Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Fruit Rot of Grapes (*Vitis vinifera*) Caused by *Cladosporium cladosporioides* in Xinjiang, China

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Xinjiang Province accounts for nearly 20% of grape (*Vitis vinifera* L., *proles orientalis*) (wine, table, and raisin combined) production, being the largest production area in China. Fruit rot is the most common disease that impacts grape quality and yield. A new disease where the ripe grape berry surfaces were coated with brownish mildew was observed, and the disease mainly occurred on whole clusters or most of the berries in the cluster. In September 2019 and 2020, 125 diseased grape clusters were collected from 10 locations in northern Xinjiang, where disease incidence was 15.3 to 27.4% [(diseased clusters/total clusters) × 100]. Symptomatic grape berries were disinfected with 1% NaClO for 2 min, followed by 70% ethanol for 30 s, and rinsed thrice in sterile distilled water. Three pieces of ~0.5-cm² diseased grape skin with partial exocarp were placed on PDA amended with streptomycin sulfate and kanamycin (50 µg/ml each). The PDA plates were incubated at 25°C under light (3,500 Lux) for 7 days. Fungal colonies emerging from the plated tissue were subcultured and single-spored three times to obtain pure cultures. From 20 strains with similar colony phenotype and gray olive hue, flocculent, felt-like surface, six isolates (Cc-Vivi-3, 7, 9, 11, 13, and 19) were chosen for further characterization after 7 days of incubation. Conidia were either single or grew in chains, with around 4 conidia/chain. Conidia were ovoid, nearly spindle, or globose with slightly smooth or irregular reticulate surface. Conidiophores were solitary, smooth, septate, erect or geniculate. These characteristics were consistent with the descriptions for *Cladosporium cladosporioides*. To confirm identification, PCR was performed on the genomic DNA using primers for internal transcribed spacer (ITS) region ITS1/ITS4, actin (ACT), and translation elongation factor (TEF) (Braun et al. 2003; Schubert et al. 2007). Amplified ITS sequences provided a 100% match to

C. cladosporioides (AY213641) in NCBI. Homology of ACT sequences to *C. cladosporioides* (HM148527 and MH047330) was 99.57 and 100%, respectively; and the homology of TEF sequences with *C. cladosporioides* (HM148258, HM148289, HM148260, and HM148266) was 97.56 to 100%. Phylogenetic analyses based on ITS, ACT, and TEF conjoint sequences from the six experimental isolates, five *C. cladosporioides* strains, and eight proximal *Cladosporium* species were analyzed. The phylogenetic tree showed that the six isolates from grapes clustered with *C. cladosporioides* strains but not other proximal *Cladosporium* species. This confirmed that all six isolates evaluated were *C. cladosporioides*. Pathogenicity tests with *C. cladosporioides* isolate Cc-Vivi-3 (ITS, MW556429; ACT, MW567144; TEF, MW567143) were carried out as follows: ripe and healthy grape clusters from cultivars Xinyu and Munag when total soluble solids were 20 to 21°Bx and 19 to 20°Bx, respectively, were detached from the vines. Five berries of three clusters of each cultivar were punctured with a sterile syringe and inoculated with 20 µl of 10⁷ conidia/ml suspension. Uninoculated, punctured berries in clusters treated with sterilized water served as controls. The experiment was repeated three times. Symptoms were recorded 15 days after incubation at 80% RH and 25°C with a 14 h light/10 h dark cycle. The olive green or blackish green mildew layer was produced on all inoculated berries. No symptoms were observed on uninoculated berries. Koch's postulates were fulfilled by reisolating *C. cladosporioides* from all symptomatic tissues and identifying them by PCR targeting the ACT gene. This is the first description of *C. cladosporioides* causing fruit rot of grape in Xinjiang, China. World-wide reports of *Cladosporium* spp. damaging crops are increasing (Briceño et al. 2008; Ding et al. 2019; Meneses et al. 2018; Robles-Yerena et al. 2019; Walker et al. 2016; Yang et al. 2021). However, relatively few methods of management including some fungicides and biocontrol agents are available (Addrah et al. 2019; Wang et al. 2018). Given the role of Xinjiang in Chinese agricultural production, that should arouse strong attention.

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