Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aspergillus niger* Causing Black Rot of Grapes in Pakistan

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In June 2015 and 2016, a postharvest survey of table grapes (*Vitis vinifera*) cultivar King's Ruby was carried out in five different commercial fruit markets of Rawalpindi (33°38'19.2"N, 73°01'45.0"E) district, Punjab Province. Black rot disease incidence on bunches averaged 12 to 17%. Symptoms appeared as brownish lesions with black sporulation on grape berries. Symptomatic tissue pieces were surface sterilized with 0.1% sodium hypochlorite for 30 s, rinsed three times with sterile distilled water, dried on filter paper for 45 s, and incubated on potato dextrose agar (PDA) at 25°C. After 3 days, dark brown to black mycelium were formed on PDA media. A total of 24 isolates were examined morphologically. The conidial head was observed radiate; vesicles were found spherical and covered with irregular metulae and phialides. Conidia were globose or subglobose, measured 3.14 ± 2.24 μ m in average diameter ($n = 50$), dark brown to black, with roughened cell walls.

The conidiophores were smooth-walled, hyaline, and became melanized toward the vesicle. These characteristics of the fungus were similar to those described for *Aspergillus niger* van Tiegh (de Hoog et al. 2000). For molecular identification, the internal transcribed spacer (ITS) region, beta-tubulin (-tub) gene region, and partial RNA polymerase II largest subunit (RPB2) gene of representative isolate Asp.n02 was amplified using primers ITS1/ITS4, BT2a/BT2b, and RPB2-6F/RPB2-7R, respectively (Glass and Donaldson 1995; Liu et al. 1999; White et al. 1990). Sequences were deposited in GenBank (ITS, MN658871; -tub, MT117924; and RPB2, MT318289). Based on BLAST analysis, sequences of the ITS -tub, and RPB2 showed 99 to 100% similarity of isolate Asp.n02 to *A. niger* (accession nos. MK307680.1, MN195121.1, MF078661.1 for ITS; MN567299.1, MK451029.1, MK451020.1 for -tub; and MK450788.1, MK450790.1 for RPB2). To complete Koch's postulates, 10- μ l aliquots of spore suspensions (10^6 spores/ml) of isolate Asp.n02 was pipetted onto three nonwounded and four wounded (5-mm diameter) asymptomatic grape berries of cultivar King's Ruby (seven berries per isolate). Sterile distilled water was applied to asymptomatic berries to serve as a negative control (Ghuffar et al. 2018; Jayawardena et al. 2018). Berries were incubated at $25 \pm 2^\circ\text{C}$ in sterile moisture chambers, and the experiment was conducted twice. Brownish lesions leading to black sporulation similar to the original symptoms were observed on both wounded and nonwounded inoculated berries after 3 days, whereas no symptoms were recorded on the negative control. The morphology of the fungus that was reisolated from each of the inoculated berries was identical to that of the original cultures. *A. niger* was reported previously in Europe and Israel causing mycotoxin (ochratoxin A) production on table grapes (Bau et al. 2006). To our knowledge, this is the first report of *A. niger* causing black rot of grapes in Pakistan. This finding will help to plan effective disease management strategies against the black rot of grapes in Pakistan.

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