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RESEARCH NOTE

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Occurrence of Cercospora Leaf Spot Caused by *Cercospora* cf. *flagellaris* on Melon in Korea

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ABSTRACT

In 2016, a cercosporoid fungus was found from leaf spot symptoms on melon in Korea. The fungus isolated from the plant was identified based on morphological characteristics and sequence analyses of five genes (ITS rDNA, translation elongation factor 1-α, actin, calmodulin, and histone H3). The fungal isolate was found to be pathogenic to melon. The results confirm that the fungus associated with leaf spot on melon was *Cercospora* cf. *flagellaris*. This is the first report of *Cercospora* cf. *flagellaris* causing Cercospora leaf spot on melon in Korea.

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Cucurbits, plants belonging to the Cucurbitaceae family, have been cultivated for edible purposes or grow wild throughout the world. Several cucurbits, such as watermelon, cucumber, melon, and pumpkin, are economically important crops cultivated in Korea. In 2018, 29,508 ha were used for cucurbit production, occupying about 10% of the total area used for vegetable production in Korea [1]. Among cucurbits, melon (*Cucumis melo* L.) is one of the most popular tropical fruits and is cultivated for its juicy and sweet taste worldwide. The cultivated area of melon in Korea has fluctuates slightly each year; however, the average area over the last decade (2009–2018) was about 1530 ha [1].

In November 2016, circular leaf spots were observed on the leaves of melon in a greenhouse located in Gochang, Korea (35°21′02.6″N, 126°32′58.8″E) (Figure 1(A)). Initially, small pale brown spots with a yellow halo were observed on the leaves; later, they coalesced to from larger irregular dark brown lesions (Figure 1(B)). The centers of the lesions became grayish white. As the disease progressed, the diseased leaves finally wilted and dried (Figure 1(C)). About 80–90% of plants presented these symptoms. The identity of the disease-causing agent was determined based on morphological characteristics, molecular analyses, and a pathogenicity test.

Small sections of leaf tissue were excised from lesions and surface-sterilized by dipping in 70% ethanol for 3 min and 1% sodium hypochlorite for 1 min, after which they were rinsed in sterile distilled water. To isolate the causal agent, the leaf tissues were placed on potato dextrose agar (PDA) plates and incubated at 25 °C. Mycelia growing out from the plant tissues were subcultured on fresh PDA plates. All cultures showed same colony morphology, and one representative fungal isolate (16–525) was selected for use in subsequent experiments. The culture was deposited in the Korean Agricultural Culture Collection as KACC 48922. Morphological features of fungal structures formed on fresh plant materials were examined and photographed using a Zeiss AXIO Zoom V16 and AXIO Imager A2 microscopes equipped with AxioCam 506 color (Carl Zeiss, Oberkochen, Germany). Colonies on the PDA were pale pinkish to light gray, with cottony aerial mycelium, and reached approximately 65 mm diameter at 25 °C after 10-day incubation (Figure 2(D)). Morphologically, stromata were poorly developed, consisting of brown hyphal cells, and were 3–10 µm in size (Figure 2(A)). Conidiophores were fasciculate, olivaceous brown, paler toward the apex, straight to slightly curved, 3–15-septate, 50–250 × 3–5 µm (Figure 2(B)). Conidia were hyaline, acicular to cylindric, truncate to subtruncate at the base, 3–17-septate, and 40–200 × 3–5 µm (Figure 2(C)). The morphological characteristics of the causal fungus were consistent with the description of *Cercospora flagellaris* Ellis & G. Martin [2–4].

Multi-gene sequence analysis was performed to identify the fungal species. An aerial mycelium

