Diseases Caused by Oomycetes

First Report of Root Rot on Mungbean Caused by *Pythium myriotylum* in China

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Mungbean (*Vigna radiata*) is a fast-growing legume and is widely planted in China. In July 2019, root rot disease symptoms were observed at seedling stage ~4 weeks after sowing in two farmers' fields located in Tongnan (30.38N; 105.77E) in Chongqing province and Nanyang (33.05N; 112.57E) in Henan province in China. The disease occurred at ~5% incidence in the two mungbean fields (~0.5 ha each). The symptomatic plants were stunted and wilted with brown and water-soaked stem lesions. In severe cases, the seedlings collapsed. Brown lesions and root rot were observed when the symptomatic plants were pulled out from soil. Five symptomatic plants were collected from each of the two fields, and one small fragment (0.5 cm long) of each plant was excised from the margin of the symptomatic root and basal stem. The fragments were surface sterilized with 2% NaOCl solution and then plated on 25% PDA plates. White mycelial colonies grew from the tissue pieces after 3 to 4 days of incubation at 25°C in dark. Eight colonies with the same morphological features were isolated, and two single-hyphal-tip cultures (CQ19-01C2 from Chongqing and HNS19-02 from Henan) were placed on PDA plates. The diameter of primary hyphae ranged from 3 to 11 (average 7.2) µm; sporangia were terminal or intercalary, filamentous, with undifferentiated and inflated lobulate elements of variable length and 9 to 18 (average 13) µm wide; oogonia were mostly globose, terminal or intercalary, ranging from 23 to 35 (average 29.4) µm in diameter. Oospores were mostly aplerotic, 11 to 25 (average 17) µm in diameter with walls 1 to 2 µm thick. DNA was extracted, and PCR amplifications of the ITS rDNA region (primers ITS1/ITS4) (White et al. 1990) and β -tubulin (TUBUF2_F/TUBUF1_R) (Kroon et al. 2004) were sequenced. The sequences were deposited in NCBI

GenBank: ITS, MT393750 and MT393751; and β -tubulin, MT416088 and MT416089. The ITS and β -tubulin sequences were 100% identical between two isolates, and a BLAST query of the obtained sequences revealed that the ITS sequences of the isolates shared 100% (736/736 base pairs) identity to *Pythium myriotylum* Py108 (KY019285.1), and β -tubulin sequence showed 99% (908/910 base pairs) similarity with *P. myriotylum* Dave1.4W (KX260348.1). The morphological and molecular characters allowed identification of the pathogen as *P. myriotylum*. To determine the pathogenicity of the two isolates and fulfill Koch's postulates, the commercial mungbean variety Sulv No.1 was inoculated with each isolate. Inoculum was prepared by transferring mycelial plugs to a sand/cornmeal mixture, which was then incubated at 20°C for 10 days. In 250-ml (70 × 52 × 85 mm) plastic cups, 40 ml (about 1 cm thick) of the inoculum was placed on top of a 150-ml layer of vermiculite. Eight seeds were sown directly on top of the inoculum mixture and then covered with 50 ml of vermiculite (Klepadlo et al. 2019). For noninoculated controls, sterile sand/cornmeal without mycelium was added at the same ratios. After watering to saturate the vermiculite, the cups were maintained in growth chamber at 25°C and 16-h/8-h light/dark photoperiod. Three weeks after inoculation, the inoculated plants were stunted and showed wilting symptoms. No disease symptoms were observed in noninoculated control plants. When symptomatic seedlings were pulled from the cups, the lower portion of the stem and roots were brown and rotted, which was similar to those observed in field. *P. myriotylum* was reisolated from sections of roots and basal stems of the symptomatic seedlings and confirmed by molecular analysis. To our knowledge, this is the first report of *P. myriotylum* causing root rot of mungbean in China. *P. myriotylum* is the main causal agent of root rot in numerous crops and is widespread in warmer regions (Wang et al. 2003; Yu et al. 2019). The findings show that *P. myriotylum* may cause potential threat during mungbean planting in the future and have implications for disease control.

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