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Colletotrichum gloeosporioides causes anthracnose on grapefruit (*Citrus paradisi*) in Mexico

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Abstract

The grapefruit (*Citrus paradisi*) is an important crop for citrus farming in Mexico. During June 2019, in a plantation in Iguala, Guerrero, Mexico, symptoms of anthracnose on grapefruit fruits were observed. Based on morphological and molecular characterisation, the fungus isolated from the anthracnose was identified as *Colletotrichum gloeosporioides*. Koch's postulates were verified and fulfilled. To our knowledge, this is the first report of *C. gloeosporioides* causing anthracnose on grapefruit fruits in Mexico.

Keywords Causal agent · Fungal disease · Rutaceae · Citrus fruit

The grapefruit (*Citrus paradisi*) is a subtropical citrus hybrid tree originated in Barbados resulting from an accidental cross (*C. maxima* and *C. sinensis*) (Morton 1987). The main grapefruit-producing countries are China, Mexico, South Africa, the United States, and Turkey (FAS 2019). Due to its bioactive compounds, the fruits are utilised in both the pharmaceutical and food industries (Rosa-Hernández et al. 2016). The grapefruit is an important crop for citrus farming in Mexico; 20,918 ha are in production yielding almost 420,000 metric tons of fruits (FAS 2019; SIAP 2019).

In international citrus production, diseases caused by *Colletotrichum* spp. are among the most important causes of economic losses (Lima et al. 2011; Guarnaccia et al. 2017). During June 2019, in Guerrero, Mexico, in a plantation of grapefruit (about 1.5 ha) in Iguala (18.359426 N, 99.508297 W) symptoms of anthracnose were observed in grapefruit fruits, with an estimated incidence of 15% in a sample of 120 fruits. Symptomatic fruits developed light-brown coloured lesions which varied in size, and covered over 50% of the surface of the fruits. When the disease progressed, lesions with grey aerial hyphae were observed on infected fruits (Fig. 1a). From eight fruits with symptoms, samples of approximately 0.5 cm in diameter were cut from the advance zone of the lesions. The fragments were superficially disinfested with 1.5% NaOCl for 1 min, then rinsed with sterile distilled water, dried with sterile filter paper and transferred to Potato Dextrose Agar (PDA) medium (BIOXON®), and incubated at 25 °C in the dark for seven days.

The isolated fungus developed colonies that initially had white mycelia, and later turned light grey, with aerial grayish-white mycelium. Superficial development of orange-coloured conidiomata occurred mainly in the centre of the colony (Fig. 2a). Conidia were unicellular, aseptate, hyaline, cylindrical to oblong with obtuse ends, occasionally slightly curved and measured ($n = 50$) 9.7 to 18.5 × 4.3 to 5.4 μm (length/width) (Fig. 2b). Based on morphology, the fungus was identified as belonging to the *Colletotrichum gloeosporioides* species complex (Sutton 1992; Weir et al. 2012). Representative isolates were deposited in the Culture Collection of Plant Pathogenic Fungi of the Department of Plant Physiology and Biotechnology Laboratory at the University

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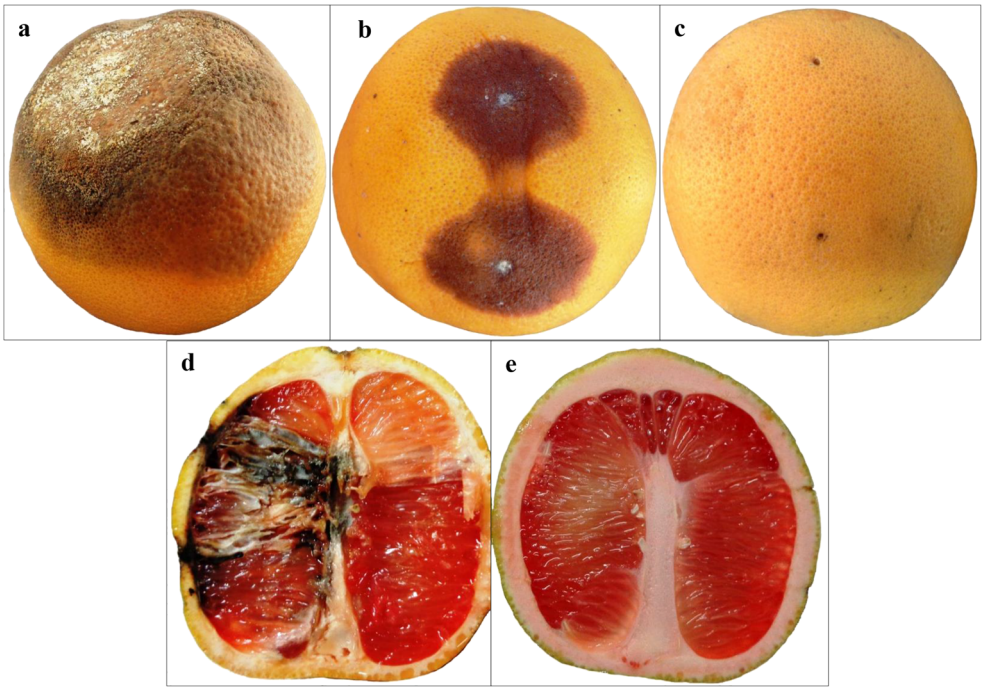

Fig. 1 Symptoms of anthracnose on grapefruit (*Citrus paradisi*) caused by *Colletotrichum gloeosporioides*. Natural symptoms of anthracnose on grapefruit fruit in field conditions (a). Symptoms induced by artificial inoculation with *C. gloeosporioides* (b). Control fruit (c). Internal lesions caused by *C. gloeosporioides* on artificially inoculated fruit (d). Control fruit (e)

Autonomous of Guerrero (state Guerrero, Mexico), under the accession number COLTOR1 and COLTOR4.

