 cob (50.1-60 %)	66.66	(PDI: 33.34-55.55)4
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	Moderately susceptible (MS)
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	(score: 5.1-7.0)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed ( $>80\%$ )	99.99	(PDI: 55.56-77.77)
			Susceptible (S)
			(score: $> 7.0$ )
			(PDI: $> 77.77$ )

\*DLA: diseased leaf area; \*\*percent disease index (PDI).

PDI =

$$\frac{\text{Sum of individual disease score}}{\frac{\text{Total number of plants scored} \times \text{Maximum disease score}}{2}} \times 100$$

#### Area under disease progress curve (AUDPC) analysis

The disease progress curve consisted of disease severity (%) recorded at 40, 50, 60 and 70 days after sowing, a 10-day intervals starting from the onset symptom. To ensure consistent field evaluation, the disease progress curve was prepared from PDI readings. AUDPC is used to quantify the epidemic onset and the time required for the blight to reach its peak. The derived disease parameter, AUDPC, was calculated using the equation (17).

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{Y_i + Y_{i+1}}{2} \times (t_{i+1} - t_i)$$

Where,  $Y_i$  = disease at the  $i^{\text{th}}$  observation,  $T_i$  = time (days) at the  $i^{\text{th}}$  observation and  $n$  = total number of observations.

#### Molecular marker analysis

Genomic DNA was extracted from three-week-old seedling using the modified CTAB method (18). The quality and quantity of extracted DNA was examined using 0.8 % agarose gel electrophoresis and a Nanodrop spectrophotometer and samples were diluted to a final concentration of 90 ng/ $\mu\text{L}$  for PCR amplification. The  $F_1$  hybridity test was performed with the *umc1042* gene-linked marker.

PCR amplification was carried out in a 10  $\mu\text{L}$  reaction mixture containing 1  $\mu\text{L}$  of 90 ng/ $\mu\text{L}$  gDNA, 0.5  $\mu\text{L}$  each of forward and reverse primers, 5  $\mu\text{L}$  of 2 $\times$  Takara green PCR Master Mix and 3  $\mu\text{L}$  of nuclease-free water, in 96-well PCR plates sealed with a thermal seal in a QI Amplifier 96 thermal cycler. The amplification profile consisted of an initial denaturation at 95  $^{\circ}\text{C}$  for 5 min, followed by 35 cycles: denaturing at 95  $^{\circ}\text{C}$  for 45 sec, annealing at 58  $^{\circ}\text{C}$  for 45 sec, extension at 72  $^{\circ}\text{C}$  for 45 sec and a final extension at 72  $^{\circ}\text{C}$  for 8 min.

The amplified PCR products were run at on 4 % agarose gel using 1 $\times$  TAE buffer at 5 V/cm for 3 hr and visualized on a UV transilluminator. PCR product sizes were determined using a 100 bp DNA ladder.

#### Genotyping of $F_2$ population

The presence of the TLB resistance gene in all  $F_2$  plants was evaluated using the gene-linked molecular marker *umc1042*. Amplicons were classified as “A” for the male parent (homozygous dominant), “B” for the female parent (homozygous recessive) and “H” for the heterozygous individuals.

#### Statistical analysis

A chi-square test was used to evaluate the segregation distortion of the SSR marker from the expected Mendelian 1:2:1 ratio in the  $F_2$  population. This test was also utilized to assess marker allele frequency, homogeneity and the distribution of marker genotypes in order to investigate the causes of segregation distortion. The chi-square value is determined using the formula provided.

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where,  $E$  = expected value and  $O$  = observed value.

