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Morphological and molecular examination of *Phytophthora* sp. to identify the causal agent of patch canker in durian (*Durio zibethinus* Murr.) in Can Tho city

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Abstract The examination and identification of *Phytophthora* species causing patch canker isolated from the Ri6 durian variety in Can Tho City were conducted. Six fungal isolates from the durian orchards were identified by their morphological and molecular phylogeny and proved pathogenicity. All isolates were proved to be *Phytophthora cinnamomi* which formed stellate or patellate colonies with hyphal swelling on PDA and CMA media. They produced obpyriform or ellipsoidal sporangia, non-papilla, and globes chlamydospores. The optimum temperature for their growth was 25 ℃. The results of Koch's postulate showed that the TD1 isolate revealed the largest lesion length with patch canker disease. *Phytophthora cinnamomi* is reported to be the first time to cause patch canker disease of durian in Can Tho City, Vietnam.

Keywords: Durian, Koch, Patch canker, *Phytophthora*

Introduction

Phytophthora species are widely distributed throughout various regions in the world and exhibit a diverse range of host plants including agricultural, industrial, and horticultural crops. Currently, over 330 species have been identified as plant pathogens within this species. Among them, *Phytophthora infestans* is known to cause potato disease, *Phytophthora parasitica* var. *nicotianae* affects tobacco plants, *Phytophthora palmivora* infects durian trees, and *Phytophthora capsici* is responsible for the diseases affecting pepper, cocoa, and chili crops. These species have been extensively studied and are considered significant threats to global agriculture and forestry industries (Drenth and Sendall, 2001). *Phytophthora* spp. usually exist in the soil as mycelia and oospores. The oospores produced by *Phytophthora* species exhibit a high level of resistance and thick walls which render them impervious to

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harsh environmental conditions. This enables the oospores to persist in the absence of a host for prolonged periods. Reproduction in *Phytophthora* is characterized by the formation of motile zoospores that possess both front and rear flagella, which facilitate movement through obstructions. Upon submergence of the roots, these spores are released and adeptly swim towards the roots, successfully penetrating and initiating infection, ultimately leading to the development of disease. In addition to penetrating roots, *Phytophthora* species exhibit the ability to spread rapidly across the garden by utilizing water currents as a means of transportation. This characteristic of *Phytophthora* sp. renders control measures challenging, resulting in severe economic losses for farmers. Further exacerbating this issue is the propensity for *Phytophthora* to cause multiple diseases on a single host. Durian plants are vulnerable to a wide range of *Phytophthora*-induced diseases, including dieback, leaf blight, root rot, patch/stem cankers, trunk cankers, as well as preharvest and postharvest fruit rots (Lim, 1990). In Vietnam, *Phytophthora*-induced fruit and root rot remain the foremost issues impacting durian crops, with stem canker regarded as a relatively minor affliction (Guest *et al*., 2004). The stem canker and leaf blight are more widely dispersed throughout Vietnam than fruit rot (Tri, 1998). Early symptoms of stem cankers in durian plants are identified by the presence of wet lesions on the bark surface, which are typically accompanied by discoloration and exude reddish-brown, resinous material. These lesions can expand leading to necrosis and impairment of water and nutrient transport to connecting branches, eventually resulting in the death of the plant (Lim, 1990). Symptoms of *Phytophthora*-induced diseases in durian plants are often concentrated on the lower branches and the base of the main trunk (Sivapalan *et al*., 1997). Stem canker has inflicted significant damage on durian crops in Soc Trang province, Vietnam, resulting in the death of approximately 50% of affected trees (Akinsanmi and Drenth, 2009). Selecting appropriate gene sequences of *Phytophthora* is a crucial step in identifying the agents responsible for durian diseases. Typically, eukaryotic genomes feature tandemly repeated rRNA genes that span hundreds to thousands of copies, comprising a small subunit (18S), a large subunit (28S), and a 5.8S region. These spacer regions are transcribed and not translated. They are more variable than the actual rRNA genes. Because these spacer regions diverge rapidly, they provide useful information in identifying a broad range of *Phytophthora* species (Grünwald *et al*., 2011). Applying the ITS sequence regions in *Phytophthora* species, ITS6 and ITS4 primers are shown very well in many cases (Cooke *et al.,* 2000). The research focused on the identification of *Phytophthora* species which causes stem canker of durians in Phong Dien District, Can Tho City.

