

# First report of postharvest root rot caused by *Rhizopus oryzae* on *Codonopsis lanceolata*

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**Abstract** Postharvest root rot of *Codonopsis lanceolata* was found at storage piles in a market in Seoul, Korea, 2012. Based on the morphological characteristics and molecular analyses of the ITS rDNA and D1/D2 region of the LSU, the causal fungus was identified as *Rhizopus oryzae*. This is the first report of *Rhizopus*-associated root rot on *Codonopsis lanceolata*.

**Keywords** Postharvest root rot · *Codonopsis lanceolata* · *Rhizopus oryzae* · ITS sequence · D1/D2 region of the LSU

*Codonopsis lanceolata*, native to East Asia, is an important medicinal root crop in Korea, second only to ginseng. This plant has long been used for therapeutic purposes as a traditional folk medicine. The root extract of *C. lanceolata* has pharmacological properties including anti-obesity, anti-inflammatory activity and anti-tumor effects (Li et al. 2007; Cho et al. 2011; Choi et al. 2013). It is widely cultivated, with 2 or 3 year old roots harvested for eating whereas 6 year old roots are highly valued and mostly used for medicinal purposes. Recently, these medicinal effects have led to its high demand as a good source of functional foods for improving physical constitution. As of 2010, production was 7,766 M/T from a cultivation area of 1,536 ha in Korea, which is increasing annually (Park et al. 2011).

*Rhizopus oryzae*, currently synonymous with *Rhizopus arrhizus*, is well known worldwide as a severe postharvest pathogen causing serious problems in successful storage and

marketing of products (Amadioha 1996, 2001). In Korea, postharvest rot caused by *R. oryzae* has been recorded on various fruits and vegetables including banana, apple, and sweet potato (Kwon et al. 2011, 2012a, b).

In May 2012, a small pile of rotted *C. lanceolata* roots was observed in a market in Seoul. Symptoms usually began in wounded or damaged parts of the root. Infected roots appeared water-soaked, and the root epidermis was easily sloughed off, showing reddish brown, discoloured tissues. In humid condition, lesions were covered with white mycelial tufts (Fig. 1a–b). As decay progressed, sporangiophores with dark pigmented sporangia grew out through infected tissues.

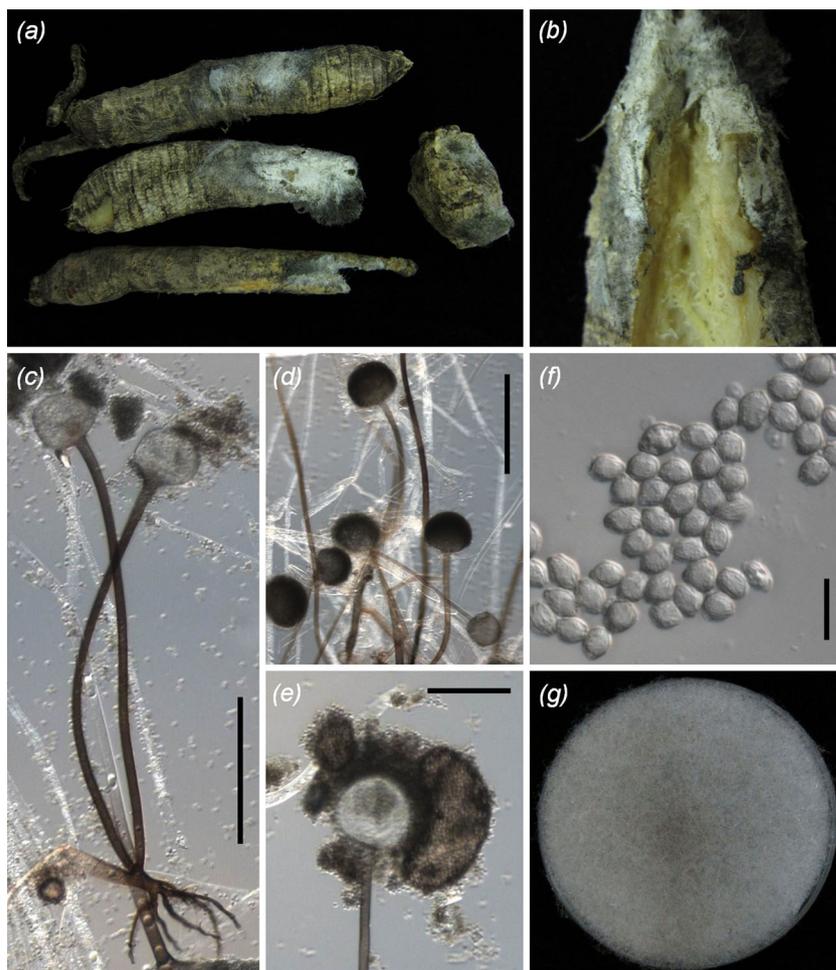
To perform the morphological observation, the fungal structures were detached from the infected lesions using watchmaker's forceps and water-mounted on glass slides. Each structure was examined in bright field- and differential interference contrast light microscopy with Olympus BX51 microscope (Olympus, Tokyo, Japan) and photographed using a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany). At least 30 measurements were made for each structure. Voucher specimens were housed in the Korea University Herbarium (KUS). The fungus associated with the symptoms was isolated on potato dextrose agar (PDA). An isolate obtained from KUS-F26796 was deposited in the Korean Agricultural Culture Collection (Accession No. KACC47222) and used for pathogenicity testing and molecular analysis.

