

First report of *Colletotrichum siamense* causing blossom blight on Thai basil (*Ocimum basilicum* L.) in Malaysia

ABSTRACT

Thai basil (*Ocimum basilicum* L.) is widely cultivated in Malaysia and commonly used for culinary purposes. In March 2019, necrotic lesions were observed on the inflorescences of Thai basil plants with a disease incidence of 60% in the Organic Edible Garden Unit, Faculty of Agriculture, in the Serdang district (2°59'05.5"N, 101°43'59.5"E) of Selangor province, Malaysia. Symptoms appeared as sudden, extensive brown spotting on the inflorescences of Thai basil that coalesced and rapidly expanded to cover the entire inflorescences. Diseased tissues (4 × 4 mm) were cut from the infected lesions, surface disinfected with 0.5% NaOCl for 1 min, rinsed three times with sterile distilled water, placed onto potato dextrose agar plates, and incubated at 25°C under 12-h photoperiod for 5 days. A total of eight single-spore isolates were obtained from all sampled inflorescence tissues. The fungal colonies appeared white and turned grayish black with age and pale yellow on the reverse side. Conidia were one-celled, hyaline, subcylindrical with rounded end, and 3 to 4 µm (width) and 13 to 15 µm (length) in size. For fungal identification to species level, genomic DNA of a representative isolate (isolate C) was extracted using a DNeasy Plant Mini Kit (Qiagen, U.S.A.). Internal transcribed spacer (ITS) region, calmodulin (CAL), actin (ACT), and chitin synthase-1 (CHS-1) were amplified using ITS5/ITS4 (White et al. 1990), CL1C/CL2C (Weir et al. 2012), ACT-512F/783R, and CHS-79F/CHS-345R primer sets (Carbone and Kohn 1999), respectively. A BLAST nucleotide search of ITS, CHS-1, CAL, and ACT sequences showed 100% similarity to *Colletotrichum siamense* ex-type cultures strain C1315.2 (GenBank accession nos.: ITS, JX010171; CHS-1, JX009865) and isolate BPD12 (CAL, FJ917505; ACT, FJ907423). The ITS, CHS-1, CAL, and ACT sequences were deposited in GenBank as accession numbers MT571330, MW192791, MW192792, and MW140016. Pathogenicity was confirmed by spraying a spore suspension (1 × 10⁶ spores/ml) of 7-day-old culture of isolate C onto 10 healthy inflorescences on five healthy Thai basil plants. Ten inflorescences from an additional five control plants were only sprayed with sterile distilled water, and the inoculated plants were covered with plastic bags for 2 days and maintained in a greenhouse at 28 ± 1°C, 98% relative humidity, with a photoperiod of 12 h. Blossom blight symptoms resembling those observed in the field developed after 7 days on all inoculated inflorescences, whereas inflorescences on control plants remained asymptomatic. The experiment was repeated twice. *C. siamense* was successfully reisolated from the infected inflorescences, fulfilling Koch's postulates. *C. siamense* has been reported causing blossom blight of *Uraria* in India (Srivastava et al. 2017) and anthracnose on dragon fruit in India and fruits of *Acca sellowiana* in Brazil (Abirami et al. 2019; Fantinel et al. 2017). This pathogen can cause a serious threat to cultivation of Thai basil, and there is currently no effective disease management strategy to control this disease. To our knowledge, this is the first report of blossom blight caused by *C. siamense* on Thai basil in Malaysia.

Keyword: Blossom blight; *Colletotrichum siamense*; Thai basil (*Ocimum basilicum* L.)