Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Nigrospora osmanthi* Causing Leaf Blight on *Orthosiphon stamineus* in Malaysia

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Orthosiphon stamineus (Java tea) is a perennial herbaceous plant in the family Lamiaceae and is cultivated extensively in Southeast Asia for its medicinal value (Arifullah et al. 2014). During October 2018, leaf blight symptoms were observed on leaves of ~210 *O. stamineus* plants grown in experimental plots of a research farm at Faculty of Engineering, Serdang, Selangor, Malaysia (3°00'30.4"N, 101°43'19.9"E), with 80% disease incidence. Initial symptoms were brown-to-black lesions on the leaves that enlarged and coalesced until the leaf withered and abscised. Diseased tissues (4 × 4 mm) of six infected leaves were excised, surface disinfected with 0.5% NaOCl for 1 min, rinsed twice with sterile distilled water, placed onto potato dextrose agar (PDA) plates, and incubated at 25°C with a 12-h photoperiod under fluorescent light for 7 days. A total of six single-spore isolates were obtained from sampled leaves. All isolates exhibited similar morphology and two representative isolates, MK and MK1, were characterized further. Colonies on PDA were initially white, then turned dark gray with age and had a pale green underside. Hyphae were branched, septate, and hyaline to pale brown. The conidia were one-celled, black, smooth-walled, spherical to sub-spherical in shape, measuring 11.0 × 16.5 µm in diameter ($n = 30$), and are borne on hyaline vesicles at the tip of each conidiophore or formed directly from the mycelia. Based on morphological characteristics, the fungal isolates were identified as *Nigrospora osmanthi* (Wang et al. 2017). Total genomic DNA of the isolates was extracted from fresh mycelium using DNeasy Plant Mini kit (Qiagen, U.S.A.) and the internal transcribed spacer (ITS), translation elongation factor 1-alpha (TEF1), and Beta-tubulin (TUB2) gene regions

were amplified using ITS5/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), and Bt-2a/Bt-2b primer sets (Glass and Donaldson 1995), respectively. BLASTn analysis showed that the ITS, TEF1, and TUB sequences of the isolates shared 99 to 100% identity with *Nigrospora osmanthi* ex-type strain CGMCC 3.18126 (GenBank accession nos. KX986010, KY019421, and KY019461). The sequences of two representative isolates (MK and MK1) were deposited in GenBank (ITS: MT645782, MW363019; TEF1: MW366861, MW366862; TUB: MW366863, MW366864). Phylogenetic analysis by the maximum likelihood method based on the concatenated ITS-TEF1-TUB sequences showed that the isolates in this study were clustered in a strongly supported group (98% maximum likelihood) with type strain *N. osmanthi* (Kumar et al. 2016). The pathogenicity of all isolates was confirmed by inoculation on 10 healthy leaves of five potted 4-week-old *O. stamineus* plants using a conidial suspension (1×10^6 spores/ml) produced on 7-day-old PDA cultures. An equal number of plants were sprayed with sterile distilled water as controls and the treated plants were kept in a growth chamber for 2 weeks at $28 \pm 1^\circ\text{C}$ and 95% relative humidity. The experiment was repeated twice. The inoculated leaves developed brown lesions that enlarged into blight symptoms similar to those observed on naturally infected leaves 5 days after inoculation, while control plants remained healthy. *N. osmanthi* was successfully reisolated from the infected leaves, confirming Koch's postulates. The fungus was not isolated from noninoculated control plants. *N. osmanthi* has been recently reported to cause leaf blight on *Ficus pandurata* (Liu et al. 2019) and *Stenotaphrum secundatum* in China (Mei et al. 2019). This disease can cause a significant threat to the cultivation of *O. stamineus*, which has been extensively grown to produce herbal Java tea. Accurate identification of this pathogen could assist in developing an effective disease management strategy to control this disease.

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