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Choanephora rot caused by *Choanephora cucurbitarum* on *Brassica chinensis* in Thailand

Chaninun Pornsuriya[1] · **Thanunchanok Chairin**[1] · **Narit Thaochan**[1] · **Anurag Sunpapao**[1]

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Abstract In July 2016, wet rot was observed on leaves of *Brassica chinensis* in a private greenhouse in Hatyai city, Songkhla province, Thailand. The fungal pathogen infected young and expanded leaves. The fungus was identified by morphological characterization and molecular analysis as *Choanephora cucurbitarum*. Pathogenicity test revealed that *C. cucurbitarum* could infect *B. chinensis*.

Keywords Molecular · Morphology · Pak-Choi · Pathogenicity

Brassica chinensis (Pak-Choi) is a type of Chinese cabbage in the family Brassicaceae, which do not form a head. The chinensis variety of *Brassica* is widely grown in southern China and Southeast Asia for use as vegetable in foods. It has smooth dark green leaves. A 100 g portion of fresh *B. chinensis* supplies 13 cal and is rich in vitamins A, C and K (Noia 2014). Several diseases have been reported as causing devastation in *Brassica* plantations. For instance, the clubroot disease caused by *Plasmodiophora brassicae* has been reported on *B. chinensis* in Korea (Kim et al. 2009). The soft rot disease caused by *Dickeya dadantii* subsp. *dieffenbachiae* has 38–56% incidence rate in *B. chinensis* in Malaysian commercial fields (Golkhandan et al. 2016).

The zygomycetes fungi are known to infect several economical plant products. The *Choanephora*, one of fungal genus of zygomycetes is known to cause devastating damage on withering floral part and fruits of many plant species (Agrios 2005). In Thailand, *B. chinensis* is cultivated widely in pots or with hydroponic systems, but little is known about its disease problems. In July 2016, wet rot was found in *B. chinensis* leaves in a private hydroponic greenhouse in Hatyai city, Songkhla, Thailand. Therefore, the aim of this research was to identify and characterize that wet rot disease on *B. chinensis* in Thailand. To identify the causal agent, morphological examination, as well as molecular analysis and pathogenicity tests were conducted.

The fungi initially infected young and expanded leaves (Fig. 1 a–c). The infected leaves became water-soaked and were covered with a dark mass of sporangiophores (Fig. 1 a–d). The fungal pathogen was isolated to a pure culture on water agar and potato dextrose agar (PDA). The fungus grew rapidly on PDA to cover the whole Petri dish (9 cm in diam.) within 48 h. After 7 days on PDA at 25 °C and under a 12 h/12 h dark/light regime the isolate produced white colonies that later turned yellow or pale brown with abundant sporangiola. Fungal structures from fresh leaf samples were mounted with water on a glass slide and observed under a light microscope (Leica DM750, Leica Microsystems, Germany). Sporangiophores were 5–13 μm in width and 1–10 mm in length, erect, solitary, unbranched, non-septate, with clavate vesicles formed at their apices. Monosporous sporangiola were brown to dark brown, ellipsoid to ovoid, 8–15 μm in width and 9–22 μm in length (Fig. 1 e). Zygosporangia were not observed. These morphometric traits were consistent with *Choanephora cucurbitarum* (Kirk 1984). Fungal specimens were deposited in the Culture Collection of Pest Management Department, Faculty of Natural Resources, Prince of Songkla University, Thailand under the accession number PSU-PM-PC01.