Identification of the fungus was confirmed by comparison of DNA sequences. DNA was extracted from 10-day old cultures of isolates COLTOR1 and COLTOR4 and the internal transcribed spacer (ITS) regions, and partial actin gene (ACT) were amplified with the primers ITS1/ITS4 (White et al. 1990), and ACT-512F/ACT-783R (Carbone and Kohn 1999), respectively, and sequenced by Macrogen, Inc. (Seoul, Korea). BLAST analysis of sequences in GenBank revealed that the ITS and ACT sequences of isolates had 100% identity with *C. gloeosporioides* strains (not shown).

Phylogenetic analysis was performed using sequences of ITS and ACT of representative isolates of *Colletotrichum* (Weir et al. 2012), and sequences of ex-type strains of *C. gloeosporioides* were aligned and concatenated in MEGA-X (Kumar et al. 2018). The phylogram tree was obtained using the Neighbor-Joining (NJ) method (Saitou and Nei 1987), and analysed based on the Kimura-2-parameter distance model (Kimura 1980). A bootstrap analysis was realised with 1000 replications of the data using the same program. The phylogram showed that the isolates COLTOR1 and COLTOR4 clustered with the ex-type strains of *C. gloeosporioides* (CBS 112999 and CBS 953.97) with 99% bootstrap values (Fig. 3).

A pathogenicity test was performed using the COLTOR1 isolate as representative. Asymptomatic grapefruit fruits were washed with running water, then disinfested with a 1% *v*/v sodium hypochlorite solution for 2 min followed by two rinses with sterile water, and dried with sterile filter paper. Ten

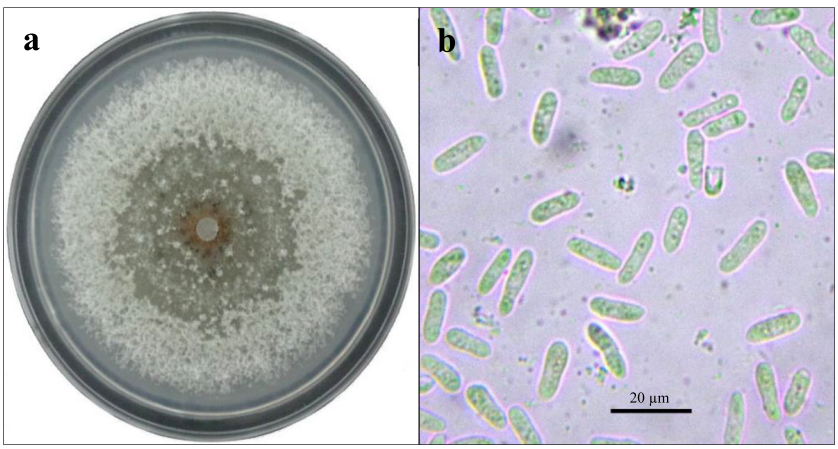

Fig. 2 Morphological characteristics of *Colletotrichum gloeosporioides* isolated from anthracnose on grapefruit (*Citrus paradisi*). Colony on PDA (a) after 7 day of incubation. Conidia (b) of *C. gloeosporioides*

