Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Soybean Stem Blight Caused by *Diaporthe phaseolorum* in Sichuan Province, China

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In September 2020, a disease resembling stem blight on soybean was found in Chengdu, Sichuan Province, southwestern China. Symptoms began as a brown lesion on the stems, usually at the nodes, then lesions expanded, darkened, and even girdled the stems, causing wilt of the above stems. In three 0.33-ha fields, a total of 300 soybean plants (20 plants/site × 5 sites/field × 3 fields) were investigated, and 3% of the plants showed the disease symptoms. The symptoms were consistent with those previously reported for stem canker and stem blight on soybean caused by *Diaporthe* complex (Cui et al. 2009; Mena et al. 2020; Santos et al. 2011). The tissues of symptomatic soybean stems were rinsed by water, disinfected by submerging them in 75% ethanol for 30 s and in 2% sodium hypochlorite solution for 2 min, then followed by washing with sterile distilled water. Small diseased tissue fragments were placed on selective potato dextrose agar (PDA) containing rifampicin and ampicillin (both 5×10^4 µg/µl). Plates were sealed and incubated at 26°C for 2 days. Developing mycelia of isolates were transferred to fresh PDA and purified by single hyphal tip. For the five obtained isolates, five markers, including the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, parts of the translation elongation factor 1-α (*TEF1*), part of the histone H3 (*HIS*) gene, the calmodulin gene (*CAL*), and the beta-tubulin gene (*TUB*), were amplified using the established primers ITS4/ITS5 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), CYLH3F (Crous et al. 2006) and H3-1b (Glass and Donaldson 1995), CAL228F/

CAL737R (Carbone and Kohn 1999), and Bt-2a/Bt-2b (Glass and Donaldson 1995), respectively, and sequenced (GenBank IDs: MW595761–MW595780 and MW624472–MW624476). Phylogenetic trees were constructed based on concatenated sequences of the five markers using the maximum-likelihood (ML) method in MEGA-5.2.2. Based on the results of morphological and phylogenetic analysis, the five isolates were identified as *Diaporthe phaseolorum*. Pathogenicity tests for the isolates were conducted on 7-day-old soybean seedlings (cv. Shangdou 1310) using a hypocotyl inoculation method. A thin slit (6 mm) was made with a sterile scalpel along the surface of the hypocotyl 2 cm below the cotyledonary node, then an agar plug bearing mycelia (3 mm × 3 mm) was placed into the slit. An agar plug without mycelia was used as a noninoculated control. Ten plants were assayed for each treatment, and inoculated plants were placed in a greenhouse at 26°C, with 14 h of light and 10 h of darkness, with humidity higher than 90% for the first 2 days. The assay was repeated at least three times. Typical brown lesions on the stems were observed 4 days after inoculation, and 20% of treated plants died. *D. phaseolorum* was reisolated from these stem lesions. No disease symptom was observed on control plants. Thus, *D. phaseolorum* was the pathogen causing the soybean stem blight in field. To our knowledge, this is the first report of *D. phaseolorum* causing this disease in Sichuan Province, China. The result may provide useful information for soybean disease control in this region of China.

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