Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum brevisporum* Causing Soybean Anthracnose in China

X.-C. Shi, S.-Y. Wang, X.-C. Duan, X. Gao, X.-Y. Zhu, and P. Laborda[†]

School of Life Sciences, Nantong University, Nantong 226019, People's Republic of China

X.-C. Shi and S.-Y. Wang contributed equally to this work.

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In March 2020, widespread anthracnose was observed on soybean (*Glycine max*) in southeastern Jiangsu (Nantong municipality; 120.53° E, 31.58° N) in China. Plants exhibited irregular brown necrotic lesions in the stem and leaves, along with pronounced wilting. The symptoms were detected in one soybean field, 0.42 ha, surrounded by healthy wheat fields. Approximately 65% of the soybean plants showed the disease symptoms, and crop yield was reduced by 28 to 35% with respect the yield achieved in previous years, when no symptoms were observed. The symptoms were consistent with those previously reported for anthracnose on soybean caused by *Colletotrichum chlorophyti*, *C. cliviae*, and *C. gloeosporioides* (Barbieri et al. 2017; Mahmodi et al. 2013; Yang et al. 2012). Diseased 3-week-old plants were collected. Small pieces, approximately 1 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) (Song et al. 2020), containing chloramphenicol (50 µg/ml), under darkness at 28 °C for 3 days. Sequencing of internal transcribed spacer (*ITS*), actin (*ACT*), β-tubulin (*TUB2*), and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) genes was performed as reported by Yang et al. (2015). Sequences were submitted to GenBank under accession numbers MT361074 (*ITS*) and MT415548 to MT415550 (*ACT*, *TUB2*, and *GAPDH*). BLAST search revealed that the amplified sequences had 100% (*ITS*; *Colletotrichum brevisporum* TCHD, MH883805), 97.66% (*ACT*; *C. brevisporum* S38, KY986905), 99.06% (*TUB2*; *C. brevisporum* PF-2, KY705061), and 100% (*GAPDH*; *C. brevisporum* LJTJ27, KP823797) matches to multiple *C. brevisporum*

strains, whereas all reported *C. chlorophyti*, *C. cliviae*, and *C. gloeosporioides* strains showed no similarity to at least two of the studied genes. A molecular phylogenetic tree constructed using MEGA7 confirmed the identity of the pathogen. *ACT* and *ITS* sequences were blasted separately in Muscle (<https://www.ebi.ac.uk/Tools/msa/muscle/>) 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 log likelihood (−1,749,2186) is shown in the supplementary materials. The *Colletotrichum* strains previously found causing anthracnose on soybean, and other relevant strains used in taxonomic analyses, were included in the phylogenetic tree. Microscope observations showed the presence of 15-µm-long cylindrical conidia and septate mycelium, and agree with those reported for the morphology of *C. brevisporum* by Damm et al. (2019). To confirm pathogenicity, the mycelia from a 2-day-old culture on PDA was collected and suspended in sterile ddH₂O (≈ 10⁶ cells/ml) to prepare the inoculum. The pathogen was spray inoculated on stem and leaves of healthy soybean plants. In control plants, sterile ddH₂O was used. Inoculated plants were maintained in growth chamber at 28°C and 50% relative humidity. Typical anthracnose symptoms were observed 20 days after inoculation. *C. brevisporum* was reported to produce anthracnose on pumpkin, papaya, mulberry, coffee, passion fruit, and pepper in China (Liu et al. 2018, 2019; Xue et al. 2019). Here, we report for the first time *C. brevisporum* causing anthracnose on soybean, an economically relevant crop in China.

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[†]Indicates the corresponding author.
E-mail: pedro@ntu.edu.cn (P. L.)