## Results

#### Field evaluation of maize landraces against TLB

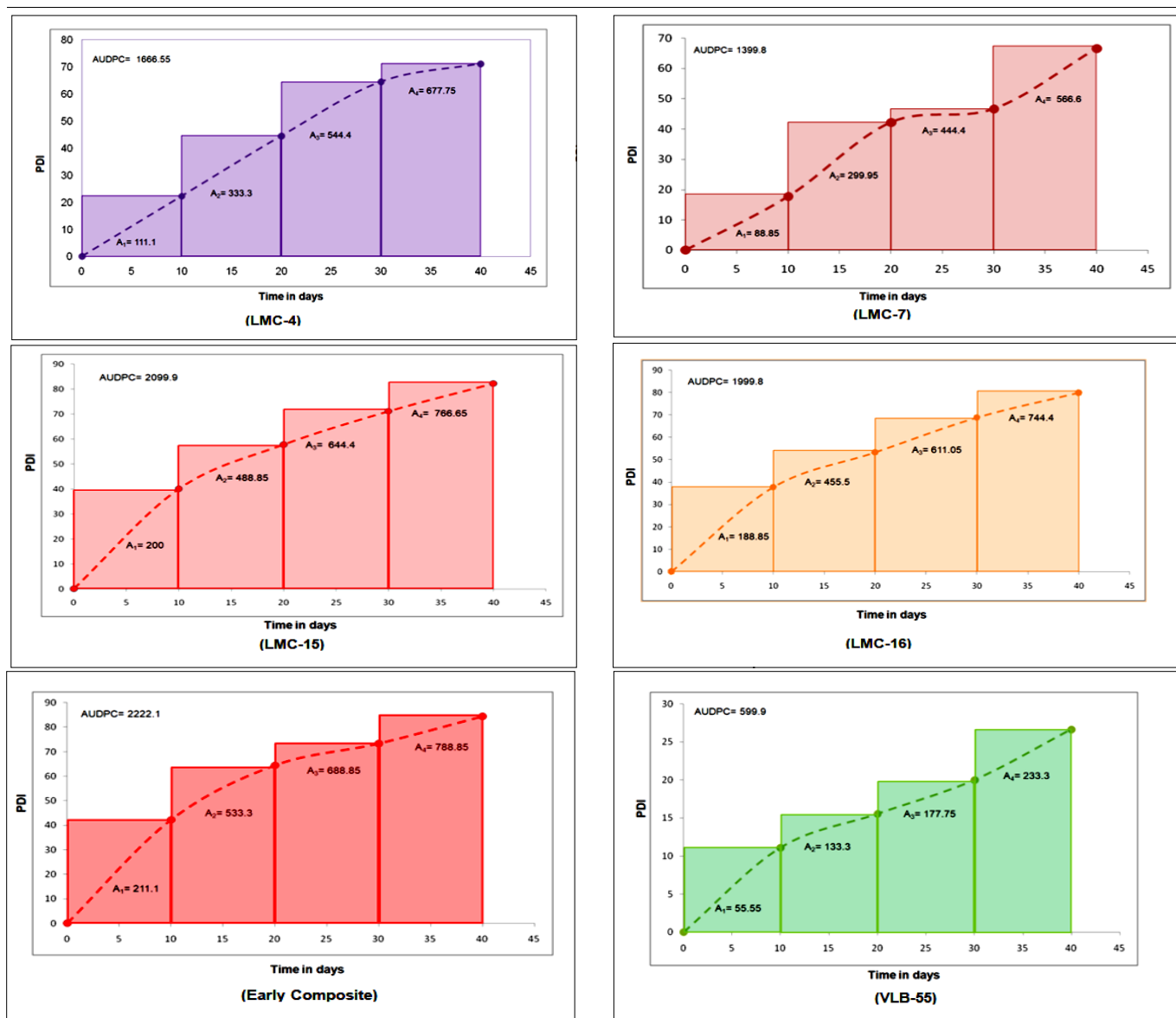
The evaluation of resistance to TLB in NEHR maize landraces was conducted during the *Kharif* season of 2023. The results showed clear and distinct responses of the landraces to the TLB pathogen under artificially inoculated conditions. Four widely recognized NEHR maize landraces, LMC-4, LMC-7, LMC-15 and LMC-16, along with two standard checks-VLB-55 (resistant check) and early composite (susceptible check)-were inoculated with *E. turicum* to evaluate their resistance to TLB.

The mean disease scores at four different growth stages of the plants were recorded and the PDI for each stage was calculated. The AUDPC values were derived from these four PDI values (Table 2). The graphs were prepared using AUDPC values (Fig. 2).

The experiment showed clear variation in disease response among the landraces. Among four landraces, two were identified as susceptible: LMC-15 (PDI 82.22 %) and LMC-16 (PDI 80 %) with a disease rating of 8 (Fig. 3). In contrast, LMC-4 (PDI 71.11 %) and LMC-7 (PDI 66.66 %) received a rating of 6 and were categorized as moderately susceptible.

