

Efficient and large-scale field screening procedure for maydis leaf blight

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Abstract: *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker is a necrotrophic fungal pathogen that causes maydis leaf blight (MLB) also known as Southern corn leaf blight (SCLB) mainly infects maize leaves. Lesions are initially small and diamond-shaped, and then become elongated as they mature. Under severe disease pressure, lesions may coalesce, blighting the entire leaf. This disease is found in almost all maize-growing regions of the world including India and significantly affects maize productivity. In the present study, an efficient and large-scale field screening procedure was developed to identify resistant maize genotypes. The plants were inoculated at the 5–6 leaf stage by placing around 10–20 sorghum grains prior infested with the fungus into the whorl of each maize plant. Primary inoculum is eventually dispersed by environmental factors, causing multiple cycles of infection that assure a high uniform disease pressure over the entire field. The intensity of the disease was recorded as disease scoring on a scale of 1–9 at the pre-flowering, tasseling and silking stage.

Keywords: *Bipolaris maydis* · Field screening · Fungal diseases · Maize · Maydis leaf blight

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as the queen of cereals because it has the highest genetic yield potential among cereals. In India, maize is the third most important food crop after rice and wheat. MLB is a major disease in the states of Jammu & Kashmir, Himachal Pradesh, Sikkim, Meghalaya, Punjab, Haryana, Rajasthan, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh, Gujrat, Maharashtra, Andhra Pradesh, Telangana, Karnataka and Tamil Nadu having warm humid temperate to the tropical climate in the cropping period.

The causal agent *Bipolaris maydis* is a member of the ascomycetes, the sac fungi which produces a toxin that attacks the mitochondria and destroys the plants' ability to capture energy from metabolism. Three races of *B. maydis* have been described. Race 'O' is considered the most common and indigenous throughout most areas where SCLB occurs. This infects the leaf blade tissue only, and forms small, tan, and parallel-side lesions with buff or brown borders (Agrios, 1997). On the other hand, race T, the cause of the 1970 SCLB epidemic in North America, is specifically virulent on Texas male sterile cytoplasm (cmsT) maize due to its ability to produce a polypeptide toxin (T toxin) to which cmsT maize is sensitive (Levings and Siedow, 1992). It attacks all above-ground parts of the maize plant. *B. maydis* releases either asexual conidia or sexual ascospores to infect maize plants. The asexual cycle is known to occur in nature and is of primary concern. Upon favourable conditions, conidia (the primary inoculum) are released from lesions of an infected corn plant and carried to plants in close proximity via wind or rain. Once conidia have landed on the leaf or sheath of a healthy plant, they will germinate on the tissue

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by way of polar germ tubes. The germ tubes either penetrate through the leaf or enter through a natural opening such as the stomata. This procedure for the mass screening of maize genotypes for MLB resistance was based on a methodology developed by Carson *et al.* (2004) and Sermons and Balint-Kurti (2018) with some modifications.

Materials and methods

Genotypes

Ten maize genotypes, LM 5, LM 11, LM 13, LM 14, LM 15, LM 16, CM 139, CM 140, CM 143 and CM 144 were sown in the *Kharif* season under a randomized complete block design arrangement. Two seeds per hill were placed by hand and later thinned 15 days after sowing to a single healthy plant per hill. Each row contained a total of 20 plants and each plot was consisted of ten rows. Spacing was 70 cm between and 20 cm within the rows covering a plot area of 14 m² with a population density of 50,000 plants/ha. Each plot was replicated thrice in the experimental field of Punjab Agricultural University, Ludhiana.

Reagents and Instruments

Pure fungal culture of *B. maydis*, KOH, HCL, glass beaker, conical flask / Erlenmeyer flask, spatula, measuring cylinder, pH meter, weighing balance, distilled water, butter paper, magnetic stirrer and pellet, pipettes and tips, petri plates and/or test tubes, hot plate, ddH₂O, ready to use potato dextrose agar media or raw potatoes, agar and dextrose, streptomycin or chloramphenicol, sorghum

grain, ethanol, tween-20, aluminium foil, cotton plug, laminar air flow, incubator, autoclave and hot air oven, muslin cloth, conical flasks, autoclave bags.