Materials and methods

Fungal isolation and culture

The isolates of *Phytophthora* were collected from the roots of durian plants in the Phong Dien District, Can Tho City (Table 1). The samples were obtained from the distinguished patch canker spot disease of the durian outer bark (Figure 1). The root and crown were also selected and washed thoroughly under tap water. Root and crown lesions were cut into small pieces. The superficial tissues were disinfected in 70% ethanol for 5 seconds. After that, each sample was disinfected with 1% sodium hypochlorite solution for 5 minutes and then washed twice in sterile distilled water (Dhingra and Sinclair, 2017). The sample was then dried on sterile paper. About 4-10 mm crown and root pieces at the junction of the healthy and necrotic tissue were placed on a P10ARP selective medium with a supply of antibiotics such as 10 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin, and 100 mg pentachloronitrobenzene (Kannwischer and Mitchell, 1978). The plates were incubated for three days in darkness at 24℃ (Erwin and Ribeiro, 1996). The pure cultures of the fungus were obtained from a hyphal tip.

Table 1. Isolates of *Phytophthora* sp. obtained from Ri6 durian variety (*Durio zibethinus* Murr.) in Phong Dien District, Can Tho City, Viet Nam

No.	Sample	Location	GPS coordinates	
			Latitude	Longitude
1	GX	Giai Xuan Village	10.0424475	105.6802958
2	NA	Nhon Ai Village	9.9967467	105.6629426
3	TD1	Phong Dien Town	10.012424	105.6511444
4	TD2	Phong Dien Town	10.0106406	105.6520841
5	TT	Tan Thoi Village	10.0418822	105.6437519
6	MK	My Khanh Village	10.0110742	105.7060519

Morphological and cultural characterization

To examine fungal growth, each isolate was transferred onto potato dextrose agar (PDA) medium from P10ARP selective medium which consisted of 10 mg pimaricin, 250 mg ampicillin, 10 mg rifampicin, and 100 mg pentachloronitrobenzene (Drenth and Guest, 2004). For each isolate, 20 ml of PDA medium was transferred with a 7 mm disk cut from mycelial pieces taken from an actively growing colony on P10ARP and incubated for seven days at 24℃. Each isolate was replicated four times and colony patterns were observed and recorded in seven days following the descriptions of Erwin and Ribeiro (1996).

Figure 1. Durian plants with symptoms of reddish-brown discoloration of the outer bark

To induce sporangia production, 5-mm-diameter fungal disks were cut from the advancing margin of a colony grown on a PDA medium. These disks were then floated on 10 ml of 1% non-sterile soil extract solution (w/v) and incubated for 4-5 days at 24 ℃ under continuous fluorescent light as described by Erwin and Ribeiro (1996). After 72 hours, hyphal portions were mounted in 3% KOH and observed under a microscope at 400X magnification using an Olympus CH-2 microscope. Isolates were measured in shape, length (L), and width (W) of 30 sporangia. The L/W was calculated. Additionally, sporangiophore width, chlamydospore diameter, and swelling hypha were also measured.

The effects of temperature on the growth of fungi were examined *in vitro* on 90 mm CMA Petri dishes at 20, 25, 30, and 35 ℃ (±0.5 ℃) in the dark. To determine the effects of temperature on colony growth, agar plugs with a diameter of 5 mm were extracted from 5-day-old colonies and placed topside down in the middle of fresh medium plates. Each isolate was repeated for five replicates. The colonies were measured twice in perpendicular directions after 96 hours, and the average diameter was determined after subtracting the original plug diameter.

Pathogenicity test

Durian seedling preparation: Durian seeds (Ri6 variety) with a length of 2-4 cm and a diameter of about 1-2 cm, are planted in rice husk ash. The seven days seedings after planting were moved into the potting soil. After 150 days of planting which was approximately 50-60 cm high, artificial inoculation was carried out. The selected seedlings for inoculation were asymptomatic plants, relatively homogeneous in plant height.

Prepare fungal inoculate: Phytophthora sp. were transferred from pure culture from the primary specimen in the PDA medium and placed in dark light alternately for 6-7 days.

