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First report from Japan of black root rot in Eustoma grandiflorum caused by Berkeleyomyces rouxiae

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Research Article

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Posted Date: March 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8820509/v1

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Version of Record: A version of this preprint was published at Journal of General Plant Pathology on May 20th, 2026. See the published version at https://doi.org/10.1007/s10327-026-01282-3.

Disease Note

First report from Japan of black root rot in *Eustoma grandiflorum* caused by *Berkeleyomyces rouxiae*

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Total number of text pages: 7

Number of table and figures: 1 table and 2 figures

Abstract

In 2025, lisianthus (*Eustoma grandiflorum* [Raf.] Shinn.) plants in Kagoshima Prefecture, Japan, exhibited stunted growth and wilting. Dark brown–black lesions were observed on the roots, and most small feeder roots did not develop. The fungus isolated from the diseased roots was identified as *Berkeleyomyces rouxiae* using morphological and genetic analyses. Pathogenicity tests confirmed *B. rouxiae* is a causal agent. This is the first report of black root rot in lisianthus caused by *B. rouxiae* in Japan.

Keywords: black root rot; *Berkeleyomyces rouxiae*; *Eustoma grandiflorum*; morphological and cultural characteristics

Lisianthus (*Eustoma grandiflorum* [Raf.] Shinn.) is native to the United States. It is now widely cultivated as a cut flower in Japan and the West due to its elegant appearance, rich colors, and long-lasting blooms (Ohkawa and Sasaki 1999). In 2025, wilting and black root rot were observed on lisianthus plants of the “Bolero White” and “Engage Lime” cultivars, grown in Kagoshima Prefecture, Japan. The disease initially manifested as brown root rot, progressing to severe decay (Figs. 1a, b, c), with the formation of black, club-shaped chains of chlamydospores. Such lesions have not been previously reported in lisianthus in Japan.

In March 2025, lesions were excised from symptomatic “Bolero White” plants in a Kagoshima field into ~5 mm segments. These were immersed in 70% ethanol for 30 s, surface-disinfected with 1% NaOCl for 1 min, thoroughly rinsed with sterile distilled water, and incubated on water agar plates. After 5 days, single hyphae from the lesions were transferred to potato dextrose agar (PDA; NISSUI Pharmaceutical, Tokyo, Japan), isolating two filamentous fungi: KY250307N1 and KY250307N3.

The morphological and cultural characteristics of the isolated fungus were determined (Nel et al. 2018). After incubation at 25 °C in the dark, colonies on PDA initially appeared white and cotton-like, later turning grayish–brown, and then to black and powdery, with aerial mycelium (Fig. 1d).

To establish the optimal temperature range, PDA cultures were grown in the dark at 25 °C for 7 days. Colonies were sampled from the periphery using a 4 mm diameter cork borer. The agar disks were placed at the center of fresh PDA plates and incubated for 10 days in the dark at temperatures: 5 °C, 10 °C, 15 °C, 17.5 °C, 20 °C, 22.5 °C, 25 °C, 30 °C, 35 °C, and 40 °C. Colony diameters were measured daily. Growth occurred between 10 °C and 30 °C, with 20 °C being optimal.

For morphological examination, the isolates were cultured on malt extract agar at 25°C for 14 days in the dark. Chlamydospores were produced in chains containing 2–5 cells

(Fig. 1e). Terminal chlamydospores were conoid, whereas the rest were short- and cylindrical; they separated at maturity. Endoconidia were unicellular, hyaline rods, blunt at both ends (Fig. 1f); they arose from cylindrical phialides (Fig. 1g). These characteristics were consistent with previous morphological descriptions of *Berkeleyomyces basicola* and *B. rouxiae* (Nel et al. 2018) (Table 1).

For molecular identification, genomic DNA from the mycelia of isolates grown on PDA for 7 days at 25 ℃ was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Four genomic regions; the internal transcribed spacers (ITS), ribosomal large subunit (*LSU*), 60S ribosomal protein RPL10 (*60S*), and DNA replication licensing factor (*MCM7*), were amplified. The primer pairs used were ITS1/ITS4 (Groenewald et al. 2018), *LROR/LR5* (Nel et al. 2018), *60s-506F/60s-908R,* and *MCM7-for*/*MCM7-rev* (Edamoto et al. 2025), respectively. The amplicons were sequenced by Eurofins Genomics K.K. (Eurofine, Tokyo, Japan). Primer design and PCR conditions for each genomic region followed the protocols described by de Beer et al. (2014) and Nel et al. (2018).