**Table 2.** Presenting AUDPC values for TLB of maize landraces and checks, derived from PDI

Sl. No.	Landraces/Genotypes	PDI (%)	AUDPC	Disease reaction
1.	LMC-4	40 <sup>th</sup> DAS: 22.22 50 <sup>th</sup> DAS: 44.44 60 <sup>th</sup> DAS: 64.44 70 <sup>th</sup> DAS: <b>71.11</b>	1666.55	Moderately susceptible
2.	LMC-7	40 <sup>th</sup> DAS: 17.77 50 <sup>th</sup> DAS: 42.22 60 <sup>th</sup> DAS: 46.66 70 <sup>th</sup> DAS: <b>66.66</b>	1399.8	Moderately susceptible
3.	LMC-15	40 <sup>th</sup> DAS: 40.00 50 <sup>th</sup> DAS: 57.77 60 <sup>th</sup> DAS: 71.11 70 <sup>th</sup> DAS: <b>82.22</b>	2099.9	Susceptible
4.	LMC-16	40 <sup>th</sup> DAS: 37.77 50 <sup>th</sup> DAS: 53.33 60 <sup>th</sup> DAS: 68.88 70 <sup>th</sup> DAS: <b>80.00</b>	1999.8	Susceptible
5.	Early composite (susceptible check)	40 <sup>th</sup> DAS: 42.22 50 <sup>th</sup> DAS: 64.44 60 <sup>th</sup> DAS: 73.33 70 <sup>th</sup> DAS: <b>84.44</b>	2222.1	Susceptible
6.	VLB-55 (resistant check)	40 <sup>th</sup> DAS: 11.11 50 <sup>th</sup> DAS: 15.55 60 <sup>th</sup> DAS: 20.00 70 <sup>th</sup> DAS: <b>26.66</b>	599.9	Resistance

**Fig. 2.** Graphical representation of disease reaction (AUDPC).





1. LMC - 15



2. Susceptible check (Early composite)



3. Resistant check (VLB - 55)

**Fig. 3.** Disease reaction of LMC-15 with resistant and susceptible checks.

### Introgression of *Ht1* resistance gene against TLB into the maize landrace

This study was conducted with the aim of introducing the *Ht1* resistance gene for Turicum Leaf Blight into susceptible maize landrace. In the *spring* season of 2024, the recurrent parent LMC-15 (female) was crossed with the donor parent BML-6 (male parent), which carries the *Ht1* gene known for TLB resistance. After confirming the  $F_1$ 's for the existence of target gene obtained from the crosses, the true  $F_1$ 's were raised and self-pollinated (Fig. 4).

### Validation of gene linked molecular markers between parents

Before starting the crossing program, the disease resistance of the parents was confirmed. In this study, both the donor and recipient parents were raised during the *spring* season of 2024 and assessed for the presence of specific resistance genes using previously identified molecular markers (Table 3). Donor and recipient parents were distinguished using polymorphic SSR markers, specifically gene-linked markers such as *umc1042*, *bnlg1721*, *bnlg198* and *bnlg1335*. The *umc1042* marker exhibited polymorphism between the recurrent and donor parents, while the other markers revealed monomorphism between the parents for the *Ht1* gene. The *umc1042* marker associated with the *Ht1* gene was amplified at 110 bp in the donor (BML-6) and a 100 bp fragment in recurrent parent LMC-15 (Fig. 5).

The molecular screening results for the target gene in the parental lines indicated that the gene-based marker effectively differentiated resistant lines from susceptible ones in a co-dominant manner, enabling the identification of homozygous and heterozygous states. As a result, plants carrying the gene in the  $F_2$  generations could be accurately identified without false positives.