Artificial inoculation on durian stems: The experiment was conducted according to the method of Tri *et al.* (2016) with modifications to suit the conditions of durian growth. Durian seedlings are planted in a pot containing sterilized soil. Preparation of fungal inoculate was conducted when the seedlings were planted for five months. All isolates with appropriate descriptive biological characteristics of *Phytophthora* were used. Ten plants were used for inoculation and ten plants were used as control. Artificial inoculation on durian stems was carried out by making wounds on the trunk. A cut of 3-4 mm long in the bark of each tree was created with a scalpel. A segment of medium 3 mm in diameter from the edge of the fungal colony was placed over the wound and covered with the dissected stem, then carefully covered with Parafilm®. The same procedure was performed in the control and the pathogen-free medium was used. Data were recorded as symptoms, time of disease appearance, the color of lesions, and lengths of lesions.

Molecular characterization

To extract genomic DNA from the oomycete, the fungus was first grown on PDA medium for five days at 25℃. Genomic DNA extraction was carried out using the method of Zelaya-Molina *et al*. (2011) with some modifications. Briefly, a 5 mm block of mycelia was cut and placed in 1.5 ml Eppendorf tubes. Lysis buffer (100 mM Tris HCl, 10 mM ethylenediaminetetraacetic acid [EDTA], 1M KCl; pH 8.0) was added to the tube, followed by gentle mixing with a pestle. The mixture was then treated with chloroform and isoamyl alcohol (24:1, v/v), vortexed until the two phases formed an emulsion, and centrifuged for 10 min at 12000 rpm. The resulting supernatant was transferred to a 1.5 ml tube and genomic DNA was precipitated by adding 1 ml of isopropanol, inverting the tube five times, and holding it at -20℃ for 15 min. The DNA pellet was then formed by centrifugation for 10 min at 12000 rpm, washed with 1 ml of 70% ethanol, air-dried for 2 hours, and resuspended in 30 µl of TE buffer (100 mM Tris/HCl, pH 8.0, 10 mM EDTA). The extracted DNA was stored at -20℃ for further analysis. Three independent DNA extractions were performed for each isolate.

Phytophthora species were identified by analyzing the ITS regions of ribosomal DNA (rDNA). The PCR method was used to amplify DNA using forward primer ITS6 (5'-GAAGGTGAAGTCGTAACAAGG-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction was conducted in a 25 µL reaction mix containing PCR buffer (1x), dNTP mix (0.2 mM), $MgCl_2$ (1.5 mM), forward and reverse primers (0.5 mM each), Taq DNA Polymerase (1 U) and 100 ng of DNA. The thermocycler conditions were 94℃

for 3 min, followed by 35 cycles of 94 ℃ for 30 seconds, 55 ℃ for 30 seconds, and 72 ℃ for 30 seconds, and then 72 ℃ for 10 min. The method used for PCR was based on Cooke *et al*. (2000).

Data analysis

The size of the genotypic DNA bands was calculated using Gel Analyzer software (Istvan, 2010). Using Excel software processed raw data. Statistical analysis of temperature parameters was SPSS 21 software.

Results

Morphological and physiological characterization

The morphology of fungal samples isolated in the Phong Dien District is shown in Figure 2. Based on the developmental morphology of fungal isolates and characteristics of the morphological structure, they were grouped into two distinct patterns: stellate (TT, and TD2) and patellae (TD1, MK, GX, and NA).

