



## SHORT RESEARCH ARTICLE

# Development of banded leaf and sheath blight-resistant maize (*Zea mays* L.) hybrids through introgression of wild progenitor alleles

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## Abstract

Banded leaf and sheath blight (BLSB), caused by *Rhizoctonia solani*, is a major constraint in maize (*Zea mays* L.) production across tropical Asia, especially during the *Kharif* season. Due to the absence of effective resistance in cultivated maize, the wild progenitor *Zea mays* ssp. *parviglumis* was utilized to develop 14 stable introgression lines (ILs) in diverse maize genetic backgrounds from 2017 to 2021. Artificial inoculation trials conducted during *Kharif* 2021 and 2022 evaluated BLSB resistance in ILs, parental lines, and teosinte. A hybrid derived from two selected ILs- $\{(LM\ 14 \times Zea\ mays\ ssp.\ parviglumis) // LM\ 14\} \times \{(LM\ 13 \times Zea\ mays\ ssp.\ parviglumis) // LM\ 13\}$  demonstrated moderate resistance to BLSB, with a disease score of 4.5. The female parent showed moderate resistance (4.5), while the male parent was moderately susceptible (6.7). Although none of the ILs matched the resistance level of teosinte (4.2), they exhibited significantly lower disease severity than their recurrent parental lines. This study underscores the potential of wild genetic resources for improving BLSB resistance in maize and lays a foundation for sustainable disease management.

**Keywords:** Banded leaf and sheath blight, disease resistance, introgression lines, maize, *Rhizoctonia solani*, Teosinte.

Maize (*Zea mays* L.), the second most important cereal in Asia after rice, is highly vulnerable to several diseases that threaten its productivity. Among them, banded leaf and sheath blight (BLSB), caused by the soil-borne fungus *Rhizoctonia solani*, and is a serious constraint in tropical Asia under warm and humid conditions. The disease typically initiates at the pre-flowering stage, with hallmark symptoms including water-soaked, straw-coloured lesions on the basal sheaths, interspersed with dark bands. In severe infestations, sclerotia develop and the cobs may be entirely destroyed (Hooda *et al.* 2018). The wide host range of *R. solani*, spanning 32 plant families, exacerbates its persistence and pathogenicity (Roy 1983; Kaur and Singh 2014). Management strategies have primarily focused on integrated approaches, including cultural practices like leaf stripping, chemical control, and development of resistant cultivars. Fungicides such as azoxystrobin and difenoconazole have been found effective in Punjab (Kumar and Kaur 2020), but genetic resistance remains the most cost-effective and environmentally sustainable option for long-term disease management (Hooda *et al.* 2018). However, the current maize cultivars exhibit inadequate resistance levels. Therefore, exploration of wild relatives, especially teosinte and its subspecies, has become vital for sourcing novel alleles for BLSB resistance.

Maize was domesticated from teosinte approximately 7500–9000 years ago in Southern Mexico, with *Zea mays* ssp. *parviglumis* was established as the closest wild ancestor (Doebley 2004). Despite significant morphological differences, maize and its progenitors remain cross-compatible and produce fertile offspring. Wild species such as *Zea mexicana*, *Zea diploperennis*, and *Tripsacum dactyloides* harbour genes conferring resistance to foliar diseases like downy mildew, northern leaf blight, and viruses (Chavan and Smith 2014; Wei *et al.* 2003; Nault and Findley 1981). For example, *Tripsacum* has been reported as

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a source of resistance to rust, northern corn leaf blight, and maize weevil (Berquist 1981; Throne and Eubanks 2015). More recently, *Z. mays* ssp. *parviglumis* has been linked to resistance loci for grey leaf spot and BLSB (Garg et al. 2019; Adhikari et al. 2021 and 2022), reinforcing its potential utility in resistance breeding.

In this context, a breeding initiative at Punjab Agricultural University (PAU), Ludhiana, aimed at developing BLSB-resistant maize hybrids through introgression of resistance genes from *Z. mays* ssp. *parviglumis*. As the Indian maize programme has developed various heterotic pools, three of them have been developed by PAU, Ludhiana. The first pair, Makki Safed x Tuxpeno, is adapted to the rainy season and represents full-season maturity types. The second pair, Indigenoux Semi-Exotic, is also adapted to the rainy season but is characterised by early maturity. The third pair, Ludhiana Lancaster x Ludhiana Stiff stalk, which is specifically adapted to the winter season, consists predominantly of germplasm of exotic origin, primarily derived from the U.S. Corn Belt (Khehra and Dhillon 1984). The breeding material originated from two distinct heterotic pools developed at PAU: Makki Safed and Tuxpeno. LM 13 and LM 14, derived from these respective pools, are elite inbred lines previously used in the development of commercial hybrids. To incorporate resistance, crosses were initiated between these lines and *Z. mays* ssp. *parviglumis* as the pollen donor. The programme followed a systematic backcrossing approach, with  $F_1$ s generated through initial crosses, followed by two successive backcrosses to the recurrent maize parent, resulting in  $BC_2F_1$  progenies. These were then selfed through four generations to obtain stable  $BC_2F_5$  introgression lines. The introgression process, carried out over four years from 2017–2021, involved growing two cycles annually under field conditions at PAU's maize research farm.

