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The New Report of Root Rot on *Fatsia japonica* Caused by *Phytophthora nicotianae* in China

Jing Zhou [1], Tingyan Xu [1], Xiaoqiao Xu [1], Tingting Dai [1,*] and Tingli Liu [2,*]

[1] Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China; zhoujing424@163.com (J.Z.); x8220110086@163.com (T.X.); qiao20010318@163.com (X.X.)
[2] School of Food Science, Nanjing Xiaozhuang University, 3601 Hongjin Avenue, Nanjing 211171, China
* Correspondence: 13770647123@163.com (T.D.); tlliu2100@163.com (T.L.)

Abstract: As an ornamental plant, *Fatsia japonica* has been widely used in gardens. From April 2021 to 2022, a disease that caused the wilting and root rot of *F. japonica* in a large area was observed, which eventually led to the plants wilting and dying, while the leaves did not fall off. This disease greatly reduced the landscape effect of plants. An oomycete species was isolated from the roots of the infected plants. This colony morphology was slightly radial to stellate, and the aerial mycelium was flocculent. Oval sporangia with papillae, apical chlamydospores and zoospores formed in sporangia were observed. The morphological characteristics were consistent with *Phytophthora*. For accurate identification, the internal transcribed spacer (ITS), cytochrome oxidase subunit II (*COXII*) and large ribosomal subunit (*LSU*) genes were amplified and sequenced. The species was identified as *Phytophthora nicotianae* using phylogenetic analysis. Finally, the disease was reproduced by inoculating healthy *F. japonica* with a zoospore suspension; the symptoms were consistent with those of natural infections, and the isolate obtained from artificially infected plants had the same morphological characteristics as the inoculated isolate. The results demonstrated that *P. nicotianae* is the pathogenic factor of root rot. of *F. japonica*. This is the first report of root rot on *F. japonica* caused by *P. nicotianae* in China.

Keywords: plant disease; first report; oomycete; *Phytophthora nicotianae*; pathogenicity; phylogenetic analysis

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1. Introduction

Fatsia japonica Decne. et Planch. (Syn. *Aralia japonica* Thunb. and *A. sieboldii* Anon.) is a member of the Araliaceae Juss family, which grows wild on Japanese islands and is widely cultivated as a decorative plant [1]. Often grown as a foliage houseplant for cooling situations, it is also a very successful shade-tolerant garden plant. *Fatsia japonica* is a well-known ornamental and potentially medicinal plant [2]. Its leaves, roots and barks have a certain medicinal value. The root bark has the medicinal efficacy of resolving phlegm and relieving cough, promoting blood circulation and removing blood stasis. Other benefits include dispersing wind and removing dampness, as well as removing stasis to ease pain; it can be used to treat traumatic injuries, cough and phlegm, rheumatic arthralgia and gout [3]. *F. japonica* is also widely planted in cities south of the Yangtze River in China [4].

The *Phytophthora*, classified in the family Pythiaceae, order Peronosporales, phylum Oomycota and kingdom Stramenopila, is distributed all around the world [5]. This genus was established by Anton de Bary in 1876 [6] and can be divided into 12 major phylogenetic branches, with many sub-branches [7–9]. In 1983, only 43 species of *Phytophthora* were identified, and later, Erwin and Ribeiro described around 60 species in 1996 [10]. By 2008, the number of species was close to 100 [11]. At present, more than three hundred species are described on www.mycobank.org (accessed on 9 May 2023). The species of the

