

First Report of Anthracnose-twister disease of Welsh onion caused by *Colletotrichum siamense* in Taiwan

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Abstract

A new disease of Welsh onion (*Allium fistulosum* L.) occurred seriously in Taiwan. The symptoms included brown necrotic spots of leaves, yellowing and blight of outer leaves, twisting and curling of new leaf, stem rot, and plant death. Three isolates (col-377, col-387 and col-413) were collected and identified as *Colletotrichum siamense* based on the morphology and molecular characteristics. The fungus caused the same leaf spot and twisting of Welsh onion after inoculation and was re-isolated from the diseased tissue. This is the first report of *C. siamense* causing anthracnose-twister disease on Welsh onion in Taiwan.

full text

Welsh onion (*Allium fistulosum* L.)- also named green onion, long green onion, bunching onion, Japanese bunching onion, and spring onion- is one of the most important vegetable crops in Taiwan. In July 2022, severe symptoms and high incidence of a Welsh onion anthracnose disease were attributable to the infection by *Colletotrichum* spp. in the central Taiwan- Taichung city, Changhua county and Yunlin county. The symptoms included brown necrotic spots of leaves, yellowing and blight of outer leaves, especially twisting and curling of new leaf, stem rot, and plant death (Fig. 1a-c).

Symptomatic (leaf spot and stem rot) plants were collected, and small pieces (5×5 mm²) of diseased tissue were cut and surface-sterilized with 0.5% sodium hypochlorite solution for seconds, then rinsed two times with sterilized distilled water, and transferred to potato dextrose agar (PDA, Difco, USA) plates or water agar (WA, Bacto Agar, Difco, USA) plates and incubated at ambient temperature (28 ± 2°C) in the laboratory. One fungus was constantly isolated from the diseased tissue, and the hyphae tip was cut and then cultured on PDA. Three isolates (respectively from three city or counties mentioned above) were selected as representative for morphological characterization, multilocus phylogenetic analysis, and pathogenicity tests. Colonies grow on PDA at 24°C were cottony, dense, with grayish white aerial mycelium and orange conidial masses (Fig. 1d). Conidia were cylindrical, hyaline, with both ends rounded (Fig. 1e), measuring (n = 40) 10.0–16.1 (13.2) × 3.4–5.6 (4.4) µm. Appressoria were ovoid, brown (Fig. 1f).

For the further identification, total DNA of the selected isolates was extracted, and fragments of internal transcribed spacer (ITS), were amplified using primer pair ITS5/ ITS-4 (White et al. 1990), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with GDR/GDF (Templeton et al. 1992), actin (ACT) with ACT-512F /ACT-783R (Carbone and Kohn 1999), and β-tubulin (TUB2) gene with T1/Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). The amplified fragments were sequenced by Tri-I Biotech Inc., Taipei, Taiwan. The assembled sequences were submitted to GenBank data-base, including isolates (col-377, col-387, col-413) with accession numbers OQ244425-OQ244427 (ITS), OQ266433-OQ266435 (GAPDH), OQ266436-OQ266438 (ACT), OQ266439-OQ266441 (TUB2). A BLAST search analyses showed that they all had over 99% identity to the ex-type strain of *C. siamense* isolate ICMP18578 (GenBank accession nos. JX010171, JX009924, FJ907423, and JX010404) (Liu et al. 2022; Weir et al. 2012). A phylogenetic

tree was constructed with MEGA11 based on neighbor-joining analysis for species showed that they belong to the species- *C. siamense* (*C. gloeosporioides* species complex) (Fig. 2).

For pathogenicity tests of these isolates, potted Welsh onion were inoculated by spraying on plants with a conidial suspension (10^6 conidia ml^{-1} from a 28-day-old culture) from each isolate and the treatment with sterile distilled water as the blank control. Plants were covered with plastic bags for 48hr and maintained at $28 \pm 2^\circ\text{C}$ with a 12h photoperiod in a growth chamber. Typical symptoms (brown necrotic spots) of anthracnose were induced 5 days post-inoculation, while the unusual symptoms (twisting and curling of new leaf, stem rot and plant death) of the inoculated plants were observed 14–21 days post-inoculation (Fig. 1g-i). No symptoms were observed in the blank treatment. *Colletotrichum siamense* was re-isolated from the inoculated symptomatic plants, completing Koch's postulates.

