

Fusarium Wilt of Korean Blackberry Caused by *Fusarium cugenangense*

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Wilt symptoms were frequently observed in Korean blackberry (*Rubus coreanus*) plants grown in farmers' fields located in Gochang and Jeongeup, Jeonbuk Province, Korea during disease surveys in July 2020. The disease occurred in 10 of the 13 fields surveyed in the two locations. The incidence of diseased plants in the fields was 5–80%. Seven isolates of *Fusarium* sp. were obtained from the diseased plants and examined for their morphological and molecular characteristics. All the isolates belonged to *Fusarium oxysporum* species complex based on the morphological characteristics but were identified as *F. cugenangense* based on the molecular characteristics. Two isolates of *F. cugenangense* were tested for pathogenicity on Korean blackberry plants by artificial inoculation. Pathogenicity of the two isolates on the plants was confirmed with the inoculation tests, which showed wilt symptoms similar to those observed in the diseased plants in the fields investigated. This is the first report of *F. cugenangense* causing Fusarium wilt in Korean blackberry.

Keywords: *Fusarium cugenangense*, Fusarium wilt, Korean blackberry, Pathogenicity, *Rubus coreanus*

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Korean blackberry (*Rubus coreanus*) belonging to family *Rosaceae* is also called Bokbunja. The native range of the plant is China and Korea (Plants of the World Online, 2021). The plant is distributed not only in China and Korea, but also in Taiwan, Japan, and Austria (Bio Resource Information Service, 2021). The plant grows mainly in mountainous areas. Fruits of the plant are edible and also used for medicinal purposes, along with leaves and roots of the plant. In the central and southern regions of Korea, some farmers grow the plant in fields as a fruit tree.

Korean blackberry is a broadleaf shrub which blooms in May and bears fruits in June. Wilt symptoms were frequently observed in the plants grown in farmers' fields located in Gochang and Jeongeup, Jeonbuk Province, Korea during disease surveys in July 2020. In the early stages of the disease,

the symptoms appeared as slight wilting of the plant leaves (Fig. 1A). The basal stems of the infected plants turned dark. In the late stages of the disease, the plants wholly wilted and blighted (Fig. 1B). Stems of the diseased plants turned black overall and rotted. Three sites were observed in a field, and twenty plants at each site were investigated for the disease incidence. The disease occurred in 10 of the 13 fields surveyed in the two locations (Table 1). The incidence of diseased plants in the fields was 5–80%.

Fungal pathogen was isolated from diseased plants of Korean blackberry collected. The 3–5 mm-long lesion pieces cut from stems of the diseased plants were plated on 2% water agar after surface-sterilizing with 1% sodium hypochlorite solution for one min. The fungal mycelia growing from the lesion pieces were transferred to potato dextrose agar slants after incubating the plates at 25°C for 2–3 days. Seven isolates of *Fusarium* sp. were obtained from the lesion pieces of the diseased plants and examined for their morphological characteristics by a light microscope. Microconidia and

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Fig. 1. Wilt symptoms of Korean blackberry plants. (A, B) Symptoms observed in the fields investigated. (C, D) Symptoms induced by artificial inoculation tests with *Fusarium cugenangense* isolates. (E) Non-inoculated plant (control).

Table 1. Occurrence of wilt disease on Korean blackberry plants grown in farmers' fields located in Gochang and Jeongeup, Jeonbuk Province, Korea in July 2020

Location investigated	No. of fields investigated	No. of fields with wilt disease	% diseased plants ^a
Gochang	7	4	5–80
Jeongeup	6	6	5–30

^aThree sites were observed in a field, and twenty plants at each site were investigated for the disease incidence.

macroconidia were produced on short monophialides (Fig. 2A, B). Microconidia were ellipsoidal to cylindrical, straight to curved, 0–1 septate, and measured 6.0–16.0×2.0–4.2 μm (av. 10.0×3.0 μm). Macroconidia were falcate, foot-shaped at both ends, 2–5 septate, and measured 18.7–42.1×2.8–

5.0 μm (av. 30.6×4.0 μm). Size of conidiophores were 3.2–13.0×1.2–3.8 μm (av. 6.8×2.4 μm). The morphological characteristics of the isolates were similar to those of *Fusarium oxysporum* described in previous studies (Booth, 1971; Domsch et al., 1993; Lombard et al., 2019).