genus *Phytophthora* are characterized by having aseptate hyphae [12]. It produces asexual organs, namely sporangia, that can be oval, inverted pear-shaped or lemon-shaped. The differentiation of the contents inside the sporangium produces zoospores with double flagella, which are finally released through the top of the sporangium. *Phytophthora* species are homothallic or heterothallic, and sexual spores are obtained by the fertilization of male and female organs, namely oospores [13]. According to some researchers [10,14], most pathogens can survive in the soil for a long time without a host and will have a certain impact on the part of the plant in contact with the ground to destroy the plant roots. The effect of *Phytophthora* on plants can be seen by its name. In Greek, *Phytophthora* means plant destroyer [14–16], which can cause diseases in herbaceous and woody plants, mostly dicotyledon plants [10,13,17–21]. Major diseases caused by *Phytophthora* spp. are crown and root rot, stem rot, foot rot, stem canker, leaf blight, fruit brown rot and late blight [22–28]. They can infect cultivated and spontaneous plants worldwide, causing serious agricultural losses and destroying natural forest ecosystems [29]. Among the ten important pathogenic oomycetes causing plant oomycete diseases in the world, *P. infestans*, *P. ramorum*, *P. sojae*, *P. capsici*, *P. cinnamomi* and *P. nicotianae* are included [30]. In the 1840s, *P. infestans* caused potato late blight, causing famine in Ireland. In 2020, Linaldeddu et al. found that *P. pini* can cause crown blight and root rot in four *Olea europaea* L. forests in the Veneto region of Italy [29]. In 2023, Hrabetova, M. et al. found that there were dark brown to black necrotic lesions in the rhizome of *Buxus sempervirens*, resulting in the death of the whole plant [31]. Some diseases caused by *Phytophthora* have reached epidemic status [13,32–34]. For example, the host range of *P. romorum* is pretty wide, and it has become an epidemic disease in many places. It can infect a variety of ornamental plants and woody trees, causing serious losses to agricultural and forestry crop production, and has been listed as a quarantine object by many countries [35]. *P. cinnamomi* has been found in Fujian [36–38], Jiangsu and Zhejiang [38], Hainan [39], Shanxi [40], Shanghai [41] and other provinces. It poses a threat to the production of chestnut, kiwifruit, blueberry, avocado, ornamental trees and the health of natural forests [42]. The annual loss of agriculture and forestry caused by epidemics worldwide is huge. Once pathogens are established in the environment, the eradication of pathogens requires huge manpower and costs, and annual economic losses and governance costs can be as high as USD 10 billion. [43].

In the past, the traditional identification of *Phytophthora* was mainly based on morphological characteristics, including sporangium, sporangium peduncle, chlamydospores, sexual organs, hyphae and colonies. Due to the large morphological variation of *Phytophthora* pathogens, the basis of some classifications and identifications is not stable, so the classification and identification of the *Phytophthora* species is difficult [44,45]. Therefore, the identification of *Phytophthora* must also be combined with molecular biology methods, and the results are more reliable. The rDNA-ITS sequence is one of the most widely used target genes for the identification of *Phytophthora*. However, for some closely related species or sister species of *Phytophthora*, it is sometimes difficult to distinguish them by a single rDNA-ITS sequence, which requires the analysis of other conserved target genes to identify the species more accurately [46]. According to the sequencing of the mitochondrial DNA (mt DNA) coding region of the *Phytophthora* species, the *COXII* (*Mitochondrially encoded cytochrome oxidase II*) gene is suitable for the broad-spectrum phylogenetic analysis of *Phytophthora* [47]. Studies have shown that the *LSU* (*Large subunit*) gene also has a good resolution for the *Phytophthora* species and is used for the study of oomycete phylogeny [46].

From April 2021 to 2022, several diseases of *Fatsia japonica* were found in the campus of Nanjing Forestry University, China. We dug up dozens of seriously diseased *F. japonica* roots under the dormitory building. The symptoms were that the whole plant was wilting, the leaves were low and dead without falling off and the roots were rotten and black after digging them up. The main purpose of this study is to isolate and identify the pathogenic factors of *F. japonica* root rot using a pathogenicity test, morphological characteristics and a phylogenetic analysis. It provides a reference for the study of diseases of *F. japonica*.

2. Materials and Methods

2.1. Disease Investigation and Isolation

In May 2022, the diseased roots of dozens of *F. japonica* were dug up under several dormitory buildings of Nanjing Forestry University (Geographic coordinates: 31°14′ N, 118°22′ E). The roots were washed thoroughly with clean water and then cut into 30 pieces of 3 mm in size, surface-disinfested by immersion in 75% ethanol for 30 s followed by 1% NaClO for 90 s, rinsed three times with sterile water, then dried on sterilized filter paper and plated onto clarified 10% V8 juice agar (cV8A) [48] that was amended with pimaricin (20 mg/L), ampicillin (125 mg/L), rifampicin (10 mg/L) and pentachloronitrobenzene (20 mg/L). They were incubated at 26 °C (Incubator MIR-553, Sanyo, Osaka, Japan) for three days. Then, these hyphae tips were transferred to a fresh V8 plate to obtain pure cultures.

2.2. Morphological Identification

Three strains of pathogenic bacteria were selected, and the colony morphology of the isolates was observed with a clear 10% V8 solid medium. The colony plugs with a diameter of 0.6 × 0.6 cm were punched on the edge with a sterile puncher and inoculated in the middle of the culture dish. The culture dish was placed in a dark incubator at 26 °C. The structure, color and morphology of the colonies were observed and recorded.

