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Colletotrichum truncatum causing anthracnose on papaya fruit (*Carica papaya*) in Brazil

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Abstract

Papaya anthracnose caused by *Colletotrichum truncatum* is reported for the first time in Brazil. The etiological agent was identified by a combination of morphological and molecular approaches. Pathogenicity was confirmed and Koch's postulates fulfilled for a selected isolate from Porto Seguro, Bahia, Brazil.

Keywords fungal disease · occurrence · postharvest

Papaya (*Carica papaya*) is among the most widely cultivated and consumed tropical fruits (Serrano and Cattaneo 2010). Among the main producers, México, Brazil, Nigeria, India and Indonesia, Brazil ranked second in 2017 for production volume, producing 1.4 tons on 30.000 ha (IBGE 2017). However, plant diseases lead to reduced productivity in the form of yield loss and have a detrimental impact on fruit quality (Ferreira et al. 2018; Martins and Blum 2014). Papaya yield losses are mainly attributable to phytopathogenic fungi, such as *Phytophthora* spp., *Phoma caricae-papayae*, *Asperisporium caricae*, *Oidium caricae*, and *Colletotrichum* spp. (Ventura et al. 2004). Anthracnose is among the most important papaya postharvest diseases due to the large losses in production, which may reach about 90% (Valenzuela et al. 2015).

In January 2017, a disease was detected causing postharvest losses on papaya in Porto Seguro, Bahia, Brazil. Symptomatic ripe fruit presented brownish, rounded, necrotic and depressed lesions. Black acervuli were produced in lesions as disease progressed and became covered by orange conidial masses. Isolates were obtained from typical anthracnose symptoms using direct isolation by transferring conidial masses from lesions to Petri dishes containing potato dextrose agar (PDA) media supplemented with streptomycin sulfate (0.03g/L). Cultures were incubated at 28°C under continuous light. Colony color was characterized seven days after incubation using Rayner's Mycological Colour Chart (Rayner 1970). Mycelial fragments from 7-day-old colonies were mounted in Shear's mounting media to assess micromorphological features, such as conidial shape and size, acervuli, setae, conidiophores, and conidiogenous cells. Pictures were taken with a DS-L3 digital camera attached to a Nikon Eclipse Ni-U transmitted light microscope using differential interference contrast illumination. Microscopic image capture and measurement were done using NIS-Elements.

Colonies on PDA presented purplish grey felt-like mycelium with white sectors in aerial view. The reverse was greyish sepia to fuscous black with buff sectors (Fig. 1 c–g). Acervuli were present in culture with abundant dark brown, smooth walled, 3–4 septate setae. Conidiophores hyaline to pale brown, septate, branched, smooth-walled. Conidiogenous cells hyaline, smooth-walled, cylindrical, collarette visible. Conidia on PDA were hyaline, aseptate, smooth-walled, falcate, 8.6– (12.5 ± 1.3) –15.6 × 3.5– (3.7 ± 0.4) –5.0 μm. Morphological features were consistent with *Colletotrichum* (Sutton 1980). The isolate LM159 was chosen as representative for further analyses. The isolate was deposited at the Culture Collection of Phytopathogenic Fungi "Prof. Maria Menezes" (CMM4878).