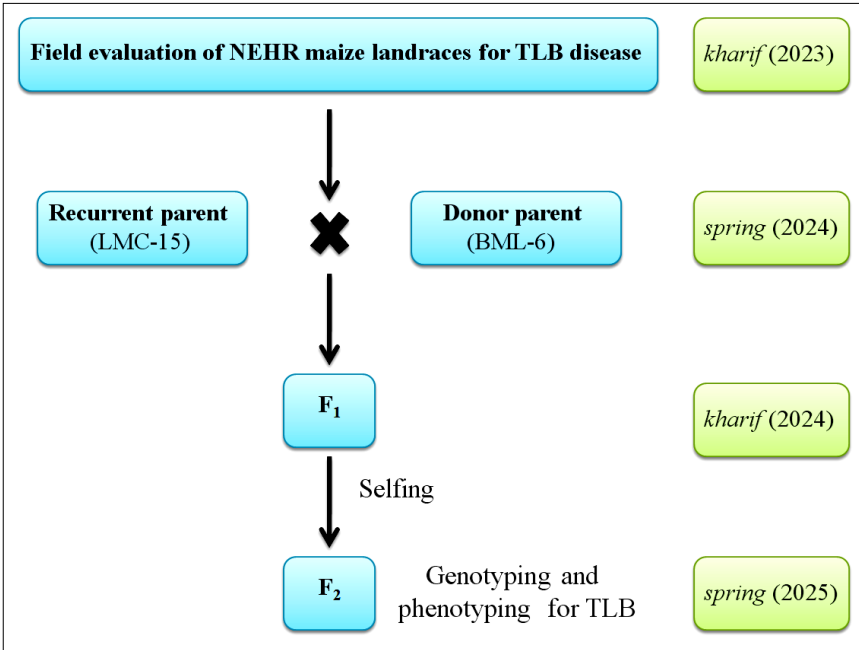
### Generation and confirmation of $F_1$ 's

From the cross between LMC-15 and BML-6, a total of 15  $F_1$  plants were generated and raised during the *Kharif* season of 2024. Leaf samples were collected at the seedling stage, DNA was isolated and each and each plant was tagged. PCR was conducted using the gene-linked marker *umc1042*, which showed polymorphism between the donor and recurrent parents.

After genotyping, 12 plants showed heterozygosity, confirming them as true  $F_1$ s, while the remaining plants showed recurrent parent bands, indicating that they were selfed. The presence of single bands revealed that those plants were not true hybrids, as SSR primers are co-dominant (Fig. 6).

### Generation of $F_2$ population and genotyping of $F_2$ population for TLB resistant genes

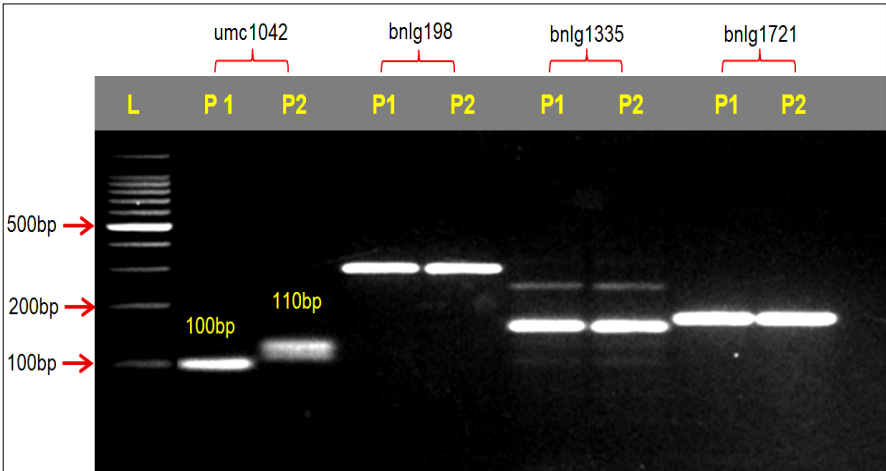
The true  $F_1$  plants with the TLB resistant gene were self-pollinated to produce the  $F_2$  generation at the research farm of the Department of



