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ORIGINAL ARTICLE

Current species of oomycetes associated with foot rot disease of black pepper in Vietnam

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Abstract

Foot rot disease caused by *Phytophthora capsici* is one of the most destructive diseases of black pepper in Vietnam and worldwide. However, other oomycete species such as *P. tropicalis* and *Pythium deliense* reported as serious threats to black pepper in India have also been recorded on this plant. The population of oomycetes occurring in black pepper plantations in Vietnam and their pathogenicity have not been investigated in the last decade. To this end, two hundred fifteen oomycete isolates were collected from the root rots and rhizospheric soil of black pepper in the Central Highlands and the Southeast region of Vietnam. Of these, 23 isolates were representatively chosen based on their origin and morphology for DNA sequence analysis of the internal transcribed spacer region, then 11 isolates were further selected for the translation elongation factor 1-alpha and the beta-tubulin gene analyses. Morphology and molecular analyses indicated that *P. capsici*, *P. cinnamomi*, *P. heveae, P. nicotianae*, *P. parvispora*, *P. tropicalis*, *Phytopythium vexans*, and a new species candidate *Phytopythium* sp. were identified among oomycete isolates. Of these, *P. capsici* and *P. tropicalis* could be the prevalent species in black pepper plantations in studied areas. The inoculation tests demonstrated that *P. capsici*, *P. nicotianae* and *P. tropicalis* were pathogenic on both leaves and roots of black pepper. *Phytopythium vexans* was pathogenic on root only. Meanwhile, *P. cinnamomi*, *P. heveae*, *P. parvispora* and *Phytopythium* sp. were non-pathogenic.

Keywords *Phytophthora capsici* · *Phytophythium vexans* · *Piper nigrum*

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Introduction

For many years, Vietnam has been the world's largest black pepper (*Piper nigrum* L.) producing and exporting country, followed by Brazil, Indonesia, and India (Yogesh and Mokshapathy 2013; Rathore and Swathi 2020; Azahari et al. 2021). To date, black pepper is cultivated mainly in the Central Highlands and the Southeast region of Vietnam with the total area increasing from 101,600 ha in 2015 to 147,500 ha in 2018, then reducing to 131,800 ha in 2020 (General statistics office 2021). Foot rot disease or "quick wilt disease" caused by *Phytophthora capsici* is the most destructive soilborne pathogen of black pepper in many countries of the world including Vietnam (Anandaraj 2000; Truong et al. 2008; Thuy et al. 2012; Farhana 2013; Kifelew and Adugna 2018; Jibat and Alo 2021; Quy et al. 2021; Kong et al. 2022). Approximately 25-30% of the vine death of black pepper was reported in Kerala (Nambiar and Sarma 1977). Anandaraj et al. (1989) reported that up to 95% of the vines showed foot rot symptoms in individual farms of this plant in many countries. In 2016, more than 10,000 ha of black pepper in Vietnam was damaged by pathogen infections and the highest-losses occurred in most cultivated regions (Trinh et al. 2019).

Phytophthora palmivora was primarily described as a causal agent of black pepper foot rot (Muller 1937; Zentmyer et al. 1977). In recent years, *Phytophthora* species infected black pepper in India were identified into two different species, *P. capsici* and *P. tropicalis*. They are closely related species with overlapping morphological characteristics and intraspecific variations but can be distinguished based on multi-locus DNA sequence analysis (Bowers et al. 2007; Jeevalatha et al. 2021; Bhai et al. 2022). Another *Phytophthora* species, *P. nicotianae*, has also been reported to cause root and stem rot on black pepper (Drenth and Guest 2004). In the *Pythium* and *Phytopythium* genera, many species were pathogenic on black pepper such as *Phytopythium vexans* (syn. *Pythium vexans*), *Pythium butleri*, *P. deliense*, *P. helicoides*, *P. irregular*, *P. middletonii* and *P. splendens* (Liu 1977; Matsuda et al. 1998). Among these species, *Pythium deliense*, causing significant damage with yellowing and drying up symptoms, was newly reported in India (Subila and Bhai 2020).

In the previous studies, *P. capsici*, *P. cinnamomi, P. nicotianae* and *Pythium* sp. were isolated from diseased tissues and rhizospheric soils of black pepper in Vietnam. Of these, *P. capsici* was the main agent causing wilt symptoms, while the pathogenicity of *P. nicotianae* was unclear and *Pythium* sp. infection was not significant (Truong et al. 2008). Dung et al. (2014) revealed that the black pepper foot rot in Dak Nong province of Vietnam was caused by *P. tropicalis* instead of *P. capsici* based on the sequence analysis of the internal transcribed spacer (ITS) region. *Phytopythium vexans* was also reported as a causal agent of black pepper root rot in Vietnam, however, the identification of this species was not confirmed (Nguyet et al. 2018). This work, therefore, aims to catalogue the species diversity and pathogenicity of current oomycete species in black pepper growing areas of Vietnam.

Materials and Methods

Sampling collection and isolation

Rhizospheric soil and root rot samples of black pepper plants showing typical symptoms of quick wilt disease were collected in the Central Highlands (Dak Lak, Dak Nong and Gia Lai provinces) and the Southeast region (Binh Phuoc and Dong Nai provinces) in 2020 and 2021. Sampling collection was conducted in two districts of each province (Fig. 1). Oomycetes from soil samples were isolated by the baiting technique (Drenth and Guest 2004) with rose petals and selective media. Thirty grams of each soil sample (60-70% humidity) were placed into a cup (12 cm high and 9 cm diameter) with 300 ml of sterilized water. After 1 hour, rose petals were dropped on the water surface and the cups were placed at room temperature (around 28° C) for 24 to 72 hours. Infected rose petals with brown symptoms were rinsed with sterilized water and pieces (3 × 3 mm) were cut from the margins of the healthy and diseased tissue and then sterilized with 70% ethanol for 30 seconds, and 5% sodium hypochlorite for 1 min, respectively. The pieces were rinsed in sterilized water and dried on sterile filter paper, then these rose pieces were placed on potato carrot agar (PCA) containing 0.01 g/L benomyl and 0.02 g/L rifampicin. The Petri dishes were incubated at 28° C for 2-3 days. Oomycete mycelia grown from rose pieces were cut from the margin of colonies and placed onto potato dextrose agar (PDA). Pure cultures of oomycetes were obtained by the hyphal tip isolation method (Tutte 1969). Oomycetes in the root rot samples were isolated according to the protocol applied for infected rose petals above.

Colony morphology and micro-morphological structures

Colony morphology of *Phytophthora* and *Phytopythium* were documented on PDA. To describe micro-morphological characteristics, oomycetes were grown on PCA Petri dishes for 3 to 5 days, and then the Petri dishes were flooded with sterile distilled water for 3 to 5 days at room temperature to induce the formation of sporangia, chlamydospores, oogonia, and antheridia. The oomycetes were mounted on glass slides

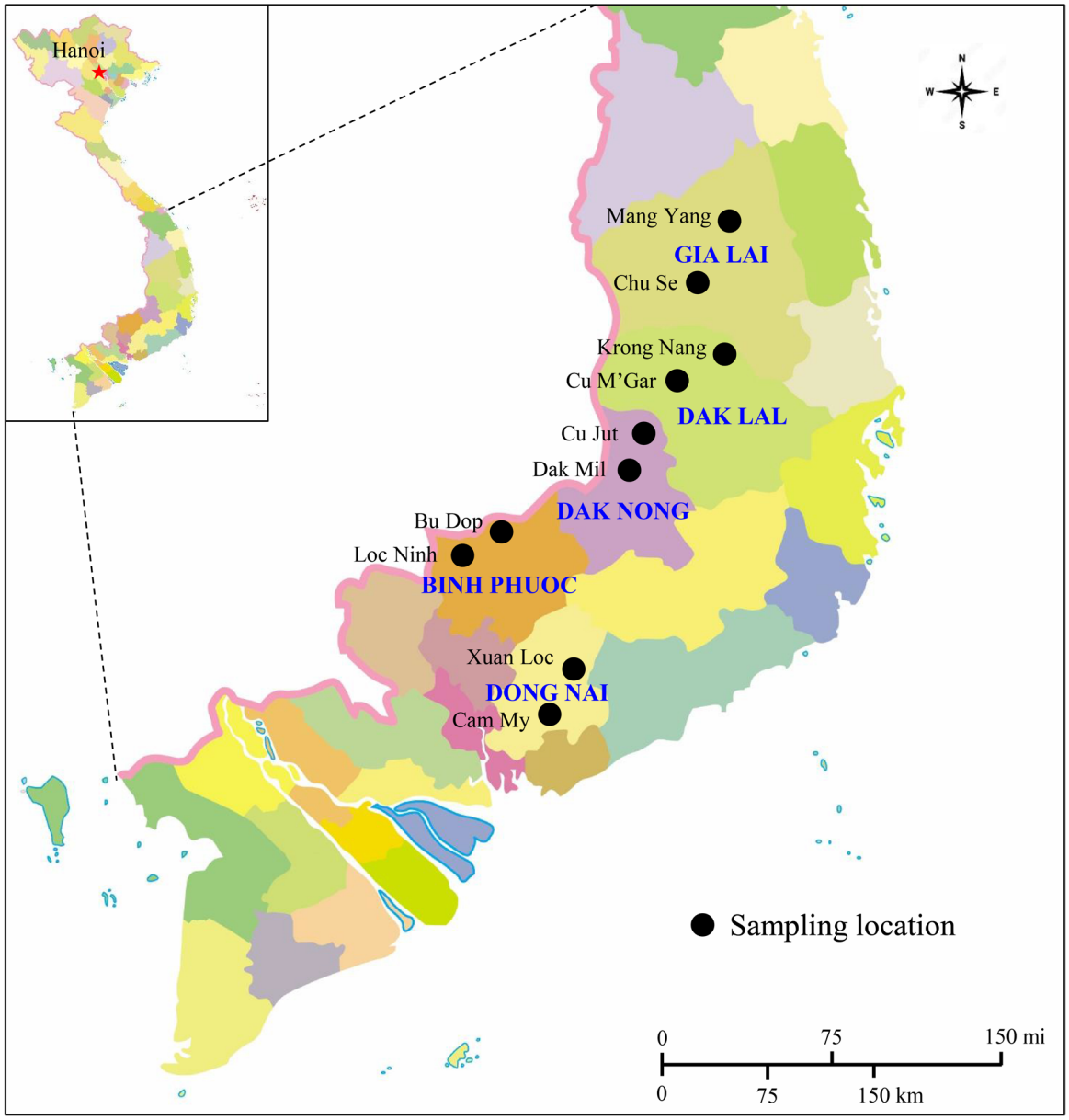

Fig. 1 Locations of original oomycete isolates collected in this study. Gia Lai (Mang Yang and Chu Se districts), Dak Lak (Krong Nang and Cu M'Gar districts), Dak Nong (Cu Jut and Dak Mil districts), Binh Phuoc (Bu Dop and Loc Ninh districts) and Dong Nai (Xuan Loc and Cam My districts)

and the micro-morphological structures were described and measured under a light microscope. At least 30 measurements of each structure were performed with means and standard deviations (SD). The ranges were described as (min–) mean-SD – mean+SD (–max) (Thao et al. 2018).

