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Botrytis cinerea as the causal agent of grey mould on floral tissue of mango in Japan

Atsushi Ajitomi[1] · Maki Yamashiro[2] · Tetsuya Takushi[1] · Atsushi Ooshiro[3]

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Abstract

In March 2016, grey mould disease was observed on mango (*Mangifera indica*) in Okinawa Prefecture, Japan. During the flowering period, the disease caused a brown coloration of the petals and peduncles accompanied by white aerial mycelium. As the disease symptoms progressed, the rot of panicle parts was accompanied by grey mould, resulting in fruit set failure. A fungus was isolated from the discoloured inflorescence into pure culture. Based on morphology and analyses of glyceraldehyde-3-phosphate dehydrogenase (*g3pdh*), heat-shock protein 60 (*hsp60*) and DNA-dependent RNA polymerase subunit II *(rpb2*) DNA sequences the fungus was identified as *Botrytis cinerea*. In an inoculation test, the isolate reproduced the symptoms observed on mango petals and peduncles and was reisolated from the flowers. This is the first report of *B. cinerea* as the causal agent of grey mould (haiirokabi-byo in Japanese) on floral tissue of mango in Japan.

Keywords *Mangifera indica* · Grey mould · *Botrytis cinerea* · *g3pdh* · *hsp60* · *rpb2*

Mango (*Mangifera indica* L.; Anacardiaceae), an evergreen plant grown thoughout the tropics and subtropics, is one of the world's most important fruit crops's most important fruit crops. In Japan, it is cultivated mainly in southwestern regions, such as Okinawa, Kagoshima and Miyazaki Prefectures (Yonemoto 2008). In March 2016, grey mould disease was observed on mango (cv. Irwin) in a greenhouse at Itoman City, Okinawa Prefecture. Initially, white mycelium appeared on lesions of petals and peduncles (Fig. 1a, b). Lesion spread from the base of the rotten peduncles, forming slightly grey spores on the lesion's surface (Fig. 1c). The surface of the infected tissues became necrotic and black, and a close-up of the spore masses at the upright conidiophores apices could be seen (Fig. 1d). As the disease progressed, the rot of panicle parts was covered with dense masses of grey spores, causing a fall of the whole inflorescence and resulting in fruit set failure (Fig. 1e). This disease differed from previously identified mango diseases in Japan, such as flyspeck, Sclerotinia rot and powdery mildew (Ajitomi et al. 2017, 2018, 2020b).

The rot of panicle parts was collected and soaked in 70% (v/v) ethanol for 30 s and 2% (v/v) sodium hypochlorous solution for two min before being rinsed in sterilised water, air-dried, and deposited on potato dextrose agar (PDA) plates (Becton, Dickinson and Co., Sparks, MD, USA). As a result, we obtained two representative isolates, which developed white aerial mycelium that turned greyish-brown on the medium and subsequently formed abundant greyish-brown aerial mycelium with conidia and conidiophores near the edge of the plates at 20 °C in seven days (Fig. 1f, h). We deposited both isolates in GeneBank, National Agriculture and Food Research Organisation as MAFF247511 and MAFF247512, respectively.

We used the two representative isolates (MAFF247511 and MAFF247512) for cultural and morphological observations. On PDA, MAFF247511 and MAFF247512 grew at 5 °C–30 °C with optimal growth of 18.5 mm and 13.8 mm at 20 °C, respectively. On PDA, isolate MAFF247511 produced flat, irregular, black sclerotia measuring 1.3–7.6 mm long, 1.1–5.0 mm wide and 0.9–2.3 mm in height after 21 days (Fig. 1g, Table 1). Isolate MAFF247512 did not develop sclerotia. Both isolates' conidiophores appeared straight or flexuous, septate, branched at the upper part and each branch end with spherical swelling carrying a cluster

✉ Atsushi Ajitomi
ajitomia@pref.okinawa.lg.jp

[1] Okinawa Prefectural Agricultural Research Centre, 820, Makabe, Itoman, Okinawa 901-0336, Japan