To observe the morphology of various spores, several colony agar blocks taken off with a sterile puncher were placed in a liquid V8 at 26 °C for 3 days with a 12/12 h light–dark cycle, then liquid V8 was replaced with sterile water and 3–5 drops of soil extract (100 g (3~10 cm deep) of surface soil from a fertile vegetable garden was collected, and 100 mL of tap water was added, fully stirred and precipitated for several hours; the supernatant was filtered with ordinary filter paper to remove the coarse particles of the soil and was repeatedly filtered twice with a 0.22 μm microporous membrane) to stimulate sporangial production [49]. Species were identified based on morphological characteristics (colony morphology, color and texture, sporangia, chlamydospores and zoospores) of the three isolates on V8. The sporangia, chlamydospores and zoospores were measured using a Zeiss Axio Imager A2 m microscope (Carl Zeiss, Oberkochen, Germany) for morphological description and size measurement (n = 50).

2.3. DNA Extraction and PCR Amplifcation

The traditional morphological identification method is uncertain due to the influence of external factors, so the identification of *Phytophthora nicotianae* must also be combined with molecular biology methods, and the results are more reliable. rDNA-ITS is one of the most widely used target genes for the identification of *P. nicotianae* because of its multiple copies and fast coding [50]. In addition to the rDNA-ITS gene, the *COXII* gene of the *Phytophthora* species can also be used as a target gene for the identification of *P. nicotianae* [51]. For molecular identification, the DNA of representative isolates was extracted from the mycelium cultured for 3 days using the CTAB method [52]. The internal transcribed spacer (ITS) region, large subunit (*LSU*) and mitochondrially encoded cytochrome oxidase II (*COXII*) genes were amplified using the primer pairs ITS1/ITS4 [53], LROR-O/LR6-O [46] and FM82/FM80 [54], respectively. The primers and PCR conditions are shown in Table 1. For PCR amplification of 50 μL, the reaction system is as follows: primers (10 μmol/L) each 2 μL, Taq DNA Polymerase 25 μL, template DNA (100 ng/μL) 2 μL and ddH_2O 19 μL. The PCR amplification products were purified with agarose gel electrophoresis and sent to Shanghai Jieli Biotechnology Co., Ltd. (Nanjing, China) for amplicon sequencing.

2.4. Phylogenetic Analyses

For further identification, the extracted DNA sequences were subjected to BLASTn search in the NCBI database to retrieve orthologous sequences with high similarity, and these sequences were submitted to NCBI/Gen Bank to obtain the registration number. Multiple gene sequence analyses used ClustalW Multiple Alignment in the bioinformatics software BioEdit ver.7.0.9.1 [55] for multiple sequence alignment. After editing and cutting,

the first base of each pair of gene sequences was the same base, and the tail was the same. To ensure maximum sequence similarity, Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses were performed based on ITS, *LSU* and *COXII* multi-locus tandem sequences in PhyloSuite ver.1.2.2 software [56]. Concatenate Sequence was used to concatenate the sequences of ITS, *LSU* and *COXII*. ML phylogenetic analysis of multi-gene tandem sequences was performed using IQ-TREE ver.1.6.8 [57]. The best nucleotide substitution model was statistically selected using ModelFinder [58] and was based on the AIC (Akaike information criterion) standard. The general time reversible (GTR) nucleotide substitution model was used, and the site difference ratio was set to invgamma. The bootstrap (BS) test was used to calculate the branch support rate with 1000 replicates. MrBayes ver.3.2.6 [59] was also used to perform BI phylogenetic analysis of interspecific relationships. Statistical selection of the best nucleotide substitution model was performed using ModelFinder and was based on the BIC (Bayesian information criterion) criteria. The Markov Chain Monte Carlo (MCMC) algorithm was used for operation. The operation lasts for more than 2×10^6 generations. Sampling is performed every 1000 generations until the average standard deviation of split frequencies is less than 0.01. The posterior probabilities (PP) of each branch were calculated. Finally, FigTree ver.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 13 May 2023)) software can be used to view the tree file and beautify it, utilizing drawing tools to trim and embellish the tree diagram.

Table 1. List of primers for PCR amplification in this article.