DNA extraction, PCR amplification and sequencing

Oomycete mycelia from 3-5 days old on V8 juice were harvested, and DNA extraction was performed using the E.Z.N.A Fungal DNA Mini Kit (OMEGA BioTek, USA), according to the manufacturer's instructions. The ITS region was amplified with the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990), following PCR conditions: initial denaturation at 95°C for 2 min; 35 cycles consisting of denaturation at 95°C for 30 s, annealing at 50°C for 50 s and extension at 72°C for 1 min; final extension at 72°C for 10 min. The translation elongation factor 1-alpha (TEF1-α) gene was amplified using the primers ELONGF1 (5'-TCACGATCGACATTGCCCTG-3') and ELONGR1 (5'-ACGGCTCGAGGATGACCATG-3') (Kroon et al. 2004),

following the amplification protocol: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 1 min; final extension at 72°C for 10 min. The beta-tubulin (TUB) gene was amplified using the primers TUBUF2 (5'-CGG TAACAACTGGGCCAAGG-3'), TUBUR1 (5'-CCTGGT ACTGCTGGTACTCAG-3') (Kroon et al. 2004), following PCR conditions: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 66°C for 1 min and extension at 72°C for 1 min 30 s; final extension at 72°C for 10 min. A PCR reaction volume (25 µL) contained 2 µL template DNA (100 ng/µL), 1 µL (4.5pMol) of each primer, 12.5 µL MyTaq HS Mix and 8,5 µL nuclease-free water. Amplifications were confirmed by gel electrophoresis. PCR products were purified by the High Pure PCR Cleanup Micro Kit (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions, and were sequenced with the amplifying primers by an ABI 3730XL automatic sequencer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis

The raw sequences were assembled with MEGA 11 and deposited to GenBank. The alignment of each dataset, including DNA sequences in this study and reference sequences from NCBI (http://blast.ncbi.nlm.nih.gov/), was produced separately. The alignments were checked manually and the poorly aligned regions at the beginning and ends of sequences were excluded. The concatenated alignment of ITS, TEF1, and TUB was performed using MEGA 11.

Phylogenetic trees were generated based on the maximum likelihood (ML) method by RAxML v8.2.4 (Stamatakis 2014) with a GTR+GAMMA+I model and 1,000 maximum likelihood bootstrap replicates to test the support of the branches. Phylogenetic trees were viewed using MEGA 11, and layouts were depicted using Adobe Illustrator software.

Pathogenicity test

Representative isolates were tested for their pathogenicity on both black pepper leaves and seedlings. Black pepper seedlings were grown from healthy seeds in plastic containers (6 cm high, 6 cm top diameter and 5 cm bottom diameter) with sterilized soil, and one seedling was maintained in a cup. Ten millilitres of the zoospore suspension (1×10^4 zoospores/ml) of *Phytophthora* isolates, or sporangia, chlamydospores and oogonia suspension (1×10^3 unit/ml) of *Phytopythium* isolates were prepared and added to each container. Each species was inoculated onto 3 seedlings. Sterilized water was used in the control. The seedlings were maintained in a greenhouse at 28°C for 4 weeks. Fresh black pepper leaves were collected from the mature plants. The leaves were washed under a running tap and then surface sterilized with 70% ethanol. One oomycete species was inoculated onto one leaf at three wound points on the upper surface. Twenty microliters of zoospore, chlamydospore, or oogonia suspensions as above were pipetted onto the wounded tissue area. The control was applied with sterilized water. The leaves were placed into an incubator at 28°C and 90% humidity in darkness for 5 days. The seedlings and leaves were monitored daily for disease symptoms. After the assessment, the seedling roots and the inoculated tissue of leaves were sampled for isolation on selective media for the detection of oomycetes. The experiment was repeated three times.

Results

Oomycete isolation

Two hundred fifteen isolates of *Phytophthora* and *Phytopythium* were isolated from rhizospheric soil and root rot samples of black pepper collected at Dak Lak, Dak Nong, Gia Lai, Binh Phuoc, and Dong Nai provinces in the years 2020 and 2021. The isolates were preserved at the Division of Plant Pathology and Phyto-immunology, Plant Protection Research Institute, Vietnam. Twenty-three representative isolates (Table 1) were selected for molecular analysis based on source, geographic origin and morphological characteristics.

Isolates obtained in this study are indicated in bold; Isolates in this study used for the multi-locus analysis (ITS, TEF1 and TUB) were indicated by $^{+}$; Ex-type and authentic strains are shown by *.

Phylogenetic analysis

Twenty-three representative isolates were selected for ITS sequencing. The ITS sequences were used for nucleotide BLAST searches on the NCBI for selecting the reference sequences. Eleven isolates were selected from the ITS phylogenetic tree (Fig. 2) for further DNA sequencing of TEF1- α and TUB genes. All assembled sequences generated in this study were submitted to GenBank (Table 1). The datasets of each locus consisted of 11 sequences in the present study and reference sequences. Most of the reference sequences were from ex-type and authentic strains. The concatenated alignment of ITS, TEF1- α, and TUB contained 898, 889, and 893 characters including gaps, respectively. The multi-locus phylogenetic tree (Fig. 3) showed that PPRI2188 and PPRI20911 isolates were clustered together with the ex-type strain of *P. capsici* (CPHST BL 33G) with a ML bootstrap value of 98%. Isolate PPRI20912 was grouped in a separate clade with *P. tropicalis* (CBS 434.91, ex-type strain), supported by a ML bootstrap value of 97%, and this species was considered as a sister of

Table 1 Representative isolates in this study and reference isolates used in the phylogenetic analysis

Species	Isolate	Source	Location	Collected date	GenBank accession numbers		
					ITS	TEF1	TUB
Pythium canariense	CBS 112353*	-	Spain	-	JX397990	-	JX397969
Pythium cystogenes	CBS 675.85*	-	Netherlands	-	AY707985	-	KJ595520
Phytopythium helicoides	CBS 286.31*	-	-	-	AY598665	EF408891	EF408881
Phytopythium sp.	**PPRI2097+**	**Soil of *Piper nigrum***	**Bu Dop, Binh Phuoc**	**Sep. 2020**	**OQ617900**	**OQ630880**	**OQ630891**
Phytopythium vexans	**PPRI20925+**	**Soil of *Piper nigrum***	**Mang Yang, Gia Lai**	**Sep. 2020**	**OQ617899**	**OQ630879**	**OQ630890**
	PPRI2098+	**Root of *Piper nigrum***	**Krong Nang, Dak Lak**	**Sep. 2020**	**OQ617901**	**OQ630881**	**OQ630892**
	PPRI21822	**Soil of *Piper nigrum***	**Mang Yang, Gia Lai**	**Aug. 2021**	**OQ617920**	**-**	**-**
	CBS 119.80*	Soil	Iran	-	GU133572	EF426555	EF426556
Phytophthora agathidicida	CPHST BL 154*	*Agathis australis*	New Zealand	2006	MG602692	MH358951	MH493902
Phytophthora capsici	**PPRI2188+**	**Root of *Piper nigrum***	**Dak Mil, Dak Nong**	**Aug. 2021**	**OQ617906**	**OQ630886**	**OQ630897**
	PPRI20911+	**Root of *Piper nigrum***	**Chu Se, Gia Lai**	**Sep. 2020**	**OQ617907**	**OQ630887**	**OQ630898**
	PPRI2092	**Soil of *Piper nigrum***	**Mang Yang, Gia Lai**	**Sep. 2020**	**OQ617910**	**-**	**-**
	PPRI2093	**Root of *Piper nigrum***	**Cu M'Gar, Dak Lak**	**Sep. 2020**	**OQ617911**	**-**	**-**
	PPRI2094	**Soil of *Piper nigrum***	**Krong Nang, Dak Lak**	**Sep. 2020**	**OQ617912**	**-**	**-**
	PPRI2086	**Root of *Piper nigrum***	**Cam My, Dong Nai**	**Aug. 2020**	**OQ617913**	**-**	**-**
	PPRI2089	**Soil of *Piper nigrum***	**Xuan Loc, Dong Nai**	**Aug. 2020**	**OQ617914**	**-**	**-**
	PPRI20910	**Root of *Piper nigrum***	**Bu Dop, Binh Phuoc**	**Sep. 2020**	**OQ617915**	**-**	**-**
	PPRI20921	**Soil of *Piper nigrum***	**Cu Jut, Dak Nong**	**Sep. 2020**	**OQ617917**	**-**	**-**
	CPHST BL 33G*	-	-	-	MG865467	MH358966	MH493915
Phytophthora castaneae	CPHST BL 47G*	*Castanea crenata*	Japan	1971	MG865470	MH358969	MH493918
Phytophthora cinnamomi	**PPRI2087+**	**Soil of *Piper nigrum***	**Cam My, Dong Nai**	**Aug. 2020**	**OQ617905**	**OQ630885**	**OQ630896**
	CPHST BL 12*	-	-	-	MG865473	MH358972	MH493920
Phytophthora cinnamomi var. *parvispora*	CBS 411.96	-	Germany	-	AY302184	EU079950	LC595877
Phytophthora cocois	CPHST BL 157*	*Cocos nucifera*	USA	1990	MG865478	MH358977	MH493925
Phytophthora heveae	**PPRI20913+**	**Soil of *Piper nigrum***	**Cu M'Gar, Dak Lak**	**Sep. 2020**	**OQ617903**	**OQ630883**	**OQ630894**
	CBS 296.29*	*Hevea brasiliensis*	Malaysia	1929	AF266770	AY564123	AY564067
Phytophthora nicotianae	**PPRI20915+**	**Soil of *Piper nigrum***	**Dak Mil, Dak Nong**	**Sep. 2020**	**OQ617904**	**OQ630884**	**OQ630895**
	CPHST BL 162*	*Gramatophyllum* sp.	Indonesia	-	OP020179	MH359042	MH493986
Phytophthora parvispora	**PPRI20920+**	**Soil of *Piper nigrum***	**Loc Ninh, Binh Phuoc**	**Sep. 2020**	**OQ617908**	**OQ630888**	**OQ630899**
	PPRI21812+	**Soil of *Piper nigrum***	**Bu Dop, Binh Phuoc**	**Aug. 2021**	**OQ617909**	**OQ630889**	**OQ630900**
	CBS 132772*	*Arbutus unedo*	Italy	2011	KC478667	MH359051	KC609402
	CPHST BL 13	-	-	-	MG865563	MH359053	MH493996
	30G9	-	-	-	EU748548	KX251820	KX251819

