

Identification of Maize (*Zea mays* L.) Inbred Lines Resistant for Late Wilt Disease caused by *Harpophora maydis*

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ABSTRACT

Late wilt disease caused by *Harpophora maydis* is one of the important diseases in maize. The disease shows its symptoms during flowering when the plants are about to tassel. Considering that host plant resistance is the most economical and eco-friendly approach to mitigate losses due to late wilt, 155 maize inbred lines were screened under field conditions by challenging them with artificial inoculation of the pathogen using specialized inoculators designed for the purpose. Of these, 9 were resistant, 108 lines were moderately susceptible, 24 were susceptible and remaining 14 lines were highly susceptible. The identified late wilt resistant inbred lines could be used for developing late wilt resistant population / inbreds or in strategic and basic research.

AMONG the several biotic stresses, post flowering stalk rot (PFSR) complex is one of the major constraints causing substantial economic losses in maize production (Khokhar *et al.*, 2013). *H. maydis* causing late wilt disease is one of the fungal pathogens involved in PFSR complex (Shekhar *et al.*, 2010). Late wilt disease reported to cause huge production losses in Asia (India), Africa (Egypt) and Europe (Hungary, Portugal, Spain) (Chalkey, 2016) and is now considered as endemic in most of the maize growing areas (Degani and Cernica, 2014). The late wilt disease incidence was first reported from Egypt in 1963 (Samra *et al.*, 1963). Subsequently, the disease was reported from Tanzania, Pakistan, Hungary and Kenya (Freeman and Ward, 2004), India (Ward and Bateman, 1999), Portugal and Spain (Molinero-Ruiz *et al.*, 2010), Romania (Bergstrom *et al.*, 2008) and Israel (Drori *et al.*, 2013). Production losses up to 51 per cent are reported from India (Johal *et al.*, 2004).

The disease is associated with wilting symptoms that occur during flowering until shortly before maturity. Due to this delay in appearance of symptoms, the disease has been designated as “late wilt” (Samra *et al.*, 1963). Leaves turn pale green; start rolling inward as though suffering from lack of water (Sabet *et al.*, 1970). Eventually, plants start drying upwards resulting in discoloration of stem inter-nodes turning them into yellow-brown hue (Sabet *et al.*, 1966). In addition to rotting, late wilt disease also causes

disintegration of stem tissues (Payak *et al.*, 1970) (Figure 1). The pathogen, *H. maydis* is both soil (Samra *et al.*, 1963) and seed borne (Sabet *et al.*, 1970; El-Shafey and Claflin, 1999). Host plant resistance is considered as the most economical and

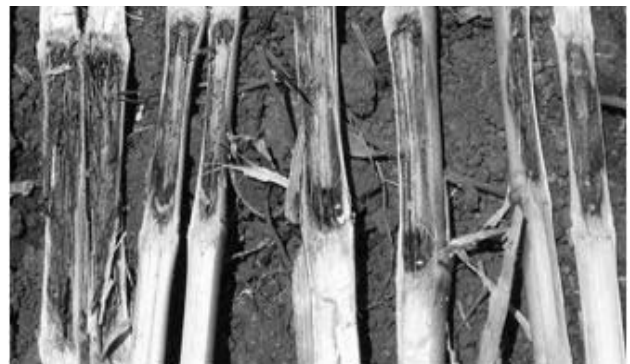


Fig 1: Typical symptoms of late wilt caused by *H. maydis* (internode discoloration and disintegration of the pith)

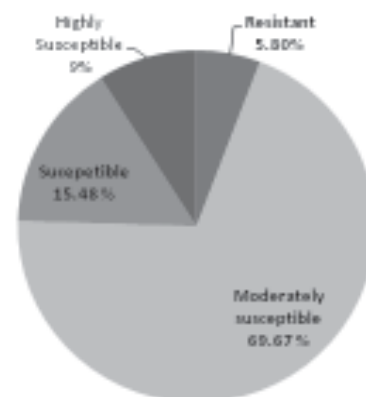


Fig 2: Per cent maize inbred lines resistant, tolerant and susceptible to late wilt disease caused by *H. maydis*

eco-friendly method of mitigating losses due to late wilt (Samra *et al.*, 1963; El-Shafey *et al.*, 1988). Identification of stable sources of resistance is a prerequisite for developing hybrids / populations with resistance to late wilt. The objective of the present investigation is to identify maize inbred lines resistant to late wilt disease.

TABLE I

Classification of maize inbred lines based on their response to late wilt disease caused by H. maydis

SlNo.	Score	Response	Number inbred lines
1	<3	Resistant	09
2	>3 – 6	Tolerant	108
3	>6 – 7	Susceptible	24
4	>7	Highly susceptible	14

A total of 155 maize inbred lines collected from Monsanto India Ltd. (MIL) constituted the experimental material. The seeds of inbred lines were dibbled at 0.3 m spacing in a single row of 3 m length with a row-to-row spacing of 0.6 m following randomized complete block design (RCBD). The experiment was carried out at Mega Breeding Station, MIL, Gowribidanur, Karnataka, India during 2014 late rainy season.

Isolation and inoculation of H. maydis : Stem pieces of maize plants infected with late wilt were surface-sterilized with 4 per cent sodium hydrochloride solution followed by thorough washing with distilled water. Surface-sterilized infected stem fiber tissues were placed on 39 per cent Potato Dextrose Agar (PDA) medium. The plates were incubated for five days in Biochemical Oxygen Demand (BOD) incubator for the development of pathogen (*H. maydis*) colonies. The colonization of *H. maydis* was confirmed based on morphological and fruiting body characteristics (Samra *et al.*, 1963). The pathogen mycelia was transferred to 24 per cent Potato Dextrose Broth (PDB) aseptically and incubated for 15 days in BOD for the growth of the pathogen. PDB containing mycelia mat of pathogen was grounded and the spore suspension was used for inoculating the healthy maize inbred lines at the second inter-node from the base using specialized injectors developed at MIL.

Phenotyping disease symptoms: Symptoms typical to late wilt disease were noticed in maize inbreds in about 20-25 days after inoculation. The stalks of the inbred lines were split open 30 days after inoculation for disease phenotyping. Disease severity and intensity was recorded using 1-9 rating scale (Payak and Sharma, 1983) based on inter-node discoloration at the point of inoculation and its movement to next inter-nodes (Shekar *et al.*, 2012). The inbred lines with a score of <3 were considered resistant; >3 to 6 as moderately susceptible and >6 to 7 as susceptible and >7 as highly susceptible.

Out of 155 maize inbred lines screened for reaction to late wilt of maize, 9 lines were found resistant (<3 score); 108 lines moderately susceptible (5 to 7 score), 24 susceptible (>6 to 7 score) and 14 highly susceptible (>7). Reports on similar work have revealed variable response of the different maize varieties, inbred lines and hybrids to infection by *H. maydis* causing late wilt (Mohamed *et al.*, 1966). Shekar *et al.*, (2010) identified a few inbred lines and populations resistant to late wilt based on screening at Hyderabad, a natural hotspot for PFSR complex.

The identified sources of resistance can be used in developing late wilt resistant open pollinated varieties and inbred lines and / or to improve the resistance level of parents of proven hybrids. Two each of highly resistant and susceptible inbred lines are being used in developing F_{2,3} populations to map genomic regions conferring resistance / tolerance to late wilt disease of maize using single nucleotide polymorphism (SNP) markers. The linked SNP markers can be used in marker-assisted breeding maize for enhanced levels of late wilt resistance with least linkage drag after their validation.

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