Anthrachnose-twister disease of onion (*Allium cepa* L.) caused by *C. siamense* has been reported in many countries (Chowdappa et al. 2015; Herath et al. 2021; Lopes et al. 2021); however, to our knowledge, this is the first report of *C. siamense* causing anthrachnose-twister disease of Welsh onion not only in Taiwan but also in the world.

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Figures

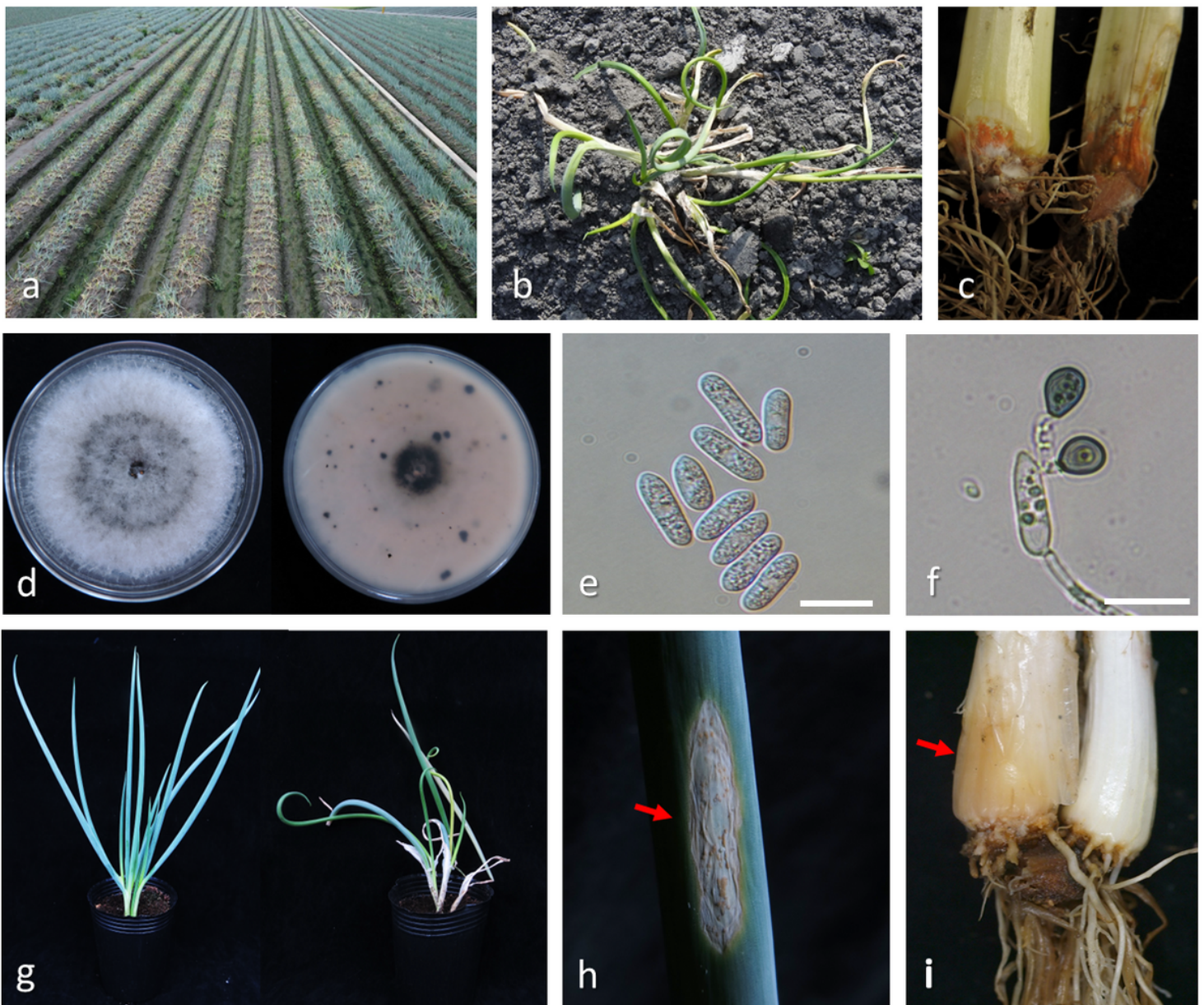


Figure 1

Symptoms of anthracnose-twister disease of Welsh onion, and the morphology of the pathogen, *Colletotrichum siamense*. **a** Anthracnose symptoms on Welsh onion in the field. **b** Twisting and curling of new leaf. **c** Stem rot. **d** Colony on PDA. **e** Conidia. **f** appressoria. **g-i** Potted Welsh onion plants presented similar symptoms after artificial inoculation with conidial suspension. Scale bar 20µm.