Table 1 (continued)

Species	Isolate	Source	Location	Collected date	GenBank accession numbers		
					ITS	TEF1	TUB
Phytophthora tropicalis	**PPRI20912**$^{+}$	**Root of *Piper nigrum***	**Chu Se, Gia Lai**	**Sep. 2020**	**OQ617902**	**OQ630882**	**OQ630893**
	PPRI2095	**Root of *Piper nigrum***	**Krong Nang, Dak Lak**	**Sep. 2020**	**OQ617916**	**-**	**-**
	PPRI21818	**Soil of *Piper nigrum***	**Dak Mil, Dak Nong**	**Aug. 2021**	**OQ617918**	**-**	**-**
	PPRI21819	**Soil of *Piper nigrum***	**Krong Nang, Dak Lak**	**Aug. 2021**	**OQ617919**	**-**	**-**
	PPRI20914	**Soil of *Piper nigrum***	**Mang Yang, Gia Lai**	**Sep. 2020**	**OQ617921**	**-**	**-**
	CBS 434.91*	*Macadamia integrifolia*	USA	-	NR_147864	LN908263	LN908272

P. capsici. Isolate PPRI20915 was segregated into a separate group with *P. nicotianae* (CPHST BL 162, authentic strain, ex-type strain not available, Abad et al. 2023), which was supported by a ML bootstrap value of 100%. Isolate PPRI20913 fell in a group with *P. heveae* (CBS 296.29, ex-type strain) supported by a ML bootstrap value of 96%. This species was a high homology with *P. agathidicida*, *P. castaneae* and *P. cocois*. Isolate PPRI2087 clustered with the ex-type strain of *P. cinnamomi* (CPHST BL 12) with a ML bootstrap value of 100%. Isolate PPRI20920 and PPRI21812 were in a group of *P. parvispora* species (CBS 132772, ex-type strain) with a ML bootstrap value of 100%, and closely related to *P. cinnamomi* species. Isolate PPRI2098 and PPRI20925 were grouped in a monophyletic clade with the reference strain of *Phytopythium vexans* (syn. *Pythium vexans*) (CBS 119.80, authentic strain, ex-type strain not available, de Cock et al. 2015) with a ML bootstrap value of 100%. Isolate PPRI2097 placed in a single clade with a distant genetic relationship from others. The ITS, TEF1- α, and TUB sequences of PPRI2097 were the highest similarity with *Phytopythium helicoides* (CBS 286.31, authentic strain, ex-type strain not available, de Cock et al. 2015), at 85.64%, 95.78% and 95.57%, respectively. Hence, isolate PPRI2097 was considered as a new species candidate of the *Phytopythium* genus.

Colony and micro-morphology

Phytophthora capsici (PPRI20911)

Colonies on PDA were depressed with limited aerial mycelia with unclear pattern to the chrysanthemum pattern, covering a 9 cm Petri dish after 7 days. Variable shapes of sporangia were observed including subglobose, ovoid, pyriform, ellipsoid, fusiform, or distorted shapes, often with papilla, (29.7–) 39.1 – 55.1 (–65.2) × (17.6–) 22.5 – 31.5 (–37.1) µm. Hyphal swellings were globose or subglobose. Chlamydospores were absent (Fig. 4).

Phytophthora cinnamomi (PPRI2087)

Colonies on PDA were white and unclear pattern, covering a 9 cm Petri dish after 7 days Sporangia were semi-papilla or non-papilla, obpyriform, ovoid or ellipsoid, (48.3–) 51.2 – 63.8 (–71.2) × (27.7–) 32.2 – 39.0 (–41.3) µm. Hyphal swellings were abundant, globose to subglobose, or coralloid. Chlamydospores were globose, terminal or intercalary, (29.4–) 31.3 – 39.1 (–43.2) µm (Fig. 5).

Phytophthora heveae (PPRI20913)

Colonies on PDA were white, often depressed with no distinct pattern, covering a 9 cm Petri dish after 7 days. Sporangia were globose, subglobose, ovoid, with papilla, (26.2–) 27.5 – 38.7 (–44.6) × (19.8–) 22.0 – 29.2 (–33.2) µm. Hyphal swellings were globose to subglobose. Oogonia were spherical or subglobose, smooth, and often tapering base, (20.1–) 23.4 – 28.8 (–31.2) × (19.8–) 22.6 – 27.2 (–29.2). Antheridia were amphigynous, spherical, or cylindrical. Chlamydospore was absent (Fig. 6).

Phytophthora nicotianae (PPRI20915)

Colonies on PDA were white and cottony, often without a pattern or unclear pattern, covering a 9 cm Petri dish after 6 days. Sporangia were globose, subglobose, ovoid, obpyriform, and irregular shapes, often with papilla, (35.4–) 36.0 – 60.0 (–90.5) × (27.7–) 29.9 – 36.3 (–42.3) µm.

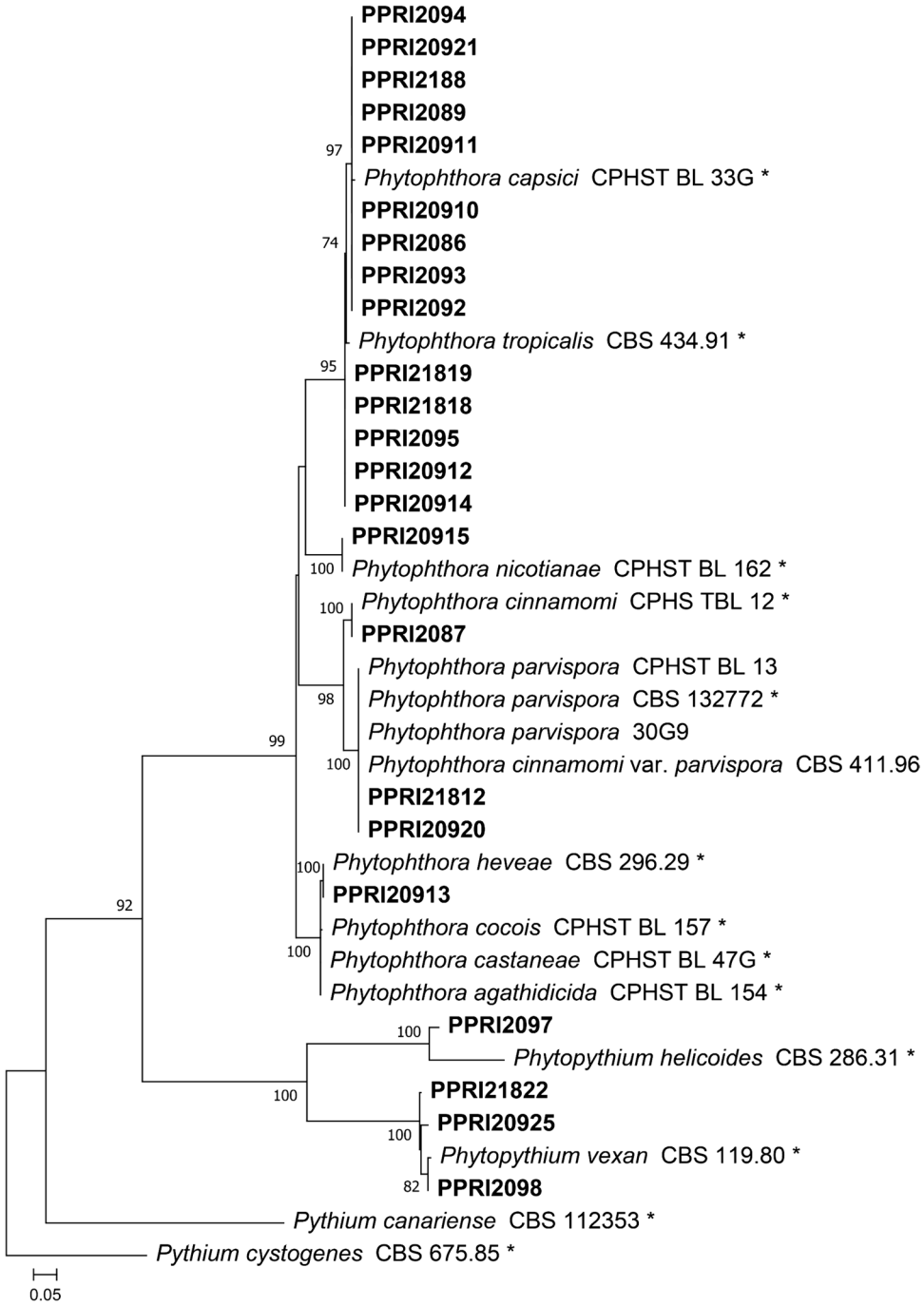

Fig. 2 Maximum likelihood tree of the ITS region. ML bootstrap support values ≥70% are presented at the nodes. The isolates in this study are in bold. Ex-type and authentic strains are indicated by an asterisk

Chlamydospores were global, terminal, or intercalary, (29.6–) 33.9 – 41.1 (–45.6) µm (Fig. 7).

Phytophthora parvispora (PPRI21812)

Colonies on PDA were white, cottony, and unclear pattern, covering a 9 cm Petri dish after 7 days. Sporangia were ovoid, obpyriform, or ellipsoid, semi-papilla or without papilla, (26.2–) 31.3 – 42.7 (–52.4) × (22.5–) 24.3 – 31.9 (–37.6) µm. Hyphal swellings were abundant, irregular, and globose to subglobose. Chlamydospores were globose, terminal, or intercalary, (19.4–) 21.5 – 25.3 (–26.5) µm (Fig. 8).

