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First report of root rot of goji (*Lycium barbarum*), caused by *Fusarium sambucinum*

Pingping SUN[1], Yuchen GUO[1], Lei ZHANG[1], Rong YANG[2], Zhengnan LI[1,*]

[1] *College of Horticulture and Plant Protection, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, China*
[2] *Inner Mongolia Academy of Forestry Sciences, Hohhot, Inner Mongolia 010010, China*
*Corresponding author. E-mail: lizhengnan@imau.edu.cn

Summary. In July 2022, root rot was observed in several goji (*Lycium barbarum*) orchards located in Qinghai Province, China. Approximately 40% of the goji plants were affected in the orchards. Morphology of fungi isolated from affected plant, phylogenetic analyses, using internal transcribed spacer (ITS), translation elongation factor 1-alpha (TEF), and trichothecene (Tri5) sequences, as well as pathogenicity assays, were conducted to characterize and identify the causing agent of goji root rot. Isolate GQGF1-3 caused typical symptoms of *L. barbarum* root rot. Fungal colony characteristics and conidium morphology, combined with ITS, TEF, and Tri5 sequences showed that isolate GQGF1-3 was *Fusarium sambucinum*. This is the first report of *F. sambucinum* causing root rot of goji.

Keywords. Pathogen identification, *Lycium barbarum*.

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ORCID:
PS: 0009-0005-6140-2307
YG: 0009-0007-1094-6317
LZ: 0000-0001-7479-952X
RY: 0009-0004-4828-8573
ZL: 0000-0003-3268-2096

INTRODUCTION

Goji (*Lycium barbarum* L.) is a perennial shrub in *Solanaceae*. Berries of this plant are rich in polysaccharides, carotenoids, and flavonoids, and are widely used as a traditional Chinese functional food (Potterat, 2010). Goji plants are tolerant to salinity, drought, and low temperatures, and are widely cultivated as medicinal or ornamental trees, or for soil amelioration in arid and semi-arid regions of China, Japan, South Korea, and southeast Europe (Zhang *et al.*, 2022).

Qinghai province is one of the most important areas in China for production of goji berries (Lu *et al.*, 2021). Continuous expansion of planting of *L. barbarum*, together with poor crop management, has caused goji root rot to become one of the most severe diseases during goji cultivation (Cariddi *et al.*, 2018). The affected roots show exposed xylem and visible dark brown vascular bundles, and these symptoms lead to reductions in fruit yields and death of whole plants. Incidence of goji root rot has been reported to be more than 30%, and the disease has caused approx. 35% reduction of goji yields (Cariddi *et al.*, 2018; Bai *et al.*, 2020).

In July 2022, root rot was observed in several goji orchards located in Qinghai Province, China (36°62'N, 101°78'E). Approximately 40% of the goji

plants in the orchards showed root rot symptoms. The affected plants had withered leaves, black rotten roots, and detached root bark, and the disease often caused death of the affected plants.

In this study potential goji root rot causal agents were isolated, characterized, and identified, using morphology, molecular identification and pathogenicity tests. This study was undertaken to provide the basic information for formulating effective management of goji root rot.

MATERIALS AND METHODS

Collection of Lycium barbarum *plants and isolation of potential pathogens*

Diseased *L. barbarum* plants were collected from several orchards in Qinghai Province, China, during July 2022, and were sent to the Laboratory of Pathogen Biology and Comprehensive Control of Horticultural Diseases at the Inner Mongolia Agricultural University. Boundaries between healthy and diseased plant tissues were cut, and then surface sterilized by immersing in 1% NaClO for 3 min, 75% ethanol for 30 s, followed by rinsing three times with sterile distilled water. The tissue pieces were then incubated on potato dextrose agar (PDA) at 28°C. Frontier mycelium of resulting fungal colonies was transferred onto fresh PDA, and incubated for 14 d. Serial dilutions of conidia from cultures were applied to PDA plates to obtain single conidium isolates. Single conidium cultures were then maintained on PDA for further tests.

