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Morphological and Molecular Identification of *Pythium* spp. from Hydroponically-Grown Lettuce

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ABSTRACT

Herein, hydroponic *Pythium* communities in Thailand were investigated. The 38 *Pythium* isolates from asymptomatic and symptomatic lettuce roots were identified using morphological and molecular features. The data indicated that *P. aphanidermatum* and *P. myriotylum* were the predominant species. Regarding the rDNA-ITS study, all isolates were identified as *P. aphanidermatum*, *P. myriotylum*, *P. deliense*, or an unidentified *Pythium* species. The reconfirmation of the three unidentified *Pythium* isolates using the cytochrome oxidase subunit I (COI) gene in comparison to the representative isolates was achieved using *P. aphanidermatum* and *P. myriotylum.* The COI phylogenetic trees were similar to that of the ITS tree. Additionally, pathogenicity of the *Pythium* representative isolates to plant seeds was evaluated in a laboratory assay. The seedlings suffered serious symptoms from *P. myriotylum* SR31 infection, with 90-100% disease severity. The other isolates presented disease severity of less than 20% when compared with uninoculated control. This study provides a comprehensive identification of *Pythium* root rot in lettuce grown on hydroponics in Thailand and provides information on beneficial microorganisms and a resistance inducer in lettuce root rot.

Keywords: Cytochrome oxidase gene; Hydroponics; ITS region; *Pythium* root rot; Pathogenicity

1. Introduction

Hydroponics is a soilless plant cultivation system that uses water, nutrient solutions and substrate. It provides higher yield and economic returns compared with traditional agriculture. *Pythium* spp. are the

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most well-known fungi of all oomycetes found in soilless cultures. It is reported to be a pathogenic [1-2] and non-pathogenic fungi in hydroponics systems. Non-pathogenic (avirulent), or low virulent (hypovirulent) strains are capable of being managed by alternative biocontrol strategies, and can also provide protection to susceptible plants against their respective pathogens [3]. Bahramisharif et al. [4] used a combination of compost and non-pathogenic *Pythium* for suppression damping-off caused by several oomycete isolates. Moreover, one of these strains, *P. oligandrum,* is reported to induce a defense response in tomato plants [5-6]. Identification of *Pythium* spp. is very difficult due to the similarities in their morphological characteristics (colony, oospore, antheridia etc.) [7]. Generally, the identification of *Pythium* species is based mainly on morphological and biological descriptions [8]. However, molecular techniques have been introduced for the identification of *Pythium* species, allowing for higher precision and resolution [9]. Comparative studies on the internal transcribed spacer (ITS) region of ribosomal DNA have been used to determine the relationships among *Pythium* spp. worldwide, because they appear to be conserved within species [7, 10-13]. The identification and detection of *Pythium* species [14] such as *P. arrhenomanes* [15], *P. longandrum* [16], and *P. rhizo-oryzae* [17] based on PCR analysis of the ITS gene has reported. Additionally, other specific primers for *Pythium* species identification, such as that for the cytochrome oxidase subunit I (COI) region have been assessed over a significant sample of oomycete genera. The COI is a mitochondrially encoded gene which is recognized as an extremely useful DNA barcode capable of accurate species identification in a broad range of eukaryotic life forms, including the oomycetes (*Phytophthora* and *Pythium*) [12, 18-19]. Therefore, the objectives of this research were to identify and characterize the *Pythium* isolates collected from hydroponics and soil planted lettuce by using molecular data in comparison to morphological appearences.

2. Materials and Methods

2.1 Morphological characteristics

Thirty-eight isolates of *Pythium* [20] from asymptomatic and symptomatic lettuce roots in hydroponic farms at Nakhon Ratchasima and Bangkok province were used in this study. In addition, some isolates were taken from infested soil. The *Pythium* isolates were characterized by culturing on potato dextrose agar (PDA). The asexual and sexual structures were induced by grass-leaf culture technique [21]. Colony morphology, together with asexual and sexual structures, were characterized for species identification based on monographs or key of Plaats-Niterink [8]. Thirty measurements were taken for each morphological characteristic. Identification of species was done based on images captured using a high-resolution digital camera (DXM 1200, Nikon). Examinations under standardized, calibrated magnification were performed using a computer-based software system (NIS-Elements version D, Nikon Canada Inc.).

