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Colletotrichum gloeosporioides s. str., the causal agent of a leaf spot disease of *Schefflera arboricola* in Iran

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Abstract: *Schefflera arboricola* is a flowering plant in the family *Araliaceae*; its common name is dwarf umbrella tree. It is an evergreen shrub and a popular ornamental plant, commonly grown as an indoor plant. During a sampling in 2015, a new leaf spot symptoms was observed on *S. arboricola* plants in several greenhouses in the Hamedan province, Iran. Most of the plants were severely damaged by this disease. The presumed causal agent was isolated from symptomatic leaves. Based on morphological and cultural characteristics, the fungus was identified as a *Colletotrichum* species, probably belonging to the *C. gloeosporioides* species complex. By means of molecular data (*TUB2* & *GAPDH*) the fungus was revealed to be *C. gloeosporioides* sensu stricto. Pathogenicity tests showed that the fungus is the causal agent of leaf spot on *S. arboricola* shrubs. To our knowledge, this is the first report of *C. gloeosporioides* sensu stricto on *S. arboricola* in Iran.

Key words: *β-tubulin*, *GAPDH*, ornamental plant, pathogenicity, *Araliaceae*

INTRODUCTION

Schefflera arboricola Hayata is a flowering plant in the family *Araliaceae*; its common name is dwarf umbrella tree. It is an evergreen shrub and a popular ornamental plant, commonly grown as an indoor plant, because of its tolerance to unfavorable growing conditions (Ohashi 1993, Xiang & Lowry 2013). Moreover, *Schefflera* spp. are often infected by fungi that cause destructive leaf spot diseases, such as *Alternaria* and *Colletotrichum* spp. (Atilano 1983, Li et al. 2017). In China, typical anthracnose symptoms were observed on the young and mature leaves of *Schefflera actinophylla* (Haung 2013). During a sampling in 2015, symptoms of brown leaf spots were observed on *S. arboricola* plants in several greenhouses in the Hamedan province, Iran. The symptoms of the disease initially appeared as small, round, water-soaked lesions. As the disease progressed, lesions rapidly enlarged and coalesced. Numerous brownish-black acervuli were produced in concentric rings on these lesions. Most of these *S. arboricola* plants were severely damaged by this disease. Our aim was to identify the causal agent of this leaf spot disease on *S. arboricola* plants.

MATERIALS AND METHODS

Sampling, isolation and identification

Symptomatic leaves were collected from different greenhouses in Hamedan province, Iran. Small leaf pieces were cut from the transition zone between diseased and healthy leave tissues, surface-sterilized with 1 % (w/v) sodium hypochlorite for 1 min, rinsed three times with sterile water, dried on sterile filter paper, transferred to PDA in Petri dishes and incubated at 25 ºC in the dark. Single conidial isolates were prepared by the method of Ho & Ko (1997) and cultivated on PDA.

All isolates were assessed morphologically using an Olympus microscope (AX70TRF, Olympus Optical, Tokyo, Japan). Colony characteristics, as well as the size and shape of conidia, were recorded after seven days. The dimensions of conidia were calculated based on 30 measurements. As cultural and morphological characters of the isolates were very similar to each other, one isolate (IRAN 2628C) was selected as a representative for molecular analysis and pathogenicity tests. The isolate deposited at the Iranian Research Institute of Plant Protection Culture Collection (Iran).

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Pathogenicity test

Pathogenicity tests were conducted under greenhouse conditions using two methods (Huang 2013). Three 1-year-old *S. arboricla* plants were inoculated with PDA plugs (0.5 cm diam) grown with the IRAN2628C isolate that were placed on 0.5-cm^2 leaf wounds and then wrapped with Parafilm. Control leaves were inoculated with PDA plugs without the fungus. Pathogenicity was also tested by spraying leaves of potted *S. arboricla* plants with 10 ml of a conidial suspension (1×10^6 conidia.ml^{-1}) prepared from 7-day-old PDA cultures. The leaves which sprayed with sterilized distilled water were used as controls. Three plants were inoculated in each of the two experiments. The inoculated plants were incubated at 25 °C ± 2 °C and 90% relative humidity in a growth chamber under light with a 12 h photoperiod.

