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DISEASE NOTE

First report of black root rot of lettuce in Japan caused by *Berkeleyomyces rouxiae*

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Abstract

In August 2016, lettuce plants (*Lactuca sativa*) cultivated in Gunma Prefecture became stunted and often wilted. Dark brown to black lesions were observed on roots of diseased plants. Most small feeder roots were lost. Fungi isolated from lesions on roots were identified as *Berkeleyomyces rouxiae* based on their morphological and genetic characteristics. Pathogenicity of this fungus on lettuce was confirmed by inoculation and reisolation. This report is the first of black root rot caused by *B. rouxiae* on lettuce in Japan.

Keywords *Chalara elegans* · *Lactuca sativa* · Soilborne pathogen · *Thielaviopsis basicola*

In August 2016 in Gunma Prefecture, a major area for lettuce (*Lactuca sativa*) production in Japan, lettuce growth was suppressed in several fields (Fig. 1a; Miki et al. 2018; Nakane et al. 2018). Those lettuce plants were stunted and often wilted. Dark brown to black bands were observed on the roots of diseased plants (Fig. 1b). The lesions were often swollen and cracked, and in severe cases, most small feeder roots were lost (Fig. 1c). Vascular discoloration on the taproot was rarely observed.

After root tissues of diseased plants were collected and washed in running tap water for 1 h, root sections were soaked in 70% (v/v) ethanol for 30 s, sodium hypochlorite solution (0.5% available chlorine) for 3 min, and sterilized distilled water. A similar fungus was frequently isolated from root sections that had been incubated on water agar plates at 25 °C. Single-spore isolates of the fungus were maintained on potato dextrose agar (PDA) (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Fungal isolates from diseased lettuce plants are listed in Table 1.

Colonies of isolated fungus on PDA were white and cottony at first, later becoming gray-brown to black and powdery with aerial mycelia (Fig. 1d). The optimum temperature for fungal growth was approximately 25 °C, while growth was negligible below 10 °C and above 30 °C (Fig. 2). Conidia of two types, chlamydospores (Fig. 1e, 1f) and endoconidia (Fig. 1g), were observed on PDA culture of the fungus. Chlamydospores were produced in chains containing 2–8 cells (Fig. 1e) and often observed directly in diseased lettuce roots (Fig. 1h). The terminal chlamydospores were conoid, and the rest were short-cylindrical and separated at maturity (Fig. 1f). The hyaline, cylindrical endoconidia arose from phialides (Fig. 1g). The dimensions of at least 50 chlamydospores, phialides, and endoconidia (Table 2) were measured and consistent with published descriptions of *Berkeleyomyces basicola* and *B. rouxiae* (Nel et al. 2018).

Parts of this paper were presented elsewhere by Nakane et al. (2018) and Miki et al. (2018).

The nucleotide sequence data reported herein are available from the DDBJ/EMBL/GenBank databases under accession numbers LC341294–LC341297, LC341302–LC341305, LC421983, LC421984, and LC430268–LC430273.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10327-019-00860-y) contains supplementary material, which is available to authorized users.

