Diseases Caused by Oomycetes

First Report of *Pythium* Cluster B2a Species Causing Root Rot in Welsh Onion in Japan

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Hiroshima Prefecture has the highest production area of hydroponically grown Welsh onions (*Allium fistulosum* L.) in Japan. Since cultivation began in 1988, root rot followed by leaf browning has caused significant economic losses. Approximately 80% (loss of 45 million JPY) plant loss occurred from May to September 2009 (Shimizu, *unpublished*), and the disease was observed again in 2020. Diseased Welsh onions (five- to six-leaf stage) were collected in 2009. Abundant nonseptate hyphae of *Pythium*-like organisms were observed in the rotted roots. Disinfected symptomatic tissue samples were placed on NARF medium (Morita and Tojo 2007) and incubated at 25°C for 3 to 7 days. Six *Pythium*-like organisms were isolated, and their morphological features on a grass blade culture, potato carrot agar (PCA) (van der Plaats-Niterink 1981), cornmeal agar (CMA), and V8 juice agar (Miller 1955) were examined. Hyphal growth rates from 1 to 46°C were measured by culturing on PCA. The ribosomal internal transcribed spacer (ITS) regions and mitochondrial *COI* of the isolates were amplified and sequenced according to Ueta and Tojo (2016). All six isolates obtained showed similar morphology, hyphal growth rates, and sequences of ITS and *COI*. Detailed descriptions are provided here for the representative isolate 72 (MAFF246451). The isolate produced asexual structures but did not form sexual structures, including oogonia, antheridia, and oospores, on all the

media used. Hyphae were up to 6.8 µm wide. Appressoria were knob-like terminations. Sporangia were filamentous and indistinguishable from the hyphae. Zoospores were formed at 5 to 25°C. The diameter of encysted zoospores ranged 7.4 to 10.1 (average 8.9) µm. Cardinal temperatures for hyphal growth on PCA were 5°C minimum, 28 to 31°C optimal, and 35°C maximum. The daily growth rate at 25°C was 15.0 mm/day. The sequence analysis of all isolates, including isolate 72 (GenBank nos. AB700596 for ITS, LC630955 for *COI*) showed the present isolates belonged to *Pythium* cluster B2a (Robideau et al. 2011). Based on these features, the six isolates were identified as *Pythium* cluster B2a sp. In the inoculation test, isolate 72 was cultured on CMA at 25°C for 5 days. Mycelium disks (5 mm diameter) obtained from the culture were placed on the primary roots of 8-day-old Welsh onion seedlings (cv. Koutou), which were grown at a density of six plants on rock wool cubes moistened with tap water in a 50-ml plastic pot. The inoculated and noninoculated plants were grown at 28°C for 7 days in a growth chamber. The experiment was repeated twice using three pots per replication. The plants inoculated with isolate 72 wilted, and their roots rotted 7 days after inoculation. No disease was found observed on the noninoculated plants. The isolate of *Pythium* cluster B2a sp. was consistently reisolated from the diseased plants, thus fulfilling Koch's postulates. *Pythium* cluster B2a sp. causing stem rot on lettuce has been recorded in Italy (Garibaldi et al. 2017). To our knowledge, this is the first report of *Pythium* cluster B2a sp. on Welsh onions. Because significant losses to root rot of Welsh onion have occurred in Japan, identification of the causal organism will enable the development of management practices to reduce losses.

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