Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium verticillioides* Causing Safflower Root Rot in Sinaloa, Mexico

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Safflower cultivation is of great socioeconomic importance worldwide. Its production is intended for the extraction of oil from the seeds. In 2021, Mexico ranked fifth in the world in safflower production with approximately 52,553.28 metric tons (SIAP 2021). In April 2022, in the north-central zone of Sinaloa, Mexico, diseased plants were reported in fields planted with safflower. Symptoms included chlorotic plants, necrosis and rot in vascular bundles, dwarfed plants, and reflexed plants bent toward the ground. The disease caused estimated losses of 15% of seed production, with respect to the production obtained from the previous year in the safflower fields surveyed. Twenty-five plants with symptoms were sampled to isolate the pathogen. Plants were cut at the base of the stem near the roots and roots cut into 5-mm² pieces. Tissue samples were superficially disinfected by immersing in 70% alcohol for 10 s, 2% sodium hypochlorite for 1 min, washed in sterile water, and placed on potato dextrose agar (PDA) at 28°C for 7 days in the dark. Twelve monosporic isolates derived from the PDA culture were morphologically characterized. Abundant white aerial mycelium and small pink to dark violet pigments in the center of the culture were observed. On 10-day-old cultures grown on carnation leaf agar medium, microconidia and macroconidia were produced. Microconidia were hyaline, had zero to two septa, were oval or ellipsoidal, and measured 4.6 to 14×1.8 to 4.2 μ m (n = 40). Macroconidia were hyaline, were slightly curved with three to five septa, and measured 26 to 69×3 to 6.1 µm (n = 40). No chlamydospores were observed. According to the morphological characteristics, the isolates were identified as Fusarium verticillioides (Leslie and Summerell 2006). DNA was extracted from one isolate, and the translation elongation factor

 $1-\alpha$ (EF1) gene was amplified and sequenced (O'Donnell et al. 2010). The sequence obtained from the isolate FV3CARCULSIN with 645 base pairs was submitted to NCBI GenBank with accession number OQ262963. The BLAST search revealed 100% similarity with the F. verticillioides isolate 13 (KM598773) (Lizárraga-Sánchez et al. 2015). Identification in FUSARIUM ID resulted in a 99.85% similarity with the F. verticillioides isolate CBS 131389 (MN534047) (Yilmaz et al. 2021). A phylogenetic tree, made with sequences of the EF1 gene, revealed that FV3CARCULSIN was most closely related to F. verticillioides (100% bootstrap). Pathogenicity tests were carried out on safflower plants (cv. Oleico) grown in sterile vermiculite. The plants were inoculated with a conidial suspension $(1 \times 10^5 \text{ conidia/ml})$ obtained from FV3CARCULSIN grown on PDA for 7 days. A total of 45 plants were inoculated by drenching the roots with 20 ml of inoculum when the plants were 20 days old. Fifteen plants served as negative controls without inoculation. The plants were kept for 60 days in greenhouse conditions; however, after 45 days, the plants began to die. The assay was conducted twice. Rotting and necrosis were observed in the roots of the plants. The pathogen was reisolated from the tissue of all the plants with symptoms and identified as F. verticillioides using morphological characteristics and EF1 sequences, fulfilling Koch's postulates. No symptoms were observed in the control plants after 60 days. This is the first report of root rot in safflower caused by F. verticillioides in Mexico. The fungus has been reported in maize (Figueroa et al. 2010), but it is unknown whether it could be the same pathogen of safflower. Identification of the pathogen is important for implementing management methods to reduce yield losses and for additional studies on the impact of the disease on oil quality extracted from safflower seeds.

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