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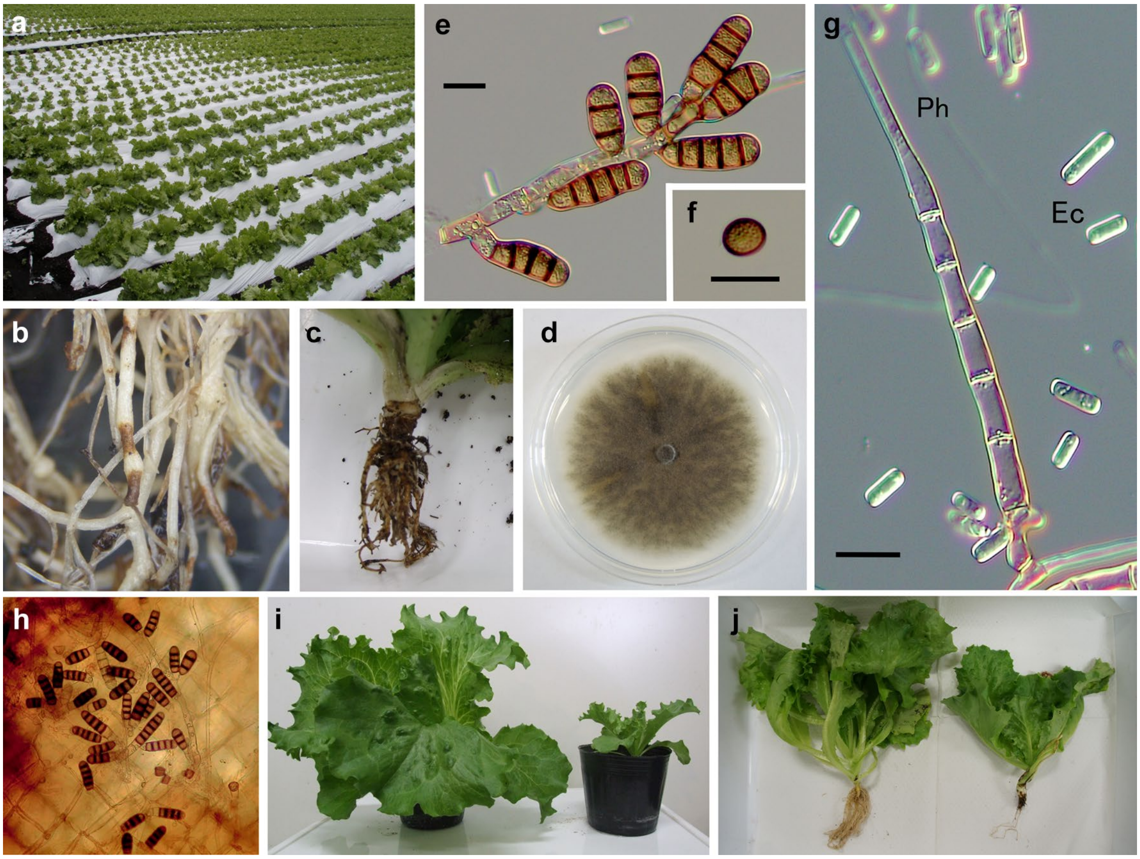

Fig. 1 **a** Typical lettuce field with stunted plants infected by *Berkeleyomyces rouxiae*. **b**, **c** Diseased roots of lettuce with necrotic lesions and lack of fine roots. **d** Colony of *B. rouxiae* LT1 on PDA after 1 week at 25 °C. **e** Chlamydospores of *B. rouxiae* LT1. **f** Chlamydospore of *B. rouxiae* LT1 separated into individual spores. **g** Terminal phialide (Ph) and endoconidia (Ec) of *B. rouxiae* LT1. **h** Chlamydospore of *B. rouxiae* on lettuce root. **i**, **j** Seedlings of lettuce cv. Summer Guy inoculated with strain LT1 (right) and non-inoculated control (left)

Table 1 Isolates of *Berkeleyomyces rouxiae* causing black root rot on lettuce

Isolate	Origin			Strain accession[a]
	Host	Country	Year	
LT1	Lettuce (cv. Summer Guy)	Japan	2016	MAFF 246781
LT2	Lettuce (cv. Tough V)	Japan	2016	MAFF 246782
LT6	Lettuce (cv. Summer Guy)	Japan	2016	MAFF 246783
LT28-122	Lettuce (cv. Summer Guy)	Japan	2016	MAFF 246784

[a]Number of isolates in culture collection of National Agriculture and Food Research Organization (NARO) Genetic Resources Center, Japan

To identify the fungal species, we analyzed nucleotide sequence of genomic regions of the ribosomal large subunit (LSU), the 60S ribosomal protein RPL10 (60S), the internal transcribed spacer region (ITS), and the minichromosome

