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First report of *Lagenaria siceraria* charcoal rot caused by *Macrophomina phaseolina* in México

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Keywords: Bottle gourd, etiology, soilborne pathogen, root rot

In February 2025, symptoms of charcoal root were detected in approximately 40% of four-month-old *Lagenaria siceraria* plants cultivated in a 2-ha commercial field located in Culiacán, Sinaloa, Mexico (24°15'06.7"N 107°11'39.3"W). Diseased plants additionally above ground: stunting, wilting, leaves yellowing and partial foliage necrosis and defoliation, and below ground: crown

and root rot. Seventy small tissue fragments (10 × 10 mm) were excised from ten randomly selected diseased plants and immediately surface-disinfected in 1% sodium hypochlorite solution, followed by two rinses in sterile distilled water, each lasting one minute. After drying on sterile absorbent paper, the tissue samples were incubated at 28 °C for seven days in Petri dishes containing Potato Dextrose Agar (PDA) supplemented with streptomycin sulfate (0.3 g L^{-1}), with five tissue fragments placed per dish. Microscopic examination revealed numerous microsclerotia of irregular shape, initially hyaline and progressively darkening with maturation. The microsclerotia measured approximately 40 µm long. These morphological features matched the description of *Macrophomina phaseolina* (Marquez et al. 2021), the causal agent of charcoal rot. Three representative isolates (MACROCH7CLNSIN, MACROCH10CULSIN and MACROCH13CULSIN) of the thirteen isolates generated were selected for molecular identification. PCR was performed using the primer sets ITS1/ITS4 and EF1-728F/EF1-986R to amplify the ITS region (White et al. 1990) and part of EF1-α gene (Carbone and Kohn 1999), respectively. The sequences were deposited in GenBank with the accession numbers PV628711, PV628712 and PV628713 for ITS, and PV651775, PV651776 and PV651777 for EF-1α, respectively. The sequences obtained from ITS and EF-1α were 99% and 100% similar to *M. phaseolina* accessions no. KJ609175 and MK447854, respectively. Phylogenetic analyses performed using the Neighbor-Joining methods with EF-1α sequences by employing a bootstrap of 1000 replications on MEGA 11 generated a well-supported clade grouped with the species *M. phaseolina*. To verify the pathogenicity of the fungus, 10 days old *L. siceraria* plants were inoculated using the toothpick method. Sterile toothpicks were previously placed in Petri dishes containing each of the three *M. phaseolina* colonies until full colonization was achieved. Subsequently, small wounds were made on the roots of ten plants per isolate using the colonized

toothpicks. For the control treatment, sterile (non-colonized) toothpicks were used. The pathogenicity assays were conducted twice. All plants were maintained in a growing chamber with 12 h photoperiod at 70 ± 5% HR and 28 ± 2 °C. After 2 weeks, inoculated plants developed symptoms similar to those observed on the commercial fields. No symptoms were observed in the control plants. *Macrophomina phaseolina* was reisolated from symptomatic root tissues, fulfilling Koch's postulates. To date, *M. phaseolina* has been reported infecting *L. siceraria* in India (Richardson 1990) and South Korea (Kim et al. 2021). To the best of our knowledge, this is the first report of *M. phaseolina* causing disease in *L. siceraria* in Mexico and the Americas. This finding provides a foundation for future studies on charcoal rot in this host and supports the development of improved management strategies for the disease.

