



First report of papaya anthracnose caused by *Colletotrichum fructicola* in India

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Papaya (*Carica papaya*) is an important fruit crop grown in tropical and subtropical regions of India. India ranks first in papaya production in the world with over 5.54 million tonnes produced annually (FAOSTAT, 2013). In August 2016, a papaya fruit showing typical anthracnose symptoms of small, sunken, dark brown lesions was purchased from a market (Fig. 1). The fruit originated from a farmer's field in Karmad village, Aurangabad Maharashtra, India, and had been harvested before disease symptoms were visible on the fruit or host plant. Symptomatic fruit were incubated to allow development of disease symptoms. In India, *Colletotrichum gloeosporioides* is reported as the causal agent of papaya anthracnose (Tasiwal, 2008). However, *C. gloeosporioides* has recently been characterised as a species complex consisting of different species (Weir *et al.*, 2012). Since *Colletotrichum* spp. are important pathogens worldwide on papaya and other fruits, it is important to identify and clarify the species on papaya in India.

Small pieces of necrotic tissue of papaya were examined under a microscope and spores were transferred onto potato dextrose agar (PDA) containing 50 mg/l streptomycin sulphate. The plates were incubated at 28°C for five to seven days. A pure culture of the *Colletotrichum* sp. isolate was obtained by sub-culturing onto fresh PDA plates. Identification of the isolate was based on morphological as well as molecular characterisation. Colonies on PDA had white aerial mycelia with an orange conidial mass (Fig. 2). The colour of the colony on the reverse side was light orange. The mycelial growth rate on the PDA plate at 28°C with a 16 h photoperiod was 15 mm per day. Conidia were single celled, cylindrical with rounded base and apex. Mean length and width of conidia was 15.3±1.5 µm and 5.3±0.1 µm, respectively (Fig. 3).

PCR amplification of ITS rDNA and the partial β-tubulin gene was done using the universal primer pair ITS 4/5 and β-tubulin gene Bt2a/b primers for molecular characterisation of the isolated *Colletotrichum* sp. Both sequences were deposited in GenBank (Accession Nos. KY007499 and KY007500, respectively) and aligned with published sequences using MEGA version 6.0 (Tamura *et al.*, 2013; Fig. 4). Blast searches in the NCBI database revealed that ITS and β-tubulin gene sequences had 99 and 100% identity with *C. fructicola* (KU366691 and KU552337, respectively). Although the ITS and β-tubulin gene sequences also showed high similarity with *C. ignotum*, *C. siamense* and *C. gloeosporioides*, the isolate obtained

from infected papaya fruit was identified as *C. fructicola* based upon these phylogenetic analyses and morphological characteristics (Weir *et al.*, 2012).

To confirm pathogenicity, papaya leaves and chilli fruits were wounded with a sterile syringe and inoculated with 10 µl of a conidial suspension (ca. 10⁵ conidia/ml). Sterile water was used as a control. Inoculated papaya leaves and chilli fruits were kept in a chamber at 28°C with 90% humidity. After seven days typical anthracnose symptoms were observed on the papaya leaves as well as on chilli fruits (Fig. 5). No symptoms were observed on the controls. *Colletotrichum fructicola* was re-isolated from symptomatic papaya, thereby fulfilling Koch's postulates.

Colletotrichum fructicola has been reported in India as a causal agent of chilli anthracnose (Sharma & Shenoy, 2014) and on papaya in Laos and Thailand (Phoulivong *et al.*, 2012). To our knowledge, this is the first report of papaya anthracnose caused by *C. fructicola* in India.

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Figure 1

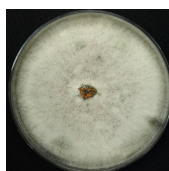


Figure 2

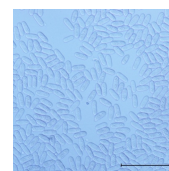


Figure 3

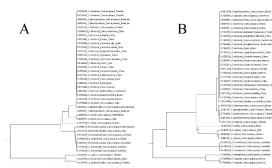


Figure 4



Figure 5

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