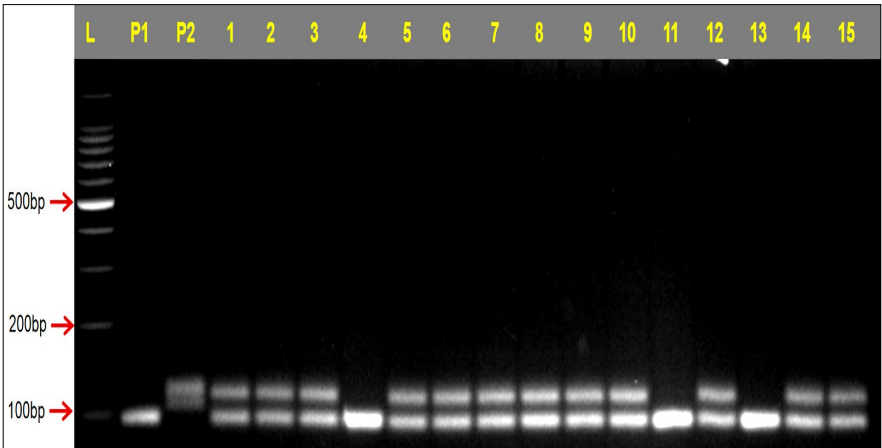
**Fig. 4.** Schematic representation for the development of TLB disease resistance lines using phenotypic and genotypic selection.

**Table 3.** Information about the molecular markers utilized for foreground selection related to the gene responsible for TLB resistance

Sl. No.	Marker	Gene	Chr. No.	References
1	<i>umc1042</i>	<i>Ht1</i>	2	(3)
2	<i>bnlg1721</i>	<i>Ht1</i>	2	(3)
3	<i>bnlg198</i>	<i>Ht1</i>	2	(28)
4	<i>bnlg1335</i>	<i>Ht1</i>	2	(28, 29)



**Fig. 5.** Validation of gene linked molecular markers between parents. L: 100 bp ladder; P1: recurrent parent (LMC-15); P2: donor parent (BML-6).



**Fig. 6.** Hybridity confirmation of the F<sub>1</sub>'s for *Ht1* gene using *umc1042* marker. L: 100 bp ladder; P1: recurrent parent (LMC-15); P2: donor parent (BML-6); 1-15: F<sub>1</sub>'s.

**Table 4.** Segregation pattern of *umc1042* marker in  $F_2$  population and their goodness of fit of genotypic ratio

Total number of $F_2$ plants	Segregation pattern						Expected ratio		
	Observed			Expected			A:H:B	$\chi^2$	$p$ -value
	A	H	B	A	H	B			
120	27	61	32	30	60	30	1:2:1	0.45	5.99

A: homozygous dominant; H: heterozygotes; B: homozygous recessive; significant value of  $p$  at 0.05 is 5.99.

Genetics and Plant Breeding, CAU, Imphal, during the *Kharif* of 2024. Genotyping of 120  $F_2$  plants was done using the *umc1042* SSR marker, which showed polymorphism between LMC-15 and BML-6. The marker *umc1042* exhibited a segregation ratio of 27:61:32 (homozygous dominant: heterozygotes: homozygous recessive) in the  $F_2$  population. The findings indicate that the chi-square value for the genotype was 0.45, which is below  $\chi^2$  ( $p \leq 0.05$ ) and thus considered non-significant. Therefore, the chi-square test demonstrated a good fitness for the 1:2:1 ratio (Table 4). Due to the study's aim of achieving durable and extensive resistance, only the 27 plants that possess the target gene in a homozygous state were selected in the field for additional assessment of their yield and agronomic characteristics (Fig. 7).