Locus	Primer	Sequence (5′-3′)	PCR Conditions	Reference
The internal transcribed spacer (ITS)	ITS1	TCCGTAGGTGAACCTGCGG	94 °C, 3 min; (94 °C, 30 s, 55 °C, 30 s; 72 °C, 45 s) × 35; 72 °C, 10 min	[53]
	ITS4	TCCTCCGCTTATTGATATGC		
Large subunit (*LSU*)	LROR-O	ACCCGCTGAACTYAAGC	94 °C, 3 min; (94 °C, 30 s; 52 °C, 30 s; 72 °C, 45 s) × 35; 72 °C, 10 min	[46]
	LR6-O	CGCCAGACGAGCTTACC		
Mitochondrially encoded cytochrome oxidase II (*COXII*)	FM82	TTGGCAATTAGGTTTTCAA-GATCC	94 °C, 3 min; (94 °C, 30 s; 52 °C, 30 s; 72 °C, 45 s) × 35; 72 °C, 10 min	[54]
	FM80	AATATCTTTATGATTTGTTGAAA		

2.5. Pathogenicity Assays

To fulfill Koch's postulates, one-year-old *F. japonica* potted seedlings (30 cm tall, $n = 12$) were placed in a greenhouse (temperature: 25 °C, 90% relative humidity, daylight: 14 h) for the pathogenicity tests. Healthy roots of *F. japonica* were dug up to expose root balls, which were wounded before inoculations with a sterile needle. Every plant was inoculated with 10 mL of zoospore suspension (10^6 zoospores/mL) that was mixed into sterile pot soil (approximately 500 g). The sterile water inoculation treatment was used as a blank control, and each treatment was repeated three times.

3. Results

3.1. Natural Symptoms

From 2021 to 2022, a survey was conducted in the Xuanwu District of Nanjing City. It was found that the disease also widely occurred in some nurseries in the Xuanwu District, including Nanjing Forestry University, with an incidence of about 40%. In the early stage of the disease, the base of the petiole drooped, but there was no obvious necrosis. In the late stage of the disease, the affected plants wilted. The leaves were shrunk and yellowed, and the underground roots rotted, but the base leaves did not fall off (Figure 1).

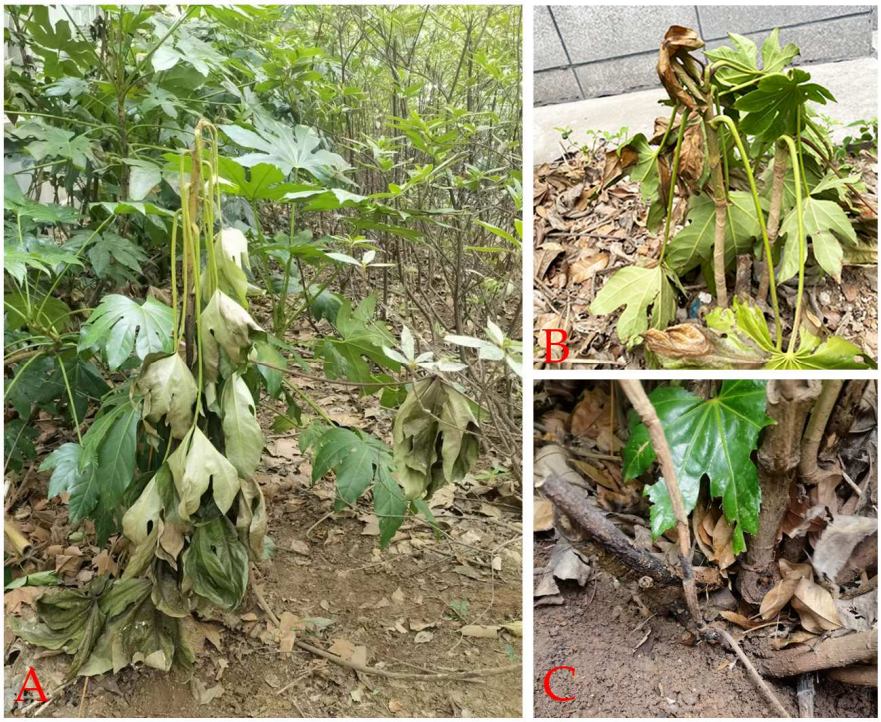

Figure 1. Symptoms of root rot on *F. japonica*. (**A**,**B**) Field infection symptoms of *F. japonica*. (**C**) Field symptoms of root and crown rot on *F. japonica*.

3.2. Morphological Characteristics

By isolation, *Phytophthora*-like mycelium appeared in 80% of the samples. The colony morphology of all isolates was slightly radial and star-shaped hyphae, an irregular colony shape; the cotton flocculent aerial mycelium was exuberant and dense, and the reverse was white (Figure 2A,B). Three representative isolates (BJP-1, BJP-2 and BJP-3) were randomly selected and preserved in the collection of Nanjing Forestry University. Sporangia produced in 10% of the liquid V8 were ovate to suborbicular or elliptic in shape, with a smooth surface, containing protoplasts and immature zoospores. Figure 2 shows the following: the sporangium was nearly spherical and 27.9 ± 6.6 µm × 25.9 ± 6.8 µm in size (n = 30) (Figure 2C); sporangia produced in 10% liquid V8 zoospores (Figure 2D,E); an empty sporangium (Figure 2E); zoospores were suborbicular (Figure 2F) and 6.9–9.3 µm in diameter (n = 30); chlamydospores were spherical, terminal and 25.2 ± 0.3 µm in diameter (n = 30) (Figure 2G).

