Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Watermelon Charcoal Rot (*Macrophomina phaseolina*) in China

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In June 2018 and 2019, charcoal rot-like symptoms and black microsclerotia suggestive of *Macrophomina phaseolina* infection were observed on the basal stems of *Citrullus lanatus* ‘Zaojia’, causing premature death. About 1 ha of Zaojia had been investigated, and disease incidence rates were almost 50%, resulting in a 40% yield loss in a single field in Shanghai, China (31°23′N, 121°33′E). A fungus was consistently isolated from infected watermelon tissues. In total, 30 cuttings from 10 infected seedlings were surface disinfected with 3% sodium hypochlorite for 3 min, washed thrice with sterile distilled water, air dried, and transferred onto potato dextrose agar (PDA). Dishes were incubated for 3 days at 27°C in the dark. In all, 24 single-hypha subcultures were obtained from these samples and were cultured for an additional 5 days at 27°C. Colonies were initially white, then became gray black. During the more advanced stages of infection, black microsclerotia were produced that were spherical or ovoid in shape. No sexual structures and conidia developed during culture on PDA. Isolate pathogenicity was assessed both in vitro and in vivo. Watermelon plants (Zaojia) were grown in growth chambers at 28°C (day) and 23°C (night), with a 16-h photoperiod. When seedlings were 20 days old, they were inoculated. Briefly, a needle was used to puncture watermelon stems, and 5-mm agar plugs containing actively growing mycelia were placed on these needle wounds, followed by

culture for 72 h at 27°C in a dark, humid chamber. In total, 10 seedlings were inoculated with 5-mm blank PDA, and the experiment was repeated three times, with the treatment being performed as described above. Seedling stems were inoculated 1 to 2 cm above the ground by puncturing them with a needle, then transferring 5-mm agar plugs containing fungi onto the wound sites. Seedlings were kept 75% humidity, then grown for 5 days at 27°C. Ten seedlings were inoculated per experiment. As a control, 10 seedlings were inoculated with 5-mm blank PDA plugs. Experiments were repeated three times. Necrotic spots around the wounds were evident on inoculated stems at 72 h. Similarly, vascular tissue necrosis and the collapse of the surrounding pith and epidermis were observed on the residual parts of seedling stems after 5 days, whereas control stems did not exhibit any disease-related symptoms. The same pathogen was then successfully reisolated and was successfully regrown in pure culture, thus fulfilling Koch’s postulates. To identify the causative pathogen, total mycelial DNA was isolated via the CTAB method (Brandfass and Karlovsky 2008), the internal transcribed spacer (ITS) rDNA regions were amplified using the ITS1 and ITS4 primers (White et al. 1990), and the genus-specific MPKFI and MpKRI primers were used for further amplification (Babu et al. 2007). *M. phaseolina* ITS sequences in this study shared 100% similarity with the ITS sequences of *M. phaseolina* from chickpea (MK757624.1). Genus-specific sequences from this isolate shared 100% homology with other *M. phaseolina* isolates (MT645816.1 and MN263167.1). As such, *M. phaseolina* was confirmed to be the pathogen responsible for watermelon charcoal rot in the present report, which resulted in the death of infected watermelon plants before maturity, causing fruit to lose their commodity value. This report is the first, to our knowledge, to identify *M. phaseolina* as a causal pathogen of watermelon charcoal rot in China.

References:

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