Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Black Spot Caused by *Penicillium citreosulfuratum* on Saffron in Chongming Island, China

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Saffron (*Crocus sativus* L.) is a world-famous source of dye and spices and an important medicinal plant, which is cultivated on a large scale on Chongming Island (31.62° N, 121.39° E) in Shanghai, China. In August 2020, a survey of saffron was conducted in this area, and black spots were observed on about 10% of plants. Characteristics of the disease were as follows: The bottom of the corm was darkened in the groove, and scattered black spots could be observed after peeling off the membranous scale leaves. The junction of lesions and healthy parts was light brown. As the lesions expanded, approximately 80% of the surface of the corm became dark brown to bluish gray. The inside of the corm was also necrotic. To isolate the pathogen, 10 diseased corms with typical symptoms were selected. All corms were first treated with 75% ethanol for 30 s, 0.1% HgCl₂ for 5 min, and then rinsed with sterile water five times. Next, tissue pieces (5 × 5 mm) at the margin of lesions were cut out and placed on potato dextrose agar (PDA) medium. After incubating at 28°C for 5 days, fungi were separated and purified by using the hyphal-tip technique. A total of six pure cultures with different colony morphologies were obtained, of which only the isolate MF3 was present in 10 diseased corms. The isolate MF3 was inoculated on the PDA and cultured at 28°C for 10 days, and characteristics of the fungus colonies were as follows: colonies subcircular, reaching 28 to 30 mm diameter, from above rough, dense, fluffy, blue-gray, with some white spots and central point raised, and outer margin forming an irregular, narrow, white ring; from below, yellow with light green. The hyphae were slender, with many septa. The

conidiophores were typically smooth walled, short, and slender and either monoverticillate with very short stipes or irregularly biverticillate. Phialides were ampulliform, five to 10 per metula, 5 to 8 × 2 to 3 μm. Conidia were smooth and globose, and they ranged in diameter from 1.4 to 1.7 μm (*n* = 50). Molecular identification of the fungus was made by PCR amplification of the internal transcribed spacer (ITS) region of rDNA and β-tubulin gene using primers ITS1/ITS4 (White et al. 1990) and TUB2Fd/TUB4Rd (Aveskamp et al. 2009), respectively. The ITS (MW881446) and β-tubulin (MW911464) sequences of pathogen were similar to the ITS (MN592912) and β-tubulin (KY469126) sequences of the epitype of *Penicillium citreosulfuratum* with 99.81 and 99.56% identity, respectively. According to the morphological and molecular characterization, the isolate MF3 was identified as *P. citreosulfuratum* (Visagie et al. 2016). For pathogenicity testing, the fungus was grown on PDA and incubated at 28°C for 5 days. Then mycelial plugs (5 mm diameter) were inoculated on the scalpel incision square wounds of surface-disinfected corms, and mock-inoculated corms received only PDA plugs. Corms were placed in sterile plastic bottles and observed after culturing at 28°C for 21 days. Each treatment had three replicates, and the experiment was repeated twice. The results showed that corms inoculated with *P. citreosulfuratum* developed disease with similar symptoms as in the field. No disease symptoms were observed on control corms. Reisolations were performed from inoculated corms, and all reisolated fungi were confirmed as *P. citreosulfuratum*, verifying the fungus as the pathogen based on Koch's postulates. To our knowledge, this is the first report of the pathogen causing black spot disease of saffron. Although the disease is not fatal to saffron, to a certain extent it will cause a reduction in the production of the crop. In addition, this pathogen has not been reported to be pathogenic to other plant species.

References:

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