Fungal pathogens cultured on PDA for 7 days were harvested for DNA extraction. Genomic DNA was extracted from

✉ Anurag Sunpapao
anurag.su@psu.ac.th

[1] Pest Management Biotechnology and Plant Physiology Laboratory, and Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hatyai 90110, Thailand

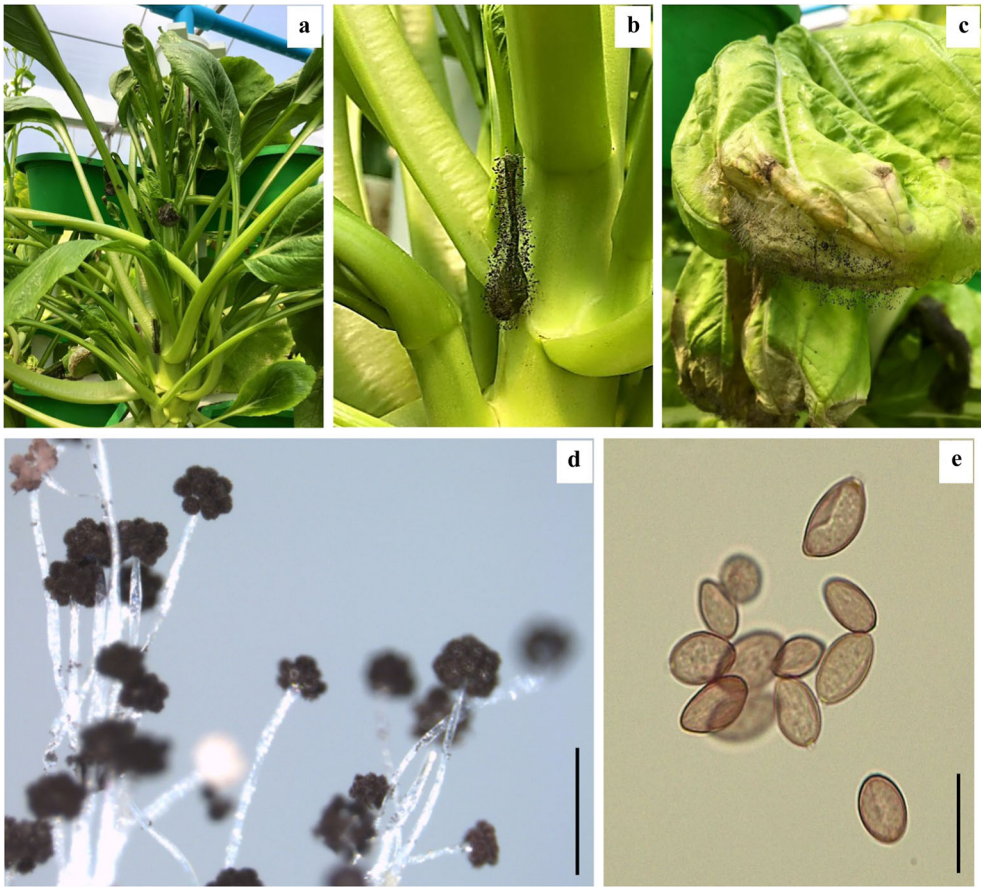

Fig. 1 Choanephora rot on *Brassica chinensis* caused by *Choanephora cucurbitarum*. Symptoms on young leaves (**a** & **b**) and on expanded leaf (**c**), sporangiophores bearing sporangiola (**d**), and monosporous sporangiola (**e**). Scale bars d = 0.5 mm, e = 20 μm

mycelia using the CTAB method (Doyle and Doyle 1987; Doyle and Dickson 1987; Cullings 1992). Inter transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by using ITS1 and ITS4 primer pair (White et al. 1990). The ITS gene regions were amplified using BIO-RAD T100™ Thermal Cycler (BioRad, Hercules, CA, USA). The PCR was carried out sequentially in a volume 50 μl containing 2× Dreamtaq Green PCR Master Mix (Thermo Scientific), 10 pmol of each primer and 50 ng of DNA template. The cycling parameters were 3 min at 95 °C, 35 cycles 30 s at 95 °C, 30s at 50 °C, an extension for 1 min at 72 °C with final extension of 10 min at 72 °C.

