



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

T.J. Saini^{1,2*}, S.G. Gupta³ and R. Anandalakshmi²

¹ Department of Biotechnology, Government Institute of Science, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, Maharashtra, India; ² Mahyco Research Centre, Dawalwadi, Jalna-431203, Maharashtra, India; ³ Government Institute of Forensic Science, Nipatniranjan Nagar, Aurangabad-431004, Maharashtra, India

*E-mail: teju.saini@gmail.com

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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 5.63 million tonnes produced annually (FAOSTAT, 2014). Anthracnose of papaya caused by *Colletotrichum* species is an important disease worldwide, resulting in post-harvest fruit decay which severely affects the marketability of the fruits (Ademe *et al.*, 2014). In August 2016, papaya fruits showing typical anthracnose symptoms of small sunken spots were observed in two different fields in Badnapur, Jalna, Maharashtra, India (Fig. 1) and around 15% of the papaya plants were affected.

The papaya fruits were semi ripe when harvested and ten fruits were incubated at 28°C in the dark for four-five days at 90% humidity to allow for further development of disease symptoms. Sunken necrotic fruit tissue was then examined under a microscope and conidia were inoculated onto potato dextrose agar (PDA) containing 50 mg/l streptomycin sulphate. The plates were incubated at 28°C with a 16/8 hr light/dark cycle for five to seven days and pure cultures were obtained by sub-culturing onto fresh PDA plates. Identification of isolates was based on morphological as well as molecular characterisation. All ten fruits were found to be infected with *Colletotrichum gloeosporioides* and among them six fruits had a mixed infection of *C. gloeosporioides* and *Colletotrichum salsolae*. Only identification of the *C. salsolae* isolate is described here.

The putative *C. salsolae* isolate had white aerial mycelia with an olive-coloured colony and a light-salmon coloured conidial mass (Fig. 2). The mycelial growth rate on PDA at 28°C with 16 hr photoperiod was 16 mm per day. Conidia obtained from incubated papaya fruits were single celled, cylindrical with a rounded base and apex. The mean length and width of conidia was 16.69 ± 1.04 µm and 5.56 ± 0.18 µm, respectively (Fig. 3). The morphological characteristics of the isolate matched the description of *C. salsolae* (Weir *et al.*, 2012). Molecular characterisation of the putative *C. salsolae* isolate involved PCR amplification of the partial β-tubulin gene using the universal primer pair Bt2a/b and GAPDH gene. Both sequences were deposited in GenBank (Accession Nos. KY401636 and KY401637 respectively) and aligned with published sequences using MEGA version 6.0 and phylogenetic analysis was done (Fig. 4). Blast searches in the NCBI database revealed that the β-tubulin and GAPDH gene sequences had 99% and 97% identity to *C. salsolae* (KC790894 and JX009916, respectively).

The pathogenicity of *C. salsolae* isolated from infected papaya fruit was confirmed on healthy papaya fruits obtained from the local market. The fruits were washed with sterile distilled water and then surface sterilised with 70% ethanol for 30 seconds. Papaya fruits were wounded with a

sterile syringe and inoculated with 10 µl of a conidial suspension (c. 10⁵ conidia/ml). Sterile water was used as a control. Inoculated papaya fruits were kept in a chamber at 28°C in the dark with 90% humidity. After seven days typical anthracnose symptoms were observed on the inoculated papaya fruit but not on the controls (Fig. 5). Conidia were re-isolated from the inoculated symptomatic papaya fruits and characterised morphologically as well as molecularly and found to be identical to the original inoculated *C. salsolae* spores, thereby fulfilling Koch's postulates.

In India, *Colletotrichum karstii* (Sharma & Shenoy, 2013), *Colletotrichum fruticola* and *C. gloeosporioides* (Saini *et al.*, 2016) along with some other unverifiable records (Farr & Rossman, 2017) have been reported to cause papaya anthracnose. To our knowledge, this is the first report of papaya anthracnose caused by *C. salsolae* in India. The pathogen has previously only been reported on *Salsola tragus* in Hungary (Weir *et al.*, 2012). As this was a mixed infection it is not known what proportion of the affected fruits was infected by *C. salsolae* in the field. However, this new finding shows that *C. salsolae* is not host-specific and may occur in more geographical locations than is currently recorded.

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Figure 1

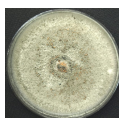


Figure 2

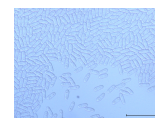


Figure 3

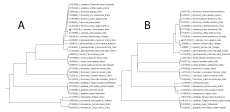


Figure 4



Figure 5

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