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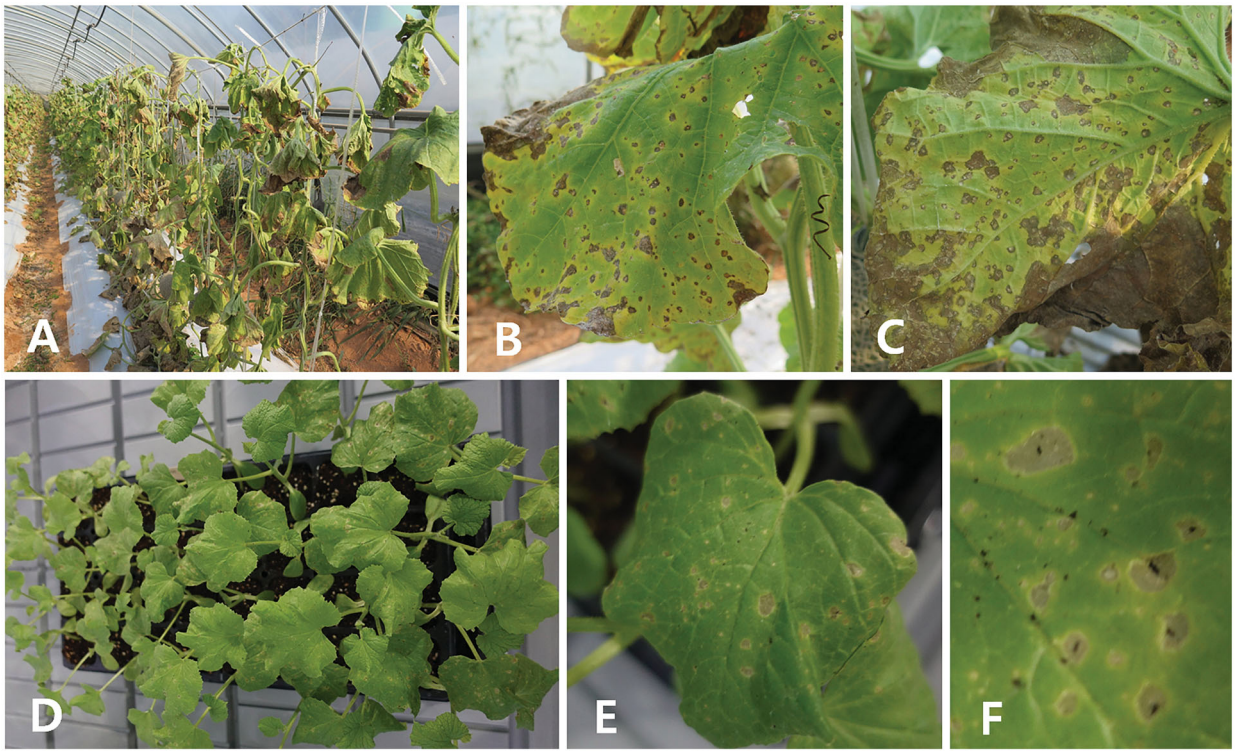

Figure 1. Cercospora leaf spot disease caused by *Cercospora* cf. *flagellaris* on melon. (A) Occurrence of Cercospora leaf spot disease on melon plants cultivated in a farm. (B,C) Leaf spot lesions on upper (B) and lower (C) sides of leaves. (D) Melon seedlings with leaf spot symptoms seven days after inoculation. (E) Symptom appearing on inoculated plant. (F) Close-up of lesions formed on young leaves of melon plant.

