Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aspergillus flavus* Causing Fruit Rot of Grapes (*Vitis vinifera*) in Pakistan

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In August 2015 and 2016, rotted grapes (about 15% of the total) of cultivar Taiffi were observed in commercial fruit markets located in Jhelum (32°56'22.3"N, 73°43'31.4"E) and Rawalpindi (33°38'19.2"N, 73°01'45.0"E) District of Punjab Province. Infected tissues were found pale in color and water-soaked, and the spores were green, powdery on the surface of berries, and easily liberated when matured. Symptomatic tissue pieces were surface disinfected in 0.1% NaOCl for 2 min, rinsed three times with sterile distilled water, dried on filter paper for 45 s, and cultivated on potato dextrose agar (PDA) medium at ~25°C. After 1 day, mycelium on PDA was shifted to a fresh PDA plate and incubated at ~25°C. Within 3 days, yellow-green colonies were fully developed on PDA medium. A total of 16 isolates were

subjected to morphological characterization. During observation, conidia were globose, relatively thin, moderately roughened walls along with conidial head found biserial, ranged in size from 400 to 800 µm. The shape of vesicles attached to the conidiophore was examined, globose to subglobose with smooth and roughened walls. Based on morphological and growth features, isolates were identified as *Aspergillus flavus* (Klich 2002). For molecular identification, the internal transcribed spacer (ITS) region and the RNA polymerase II subunit (RPB2) and translation elongation factor-1 alpha (TEF-1α) genes of representative isolates (Asp.f 01 and Asp.f 02) were amplified using primers ITS1/ITS4, RPB2-5F/RPB2-7cR, and A-EF_F/A-EF_R, respectively (Liu et al. 1999; Perrone et al. 2011; White et al. 1990). Sequences of Asp.f 01 and Asp.f 02 were deposited in GenBank (ITS, KY348332 and MT268536; RPB2, MT318292 to MT318293; and TEF-1α, MT318315 to MT318316). BLAST analysis, for sequences of the ITS region, RPB2, and TEF-1α genes identified 99 to 100% similarity with the sequences of *A. flavus* isolates (KX253943.1 for ITS gene, MG517926.1 for RPB2 gene, and KP054375.1 for TEF-1α gene). To complete Koch's postulates, 10-µl aliquots of spore suspensions (10⁶ spores/ml) of isolates (Asp.f 01 and Asp.f 02) were pipetted onto three nonwounded and four wounded asymptomatic grape berries, cultivar Taiffi (seven berries per isolate). Sterile distilled water was applied to asymptomatic berries to serve as a negative control. Berries were incubated at 25 ± 2°C in sterile moisture chambers, and the experiment was conducted twice. Pale yellow to green mycelium with the original symptoms was observed on both wounded and nonwounded inoculated berries after 3 days, whereas no symptoms were recorded on the negative control. The pathogen was reisolated from the infected grape berries and identified as *A. flavus*, fulfilling Koch's postulates. Although aflatoxin B1 production from *A. flavus* has been reported previously on grapes in Tunisia (Fredj et al. 2009), to the best of our knowledge, this is the first report of *A. flavus* detected causing fruit rot of table grapes in Pakistan. It would be instructive to further investigate the extent of occurrence of the fungus and associated mycotoxins that are of concern to food safety and quality.

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