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Fusarium oxysporum f. sp. *ginseng*, a new *forma specialis* causing *Fusarium* root rot of *Panax ginseng*

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Summary. *Panax ginseng* is a valuable medicinal plant which is affected by many diseases during its long cultivation period. Ginseng root rot, caused by *Fusarium oxysporum*, has become severe in China. This soilborne pathogen comprises many *formae speciales* based on host specificity. Ten representative isolates from diseased ginseng rot root showed pathogenicity on ginseng. To identify the *forma specialis* of the *F. oxysporum* strains, host range tests of three representative isolates were carried out on nine plant species. All three isolates caused severe symptoms only on ginseng, but only slight or no visual symptoms on the other eight hosts, indicating that the strains were host-specific to ginseng. Phylogenetic trees were constructed based on sequences of the translation elongation factor 1-α (*EF-1α*) gene, two endopolygalacturonase genes (*Pg1*, *Pg5*) and two exopolygalacturonase genes (*Pgx1* and *Pgx4*). The ten *F. oxysporum* isolates from ginseng clustered into a unique group clearly separated from other *formae speciales* already described. Based on the host range tests and phylogenetic analyses, the isolates of *F. oxysporum* derived from *ginseng* have been identified as a new *forma specialis*, designated *Fusarium oxysporum* f. sp. *ginseng*. This is the first report of *forma specialis* of *F. oxysporum* on ginseng.

Keywords. Soilborne pathogen, host specificity, pathogenicity, *EF-1α*, endopolygalacturonase, exopolygalacturonase.

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INTRODUCTION

Panax ginseng Meyer, a perennial herbaceous plant (*Araliaceae*), is cultivated widely in Northeast China and Korea because of its well-known medicinal value (Yun, 2001; Park *et al.*, 2012). The long cultivation period makes ginseng roots vulnerable to a variety of pathogens (Durairaj *et al.*, 2019). Ginseng root rot caused by *Fusarium* spp. can occur during the whole growth period of ginseng, especially after the third or fourth year (Reeleder *et al.*, 2002; Lee, 2004; Durairaj *et al.*, 2019). In China, incidence of ginseng root rot is usually 10-30%, and as high as 80% in severe cases (Ma *et al.*, 2021).

Fusarium spp. make ginseng roots dark brown, with wet rot, later decaying leaving hollowed-out roots in the soil, and then the inner parts of the roots disintegrate (Lee, 2004). However, the symptoms of infected roots are not obvious in the initial stage. Along with root rot development, the leaves turn yellow in the middle and later stages, and whole plants wilt and die (Lee, 2004; Punja *et al*., 2008). Ginseng root rot is very difficult to control because symptoms on above ground stems and leaves are not obvious in the early stages of the disease.

Fusarium root rot of ginseng was mainly caused by *F. oxysporum* and *F. solani* (Lee, 2004). Pathogenic *F. oxysporum* is a common fungus, causing vascular wilt or root rot symptoms of many plants (Michielse and Rep, 2009; Gordon, 2017). Generally, each strain is pathogenic to one or a few host species. Therefore, *F. oxysporum* pathogenic strains have been grouped into *formae speciales* based on specificity to host species (Gordon and Martyn, 1997; Rana *et al*., 2017). To date, approximately 150 different *formae speciales* of *F. oxysporum* have been described (Armstrong and Armstrong, 1981; Gordon and Martyn, 1997; Edel-Hermann and Lecomte, 2019). Some of these have been further ascribed to races, according to cultivar specificity (Armstrong and Armstrong, 1981; Gordon and Martyn, 1997; Edel-Hermann and Lecomte, 2019).

Identification of *formae speciales* has been based on host range tests using a set of inoculated plant hosts. However, this method is affected by factors in the process of inoculation, including temperature, soil moisture, and light. Molecular identification techniques have advantages compared to the traditional methods, and several useful molecular markers have been developed to identify the *formae speciales* of *F. oxysporum*. These include the translation elongation factor (EF) (Lievens *et al*., 2009), the secreted in xylem (SIX) effector genes (Fraser-Smith *et al*., 2014) and polygalacturonase genes (Hirano and Arie, 2009). Polygalacturonases are important in the process of pathogen infection, especially in the process of hyphae degrading cell walls and infecting plants (Pietro *et al*., 2003). Hirano and Arie (2009) and Ortu *et al*. (2013) demonstrated that polygalacturonase sequences play an important role in the identification of *formae speciales* of *F. oxysporum*. Several new *formae speciales* have been identified by phylogenetic analyses based on endopolygalacturonase and exopolygalacturonase genes. These include *F. oxysporum* f. sp. *crassulae* on *Crassula ovata* (Ortu *et al*., 2013), *F. oxysporum* f. sp. *echeveriae* on *Echeveria agavoides* (Ortu *et al*., 2015a), *F. oxysporum* f. sp. *papaveris* on *Papaver nudicaule* (Ortu *et al*., 2015b), and *F. oxysporum* f. sp. *lavandulae* on hybrid of *Lavandula dentata* and *L. latifolia* (Ortu *et al*., 2018).

With increased ginseng planting area, ginseng root rot has increased in severity, and the control of this disease mainly relies on the use of fungicides. However, control efficiencies were usually low due to the lack of effectiveness of available fungicides and untimeliness of applications. Localization of the pathogen in host vascular systems makes disease management difficult and requires early applications. In addition, the control methods for different species of *Fusarium* and *forma specialis* of *F. oxysporum* are distinct.

There are no reports of identification of the *forma specialis* of *F. oxysporum* causing root rot of ginseng. In this study, host range tests combined with molecular biology technology were used to determine a new *forma specialis* of *F. oxysporum*, *Fusarium oxysporum* f. sp. *ginseng*. This knowledge will provide a foundation for the assessing pathogenic mechanisms of *F. oxysporum*, and control of root rot of ginseng.

MATERIALS AND METHODS

Fungus strains and plants

Fusarium oxysporum isolates were obtained from ginseng root tissues with root rot symptoms, in Jilin Province, China. Other *formae speciales* of *F. oxysporum* were provided by the Agricultural Culture Collection of China (Table 1).