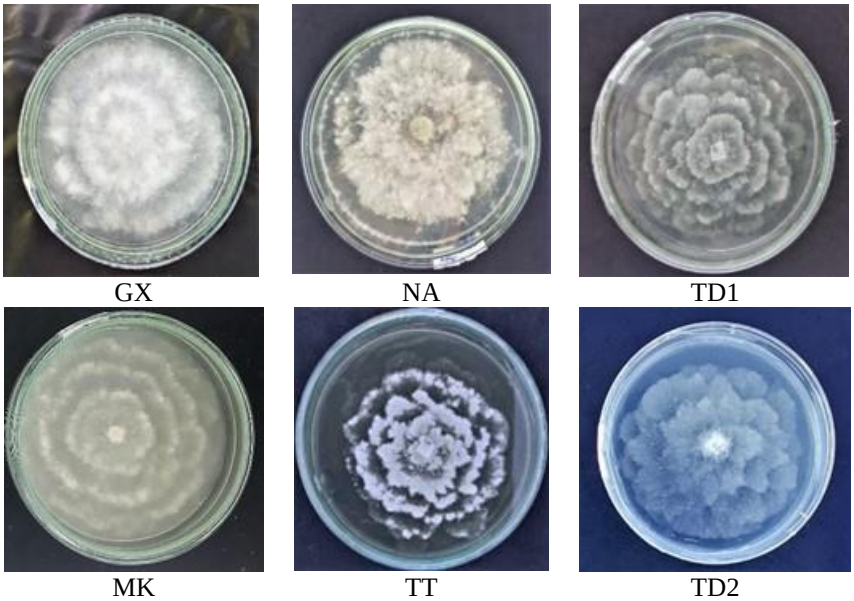

Figure 2. *Phytophthora* sp. isolates exhibiting colony morphology on PDA medium

Morphometric characteristics of 6 isolates of *Phytophthora* sp. isolated from durian were shown in Table 2. The isolated fungal samples had the length-to-width ratio of the sporangia in the range of 1.65-1.78. The L/W ratio of the

TT sample was the highest among examined samples (1.78±0.40), while the one with the lowest L/W ratio GX was 1.65±0.17. The remaining samples had the L/W ratio ranging from 1.67±0.11 to 1.76±0.12. All the isolated fungal samples had sporangium lengths greater than 50 µm, with the average ranging from 70.08±8.11 µm to 90.80±20.68 µm.

Table 2. Morphometric characteristics of 6 isolates of *Phytophthora* sp. isolated from durian plants with symptoms of patch canker

Isolate	Sporangium size			Diameter of chlamydospores (µm)
	Length (µm)	Width (µm)	L/W	
GX	82.73±23.86^{ab}	50.04±13.43^{ab}	1.65±0.11^{b}	36.37±5.11^{b}
NA	70.08±8.11^{c}	39.89±4.41^{c}	1.76±0.12^{ab}	36.08±7.29^{a}
TD1	90.80±20.68^{a}	55.58±14.80^{a}	1.65±0.17^{b}	34.52±5.57^{a}
TD2	76.50±16.18^{bc}	45.57±9.41^{bc}	1.68±0.13^{ab}	36.53±5.39^{a}
TT	71.07±12.55^{c}	40.84±8.14^{c}	1.78±0.40^{b}	35.22±6.94^{c}
MK	89.06±24.18^{a}	53.28±13.60^{a}	1.67±0.11^{b}	30.17±2.95^{a}
F	*	*	*	*
CV (%)	23.19	23.68	11.91	16.64

Mean values in the same column followed by the same lowercase letter are not different at the 5% level of significance by Duncan's test; "*" Statistically significant difference 5%; Mean ± SD.

The morphological characteristics of fungi in PDA and CMA media showed that their coenocytic hyphae which belong to *Pythiaceae*. The patellae mycelium was recognized with swollen hyphae and rounded chlamydospores with a thin wall forming clusters. Many obpyriform and ellipsoid zoosporangia were observed. They have apical pores and wide pores without papilla formation. The thin, long, and no branching sporangiophores were observed. The oospore was not found (Figure 3 and Table 2).

Table 3. Characteristics of papillae, shape, and proliferation of six isolates

Isolate	Papillation	Shape of sporangia	Proliferation
GX	Non-papillate	Obpyriform	Internal & extended proliferation
NA	Non-papillate	Obpyriform	Internal & extended proliferation
TD1	Non-papillate	Ellipsoid	Internal & extended proliferation
TD2	Non-papillate	Ellipsoid	Internal & extended proliferation
TT	Non-papillate	Ellipsoid	Internal & extended proliferation
MK	Non-papillate	Ellipsoid	Internal & extended proliferation

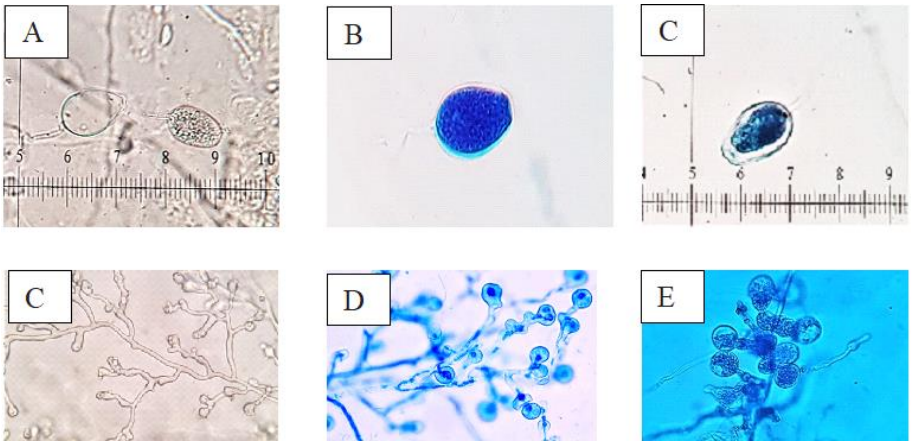

Figure 3. Morphological feature dyed with lactophenol cotton blue of *Phytophthora* sp. isolated from durian: Obpyriform to ellipsoids sporangia without papillae with long pedicels (A-C), Coralloid type mycelium with Hyphal swellings (D-E), chlamydospores (F)

Table 4. Temperature responses of six isolates of *Phytophthora* sp.