Molecular analyses

Genomic DNA was extracted from mycelium grown on PDA as described by Sharma et al. (2002). A partial sequence of the β-tubulin gene (*TUB2*) and a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) of IRAN2628C isolate were amplified using the primer pairs T1 (O'Donnell & Cigelnik 1997) and Bt-2b (Glass & Donaldson 1995) and GDF1 and GDR1 (Guerber et al. 2003), respectively. The PCR reactions were performed in a TC-512 thermocycler (Techne, Germany) in a total volume of 25 µl. The PCR reactions contained 10 ng of genomic DNA, 1 µM of each primer, 0.2 mM of dNTPs (CinnaGen, Iran), 2.5 µL 10X PCR buffer, 2.5 mM $MgCl_2$ and 1 U Taq DNA polymerase (CinnaGen, Iran). PCR conditions for *TUB2* and *GAPDH* included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were visualized by agarose gel electrophorese and sequenced by Bioneer Company (South Korea). The sequences were deposited in GenBank, under accession numbers. Clustal W (Thompson et al. 1994) was used for sequence alignments. A phylogenetic tree was constructed in MEGA6 (Tamura et al. 2013) using the neighbor-joining (NJ) method (Saitou and Nei 1987) with the Kimura-2-parameter distance model (Kimura 1980) based on the concatenated *β-tubulin* and *GAPDH* sequences of the isolate IRAN2628C and *Colletotrichum* reference sequences obtained from GenBank. Confidence values for individual branches were determined by 1000 replication bootstrap analyses (Felsenstein 1985).

RESULTS

In this study, 24 isolates were obtained from the symptomatic leaves. The examined isolate (IRAN 2628C) produced white aerial mycelium on PDA that turned gray to grayish black with age and orange conidial masses. Conidia were hyaline, aseptate, straight, subcylindrical, slightly constricted in the middle, rounded at each end and measured 13–17 × 4–5.5 µm (Fig. 1). Morphological characteristics of these isolates were similar to *C. gloeosporioides s. str.*, but also to several other species belonging to the *C. gloeosporioides* complex (Weir et al. 2012).

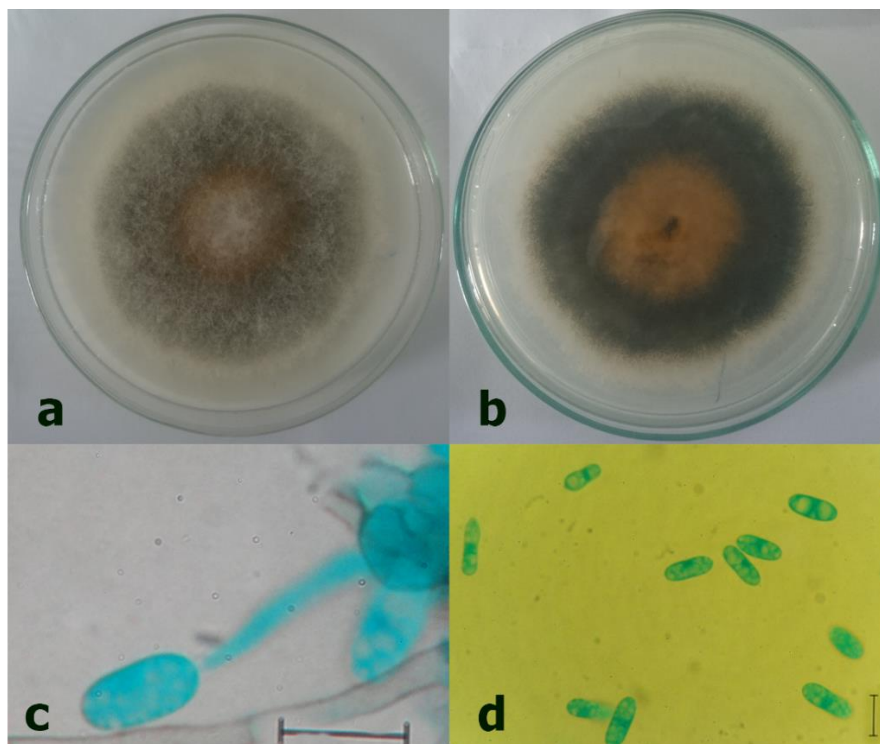

Fig. 1. *Colletotrichum gloeosporioides* IRAN 2628C after 7 days on PDA. **a.** surface of colony; **b.** underside of colony; **c.** Conidiophore. —Scale bar = 100 µm; **d.** conidia —Scale bar = 10 µm.

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Blastn searches on NCBI GenBank (www.ncbi. nlm.nih.gov/ genbank/) showed that the *TUB2* (KY271052, 426 bp), and *GAPDH* (KY271051, 266 bp) sequences of isolate IRAN 2628C were 99% (query cover: 100%, one nucleotide difference to GQ849434) and 100% (query cover: 100%, JX010056), respectively, identical with those of IMI 356878, the ex-type strain of *C. gloesporioides s. str.* (Cannon et al. 2008). The phylogeny analysis showed that isolate IRAN2628C, obtained from the leaf spot on *Schefflera arboricola*, clustered with authentic isolates of *C. gloeosporioides* (Fig. 2).

With the first method of pathogenicity tests, foliar lesions were formed after seven days on inoculated leaves closely resembling those observed on naturally infected leaves. With the second method, seven days after inoculation, tiny brown spots started to develop on all inoculated leaves. The progression of symptom development was similar to that observed on naturally infected leaves. With both methods, no symptoms developed on control leaves, and the inoculated fungus was consistently re-isolated from infected leaves (Fig. 3).

