Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium proliferatum* Causing Root Rot Disease in *Salvia miltiorrhiza* in China

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Salvia miltiorrhiza Bunge is an herb plant used as a traditional Chinese medicine to cure cardiovascular disease. In December 2018, a root rot disease was observed on *S. miltiorrhiza* in four surveyed counties (Song, Yuzhou, Fangcheng, and Mianchi) in Henan province in China. The disease incidence ranged from 15 to 50% in 12 surveyed fields. At the early stage, the diseased plants were wilting with purple leaves. Leaves and branches became withered, and fibrous roots became brown and rotted. The main roots of severely diseased plants also became rotted. The color of the stem surface turned from red to black, and the color of the stem xylem and phloem turned from dark red to brown. Eventually, the roots of diseased plants became completely rotted and whole plants became dead, but there was no stink, which is different from *Fusarium solani* (Mart.) Sacc. (Yuan et al. 2015). Diseased root tissues (5 × 5 × 5 mm in size) were cut from diseased plants, surface sterilized with 1% sodium hypochlorite for 1 min followed by dipping in 75% alcohol for 30 s, rinsed in sterile distilled water three times, air dried on a sterilized filter paper in a laminar flow hood, placed on potato dextrose agar (PDA) containing 250 mg/liter of streptomycin sulfate, and incubated at 28°C. Five isolates of *Fusarium* were obtained and purified using the single-spore isolation method. On PDA plates, the colonies were purple in color with formation of white aerial mycelia and reached 50 to 60 mm in diameter after incubation for 5 days. The colonies produced abundant microconidia on the colonies. The microconidia were 4.3 to 12.3 (10.0) × 2.1 to 3.5 (3.1) μm in size (*n* = 40), hyaline, ovoid or elliptical in shape. The conidiogenous cells were polyphialides. On mung bean medium, the isolates formed macroconidia with three to six

septa, fusiform in shape, slightly curved, 21.8 to 32.7 (31.4) × 2.6 to 4.3 (3.4) μm in size (*n* = 50). The morphological features of the five isolates were consistent with the description for *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg (Leslie and Summerell 2006). To further define the identity of the five isolates, molecular phylogenetic analysis was performed. The genomic DNA was extracted from all five isolates using the CTAB method. Five genes (nuclear ribosomal internal transcribed spacer [ITS] region, translation elongation factor 1-α [EF1α], β-tubulin gene, partial sequence for calmodulin [PRO], and RNA-dependent DNA polymerase II subunit [RPB2]) in *F. proliferatum* were amplified using primers pairs ITS1/ITS4, EF1T/2T, β-tubulin 2a/b, PRO1/2, and RPB2F/R, respectively (Glass and Donaldson 1995; Liu et al. 1999; Mulè et al. 2004; O'Donnell et al. 1998, 2010). The sequences (GenBank nos. MT371373, MT371384, MT925651, MT925652, and MT934441, respectively) showed 99.6 to 100% identities to the corresponding DNA sequences in *F. proliferatum* (GenBank nos. MK243486, MN245720, KJ12896, MN245721, and MK144327, respectively). All five isolates were tested for pathogenicity to fulfill Koch's postulates. The 45-day-old healthy plants of *S. miltiorrhiza* grown in sterilized soil in pots (20 cm in diameter), one plant in one pot, were inoculated with conidial suspensions (1.0 × 10⁷ CFU/ml) by pouring 10 ml of conidial suspension around the stem base in one pot. For each isolate, four plants were inoculated. Four plants were treated with sterilized water in the same volume as a control. The tested plants were placed in a growth room at 25°C (>60% RH) with a 12-h photoperiod of fluorescent light. The pathogenicity assay was repeated three times. Similar wilt symptoms were observed on the roots in the inoculated plants 30 days after inoculation but were not observed in the control plants. *F. proliferatum* was reisolated from the infected roots, and its identity was confirmed by PCR with the primers described above. To our knowledge, this is the first report of *F. proliferatum* causing root rot disease on *S. miltiorrhiza* in China.

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