Phylogenetic relationships were inferred using MEGA version 7.0 (Sudhir et al. 2016). Bootstrap analysis was performed with 1,000 replicates to assess nodal support in the neighbor-joining tree (Saitou and Nei 1987). Molecular phylogenetic analyses included eight strains: i) the lisianthus isolates; ii) two *B. rouxiae* strain, CMW5472 and CMW14219; iii) the *B. basicola* strain, Chaina; iv) three *B. basicola* strains, CMW25440, CMW6714 and CMW49352; and v) the outgroup, the *Chalaropsis thielavioides* strain CMW22736. The results revealed high bootstrap values of 100, indicating that the lisianthus isolates are closely related to *B. rouxiae* (Fig. 2). The sequence of the isolate KY250307N1 was deposited in the NARO Genebank with accession number MAFF248283. ITS, *LSU*, *60s* and *MCM7* sequences from isolate was submitted to the GenBank (accession number LC919100 for ITS, LC919101 for *LSU*, LC919102 for *60s* and LC919103 for *MCM7*).

Pathogenicity was tested in “Bolero White” and “Engage Lime” plants obtained from the Kagoshima Prefecture. The roots of four 4-month-old potted plants of seach cultivar were inoculated by immersion in a conidial suspension 10^5 spores/ml for 30 min. Additionally, four plants were mock-inoculated with sterile distilled water and served as controls. Three weeks after inoculation, black root rot symptoms developed in all inoculated plants, whereas control plants remained asymptomatic. The fungus was reisolated from the root lesions of the symptomatic plants (Figs. 1h, i, j), thereby fulfilling Koch’s postulates.

This study identified isolates KY250307N1 and KY250307N3 as *B. rouxiae*, a phytopathogen with a host range of >230 species. It is destructive to numerous crops and ornamental plants, including cotton (Gossypium barbadense L.), tobacco (Nicotiana tabacum L.), and lettuce (Lactuca sativa L.) (Duy et al. 2022; Grazyna et al. 2024; Nakae et al. 2019). Although eustoma root rot caused by *B. basicola* has been reported in China (Huang et al. 2021), this study reports the first case of lisianthus root rot caused by *B. rouxiae* in Japan. Thus, we name the disease “eustoma black root rot (kurone-byo).”

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Acknowledgments

The author expresses gratitude to Dr. Toshiyuki Usami (Chiba University). The author thanks the Kagoshima Prefectural Economic Federation of Agricultural Co-operatives and Food Research Institute for their cooperation.

Ethics declarations

Conflict of interest

The author declares that they have no conflict of interest.

Ethical approval

This article does not report the findings of any studies with human participants or animals.

Table 1 Morphological comparison of isolates KY250307N1 and KY250307N3 with previously described *Berkeleyomyces* species.

Isolate	Chlamydospore segments		Phialides			Endoconidia	
	Length	Width at center	Length	Width at tip	Width at base	Length	Width at center
KY250307N1	8.2–13.1[a)]	5.6–11.4	45.2–78.0	3.0–5.2	4.3–7.4	10.5–31.4	3.3–4.7
KY250307N3	10.2–13.3	6.2–11.8	47.1–78.9	3.0–4.4	4.7–6.9	11.3–26.9	3.4–5.4
B. rouxiae ThL001[b)]	6.3–12.9	7.1–12.4	31.8–90.3	2.8–4.8	4.1–7.9	8.5–30.0	3.3–6.3
B. rouxiae LT1[c)]	5.3–9.1	8.5–13.2	49.1–69.6	3.3–5.0	4.8–7.4	8.4–20.4	2.2–6.3
B. rouxiae[d)]	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
B. basicola[d)]	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

a) Minimum value (μm)-Maximum value (μm).
b) Description by Sasaki et al.
c) Description by Nakane et al.
d) Description by Nel et al.

