Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium proliferatum* Causing Fruit Rot on Muskmelon (*Cucumis melo*) in China

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Muskmelon (*Cucumis melo* L.) is an economically important crop worldwide, especially in China, the largest producer of muskmelon with an annual output up to 12.7 million tons (Gómez-García et al. 2021). Since 2018, fruit rot has been observed on muskmelon in Malianzhuang Base, the main muskmelon-producing area in Shandong Province, where disease incidence was ~25 to 30%. Water-soaked dark-brown spots initially appeared on the side of the fruit near the ground, then gradually expanded and covered fruit with white mold over time. To isolate the pathogens, 10 muskmelon fruits with typical symptoms were collected from different greenhouses in the base. Small samples taken from the edge of the diseased and healthy tissue were immersed in 1% NaClO for 2 min, then soaked in 75% ethanol for 30 s, and rinsed three times with sterile distilled water (SDW). Sterilized tissues were naturally dried and placed on PDA amended with streptomycin sulfate (50 mg/liter) for 7 days at 28°C. Emerging fungal mycelia were transferred to fresh PDA using hyphal tip technology. Ten colonies were purified by a single-spore method and cultured on PDA for 7 days at 28°C in the dark for morphological and molecular analyses. All colonies were flocculent with abundant white to light-purple aerial hyphae, and the undersides of the colonies were observed to change from white to purple over time. Microconidia produced on PDA were hyaline, fusiform, ovoid, single cell without septum, and 4.5 to 12.7 by 2.0 to 3.6 µm in size ($n = 50$). Macroconidia produced on carboxymethylcellulose agar were slightly curved at both ends with three to five septa and 17.6 to 35.7 by 2.8 to 4.0 µm in size ($n = 30$). According to their morphological characteristics,

these isolates were preliminarily identified as *Fusarium* spp. (Leslie and Summerell 2006). To further identify the isolates, genomic DNA of five isolates was extracted by the CTAB method (Wu et al. 2001). The ITS region of ribosomal DNA, *TEF1* region, and *RPB2* were amplified by PCR amplification with primers ITS1/ITS4, EF-1/EF-2, and RPB2-5F2/IRPB2-7cR, respectively (Liu et al. 1999; O'Donnell et al. 2008; White et al. 1990). Sequences of the five isolates were identical. The ITS, *EF1-α*, and *RPB2* gene sequences of isolate NEAU-Mf-10-2 were submitted to NCBI GenBank as MZ950914, MZ960928, and MZ960929, respectively, having 100% similarity to those of *Fusarium proliferatum* (MK372368, MK952799, and MN245721). Phylogenetic trees were constructed based on the concatenated sequences of *TEF1* and *RPB2* genes using neighbor-joining and maximum-likelihood algorithms with MEGA 7.0. Two similar tree topologies showed isolate NEAU-Mf-10-2 clustered with *F. proliferatum* NRRL 43665. Therefore, isolate NEAU-Mf-10-2 was identified as *F. proliferatum* based on morphological characteristics and phylogenetic analysis. To fulfill Koch's postulates, 10 muskmelon fruit ('Tianbao') were soaked in 2% NaClO for 2 min, then washed three times with SDW. Muskmelon fruit were inoculated by injecting a conidial suspension (200 µl, 1×10^6 spores/ml) with a sterile injector. Ten other surface-sterilized muskmelon fruit inoculated with sterile water were used as control. The fruit were placed in a light incubator at 28°C with 12-h light cycles for 7 days. All inoculated fruit showed symptoms highly similar to those of infected muskmelon fruit observed in the field. No symptoms were observed on fruit used as control. *Fusarium* isolates were successfully reisolated from the symptomatic fruit and identified based on above morphological and molecular biological methods. Previous studies have reported that *F. proliferatum* can infect *Polygonatum cyrtoneura*, *Salvia miltiorrhiza*, *Allium cepa*, *A. sativum*, and so on. To our knowledge, this is the first report of *F. proliferatum* causing fruit rot on muskmelon in China, which will provide basic information for designing effective prevention and control strategies on this disease.

References:

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The author(s) declare no conflict of interest.

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