[2] Okinawa Prefectural Department of Agriculture, Forestry and Fisheries, Chubu Agricultural Development Centre, 1-6-34 Mihara, Okinawa, Okinawa 904-2155, Japan

[3] Okinawa Prefectural Agricultural Research Centre Nago Branch, 4605-3, Nago, Nago, Okinawa 905-0012, Japan

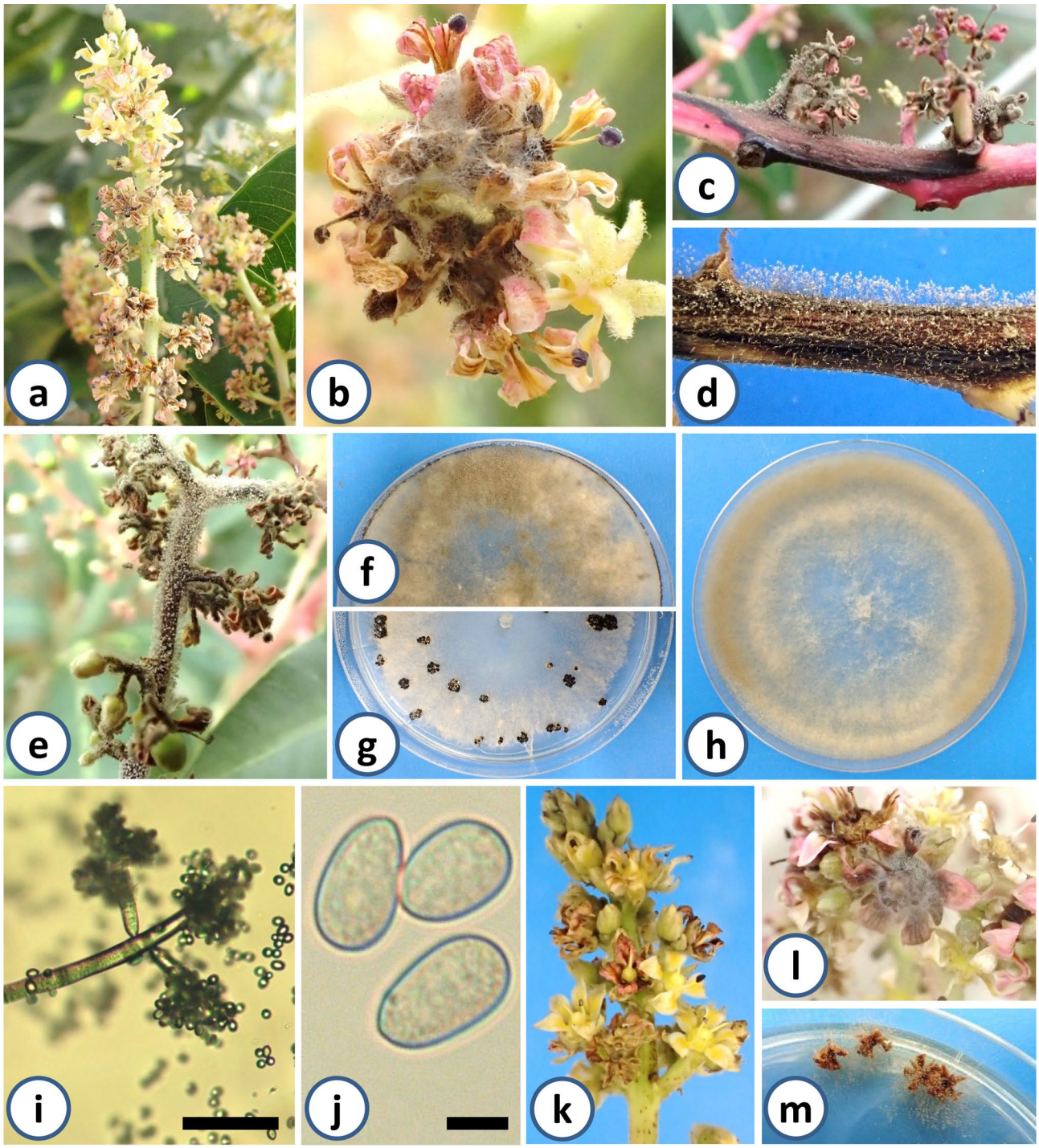

Fig. 1 Symptoms of the grey mould on mango flowers caused by *Botrytis cinerea* in the field **a**, **b** Lesions on petals and peduncles with a thin aerial mycelium. **c** Lesion spread from the base of the rotten peduncles, forming slightly grey spores on the lesion's surface. **d** The surface of the infected tissues became necrotic and black, and a close-up of the spore masses at the upright conidiophores apices can be seen. **e** As the disease progressed, the rot of panicle parts was covered with dense masses of grey spores, causing a fall of the whole inflorescence. **f, g** Colonies of the isolated MAFF247511 cultured on PDA in the dark at 20° were accompanied by aerial mycelium after seven days and formed sclerotia after 21 days. **h** Colonies of the isolate MAFF247512 cultured on PDA in the dark at 20° were accompanied by aerial mycelium after seven days but had not formed nuclei after 21 days. **i** Conidiophores appeared straight or flexuous, septate, branched at the upper part and each branch end with spherical swelling carrying a cluster of conidia. **j** Conidia were hyaline, mostly ellipsoid or ovoid. **k, l** Three days after inoculation, aerial grey mycelium developed on the inoculated petals and peduncles. **m** Reproduced symptoms and signs by spray inoculation with conidia of isolate BCM1 (*Bars*: 60 µm for **i**, 10 µm for **j**)

of conidia, 0.3–3.0 mm long and 7.3–15.8 µm wide. Both isolates' conidia were hyaline, mostly ellipsoid or ovoid and they ranged in size from 6.7–17.3 µm to 5.8–10.3 µm (Fig. 1i, j). Several researchers compared these morphological characteristics with descriptions of *B. cinerea* (Aktaruzzaman et al. 2017; Ellis 1971; Nagashima et al. 2021), and the results indicated that the morphology of the sclerotia and conidia were similar, although the length of the conidiophores was inconsistent by researchers (Table 1).