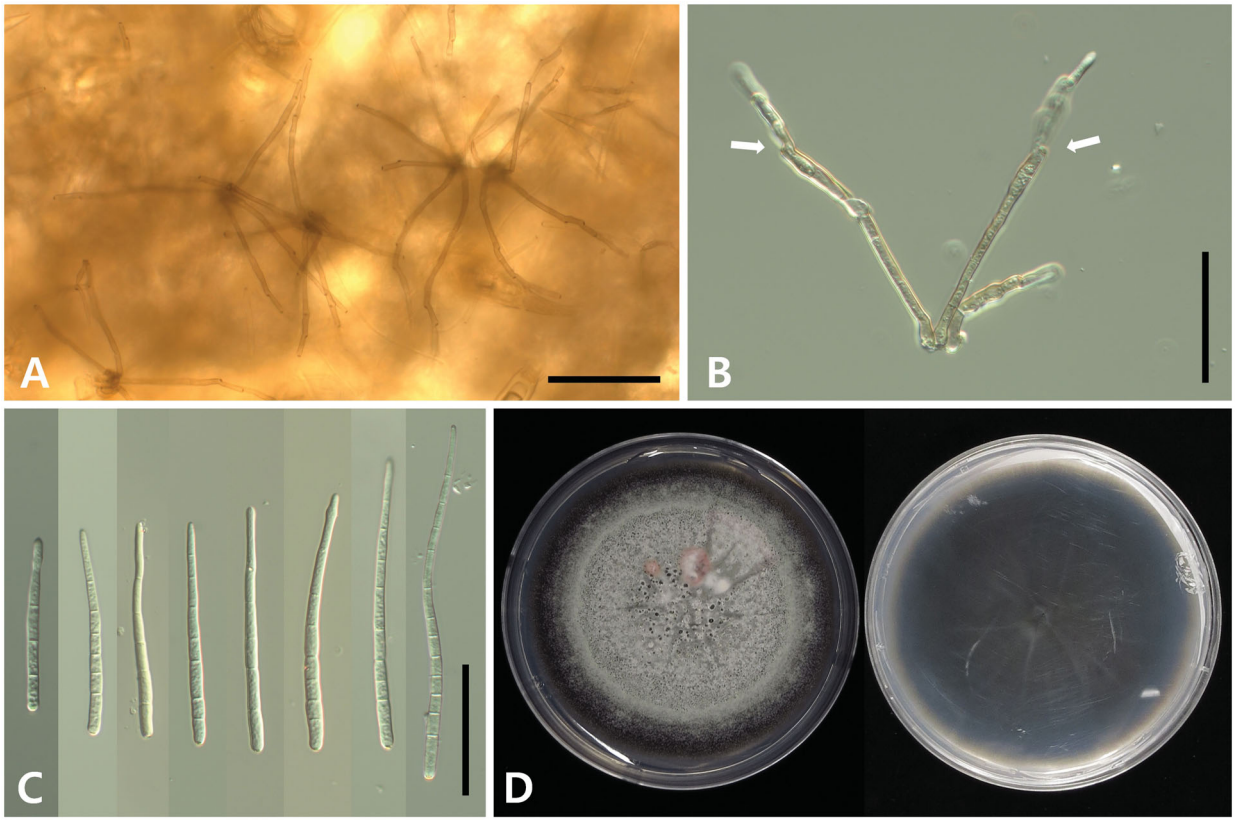

Figure 2. Morphological and cultural features of *Cercospora* cf. *flagellaris* causing leaf spot on melon. (A) Stromata. (B) Conidiophores (arrows indicate conidiogenous loci). (C) Conidia. (D) Upper and reverse sides of colony grown on PDA after incubation for 10 days. Scale bars: A–C = 50 μm.

Table 1. Information on sequence data of *Cercospora* cf. *flagellaris* analyzed in this study.

Isolate No.	Host Speceis	Host Family	Country	GenBank Accession No.				
				ITS	TEF	ACT	CAL	HIS
KACC 48922	*Cucumis melo*	Cucurbitaceae	Korea	MN945227	MN945228	MN945229	MN945230	MN945231
CBS 132648	*Amaranthus patulus*	Amaranthaceae	Korea	JX14302	JX143360	JX143114	JX142868	JX142622
CPC 5441	*Amaranthus* sp.	Amaranthaceae	Fiji	JX143611	JX143370	JX143124	JX142878	JX142632
CBS 143.51	*Bromus* sp.	Poaceae		JX143607	JX143365	JX143119	JX142873	JX142627
CBS 132667	*Celosia argentea* var. *cristata*	Amaranthaceae	Korea	JX143604	JX143362	JX143116	JX142870	JX142624
CBS 132646	*Cichorium intybus*	Asteraceae	Korea	JX143601	JX143359	JX143113	JX142867	JX142621
CCTU 1162	*Citrullus lanatus*	Cucurbitaceae	Iran	KJ886496	KJ886335	KJ886013	KJ885852	KJ886174
CBS 115482	*Citrus* sp.	Rutaceae	South Africa	AY260070	DQ835095	DQ835114	DQ835141	DQ835168
CPC 4411	*Citrus* sp.	Rutaceae	South Africa	AY260071	DQ835098	DQ835118	DQ835145	DQ835172
MUCC 127	*Cosmos sulphureus*	Asteraceae	Japan	JX143612	JX143371	JX143125	JX142879	JX142633
CCTU 1029	*Cucurbita maxima*	Cucurbitaceae	Iran	KJ88640	KJ886299	KJ885977	KJ885816	KJ886138
CCTU 1136	*Cucurbita pepo*	Cucurbitaceae	Iran	KJ886478	KJ886317	KJ885995	KJ885834	KJ886156
CBS 132653	*Dysphania ambrosioides*	Chenopodiaceae	Korea	JX143603	JX143361	JX143115	JX142869	JX142623
CBS 113127	*Eichhornia crassipes*	Pontederiaceae	USA	DQ835075	AF146147	DQ835121	DQ835148	DQ835175
MUCC 735	*Hydrangea serrata*	Hydrangeaceae	Japan	JX143613	JX143372	JX143126	JX142880	JX142634
MUCC 831	*Hydrangea serrata*	Hydrangeaceae	Japan	JX143614	JX143373	JX143127	JX142881	JX142635
CBS 132674	*Phytolacca americana*	Phytolaccaceae	Korea	JX143606	JX143364	JX143118	JX142872	JX142626
CPC 10124	*Phytolacca americana*	Phytolaccaceae	Korea	JX143608	JX143366	JX143120	JX142874	JX142628
CPC 10684	*Phytolacca americana*	Phytolaccaceae	Korea	JX143610	JX143369	JX143123	JX142877	JX142631
CPC 1051	*Populus deltoides*	Salicaceae	South Africa	AY260069	JX143367	JX143121	JX142875	JX142629
CBS 132670	*Sigesbeckia pubescens*	Asteraceae	Korea	JX143605	JX143363	JX143117	JX142871	JX142625
CBS 132637	*Trachelium* sp.	Campanulaceae	Israel	JX143600	JX143358	JX143112	JX142866	JX142620