Phytophthora tropicalis (PPRI20912)

On PDA, the colonies were unclear pattern to the chrysanthemum pattern, often depressed with limited aerial mycelia, covering a 9 cm Petri dish after 7 days. Sporangia were

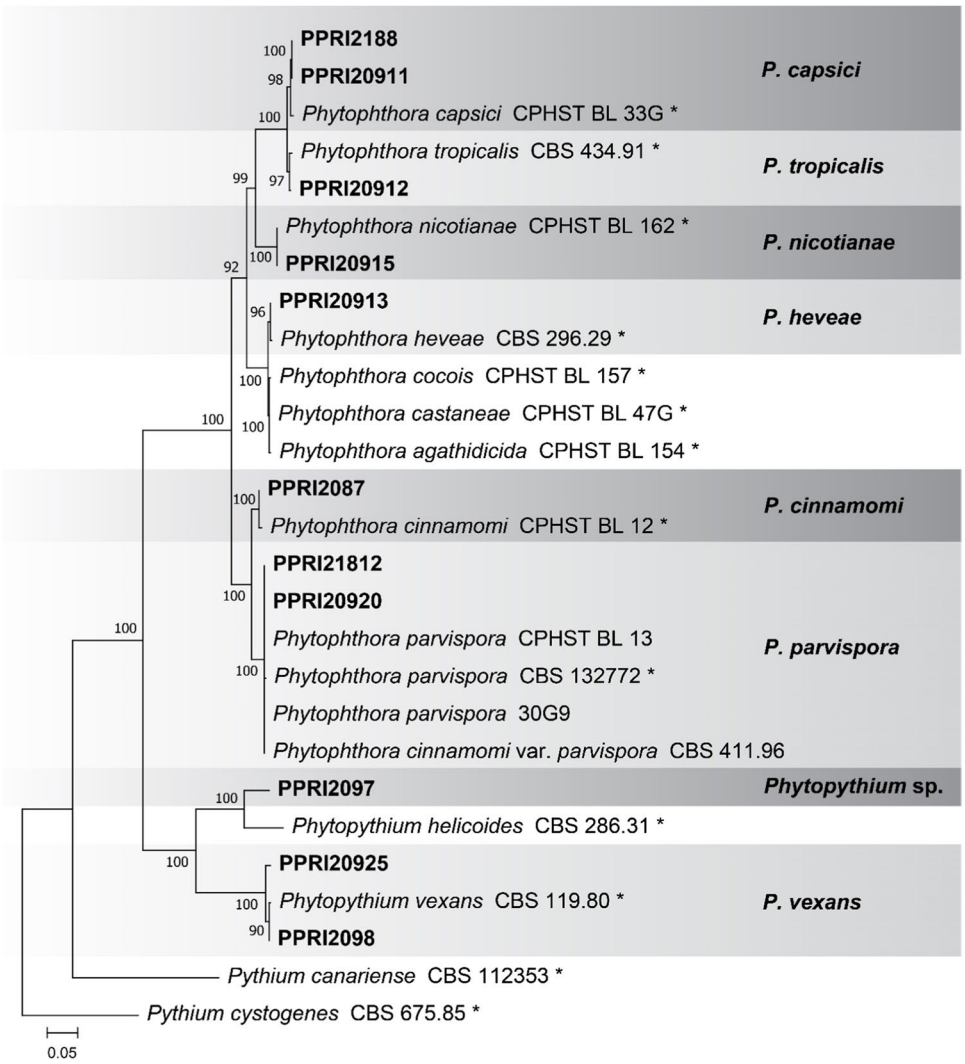

Fig. 3 Maximum likelihood tree of multi-locus sequences (ITS, TEF1- α, and TUB). ML bootstrap support values ≥70% are presented at the nodes. The isolates in this study are in bold. Ex-type and authentic strains are indicated by an asterisk

subglobose, ovoid, pyriform, ellipsoid, fusiform, or distorted shapes, often with papilla, (34.5–) 39.7 – 53.5 (–62.4) × (25.8–) 29.0 – 35.2 (–38.5) µm. Chlamydospores were globose, terminal or intercalary, (23.5–) 27.9 – 34.7 (–37.2) µm (Fig. 9).

Phytopythium vexans (PPRI2098)

Colonies on PDA were white, cottony, unclear pattern, rich in aerial mycelia and fast-growing, reaching the margin of 9 cm Petri dishes after 4 days. Sporangia were globose, subglobose, ovoid, or pyriform, with or without papilla, (16.3–) 18.5 – 24.1 (–27.8) × (15.3–) 17.7 – 21.5 (–24.8) µm. Oogonia were spherical, smooth and terminal, (15.1–) 17.2 – 20 (–21.5) µm. Antheridia were cylindrical, often monoclinous and broadly attached to the oogonia. Zoospores were released from sporangia via a short exit tube. (Fig. 10).

Phytopythium sp. (PPRI2097)

Colonies on PDA were white, chrysanthemum pattern and fast-growing, covering a 9 cm Petri dish after 4 days. Sporangia were globose, subglobose, ovoid, with or without papilla, (19.2–) 22.5 – 28.9 (–32.5) × (14.5–) 17.3 – 21.3 (–23.5) µm. Papilla developed at maturity to form a long discharge tube. Chlamydospores were globose, terminal or intercalary, (21.3–) 22.7 – 25.1 (–26.1) µm. (Fig. 11).

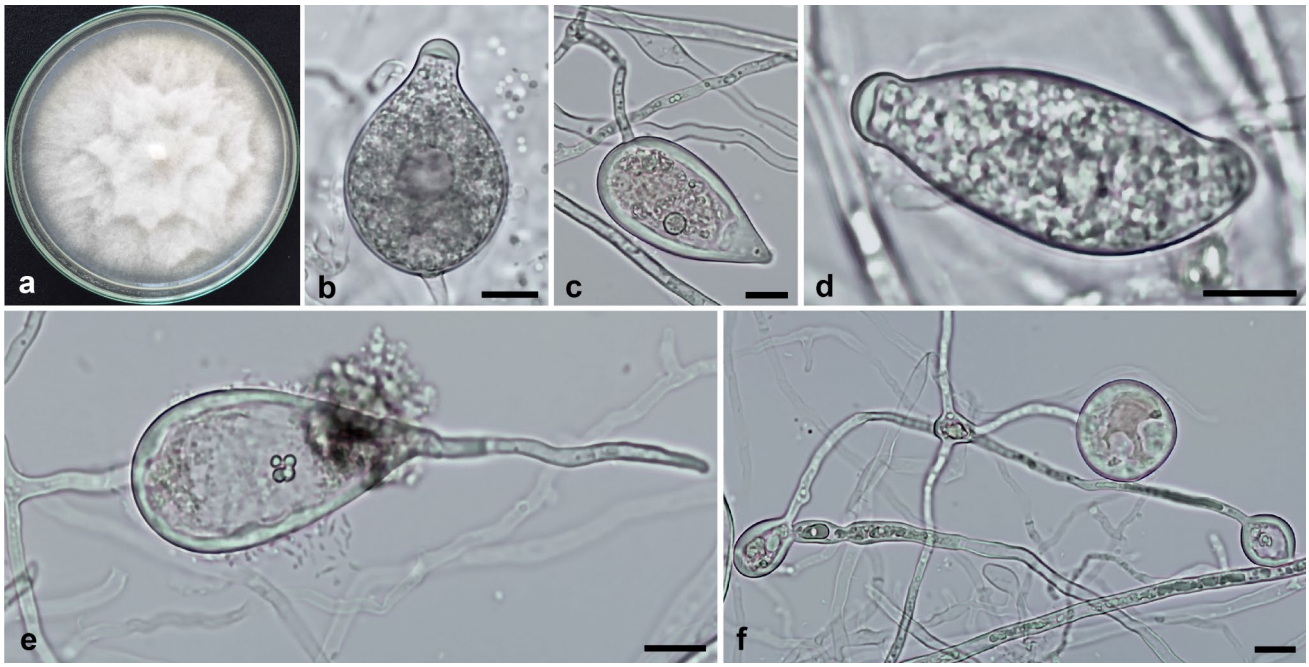

Fig. 4 *Phytophthora capsici* PPRI20911. a. Colony on PDA; b-e. Sporangia; f. Hyphal swellings; Scale bars = 10 µm

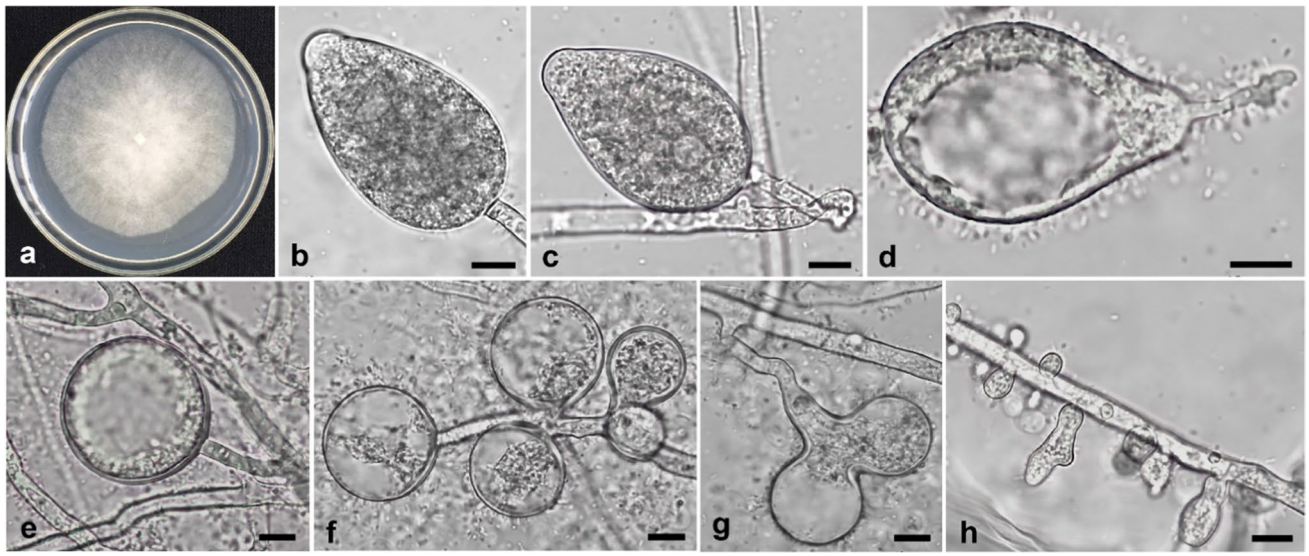

Fig. 5 *Phytophthora cinnamomi* PPRI2087. a. Colony on PDA; b-d. Sporangia; e. Chlamydospore; f-h. Hyphal swellings; Scale bars = 10 µm

Pathogenicity

Eight representative isolates including PPRI20911 (*P. capsici*), PPRI2087 (*P. cinnamomi*), PPRI20913 (*P. heveae*), PPRI20915 (*P. nicotianae*), PPRI21812 (*P. parvispora*), PPRI20912 (*P. tropicalis*), PPRI2098 (*Phytopythium vexans*), and PPRI2097 (*Phytopythium* sp.) were used to inoculate on black pepper leaves and seedlings. The pathogenicity tests revealed that the leaves inoculated with PPRI20911, PPRI20912 and PPRI20915 isolates showed diseased symptoms with dark brown lesions after 3 days. The seedlings inoculated with PPRI20911, PPRI20912, PPRI20915 and PPRI2098 isolates showed wilt and root rot symptoms at 10 days to 30 days post-inoculation. While, the other isolates of *P. cinnamomi*, *P. heveae*, *P. parvispora* and *Phytopythium* sp. did not produce any diseased symptoms (Figs. 12 and 13).