Glyceraldehyde-3-phosphate dehydrogenase (*g3pdh*), heat-shock protein 60 (*hsp60*) and DNA-dependent RNA polymerase subunit II (*rpb2*) from both isolates were sequenced and analysed to confirm the species identity. We used a ZR Fungal/Bacterial DNA kit (Zymo Research, Irvine, CA, USA) to extract genomic DNA. *g3pdh*, *hsp60* and *rpb2* genes were amplified with the primer pairs G3PDHfor/G3PDHrev, HSP60for/HSP60rev and RPB2for/RPB2rev (Staats et al. 2005), respectively. Furthermore, we determined DNA sequences of the amplified products using a BigDye Terminator v1.1 Cycle Sequencing Kit (Life Technologies, Foster City, CA, USA) and DNA sequencer (ABI3130, Life Technologies) following the manufacturer's instructions. We deposited the DNA sequences obtained in this study in the DDBJ/EMBL/GenBank database with accession numbers LC651615–LC651620, respectively. BLAST (Altschul et al. 1990) analysis revealed that both isolates had high similarity to *B. cinerea* (ex-type strain: MUCL87) (Staats et al. 2005), with the *g3pdh*, *hsp60* and *rpb2* regions showing 100%, 98%–100% and 99% similarity with AJ745676, AJ716065 and AJ705004, respectively. Moreover, the combined *g3pdh*, *hsp60* and *rpb2* sequences of each isolate with reference sequences obtained from GenBank, were aligned using ClustalW and a neighbour-joining phylogenetic tree was constructed using MEGA version 7 (Kumar et al. 2016). The reference sequence strains used were 51 strains, including 49 strains of 22 species of *Botrytis* and two outgroup strains, namely *Sclerotinia sclerotiorum* (strain: 484) and *Monilinia fructigena* (strain: 9201). The numbers at selected nodes represent the support level using 1000 bootstrap replicates. Both mango isolates were clustered into a group with *Botrytis* spp. and putative species of the genus and placed on a branch next to one containing *B. cinerea*, as reported by Staats et al. (2005) (Fig. 2).

