

First report of damping-off caused by *Pythium aphanidermatum* in the seedlings of wax gourd grown in the seedling period

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Research Article

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Abstract

In February 2023, seedlings of wax gourd (*Benincasa hispida* (Thunb.) Cogn.) developed water-soaked rot on hypocotyls in the seedling period in Japan. The diseased seedlings eventually displayed damping-off. A *Pythium* sp. was isolated from the hypocotyls and was identified as *Pythium aphanidermatum* based on the morphological characteristics and internal transcribed spacers region of DNA sequences. The same symptoms were observed when seedlings were inoculated with the isolated fungus. Moreover, the same fungus was reisolated from the damping-off seedlings. This is the first report of damping-off in wax gourd seedlings caused by *P. aphanidermatum* during the seedling period in Japan.

Main Text

Wax gourd (*Benincasa hispida* (Thunb.) Cogn.), also known as white gourd, ash gourd, winter gourd, and winter melon, is a plant species native to South and Southeast Asia. This vegetable is a creeping plant that produces large fruits, which are used in a variety of dishes. In Japan, Aichi Prefecture is a major wax gourd production area.

In February 2023, seedlings of wax gourd (cv. NRT-102) developed water-soaked rot on the hypocotyl while extending its cotyledon in the seedling box (30 × 60 × 3 cm), which was kept warm via electric heat at 30 °C in a commercial greenhouse (Fig. 1a). The diseased seedlings finally developed damping-off.

Running tap water was used to wash the infected hypocotyls, and filter paper was used to wipe off excess water. Then, 1% (v/v) sodium hypochlorite was used to sterilize 5-mm segments of the hypocotyls for 1 min. Subsequently, hypocotyls were washed three times using sterile distilled water. The excess water was removed separately on sterilized filter paper; then the segments were placed on water agar plates (15 g agar/L distilled water). After 1–3 days of incubation at 25 °C under dark conditions, single hyphal tips were transferred to corn meal agar (CMA) slants (17 g BBL corn meal agar/L distilled water) for obtaining pure cultures. Three fungal cultures named ASCWg1, ASCWg2, and ASCWg3 were obtained and placed on CMA slants at 23 °C. ASCWg1 was deposited as a representative isolate under the accession number MAFF 247814 in the NARO Genbank Project, National Agriculture and Food Research Organization, Japan.

Three seeds of wax gourd (cv. Okinawa kogata tougan) were sown in 7.5-cm pots, each containing 180 mL of soil mixture (Tanemaki Baido, Komeri, Niigata, Japan), in a greenhouse. Seedlings with cotyledons were used for inoculation. Each inoculum was prepared as described by Tojo et al. (1993) with modifications. A 300-mL flask containing 4 g of autoclaved bentgrass seeds in 20 mL of distilled water was inoculated with mycelial plugs obtained from the CMA culture of ASCWg1 isolate. The colonized bentgrass seeds were homogenized using a sterilized mortar and pestle in 200 mL of distilled water after incubation for 7 days at 25 °C under dark conditions. Fluffy-looking colonies with numerous aerial aseptate hyphae were formed by the ASCWg1 isolate. Further, 50 mL of the inoculum or distilled water as a negative control was used to inoculate each seedling, after which the seedlings were

incubated at 27 °C under a 16-h photoperiod (photon flux density 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and at 24 °C under a 8-h dark period in a growth chamber (CLE-303, Tomy Seiko, Tokyo, Japan). After inoculation for 3 days, water-soaked rot appeared on all hypocotyls in the seedlings (Fig. 1b, c), and no symptoms were noted on the control seedlings (Fig. 1b, d). The inoculated *Pythium* sp. was reisolated from the water-soaked rot on the hypocotyls, but not from the control seedlings. Each pathogenicity experiment was performed using 12 seedlings, and the test was conducted twice.

Grass blade cultures (Waterhouse 1967) were used to observe morphological characteristics such as zoospore formation and sexual organs. The ASCWg1 isolate was inoculated on CMA plates. Subsequently, the autoclaved bentgrass blades were placed on the same plates for 2 days at 25 °C under dark conditions. A total of 7 mL of autoclaved pond water (a 1:2 solution of pond water and distilled water) in a 9-cm Petri dish was used to form zoospore and sexual organs in the same colonized bentgrass blades, which were observed for 1–7 days at 25 °C. Filamentous and inflated sporangia (Fig. 2a) and zoospores in vesicles (Fig. 2b) were formed on the colonized bentgrass blades. Oogonia, smooth and 20.0–27.0 μm (mean, 23.9 μm) in diameter, were formed in a single culture (Fig. 2c). Antheridia were mostly intercalary, sometimes terminal, monoclinal or diclinal, and broadly sac-shaped; one or rarely two antheridia were formed per oogonium (Fig. 2c). The oospores were 18.0–22.0 μm (mean, 19.3 μm) in diameter, aplerotic, and spherical (Fig. 2d).