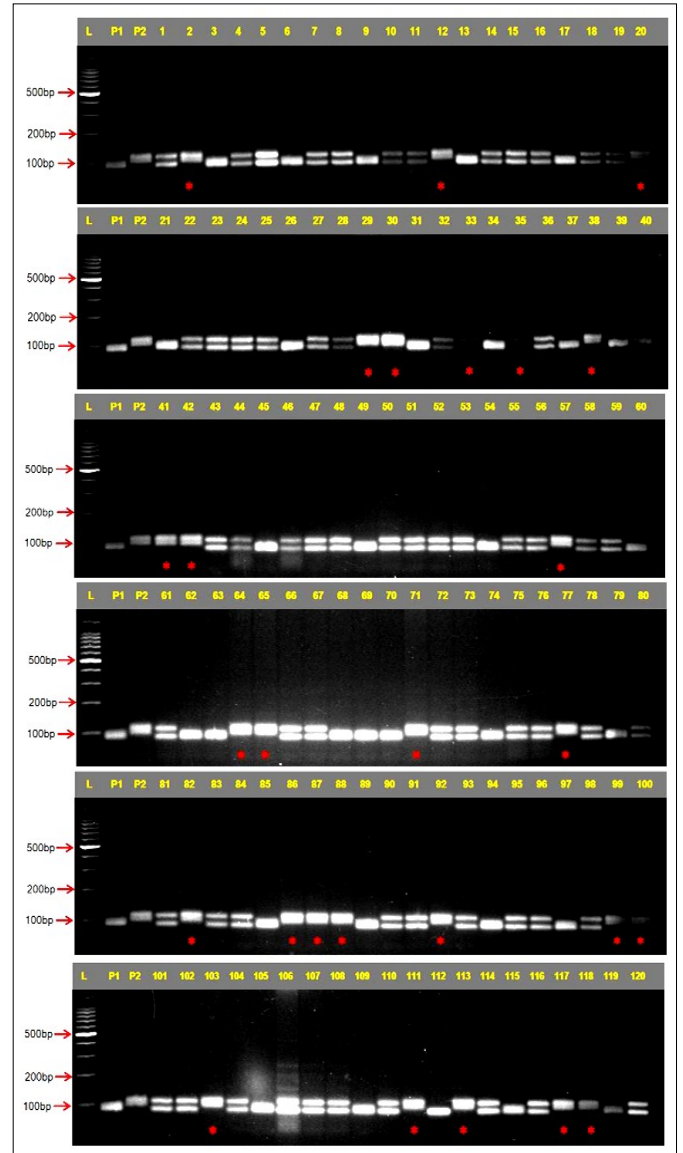
#### Phenotyping of $F_2$ mapping population

A total of 120  $F_2$  plants were evaluated for TLB disease under field conditions. Among them, 12 plants showed resistance reactions, 76 plants exhibited moderate resistance, while 27 plants showed moderate susceptible and 5 plants showed susceptibility. This distribution resulted in a phenotypic ratio of 3R:1S (88 resistance:32 susceptible), suggesting that TLB resistance is governed by a dominant gene (Table 5).

#### Discussion

Field screening of maize under natural epiphytotic conditions revealed significant variation in resistance to TLB caused by *E. turicum*. The field conditions in Imphal, characterized by high relative humidity and moderate temperatures during the crop growth period, were favourable for the development of TLB. The differential host response observed in this study indicate the presence of moderately susceptible and susceptible landraces in the tested materials. Such variability has also been reported in other maize-growing regions of India and abroad, underscoring the availability of valuable resistance sources for breeding (19, 20). The present research found that LMC-15 and LMC-16 were susceptible, while LMC-4 and LMC-7, were classified as moderately susceptible (21-23).

The present study demonstrates the successful introgression of the *Ht1* gene, which confers resistance to TLB caused by *E. turicum*, into a susceptible maize background and its validation in the  $F_2$  segregating population through MAS. The SSR marker *umc1042* demonstrated polymorphism between the two

**Fig. 7.** Foreground selection in  $F_2$  plants for the *Ht1* gene, along with the parental lines, utilizing the *umc1042* marker. L: 100 bp ladder; P1: recurrent parent (LMC-15); P2: donor parent (BML-6);  $F_2$ : 1-120.

parental lines, amplifying a 110 bp allele in the resistant parent and 100 bp allele in the susceptible parent. Chi-square analysis of the genotypic data from 120  $F_2$  plants amplified using the *umc1042* marker showed a non-significant deviation, indicating a good fit to the expected 1:2:1 segregation ratio.

**Table 5.** Segregation pattern of individual  $F_2$  population regarding TLB disease resistance and their goodness of fit of phenotypic ratio

Total number of $F_2$ plants	Reaction to TLB disease				Expected ratio		
	Observed		Expected		R:S	$\chi^2$	$p$ -value
	R	S	R	S			
120	88	32	90	30	3:1	0.17	3.84

R: number of resistance plants; S: number of susceptible plants; significant value of  $p$  at 0.05 is 3.84.