We prepared a conidial suspension (1×10^4 cfu/mL) of isolate MAFF247511 by flooding the colony on PDA with 10 mL of sterilised water and scraping the colony's surface with a bacterial spreader. Then, we sprayed the suspension onto two healthy mango panicles (cv. Irwin) cut from branches just before inoculation. Furthermore, two healthy panicles sprayed with sterilised water served as the control. Treated panicles were water-cultivated in a growth chamber regulated at 20℃ in the dark and covered with a plastic bag to maintain high humidity conditions. Three days after inoculation, aerial grey mycelium developed on the inoculated petals and peduncles (Fig. 1k). A fungus similar to the inoculum was reisolated from the aerial mycelium on inoculated panicles (Fig. 1l, m), whereas no symptoms were observed on the controls. Thus, the isolate was demonstrated to be the cause of the disease.

We deduced that *B. cinerea* caused this disease based on our results. Mango blossom blight caused by *B. cinerea* has already been reported in Brazil, New Zealand, Pakistan and Hawaii (Farr and Rossman 2021), and it has also been reported in Japan as 'grey mould of mango' caused by *Botrytis* sp. in a Japanese handbook (Tokashiki 1995). From the symptoms and signs, it seems the same as the current disease, although the previous report excludes any identification of the species of pathogen or the results of inoculation tests. Therefore, we formally designated this disease in this study as grey mould (haiirokabi-byo in Japanese) caused by *B. cinerea* on floral tissue of mango in Japan. Stem-end rot is another symptom of *B. cinerea* caused mango disease that has been reported in Egypt and Pakistan (Abdallah et al. 2003, Alam et al. 2017). In Japan, numerous pathogens cause mango stem-end rot disease, such as *Diaporthe* spp., *Lasiodiplodia theobromae* sensu stricto (Pat.) Griffon and Maubl and *Neofusicoccum parvum* (Pennycook and Samuels) Crous, Slippers & A.J.L. Phillips (Ajitomi et al.

Table 1 Morphological characteristics of the two fungal isolates from mango and published descriptions *of Botrytis cinerea*

Isolates	Sclerotia L×W (mm)	Conidiophores L (mm)×W (µm)	Conidia L×W (µm)	L/W ratio
MAFF247511	1.3–7.6×1.1–5.0	0.6–3.0×7.9–15.8	6.7–17.3×5.8–10.3	1.05–2.24
MAFF247512	nf	0.3–1.6×7.3–12.1	9.2–16.1×5.9–10.3	1.13–2.42
B. cinerea[a]	1.2–4.3×1.1–3.5	0.1–0.4×12.1–26.3	5.1–8.5×5.2–9.8	nr
B. cinerea[b]	nr	>2 or more×16–30	6–18×4–11	1.35–1.5
B. cinerea[c]	1.3–7.0×1.3–5.0	0.7–2.6×nr	6.6–12.6×4.1–8.0	1.56–1.62

L length, *W* width, *Diam* diameter, *nf* not formed, *nr* not reported

[a] Aktaruzzaman et al. (1993)

[b] Ellis (1971)

[c] Nagashima et al. (2021)