The following nine plant species were evaluated in host range tests: *P. ginseng* 'Damaya', *P. notoginseng* 'Wenshan', *Acanthopanax senticosus* 'Lianguanshan', *Citrullus lanatus* 'Zaojia8424', *Gossypium hirsutum* 'Qianjinwang', *Capsicum annuum* 'Baopi', *Solanum lycopersicum* 'Zhongsu 4', *Pisum sativum* 'Qizhen 76', and *Linum usitatissimum* 'Lanhua'.

Pathogenicity and host range tests

Host planting. Seeds of six plant species (*Citrullus lanatus* 'Zaojia8424', *Gossypium hirsutum* 'Qianjinwang', *Capsicum annuum* 'Baopi', *Solanum lycopersicum* 'Zhongsu 4', *Pisum sativum* 'Qizhen 76', and *Linum usitatissimum* 'Lanhua') were washed and disinfested with 5% sodium hypochlorite for 20 min, and then rinsed three times with sterile water. The seeds were then sown into pots filled with sterilized soil. When the plants grew two pieces of euphylla, the roots of the plants were dug out for inoculation. In the spring, 2-year-old seedlings of *P. ginseng*, *P. notoginseng*, *A. senticosus* were washed and disinfected with 5% sodium hypochlorite for 20 min, and then rinsed three times with sterile water. The roots

Table 1. *Fusarium oxysporum* isolates from *ginseng* and six other *formae speciales* used for pathogenicity and host range tests.

Strains	*Formae speciales*	Hosts	Sources
ACCC30222	*Fusarium oxysporum* f. sp. *vasinfectum*	*Gossypium herbaceum*	Agricultural Culture Collection of China
ACCC31037	*F. oxysporum* f. sp. *pisi*	*Pisum sativum*	Agricultural Culture Collection of China
ACCC36175	*F. oxysporum* f. sp. *niveum*	*Citrullus lanatus*	Agricultural Culture Collection of China
ACCC36465	*F. oxysporum* f. sp. *lycopersici*	*Solanum lycopersicum*	Agricultural Culture Collection of China
ACCC36472	*F. oxysporum* f. sp. *capsicum*	*Capsicum annuum*	Agricultural Culture Collection of China
BNCC85312	*F. oxysporum* f. sp. *linum*	*Linum usitatissimum*	Agricultural Culture Collection of China
0083	*F. oxysporum* f. sp. *ginseng*	*Panax ginseng*	In this study
0414	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
DH10-8	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
FS03-2	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
FS03-5	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
FS04-1	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
FS13-3	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
FS15-1	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
TH08	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study
TH10-2	*F. oxysporum* f. sp. *ginseng*	*P. ginseng*	In this study

were planted in pots filled with sterilized soil. All pots with seeds or roots of plants were cultured in a greenhouse (25 to 28°C, 12 h light/12 h dark cycle). The pots planted with roots were placed under black shading net.

Culture of Fusarium inoculum. The *Fusarium* strain was first activated on PDA medium for 7 d at 25°C. Mycelium plugs at the edges of resulting colonies were taken with a sterile punch, and were placed into 100 mL of potato dextrose broth, which was then incubated for 3 d in an incubation shaker (25°C, 150 r min^{-1}). The resulting conidium suspension was then filtered and adjusted to a final concentration of 1.0×10^6 conidia mL^{-1} to inoculate the plants.

Inoculation methods. For *in vitro* inoculations, the surfaces of 2-year-old healthy ginseng taproots were washed and disinfested with 75% alcohol, and then rinsed with sterile water. The roots were then wounded with a sterilized steel needle to 2 mm depth, and were then each inoculated with 0.5 cm^2 mycelium plugs of one of the ten representative *F. oxysporum* isolates (isolates 0083, 0414, DH10-8, FS03-2, FS03-5, FS04-1, FS13-3, FS15-1, TH08, and TH10-2), which had been derived from ginseng. The inoculated roots were then incubated in a sealed plastic container. Lesion development was observed after 5 to 7 d. Sterile PDA plugs were put on the ginseng taproot as inoculation controls. For *in vivo* inoculation and host range tests, the roots of ginseng and other plant seedlings (*P. notoginseng*, *A. senticosus*, *C. lanatus*, *G. hirsutum*, *C. annuum*, *S. lycopersicum*, *P. sativum*, and *L. usitatissimum*) were each wounded, and then immersed in 1.0×10^6 mL^{-1} conidium suspension of the ten representative isolates (for *in vivo* inoculation) or three representative isolates (0083, 0414, DH10-8, for host range tests), for 30 min (ten replicate plants per strain). The inoculated plants were then transplanted into new pots filled with sterile soil, and these were maintained

in a greenhouse (25° to 28°C, 12 h light/12 h dark cycle). Non-inoculated plants were dipped in sterilized water as experimental controls. All treatments were repeated three times. The whole experiment was repeated for two years.

Assessment methods. Final observations of disease development were carried out 14 to 21 d after inoculation. The roots of plants were dug out of the soil and washed with clean water. A disease index of each plant was evaluated using a 0 to 5 scale, based on that of Song *et al.* (2014) with modification: where 0 = visible lesions; 1 = lesions covering 1-10% of the root surface; 2 = lesions covering 10-20% of the root surface; 3 = lesions covering 20-30% of the root surface; 4 = lesions covering 30-50% of the root surface; and 5 = lesions covering 50-100% of the root surface. Disease severity (DS) for each treatment was calculated using the formula:

$DS = [(0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3 + 4 \times n_4 + 5 \times n_5)/5 \times N)] \times 100$, where n_{0-5} = the number of plants exhibiting the scores of, respectively, 0, 1, 2, 3, 4 or 5, and N = total number of plants tested (Chiang *et al.*, 2017). Disease severity of nine species of plants was assayed, and the experiments were replicated three times. To confirm the presence of *F. oxysporum*, the tissues of diseased plants were cut into small pieces. These were then surface-disinfected with 70% ethanol for 30 s and then in 1.5% (v/v) NaOCl for 1 min, and were then washed three times with sterile distilled water. The tissue pieces were then placed onto a PDA in Petri dishes and incubated at 25°C for 3 to 4 days. New mycelium and single conidia were transferred onto a new PDA medium to identify the resulting fungi.

