Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Target Spot Caused by *Rhizoctonia solani* AG-6 in Tobacco in China

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Funding: Funding was provided by Guizhou Science Technology Foundation (ZK[2021]Key036), China National Tobacco Corporation (110202001035 (LS-04), 110202101048(LS-08)), National Natural Science Foundation of China (award nos. 31960550 and 32160522), International Science and Technology Cooperation Base ([2020]4102), Guizhou Provincial Academician Workstation of Microbiology and Health ([2020]4004), and Guizhou Tobacco Company Project (nos. 201914, 2020XM03 and 2020XM22). Plant Dis. 106:2761, 2022; published online as <https://doi.org/10.1094/PDIS-09-21-2077-PDN>. Accepted for publication 3 March 2022.

Tobacco is an annual solanaceous crop that is widely produced in China. In July 2020, tobacco target spot was observed on 50% of tobacco plants in a 5-ha commercial field in Bijie (27.32° N, 105.29° E), Guizhou province, China. Typical symptoms first appeared on the old leaves as round, watery spots. The spots grew to a diameter of 2 to 20 cm, with concentric ring lines and dead spots. Fifteen small pieces (5 × 5 mm) of leaf tissue were cut from the edge of lesions, surface sterilized, and placed on potato dextrose agar (PDA) medium amended with kanamycin (0.1 mg/ml). Isolate J136, one of five isolates with similar morphology, was selected for pathogen identification. The culture of the isolate on PDA after incubation at 28°C in darkness for 5 days was brown and exhibited radial mycelial growth. Hyphae of the fungus were white at the beginning, turned light brown to brown at the later stages, and finally became thick and separated. Sclerotia were brown and produced on PDA after 25 days of incubation in the dark. These characteristics were similar to the colony characteristics of *Rhizoctonia solani*. The genomic DNA of isolate J136 was extracted using the CTAB method. PCR analysis was conducted using the following primers specifically designed for the detection of individual AGs or subgroups of *R. solani*: AG-1 IA, IB, and IC

(Kuninaga 2003); AG-2-1, AG-2-2, IIIB, IV, and LP (Carling et al. 2002); AG-3 PT (Misawa 2015); AG-4 HG-I and HG-II (Kuninaga 2003); and AGs-5-6 (Arakawa and Inagaki 2014). Among the 12 specific primer pairs, only AG-6-specific primers amplified a fragment of ca. 230 bp product, indicating that the tested strain belonged to *R. solani* AG-6. The sequence was deposited in GenBank with accession no. MZ379468. Using a BLASTn search, the sequence of the gene was aligned with the voucher specimen, *R. solani* AG-6. A phylogenetic tree was constructed based on these sequences. Afterwards, isolate J136 was tested for the hyphal anastomosis reaction using the *R. solani* AG-6 standard strain according to the method described by Ogoshi (1987). The hyphal diameter at the point of anastomosis was reduced, with an obvious anastomosis point, and the death of adjacent cells, indicating their anastomosis reactions (Anderson 1982). Thus, based on the morphological and genetic analyses, the fungus was identified as *R. solani* AG-6. To verify its pathogenicity, six plants (cv. Yunyan87) at the 5- to 6-leaf stage were inoculated with mycelial PDA plugs (5 mm in diameter). Leaves inoculated with PDA-only plugs served as the controls. Treated tobacco plants were maintained at a temperature of 15 to 25°C in a greenhouse with 85% relative humidity. After 5 days of inoculation, typical symptoms were observed on the inoculated leaves, whereas no symptoms were observed on the control leaves. Koch's postulates were fulfilled by reisolation of the pathogen from the diseased leaves. *R. solani* AG-3 is the major previously reported group of *R. solani* that causes tobacco target spot in the field (Gonzalez et al. 2011). Therefore, to our knowledge, this is the first report of *R. solani* AG-6 causing target spot of tobacco in the field in China. Since considerable losses caused by the disease have frequently happened in this region, the addition of this new group pathogen in the disease pool can be problematic. Proper disease control strategies need to be developed to prevent further losses.

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The author(s) declare no conflict of interest.

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Keywords: hyphal anastomosis reaction, identification, *Rhizoctonia solani* AG-6, tobacco target spot

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