Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Macrophomina phaseolina* Causing Charcoal Rot of Peanut (*Arachis hypogaea*) in Mexico

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Peanut (*Arachis hypogaea* L.) is the third most important oilseed crop in the world. The cultivated area in Mexico is currently 52,046 ha with a production of 91,109 t in 2018 (FAO 2020). Puebla state ranks third in the national production with 9,313 t (SIAP 2020). In September 2019, typical symptoms of charcoal rot (*Macrophomina phaseolina* [Tassi] Goid.) were observed in about 50% of cultivar Champs peanuts, affecting 1.5 ha located in Chietla (18° 27' 39" N; 98° 37' 11" W), Puebla, Mexico. Diseased plants showed brown discoloration in stem and root rot, with chlorotic foliage, dark microsclerotia on the stem, and premature dying. To isolate the causal agent of these symptoms, 20 infected plants were recovered and processed in the laboratory. Ten pieces of stem and root tissue were selected from each plant, cut into small pieces 5 mm in length, and superficially disinfested with 1% sodium hypochlorite for 3 min followed by three rinses with sterile distilled water. Later, they were dried on sterile paper and placed on Petri plates containing potato dextrose agar (PDA) medium, which were kept at 28°C for 7 days (12-h light and 12-h dark). Four colonies were purified via hyphal-tip culture, and fungus was consistently isolated from the analyzed tissues; additional microcultures were prepared to observe phenotypic characteristics. Colonies showed dense growth, with a gray initial mycelium, becoming black after 7 days. Microsclerotia with spherical to oblong in shape were observed after 5 days on PDA, with a black coloration, measuring an average of 74 µm width × 110 µm length ($n = 40$). Phylogenetic analysis was conducted by amplification and sequencing of the internal transcribed spacer (ITS)

region with the ITS5 and ITS4 primers (White et al. 1990). The obtained sequences were deposited in GenBank database under accession numbers MW585378, MW585379, MW585380, and MW585381 containing approximately 601 bp of the ITS1-5.8S-ITS2 region (complete sequence); they were 99% identical to the reference sequence of *M. phaseolina* (GenBank accession KF951698) on *Phaseolus vulgaris* from Mexico. Based on the symptoms in the field, colony morphology, color and shape of the microsclerotia, and molecular identification, the fungus was identified as *M. phaseolina*. The pathogenicity test was performed on peanut plants of cultivar Champs grown on plastic pots with an autoclaved peat/soil mixture under greenhouse conditions (70% relative humidity and 28°C). Fifty 2-month-old peanut plants were inoculated using the toothpick method. The toothpicks were previously sterilized and then placed in Petri plates with each of the four colonies of *M. phaseolina* until colonization. Small wounds were made with those toothpicks in the roots, and a sterile toothpick was used in the control plants; the assays were performed twice. After 3 weeks, the inoculated plants exhibited symptoms of wilting chlorosis on the leaves and brown to dark brown discoloration of the vascular ring, whereas control plants remained healthy. *M. phaseolina* was reisolated from symptomatic root tissues and identified by phylogenetic approach, fulfilling Koch's postulates. To date, this fungus affects at least 372 hosts globally, causing yield losses. In Mexico this fungus has been documented in *Glycine max*, *Ipomoea batatas*, *P. vulgaris*, *Physalis ixocarpa*, *Saccharum officinarum*, *Sesamum indicum*, *Solanum melongena*, *S. tuberosum*, and *Sorghum bicolor* (Farr and Rossman 2021). However, there are no reports of *M. phaseolina* as a potential pathogen on peanut; therefore, according to our knowledge, this is the first report of this fungus affecting *A. hypogaea* in Mexico.

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