Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium equiseti* Causing Fruit Rot Disease of Watermelon in Malaysia

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Watermelon (*Citrullus lanatus*) accounts for almost 13% of all tropical fresh fruit production in Malaysia. They are grown, mostly in Johor, Kedah, Kelantan, Pahang, and Terengganu areas of Malaysia on 10,406 ha and yielding 172,722 Mt. In 2019, a new fruit rot disease was observed in two major production areas in Peninsular Malaysia. Disease symptoms included water-soaked brown lesions on the fruit surface in contact with the soil. The lesions enlarged gradually and ultimately covered the whole fruit with white mycelium, leading to internal fruit decay. Disease surveys were conducted in December 2019 and November 2020 in fields at Kuantan, Pahang, and Serdang, Selangor. Disease incidence was 10% in 2019 and 15% in 2020. Infected fruits were collected and washed under running tap water to wash off adhering soil and debris. Fruit tissue sections 1 to 2 cm in length were surface sanitized with 0.6% sodium hypochlorite (NaOCl) for 3 min and washed twice with sterile distilled water. The disinfected air-dried tissues were then transferred onto potato dextrose agar medium and incubated at 25 ± 2°C for 3 days. Fungal colonies with whitish mycelium and pink pigment were isolated using single-spore culture. The pure cultures were placed onto carnation leaf agar (CLA), and the culture plates were incubated at 25 ± 2°C for 15 days for morphological characterization. On CLA, macroconidia were produced from monophialides on branched conidiophores in orange sporodochia. Macroconidia were thick-walled, strong dorsiventral curvature, five to seven septate with a tapered whip-like pointed apical cell and characteristic foot-shaped basal cell, 21.9 to 50.98 µm long, and 2.3 to 3.60 µm wide. Typical verrucose thick chlamydospores with rough walls were profuse in chains or clumps,

subglobose or ellipsoidal. Based on morphological characteristics they were identified as *Fusarium equiseti* (Leslie and Summerell 2006). Molecular identification of both U4-1 and N9-1 pure-culture isolates were carried out using two primer pair sets: internal transcribed spacer (ITS) ITS-1/ITS-4 and translation elongation factor 1 alpha (TEF1-α) (EF-1/EF-2). A BLASTn analysis of the ITS gene sequence of U4-1 (MW362286) and N9-1 (MW362287) showed >99% similarity index to the reference gene sequence of *F. equiseti* isolate 19MSr-B3-4 (LC514690). The TEF1-α sequences of U4-1 (accession no. MW839563) and N9-1 (accession no. MW839564) showed 100% identity, with an e-value of zero, to the reference gene sequence of *F. equiseti* isolate URM: 7561 (accession no. LS398490). Each isolate also had a >99% identity with isolate NRRL 34070 (accession no. GQ505642) in *Fusarium* MLST database that belongs to the *F. incarnatum-equiseti* species complex (O'Donnell et al. 2015). Based on phylogenetic analysis of the aligned sequences (TEF1-α) by the maximum likelihood method, the U4-1 and N9-1 isolates were confirmed to be *F. equiseti* as was reported in Georgia, U.S.A. (Li and Ji 2015) and in Harbin, Heilongjiang Province, China (Li et al. 2018). Finally, the two pure-culture isolates of U4-1 and N9-1 were used to fulfill Koch's postulates. Stab inoculations of five healthy watermelon fruits (cv. 345-F1 hybrid seedless round watermelon) were performed with a microconidial suspension of individual isolates (4 × 10⁶ spores/ml). Five control fruits were stabbed with double-distilled water. The inoculated fruits were incubated under 95% relative humidity at a temperature of 25 ± 2°C for 48 h followed by additional incubation inside an incubator at 25 ± 2°C for 8 days. Ten days postinoculation, the control fruits showed no disease symptoms. However, inoculated fruits exhibited typical symptoms of fruit rot disease like water-soaked brown lesions, white mycelium on the fruit surface, and internal fruit decay, which is similar to the farmer's field infected fruits. The suspected pathogen was successfully reisolated from the symptomatic portion of inoculated fruit and morphologically identified for verification. To our knowledge, this is the first report of *F. equiseti* causing fruit rot of watermelon in Malaysia. Malaysia exports watermelon year-round to many countries around the world. The outbreak of this new fruit rot disease could potentially pose a concern to watermelon cultivation in Malaysia.

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