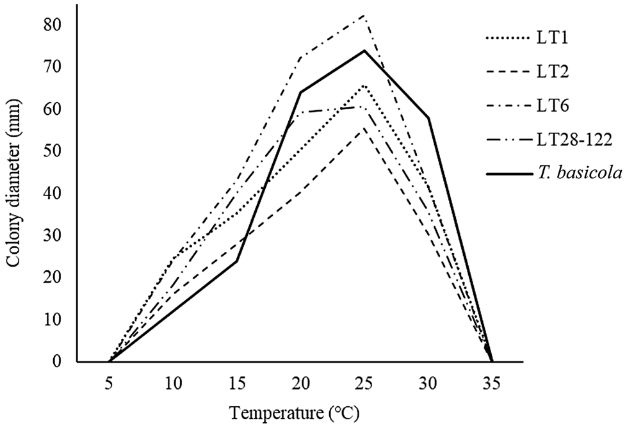

Fig. 2 Colony diameters of respective isolates after incubation on PDA for 2 weeks at various temperatures. Five plates were used for each isolate. The average was calculated. Data for a strain of *Thielaviopsis basicola* (synonym of *Berkeleyomyces* species) reported by Kobayashi and Kotani (1987) are included

Table 2 Morphological characteristics of present lettuce isolates of *Berkeleyomyces* and previously reported dimensions

Isolate	Chlamydospore segments, L × W (µm)	Phialides			Endoconidia, L × W (µm)
		Length (µm)	Width		
			Tip (µm)	Base (µm)	
LT1	5.3–9.1 × 8.5–13.2	49.1–69.6	3.3–5.0	4.8–7.4	8.4–20.4 × 2.2–4.3
LT2	5.1–10.3 × 10.5–14.1	56.0–70.4	3.1–5.8	4.7–7.1	11.3–18.9 × 3.5–5.4
LT6	6.9–9.6 × 10.4–13.8	56.8–70.9	3.8–6.0	5.3–8.5	11.5–30.9 × 2.7–4.4
LT28-122	7.4–10.3 × 8.9–12.7	55.3–71.0	3.7–4.9	5.3–7.2	12.0–23.4 × 3.3–4.7
B. basicola[a]	6.2–14.3 × 7.8–13.8	13.8–89.0	2.9–5.6	3.2–7.0	7.2–29.7 × 3.0–5.0
B. rouxiae[a]	6.0–12.6 × 8.4–13.6	16.7–81.5	3.0–5.3	3.5–6.9	8.4–28.9 × 3.5–5.7

[a] Nel et al. (2018)

maintenance complex component 7 (*MCM7*). Genomic DNA of each fungal isolate was extracted as described by Usami et al. (2002). The primer sequence and PCR condition for amplification of each genomic region was as described by de Beer et al. (2014) and Nel et al. (2018). Phylogenetic analyses were conducted using software (MEGA 6; https://www.megasoftware.net/, Tamura et al. 2013). A maximum likelihood (ML) tree (combined LSU, 60S, ITS, and *MCM7*) of known fungal species and isolates from diseased lettuce plants is presented in Fig. 3. Detailed information of each fungal isolates and their accession numbers for their nucleotide sequences are presented in Table S1. Two *Berkeleyomyces* species, *B. basicola* and *B. rouxiae*, were stated as morphologically indistinguishable (Nel et al. 2018). However, the fungal isolates from diseased lettuces were identified as *B. rouxiae* based on their phylogenetic position (Fig. 3).