To confirm the morphological identification of the seven isolates, DNA sequencing of the partial translation elongation factor 1-alpha (TEF) and RNA polymerase II largest subunit (RPB2) genes was analyzed. Genomic DNA was extracted from mycelium powder using the Maxwell RSC Cell DNA Purification kit (Promega, Madison, WI, USA). TEF and RPB2 genes were amplified from genomic DNA using primer sets of EF1/EF2 and 7cf/11ar, respectively (O'Donnell et al., 2010). Multiple sequence alignments of the two genes were carried out using Clustal W, and Neighbor-Joining tree for the combined dataset (total 1,486 bp) was generated us-

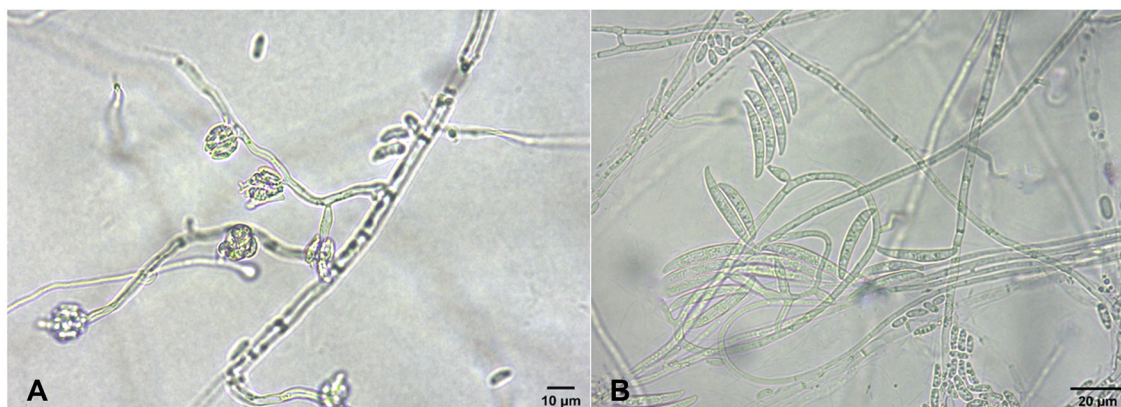


Fig. 2. Morphological features of *Fusarium* sp. isolates from diseased Korean blackberry plants. (A) Microconidia, monophialides, and hyphae of the isolate. (B) Microconidia, macroconidia, monophialides, and hyphae of the isolate.

ing MEGA X software (Kumar et al., 2018). The phylogenetic tree based on loci TEF and RPB2 combined sequence data showed that all the isolates were clustered in a group with *Fusarium cugenangense* strains (Fig. 3). The sequences of the two loci genes from the isolates were 99–100% identical to those of the reference strains of the GenBank database. The nucleotide sequences of TEF and RPB2 genes obtained from

the seven isolates were deposited in NCBI GenBank with accession numbers of OK041485–OK041491 and OK041478–OK041484, respectively.

The genus *Fusarium* contains many species complexes (O'Donnell et al., 2015). *F. oxysporum* is known to be a species complex, comprising of many species and clades (Achari et al., 2020; Baayen et al., 2000; O'Donnell et al., 1998). The