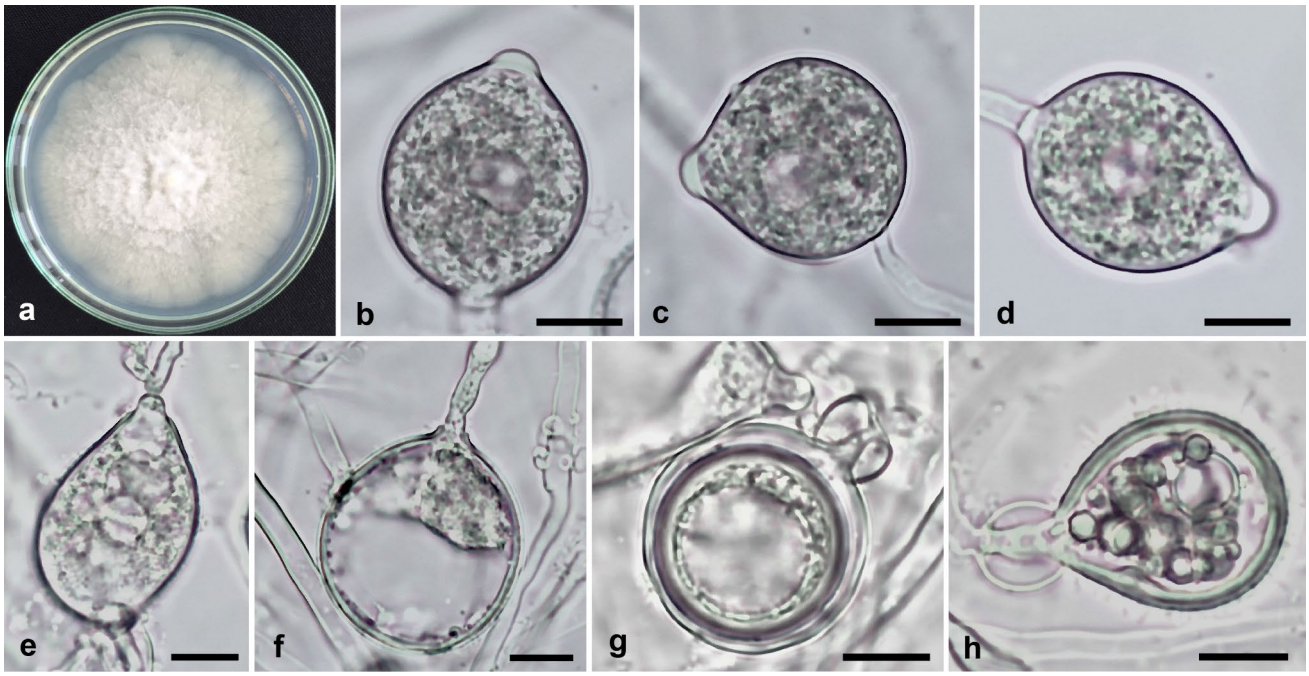

Fig. 6 *Phytophthora heveae* PPRI20913. a. Colony on PDA; b-f. Sporangia; g-h. Oogonia with antheridia; Scale bars = 10 µm

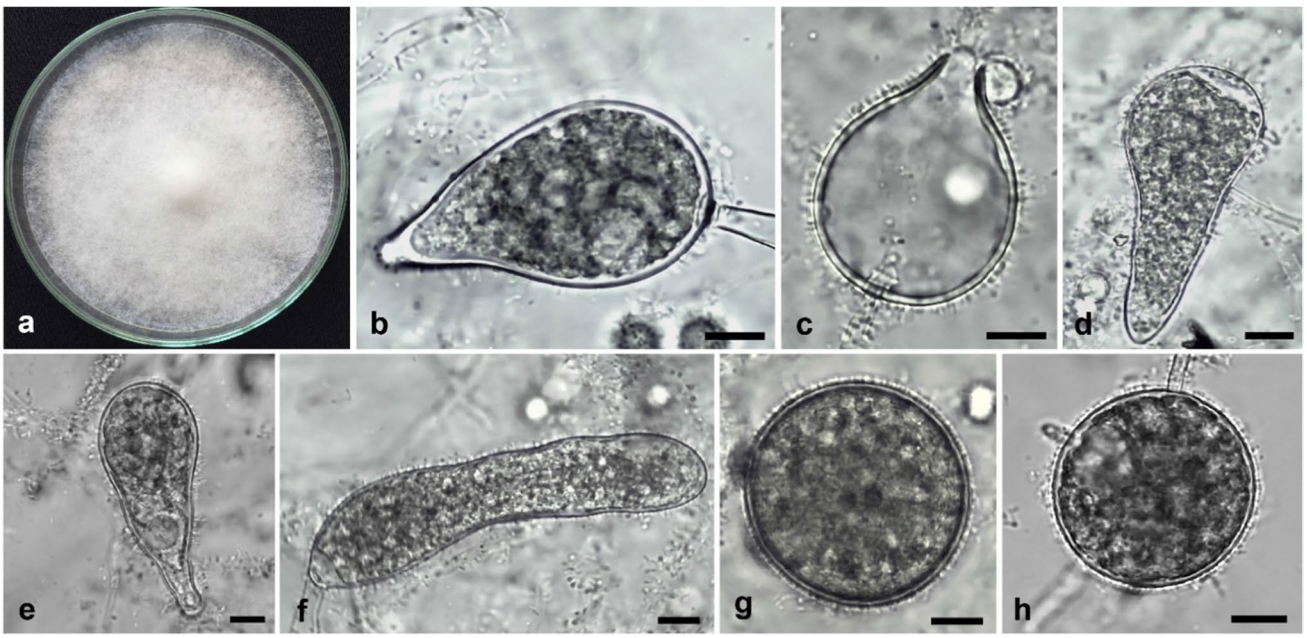

Fig. 7 *Phytophthora nicotianae* PPRI20915. Colony on PDA; b-f. Sporangia; g-h. Chlamydospore; Scale bars = 10 µm

The causal agents of diseased tissues of leaves and roots were re-isolated and the same original oomycetes were obtained. Non-symptom leaves and roots of healthy seedlings in the controls were re-isolated and no oomycetes were obtained. The result indicated that *P. capsici*, *P. nicotianae* and *P. tropicalis* were infected with both leaves and roots of black pepper. While *Phytopythium vexans* infected roots only.

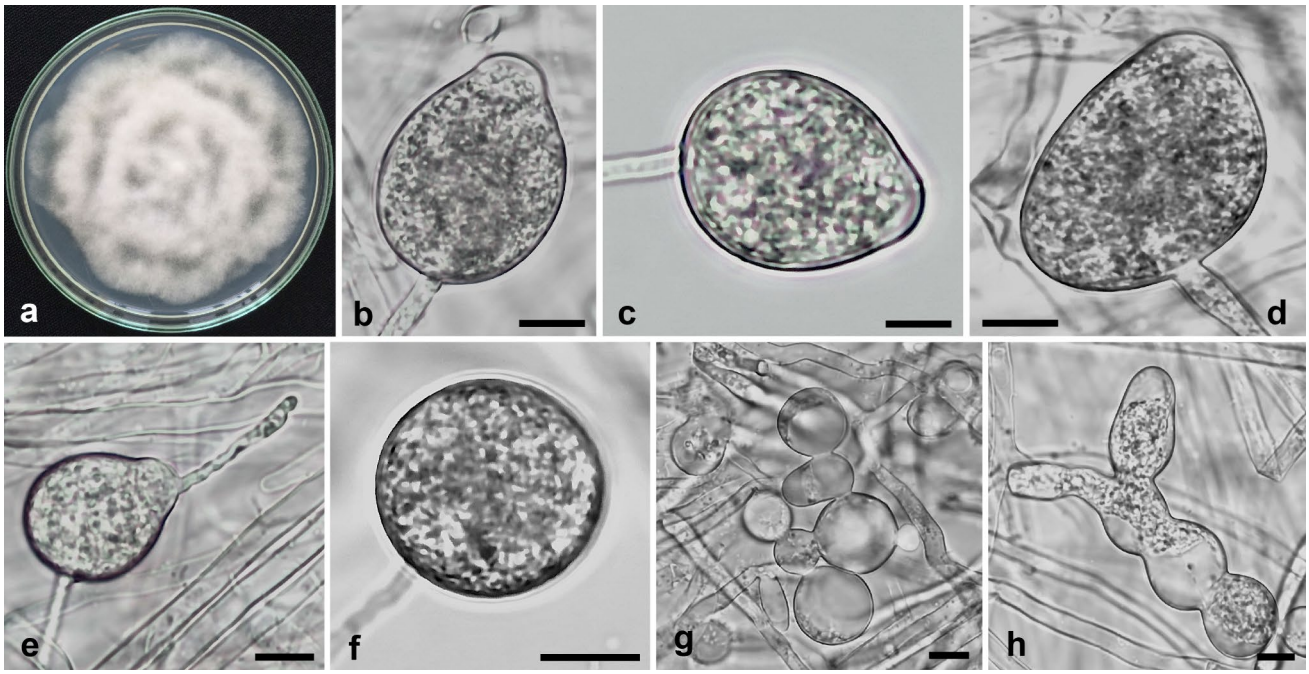

Fig. 8 *Phytophthora parvispora* PPRI21812. a. Colony on PDA; b-e. Sporangia; f. Chlamydospore; g-h. Hyphal swellings; Scale bars = 10 µm