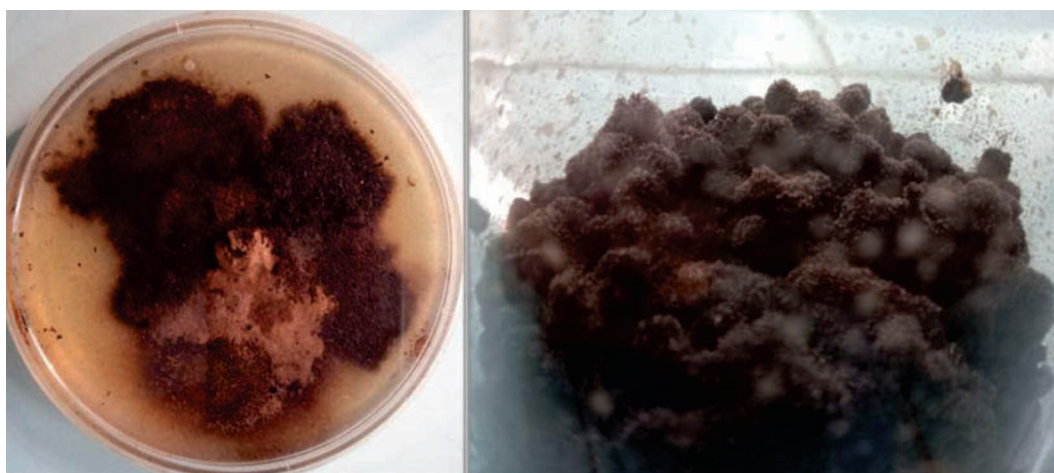
Preparation of media

The fungal strain was cultured on a full strength of PD media that were prepared by autoclaving conical flask containing 10 g PDA powder in 500 ml double distilled water (ddW). After autoclaving, the molten PDA media could cool to about and 0.1% streptomycin was added to eliminate bacterial contaminant. To each glass Petri dish, 18 to 20 ml molten PDA media was poured and allowed to cool. The sorghum grain media was prepared by soaking 100 g clean sorghum grains for 24 hours in ddW. After draining off excess water from the flasks, a pinch of sucrose added to the moistened grains and then autoclaved at 121°C (15 psi) for 30 min. Sorghum grain flasks were kept at 4°C till further use.

Culture inoculation

Maintenance and inoculation of the fungal pathogen of *B. maydis* were done according to the methodology developed by Carson *et al.* (2004). PDA plates were inoculated (inside a laminar flow hood) by placing 1 cm round cork from the 25 days old fungal colony and dabbing it over the surface of a fresh PDA plate. After inoculation, plates were sealed with the parafilm and placed into an incubator at 25±1 °C, under 12 hours light/dark cycle. Culture at a growing stage, was cut into the smaller used to inoculate previously autoclaved sorghum grain conical flasks under laminar air flow conditions and was kept in an incubator at 30±1 °C. Fungal growth was

Figure 1. Culture growth of *B. Maydis* on PDA media plate (left) and sorghum grains covered with the fungal growth (right)



observed 3–4 days after inoculation and allowed to continue for 14 days (Figure 1). This grain inoculum was collected in a plastic container covered with a plastic bag by a sterile spatula. The inoculum was allowed to dry either by using a fan or by simply placing it under the shed for 1–2 hours.

Inoculation of maize plants

Maize plants at the 5–6 leaf stage was inoculated by *B. maydis* in the evening by dropping about ten to twenty grains of inoculum directly into the whorl. After two weeks of inoculation, the secondary inoculum was spread all over the plots creating a high, uniform disease pressure. Humidity or light irrigation is required in order to produce secondary inoculum for dispersion and successful germination of fungal spores. Upon germination, it gives rise to conidiophores which, upon favorable conditions, can either further infect the original host plant (kernels, husks, stalks, leaves) or release conidia to infect other nearby plants thus completing the disease cycle (Figure

2). For inoculum to grow and proliferate, moisture was maintained by irrigation the next day after inoculation. Initial SLB symptoms were appeared within one week and they spread over all field within the next week.