2.2 DNA extraction and PCR amplification

The *Pythium* genomic DNA was extracted from mycelium cultured in 50 ml of 20% V8 juice broth for five days. The extraction method was performed according to the procedure done by Matsumoto et al. [14]. The quantity and quality of extracted DNA were verified using absorbance readings at 260 and 280 nm. DNA was separated for measurement by 0.8 % agarose gel electrophoresis and stored at -20 .ºC

2.3 PCR amplification

The ITS regions of all *Pythium* isolates were amplified using the universal primers ITS1 and ITS4 [22]. The oomycete-specific primers were used to amplify fragments of

COI mitochondrial DNA [18]. A total volume of 50 µl of reaction mixture contained 0.2 µM of each primer, 1.25 units of *Taq* polymerase (Thermo Scientific, China), 0.2 mM dNTP mix, 1X PCR buffer (10 mM Tris-HCl; pH 8.8, 50 mM KCl), 1 mM $MgCl_2$, and 2 µl of 50 ng/µl of DNA template. The reaction was carried out with a thermal cycler (Biometra GmbH, Germany). The temperature cycling parameters were programmed for one cycle of 3 min at 94 °C, followed by 30 cycles of 45 s at 94 ºC, 45 s at 55 ºC, 50 s at 72 °C, and one cycle of 10 min at 72 °C. The reaction for the COI gene was the same except that the PCR profile consisted of one cycle of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C, 50 s 72 °C, and a final extension for 5 min at 72 °C. The PCR product sizes were confirmed by electrophoresis on 0.8% (w/v) agarose gels (Vivantis, Malaysia). The targeted bands were cut out and purified with GenepHlow™ Gel Extraction Kit (Geneaid, Taiwan). Four reactions of each isolate were sent out for sequence analysis (Macrogen, Korea) including two reactions for forward and two reactions for reverse primer. The ITS amplification was repeated two times separately.

2.4 Phylogenetic analysis

The sequences were edited using the BioEdit version 7.2.2 [23]. The consensus sequences were used for alignment analysis using CLUSTAL OMEGA. The DNA sequence data were deposited in the DDBJ (DNA Data Bank of Japan). The sequences for the outgroup *Pythium intermedium* were obtained from GenBank (accession no. KF780558 for ITS and KT692880 for COI) and included in the analysis. All the ITS sequences were analyzed by using UPGMA (Unweighted Pair Group Method with Arithmetic mean) and MEGA version 7 program for phylogenetic analysis [24]. The bootstrap analysis was achieved using 1,000 replicates of heuristic searches to determine the confidence levels of the inferred phylogenies. The contiguous sequence of each isolate was applied to BLAST alignment program for a similarity search against species sequences available in the GenBank database at NCBI (the National Center for Biotechnology Information).

2.5 Pathogenicity test

The pathogenicity of three-selected representative isolates of non to weakly, moderate, and virulent *Pythium* (ASR23, SR36, and SR31) from our previous study [20] were tested using seeds of six plants including butterhead, green oak, and red oak lettuce (HI-Q Agricultural Co., LTD., Thailand), kale, cucumber, and tomato (Chia Tai Co., LTD., Thailand). The pathogenicity test used was modified from the test used by Littrell and McCarter [25]. Seeds were surface sterilized with 6% sodium hypochlorite for 5 min and washed thoroughly 3 times with sterilized water. Sterilized seeds were dried on sterilized tissue paper and then immersed in inoculum for 10 min. Infected seeds were placed on a moistened paper towel in a Petri-dish for seed germination and incubated at 26 ºC. For non-inoculated control, seeds were immersed in sterilized water. The experiment was set up in a completely randomized design with 7 replicates of 10 seeds and repeated twice. Then, the percentage of seed germination was determined and seedlings were scored for disease severity and measured for root length. The rating scale used is as follows: 0 = asymptomatic root, 1 = 1 to 20% of root rot, 2 = 21 to 40% of root rot, 3 = 41 to 60% of root rot, 4 = 61 to 80% of root rot, and 5 = 81 to 100% of root rot or dead seedling. The disease severity was calculated using the equation according to Talubnak et al. [20].

Table 1. Morphology characterization of *Pythium* isolates collected from hydroponically-grown lettuce in Thailand.