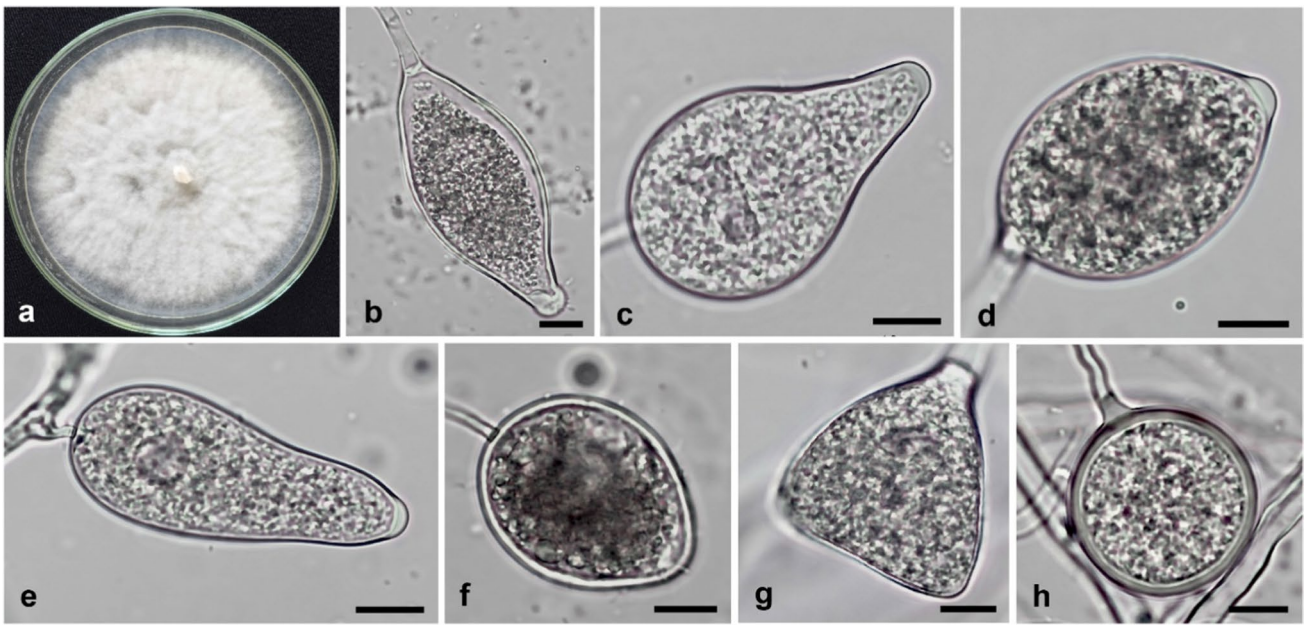

Fig. 9 *Phytophthora tropicalis* PPRI20912. a. Colony on PDA; b-g. Sporangia; h. Chlamydospore; Scale bars = 10 µm

Discussion

Among oomycete isolates obtained from black pepper plantations in five provinces of Vietnam, six *Phytophthora* species (*P. capsici*, *P. cinnamomi*, *P. heveae*, *P. nicotianae*, *P. parvispora* and *P. tropicalis*) and two *Phytopythium* species (*Phytopythium vexans* and *Phytopythium* sp.) were identified based on molecular analyses with the supporting data of morphology. *Phytophthora capsici* has been known as the most important agent of

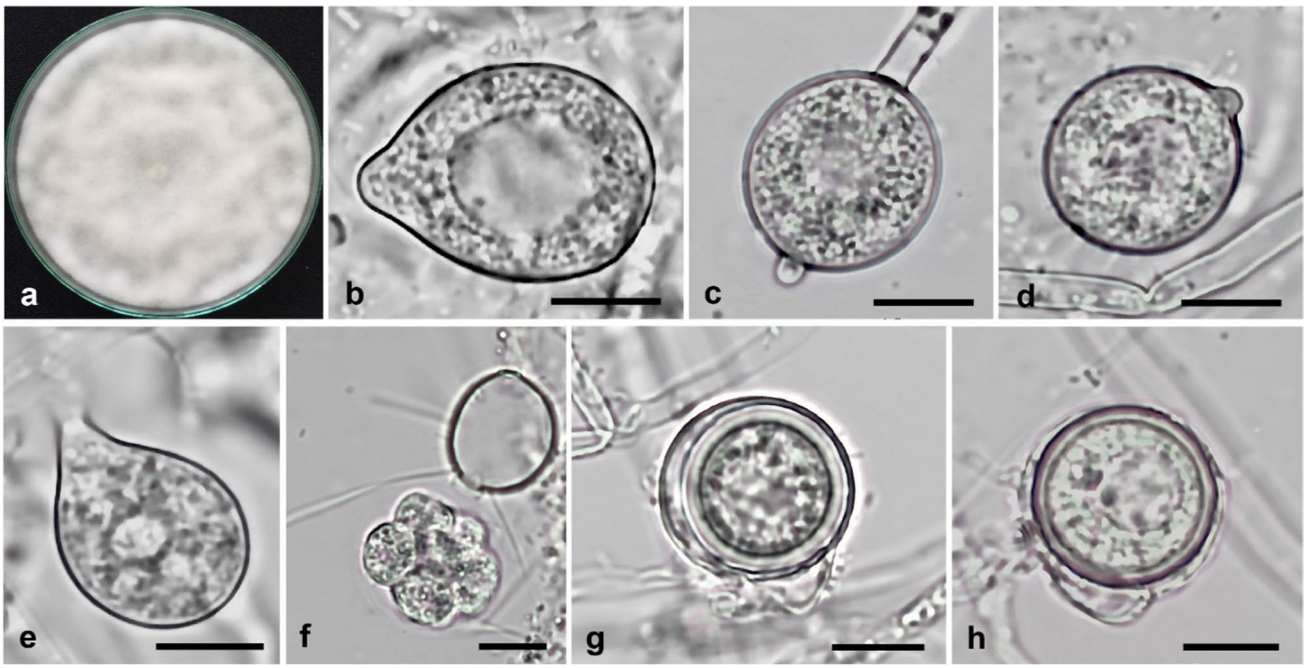

Fig. 10 *Phytopythium vexans* (PPRI2098). a. Colony on PDA; b-f. Sporangia; f. Release of zoospores; g-h. Oogonia with antheridia; Scale bars = 10 µm

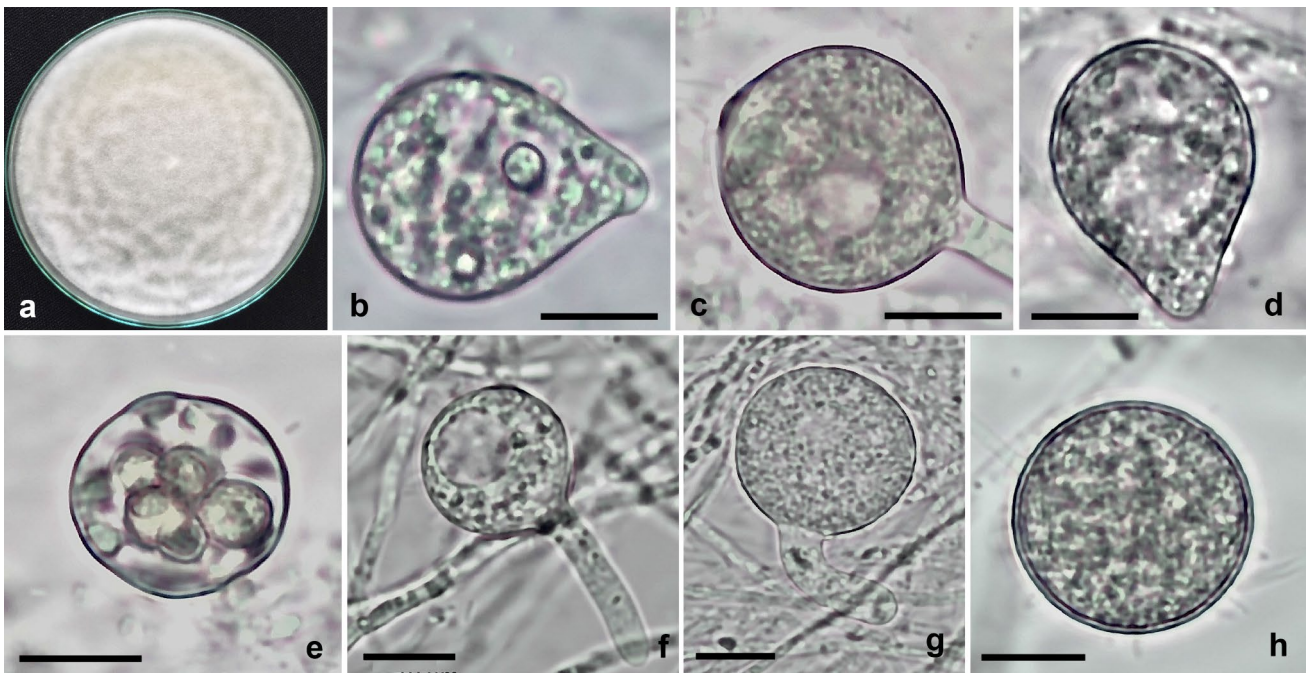

Fig. 11 *Phytopythium* sp. PPRI2097. a. Colony on PDA; b-g. Sporangia; f-g. Formation of long discharge tubes; h. Chlamydospore; Scale bars = 10 µm

quick wilt disease in Vietnam (Truong et al. 2008; 2010; 2012; Nguyen 2015; Quy et al. 2021). However, the results of this study revealed that not only *P. capsici*, but the other species including *P. tropicalis*, *P. nicotianae* and *Phytopythium vexans* also infected black pepper in locations sampled. According to the results of analysed samples,