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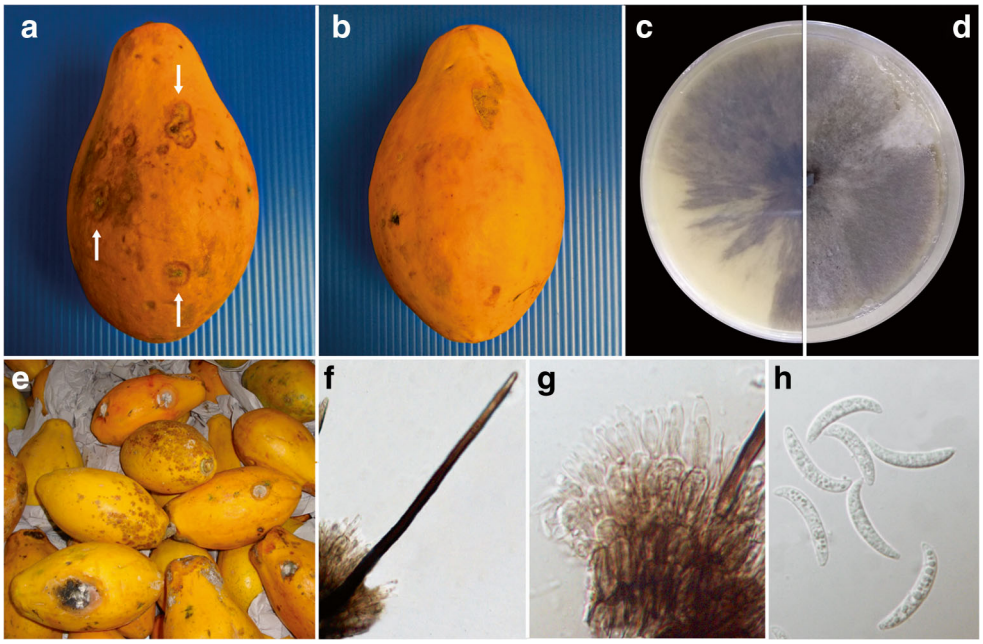

Fig. 1 Papaya anthracnose caused by *Colletotrichum truncatum*: **a** typical papaya anthracnose symptoms 4 days after inoculation, **b** negative control. *Colletotrichum truncatum* LM159 isolate: 7-day-old culture on PDA (**c** reverse view, **d** aerial view), **e** papaya fruit naturally infected by *Colletotrichum* spp., showing typical anthracnose symptoms, **f** acervuli, **g** conidiophores, **h** conidia. Bar, 10 μm

Genomic DNA was extracted from LM159 following the CTAB (cetyl trimethyl ammonium bromide) protocol described by Doyle and Doyle (1990). Partial sequences of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank accession code MK135782) and β-tubulin (TUB2, MK135783) were PCR amplified using GoTaq® Master Mix, (Promega Wisconsin, USA). Nucleotide sequences were queried against the NCBI sequence database in order to determine the species complex to which LM159 should be assigned using the BLAST algorithm. LM159 was considered to be in the same species complex as sequences with the highest sequence identity and lowest e-value. Phylogenetic analysis was performed in order to accurately assign LM159 to species. Previously published sequences of ex-type and representative isolates from species within the respective species complex were retrieved from GenBank and aligned using MEGA 7 (Kumar et al. 2016). Individual gene alignments were concatenated in SequenceMatrix v.1.8 (Vaidya et al. 2011) and a maximum likelihood phylogenetic analysis was performed. Phylogenetic analysis was carried out using RAxML 8.2.12 (Stamatakis 2014) under the GTRGAMMA model and support values estimated with 1000 bootstrapped