The segregation pattern in the  $F_2$  generation revealed the expected distribution of resistant and susceptible individuals, consistent with the monogenic inheritance model (24, 25). These results confirm that *Ht1* was effectively introgressed and expressed in the target genetic background. MAS ensure precise and early detection of resistant individuals, thereby accelerating the breeding cycle and reducing the cost and time associated with multiple generations of field testing. Similar benefits of MAS in the selection of disease resistance genes in maize have been reported and validated in subsequent studies (26, 27). Overall, the findings confirm that MAS is an efficient and reliable approach for introgressing *Ht1* into susceptible maize lines. The  $F_2$  segregating population demonstrated clear evidence of resistance, validating both the effectiveness of *Ht1* and the utility of marker-based selection. Moving forward, integrating MAS with conventional backcrossing, pyramiding *Ht1* with other resistance sources and monitoring pathogen variability will be essential for developing durable, high-yielding, TLB-resistant maize hybrids.

## Conclusion

The study focused on improving maize landraces from the NEHR of India that are highly valued by farmers but remain susceptible to TLB. Four landraces were screened under artificial inoculation and two (LMC-15 and LMC-16) were identified as highly susceptible, while the other two showed moderate susceptibility. To introduce resistance, the *Ht1* gene from a resistant donor parent (BML-6) was crossed into the susceptible landrace LMC-15. The resulting  $F_1$  plants were validated using a co-dominant SSR marker (*umc1042*), confirming the presence of the target gene. The integration of phenotypic evaluation with marker-assisted selection proved to be an effective approach for incorporating resistance genes. This study successfully demonstrated the introgression of the TLB-resistant gene *Ht1* into a NEHR maize landrace. Moreover, the developed material can be advanced using the single seed descent (SSD) method to create recombinant inbred lines (RILs) for further genetic analysis and breeding.

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## Authors' contributions

RSC conducted the complete research process, including drafting and preparing the initial version. PK supplied the resources, oversaw the study, and revised the manuscript. TRD, PS, NN, BS, SK and VC were involved in the research methodology, manuscript creation and editing. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

## References

1. Ansari MA, Prakash N, Kumar A, Jat SL, Baishya LK, Sharma SK, et al. Maize production technology highlighted in North East India. Training Manual RCM-05. ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal; 2015.
2. Natesan S, Singh TS, Duraisamy T, Chandrasekharan N, Chandran S, Adhimoolam K, et al. Characterization of crtRB1 gene polymorphism and  $\beta$ -carotene content in maize landraces originated from North Eastern Himalayan Region of India. *Front Sustain Food Syst*. 2020;4:78. <https://doi.org/10.3389/fsufs.2020.00078>
3. Puttarach J, Puddhanon P, Siripin S, Sangtong V, Songchantuek S. Marker-assisted selection for resistance to northern corn leaf blight in sweet corn. *SABRAO J Breed Genet*. 2016;48(1):72-9.
4. Hooda KS, Bagaria PK, Khokhar Kaur M, Harleen, Sujay R. Mass screening techniques for resistance to maize diseases. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana; 2018. p. 93.
5. Khatri NK. Influence of temperature and relative humidity on the development of *Exserohilum turcicum* on maize in Western Georgia. *Indian J Mycol Plant Pathol*. 1993;23:35-7.
6. Navarro BL, Campos RA, Gasparoto MC, Tiedemann AV. *In vitro* and in planta studies on temperature adaptation of *Exserohilum turcicum* isolates from maize in Europe and South America. *Pathogens*. 2021;10(2):154. <https://doi.org/10.3390/pathogens10020154>
7. Lal. Genetics of *Helminthosporium* leaf blight resistance in maize. Maize Genetics Prospectus Symposium in the TNAU. Coimbatore; 1991. p. 231-7.
8. Sivanesan A, Abdullah SK, Abbas BA. *Exserohilum curvisporum* sp. nov., a new hyphomycete from Iraq. *Mycol Res*. 1993;97:1486-8. [https://doi.org/10.1016/S0953-7562\(09\)80222-0](https://doi.org/10.1016/S0953-7562(09)80222-0)
9. Hooker AL. *Helminthosporium turcicum* as a pathogen of corn. *Post-IAMS Japanese*; 1975. p. 115-25.
10. Gevers HO. A new major gene for resistance to *Helminthosporium turcicum* leaf blight of maize. *Plant Dis Rep*. 1975;59:296-9.
11. Hooker AL. Monogenic resistance in *Zea mays* L. to *Helminthosporium turcicum* Pass. *Crop Sci*. 1963;3(5):381-3. <https://doi.org/10.2135/cropsci1963.0011183X000300050002x>
12. Hooker AL. A second major gene locus in maize for resistance to *Helminthosporium turcicum*. *Crop Sci*. 1977;17(1):132-5. <https://doi.org/10.2135/cropsci1977.0011183X001700010035x>
13. Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK. An introduction to markers, quantitative trait loci mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*. 2005;142(1-2):169-96. <https://doi.org/10.1007/s10681-005-1681-5>
14. Hurni S, Scheuermann D, Krattinger SG, Kessel B, Wicker T, Herren G, et al. The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc Natl Acad Sci USA*. 2015;112(28):8780-5. <https://doi.org/10.1073/pnas.1502522112>
15. Joshi LM, Goel LB, Renfro BL. Multiplication of inoculum of *Helminthosporium turcicum* on sorghum seeds. *Indian Phytopathol*. 1969;22:146-8.
16. Wheeler BEJ. An introduction to plant diseases. London: Wiley and Sons; 1969. p. 374.
17. Madden LV, Hughes G, Van Den Bosch F. The study of plant disease epidemics. St. Paul (MN): American Phytopathological Society; 2007.
18. Doyle JJ, Doyle JL. A rapid total DNA preparation procedure for fresh plant tissue. *Focus*. 1990;12:13-5.
19. Ogliari JB, Guimaraes MA, Camargo LEA, Schuster I. Genetic variability for resistance to *Exserohilum turcicum* in tropical maize populations. *Euphytica*. 2005;142(3):227-35.