Isolate (A)	Diameter of *Phytophthora* sp. in different temperature levels (B)				Average (A)
	20 ℃	25 ℃	30 ℃	35 ℃	
GX	3.20 ±0.82^{fg}	8.56 ±0.09^{a}	3.23 ±0.45^{fg}	0.74 ±0.14^{j}	**3.93 ±2.93^{D}**
NA	4.18 ±0.33^{e}	7.94 ±0.11^{bc}	3.17 ±0.19fg	1.60 ±0.85^{h}	**4.22 ±2.41^{ABC}**
TD1	4.94 ±0.30^{d}	8.16 ±0.32^{ab}	3.52 ±0.25^{g}	0.90 ±0.25^{j}	**4.38 ±2.67^{A}**
TD2	4.27 ±0.57^{e}	7.73 ±0.75^{bc}	3.39 ±0.34^{fg}	1.09 ±0.35^{j}	**4.12 ±2.47^{BCD}**
TT	4.01 ±0.54^{e}	7.53 ±0.26^{c}	3.36 ±0.20^{fg}	1.13 ±0.14^{ij}	**4.01 ±2.35^{CD}**
MK	4.30 ±0.93^{e}	8.47 ±0.20^{a}	2.99 ±0.25^{g}	1.54 ±0.31^{hi}	**4.33 ±2.66^{AB}**
Average (B)	**4.15 ±0.79^{B}**	**8.06 ±0.51^{A}**	**3.28 ±0.33^{C}**	**1.17 ±0.51^{D}**	
	$F_{(A)}$*; $F_{(B)}$*; $F_{(AXB)}$*				
	CV = 10.63 (%)				

Mean values in the same column and row followed by the same lowercase letter are not different at the 5% level of significance by Duncan's test; Mean values in the same column or row followed by the same capital letter are not different at the 5% level of significance by Duncan's test; "*" Statistically significant difference 5%; Mean ± SD.

The colony diameters of 6 fungal isolates at four temperatures are presented in Table 4. The morphology of the colony was observed at temperatures of 20 ℃ and 30 ℃. After five days of incubation, the mycelia grew closer to the agar surface and thinner at 25 ℃; white spongy mycelia with rosaceous, chrysanthemum, or stellate shape. When the temperature reached 35 ℃, the mycelia did not grow. The sample

with the lowest diameter of mycelia was GX, with an average of 3.93±2.93 cm. The remaining fungal isolates had diameters ranging from 4.01±2.35 cm to 4.38±2.67 cm. Observation of the colonies on the fourth day after incubation showed that at 25 ℃, the fungal isolates had the largest diameter of 8.47±0.49 cm. In contrast, at 35 ℃, the colony diameters of the fungal isolates were the smallest, only 1.24±2.54 cm. The average diameters of all isolates at 20 ℃ and 30 ℃ were 4.20±0.78 cm and 3.25±0.34 cm, respectively. Thus, the optimum temperature range for the growth of all isolates was 20-30 ℃, and the most appropriate one was 25 ℃.

The fungal isolates were characterized by morphological features such as length, width, chlamydospore, papillate formation, and optimal temperature. The classification was based on Ho's classification key, and the results showed that the isolates had varied degrees of similarity to different *Phytophthora* species. Specifically, the isolates were found to be 100% similar to *P. cinnamomi*, 83.33% similar to *P. palmivora*, and 50% similar to *P. botryose* (Table 5).

Table 5. Comparison of fungal isolates from Phong Dien District to Ho's classification key (1981)

Isolates	**Criteria**	***P. cinnamomi***	***P. palmivora***	***P. botryosa***
GX, NA, TD1, TD2, TT, MK	Length	1	1	0
	Width	1	1	0
	L/W	1	1	1
	Chlamydospores	1	1	1
	Non-papillate	1	0	0
	Temperature	1	1	1
Total		6	5	3
Similarity coefficient (%)		**100.00**	**83.33**	**50.00**

"1" represents the character that matches Ho's classification key and "0" represents the character that does not match Ho's classification key.