DISCUSSION

There are several previous reports of *Colletotrichum* species from *Schefflera* spp., including *C. karstii*, *C. gloeosporioides* and *C. siamense* (e. g. Huang 2013, Li et al. 2017). *Colletotrichum gloeosporioides* was previously reported from *S. actinophylla* and *S. arboricola* in China, *S. arboricola* in South Korea and Japan and *S. elliptica* in Indonesia (Chi et al. 2016, Farr et al. 2017, Huang 2013, Kim et al. 1991, Sato et al. 2003, Xi et al. 2000). However, while *C. karstii* and *C. siamense* had been identified by means of multi-locus sequence data, the reports of *C. gloeosporioides* are based on morphology or ITS sequences only. Due to a previous revision of the genus *Colletotrichum* by von Arx (1957), who synonymized about 600 species in *C. gloeosporioides*, the circumscription of this species had been very blurred and *C. gloeosporioides* was regarded as occurring worldwide on nearly every host. Therefore, species identified by morphology as *C. gloeosporioides* in the past could refer to various species. However, after the epitypification of the species by Cannon et al. (2008) and the comprehensive study on the *C. gloeosporioides* species complex by Weir et al. (2012), we know that host range and distribution of *C. gloeosporioides* s. str. are narrower than previously assumed. For this reason, generally all reports of *C. gloeosporioides* before 2012 or those based on morphological or ITS sequence data only are not reliable. *Colletotrichum gloeosporioides* s. str. can be identified based on all loci analyzed in the study of Weir et al. (2012) including *TUB2*, *GAPDH* and ITS. By means of comparison of the ITS sequences with those of ex-type strains in GenBank, we revealed that the causal organism of leaf spot diseases of *Schefflera* in the studies of Chi et al. (2016), Huang et al. (2013) and Kim et al. (1991) was not *C. gloeosporioides* s. str.; the ITS sequences were 100 % identical with those of other species in the *C. gloeosporioides* species complex. The strain in the study of Sato et al. (2003) had later been re-identified as *C. tropicale* by T. Sato based on multi-locus sequence data (http://www.gene. affrc.go.jp/databases micro_search_en.php). In contrast, based on morphology and *TUB2* and *GAPDH* sequences data, the isolated fungus from leaf spot symptoms of *S. arboricola* in this study was identified as *C. gloeosporioides* s. str. This is the first report of a leaf spot disease of *S. arboricola* caused by *C. gloeosporioides* s. str. in Iran.

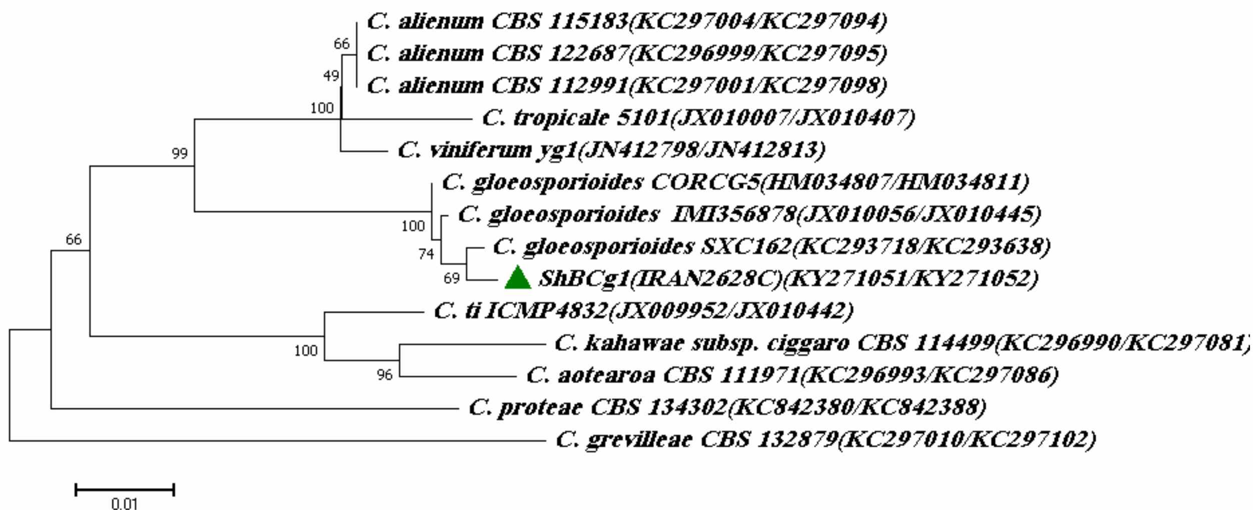

Fig. 2. The optimal neighbor-joining phylogenetic tree based on concatenated *β-tubulin* and *GAPDH* sequences of *Schefflera* isolate IRAN2628C and other *Colletotrichum* isolates obtained from GenBank. The GenBank accession numbers are given in parentheses behind the species names and strain numbers. Numbers at the nodes are the bootstrap values obtained for 1000 replicates. The tree is rooted to *C. grevilleae* CBS 132879.