The colonies were at first white and cottony, then became grey to black with the appearance of sporangia (Fig. 1g). Sporangiophores were mostly erect, unbranched, subhyaline to brown, singly or in groups, and 180 to 1,500 µm long, 10 to 15 µm wide (Fig. 1c). Sporangia were globose to subglobose, greyish brown to black, and 65 to 200 µm in diameter (Fig. 1d–e). Columella were subglobose to ovoid, subhyaline

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**Fig. 1** Root rot of *Codonopsis lanceolata* infected with *Rhizopus oryzae*. (a) Infection of the root covered with white mycelial tufts. (b) Longitudinal section of infected root showing water-soaked symptoms (c) Conidiophores with rhizoids. (d) Conidiophores with sporangia. (e) Columellum with numerous sporangiospores. (f) Sporangiospores. (g) One-week-old colony of *R. oryzae* growing on a potato dextrose agar. Scale bars = 200  $\mu\text{m}$  for c-d, 100  $\mu\text{m}$  for e and 10  $\mu\text{m}$  for f



to greyish brown, and 35 to 100  $\times$  50 to 120  $\mu\text{m}$ . Sporangiospores were ovoid, angular, striate, pale greyish to brown, and 5 to 8  $\times$  3 to 5  $\mu\text{m}$  (Fig. 1f). Chlamydo spores and zygo spores were not observed. The fungus was identified as *Rhizopus oryzae* based on the morphological characteristics and growth temperature (Liou et al. 2007).

To confirm the identity of the causal fungus, genomic DNA was extracted with DNeasy Plant Mini Kits (Qiagen Inc., Valencia, CA). The internal transcribed spacer (ITS) region of rDNA and the D1/D2 region of the large subunit (LSU) were amplified with primers ITS1/ITS4 (White et al. 1990) and primers NL1/LR3 (O'Donnell 1993) and sequenced. The resulting 588 bp ITS sequences and 655 bp D1/D2 sequences were deposited in GenBank under accession numbers KF218955 and KF218956. A GenBank BLAST search with the present data revealed that the ITS sequences showed 100 % similarity with that of *R. oryzae* (JX120700) and D1/D2 sequences also showed 100 % identity with that of *R. oryzae* (JQ745263).

A conidial suspension ( $2 \times 10^6$  conidia/ml) was sprayed over five three-year-old roots of *C. lanceolata* wounded with a fine needle and kept in plastic containers (25  $^{\circ}\text{C}$  and 100 % RH). Five roots of the same

age sprayed with sterile water served as controls. After 3 days, typical root rot symptoms, identical to the ones observed in the market, developed on the inoculated roots. No symptoms were observed on controls. *Rhizopus oryzae* was reisolated from the lesions of inoculated roots, fulfilling Koch's postulates.

Root rots of *C. lanceolata* caused by *Fusarium oxysporum* and *Sclerotium rolfsii* (= *Athelia rolfsii*) in fields are known to occur in Korea (Kim et al. 1998; Farr and Rossman 2014). Such pathogens can be associated with postharvest root rots. However, no *Rhizopus*-associated root rots were reported. To our knowledge, this is the first report of *R. oryzae* as a postharvest pathogen on *C. lanceolata* roots globally as well as in Korea. Our observations in storage rooms and in markets suggest that preventing wounds on roots as well as maintaining good hygiene during handling might be the main strategies for controlling this postharvest root rot.

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