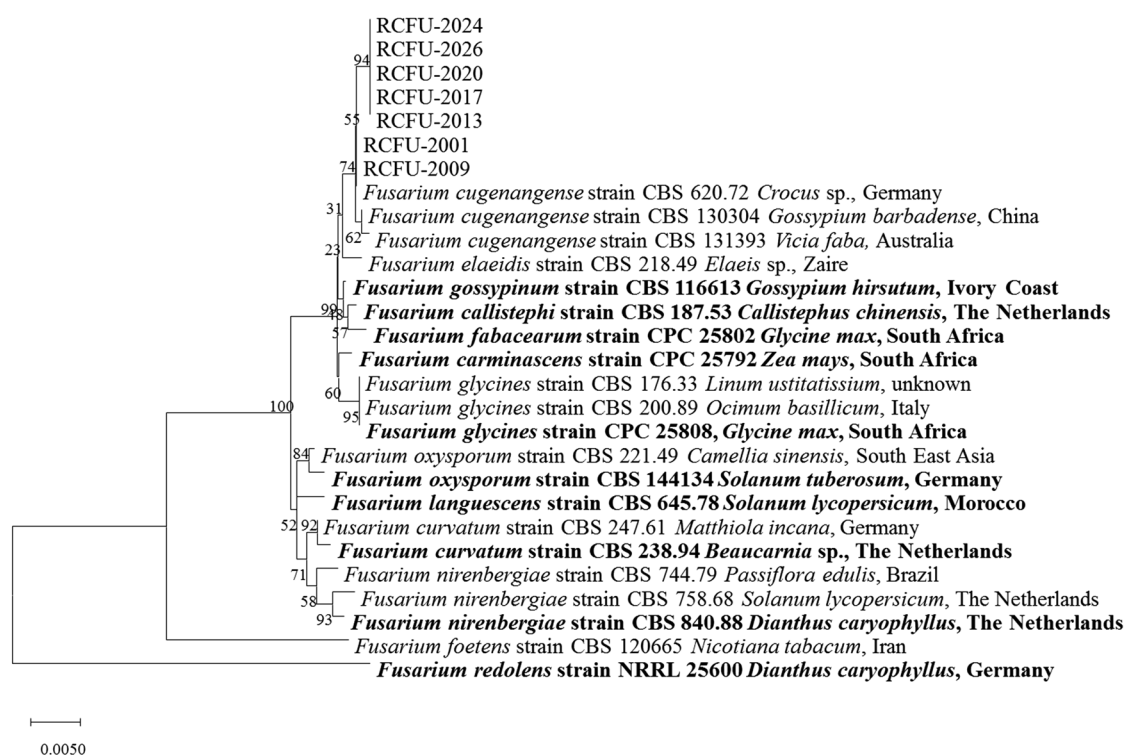


Fig. 3. Phylogenetic tree based on the elongation factor 1 alpha (TEF) and RNA polymerase second largest subunit (rpb2) genes sequence dataset for *Fusarium cugenangense* isolates from diseased Korean blackberry and reference species. Sequence data were obtained from the NCBI GenBank database. The tree was generated using neighbor-joining analysis and Kimura 2-parameter model. The bar represents the number of nucleotide substitutions per site. Ex-type and Epitype cultures are indicated in bold.

epitype of *F. oxysporum* was newly established using multi-locus phylogenetic inference and subtle morphological differences (Lombard et al., 2019). In this study, *Fusarium* sp. isolates from diseased plants of Korean blackberry belonged to *F. oxysporum* species complex based on the morphological characteristics but were identified as *F. cugenangense* based on the molecular characteristics.

Among the seven isolates of *F. cugenangense*, two were tested for pathogenicity on Korean blackberry plants using artificial inoculation. Each isolate was cultured on cornmeal-sand medium (23 g cornmeal : 210 g sand : 60 ml distilled water) in 500 ml-flasks for 50 days to prepare the inoculum. The inoculum was inoculated to two-month-old Korean blackberry plants that were grown in circular plastic pots (height, 15 cm; upper diameter, 17 cm; lower diameter, 10 cm) in a vinyl greenhouse. For the inoculation test, surface soil around the plant was dug at a depth of 2–3 cm, and 60 g of each inoculum was placed around the stem. The inoculated plant part was covered with the original soil. The same quantity of cornmeal-sand medium was used for the control plant. The inoculated plants were cultivated in a greenhouse at 24–30°C. The inoculation test was performed in triplicate. Virulence of the isolates was rated based on the degree of wilt symptoms induced 15 days after inoculation.

The tested isolates of *F. cugenangense* induced wilt symptoms in the inoculated plants (Fig. 1C, D), whereas no symptoms were observed in the control plant (Fig. 1E). The symptoms induced by the artificial inoculation of plants were similar to those observed in the diseased plants in the fields investigated. The isolates that induced symptoms on the plants were re-isolated from the stem lesions.

It has been reported that *F. oxysporum* causes Fusarium wilt in blackberry (*Rubus* subgenus *Rubus*) (Gorden et al., 2016; Martin et al., 2017). In addition, the pathogen causing Fusarium wilt of blackberry was reported to have a forma specialis (Pastrana et al., 2017). *F. cugenangense* was first reported based on the phylogeny and genetic diversity of banana Fusarium wilt pathogen in Indonesia (Maryani et al., 2019). The pathogen has also been reported to cause diseases in *Gossypium barbadense*, *Vicia faba*, *Crocus* sp., and *Musa* sp. (Farr and Rossman, 2021). However, there has been no report on disease occurrence caused by the pathogen in Korean blackberry. This is the first report of *F. cugenangense* causing Fusarium wilt in Korean blackberry.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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