

Investigations into the symptoms and pathogenicity of late wilt disease caused by *Magnaporthiopsis maydis*

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Abstract

Late wilt disease of maize, caused by *Magnaporthiopsis maydis*, is characterized by progressive wilting and vascular discoloration. The present study documented disease symptoms and confirmed the pathogenicity of the associated pathogen. Naturally infected plants showed initial yellowing and drying of lower leaves at flowering, followed by severe wilting, vascular and pith discoloration, and reddish-brown to yellowish streaks in the lower internodes. Pathogenicity was evaluated on 30-day-old susceptible maize hybrid CP828 using toothpick and syringe inoculation methods under greenhouse conditions. Initial symptoms appeared 15–20 days after inoculation, followed by progressive leaf drying, wilting and vascular browning, with complete plant death at 35–45 days. No symptoms developed in uninoculated controls. The pathogen was successfully re-isolated from symptomatic vascular tissues, and the re-isolates showed characteristics comparable to the original isolate. Reproduction of characteristic symptoms and successful re-isolation confirmed the pathogenicity of *M. maydis* and fulfilled Koch's postulates.

Keywords: Maize, *Magnaporthiopsis maydis*, symptomatology

Introduction

Maize (*Zea mays* L.) is an important cereal crop cultivated worldwide because of its high productivity, wide agro-ecological adaptability and diverse uses as food, feed and industrial raw material. As a C₄ crop, its high photosynthetic efficiency contributes to substantial biomass production and yield potential. Maize grain is rich in carbohydrates, proteins and oil and is widely used for human consumption, livestock feed and industrial products such as starch, syrup, dextrose and oil (Aldrich *et al.*, 1975^[1]; Downswell *et al.*, 1996). In India, maize is cultivated under diverse agro-climatic conditions during *kharif*, *rabi* and summer seasons. During 2023-24, it occupied 108.87 lakh hectares with a production of 356.73 lakh tonnes, reflecting its importance to Indian agriculture and the food, feed and industrial sectors (Anon., 2024).

Despite its adaptability and economic importance, maize production is constrained by several biotic stresses, among which diseases are important yield-limiting factors. More than 112 diseases have been reported on maize worldwide, with approximately 35 diseases occurring in India (Kumar *et al.*, 2013)^[7]. Fungal diseases, in particular, constitute a major constraint to maize production and include downy mildews, leaf blights, stalk rots, ear rots, rusts and wilt diseases. The incidence and severity of these diseases vary depending on the maize genotype, pathogen population and prevailing environmental conditions (Stukenbrock and Gurr, 2023)^[11].

Among the important fungal diseases of maize, late wilt disease (LWD) is caused by the soil- and seed-associated pathogen *Magnaporthiopsis maydis* (Samra *et al.*, 1962)^[10], formerly known as *Cephalosporium maydis* and *Harpophora maydis*. The disease causes wilting and plant decline during the later stages of crop development and was first reported from Egypt in the 1960s. It has subsequently been reported from several maize-growing regions,

including India, Hungary, Romania, Spain, Portugal, Israel and Nepal (Payak *et al.*, 1970; Pecs and Nemeth, 1998; Bergstrom *et al.*, 2008; Drori *et al.*, 2013; Degani *et al.*, 2021)^[3, 4, 6, 8, 9]. The pathogen can survive in soil, crop residues and seed and may colonize resistant maize plants and alternative hosts without producing conspicuous symptoms, indicating possible symptomless or latent associations (Degani *et al.*, 2021)^[4]. These characteristics emphasize the importance of accurate pathogen identification and pathogenicity confirmation for understanding and managing LWD.

Material and methods

1. Symptomatology

Maize plants showing symptoms suspected to be associated with late wilt disease were surveyed and examined at different stages of crop development. Symptomatic plants were selected based on visible disease characteristics, and the progression of symptoms was recorded from the initial appearance to advanced stages of disease development. Observations were made on the leaves, stem, vascular tissues and roots, with particular attention to leaf yellowing, wilting, drying, premature senescence and plant death. The affected plants were photographed, and representative symptomatic plant parts were collected in clean, labelled polyethylene bags and transported to the laboratory for further examination and isolation of the associated pathogen. The symptoms observed in the field were compared with previously described symptoms of late wilt disease caused by *Magnaporthiopsis maydis*.