Genomic DNA extraction, PCR amplification and sequencing

The genomic DNA of *F. oxysporum* strains isolated from *P. ginseng* was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux) according to the manufacturer's instructions. The *EF-1 α* gene, two endopolygalacturonase genes (*Pg1* and *Pg5*) and two exopolygalacturonase genes (*Pgx1* and *Pgx4*) were amplified with the primers reported previously (O'Donnell *et al.*, 1998; Hirano and Arie, 2009) (Table 2). The PCR reactions were each carried out in 20 μL volumes, containing 50 ng of gDNA, 1 μL (10 mM) of each primer, 1 unit of Taq DNA polymerase (TaKaRa), 2 μL of Taq DNA polymerase buffer, 1 μL of dNTPs mix with the following program: an initial denaturing step at 94°C for 5 min, 40 cycles each with denaturation at 94°C for 1 min, annealing at 52°C (*Pg1*, *Pg5*, *Pgx1*, and *Pgx4* genes) or 57°C (*EF-1α* gene) for 1 min, extension at 72°C for 1 min (*EF-1α* gene) or 2 min (*Pg1*, *Pg5*, *Pgx1*, and *Pgx4* genes), and final extension at 72°C for 10 min. A negative control (no template DNA) was included in all experiments. PCR profiles were analyzed on 1% agarose gel. After purification with PCR purification kits (Tian-Gen), purified PCR products were sequenced in both directions by Sangon Biotechnology Corporation. The sequences were deposited at GenBank with the accession numbers shown in Table 3.

Phylogenetic analyses

According to the *EF-1α*, *Pg1*, *Pg5*, *Pgx1*, and *Pgx4* genes, specific sequences of different *formae speciales* of *F. oxysporum* were downloaded from GenBank (Table 3). The sequences obtained were used for multiple sequence alignment using the MAFFT program (Katoh and Standley, 2013). Manual corrections were performed in Partition Finder 2 for each alignment in order to delete trimmer regions outside and discard incomplete sequences (Lanfear *et al.*, 2017). Phylogenetic trees were generated for single *EF-1α* gene and multiple *Pg1*, *Pg5*,

Table 2. Primers used in this study for PCR amplification of *EF-1α* and polygalacturonase genes.

Target genes	Primers	Primer sequence (5'-3')	References
EF-1α	Ef1	ATGGGTAAGGAAGACAAGAC	O'Donnell *et al.*, 1998
	Ef2	GGAAGTACCAGTGATCATGTT	
Pg1	endoF	CCAGAGTGCCGATACCGATT	Hirano and Arie, 2009
	endoR2	GCTTAGYGAACAKGGAGTG	
Pg5	PG2F	AGATGCAAGGCCGATGATGT	Hirano and Arie, 2009
	PG2R	TCCATGTACTTCTCCTCACC	
Pgx1	PgxF	TCGTGGGGTAAAGCGTGGT	Hirano and Arie, 2009
	PgxR	TTACTATAGGTCGATCAGCC	
Pgx4	exoF2	TTACTGTCCACGAATGAGAAG	Hirano and Arie, 2009
	exoR	ACCCCAACCCCCCTCATCT	

Table 3. *Fusarium* strains used in the phylogenetic analysis of single *EF-1α* gene and combined polygalacturonase genes.