The hyphal growth of the ASCWg1 isolate was measured on three replicate CMA plates. A 5.5-mm mycelial disk was obtained from the margin of a colony on CMA plates inoculated and incubated at various temperatures ranging from 5 °C to 45 °C under dark conditions (Fig. 3). The diameter of each colony was measured daily, and this test was performed thrice. The mycelial growth was confirmed at temperatures of 10 °C–42 °C, and the optimum temperature was 35 °C (Fig. 3). The radial growth rate at 35 °C was 37.4 mm/day (Fig. 3). The morphological characteristics and growth temperatures were similar to those of *P. aphanidermatum* (van der Plaats-Niterink 1981).

DNA extraction, PCR, and sequencing were performed as described by Miyake et al. (2022). The standard primers ITS4 and ITS5 (White et al. 1990) were used to amplify the internal transcribed spacers (ITSs), including the 5.8S rRNA from the ASCWg1 isolate. The ITSs of the DNA sequences were registered in the DDBJ/EMBL/GenBank databases under the accession number LC769377. BLAST (Altschul et al. 1990) revealed that the nucleotide sequence of the isolate ASCWg1 shared 99.9% identity with that of *P. aphanidermatum* CBS118.80 accession number AY598622. Therefore, the isolate ASCWg1 was identified as *P. aphanidermatum* (Edson) Fitzpatrick.

In Japan, the vegetable and flower diseases caused by *P. aphanidermatum* in fields and commercial greenhouses have recently spread to many districts (Miyake et al. 2014). For example, *P. aphanidermatum* causes seedling blight in *Glycyrrhiza uralensis* (Ishiguro et al. 2014), damping-off in Cape gooseberry (Miyake et al. 2015), and root rot in sweet pepper (Hayano et al. 2021). With its high temperature tolerance, *P. aphanidermatum* can grow at temperatures exceeding 40 °C, and its optimal temperature is 35 °C. The *Pythium* root rot caused by *P. aphanidermatum* was most severely pathogenic

to poinsettia at 35 °C, which was also confirmed to be the optimal temperature for hyphal growth (Miyake et al. 2014).

The seedling period in which cultivated wax gourd was affected by the damping-off was kept warm for 7 days. The soil temperature in the seedling box was maintained at 30 °C via electric heat in the infected greenhouse. *P. aphanidermatum* caused more damage at high temperature than at low temperature (Miyake et al. 2014). Moreover, high humidity was maintained in the soil after seeding to promote the germination of seeds and improve growth during the seedling stage. Thus, high temperature and humidity were considered optimal for *P. aphanidermatum* to infect the seedlings because high soil moisture is necessary for the development of the disease caused by *Pythium* species (Hendrix and Campbell 1973). Therefore, these factors contribute to the development of damping-off in wax gourd seedlings.

To the best of our knowledge in Japan, this is the first report of damping-off caused by *P. aphanidermatum* in the seedlings of wax gourd during the seedling period. We propose “damping-off” (Japanese name: nae-tachigare-byo) as a common name for the new disease.