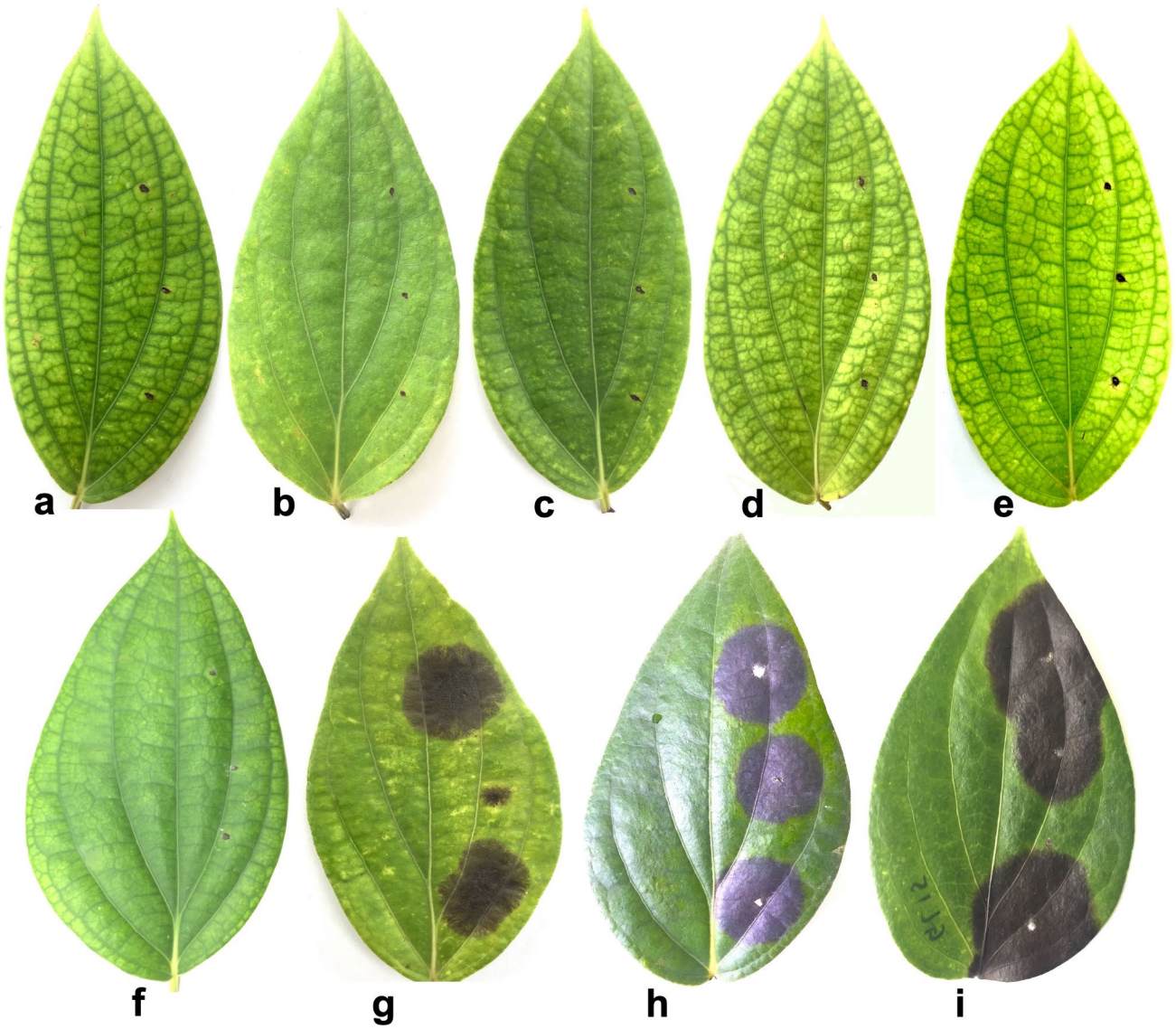

Fig. 12 Pathogenicity test of oomycetes on black pepper leaves. a-f. No disease symptoms, a. Control, b. PPRI20913 (*P. heveae*), c. PPRI2087 (*P. cinnamomi*), d. PPRI21812 (*P. parvispora*), e. PPRI2097 (*Phytopythium* sp.) and f. PPRI2098 (*Phytopythium vexans*); g-i. Disease symptoms, g. PPRI20915 (*P. nicotianae*), h. PPRI20912 (*P. tropicalis*), and i. PPRI20911 (*P. capsici*)

Phytophthora capsici (9 isolates) was the most prevalent pathogenic species, followed by *P. tropicalis* (6 isolates), *Phytopythium vexans* (3 isolates) and *P. nicotianae* (1 isolate). *Phytophthora capsici* was present in all five provinces, in the soil and root of black pepper. Meanwhile, *P. tropicalis* was found only in the Central Highlands (Dak Lak, Dak Nong and Gia Lai provinces), in the soil and root. *Phytopythium vexans* was isolated from the soil and root in Dak Lak and Gia Lai provinces. The other pathogenic species, *P. nicotianae*, was obtained from the soil in Dak Nong province. To investigate the presence and distribution of the identified species more samples need to be collected and analysed.

Phytophthora capsici was first described by Leonian (1922) on chili pepper in New Mexico. The species was a devastating pathogen, infecting the root, crown, stem, leaf and fruit of host plants such as papaya, cacao, rubber, macadamia, black pepper, and numerous species of solanaceous and cucurbitaceous (Erwin et al. 1996; Ristaino and Johnston 1999; Hausbeck and Lamour 2004; Parada-Rojas et al. 2021). While *P. tropicalis,* named by Aragaki and Uchida (2001), was often recorded from woody perennial hosts such as cacao, breadfruit and citrus, and not usually reported on the common host plant of *P. capsici* such as pepper, tomato and cucurbits (Donahoo and Lamour 2008; Farr et al 2023). The different host ranges of these

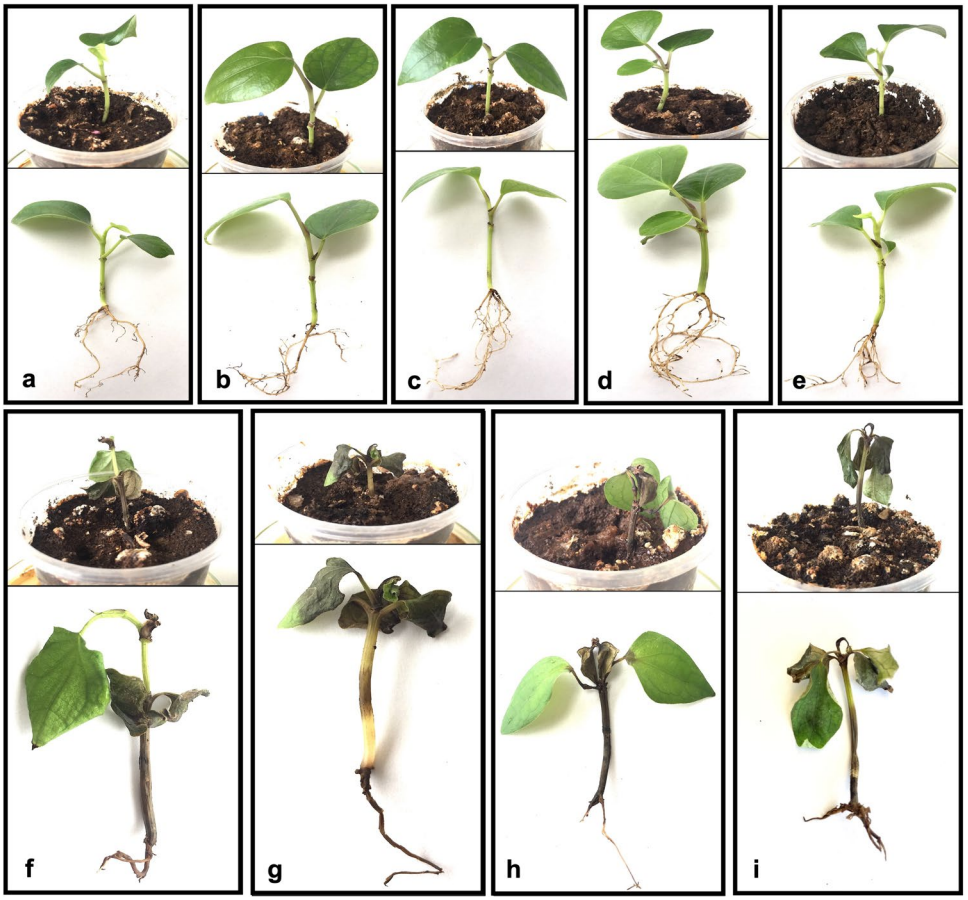

Fig. 13 Pathogenicity test of oomycetes on black pepper seedlings. a-e. No disease symptoms, a. Control, b. PPRI20913 (*P. heveae*), c. PPRI2087 (*P. cinnamomi*), d. PPRI21812 (*P. parvispora*) and e. PPRI2097 (*Phytopythium* sp.); f-i. Disease symptoms, f. PPRI2098 (*Phytopythium vexans*), g. PPRI20915 (*P. nicotianae*), h. PPRI20912 (*P. tropicalis*), and i. PPRI20911 (*P. capsici*)

species may lead to a difference in the epidemiology of the pathogens. Current and previous studies indicate that *P. capsici* and *P. tropicalis* showed significant variation in their morphology, and chlamydospores were present in *P. tropicalis* isolates, while they were absent in *P. capsici* isolates (Bhai et al. 2022). However, hyphal swellings observed in *P. capsici* in this study were highly similar to chlamydospores. Therefore, the morphological characteristics could not be used to separate these two species and they were accurately identified based on multi-locus analysis of ITS, TEF1, and TUB.

Phytopythium vexans and *P. nicotianae* were serious pathogen species and were frequently present in avocado orchards in many countries (Machado et al. 2013; Rodriguez-Padron et al. 2018; Hernández-Pérez et al. 2019). These species also attacked citrus (Benfradj et al. 2017; Wu et al. 2020) and rubber (Zheng and Ward 1998; Zeng et al. 2005). *Phytopythium vexans* was recently reported as a causal agent of durian root rot in the Central Highlands (Thao et al. 2020) and *P. nicotianae* frequently infected citrus plants in Southern Vietnam (Patrizio et al. 2013). The host plants mentioned herein are often cultivated close to, or intercropped with black pepper, and the areas of avocado, durian and citrus are dramatically increasing. This will become a favorable condition for the infection of *P. nicotianae* and *Phytopythium vexans*.

The results of this study suggest that *P. capsici* and *P. tropicalis* could be the prevalent species in black pepper growing areas of Vietnam. *Phytophthora nicotianae* and *Phytopythium vexans* could become widely spread pathogens to black pepper and other host plants in the future. These findings are meaningful information for developing strategies to control the disease. On the other hand, multiple infections of different oomycete species and the presence of non-pathogenic species to black pepper (*P. cinnamomi*, *P. heveae*, *P. parvispora* and *Phytopythium* sp.) in their community are still unclear.