To assess the pathogenicity of the fungal isolates, each isolate was first shake-cultured in potato sucrose broth (PSB, made from fresh potatoes) at 25 °C in the dark. After filtering through cheesecloth, conidia were collected by centrifugation (1500×*g*), then resuspended in sterilized distilled water. Roots of 3-week-old seedlings of lettuce (cv. Summer Guy) were soaked in the conidial suspension (10^7 conidia/ml) for a few minutes. Inoculated and noninoculated lettuce plants were planted in commercial potting soil and were maintained in a growth chamber at 25 °C with a 12-h photoperiod. Three weeks after inoculation, lettuce plants that were inoculated with the respective fungal isolates were stunted and wilted (Fig. 1i). Root symptoms (dark brown to black lesions and loss of small feeder roots, Fig. 1j) were also observed on inoculated plants. None of these symptoms were observed on noninoculated plants. The inoculated fungus was reisolated from the root lesions of inoculated plants. These results indicate that this fungus is the causal agent of the lettuce disease.

Based on the results presented above, we concluded that *B. rouxiae* causes the lettuce disease in Gunma Prefecture. Black root rot of lettuce caused by *Thielaviopsis basicola*

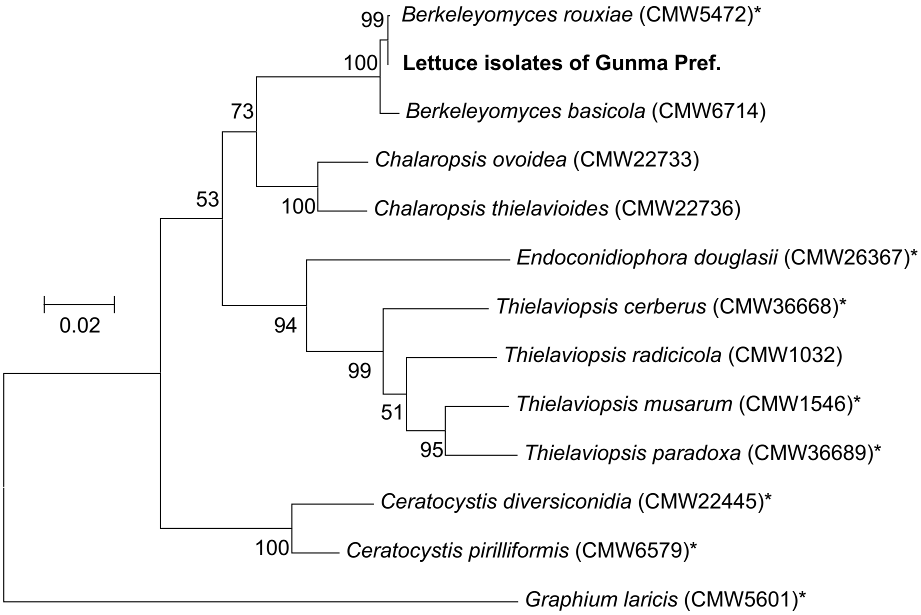

Fig. 3 Maximum likelihood tree derived from the combined nucleotide sequences of LSU, 60S, ITS, *MCM7*. Bootstrap values (1000 replicates) are shown on branches. Names of strains are in parentheses after scientific names. Accession numbers of DNA sequences deposited in the DDBJ/EMBL/GenBank database for each strain are in Table S1. Asterisks denote ex-type strains

(= *Chalara elegans*) was reported in Australia (O'Brien and Davis 1994) and in the United States (Koike 2008). Based on genetic characteristics, Nel et al. (2018) recently classified *T. basicola* in the new genus *Berkeleyomyces*, proposing that *T. basicola* includes two genetic lineages, and thus they divided *T. basicola* into two species, *B. basicola* and a new species, *B. rouxiae*. However, species identification of the pathogen that causes black root rot in Australia and the United States has been unclear.

In Japan, black root rot of various plant species caused by *T. basicola* (= *C. elegans*) has been reported (Anonymous 2018; Horita and Ohgami 2015; Kobayashi and Kotani 1987; Nishikawa 2007). These pathogens were recently reidentified as *B. rouxiae* (Anonymous 2018). Nevertheless, no report has described a lettuce disease caused by *B. rouxiae* in Japan. This report is therefore the first of black root rot (kurone-byo in Japanese) on lettuce caused by *B. rouxiae* in Japan.

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Compliance with ethical standards

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

Human and animal rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

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