3.3. Molecular Biology Identification

The genomic DNA of three representative isolates was amplified using three genes, and the bands obtained with gel electrophoresis were in line with the expected size. Then, the amplified sequences were subjected to BLAST alignment analysis in NCBI, and the results are listed in Table 2. These sequences amplified in this study have been registered in GenBank (http://www.ncbi.nlm.nih.gov (accessed on 5 May 2023)). A total of 14 closely related species and 17 isolate sequences were downloaded as references. The GenBank accession of the sequences of *Phytophthora* are shown in Table 3. Based on the tandem sequences of the three genes, under the AIC standard, the Maximum Likelihood method development tree was constructed, and the Bayesian development tree was constructed under the BIC standard. The tree structure of the two is the same.

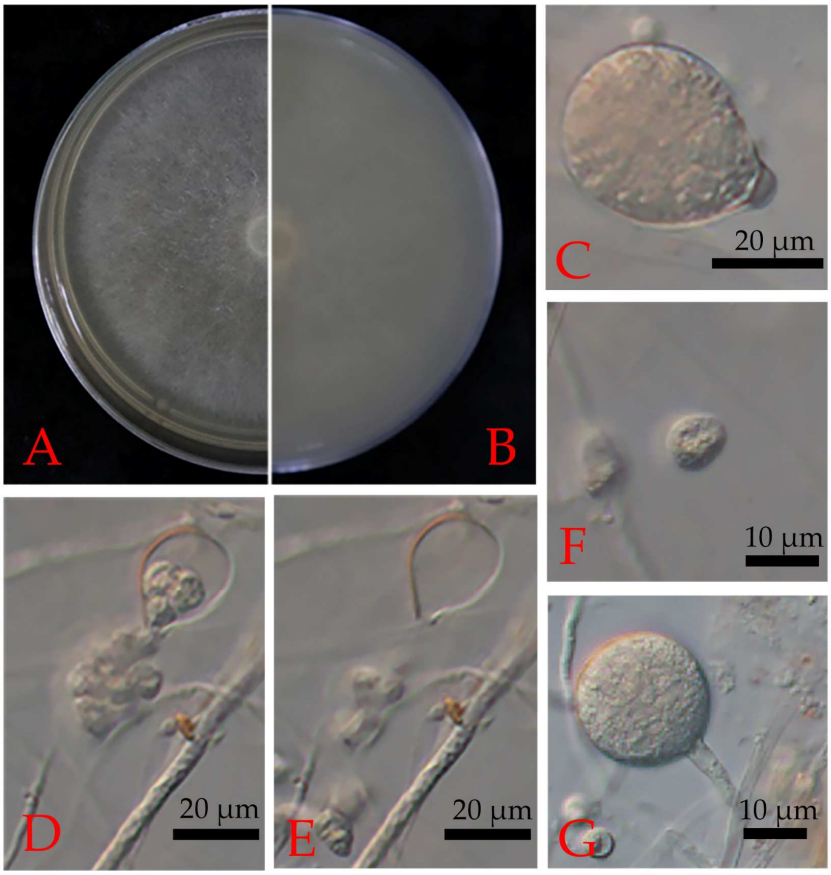

Figure 2. Morphological characters of *P. nicotianae* from *F. japonica*. (**A**,**B**) Colony morphology of three-day-old isolate BJP-1 grown on V8A. (**C**) Sporangium. (**D**) Sporangium releasing zoospores. (**E**) Empty sporangium. (**F**) Zoospore. (**G**) Chlamydospore.

Table 2. BLAST results based on the ITS, *LSU* and *COXII* gene amplification sequences of three representative isolates in this experiment.