2. Pathogenicity test

The pathogenicity of the fungal pathogen was confirmed by applying Koch's postulates. A tooth pick method was used for this purpose. Seeds of the highly susceptible maize hybrid CP828 were sown in pot and thirty-day-old plants

were used for inoculation. An oblique hole was made in the second or third internode of the stalk above ground level using a tooth pick and a syringe containing the culture suspension of *M. maydis* isolate was inserted into the hole to inoculate the plant, ensuring adequate moisture for infection. The inoculated plants were observed regularly and symptom development was recorded 30 days after inoculation. After symptom expression, the pathogen was re-isolated from infected stalk tissues and the resulting cultures were compared with the original isolates to confirm pathogenicity. The morphological characteristics of the re-isolated fungus were examined under an Olympus microscope and compared with the original culture for confirmation.

Results and Discussion

1. Disease Symptomatology

Late wilt symptoms were first observed during the flowering stage of the maize crop. Initially, the lower leaves exhibited rapid drying, followed by gradual loss of green colour, dehydration and drying. The affected plants showed impaired water movement resulting in severe wilting symptoms. Internal examination of infected stalks revealed distinct vascular discoloration, with the vascular bundles changing from white or pale yellow to dark yellow and

finally, brown. Diagonal transverse sections of diseased stalks showed characteristic discoloration of the pith region, while longitudinally split stalks exhibited reddish-brown to yellowish streaks in the lower internodes (Plate 1).

The lower stem tissues became dry, shrivelled and hollow, particularly at the internodal regions, indicating extensive colonization of the vascular tissues by the pathogen. In advanced stages of infection, the stalks lost their mechanical strength, became weak and brittle. Severely infected plants produced fewer and smaller cobs with poor ear development and the kernels were shrivelled, immature and poorly filled due to restricted translocation of water and nutrients. These characteristic external and internal symptoms were consistently observed in naturally infected maize plants affected by late wilt disease during the survey (Plate 1).

The symptoms observed during the present investigation closely agreed with those reported by Degani *et al.* (2021)^[4] for late wilt disease of maize. The infected stalks exhibited progressive vascular discoloration from white or pale yellow to dark brown, accompanied by pith discoloration and characteristic reddish-brown to yellowish streaks in the lower internodes which was in accordance with the classical description of late wilt symptoms reported by Samra *et al.* (1962)^[10].



Plate 1: Development of yellow to reddish-brown streaks on leaves and drying of stem caused by *M. maydis*

2. Pathogenicity test

The pathogenicity of the isolated fungus was confirmed by artificial inoculation of healthy maize plants under greenhouse conditions. Thirty-day-old maize plants grown in pots were inoculated with actively growing cultures of *Magnaporthiopsis maydis* using the tooth pick and syringe inoculation methods, while uninoculated plants served as controls. The inoculated plants were maintained under greenhouse conditions and observed regularly for symptom development.

The initial symptoms appeared 15-20 days after inoculation as slight yellowing and rolling of the lower leaves, followed by gradual wilting. As the disease progressed, the leaves lost their turgidity, dried from the lower to the upper portion of the plant and eventually became straw coloured. Longitudinal splitting of the basal stalk revealed characteristic brown discoloration of the vascular tissues, indicating colonization of the xylem by the pathogen. Complete wilting and death of the inoculated plants were observed 35-45 days after inoculation, closely resembling the symptoms observed under natural field conditions during the survey. No disease symptoms were observed in the uninoculated control plants, which remained healthy

throughout the experimental period (Plate 3).