Koch's postulates

The obtained wound length had a significant difference at 5% level (Table 6 and Figure 4). Results on the stem showed that the lesion was dark brown that expanded on the cortical tissue where the artificial wound was infected ten days after inoculation.

The big wound lengths were examined on GX and TD1 isolates. Their wound lengths were 14.04±5.13 cm and 16.61±4.66 cm. The other isolates with smaller wound lengths included NA, TD2, and MK. Their wound lengths were 10.09 cm to 11.08 cm. The fungus was re-isolated from the artificially inoculated wound and found to be the same isolate of *Phytophthora* sp.

Table 6. The wound length of artificial inoculation of fungal isolates

No.	Isolate	Wound length (cm)
1	GX	14.04 ± 5.13^{ab}
2	NA	11.08 ± 4.44^{b}
3	TD1	16.61 ± 4.66^{a}
4	TD2	10.89 ± 5.13^{b}
5	TT	11.01 ± 4.79^{b}
6	MK	10.09 ± 3.15^{b}
7	Control	-
F		*****
CV (%)		**37.66**

Mean values in the same column followed by the same lowercase letter are not significantly different at the 5% level by Duncan's test; "-" not detect the wound growth "*" Statistically significant difference at 5% level; Mean ± SD.

Figure 4. Koch-postulate assays: durian stems were detached after 7 day-incubation, (A) Control - using sterilized water (B) using spore of TD1 isolate

Molecular identification

The DNA band size was calculated using GelAnalyzer software. The results of DNA sequence electrophoresis of the ITS gene region using primers ITS6 and ITS4 of six fungal isolates are shown in Figure 5 with sizes from 916-938 bp.

The maximum likelihood phylogenetic tree was constructed from the consensus sequence of six isolates, and their six corresponding species were *Phytophthora* sp. which *Pythium aphanidermatum* and *Pythium ultimum* are compared as outgroups (Figure 6). These isolates were identified as *Phytophthora* sp., all joined to the branch including *Phytophthora cinnamomi.*

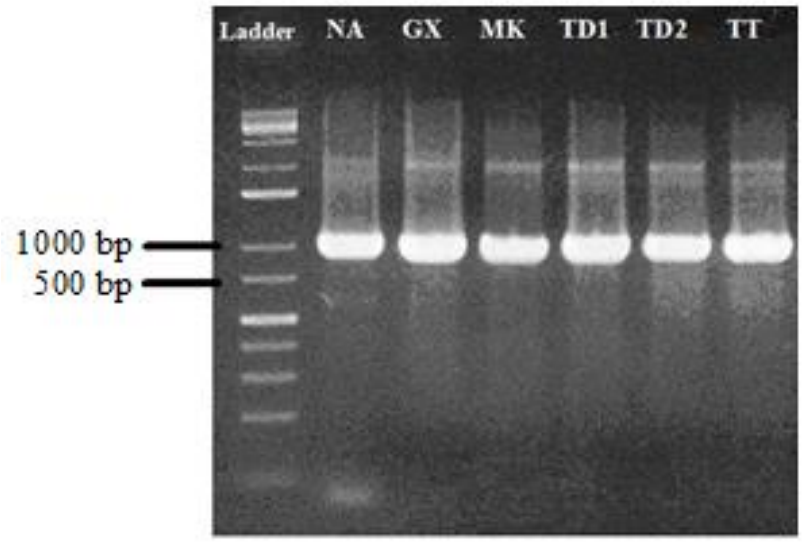

Figure 5. PCR amplification of DNA prepared from *Phytophthora* sp. PCR was performed using ITS6 and ITS4 primers. The amplified products were analyzed by 1.5% agarose gel electrophoresis

Discussion

On the PDA medium, the isolated samples were similar to the description of *Phytophthora* sp. (Drenth and Guest, 2004), which are all ivory-white to cotton-white colonies, with a stellate or patellate shape. The morphological characteristics, the ratio of length to width of sporangia (L/W), papilla length, and other microscopic features are considered the criteria for the classification of the fungal isolates to the species level.