Pathogenicity test

Pathogenicity of isolates was assessed by inoculating each isolate into healthy goji seedlings. To produce large quantities of conidia, the isolates were inoculated into potato dextrose broth, which was then incubated on a rotary shaker (180 rpm) at 28°C. After 7 d, mycelium with conidia and broth were filtered through three layers of sterilized gauze to remove the hyphae. Conidium concentration was adjusted to approx. 10^7 conidia mL^{-1}. Healthy goji seedlings were grown in plastic pots containing sterilized peat soil and sand (1:1 volume). For inoculations, four holes were made in the potting medium in each pot at approx. 3 cm from the seedlings. A scalpel was used to cut the roots of each plant. Ten mL of conidium suspension was poured into the holes. Six replicate pots were inoculated with each isolate, and sterilized water was similarly applied as the inoculation control. The treated plants were transferred to a greenhouse with temperature between 20 and 28°C, and were watered every second day. Disease symptoms were observed at 15 to 20 d post inoculation.

Identification of pathogenic isolates

Morphological and molecular methods were used to identify the isolates obtained from diseased plants. Isolates were transferred onto PDA, mung bean residue agar (MBRA: 20 g mung bean, 1 L water, autoclaved for 30 min, mixed by a blender, 20 g agar, autoclaved for 20 min at 121°C), carnation leaf agar (CLA), and synthetic low nutrient medium (SNA), and were cultured at 28°C in an incubator. Macroconidia were observed on PDA, MBRA, and CLA, and microconidia were observed on SNA medium.

Mycelia of one selected isolate were collected, and ground with liquid nitrogen. The fungal DNA extraction kit (Sangon Biotech) was used to extract DNA. With DNA as a template, universal primers ITS1/ITS4 were used to amplify the internal transcribed spacer (ITS) regions (White *et al.*, 1990). The PCR reaction system was as follows: 10 μL of 2× M5 HiPer plus Taq HiFi

Table 1. Details of amplification conditions, including sequence primers, annealing temperatures, and extension timse.

Target	Primers	Sequence 5'→3'	PCR conditions	Product size (bp)	References
ITS	ITS1/ITS4	TCCGTAGGTGAACCTGCGG TCCTCCGCTTATTGATATGC	55°C, 40 s	~550	White *et al.* (1990)
Fusarium sambucinum	FSF1/FSR1	ACATACCTTTATGTTGCCTCG GGAGTGTCAGACGACAGCT	58°C, 30 s	315	Mishra *et al.* (2003)
TEF	ef1'/ef2'	ATGGGTAAGGAAGACAAGAC GGAGGTACCAGTGATCATGTT	58°C, 55 s	700	Du *et al.* (2012)
Tri5	Tri5F/Tri5R	AGCGACTACAGGCTTCCCTC AAACCATCCAGTTCTCCATCTG	60°C, 30s	545	Nicholson *et al.* (2004)

PCR Mix, 7 µL of dd H_2O, 1 µL of DNA template, 1 µL of each upstream and downstream primers; The reaction conditions were: 95°C for 3 min; 35 cycles of 94°C for 25 s, 55°C for 30 s, and 72°C for 40 s, and then 72°C for 10 min.

Based on the resulting ITS sequence, *Fusarium sambucinum* species-specific primer pair FSF1/FSR1 (Mishra *et al*., 2003) was used. The ef1'/ef2' (Du *et al*., 2012) were used for amplification of the partial translation elongation factor 1-alpha (TEF).

Potential ability by cultures to produce trichothecenes was tested using primers specific for the gene Tri5 (Nicholson *et al*., 2004). Sequences of all primers, product sizes, annealing temperature and extension time for each assay are presented in Table 1. The PCR amplification products were detected by 1.2% agarogel electrophoresis, and were recovered and purified using an agarogel DNA recovery kit (Tiangen). The products were sent to Sangon Biotech (Shanghai, China) for sequencing. The homologous sequence was retrieved by NCBI using BLASTn to determine its taxonomic status.

RESULTS

A total of 18 fungal isolates were obtained from goji samples. Pathogenicity tests showed that *L. barbarum* plants inoculated with isolate GQGF1-3 showed typical symptoms of root rot 3 weeks after inoculation, while the control plants and the plants inoculated with other isolates were asymptomatic. The pathogenicity test was repeated three times, with similar results. Leaves of the infected plants were chlorotic and wilted, and the roots of inoculated plants were rotted, with brown vascular bundles (Figure 1).