The site, located at 30°55' N latitude and 75°54' E longitude at an altitude of 247 m, offers favourable conditions for BLSB screening, particularly in the *kharif* due to average temperatures around 28°C and relative humidity above 80%. Disease development was further ensured by artificial inoculation using the virulent *R. solani* isolate *Rs-12* cultured on maize-sand medium, as described by Kumar and Kaur (2020). Inoculation was performed by placing colonised grains in the basal leaf sheaths of 30-day-old plants, followed by irrigation and humidity maintenance to promote disease development.

A total of 14 introgression lines (ILs), representing nine from the LM 13 background and five from the LM 14 background, were evaluated under replicated trials in *Kharif* 2022. Each line was assessed for disease severity 45 days after inoculation using a standard 1–9 scale (Hooda et al., 2018). Based on disease scores, genotypes were classified into resistant ( $\leq 3.0$ ), moderately resistant (3.1–5.0), moderately

**Table 1. Disease reaction of parental and introgression lines of maize**

S. No.	Introgression line	Disease score*	Disease reaction
1	LM 13	6.7	MS
2	Teosinte	4.2	MR
3	LM 14	7.8	S
4	(LM13xTeosinte)/LM13*3- $\otimes$ L-1	7.4	S
5	(LM13xTeosinte)/LM13*3- $\otimes$ L-2	6.6	MS
6	(LM13xTeosinte)/LM13*3- $\otimes$ L-3	8.1	S
7	(LM13xTeosinte)/LM13*3- $\otimes$ L-4	5.7	MS
8	(LM13xTeosinte)/LM13*3- $\otimes$ L-5	5.2	MS
9	(LM13xTeosinte)/LM13*3- $\otimes$ L-7	8.4	MS
10	(LM14xTeosinte)/LM14*3- $\otimes$ L1	5.4	MS
11	(LM14xTeosinte)/LM14*3- $\otimes$ L4	5.6	MS
12	(LM14xTeosinte)/LM14*3- $\otimes$ L6	6.1	MS
13	(LM14xTeosinte)/LM14*3- $\otimes$ L7	7.1	S
14	(LM14xTeosinte)/LM14*3- $\otimes$ L8	7.5	S

\*1-3.0: Resistant; 3.1-5.0: Moderately Resistant; 5.1-7.0: Moderately Susceptible; 7.1-9.0: Susceptible

**Table 2. Disease reaction of parents and  $F_1$  generated from introgression lines**

S. No.	Entry tested	Disease score*	Disease reaction
P 1	LM 14	7.7	S
P 2	LM 13	6.9	MS
Wild progenitor	Teosinte( <i>Zea mays</i> ssp. <i>parviglumis</i> )	4.2	MR
$F_1$	{(LM 14 X <i>Zea mays</i> ssp. <i>parviglumis</i> )/LM 14*3- $\otimes$ L1} x {(LM 13 X <i>Zea mays</i> ssp. <i>parviglumis</i> )/LM 13*3- $\otimes$ L5}	4.5	MR

\*1-3.0: Resistant; 3.1-5.0: Moderately Resistant; 5.1-7.0: Moderately Susceptible; 7.1-9.0: Susceptible

susceptible (5.1–7), and susceptible ( $>7.0$ ) categories (Table 1). The wild donor parent, *Z. mays* ssp. *parviglumis*, recorded a disease score of 4.2, categorising it as moderately resistant. In contrast, the recurrent parents LM 13 and LM 14 exhibited higher scores of 6.7 and 7.8, respectively, indicating moderate to high susceptibility. Among the 14 ILs, disease scores ranged from 5.2 to 8.4. The two best-performing ILs were {(LM 14 x *Zea mays* ssp. *parviglumis*)/LM 14- $\otimes$ 3-L5--f}(5.2) and {(LM 13 x *Zea mays* ssp. *parviglumis*)/LM 13}- $\otimes$ 3-L1--f}(5.4), demonstrating improved resistance over their recurrent parents. While the introgressed lines did not surpass the resistance level of the donor, the reduction in severity by 1.3–2.4 underscores partial transfer of resistance genes into the cultivated maize background. A hybrid derived from two selected ILs demonstrated moderate resistance to BLSB (Table 2).

These outcomes demonstrate the feasibility of enhancing BLSB resistance in elite maize backgrounds through wild introgression. Although the complete resistance of the wild progenitor could not be fully recovered, a significant improvement in disease response was achieved in selected lines. The hybrids developed using these lines represent a promising genetic base for further improvement.

This research highlights the strategic advantage of harnessing wild germplasm for strengthening maize disease resistance. Given the limited resistance in commercial maize varieties, exploiting the genetic wealth of *Z. mays* ssp. *parviglumis* offers a powerful tool in diversifying the resistance gene pool. Continued backcrossing and marker-assisted selection could further refine these lines, stabilise resistance traits, and expedite their deployment in hybrid development pipelines. This approach is not only relevant to managing BLSB but also paves the way for developing broadly disease-resistant maize varieties capable of sustaining yield under changing climatic conditions.

#### Authors' contribution

Conceptualization of research (SKS); Designing of the experiments (SKS); Contribution of experimental materials (SKS); Execution of field/lab experiments and data collection (NY, SK); Analysis of data and interpretation (SKS, NY); Preparation of the manuscript (SKS, NY, HK).

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