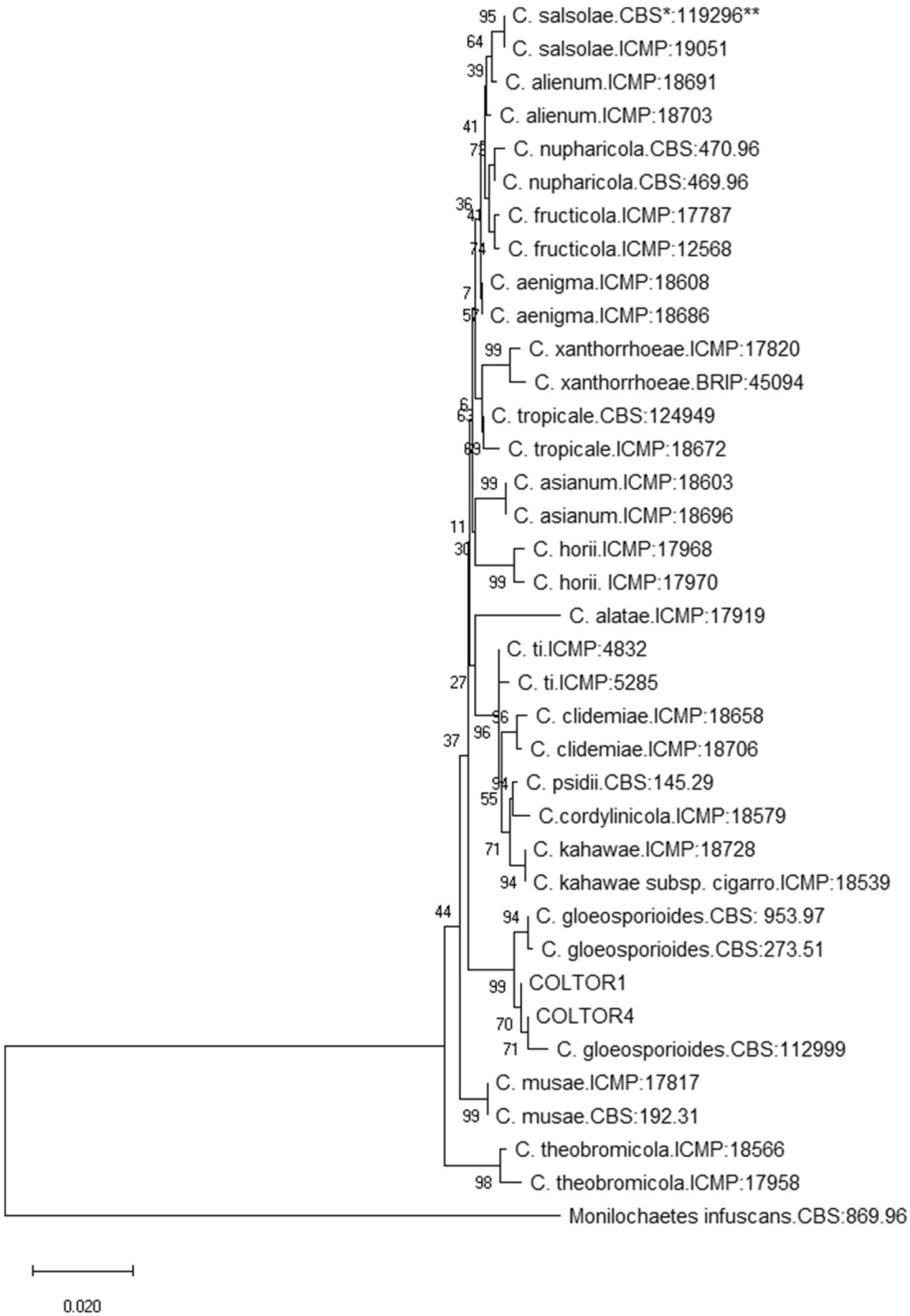

Fig. 3 Phylogram tree using Neighbor-Joining (NJ) method, with analysis based on the Kimura-2-parameter distance model. A bootstrap analysis was conducted with 1000 replications. The phylogram was constructed from a combined dataset of ITS and ACT sequences showing phylogenetic relationships among *Colletotrichum gloeosporioides* isolated from grapefruit fruits (COLTOR1 and COLTOR4), and representative species of *C. gloeosporioides* species complex. *Monilochaetes infuscans* was used as the outgroup. Species names are followed by culture collection. *CBS = Centraalbureau voor Schimmelcultures, ICMP = International Collection of Microorganisms from Plants, and BRIP = Queensland Plant Pathology Herbarium. **Culture number

grapefruit fruits were inoculated at two equidistant points/fruit. A small injury was made with a sterile toothpick (2 mm diameter), and each was inoculated with 10 µl of a spore suspension (10^6 conidia/ml). As a control, five grapefruit fruits were inoculated with sterile distilled water. Inoculated fruits were incubated in a closed chamber at 85% RH and 28 °C. Fruits of grapefruit inoculated with the COLTOR1 isolate showed typical anthracnose symptoms at all inoculation sites after eight days (Fig. 1b); these symptoms were similar to those observed in the field (Fig. 1a). Artificially inoculated grapefruit fruits developed severe internal necrosis at the inoculated point (Fig. 1d). From these artificially inoculated grapefruit fruits, the causal agent was re-isolated and identified morphologically as *C. gloeosporioides*, thus fulfilling Koch's postulates. The control grapefruit fruits showed no symptoms (Fig. 1c, e).

In Mexico, there are reports that *C. acutatum* and *C. gloeosporioides* cause devastating diseases in fruits of Mexican lime (*Citrus aurantiifolia*) (Ruiz et al. 2014; Rojo-Báez et al. 2017). In diverse countries such as Australia, Cuba, Italy, Portugal, Sierra Leone, South Africa, Spain,

Trinidad and Tobago, *C. gloeosporoides* has been reported to cause anthracnose on *C. paradisi* (Farr and Rossman 2020); but, it had not been reported in Mexico. To our knowledge, this is the first report of *C. gloeosporioides* as causing anthracnose on *C. paradisi* fruits in Mexico (Farr and Rossman 2020). Given the importance of this crop for Mexican citrus farming, this research provides valuable information for the design of effective management for this serious problem for grapefruit field producers.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Experiments do not involve human participants or animals.

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