

First Report of *Colletotrichum siamense* causing Anthracnose on White Frangipani (*Plumeria alba* L.) in Malaysia

ABSTRACT

Plumeria alba L. is a flowering plant in the family Apocynaceae and widely cultivated in Malaysia as a cosmopolitan ornamental plant. In January 2020, anthracnose lesions were observed on leaves of *Plumeria alba* planted in Agricultural Farm, Universiti Putra Malaysia, in Selangor state, Malaysia. The disease mainly affected the leaves with symptoms occurring with approximately a 60% disease incidence. Ten symptomatic leaves were sampled from 3 different trees in the farm. Symptoms initiated as small circular necrotic spots that rapidly enlarged into black lesions with pale brown borders. Diseased tissues (5×5 mm) were surface-sterilized with 70% ethanol for 1 min, rinsed three times with sterile distilled water, dried on sterile filter papers, plated on PDA and, incubated at 25 °C with a 12-h photoperiod. A total of seven single-spore isolates with similar colony morphologies were obtained from tissue samples. After 7 days, the colonies raised the entire margin and showed white-to-gray aerial mycelium, orange conidial masses in the center and appeared dark brown at the center of the reverse view. The conidia were 1-celled, hyaline, smooth-walled, cylindrical with narrowing at the center, averaged (13-15 µm × 3 - 4 µm) (n=40) in size. Morphological characteristics of the isolates were similar to those detailed in taxonomic description of *Colletotrichum* sp. (Prihastuti et al. 2009). For molecular identification, genomic DNA of two representative isolates, PL3 and PL4 was extracted from fresh mycelium using DNeasy Plant Mini Kit (Qiagen, USA). The internal transcribed spacer (ITS) region, actin (ACT) and calmodulin (CAL) genes were amplified using ITS5/ITS4 (White et al. 1990), ACT-512F/783R (Carbone and Kohn 1999) and CL1C/CL2C primer sets (Weir et al. 2012). A BLAST nucleotide search of GenBank using ITS sequences showed 100% identity to *Colletotrichum siamense* ex-type culture ICMP 18578 (GenBank accession no. JX010171). ACT and CAL sequences showed 100% identity with *C. siamense* ex-type isolate BPD-I2 (GenBank accession no. FJ907423 and FJ917505). The sequences were deposited in GenBank (ITS: accession nos. MW335128, MT912574), ACT: accession nos. MW341257, MW341256, CAL: accession nos. MW341255 and MT919260). Based on these morphological and molecular characteristics, the fungus was identified as *C. siamense*. Pathogenicity of PL3 and PL4 isolates was verified using four healthy detached leaves of *Plumeria alba*. The leaves were surface-sterilized using 70% ethanol and rinsed twice with sterile water before inoculation. The leaves (three inoculation sites/leaf) were wounded by puncturing with a sterile needle through the leaf cuticle and inoculated in the wound site with 10-µl of conidial suspension (1×10⁶ conidia/ml) from 7-days-old culture on PDA. Four leaves were used as a control and were inoculated only with 10-µl of sterile distilled water. Inoculated leaves were kept in humid chambers for 2 weeks at 25 °C with 98% relative humidity on a 12-h fluorescent light/dark period. The experiment was repeated three times. Anthracnose symptoms were observed on all inoculated leaves after 3 days, whereas controls showed no symptoms. Fungal isolates from the diseased leaves showed the same morphological characteristics as isolates PL3 and PL4, confirming Koch's postulates. *C.*

siamense has been reported causing anthracnose on rose (*Rosa chinensis*) in China (Feng et al. 2019), *Coffea arabica* in Thailand (Prihastuti et al. 2009) and mango leaf anthracnose in Vietnam (Li et al. 2020). To our knowledge, this is the first report of *Colletotrichum siamense* causing leaf anthracnose on *Plumeria alba* in Malaysia. Accurate identification of this pathogen provides a foundation in controlling anthracnose disease on *Plumeria alba*.

Keyword: *Colletotrichum siamense*; *Plumeria alba* L.; Anthracnose; White frangipani