167 Figure legends

168 Fig. 1 Symptoms of Eustoma black root rot and the pathogen *Berkeleyomyces rouxiae*.

169 a, b Natural symptoms. c Chlamydospore of eustoma root. d Colony cultured on PDA at 25 °C

170 for 8 days (left, surface view; right, reverse view). e Chlamydospores of B. rouxiae

171 KY250307N1. f Endoconidia of *B. rouxiae* KY250307N1 produced in chains. g Terminal

172 phialide of *B. rouxiae* KY250307N1. h–j Lesions produced on eustoma post-inoculation with

173 KY250307N1. h, i Plants of the cultivar “Bolero White” inoculated with KY250307N1

174 (right), noninoculated control (left). j Diseased roots of eustoma with necrotic lesions.

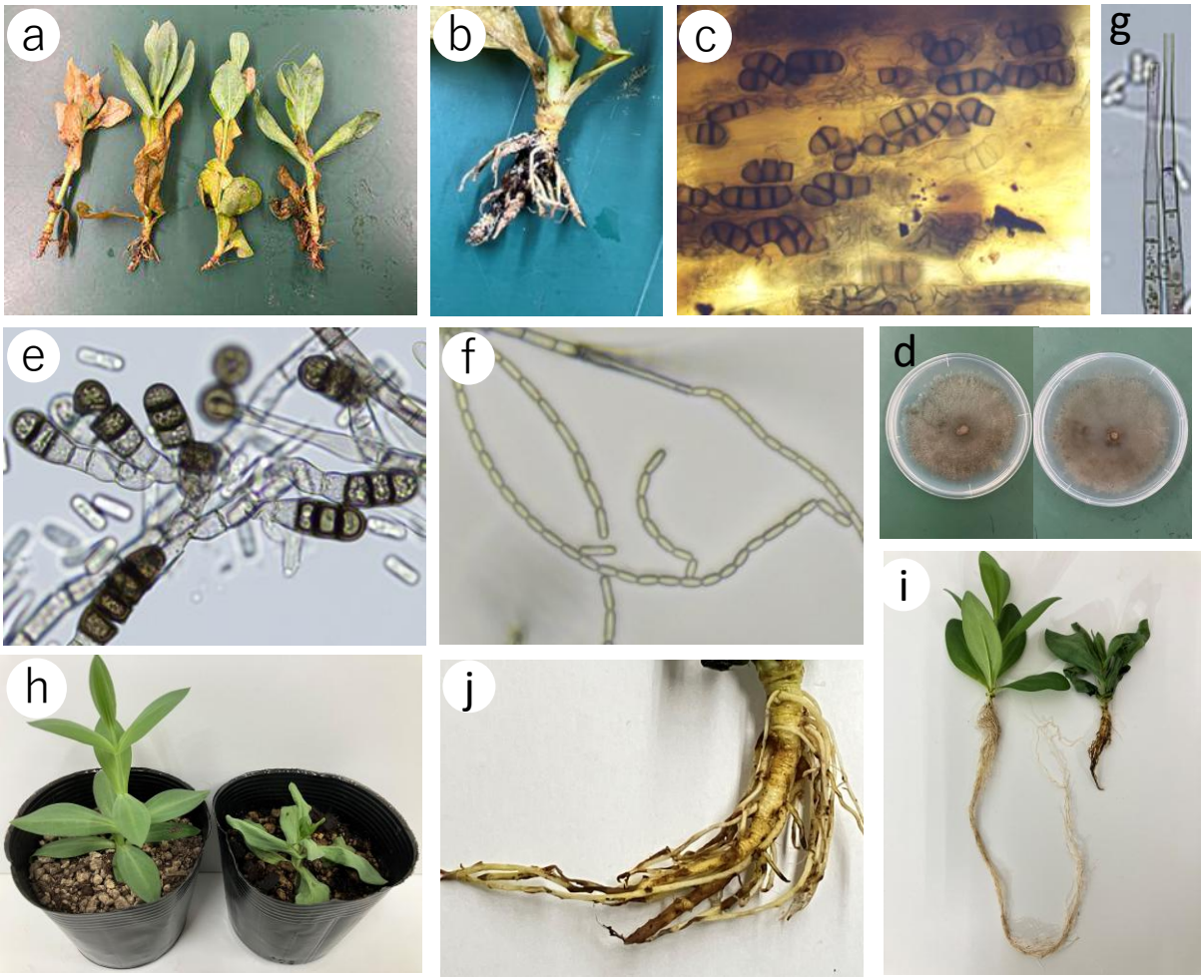
