Diseases Caused by Fungi and Fungus-Like Organisms

Konjac Anthracnose Is Caused by *Colletotrichum gloeosporioides* in Yunnan Province of China

Cheng Li[†]

Key Laboratory of Agricultural Microbiology, College of Agriculture, Guizhou University, Guiyang 550025, Guizhou, China

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Konjac (*Amorphophallus konjac*) is an economically important traditional crop in Fengqing County, Yunnan Province, China. Anthracnose symptoms were observed on this crop in June and July of 2020. The plants developed round, oval, or irregular leaf spots with brown edges and central taupe spots, and exhibited serious defoliation and tree weakness. Disease incidence in affected fields reached up to 35%. Anthracnose caused significant economic losses in konjac production and became a limiting factor of the konjac industry in Fengqing County. To date, no control measures of konjac anthracnose have been reported and tested in China. To determine the causal pathogen, symptomatic leaves were collected and cut into 5-mm² pieces. The leaf pieces were surface sterilized in 70% ethanol for 10 s, followed by treatment with 0.1% mercuric chloride for 3 min and three rinses in sterile distilled water. The tissue pieces were transferred onto potato dextrose agar (PDA) and incubated at 28°C. After 4 days of incubation, hyphal tips from leaf pieces were transferred to new PDA to generate pure cultures. The hyphae were initially white and then became dark green; red-orange conidial masses were observed on the mycelium plate surface at a growth rate of 13.14 mm/day. The conidia, observed under 400× magnification, were colorless, long-oval to fusiform, one-celled, and 15.4 to 18.2 × 3.3 to 5.9 μm in size. To identify the isolate, the genomic DNA of the pathogen was extracted using the CTAB method. The internal transcribed spacer (ITS) region, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, and β-tubulin (TUB) gene were PCR amplified using the ITS1/ITS4, GDF/GDR, and Bt2a/Bt2b primer pairs, respectively (Lee et al. 2020). BLASTn search of the obtained 536-bp ITS

fragment (GenBank accession no. MT785772), 229-bp GAPDH sequence (MW187543), and 717-bp TUB sequence (MW187544) revealed 99.44 to 99.63% sequence homology (100% query cover) with ITS (JQ005152, 99.44%), GAPDH (JQ005239, 99.63%), and TUB (JQ005587, 99.60%) sequences of the *Colletotrichum gloeosporioides* type strain CBS 112999, respectively. The highest homology with other *Colletotrichum* species was only 98.16%, including *C. siamense*, the causal agent of anthracnose in *A. paeoniifolius* and *A. konjac* (Prasad et al. 2017; Wu et al. 2020). To complete Koch's postulates, leaves of 3-month-old konjac plants grown in the field were sprayed with a conidial suspension (10⁶ spores/ml) of the isolate YNFQ-1 (sterile water was used as a negative control). Approximately 5 days after inoculation with YNFQ-1, symptoms similar to those in natural conditions appeared, whereas the negative control plants and fruits inoculated with sterile water had no disease. The pathogen was reisolated (strain YNFQ-1) from inoculated leaf tissues, and its identity was confirmed with both morphological and molecular (DNA sequences) tools, thus fulfilling Koch's postulates. The culture properties, morphological characteristics, and molecular identification confirmed the identity of the pathogen as *C. gloeosporioides*. There have been many reports about anthracnose of *Amorphophallus*; *C. siamense* causes anthracnose on *A. paeoniifolius* in India (Prasad et al. 2017) and *A. konjac* in Hubei, China (Wu et al. 2020), and *C. gloeosporioides* causes anthracnose of *A. muelleri* in Yunnan, China (Yang et al. 2020). To the best of our knowledge, this is the first report of *C. gloeosporioides* causing anthracnose on *A. konjac* in Fengqing County, China. The results are expected to have important implications in the diagnosis, control, and future research of anthracnose on *A. konjac*.

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The author(s) declare no conflict of interest.

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[†]Indicates the corresponding author.
C. Li; licheng20070242@126.com