According to Ho (1981), these fungal isolates could be one of the following species: *P. cajani, P. cambivora, P. cinnamomi, P. erythroseptica* var. *erythroseptica, P. fragariae, P. quinea* or *P. syringae.* The average width of the samples ranged from 39.89±4.41 µm to 55.58±14.8 µm. The sporangium width of the isolated fungal samples was similar to the sporangium width of the *Phytophthora* species that was described by Gerettson-Cornell (1989). Based on

the sporangium width, the isolated fungi could be *P. cinnamomi*, *P. palmivora*, *P. primulae*, *P. cambivora*, *P. capsici*, or *P. citricola*. The L/W ratios showed that these ratios of the isolates were different at the 5% statistical significance level.

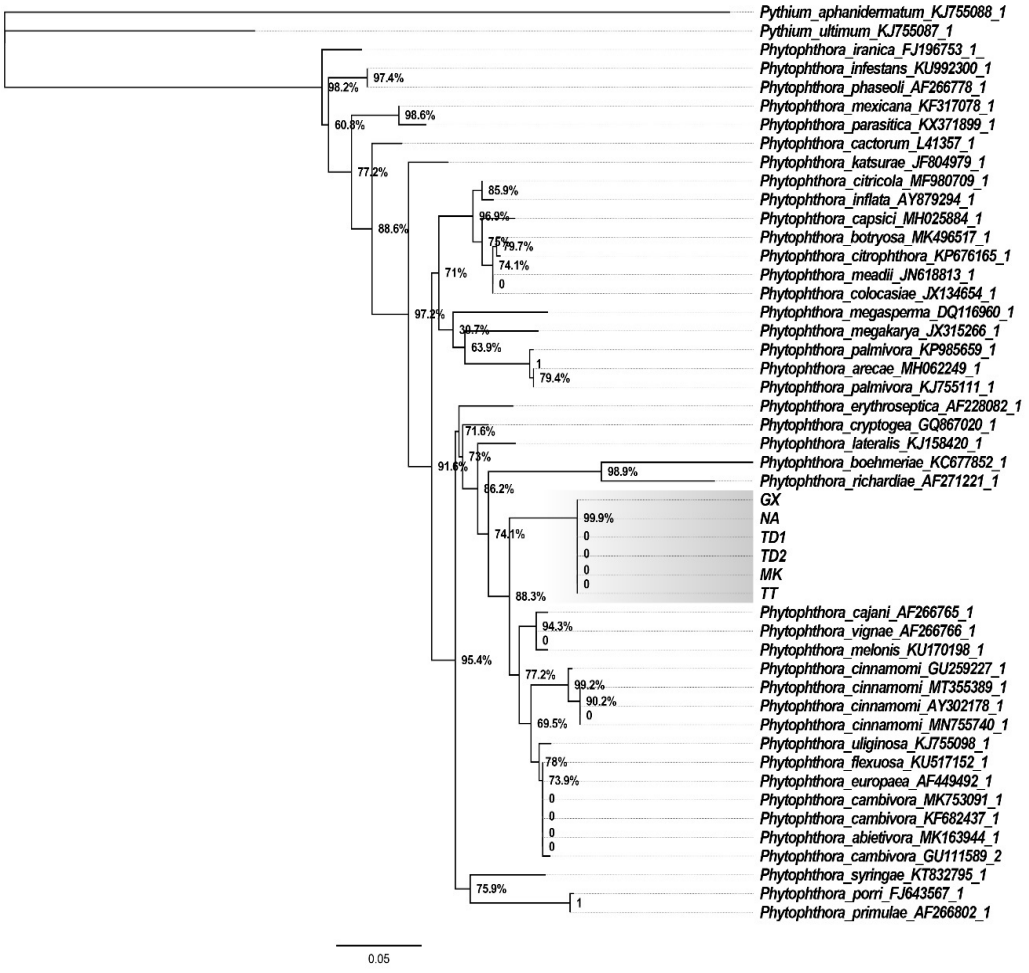

Figure 6. Phylogeny analysis derived from Maximum likelihood inference of concatenated ITS-nrDNA NCBI sequence data from *Phytophthora* sp. with *Pythium aphanidermatum* and *Pythium ultimum* as outgroups: Maximum likelihood bootstrap values in percentages are indicated in the branch points, respectively. The scale bar indicates 0.05 substitutions per site per branch

The microscopic sizes of 7 samples were similar to the results of Latifah (2018), which had described that *Phytophthora* species on durian had the L/W ratio of sporangia at about 1.54-1.69, with an average of 1.62. According to Ho