Isolate	DNA Target	GenBank Accession No.	Blast Match Sequence	
			Reference Accession No.	Sequence Identity (%)
BJP-1	ITS	OP735506	*P. nicotianae* B2 (MT472132.1)	99.88% (801/802)
	LSU	OP738518	*P. nicotianae* 22F9 (KX250514.1)	100% (1246/1246)
	COXII	OP743911	*P. nicotianae* P6303 (GU318304.1)	99.73% (733/735)
BJP-2	ITS	OP735507	*P. nicotianae* B2 (MT472132.1)	99.63% (802/805)
	LSU	OP738516	*P. nicotianae* 22F9 (KX250514.1)	100% (1248/1248)
	COXII	OP743912	*P. nicotianae* P6303 (GU318304.1)	99.66% (880/883)
BJP-3	ITS	OP735526	*P. nicotianae* B2 (MT472132.1)	100% (803/803)
	LSU	OP738517	*P. nicotianae* 22F9 (KX250514.1)	100% (1245/1245)
	COXII	OP743913	*P. nicotianae* P6303 (GU318304.1)	99.61% (758/761)

ML and BI analysis produced a basically consistent tree topology, indicating that the evolutionary relationship of *Phytophthora* isolates was statistically supported. A consensus tree with RAxML bootstrap ratio (BP) and Bayesian posterior probability (BPP) was generated from ML and BI (Figure 3). Phylogenetic analysis showed that the isolates BJP-1, BJP-2 and BJP-3 were clustered on the branch of P6303 (BP/BPP = 100%/1).