scraped from a 7-day-old culture was used to extract genomic DNA. Sequences of five genes; the internal transcribed spacer (ITS) region including 5.8S rDNA, translation elongation factor 1-α (TEF), actin (ACT), calmodulin (CAL), and histone3 (HIS), were amplified and sequenced using the primer pairs described by Groenewald et al. [4]. The sequences derived from this study were registered in GenBank (Table 1). Reference sequences of *Cercospora* spp., including *C.* cf. *flagellaris*, were downloaded from GenBank (Table 1), and used to construct a phylogenetic tree. A neighbor-joining (NJ) tree was generated based on a concatenated five-locus dataset using MEGA7 [5] (Figure 3). *Septoria provencialis* (CBS 118910) was used as an outgroup. Phylogenetic analysis revealed that the present isolate from melon formed a well-supported clade together with isolates of *C.* cf. *flagellaris* obtained from diverse host plants with a bootstrap value of 98%.

The pathogenicity of the present isolate from melon was tested in a glasshouse on melon seedlings. The leaves of young plants at the second-leaf stage were spray-inoculated with mycelial suspension from the fungal isolate following growth on PDA for 10 days. Plants without fungal inoculum served as the control. After inoculation, plants were sealed in plastic bags, transferred to a growth chamber at 25 °C, and maintained for 48 h. Leaf spot symptoms appeared on the inoculated plants 7 days after inoculation (Figure 1(D–F)). The symptoms were not visible on non-inoculated control plants. The fungus was re-isolated from the symptomatic tissues of inoculated plants. A pathogenicity test revealed that the present isolate was pathogenic to melon seedlings, thus fulfilling Koch's postulates.

The genus *Cercospora* includes important phytopathogens that cause leaf spot diseases on many host plants worldwide [6,7]. Currently, polyphasic approaches based on ecology, morphology, cultural characteristics, and molecular phylogeny, are used to identify *Cercospora* species following the consolidated species concept [8]. Multi-gene phylogeny inferred from the sequence data of five genes (ITS rDNA, TEF, ACT, CAL, and HIS) has been used to identify and delimit *Cercospora* species [4,9–13]. More recently, three genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase II second largest subunit (RPB2), and β-tubulin (TUB), were found to be useful for improving the phylogenetic resolution of *Cercospora* species complexes, including *C. apii*, *C. armoraciae*, *C. beticola,* and *C.* cf. *flagellaris* [14]. Members of the *C.* cf. *flagellaris* species complex were phylogenetically separated into three distinct clades but were indistinguishable by morphology or host range [14].

