Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Puccinia thaliae* Causing Leaf Rust on *Canna indica* in Malaysia

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Canna indica L. (family Cannaceae), locally known as bunga kana, is a perennial plant grown as a source of starch and for ornamental purposes in Malaysia. During June 2021, bunga kana with rust symptoms and signs were collected from the Universiti Malaysia Sabah in the province of Sabah. The severity was 95%, and the incidence was 90%. Yellow uredinia were observed primarily on the abaxial surface of the leaves. As the disease progressed, leaves were covered with coalescing pustules, and chlorosis and brown necrosis developed. Microscopic examination of pustules revealed the presence of urediniospores and teliospores. Urediniospores were round to ovoid in shape, yellow, echinulate, 17.7 to 24.6 × 26.8 to 45.2 µm, with two equatorial pores. Teliospores were elongate-clavate, with a rounded apex, yellow contents, 18.3 × 20.2 to 45.8 × 53.9 µm, with a short pedicel. Yellow urediniospores were collected using a fine brush, and genomic DNA was extracted using a lysis buffer (Tris-HCl [0.1 M, pH 9.5], NaCl [1 M], and EDTA [0.5 M, pH 8]) prior to heating at 95°C for 10 min. KOD One PCR Master Mix containing hot-start modified KOD DNA polymerase was used for PCR amplification. The 28S ribosomal RNA gene was amplified using

primers Rust28SF (Aime et al. 2018) and LR5 (Vilgalys and Hester 1990). PCR products with positive amplicons were sent to Apical Scientific Sdn. Bhd. for sequencing. BLASTn analysis of the newly generated 28S ribosomal RNA gene (OK462969) in GenBank revealed a 99% sequence identity to *Puccinia thaliae* Dietel (JX206994 of 28S ribosomal RNA gene). The morphological and molecular characterization of the rust fungus matched *P. thaliae* described by Padamsee and McKenzie (2012). Koch's postulates were performed with spray inoculations of urediniospores suspended in water (10⁶ spores/ml) on leaves of three healthy bunga kana plants, while water was sprayed on three additional bunga kana plants as a control. The inoculated bunga kana plants were covered with plastic for 48 h at 25°C in the dark and then placed in a greenhouse. Symptoms and signs similar to those of the field collection occurred after 13 days postinoculation. No symptoms occurred on controls. Leaf rust on bunga kana plants caused by *P. thaliae* has been reported in Europe (Talhinhas et al. 2016), Hawaii (Nelson 2013), India (Gopi et al. 2014), Mexico (Cedas de Jesús et al. 2018), Nepal (Adhikari and Durrieu 2016), New Zealand (Padamsee and McKenzie 2012), Singapore (Neo and Tham 2010), and South Africa (van Jaarsveld et al. 2006) over the past 15 years. To our knowledge, this is the first report of *P. thaliae* causing leaf rust on *C. indica* in Malaysia. Our findings expand the geographic range of *P. thaliae* and indicate it could be a potential threat that limits the starch production of *C. indica* in Malaysia.

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