The pathogen was successfully re-isolated from the discoloured vascular tissues of the artificially inoculated plants on potato dextrose agar (PDA). The re-isolated fungus exhibited cultural and morphological characteristics identical to those of the original isolate. The successful reproduction of typical late wilt symptoms followed by re-isolation of the pathogen fulfilled Koch's postulates and confirmed *Magnaporthiopsis maydis* as the causal agent of late wilt disease of maize.

The present findings are in accordance with those of Samra *et al.* (1962)^[10], who first established the pathogenicity of *Cephalosporium maydis* (currently *Magnaporthiopsis maydis*) by inoculating healthy maize plants with isolates obtained from diseased plants. The inoculated plants developed typical late wilt symptoms and the pathogen was successfully re-isolated from infected tissues, thereby fulfilling Koch's postulates and confirming *C. maydis* as the causal organism of late wilt disease in maize. The successful confirmation of pathogenicity in the present study further validates the identity of the isolated pathogen and provides a reliable foundation for subsequent studies on its morphological, cultural and molecular characterization.



Plate 2: Symptoms of late wilt of maize on different parts of the plant

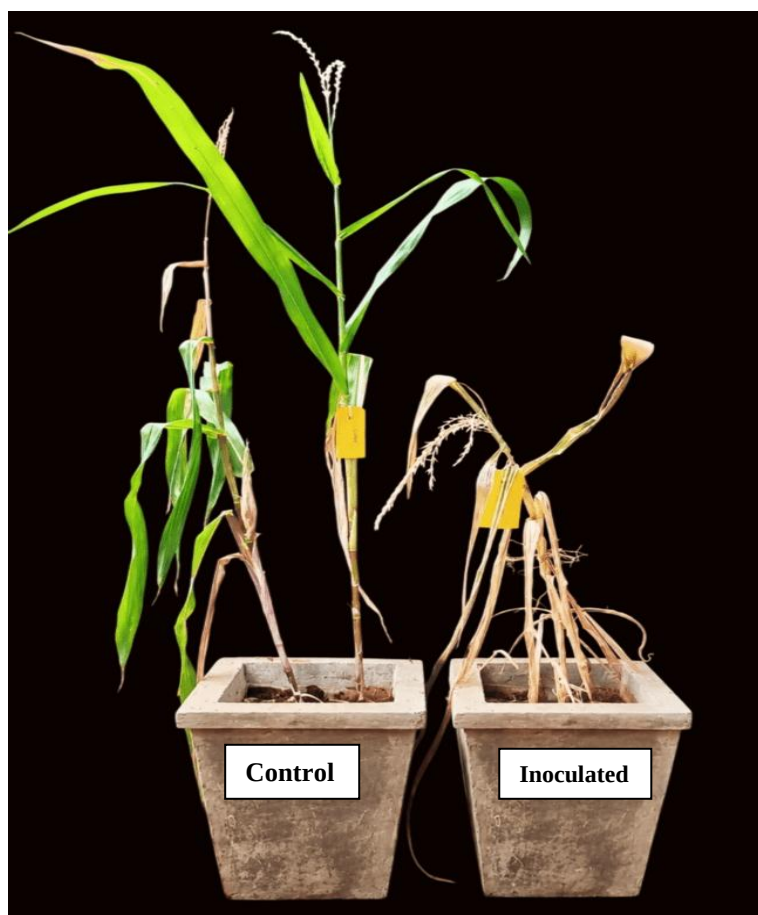


Plate 3: Pathogenicity against late wilt disease under greenhouse condition

Conclusion

The present study established the characteristic symptomatology and pathogenicity of *Magnaportheopsis maydis* associated with late wilt disease of maize. Natural infection was characterized by progressive drying and wilting of the lower leaves, vascular and pith discoloration, and reddish-brown to yellowish streaks in the lower internodes. Artificial inoculation reproduced comparable disease symptoms in susceptible maize plants, while uninoculated plants remained symptomless. The successful re-isolation of the pathogen from

artificially infected plants, with characteristics comparable to the original isolate, fulfilled Koch's postulates and confirmed *M. maydis* as the causal agent of late wilt disease. These findings provide a basis for further studies on the identification, epidemiology and management of late wilt disease in maize.

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