Formae speciales	Strains	Hosts	Accession numbers on GenBank				
			EF-1α	*Pg1*	*Pg5*	*Pgx1*	*Pgx4*
Fusarium oxysporum f. sp. *ginseng*	0083	*Panax ginseng*	ON316841	MW582553	MW582563	MW505948	MW505957
F. oxysporum f. sp. *ginseng*	0414	*Panax ginseng*	MW532127	MW582554	MW582564	MW505949	MW505958
F. oxysporum f. sp. *ginseng*	DH10-8	*Panax ginseng*	MK962137	MW582555	MW582565	MW505950	MW505959
F. oxysporum f. sp. *ginseng*	FS03-2	*Panax ginseng*	MH698989	MW582556	MW582566	MW505951	MW505960
F. oxysporum f. sp. *ginseng*	FS03-5	*Panax ginseng*	MH698990	MW582557	MW582567	MW505952	MW505961
F. oxysporum f. sp. *ginseng*	FS04-1	*Panax ginseng*	MK962130	MW582558	MW582568	MW505953	MW505962
F. oxysporum f. sp. *ginseng*	FS13-3	*Panax ginseng*	MH748098	MW582562	MW582572	MW505956	MW505966
F. oxysporum f. sp. *ginseng*	FS15-1	*Panax ginseng*	MH748099	MW582559	MW582569	MW532128	MW505963
F. oxysporum f. sp. *ginseng*	TH08	*Panax ginseng*	MH698987	MW582560	MW582570	MW505954	MW505964
F. oxysporum f. sp. *ginseng*	TH10-2	*Panax ginseng*	MH698986	MW582561	MW582571	MW505955	MW505965
F. oxysporum f. sp. *colocasiae*	MAFF744032	*Colocasia esculenta*	–	AB256751	AB256841	AB256883	AB256800
F. oxysporum f. sp. *conglutinans*	MAFF744001	*Brassica oleracea*	–	AB256754	–	AB256886	AB256803
F. oxysporum f. sp. *cucumerinum*	MAFF103054	*Cucumis sativus*	–	AB256755	AB256843	AB256887	AB256804
F. oxysporum f. sp. *cucumerinum*	MAFF744005	*Cucumis sativus*	–	AB256756	AB256844	AB256888	AB256805
F. oxysporum f. sp. *dianthi*	MAFF103072	*Dianthus caryophyllus*	–	AB256757	AB256845	AB256889	AB256806
F. oxysporum f. sp. *fragariae*	MAFF727510	*Fragaria ananassa*	–	AB256759	AB256848	AB256892	AB256809
F. oxysporum f. sp. *lactucae*	MAFF744028	*Lactuca sativa*	–	AB256761	AB256850	AB256894	AB256811
F. oxysporum f. sp. *lagenariae*	MAFF744002	*Lagenaria siceraria*	–	AB256764	AB256853	AB256897	AB256814
F. oxysporum f. sp. *lycopersici*	Saitama ly1	*Solanum lycopersicum*	–	AB256767	AB256856	AB256900	AB256795
F. oxysporum f. sp. *melongenae*	MAFF103051	*Solanum melongena*	–	AB256776	AB256865	AB256909	AB256823
F. oxysporum f. sp. *melonis*	MAFF305122	*Cucumis melo*	–	AB256777	AB256866	AB256910	AB256824
F. oxysporum f. sp. *melonis*	MAFF305544	*Cucumis melo*	–	AB256778	AB256867	AB256911	AB256825
F. oxysporum f. sp. *niveum*	MAFF305543	*Citrullus lanatus*	–	AB256779	AB256868	AB256912	AB256826
F. oxysporum f. sp. *niveum*	NBRC9969	*Citrullus lanatus*	–	AB256780	AB256869	AB256913	AB256827
F. oxysporum f. sp. *phaseoli*	MAFF235727	*Phaseolus vulgaris*	–	AB256781	AB256870	AB256914.	AB256828
F. oxysporum f. sp. *phaseoli*	NBRC9970	*Phaseolus vulgaris*	–	AB256782	AB256871	AB256915	AB256829
F. oxysporum f. sp. *radicis-lycopersici*	KEF-2R1	*Solanum lycopersicum*	–	AB208068	AB208072	AB208080	–
F. oxysporum f. sp. *raphani*	MAFF103058	*Raphanus sativus*	–	AB256787	AB256876	AB256920	AB256833
F. oxysporum f. sp. *raphani*	MAFF305124	*Raphanus sativus*	–	AB256788	–	AB256921	AB256834
F. oxysporum f. sp. *spinaciae*	MAFF103059	*Spinacia oleracea*	–	AB256789	AB256877	AB256922	AB256835
F. oxysporum f. sp. *spinaciae*	MAFF731044	*Spinacia oleracea*	–	AB256790	AB256878	AB256923	AB256836
F. oxysporum f. sp. *tracheiphilum*	MAFF235725	*Vigna unguiculata*	–	AB256791	AB256879	AB256924	AB256837
F. oxysporum f. sp. *tracheiphilum*	MAFF235726	*Vigna unguiculata*	–	AB256792	AB256880	AB256925	AB256838
F. oxysporum f. sp. *tulipae*	MAFF235105	*Tulipa gesneriana*	–	AB256793	AB256881	AB256926	AB256839
F. oxysporum f. sp. *tulipae*	MAFF235109	*Tulipa gesneriana*	–	AB256794	AB256882	AB256927	AB256840
F. oxysporum f. sp. *asparagi*	FOA50	*Asparagus officinalis*	AY337434	–	–	–	–
F. oxysporum f. sp. *melonis*	0348	*Cucumis melo*	DQ016282	–	–	–	–
F. oxysporum f. sp. *lactucae*	BMP1880	*Lactuca sativa*	DQ837670	–	–	–	–
F. oxysporum f. sp. *lactucae*	BMP1375	*Lactuca sativa*	DQ837673	–	–	–	–
F. oxysporum f. sp. *callistephi*	NRRL22536	*Callistephus chinensis*	DQ837679	–	–	–	–
F. oxysporum f. sp. *vasinfectum*	NRRL25231	*Anemone vitifolia*	DQ837680	–	–	–	–
F. oxysporum f. sp. *cepae*	NRRL22538	*Allium cepa*	DQ837681	–	–	–	–
F. oxysporum f. sp. *matthiolae*	NRRL22545	*Matthiola incana*	DQ837682	–	–	–	–
F. oxysporum f. sp. *medicaginis*	NRRL22546	*Medicago sativa*	DQ837690	–	–	–	–
F. oxysporum f. sp. *lycopersici*	FOLR2	*Solanum lycopersicum*	DQ837692	–	–	–	–
F. oxysporum f. sp. *lactucae*	F9501	*Lactuca sativa*	DQ837693	–	–	–	–
F. oxysporum f. sp. *lactucae*	FK09701	*Lactuca sativa*	DQ837694	–	–	–	–

(Continued)

Table 3. (Continued).

Formae speciales	Strains	Hosts	Accession numbers on GenBank				
			EF-1α	*Pg1*	*Pg5*	*Pgx1*	*Pgx4*
F. oxysporum f. sp. *radicis-cucumerinum*	14	*Cucumis sativus*	EF056779	–	–	–	–
F. oxysporum f. sp. *radicis-cucumerinum*	30	*Cucumis sativus*	EF056781	–	–	–	–
F. oxysporum f. sp. *cucumerinum*	ATCC 16416	*Cucumis sativus*	EF056783	–	–	–	–
F. oxysporum f. sp. *lycopersici*	MUCL 14159	*Solanum lycopersicum*	EF056784	–	–	–	–
F. oxysporum f. sp. *conglutinans*	Apr-81	*Brassica oleracea*	EF056786	–	–	–	–
F. oxysporum f. sp. *gladioli*	NRRL 26993	*Gladiolus gandavensis*	EF056787	–	–	–	–
F. oxysporum f. sp. *lilii*	NRRL 28395	*Lilium brownii*	EF056788	–	–	–	–
F. oxysporum f. sp. *melonis*	CBS 423.90	*Cucumis melo*	EF056789	–	–	–	–
F. oxysporum f. sp. *melonis*	CBS 420.90	*Cucumis melo*	EF056790	–	–	–	–
F. oxysporum f. sp. *lilii*	Fol-11	*Lilium brownii*	EU220403	–	–	–	–
F. oxysporum f. sp. *cepae*	NL106-2	*Allium cepa*	EU220404	–	–	–	–
F. oxysporum f. sp. *canariensis*	2675A	*Phoenix canariensis*	FJ895287	–	–	–	–
F. oxysporum f. sp. *canariensis*	4873C	*Phoenix canariensis*	FJ895290	–	–	–	–
F. oxysporum f. sp. *passiflorae*	NRRL 38273	*Passiflora caerulea*	FJ985362	–	–	–	–
F. oxysporum f. sp. *canariensis*	NRRL 38338	*Phoenix canariensis*	FJ985388	–	–	–	–
F. oxysporum f. sp. *cepae*	NRRL 38481	*Allium cepa*	FJ985399	–	–	–	–
F. oxysporum f. sp. *raphani*	NRRL 53154	*Raphanus sativus*	FJ985441	–	–	–	–
F. oxysporum f. sp. *conglutinans*	NRRL 53156	*Brassica oleracea*	FJ985442	–	–	–	–
F. oxysporum f. sp. *conglutinans*	NRRL 53158	*Brassica oleracea*	FJ985443	–	–	–	–
F. oxysporum f. sp. *radicis-lycopersici*	CL-0620	*Solanum lycopersicum*	HM057325	–	–	–	–
F. oxysporum f. sp. *radicis-lycopersici*	CL-06202	*Solanum lycopersicum*	HM057332	–	–	–	–
F. oxysporum f. sp. *lycopersici*	OSU451	*Solanum lycopersicum*	HM057335	–	–	–	–
F. oxysporum f. sp. *canariensis*	PLM-385B	*Phoenix canariensis*	HM591537	–	–	–	–
F. oxysporum f. sp. *canariensis*	PLM-511A	*Phoenix canariensis*	HM591538	–	–	–	–
F. fujikuroi	NRRL 66453	*Vitis vinifera*	KX656207	–	–	–	–

