Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium thapsinum* Causing Maize Stalk Rot in China

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Maize (*Zea mays* L.) is the most widely grown crop in China; 41.28 million hectares was planted in 2019 (<https://data.stats.gov.cn/>). Several fungal diseases of maize are reported, of which stalk rot has become one of the most destructive diseases in China. The average yield losses affected by the disease are estimated at 10 to 20% (Yu et al. 2017). From 2017 to 2019, a survey was conducted to determine the population diversity of *Fusarium* species associated with maize diseases in 18 cities across Henan province. *Fusarium* stalk rot of maize with disease incidence more than 25% was observed in two continuous maize fields at Xuchang city. The diseased stem tissues from junctions in health and disease were chopped into small pieces (3 × 8 mm), superficially disinfected (70% ethyl alcohol for 1 min), placed onto potato dextrose agar (PDA) amended with L-(+)-lactic-acid (1 g/liter), poured in Petri plates, and incubated at 25°C for 4 days. Mycelia showing morphological characteristic of *Fusarium* spp. were subcultured from a single conidium. The pure fungal isolates produced fluffy colonies, white aerial mycelium with yellow pigment in agar. The radial mycelial growth was measured and calculated at an average growth rate 10.9 mm/day at 25°C. Macroconidia produced on carnation leaf agar (CLA) were relatively slender, slightly curved, and thick-walled, mostly three to five marked septa, with a curved and

tapering apical cell and poorly developed foot cell, $46.9 \pm 5.6 \times 4.9 \pm 0.2$ μ m. Microconidia formed abundantly and were generally oval on CLA, $8.2 \pm 0.5 \times 3.4 \pm 0.1$ μ m. No chlamydospores were observed. Morphological characteristics of the isolates matched the description of *Fusarium thapsinum* (Leslie and Summerell 2006). To further get the phylogenetic evidence, translation elongation factor (*EF1- α*), the largest subunit of RNA polymerase II (*RPB1*), and the second largest subunit of RNA polymerase II (*RPB2*) were amplified with primer pairs EF1/EF2 (O'Donnell et al. 1998), thapR1F (5'-TTTTCCTCACAAAGGAGCAAATCATG-3')/thapR1R (5'-GTTACCC AAGATATGGTCGAAAGCC-3'), and thapR2F (5'-ACTCTTTACATTTG CGCCGAAC-3')/thapR2R (5'-CGGAGCTTTCGTCCAGTGTGAC-3'), respectively, and sequenced. The BLAST search of the sequences of *EF1- α* , *RPB1*, and *RPB2* shared 99.87 to 100% identity with those of *F. thapsinum* strains deposited in GenBank. Sequences from two different isolates were deposited in GenBank (accession nos. MT550014, MT997082 for *EF1- α* ; MT550011, MT997087 for *RPB1*; and MT550008, MT997091 for *RPB2*). The phylogenetic relationships based on analysis of the partial sequences showed the representative isolates clustered together with *F. thapsinum* at 96% bootstrap values. Combined with the results of morphological characteristics and phylogenetic analysis, the strain designated as *F. thapsinum*. To complete Koch's postulates, the pathogenicity of the isolates was tested using silking-stage plants in a greenhouse based on a previously described method with modification (Zhang et al. 2016). An 8-mm-diameter wound hole was created at the second or third internode of the plant above the soil surface and injected with 0.5 ml of mycelia plug. The inoculated stalk exhibited internal dark brown necrotic regions, and the brown area elongated obviously around the insertion at 14 days postinoculation (dpi). At 30 dpi, the stalks turned soft, hollow, and even showed lodging of the plants for those severe ones, which are similar to those observed on naturally infected maize plants in the field. When the roots of the three-leaf-stage seedlings were inoculated with 1×10^6 macroconidia solution (Ye et al. 2013), root rot and leaf wilting symptoms were observed, whereas control plants that were inoculated with only sterile water showed no disease symptoms. The pathogen was reisolated from the inoculated tissues, and the identity was confirmed by the morphological characters. *F. thapsinum* had been described as causal agent of maize stalk rot in Pakistan (Tahir et al. 2018). To our knowledge, this is the first report of *F. thapsinum* associated with maize stalk rot in China. The discovery will strengthen the theoretical foundation of maize stalk rot disease management.

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