175

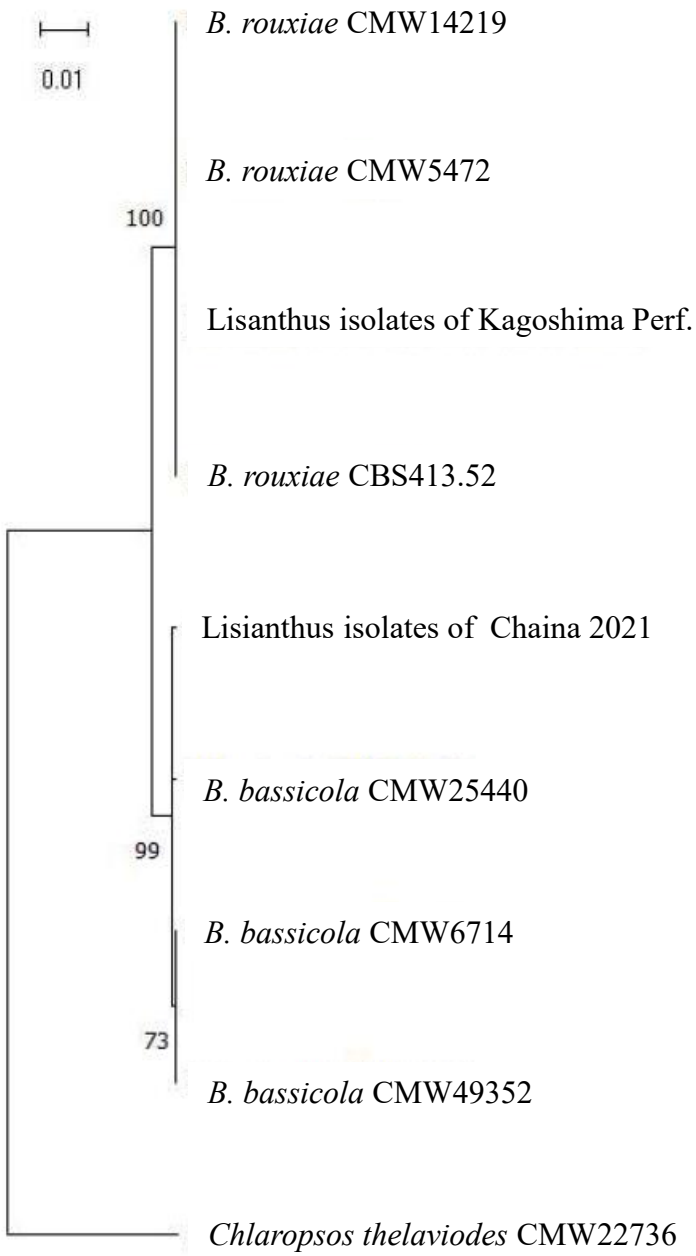
176 Fig. 2 Neighbor-joining phylogenetic tree of the isolated fungus and *Berkeleyomyces* spp.

177 Combined ITS, LSU, 60S and MCM7 sequences of the isolated fungus and members of the

178 *B. rouxiae* and *B. basicola* species complexes, with *Chalaropsis thielavioides* as the outgroup.

179 Numbers at nodes indicated bootstrap values (%) based on 1,000 replicates.


a
b
c
g
e
f
d
i
h
j


0.01
B. rouxiae CMW14219
B. rouxiae CMW5472
100
Lisanthus isolates of Kagoshima Perf.
B. rouxiae CBS413.52
Lisianthus isolates of Chaina 2021
B. bassicola CMW25440
99
B. bassicola CMW6714
73
B. bassicola CMW49352
Chlaropsos thelaviodes CMW22736