Pgx1, and *Pgx4* genes using the Bayesian Inference (BI) method through the PhyloSuite platform (Ronquist *et al*., 2012; Zhang *et al*., 2020).

RESULTS

Pathogenicity assays

The samples of diseased ginseng roots were collected, and a total of 272 isolates of *F. oxysporum* were obtained in pure cultures. In the *in vitro* experiment, after 5–7 d, all of the ginseng roots inoculated with the ten representative isolates showed water-soaked root rot lesions (Figure 1, A and B), whereas no lesions were observed in the control roots (Figure 1, C and D). For the *in vivo* inoculations of whole plants in the greenhouse, typical root rot symptoms were observed 14 d after inoculation with the ten representative isolates. The symptoms included yellowing of leaves and brown necrotic lesions on the root surfaces. As the symptoms developed, all of the infected ginseng plants were dead at 21 d post-inoculation, with dark brown and soft root rot (Figure 1, E and F). No symptoms were observed on the control ginseng plants (Figure 1, G and H). The same pathogens were re-isolated from the diseased ginseng roots, fulfilling the Koch's postulates.

Greenhouse host range tests

In the host range tests, three representative *F. oxysporum* strains derived from ginseng roots were inoculated onto nine species of plants. The three ginseng isolates (0083, DH10-8, and 0414) caused severe rot symptoms on ginseng roots, with disease mean severities ranging from 86.67 to 96.67. However, the isolates 0083, 0414, and DH10-8 only slightly infected *A. senticosus*, *P. notoginseng*, *P. sativum*, *C. lanatus*, and *S. lycopersicum*, with disease severities less than 7.00 (Figures 2 and 3). Six *formae speciales* of *F. oxysporum* from other hosts (Table 1) did not infect, or only slightly infected ginseng,

Figure 1. Pathogenicity of *Fusarium oxysporum* derived from ginseng on 2-year-old ginseng taproots (A, B) and whole plants (E, F). Healthy controls inoculated with PDA plugs (C, D) and sterilized water (G, H) are also shown.

with disease severities less than 11.00. These isolates also caused obvious symptoms on corresponding host plants with disease severities greater than 73.00. For example, *F. oxysporum* f. sp. *pisi* (ACCC31037) was only highly pathogenic to *P. sativum* (disease severity = 92.67), while *F. oxysporum* f. sp. *capsicum* (ACCC36472) was only highly pathogenic to *C. annuum* (disease severity = 96.00) (Figures 2 and 3). These results indicated that the *F. oxysporum* isolates from ginseng root were host-specific to ginseng.

Phylogenetic analyses

The amplification products of the *EF-1α*, *Pg1*, *Pg5*, *Pgx1*, and *Pgx4* genes were, respectively, approx. 750, 1560, 1800, 1800 and 1400 base pairs (bp) long. The nucleotide sequences were submitted and deposited in GenBank, and the GenBank accession numbers are shown in Table 3. Phylogenetic analyses were carried out using the single gene *EF-1α* sequences, and a combined *Pg1*, *Pg5*, *Pgx1* and *Pgx4* gene sequences, from the ten isolates from ginseng and those of other *formae speciales* of *F. oxysporum* from the GenBank database (Table 3). In the phylogenetic trees constructed with the single gene *EF-1α* sequences, the ten isolates from ginseng formed a single and distinct clade, separate from the other *formae speciales* of *F. oxysporum* (Figure 4). Similarly, the phylogenetic tree constructed using the combined sequences of the genes *Pg1*, *Pg5*, *Pgx1*, and *Pgx4* also clearly clustered the ginseng isolates in an independent clade distinguished from the other *formae speciales* of *F. oxysporum* isolates (Figure 5). These results showed that the ten isolates obtained from *P. ginseng* were clustered together to form a unique clade which was clearly separated from other *formae speciales* of *F. oxysporum*.