C. cf. *flagellaris* remains an unresolved species complex. It has broad host ranges and has been associated with members of more than 20 plant families including Cucurbitaceae members [4,13,14]. *C.* cf. *flagellaris* has been identified around the world, except in European countries [4,14,15]. In Asian countries, the fungus has been reported as a plant pathogen from Korea, China, and Japan [15]. In Korea, seven plant hosts of *C.* cf. *flagellaris* have been found; *Amaranthus patulus* (Amaranthaceae), *Celosia argentea* var. *cristata* (Amaranthaceae), *Cichorium intybus* (Asteraceae), *Siegesbeckia pubescens* (Asteraceae), *Dysphania ambrosioides* (Chenopodiaceae), *Phytolacca americana,* and *P. esculenta* (Phytolaccaceae) [3,4,16]. However, there have been no previous records of leaf spot associated with *C.* cf. *flagellaris* on *Cucumis melo*

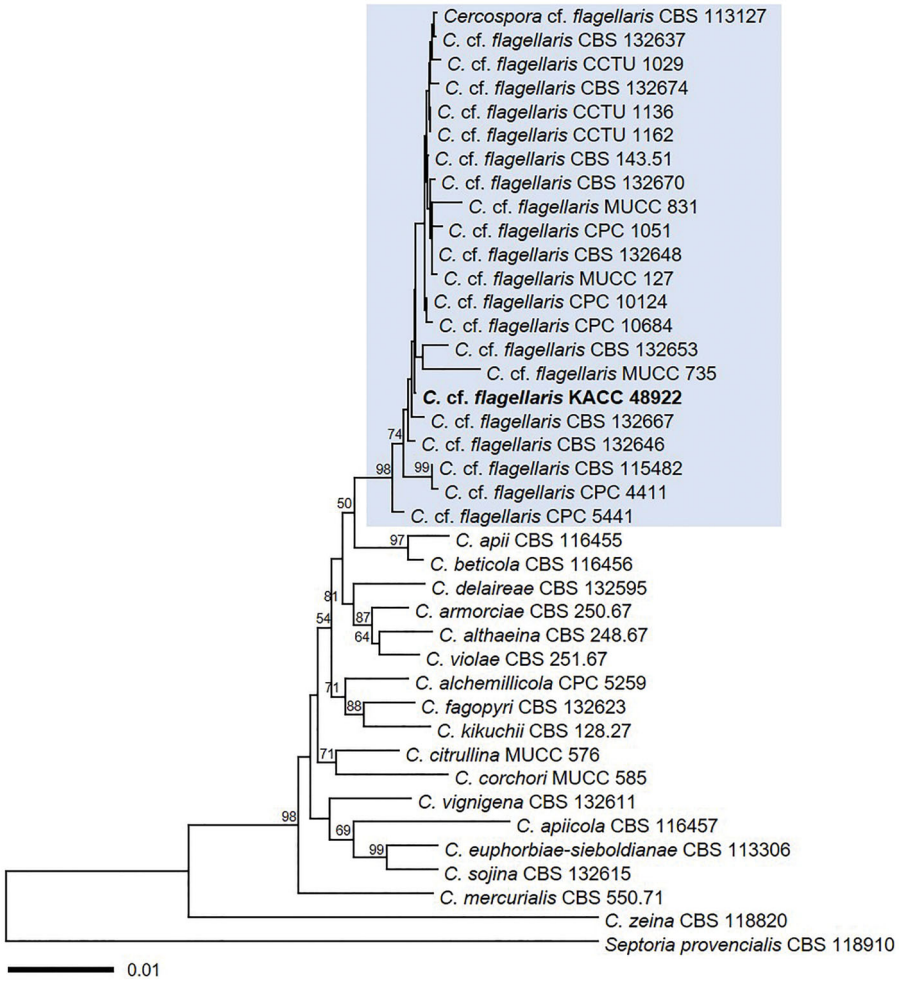

Figure 3. A neighbor-joining tree based on the concatenated alignment of sequence data of five genes, ITS rDNA, translation elongation factor 1-a, actin, calmodulin and histone H3, showing phylogenetic affinities of one isolate obtained from this study with other members of *Cercospora* cf. *flagellaris*. *Septoria provencialis* was designated as outgroup. Isolate in boldface was sequenced in this study. Bootstrap values above 50% are shown at the nodes. The scale bar represents 0.01 nucleotide substitutions per site.

(Cucurbitaceae) in Korea or other countries. As cucurbitaceous plants, *Citrullus lanatus*, *Cucurbita maxima*, *Cucurbita pepo*, and *Ecballium elaterium* have been recorded as plant hosts infected by the fungal pathogen in Iran [13,14]. Therefore, this is the first report of Cercospora leaf spot on melon caused by *C.* cf. *flagellaris* in Korea.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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