Isolate GQGF1-3 had yellow to light grey, dense and floccose aerial mycelium, and yellow mycelium on the reverse sides of PDA culture plates. On MBRA, the isolate had white, fluffy aerial mycelium, and yellow to brown colouration on reverse sides (Figure 2, A to D). Macrospores of the isolate were sickle-shaped, with 3 to 4 septa, and measured 11 to 25 × 2.5 to 4.5 µm on PDA (Figure 2, E), 15 to 26 × 1.5 to 3.5 µm on MBRA (Figure 2, F), and 15 to 19 × 1.5 to 3 µm on CLA (G). Single-celled and ovoid microspores measured 4.0 to 7.5 × 0.8 to 1.2 µm on SNA (Figure 2, H).

Isolate GQGF1-3 was morphologically identified as a *Fusarium* sp. The internal transcribed spacer (ITS) region of the isolate was amplified, and the obtained sequence was submitted to GenBank, with accession number of OR342306. Blastn analysis of the isolate ITS sequence revealed 100% similarity to 17 isolates of *Fusarium sambucinum*, which had been isolated in China, India, Eygpt, or Canada. The phylogenetic tree based on the ITS sequence showed that GQGF1-3 clustered with *F. sambucinum* strains (Figure 3, A). *Fusarium sambucinum* species-specific primer pair FSF1/FSR1 amplified GQGF1-3, and gave a 350 bp sequence (Figure. 3, B). Phylogenetic trees based on TEF (Genbank accesion number: PP294739) and Tri5 (Genbank accesion number: PP294738) both showed that isolate GQGF1-3 clustered with *Fusarium sambucinum* (Figure 4).

Based on colony characteristics, conidium morphology, and ITS, TEF and Tri5 sequences, isolate GQGF1-3 was identified as *F. sambucinum*. In addition, re-isolation of this fungus from inoculated goji roots in the pathogenicity test confirmed Koch's postulates for the pathogen.

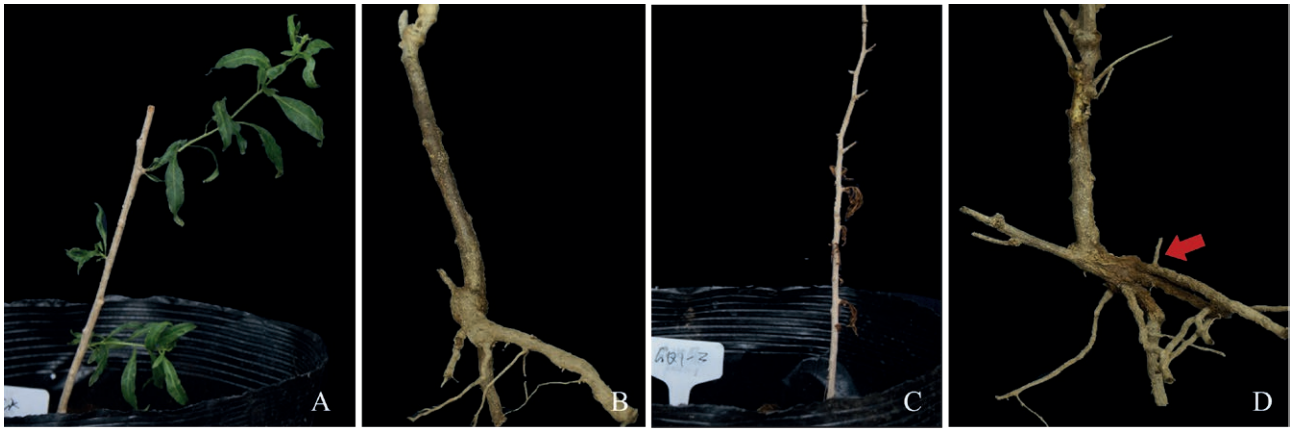

Figure 1. Symptoms on goji plants inoculated with isolate GQGF1-3. A and B show symptoms of control (non-inoculated) plants, C and D are plants that were inoculated with isolate GQGF1-3.