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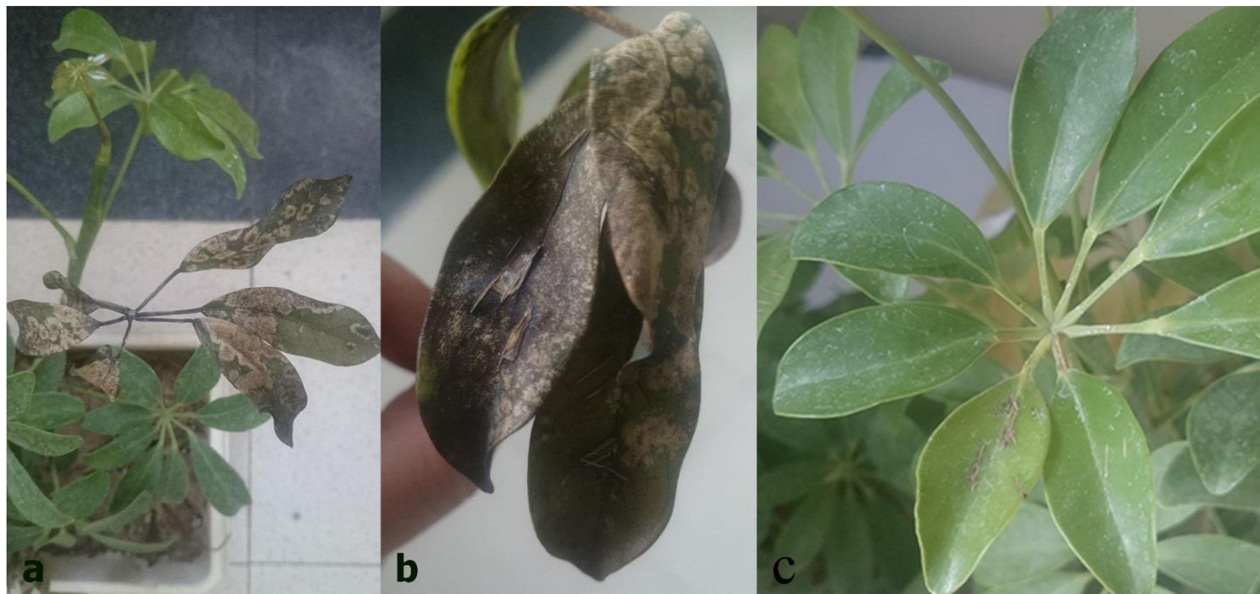

Fig. 3. Leaf spot caused by *Colletotrichum gloeosporioides* s. str. on *Schefflera arboricola*.**a.** after natural infection; **b.** in greenhouse seven days after inoculation with *C. gloeosporioides*; **c.** control plant .

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قارچ Colletotrichum gloeosporioides sensu stricto عامل بیماری لکه برگی *Schefflera arboricola* در ایران

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چکیده: گونه *Schefflera arboricola* گیاهی گلدار از خانواده *Araliaceae* با نام رایج درخت چتری است .شفلرا گیاهی درختچه_ای، همیشه سبز و زینتی است که معمولا به عنوان یک گیاه خانگی رشد می کند. طی نمونه‌برداری در سال ۱۳۹۴، علائم لکه برگی روی گیاهان شفلرا در چندین گلخانه استان همدان مشاهده شد. اکثر گیاهان به شدت از این بیماری آسیب دیده بودند. عامل احتمالی آن از برگ‌های دارای علائم جدا شد. براساس مورفولوژی و ویژگی‌های کشت، قارچ جدا شده به عنوان یکی از گونه‌های *Colletotrichum* شناسایی شد که احتمالا متعلق به کمپلکس گونه ای *C. gloeosporioides* بود. با استفاده از داده های مولکولی (توالی نواحی ژنی *TUB2* و *GAPDH*) قارچ به عنوان *C. gloeosporioides* sensu stricto شناسایی شد. آزمون‌های بیماری‌زایی نیز نشان داد که عامل بیماری لکه برگی در درختچه‌های *S. arboricola* قارچ *C. gloeosporioides* sensu stricto می‌باشد. با توجه به دانش ما، این اولین گزارش از وجود این قارچ روی *S. arboricola* در ایران است.

کلمات کلیدی: *β-tubulin*، *GAPDH* ، گیاه زینتی، بیماری زایی، *Araliaceae*

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