Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum viniferum* Causing Ripe Rot of Grape Berry in Taiwan

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Funding: Funding was provided by Council of Agriculture, Taiwan (grant no. 104AS-8.4.1-PI-P1-3). Plant Dis. 106:764, 2022; published online as <https://doi.org/10.1094/PDIS-06-21-1336-PDN>. Accepted for publication 26 August 2021.

Grape (*Vitis* spp.) is one of the most profitable fruit crops in Taiwan because of its delicacy and high nutritious value. Fruits of grape are harvested two times a year (summer and winter). In July 2015, a ripe rot disease was observed on grape berries (cv. Black queen) planted in a vineyard in Erlin Township of Changhua County (23°53'19" N, 120°24'40" E). The problem caused great concerns to the vine farmers because of its wide distribution and serious damage on berries, especially in rainy weather. Symptoms observed on ripe and nearly ripe berries showed reddish brown, irregular lesions covered with salmon-colored spore masses. Four fungal isolates were single spore isolated from four diseased berries by a handmade glass needle. Fungal isolates were grown on potato dextrose agar (PDA) at 24 to 28°C with diffused light. All four strains produced salmon-colored conidial masses with few whitish mycelia around the colony on PDA. The conidia were hyaline, single-celled, round cylindrical on both ends, thin-walled, and the contents guttulate. The conidia were 13.0 ± 0.2 (11.0 to 15.0) $\times$ 4.5 ± 0.1 (3.0 to 5.0) μm (L/W ratio = 3.0 ± 0.1 , $n = 40$). DNA was isolated from GC9 and used for amplification of partial sequences of the internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin (ACT), β -tubulin (TUB2), chitin synthase 1 (CHS-1), and *apn2*/MAT1-2-1 (ApMAT) genes (Silva et al. 2012; Weir et al. 2012). A BLAST search against the NCBI database revealed that GC9 gene sequences (GenBank accession nos. MT613359 [ITS], MT648518 [GAPDH], MT815915 [ACT], MT648525 [TUB2], MW684718

[CHS-1], MT648530 [ApMAT]) displayed 99.6, 100.0, 99.5, 99.5, 99.2, and 100.0% nucleotide identity to the respective gene sequences of *Colletotrichum viniferum* GZAAS5.08601 (JN412804, JN412798, JN412795, JN412813, JX009413) and GZAAS5.08608 (KJ623242). Bayesian inference analysis (Noireung et al. 2012) of the concatenated sequences of ITS, GAPDH, ACT, CHS-1, and TUB2 revealed that isolate GC9 and *C. viniferum* GZAAS5.08601 were grouped in the same clade, which was clearly separated from the other five closely related species of *Colletotrichum*. Conidial suspensions (1×10^6 conidia/ml) were prepared from a mixture of the four isolates of *C. viniferum* and inoculated by spraying onto detached, ripe, healthy, non-wounded, and surface-disinfected grape berries (cv. Kyoho, $n = 4$). Four bunches of berries were sprayed with sterile water as control. Berries were kept in a moist chamber (>90% relative humidity, 24 to 28°C) for 24 h and maintained in the lab for an additional 5 days. The inoculated fruit showed small light brown spots, which eventually developed into brown, water-soaked lesions, similar to the symptoms in the vineyard. No symptom was observed on berries treated with water. *C. viniferum* was reisolated from symptomatic fruit, showing similar morphological characteristics to those collected from the field, thus fulfilling Koch's postulates. The experiment was repeated once showing similar results. The GC9 isolate of *C. viniferum* with the identification number BCRC FU31518 has been deposited at Taiwan Bioresource Collection and Research Center. *C. viniferum* has been reported to infect grape in China, Korea, Brazil, and Japan (Farr and Rossman 2021). To our knowledge, this is the first report of *C. viniferum* causing grape ripe rot in Taiwan.

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The author(s) declare no conflict of interest.

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Keywords: *Colletotrichum viniferum*, grape, ripe rot, Taiwan

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