Declarations

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Declarations

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figures

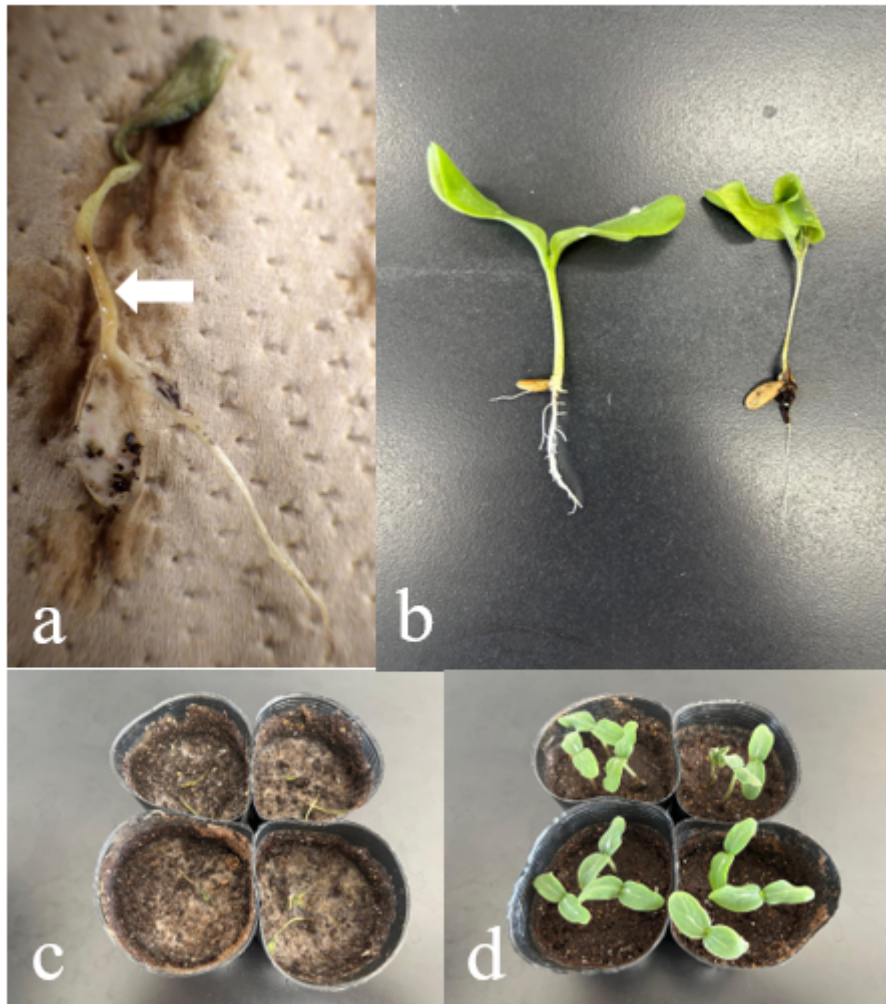


Figure 1

Damping-off and water-soaked rot symptoms on wax gourd seedlings. **a** Damping-off and water-soaked rot on the hypocotyl (arrow) after natural infection. **b** Healthy uninoculated seedling (left) and damping-off in an inoculated seedling (right). **c** Damping-off and water-soaked rot after 3 days of inoculation. **d** Healthy, uninfected seedlings after 3 days of growth with distilled water.

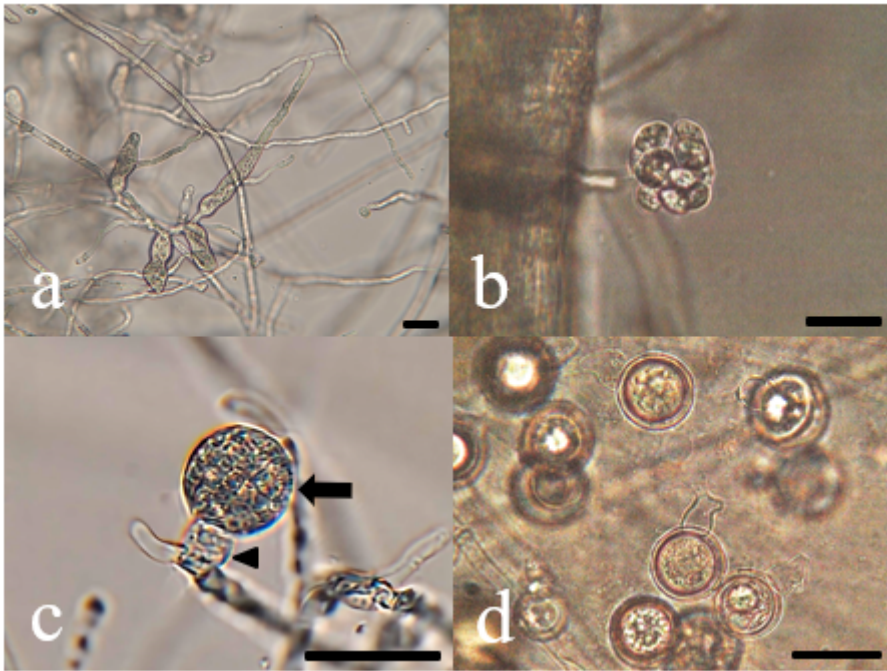


Figure 2

Morphology of *Pythium aphanidermatum* (isolate ASCWg1). **a** Filamentous, inflated sporangia. **b** Zoospores in a vesicle. **c** Oogonium (arrow) with one intercalary sac-shaped antheridium (arrowhead). **d** Oogonium with an aplerotic oospore. Bars: 30 μm

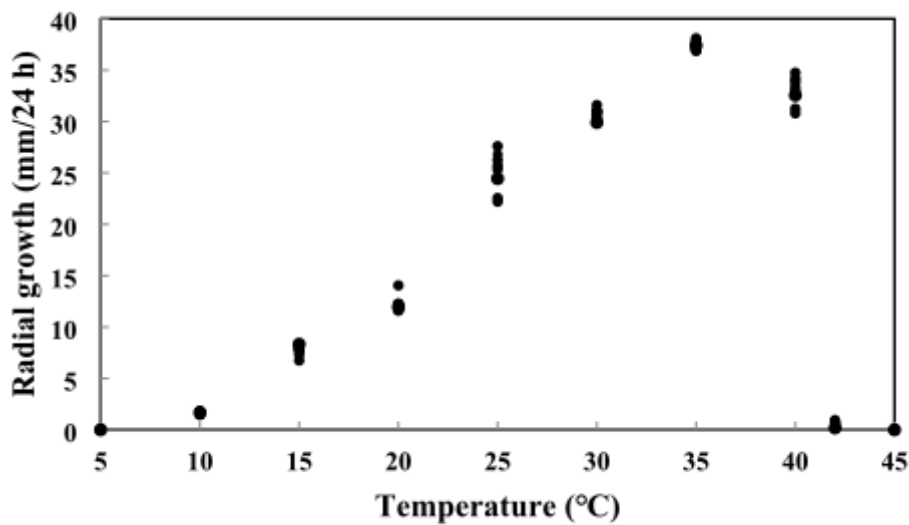


Figure 3

Growth rates of the representative isolate ASCWg1 of *Pythium aphanidermatum* on corn meal agar at different temperatures in the dark.