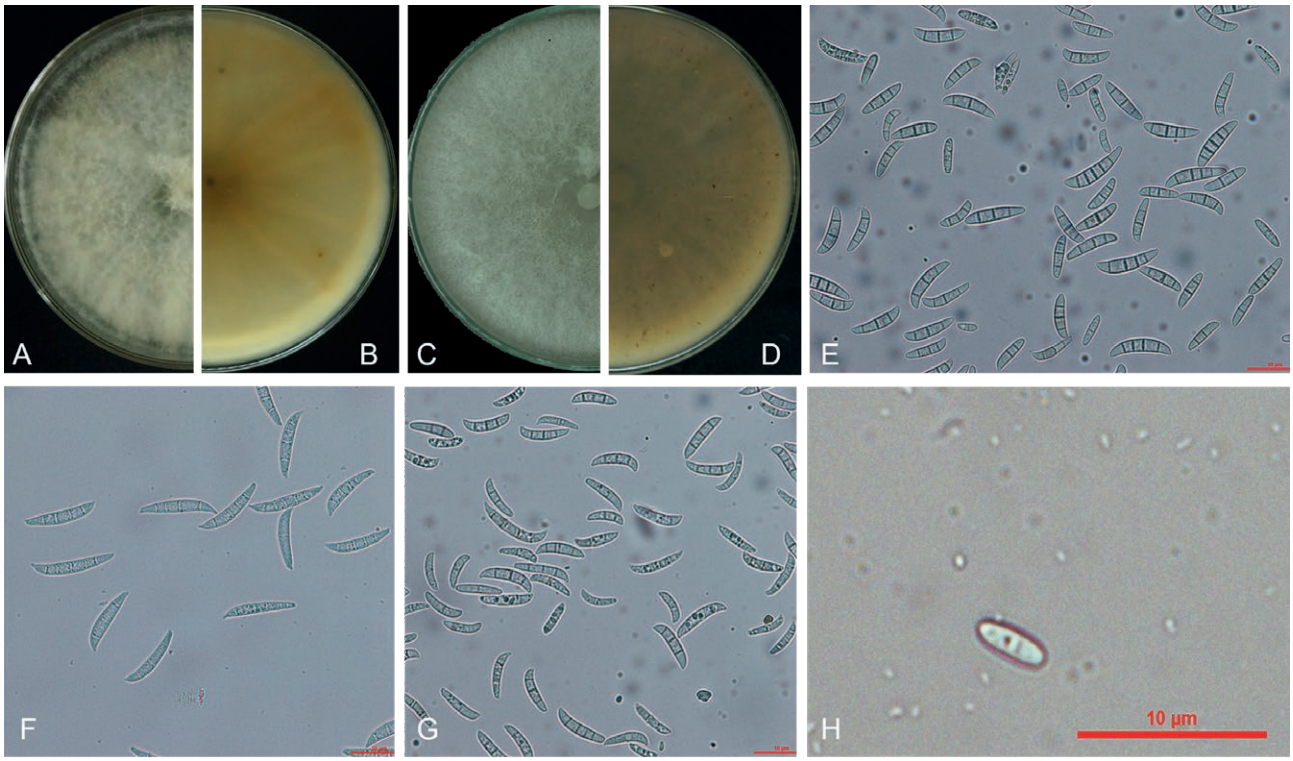

Figure 2. Colony and conidium morphology of isolate GQGF1-3. A, upper and B, reverse side of a GQGF1-3 colony on PDA, and C, upper and D, reverse side on MBRA. Macroconidia of GQGF-3 on PDA (E), MBRA(F), and CLA (G), and a microspore on SNA (H). Scale bars in E to H indicate 10 μm.

DISCUSSION

Isolate *Fusarium sambucinum* GQGF1-3 caused typical root rot symptoms on *L. barbarum* plants. Pathogens previously found on *L. barbarum* include: *Fusarium oxysporum*, *F. solani*, *F. concolor*, *F. moniliforme*, *F. equiseti*, *F. incarnatum, F. culmorum*, *F. tricinctum*, *Phytophthora nicotianae* var. *parasitica*, and *Rhizoctonia solani*, of which *F. oxysporum* is the prevalent species (Bai *et al.*, 2020; Cariddi *et al.*, 2018; Chen *et al.*, 2021; Zhu *et al.*, 2023; Jia *et al.*, 2023).