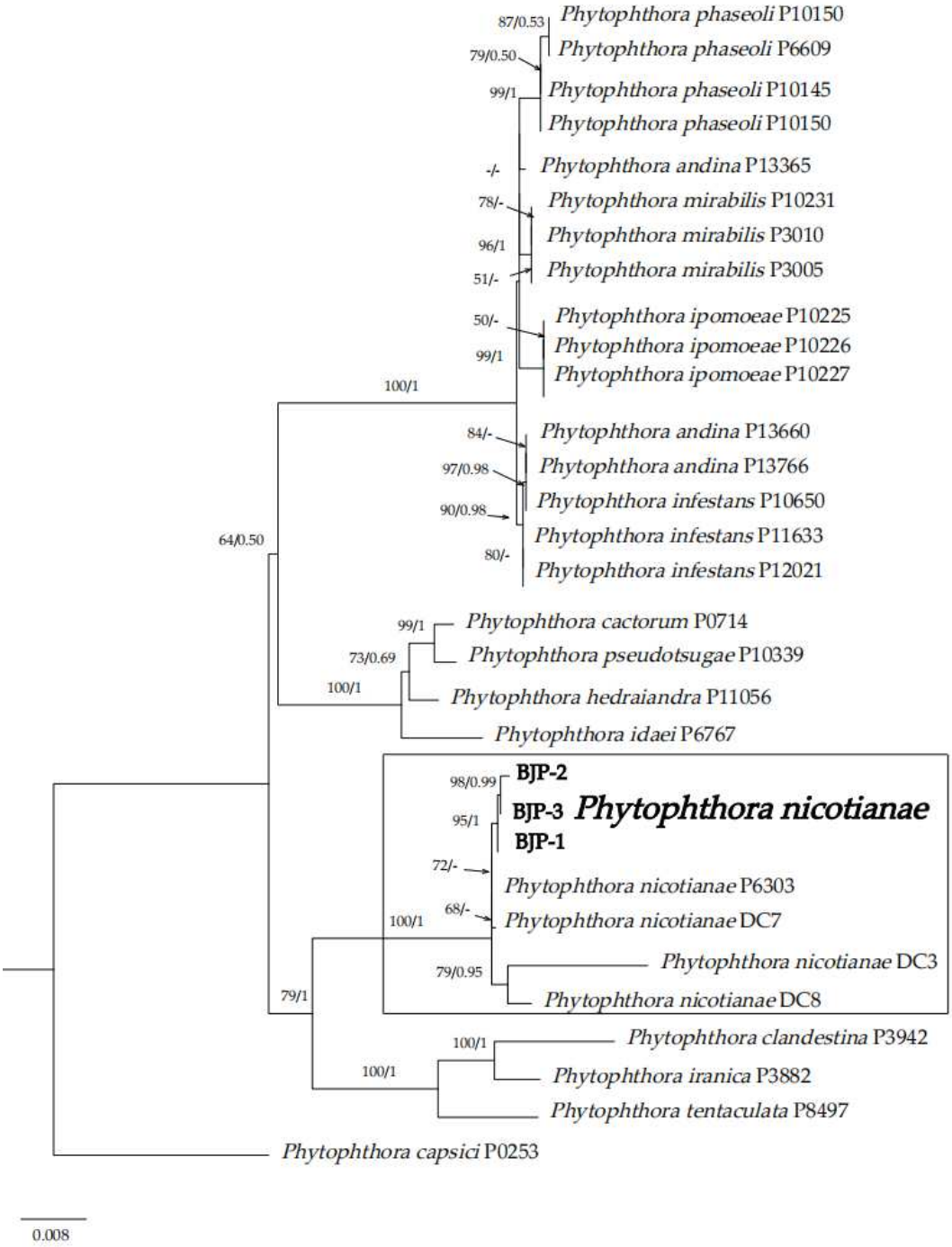

Figure 3. Maximum Likelihood and Bayesian analyses of *Phytophthora* species constructed using the concatenated dataset (ITS, *LSU* and *COXII*). *Phytophthora nicotianae* (BJP-1, BJP-2 and BJP-3) found in this study formed a monophyletic clade with other isolates of the same species. Bootstrap support values (ML $\geq$ 50) and Bayesian posterior probability (PP $\geq$ 0.50) were shown at the nodes. The scale bar shows the predicted number of substitutions per nucleotide position. *Phytophthora capsici* was used as an outgroup.

Table 3. NCBI accession numbers for sequences used in phylogenetic study.

Phytophthora Species	Isolate	GenBank Accession Numbers [1]		
		ITS	LSU	COXII
P. nicotianae	P6303	JN699566	EU080603	GU318304
P. nicotianae	dc3	MZ557793	MZ573546	MZ573545
P. nicotianae	dc7	MZ519893	MZ573547	MZ540768
P. nicotianae	dc8	MZ557794	MZ573549	MZ573548
P. cactorum	P0714	HQ261514	EU080282	GU221951
P. hedraiandra	P11056	FJ802065	EU080077	JF771449
P. idaei	P6767	HQ261579	EU080134	GU222032
P. pseudotsugae	P10339	HQ261654	EU080431	GU222121
P. clandestina	P3942	HQ261538	EU079871	GU221981
P. iranica	P3882	HQ261598	EU080116	GU222048
P. tentaculata	P8497	HQ261717	EU079960	GU222150
P. andina	P13365	FJ801734	EU080187	GU318297
	P13660	FJ801748	-	GU221934
	P13766	FJ801753	-	JQ439407
P. infestans	P10650	HQ261589	EU079630	GU318302
	P11633	FJ802075	-	JF771479
	P12021	GU258555	-	JF771480
P. ipomoeae	P10225	HQ261597	EU080835	GU222045
	P10226	HQ261596	EU080842	GU222046
	P10227	HQ261595	EU080849	GU222047
P. mirabilis	P3005	HQ261622	EU079780	GU222077
P. phaseoli	P6609	HQ261640,	EU079918	GU222106
	P10145	HQ261642	EU080753	GU222104
	P10150	HQ261641	EU080766	GU222105
P. capsici	P0253	FJ801244	EU080856	GU318299

[1]: ITS: internal transcribed spacer region of the rDNA; *LSU*: large subunit; *COXII*: mitochondrially encoded cytochrome oxidase II.

3.4. Pathogenicity Tests of Isolates

The results showed that 25 days after inoculation, all inoculated seedlings (n = 9) showed the same root rot symptoms as those observed in plants with natural infections (Figure 4A,C,E). In contrast, the control seedlings (n = 3) did not show symptoms (Figure 4B,D,F). The pathogen was re-isolated from all inoculated plants, and the experiment was repeated three times. Based on the morphological and molecular characters, the isolates were identified as *P. nicotianae*.

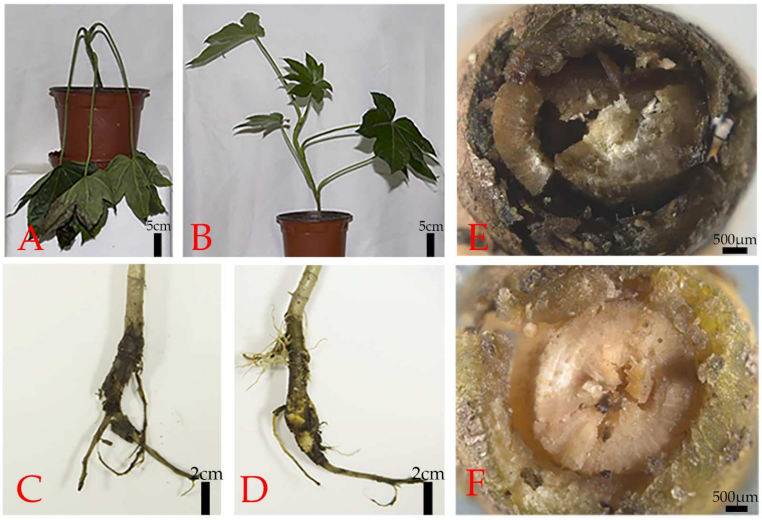

Figure 4. Pathogenicity of *P. nicotianae* on the roots of *F. japonica* inoculated artificially with zoospores. (**A**) Symptoms of *F. japonica* 25 days post inoculation of roots with a zoospore suspension. (**B**) Control plant treated with sterile water. (**C**) Root rot symptoms after inoculation with *P. nicotianae*. (**D**) Healthy root tissues of a control plant. (**E**) Cross-section of basal stem of a diseased plant inoculated with zoospore suspension of *P. nicotianae*. (**F**) Cross-section of basal stem of a healthy control plant.

