Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Botryosphaeria dothidea* Causing Stem Canker on Soybean in China

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In September 2020, widespread stem canker on soybean (*Glycine max*) was detected in southeastern Jiangsu (Nantong municipality; 120.76° E, 32.23° N) in China. Mature plants, 14 weeks of cultivation, exhibited brown necrotic lesions and a dried-up stem. The symptoms were observed in 11 soybean fields, 1.6 ha in total, and approximately 80% of the plants were symptomatic. The symptoms were consistent with those previously reported for stem canker on soybean caused by *Diaporthe aspalathi*, *D. caulivora*, and *D. sojae* (Ghimire et al. 2019; Mena et al. 2020). Small pieces, approximately 0.4 cm² in size, of symptomatic tissue were surface sterilized in 1.5% NaOCl for 1 min and washed twice with sterile ddH₂O. The pathogen was isolated and cultured on potato dextrose agar (PDA), containing chloramphenicol (50 µg/ml), under darkness at 28°C for 7 days. Amplification of internal transcribed spacer (*ITS*), elongation factor 1-α (*EF1-α*), and β-tubulin (*TUB2*) genes was performed using ITS1/ITS4, EF1-728F/EF1-986R, and Bt2a/Bt2b primers, respectively (Jia et al. 2019). Sequences were submitted to GenBank under accession numbers MW130133 (*ITS*), MW147481 (*EF1-α*), and MW147482 (*TUB2*). BLAST search revealed that the amplified sequences had 99.65% (*ITS*; *Botryosphaeria dothidea* JZB310202, MN945381), 100% (*EF1-α*; *B. dothidea* ZB-77, MH726166), and 99.75% (*TUB2*; *B. dothidea* ZB-1, MN642587) matches to multiple *B. dothidea* strains, whereas all reported *Diaporthe* strains showed no nucleotide identity to the amplified sequences. A molecular phylogenetic tree was constructed using MEGA7 to confirm the identity of the pathogen. *ITS*, *EF1-α*, and *TUB2* sequences were blasted separately in Musclev (https://www.ebi.ac.uk/Tools/msa/musclev/) and then

combined together to make the phylogenetic tree. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura three-parameter model, and the tree with the highest likelihood (−4,291.3981) is shown in the supplementary material. *Diaporthe* strains found causing stem canker on soybean, some *Phytophthora sojae* strains (which also cause a dried-up stem on soybean) (Yang et al. 2019), and *B. dothidea* strains found in China in other hosts were included in the phylogenetic tree. To confirm pathogenicity, a sterilized spatula was used to make wounds (3 mm diameter, 1 mm depth) on the stem of 2-week-old soybean plants. Mycelial plugs from a 7-day-old culture on PDA were placed on the wounds and covered with Parafilm. Sterilized PDA plugs were used as a control. Inoculated plants were maintained in a growth chamber at 28°C and 60% relative humidity. Typical stem canker symptoms were observed 5 days after inoculation. Microscope observations showed the presence of septate mycelium, fusiform conidia, and round conidiomata, and agreed with those previously reported for the morphology of *B. dothidea* strains (Phillips et al. 2013). During recent months, *B. dothidea* was reported to cause stem canker and leaf wilt on a number of plant species in China (Huang et al. 2021; Ju et al. 2021; Wang et al. 2020a, 2020b, 2021), confirming the expansion and host promiscuity of this pathogen. Stem canker on soybean has been thoroughly associated to *Diaporthe* strains; however, this is the first report of *B. dothidea* causing this disease. We believe that our results will help to better understand the pathogens affecting soybean production in China.

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