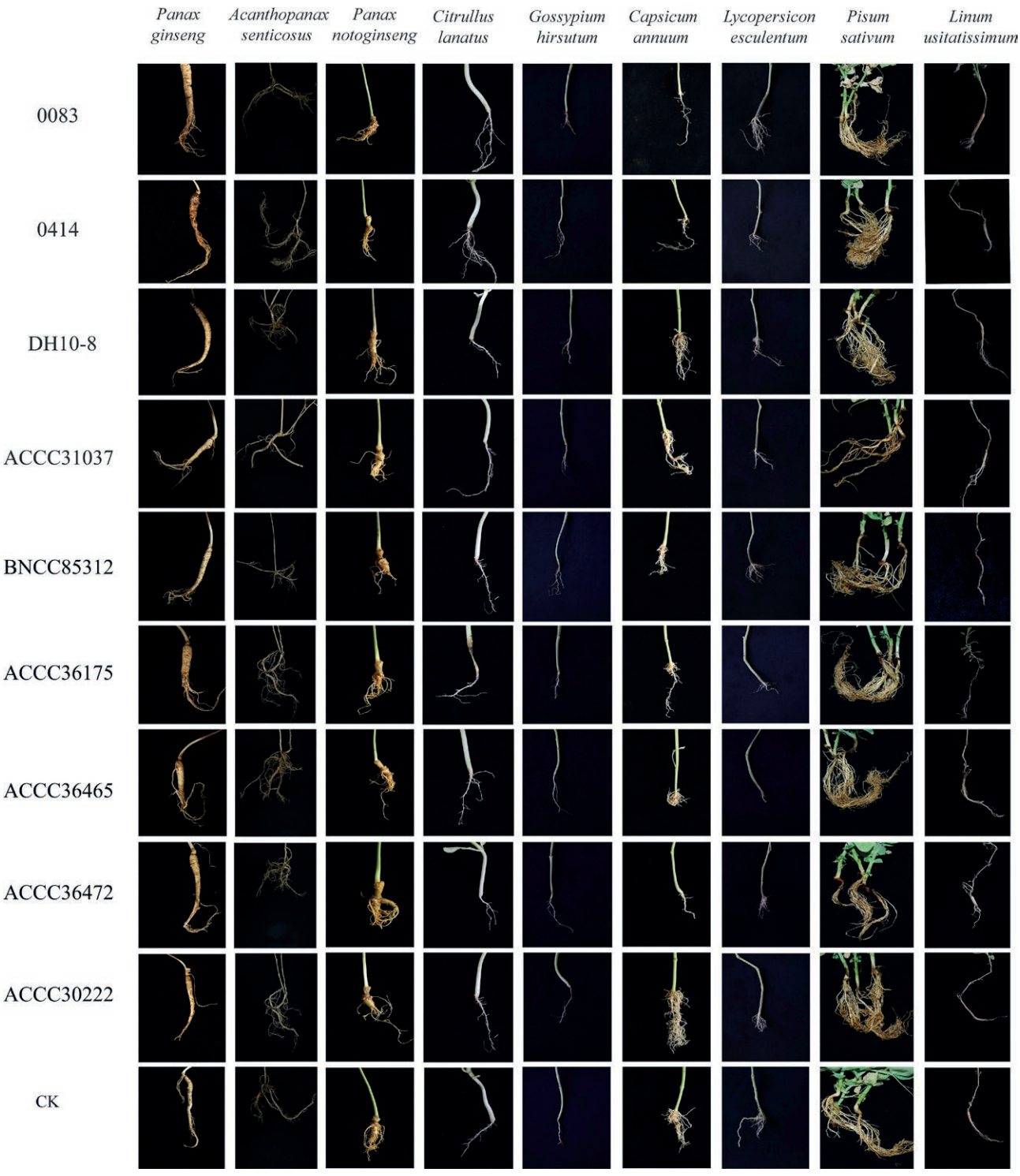

Figure 2. Symptoms on nine species of plants inoculated with *Fusarium oxysporum* derived from ginseng and six other *F. oxysporum formae speciales*.

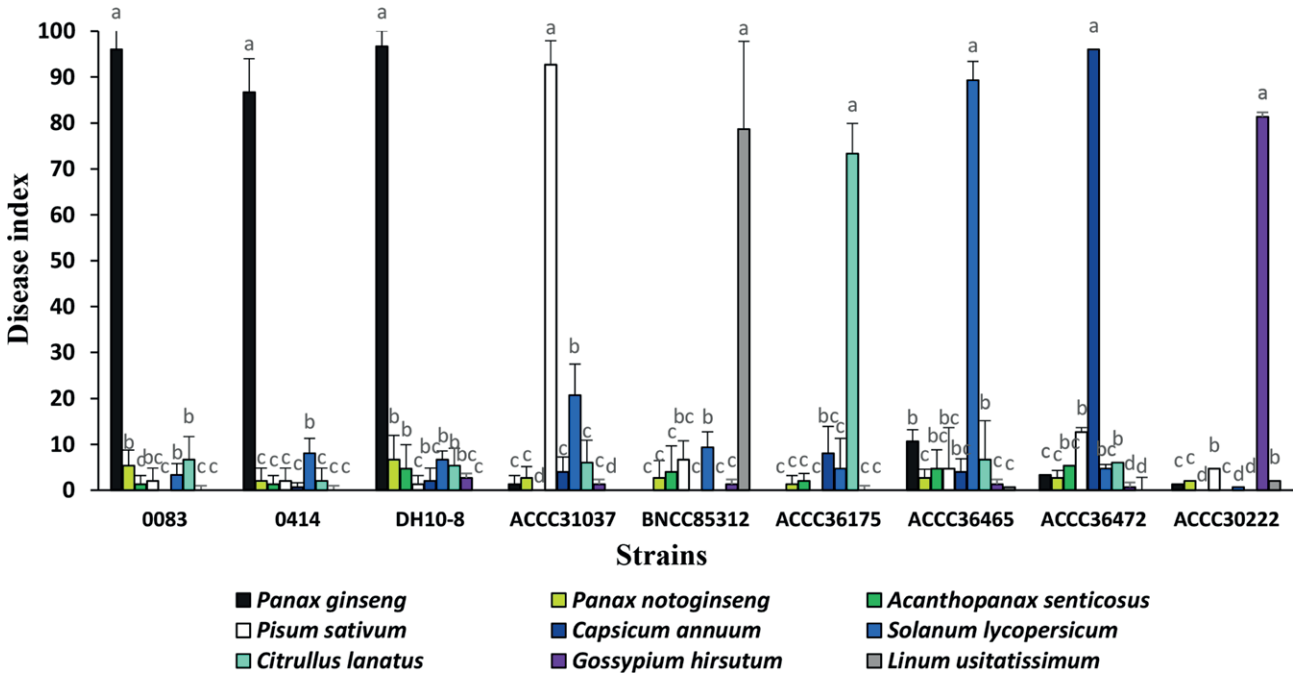

Figure 3. Mean disease severity indices of plant root rot caused by *Fusarium oxysporum* derived from ginseng, and six other *F. oxysporum formae speciales*. Means accompanied by different lowercase letters among treatments within a given strain are different ($P < 0.05$).