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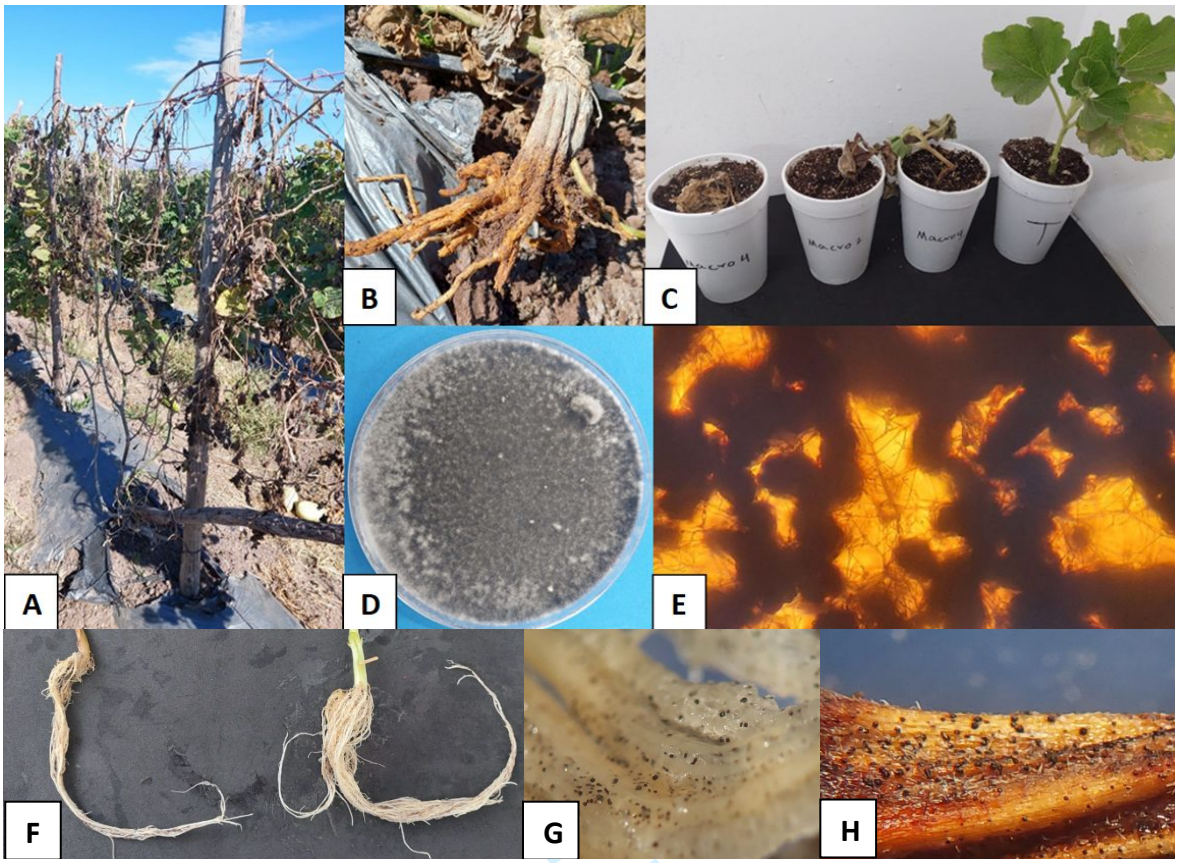
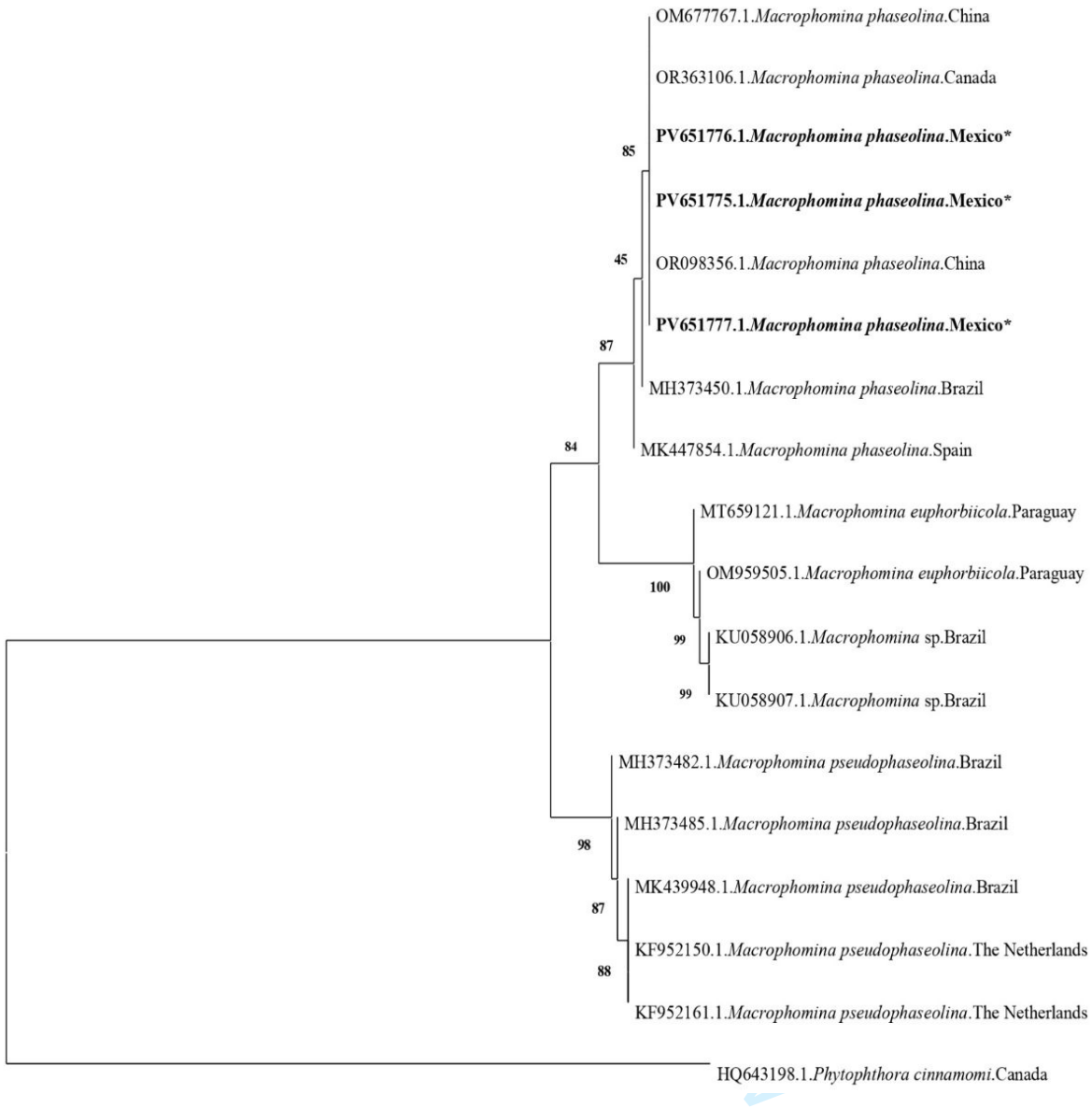


Symptoms and morphological traits of *Lagenaria siceraria* charcoal rot associated with *Macrophomina phaseolina*. A = Diseased plants in the commercial field. B = Root rot symptoms. C = *Lagenaria siceraria* plants without and with *M. phaseolina* inoculation. D = Top view of *M. phaseolina* on PDA medium. E = Dark *M. phaseolina* microsclerotia. F = Diseased (left) and healthy (right) *L. siceraria* roots. G = Microsclerotia on roots of inoculated plants. H = Microsclerotia on stems of inoculated plants.



Phylogenetic dendrogram constructed using the Neighbor-Joining method based on the alignment of partial EF-1α nucleotide sequences of *Macrophomina phaseolina* isolates: MACROCH7CLNSIN (PV628711), MACROCH10CULSIN (PV628712), and MACROCH13CULSIN. Values at the nodes represent the percentage bootstrap scores (1000 replicates). *Phytophthora cinnamomi* was used as outgroup.