Disease scoring

Disease scoring was done at the pre-flowering stage (approximately three weeks after inoculation) tasseling and silking stage by observing the ear leaf and the leaf above, then rating the symptoms on a 1 to 9 scale where the maximum score of nine indicates complete death of the plant and the minimum score of one indicates healthy plants without disease symptoms (Kump *et al.*, 2011) (Table 1).

Data analysis

For the disease scoring analysis, data sets were subjected to factorial ANOVA (Analysis of variance) in accordance with the RCBD experimental design using SPSS version

Figure 2. Disease cycle of *B. maydis*

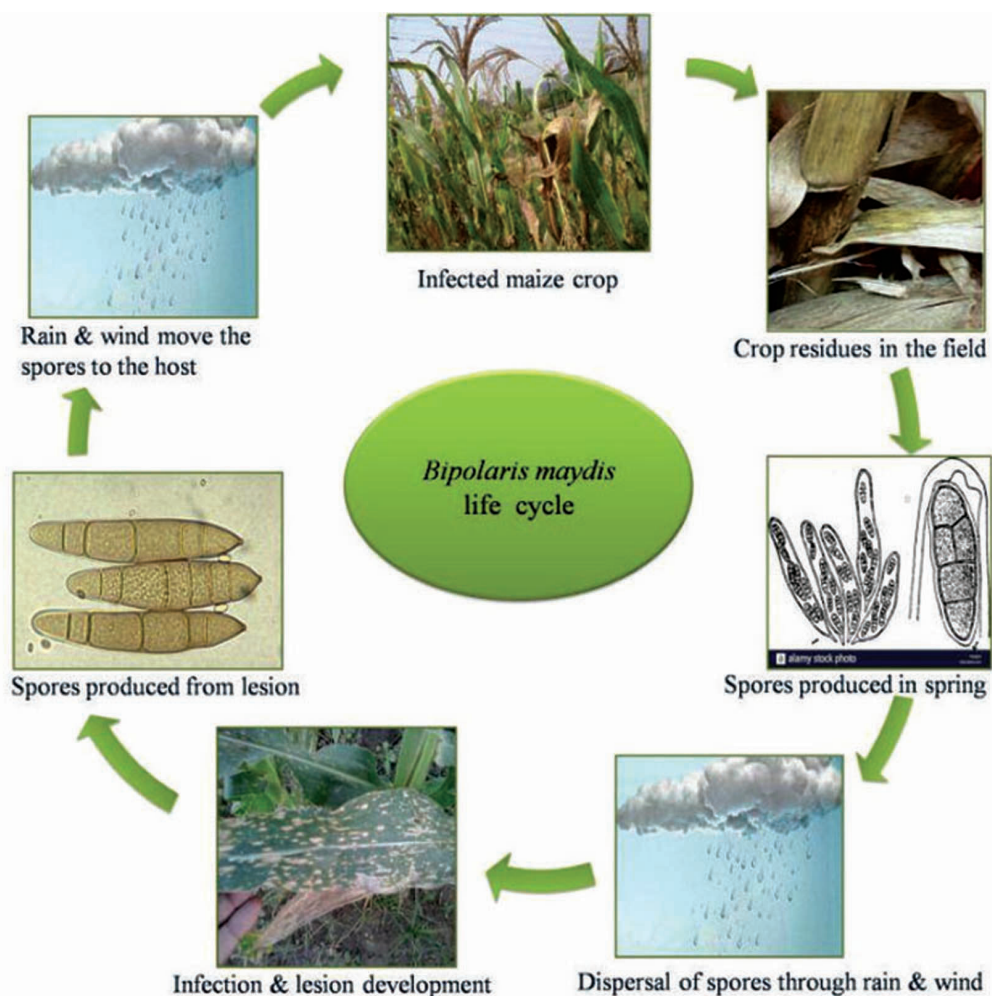


Table 1. SCLB disease rating scale (1–9)