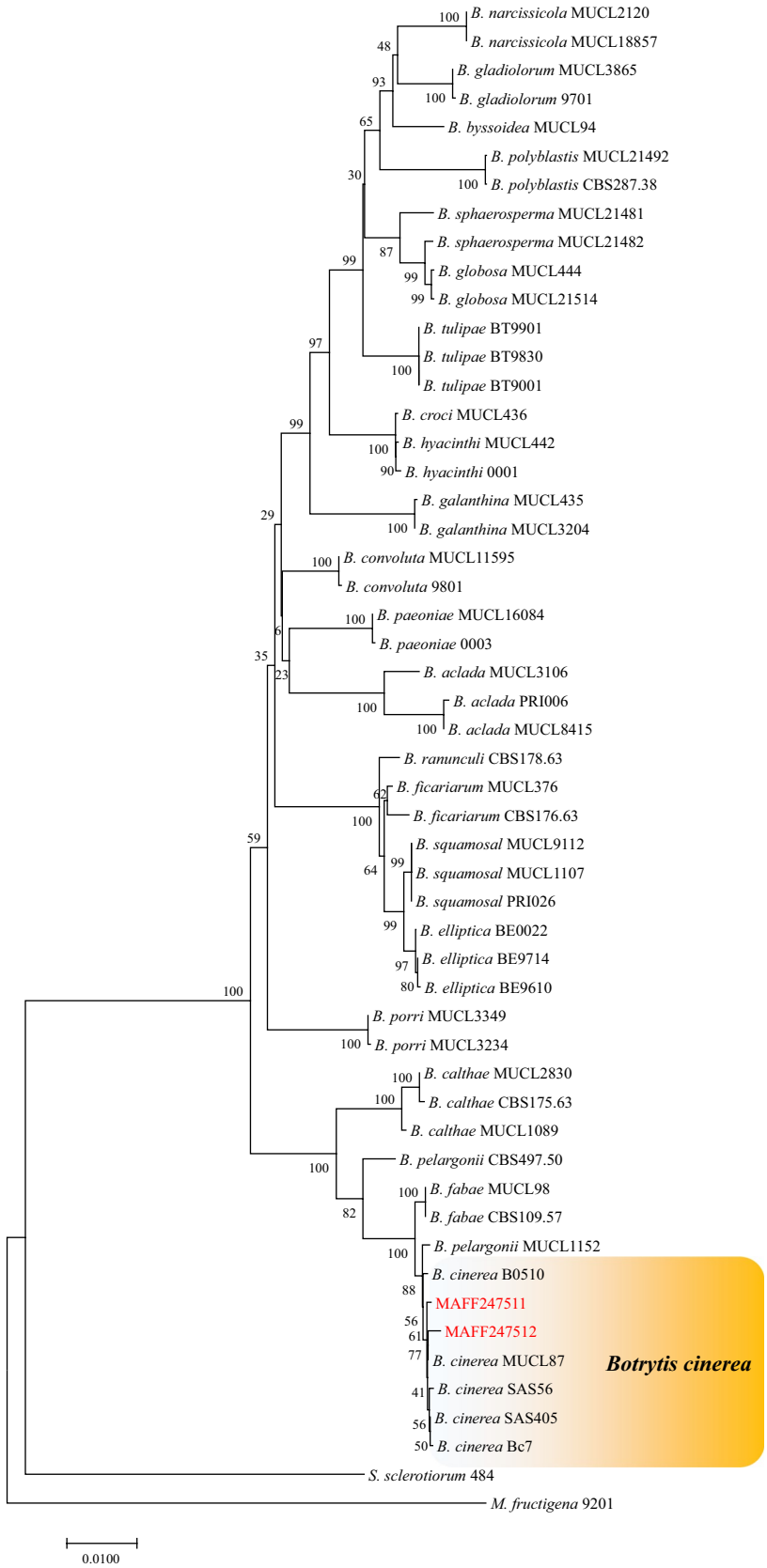

Fig. 2 Neighbour-joining phylogenetic trees were constructed using MEGA v.7 based on the nucleotide sequences of *g3pdh*, *hsp60* and *rpb2* regions for the *Botrytis* spp. The reference sequence strains used were 51 strains, including 49 strains of 22 species of *Botrytis* and two outgroup strains, namely *Sclerotinia sclerotiorum* (strain: 484) and *Monilinia fructigena* (strain: 9201). Numbers after taxa are strain numbers

2020a; Takushi et al. 2013, 2017). So far, *B. cinerea* caused mango stem-end rot disease has not been confirmed in Japan. We could not determine the cause of the occurrence of stem-end rot caused by *B. cinerea* wound due to different cultivation methods, and the cultivars of mango differ between Japan and other countries; however, it is necessary to pay attention to the occurrence in Japan in the future.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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