DISCUSSION

Fusarium oxysporum, is a major pathogen causing plant vascular wilts and root rots, but strains of this fungus often infect only one or a few plant species, indicating that they are usually host-specific. Therefore, based on host specificity, pathogenic strains of *F. oxysporum* have been grouped into *formae speciales* (Gordon and Martyn, 1997). In the present study, host range tests were used to identify a new *forma specialis* of *F. oxysporum* isolated from ginseng. Phylogenetic analysis based on the *EF-1α* gene and four polygalacturonase genes (*Pg1*, *Pg5*, *Pgx1*, and *Pgx4*) confirmed the results of host range tests, suggesting the presence of a new *forma specialis* of *F. oxysporum*. A novel *forma specialis*, *F. oxysporum* f. sp. *ginseng*, is therefore proposed, which causes root rot of ginseng.

Traditional identification of *formae speciales* mainly through pathogenicity tests on particular hosts or groups of hosts. In the present study, *P. ginseng*, *P. notoginseng*, *A. senticosus*, *C. lanatus*, *G. hirsutum*, *C. annuum*, *S. lycopersicum*, *P. sativum*, and *L. usitatissimum* were selected for host range tests. The results showed that *F. oxysporum* isolated from ginseng could only cause severe root rot of ginseng, and did not infect other hosts or infected some only slightly. In addition, low levels of root rot symptoms were observed in some plants inoculated with *formae speciales* of *F. oxysporum* nonpathogenic to them. It is likely that the artificial inoculation method in a greenhouse enhances host infection, and infections could be less under field conditions. In addition, the results of the host range tests could also be influenced by environmental conditions and the species (and/or cultivar) of host plants.

Because of abundance of plant species, host range tests are time-consuming and laborious (Correll, 1991). With the development of molecular biology and sequencing technologies, some DNA markers and transposon insertions based on genomic DNA sequences have been developed as alternative methods for identification of the *formae speciales* of *F. oxysporum*. Phylogenetic analysis based on the mitochondrial small subunit (*mtSSU*) ribosomal RNA gene, the rDNA intergenic spacer (IGS) region, and the *EF-1α* gene have helped to reveal the genetic and evolutionary relationships within and among *formae speciales* of *F. oxysporum* (Lievens *et al.*, 2008). Furthermore, some pathogenicity-related genes, such as secreted in xylem (*SIX*) effector (Lievens *et al.*, 2009) and polygalacturonase genes (Hirano and Arie, 2009) have become important tools used for high resolution discrimination of different *formae speciales* of *F. oxysporum*. Several new *formae speciales* have recently been identified by phylogenetic analyses based on *EF-1α* and polygalacturonase genes (Ortu *et al.*, 2013; Ortu *et*