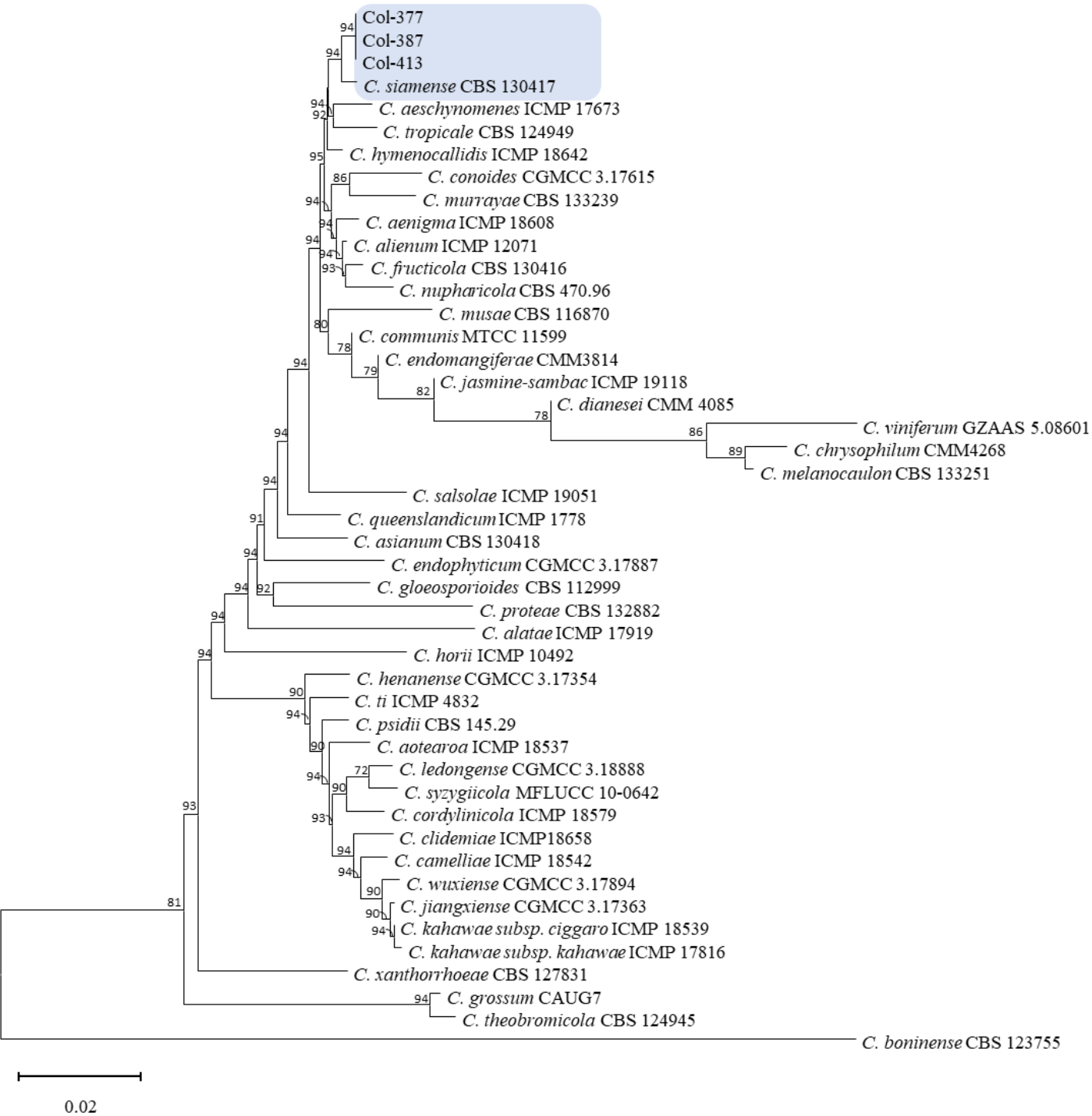


Figure 2

Neighbor-joining phylogenetic tree of the concatenated dataset (ITS, GAPDH, ACT and TUB2) showing phylogenetic relationship among *Colletotrichum gloeosporioides* species complex. *Colletotrichum boninense* was used as the outgroup.