20. Welz HG, Geiger HH. Genes for resistance to *Exserohilum turcicum* in diverse maize populations. *Plant Breed.* 2000;119(1):1-14. <https://doi.org/10.1046/j.1439-0523.2000.00462.x>
21. Yousuf N, Dar SA, Lone AA, Ahanger MA, Dar ZA, Bhat MA, et al. Field screening of maize (*Zea mays* L.) landraces for resistance against turcicum leaf blight under temperate conditions. *Int J Chem Stud.* 2018;6(1):333-7.
22. Meghana SP, Harlapur SI, Patil PV, Kachapur RM. Screening of maize germplasm against turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. *Environ Ecol.* 2022;40(3B):1497-503.
23. Keerthana D, Haritha T, Sudhir Kumar I, Ramesh D. Field screening of maize (*Zea mays* L.) genotypes against turcicum leaf blight under artificial conditions. *Int J Plant Soil Sci.* 2023;35(7):1-11. <https://doi.org/10.9734/ijpss/2023/v35i72856>
24. Hooker AL. Additional sources of monogenic resistance in corn to *Helminthosporium turcicum*. *Crop Sci.* 1978;18(5):787-8. <https://doi.org/10.2135/cropsci1978.0011183X001800050024x>
25. Hooker AL. Resistance to *Helminthosporium turcicum* from *Tripsacum floridanum* incorporated into corn. *Maize Genet Coop Newsl.* 1981;55:87-8.
26. Zaitlin D, De Mars S, Gupta M. Linkage of a gene controlling resistance to *Exserohilum turcicum* (ht1) with molecular markers in maize. *Theor Appl Genet.* 1992;83(5):531-8.
27. Zhu M, Ma J, Liu X, Guo Y, Qi X, Gong X, et al. High-resolution mapping reveals a novel QTL and candidate genes associated with resistance to northern corn leaf blight in maize. *Front Genet.* 2022;13:1038948. <https://doi.org/10.3389/fgene.2022.1038948>
28. Min J, Chunyu Z, Khalid H, Suwen W, Feng L. Pyramiding resistance genes to northern leaf blight and head smut in maize. *Int J Agric Biol.* 2012;14:430-4.
29. Naeem S, Tahir M, Khan K, Hassan A, Ahmad R. Morphological and molecular markers based screening of maize hybrids against northern corn leaf blight. *Pak J Bot.* 2021;53(1):301-8. [https://doi.org/10.30848/PJB2021-1\(5\)](https://doi.org/10.30848/PJB2021-1(5))

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