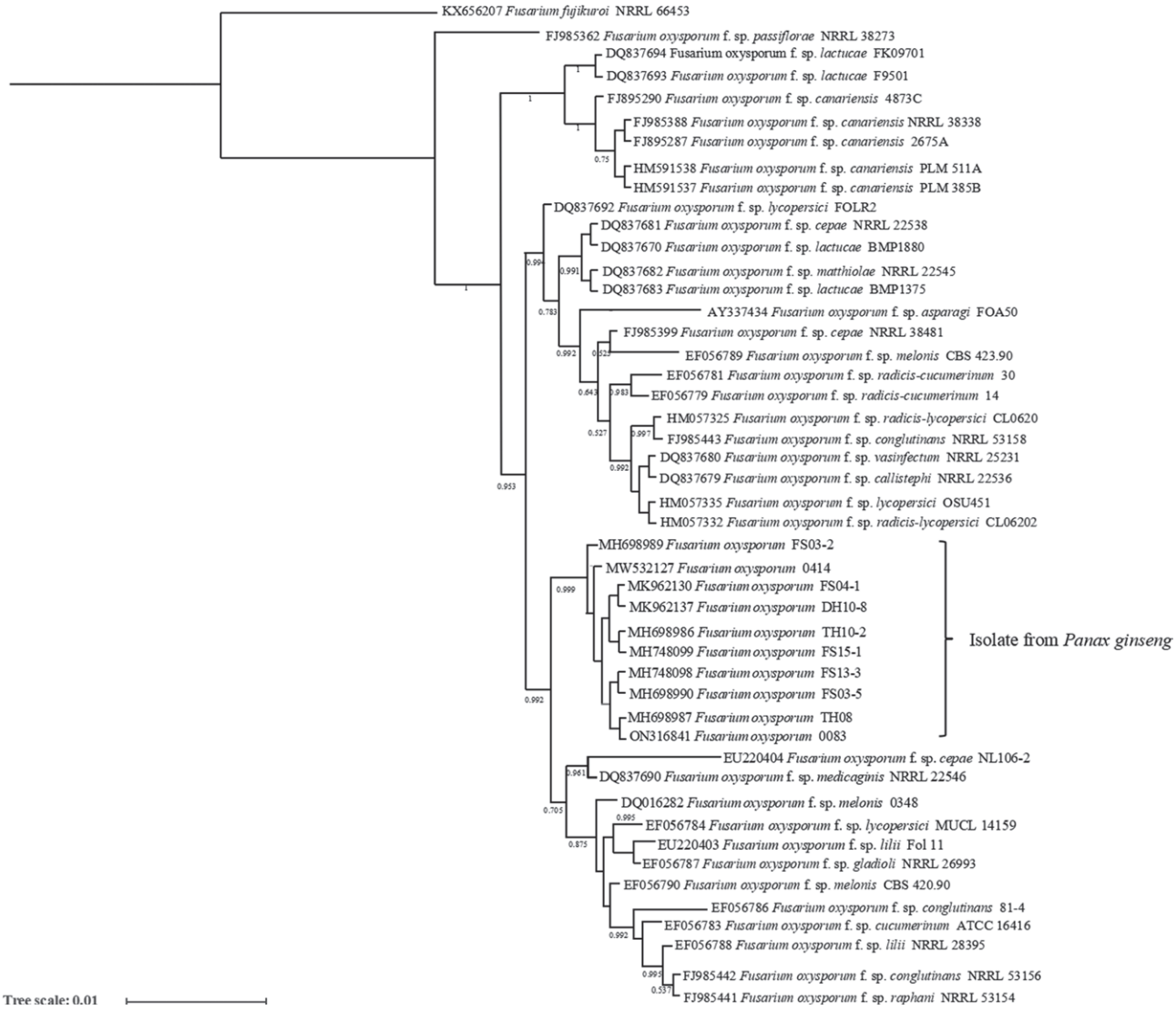

Figure 4. Phylogenetic tree based on *EF-1α* gene sequences of different *formae speciales* of *Fusarium oxysporum*, using Bayesian inference (BI) methods in the PhyloSuite platform. *Fusarium fujikuroi* was used as an outgroup. Numbers under branches indicate a posterior probability > 0.5.

al., 2015a; Ortu *et al*., 2015b; Ortu *et al*., 2018). Phylogenetic analysis based on these genes has demonstrated the usefulness of these regions for the identification of *F. oxysporum forma speciales*.

Compared with traditional pathogenicity testing, molecular identification of *forma specialis* of *F. oxysporum* based on the genomic DNA has many advantages. It is highly specific and sensitive, and is rapid and easy to operate in a laboratory. However, molecular identification of *forma specialis* combined with traditional identification methods such as morphology and pathogenicity tests, increases precision of results. Therefore, identification of *formae speciales* is commonly based on pathogenicity assays and is supported by molecular identification tools. For soilborne pathogens such as *F. oxysporum*, it is important to develop diagnosis tools to discriminate between species and *formae speciales*, because these will detect the risk of the infection, and provide information to guide corresponding disease management treatments.

ACKNOWLEDGEMENT

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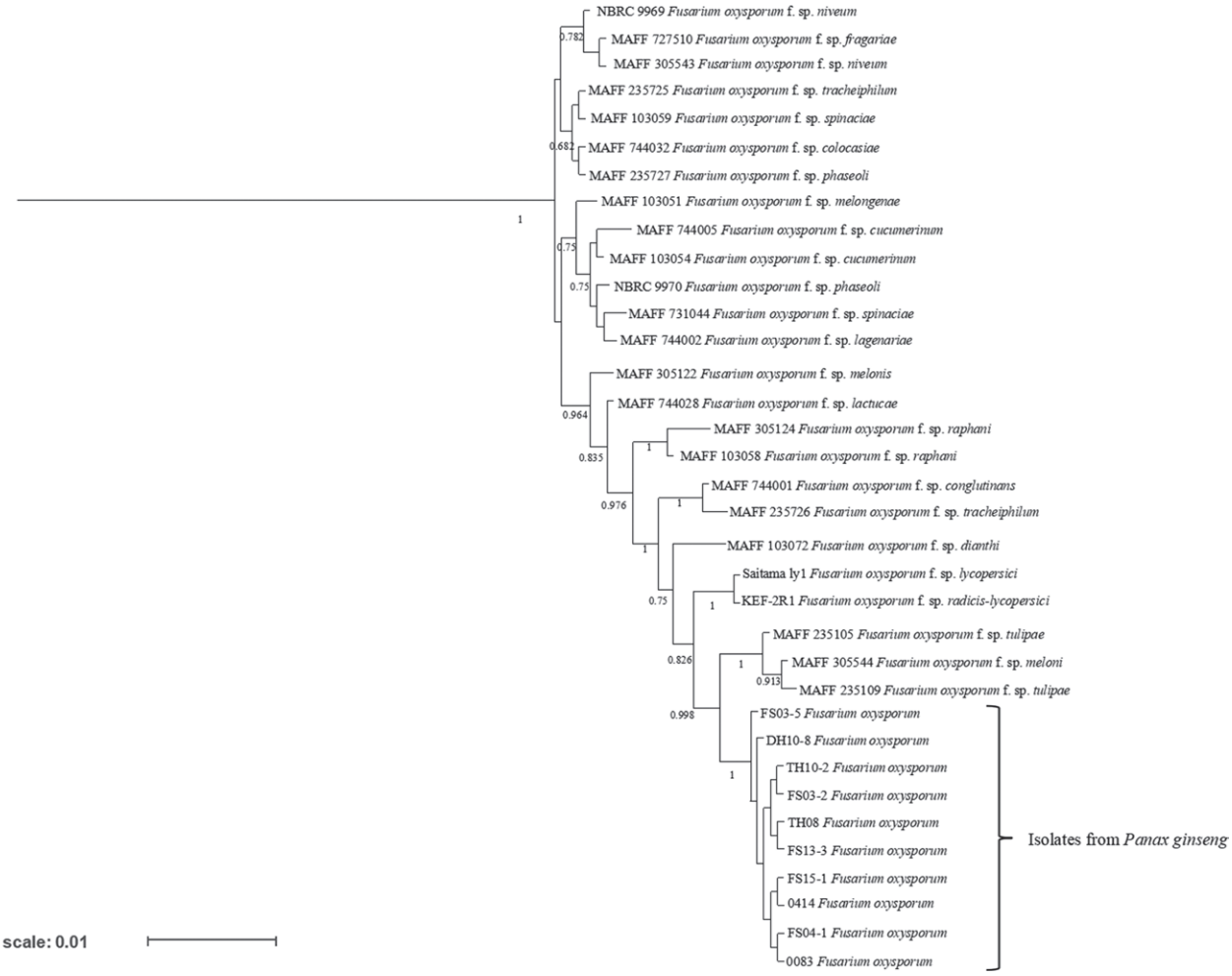

Figure 5. Phylogenetic tree based on combined *Pg1*, *Pg5*, *Pgx1* and *Pgx4* gene sequences of different *formae speciales* of *Fusarium oxysporum*, using Bayesian inference (BI) methods in the PhyloSuite platform. Numbers under branches indicate a posterior probability > 0.5.

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