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Data availability The sequence data obtained in this study were deposited in GenBank with accession numbers listed in Table 1.

Declarations

Conflicts of interest The authors declare that there is no conflict of interest.

References

Abad ZG, Burgess T, Redford AJ, Bienapfl JC, Mathew R, Srivastava SK, Jennings KC (2023) *IDphy*: An international online resource for molecular and morphological identification of *Phytophthora*. Plant Disease 107:987–998

Anandaraj M (2000) Diseases of black pepper. Black pepper (*Piper nigrum*). Harwood Academic Publishers, Amsterdam, The Netherlands, pp 239–267

Anandaraj M, Abraham J, Balakrishnan R (1989) Crop loss due to foot rot disease of black pepper. Indian Phytopathology 42:473–476

Aragaki M, Uchida JY (2001) Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. Mycologia 93:137–145

Azahari DH, Purba HJ, Erwidodo Darwis V, Dabukke FBM, Hestina J, Yusuf ES (2021) The competitiveness of Indonesia's pepper export and its challenges. IOP Conference Series Earth and Environmental Science 892:012074

Benfradj N, Migliorini D, Luchi N, Santini A, Boughalleb-M'Hamdi N (2017) Occurrence of *Pythium* and *Phytopythium* species isolated from citrus trees infected with gummosis disease in Tunisia. Archives Phytopathology and Plant Protection 50:286–302

Bhai RS, Jeevalatha A, Biju CN, Vinitha KB, Cissin J, Rosana OB, Fayad A, Praveena R, Anandaraj M, Eapen SJ (2022) Sympatric occurrence of sibling *Phytophthora* species associated with foot rot disease of black pepper in India. Brazilian Journal of Microbiology 53:801–818

Bowers JH, Martin FN, Tooley PW, Luz EDMN (2007) Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. Phytopathology 97:492–503

de Cock AWAM, Lodhi AM, Rintoul TL, Bala K, Robideau GP, Abad ZG, Coffey MD, Shahzad S, Lévesque CA (2015) *Phytopythium*: molecular phylogeny and systematics. Persoonia 34:25–39

Donahoo RS, Lamour KH (2008) Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. Mycologia 100:911–920

Drenth A, Guest DI (2004) Diversity and management of *Phytophthora* in Southeast Asia. ACIAR monograph no. 114:1238

Dung PN, Cuong HV, Tuat NV, Matsumoto M (2014) Analysis of internal transcribed spacer (ITS) region of *Phytophthora tropicalis* causing quick wilt disease of black pepper in Vietnam. Archives of Phytopathology and Plant Protection 47:842851

Erwin DC, Ribeiro OK (1996) Phytophthora diseases worldwide. The American Phytopathological Society, St. Paul, Minnesota

Farhana MDSN, Bivi MR, Khairulmazmi A, Wong SK, Sariah M (2013) Morphological and molecular characterization of *Phytophthora capsici*, the causal agent of foot rot disease of black pepper in Sarawak, Malaysia. International Journal of Agriculture & Biology 15:1083–1090

Farr DF, Rossman AY. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Available at: https://nt.ars-grin.gov/fungaldatabases/. Accessed on May 20, 2023

General statistics office (2021) Statistical yearbook of Viet Nam 2021. Statistical publishing house, Hanoi

Hausbeck MK, Lamour KH (2004) *Phytophthora capsici* on vegetable crops: research progress and management challenges. Plant Disease 88:1292–1303

Hernández-Pérez A, Cerna-Chávez E, Delgado-Ortiz JC, Beltrán-Beache M, Tapia-Vargas LM, Ochoa-Fuentes YM (2019) First report of *Phytopythium vexans* causing the "avocado sadness" in Michoacan, Mexico. Revista Internacional de Botánica Experimental 88:11–13

Jeevalatha A, Biju CN, Bhai RS (2021) Ypt1 gene-based recombinase polymerase amplification assay for *Phytophthora capsici* and *P. tropicalis* detection in black pepper. European Journal of Plant Pathology 159:863–875

Jibat M, Alo S (2021) Characterization of *Phytophthora capsici* foot rot disease of black pepper in Ethiopia. Journal of Plant Pathology & Microbiology 12:542

Kifelew H, Adugna G (2018) Study on *Phytophthora capsici* cause of foot rot disease on black pepper (Piper Nigrum. L.), Tepi, Ethiopia. International Journal of Research Studies in Agricultural Sciences (IJRSAS) 4:1–7

Kong JC, Thanh TAV, Yeo FKS, Kueh KH (2022) Characterisation of *Phytophthora capsici* causing foot rot of black pepper (*Piper nigrum* L.) in Julau, Sarawak. Archives of Phytopathology and Plant Protection 55:1686–1712

Kroon LP, Bakker FT, van den Bosch GB, Bonants PJ, Flier WG (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. Fungal Genetics and Biology 41:766–82

Leonian LH (1922) Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopathology 12:401–408

Liu PSW (1977) Diseases caused by *Phytophthora* and *Pythium* in Sabah. Department of Agriculture Sabah, Malaysia, Malaysia

Machado M, Collazo C, Peña M, Coto O, López MO (2013) First report of root rot caused by *Phytophthora nicotianae* in avocado trees (*Persea americana*) in Cuba. New Disease Reports 28:9

Matsuda A, Moya Franco JD, Gonzalez JL, Watanabe T (1998) Root rots of black pepper caused by *Pythium splendens* in the Dominican Republic. Annals of the Phytopathological Society of Japan 64:303306

Muller HRA (1937) Het *Phytophthora*-voetrot van peper (*Piper nigrum* L.) in Nederlandsch-Indie. Landsdrukkerij, Batavia

Nambiar KKN, Sarma YR (1977) Wilt diseases of black pepper. Journal of Plantation Crops 5:92–103

Nguyen VL (2015) Spread of *Phytophthora capsici* in black pepper (*Piper nigrum*) in Vietnam. Engineering 7:506–513

Nguyet NTA, Cham LTM, Trang TT, Xo DH (2018) Evaluation of *Trichoderma* spp. and *Bacillus subtilis* against *Pythium vexans* causes root rot on black pepper. Ho Chi Minh City Open University Journal of Science 13:140–151

Parada-Rojas CH, Granke LL, Naegele RP, Hansen Z, Hausbeck MK, Kousik S, McGrath MT, Smart C, Quesada-Ocampo LM (2021) A diagnostic guide of *Phytophthora capsici* infecting vegetable crops. Plant Health Progress 22:404–414

Patrizio AD, Prigigallo MI, Cacciola SO, Olsson CH, Faedda R, Ramstedt M, Wright SA, Chau NL, Hoa N, Pane A, Schena L, Lio GM (2013) Identification of *Phytophthora* species infecting citrus in Vietnam. Journal of Plant Pathology 95(S4):41

Quy PT, Hai NTT, My TTA, Bui TQ, Tran Hoa TT, Phu NV, Loan HTP, Nhung NTA (2021) A theoretical study on inhibitability of silver(I) N-heterocyclic carbene and dimer silver(I) N-heterocyclic carbene complexes against *Phytophthora capsici* and *Fusarium sporotrichioides* in *Piper nigrum* L. Vietnam Journal of Chemistry 59:405–415

Rathore T, Swathi VLA (2020) Black pepper export scenario in India (2001–2017). Journal of Xi'an University of Architecture & Technology 12:4153–4164

Ristaino JB, Johnston SA (1999) Ecologically based approaches to management of Phytophthora blight on bell pepper. Plant Disease 83:1080–1089

Rodriguez-Padron C, Siverio F, Perez-Sierra A, Rodriguez A (2018) Isolation and pathogenicity of *Phytophthora* species and *Phytopythium vexans* recovered from avocado orchards in the Canary Islands, including *Phytophthora niederhauserii* as a new pathogen of avocado. Phytopathologia Mediterranea 57:89–106

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313

Subila KP, Bhai RS (2020) *Pythium deliense*, a pathogen causing yellowing and wilt of black pepper in India. New disease reports 42:6

Thao LD, Hien LT, Liem NV, Thanh HM, Khanh TN, Binh VTP, Trang TTT, Anh PT, Tu TT (2020) First report of *Phytopythium vexans* causing root rot disease on durian in Vietnam. New Disease Reports 41:2

Thao LD, Zaw M, Matsumoto M (2018) *Diaporthe* species complex occurring on *Asparagus kiusianus* in Japan. Journal of Plant Pathology 101:161–167

Thuy TTT, Yen NT, Tuyet NTA, Te LL, Waele DD (2012) Plant-parasitic nematodes and yellowing of leaves associated with black pepper plants in Vietnam. Archives of Phytopathology and Plant Protection 45:1183–1200

Trinh THT, Wang SL, Nguyen VB, Tran MD, Doan CT, Vo TPK, Huynh VQ, Nguyen AD (2019) A potent antifungal rhizobacteria *Bacillus velezensis* RB.DS29 isolated from black pepper (*Piper nigrum* L.). Research on Chemical Intermediates 45:5309–5323

Truong NV, Burgess LW, Liew ECY (2008) Prevalence and aetiology of Phytophthora foot rot of black pepper in Vietnam. Australasian Plant Pathology 37:431–442

Truong NV, Burgess LW, Liew ECY (2012) Cross-infectivity and genetic variation of *Phytophthora capsici* isolates from chilli and black pepper in Vietnam. Australasian Plant Pathology 41:439–447

Truong NV, Liew ECY, Burgess LW (2010) Characterisation of *Phytophthora capsici* isolates from black pepper in Vietnam. Fungal Biology 114:160–170

Tutte J (1969) Plant pathological methods: Fungi and bacteria. Burgess publishing company, USA

White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322

Wu J, Handique U, Graham J, Johnson E (2020) *Phytophthora nicotianae* infection of citrus leaves and host defense activation compared to root infection. Phytopathology 110:1437–1448

Yogesh MS, Mokshapathy S (2013) Production and export performance of black pepper. International Journal of Humanities and Social Science Invention 2:36–44

Zeng HC, Ho HH, Zheng FC (2005) *Pythium vexans* causing patch canker of rubber trees on Hainan Island, China. Mycopathologia 159:601–606

Zentmyer GA, Kaosiri T, Idosu G (1977) Taxonomic variants in the *Phytophthora palmivora* complex. Transactions of the British Mycological Society 69:329–332

Zheng FC, Ward E (1998) Variation within and between *Phytophthora* species from rubber and citrus trees in China, determined by polymerase chain reaction using RAPDs. Journal of Phytopathology 146:103–109