Sequencing was performed by Macrogen Sequencing Service (Macrogen, Seoul, Korea) with the same primers used for PCR amplification. The sequence of ITS was blasted in the database of NCBI (National Center for Biotechnology Information). Nucleotide sequences were aligned with MEGA version 6.0 (Tamura et al. 2013). Phylogenetic analysis was performed using the maximum likelihood with 1000 bootstrap replications.

PCR amplification of the ITS gene region gave fragments about 569 base pairs (bp) and the sequence was deposited in GenBank under the accession number LC189371. BLAST search indicated that the ITS sequence was 99% similar to *C. cucurbitarum* available in GenBank (accession number KU316934, KX790359). A phylogenetic analysis showed the isolated sample was conspecific with other members of *C. cucurbitarum* (Fig. 2). The isolate LC189371 and *C. cucurbitarum* (KX790359, KY080447, KU316934) were clustered together with 96% bootstrap support (Fig. 2).

The pathogenicity tests were carried out in young healthy *B. chinensis* plants (30-day-old). The isolated fungal pathogens were cultured on PDA for 14 days at room temperature (28 ± 2 °C) and a spore suspension was prepared with sterile

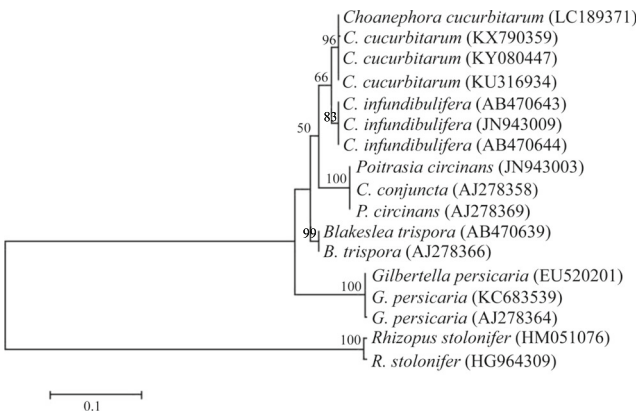

Fig. 2 Phylogenetic tree of *Choanephora cucurbitarum* including related species from GenBank, based on rDNA ITS gene sequences. *Rhizopus stolonifer* is used as an outgroup

distilled water (1×10^6 spores/mL). Ten healthy plants were sprayed with the spore suspension, while plants sprayed with water alone served as the negative control. The inoculated plants were covered with plastic bags for 48 h and kept in a greenhouse (30 °C and 75–90% relative humidity). Water-soaked lesions emerged on the leaves 3 days after inoculation, and these then rotted within 7 days. All the inoculated leaves of *B. chinensis* plants showed the typical disease symptoms within 10 days, whereas the control remained healthy. *Choanephora cucurbitarum* was re-isolated from the inoculation induced lesions and was morphologically identical to the original isolates. No symptoms developed on the stems or on other parts of *B. chinensis* plants, with the exception of leaves. These results confirmed that *C. cucurbitarum* could infect *B. chinensis* and cause the wet rot disease on its leaves.

It has been documented that *C. cucurbitarum* causes wet rot on cabbage plug seedlings in Japan (Kubota and Abiko 2001). This pathogen has been also found to cause the soft rot on eggplants in Korea (Kwon and Jee 2005). Furthermore, Choanephora flower rot on *Abelmoschus manihot* caused by *C. cucurbitarum* has also been recently reported in Korea (Park et al. 2015). However, there is no prior report of *C. cucurbitarum* causing wet rot disease on *B. chinensis*. This is the first report of Choanephora rot of *B. chinensis* being caused by *C. cucurbitarum,* in Thailand or elsewhere. There is an increasing trend in Choanephora rot disease prevalence in several parts of Asia, probably associated with the long period of high rainfall, high humidity, and tropical temperatures (Kwon and Jee 2005). An epidemic of Choanephora rot in Asia could cause devastating damage to the plantation crops, and the farmers need to find appropriate ways to manage this disease in the near future.

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