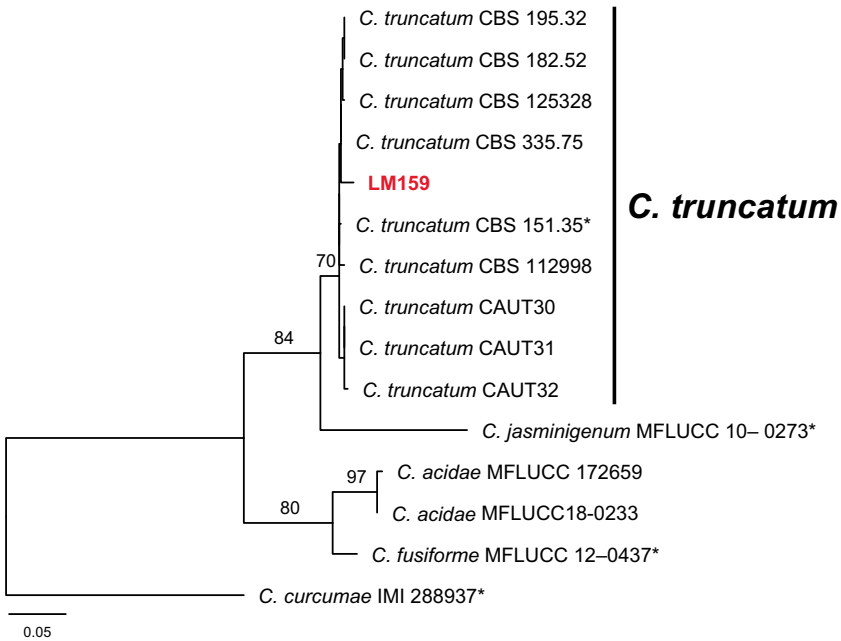

Fig. 2 Maximum likelihood tree showing relationships among *Colletotrichum* spp. from the *C. truncatum* species complex. The tree is rooted with *C. curcumae*. Bootstrap supports are shown above the branches. Isolate from *Carica papaya* is highlighted in red

pseudoreplicate datasets (-m GTRGAMMA -p 12345 -k -f a -N 1000 -x 12345).

BLAST searches reveal that the sequences of LM159 shared a high sequence identity with species in the *C. truncatum* species complex. The GAPDH sequence shared 100% sequence identity (e-value = 3e-111) with *C. truncatum sensu stricto* LJTJ45 (KP943554), whereas TUB2 sequences were 100% similar (e-value = 0) to *C. truncatum s. s.* COUFAL0018 (MG543283). The papaya isolate grouped in a clade containing the ex-type and representative isolates of *C. truncatum* with 70% support in a concatenated analysis (Fig. 2), which confirms LM159 is conspecific with *C. truncatum*. Morphological features were largely concordant with the description of *C. truncatum* with the exception of conidial length. LM159 had shorter conidia than the type of *C. truncatum* (21.8 ± 1.9 on synthetischer nährstoffarmer agar – SNA; 22.9 ± 1.6 on *Anthrischus* stem) (Damm et al. 2009). This variation may be due to the production of LM159 conidia on PDA instead of SNA or *Anthriscus* stems, or simply represents the variation in conidial dimensions within the species, which is commonly reported among isolates from the same *Colletotrichum* species.

Koch's postulates were fulfilled to confirm the pathogenicity of LM159. Fruit were washed in running water and surface sterilized in 1.5% sodium hypochlorite for 3 min, rinsed in sterile distilled water, and air dried. Ten microliters of conidial suspension (10^6 conidia/mL) were deposited at three points on the surface of three papaya fruit. The negative control was represented by fruit inoculated with 10 μL of sterile distilled water. Inoculated fruit were kept in a humid chamber for 48 h, 25 °C, and a 12-h photoperiod. The experiment was repeated 2 times. Typical anthracnose symptoms were observed on the papaya fruit four days after inoculation (Fig. 1a, b). No symptoms were observed on the negative control. The pathogen was reisolated from symptomatic fruit and was morphologically identical to LM159, fulfilling Koch's postulates.

Papaya fruit anthracnose has been associated with *C. acutatum* (Peres et al. 2002), *C. brevisporum* (Vieira et al. 2013) and *C. karstii* (Damm et al. 2009) in Brazil. *Colletotrichum truncatum* was previously reported causing papaya anthracnose in Korea (Aktaruzzaman et al. 2017), Mexico (Torrez-Calzada et al. 2018) and Trinidad and Tobago (Rampersad 2011), but to our knowledge, this is the first report of *C. truncatum* causing papaya anthracnose in Brazil. Knowledge about new *Colletotrichum* species in association with papaya anthracnose is important for disease management because different species may react differently according to the control strategies adopted.

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