4. Discussion

Fatsia japonica is not only a widely planted ornamental foliage plant but also has great value in medical medicine. With the extensive cultivation of *F. japonica*, several diseases have emerged, which caused a reduction in its beauty, vitality and longevity. Anthracnose on *F. japonica* caused by *Colletotrichum fructicola*, *C. karstii* and *C. gloeosporioides* has been reported in China [13,60,61]. *Botryosphaeria dothidea* caused stem canker and leaf wilt on *F. japonica* in Iran and China, respectively [62,63]. Leaf blight on *F. japonica* caused by *Alternaria panax* and *P. cactorum* has been reported in Europe and Korea [64,65]. But, there are few reports on root diseases of *F. japonica*.

Phytophthora nicotianae was first described by De Haan in 1896. In the past, *P. nicotianae* was considered to infect tobacco only under natural conditions and could not infect other plants. Subsequent researchers isolated *P. nicotianae* from fruit trees, crops, herbs, ornamental plants, shrubs and other plants, indicating that *P. nicotianae* is not specific to tobacco and can infect a variety of host plants other than tobacco under certain conditions [35]. With a wide host range, more than 255 species have become one of the most destructive plant pathogens of oomycetes in the world [66]. *P. nicotianae* was also reported under the name of *P. parasitica*, and these two names are often used as synonyms [67]. It preferentially infects roots and the stem basal region of the plant, although all the parts of the plant can be infected [68]. For instance, *P. nicotianae* was reported to cause asparagus spear and root rot in China [69], cherry stem rot and leaf necrosis in China [70], strawberry crown and leather rot in Florida [71], foot rot of citrus in Texas [72], *Dianthus chinensis* root rot and foliage blight in China [73], brown rot of citrus fruits in California [74], bud rot disease of *Washingtonia palms* in Saudi Arabia [75], *Catharanthus roseus* leaf blight in Bangladesh [76] and Phytophthora blight disease on konjac in Yunan [51]. Recently, it has been reported to cause root and crown rot of paulownia and sago palms in Italy [77,78]. These last reports further expand the list of known hosts of *P. nicotianae*. The above examples indicate that *P. nicotianae* poses a potential threat to many plant species in nature, and there may be many natural hosts in nature that have not been discovered yet.

In this study, we investigated several areas in the Xuanwu District of Nanjing City and found that 40% of *Fatsia japonica* were also persecuted by root rot. This disease is a serious threat to the cultivation of *F. japonica*, which can lead to the death of the whole plant, reduce the ornamental value of *F. japonica*, and even affect the growth of the plant. According to the investigation of many places in Nanjing, the isolation and identification data of samples were collected. The isolation rate of *Phytophthora nicotianae* can reach 60%, in the samples that were collected. It was found that the disease was prone to occur in humid conditions with insufficient light, especially after rain. Although its natural transmission rate is very slow at present, with the increase in the number of plants, the incidence of the disease is expected to increase in the next few years, which will have a serious impact on the ecological and economic value of *F. japonica*. Therefore, based on the above reasons, we must attach great importance to the occurrence of this disease and take appropriate strategies to prevent the spread of the disease and its harm.

5. Conclusions

In summary, this study clarified the cause of disease in *Fatsia japonica*. Through the preliminary observation of symptoms of the whole plant, the diseased tissue was isolated; the isolated pathogen was identified with the morphology of mycelium, sporangia and chlamydospores; and the phylogenetic tree was constructed using ITS, *COXII* and *LSU* multi-gene series. Finally, it was determined that the disease was caused by *Phytophthora nicotianae*. The results of this study will contribute to a comprehensive and systematic understanding of the disease. The detailed descriptions, molecular data and pathogenicity studies of new diseases can provide new disease resources for plant pathologists and mycologists and can help identify diseases more accurately. On the other hand, this study can provide a theoretical basis for the future study of the pathogenic mechanism and prevention of *P. nicotianae*.

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