This is the first report of root rot caused by *F. sambucinum* on *L. barbarum*. *Fusarium sambucinum* is a common pathogen for agricultural plants, causing head blight of wheat, maize, and barley, and dry rot of potato tubers, soybean, squash, and chilli (Alejandra *et al.*, 2019; Iwase *et al.*, 2020; Yikilmazsoy and Tosun, 2021; Kitabayashi *et al.*, 2022).

Identifying the causal agent of root rot of *L. barbarum* will support efforts for the future control and management of this disease of goji, which is an economically important perennial Solanaceous shrub.

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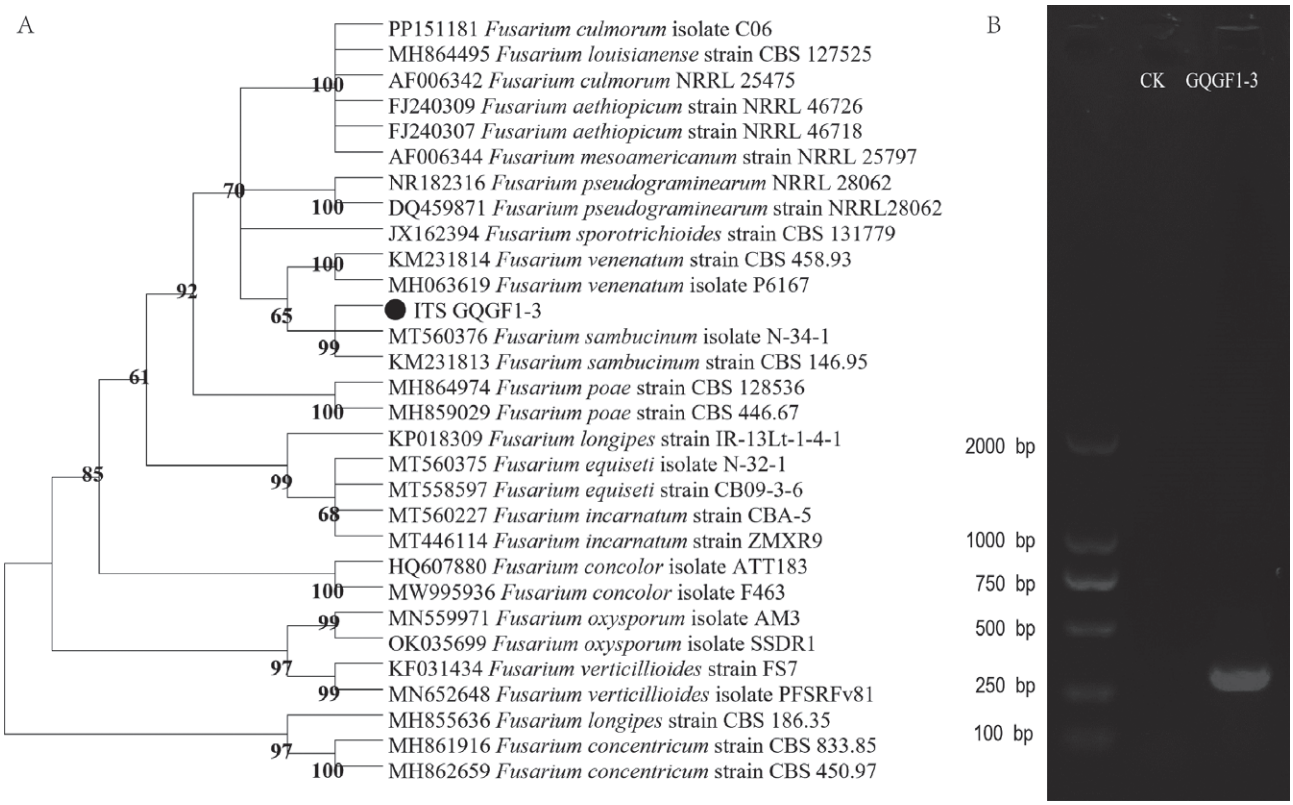

Figure 3. A, Phylogenetic tree for isolate GQGF1-3, based on ITS sequences, and B, amplification of GQGF1-3 with the species-specific primer pair FSF1/FSR1.