Rating scale	Degree of infection
1.0	Nil to very slight infection (<10%). Very slight to slight infection, one or two to few scattered lesions on lower leaves
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1–20%).
3.0	Light infection, a moderate number of lesions scattered on four lower leaves (20.1–30%).
4.0	Light infection, a moderate number of lesions scattered on lower leaves, and a few lesions scattered on middle leaves below the cob (30.1–40%).
5.0	Moderate infection, an abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1–50%).
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%).
7.0	Heavy infection, an abundant number of lesions scattered on lower and middle leaves, and a moderate number of lesions on two to four leaves above the cob (60.1–70%).
8.0	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1–80%).
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant prematurely dried and killed (>80%).

23.0 (IBM, Armonk, NY) in triplicates to evaluate the source of variation and interactions within and between genotypes and disease scoring.

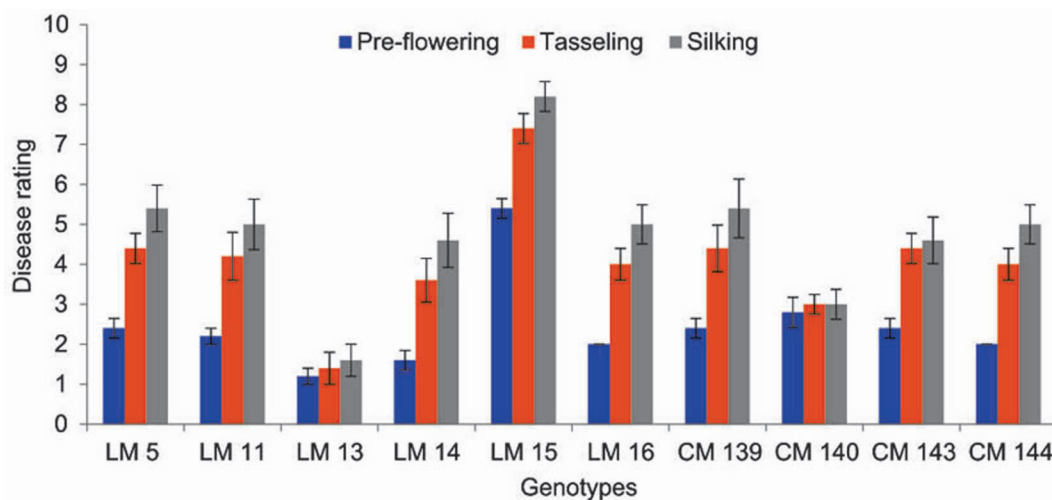
Results and discussion

Here we presented the detailed methodology used for the mass screening of maize genotypes against MLB. The first disease score was recorded at the pre-flowering stage only after the consistent spread of disease through the field mainly due to secondary inoculum. Disease scoring for the screening of genotypes for MLB was reported by Vasmatkar *et al.* (2021), on a rating scale of 1–5. The disease score on a scale of 1–9 was recorded at the pre-flowering, tasseling and silking stage and all the stages of LM 15 showed higher disease scores as compared to

other genotypes. However, LM 13 was the least affected genotype, showing the lowest MLB scoring (Figure 3).

The detailed biochemical profiling of these genotypes under MLB infestation was reported by Vasmatkar *et al.* (2019). Symptoms were found to be aggravated at the silking stage in all studied genotypes except CM 140. The highest disease score of 8.2 was observed in LM 15 at silking stage (Figure 4). There was moderate rain three days after the inoculation due to which disease in the field plots was evenly spread thus during the scoring whole plot was showing a similar disease score. We wanted to record the disease score for five times after the inoculation at ten days intervals but due to unusually hot and humid weather decrease plant specifically, LM 15 genotype has started to senesce because of severe necrosis thus it was no longer possible to score disease. Goudar and Harlapur

Figure 3. Disease score rating of different maize genotypes for maydis leaf blight