(1981), the isolates belonged to the group with L/W from 1.6 to 1.9 including the following species: *P. botryosa, P. capsici, P. cinnamomi, P. colocasiae, P. cryptogea, P. drechsleric, P. drechsleric* var. *cajani, P. erythroseptica* var. *erythroseptica, P. hibernalisc, P. inflata, P. meadii, P. palmivora, P. primulae, P. quinea, P. richardiae* and *P. vignae*. According to Gerettson-Cornell (1989), the L/W ratio of sporangia varied from 1.1-2.6, with an average of 1.3-1.9, belonging to *Phytophthora cinnamomi.*

The isolated samples had chlamydospores with diameters ranging from 27.30±4.12 µm to 36.37±5.11 µm, which was compatible with the description by Gerettson-Cornell (1989) about *P. cinnamomi*, whose chlamydospores have an average diameter of about 27-45 µm. Two samples of fungi GX and NA had an ovoid shape. Five samples TD1, TD2, TT, and MK had ellipse sporangia. Based on the Synoptic keys of Ho (1981), the examined *Phytophthora* samples may belong to one of the following species: *P. botryosa, P. capsici, P. cinnamomi, P. colocasiae, P. cryptogea, P. drechsleric, P. erythroseptica, P. hibernalisc, P. inflata, P. meadii, P. palmivora, P. primulae, P. quinea, P. richardiae* or *P. vignae*.

All the sporangia are non-papillate and the shapes are obpyriform or ellipsoid, which indicated that the isolated samples could be *P. cinnamomi* (Gerettson-Cornell, 1989). All 6 examined isolates experienced internal and extended proliferation, which according to Ho (1981), was found to be one of the following species: *P. cajani, P. cambivora, P. cinnamomi, P. drechsleric, P. erythroseptica, P. fragariaec, P. lateralis, P. meadii, P. megasperma, P. quinea* and *P. richardiae.* Six fungal isolates from the Phong Dien District had similar characteristics to *Phytophthora* sp. (Ho, 1981; Erwin and Ribeiro, 1996).

After the inoculation of *Phytophthora* sp. on susceptible plants, typical symptoms including wilting and brown discoloration of the stem were observed. Control plants showed no symptoms of the disease. To confirm the causative agent of the disease, re-isolation of *Phytophthora* sp. was carried out from the infected plants. The presence of the pathogen was detected in all aerial parts of the infected plants, fulfilling Koch's postulates. In contrast, *Phytophthora* colonies did not find in the control plants, confirming that the symptoms were due to the inoculation of *Phytophthora* sp.

According to Drenth and Sendall (2001), most species of *Phytophthora* grow optimally in the temperature range of 15-25℃. Furthermore, the morphological and physical characteristics of isolated fungus samples indicated a 100% similarity coefficient to *Phytophthora cinnamomi*, based on the Ho classification key (Ho, 1981).

In general, six *Phytophthora* isolates that were artificially inoculated on durian seedlings showed disease symptoms. The results of DNA sequence electrophoresis of the ITS gene region using primers ITS6 and ITS4 of six fungal samples with sizes from 916-938 bp are consistent with the study of Cooke *et al.* (2000). The ITS sequence region contains information that can distinguish most *Phytophthora* species. Research by Lee and Taylor (1992) indicated that the ITS sequence region is one of the regions that help to classify. *Phytophthora* spp. such *as P. palmivora, P. megakarya, P. capsici, P. citrophthora, and P. cinnamomi.*

Before the revolution of molecular biology, fungi, and oomycetes were identified morphologically. Nowadays, with the advance of technology, the study of microorganisms through their DNA has many applications, including the identification of species, the observation of the relationships between groups of organisms, and even the detection of pathogens in water and soil without resorting to isolation in culture medium (Cooke *et al.,* 2000).

P. cinnamomi, in contrast, shares mutual morphological and physiological characteristics with the isolated samples. *P. palmivora* and *P. nicotanae* are commonly described as causal agents for diseases in durian, but in Indonesia, the existence and destruction of *P. cinnamomi* in durians were confirmed (Santoso *et al.,* 2015). The existence of *P. cinnamomi* in Vietnam has been described by Tri *et al.* (2016) attacked to avocado. Additionally, *P. cinnamomi* is an aggressive pathogen with the broadest host range of all *Phytophthora* species (Robin *et al.,* 2012).

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