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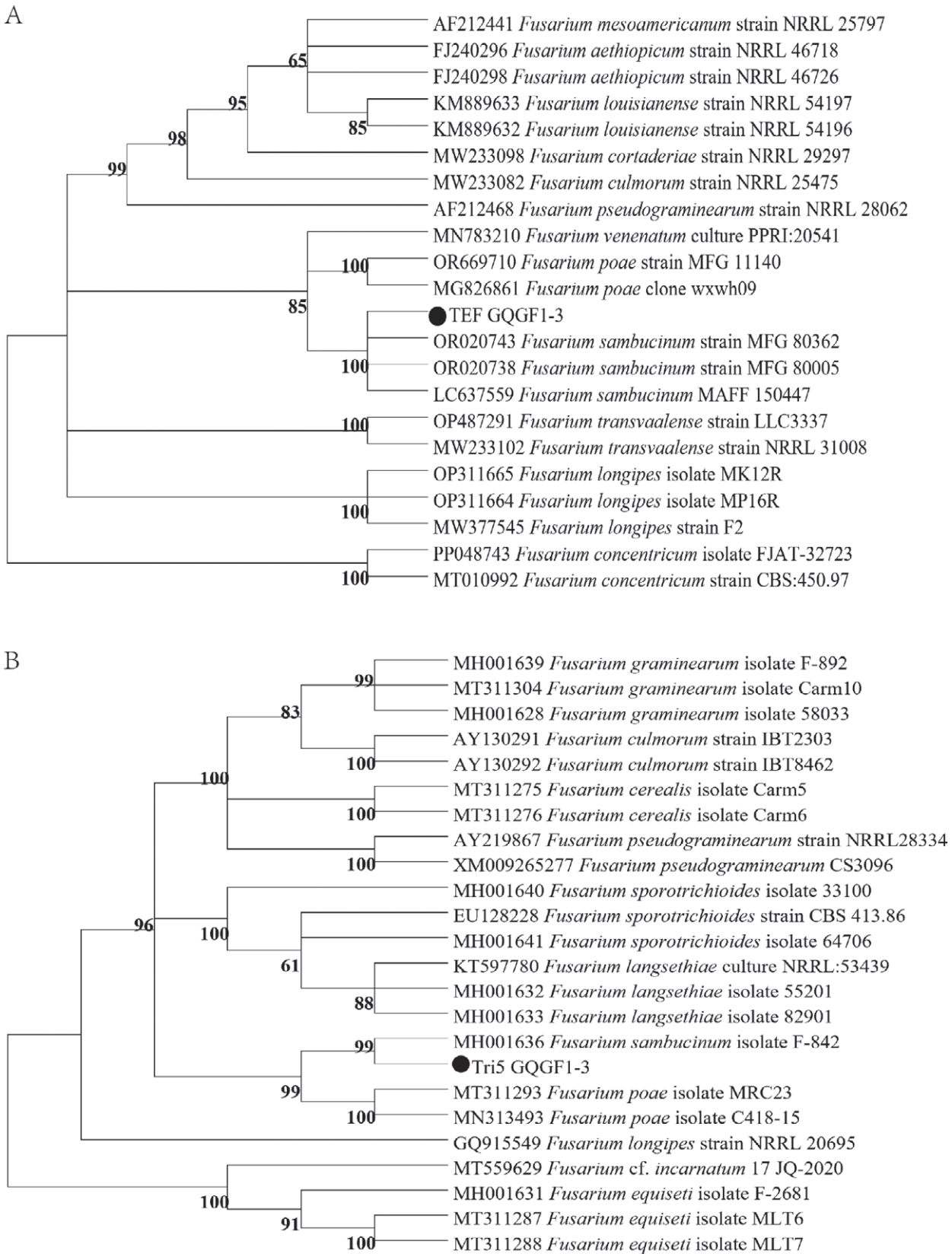

Figure 4. Phylogenetic trees for isolate GQGF1-3, based on TEF (A) and Tri5 (B) sequences.

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