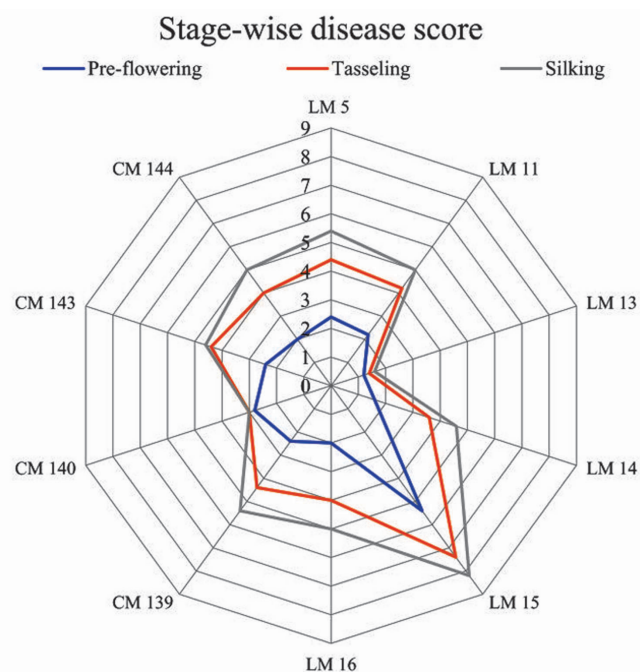


Figure 4. Stage-wise disease score rating of different maize genotypes for maydis leaf blight

(2019) also screened the maize genotypes against MLB. The least disease severity in LM 13 lead to high grain yield which might attribute to its higher resistance whereas in LM 15 highest disease severity and lowest grain yield could be attributed to the susceptibility toward MLB (Vasmatkar *et al.*, 2021).

We observed that, the plants with asynchronous ear development had a significant effect on the disease development and appearance of symptoms. The disease pressure was uniform across the field, various factors such as soil type soil moisture, wind velocity, temperature, and humidity may cause the variation in disease symptoms development at least somewhat in some genotypes. The effect of these factors on disease development needs to be studied further to minimize experimental error.

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References

- Agrios, G. N. (1997). Control of plant diseases. In *Plant Pathology*, 4th Edition, Academic Press, San Diego, pp 200–216.
- Carson, M. L., Stuber, C. W. & Senior, M. L. (2004). Identification and mapping of quantitative trait loci conditioning resistance to southern leaf blight of maize caused by *Cochliobolus heterostrophus* race O. *Phytopathology*, **94**(8): 862–867.
- Goudar, S. V. & Harlapur, S. I. (2019). Studies on loss assessment of maydis leaf blight (*Bipolaris maydis*. Nisikado) Shoemaker in maize. *International Journal of Chemical Studies*, **7**: 1831–1833.
- Kump, K. L., Bradbury, P. J., Wisser, R. J., Buckler, E. S., Belcher, A. R., Oropeza-Rosas, M. A., Zwonitzer, J. C., Kresovich, S., McMullen, M. D., Ware, D., Balint-Kurti, P. J. & Holland, J. B. (2011). Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nature Genetics*, **43**(2): 163–168.
- Levings, C. C. S. & Siedow, J. N. (1992) Molecular basis of disease susceptibility in the Texas cytoplasm of maize. *Plant Molecular Biology*, **19**: 135–147.
- Sermans, S. M. & Balint Kurti, P. J. (2018). Large scale field inoculation and scoring of maize southern leaf blight and other maize foliar fungal diseases. *Bio-protocol*, 2745.
- Vasmatkar, P., Kaur, K. & Pannu, P.P.S. (2021). Field based assessment of yield-related traits and flowering response in *Zea mays* towards Southern corn leaf blight. *Indian Phytopathology*, **74**: 969–979.
- Vasmatkar, P., Kaur, K., Pannu, P.P.S., Pannu, Kaur, G. & Kaur, H. (2019). Unraveling the metabolite signatures of maize genotypes showing differential response towards southern corn leaf blight by ¹H-NMR and FTIR spectroscopy. *Physiological and Molecular Plant Pathology*, **108**: 101441.