Isolates	Occurrence		Morphological descriptions							Accession number
	Host	Geographical origin	Colony	Hyphal width (µm)	Sporangia (µm)	Oogonia diameter (µm)	Antheridia	Oospores		
								Diameter (µm)	Wall (µm)	
ASR1	Asymptomatic Red oak	Bangkok	No pattern	5.66± 0.76	8.27± 1.35	23.14± 2.85	1	17.59± 2.12	1.81± 0.34	LC176451
ASR2	Asymptomatic Red oak	Bangkok	No pattern	6.29± 0.63	8.13± 2.23	24.51± 2.20	1	15.85± 1.35	2.36± 0.39	LC176452
ASR3	Asymptomatic Red oak	Bangkok	No pattern	6.14± 0.95	10.49± 2.2	22.53± 1.40	1	15.54± 1.04	1.78± 0.25	LC176453
ASR4	Asymptomatic Butter head	Bangkok	No pattern	6.03± 1.16	7.45± 1.40	20.55± 1.77	1	15.05± 1.64	1.61± 0.39	LC176454
ASR5	Asymptomatic Butter head	Bangkok	No pattern	5.53± 0.78	6.15± 1.60	22.77± 1.19	1	17.49± 1.79	1.54± 0.24	LC176455
ASR6	Asymptomatic Green oak	Bangkok	No pattern	6.06± 0.75	8.76± 1.58	22.02± 1.99	1	16.52± 2.00	1.57± 0.35	LC176456
ASR7	Asymptomatic Green oak	Bangkok	No pattern	6.18± 1.05	7.89± 2.62	21.91± 1.46	1	15.97± 1.51	1.43± 0.30	LC176457
ASR8	Asymptomatic Red coral	Bangkok	No pattern	6.90± 1.15	7.50± 2.13	20.95± 1.34	1	14.74± 1.33	1.43± 0.34	LC176458
ASR9	Asymptomatic Red coral	Bangkok	No pattern	4.95± 0.70	9.03± 3.16	22.22± 1.63	1	16.26± 2.09	1.58± 0.30	LC176459
ASR10	Asymptomatic Red oak	Bangkok	No pattern	6.55± 1.41	7.98± 2.72	33.15± 6.93	4-10	23.88± 3.19	1.69± 0.28	LC176460
ASR11	Asymptomatic Red oak	Bangkok	No pattern	5.83± 1.17	7.81± 2.53	23.28± 2.92	1	16.26± 0.98	1.76± 0.25	LC176461
ASR12	Asymptomatic Red oak	Bangkok	No pattern	5.01± 1.11	6.83± 3.18	25.67± 4.76	1	17.23± 1.93	1.41± 0.19	LC176462
ASR13	Asymptomatic Butter head	Bangkok	No pattern	5.26± 0.75	7.82± 3.15	23.08± 2.68	1	17.78± 2.10	1.19± 0.38	LC176463
ASR14	Asymptomatic Butter head	Bangkok	No pattern	5.27± 0.62	8.81± 2.42	22.83± 1.26	1	16.44± 1.95	1.69± 0.23	LC176464
ASR15	Asymptomatic Butter head	Bangkok	No pattern	6.35± 1.01	7.16± 1.42	24.21± 2.24	1	17.21± 1.58	1.73± 0.34	LC176465
ASR16	Asymptomatic Butter head	Bangkok	No pattern	5.42± 0.91	9.38± 1.14	24.76± 2.71	1	18.15± 1.70	1.16± 0.36	LC176466
ASR17	Asymptomatic Green oak	Bangkok	Rosette pattern	No Data	No Data	No Data	No Data	No Data	No Data	LC176467
ASR18	Asymptomatic Green oak	Bangkok	No pattern	5.88± 1.06	7.89± 2.26	19.88± 1.51	1	16.01± 1.28	1.28± 0.23	LC176468
ASR19	Asymptomatic COS	Bangkok	No pattern	5.34± 1.07	8.30± 1.78	22.56± 3.59	1	16.25± 7.54	1.44± 0.21	LC176469
ASR20	Asymptomatic COS	Bangkok	No pattern	4.75± 0.87	8.87± 2.12	34.27± 5.97	4-10	23.85± 2.56	1.52± 0.28	LC176470
ASR21	Asymptomatic COS	Bangkok	No pattern	6.84± 1.41	8.54± 3.33	35.20± 4.14	4-10	21.84± 4.55	1.12± 0.20	LC176471
ASR22	Asymptomatic Red coral	Nakhon Ratchasima	No pattern	6.40± 1.44	7.87± 2.27	40.32± 3.31	4-10	25.68± 4.30	1.35± 0.50	LC176472
ASR23	Asymptomatic Butter head	Nakhon Ratchasima	No pattern	4.81± 0.69	6.99± 2.14	21.64± 1.97	1	17.42± 1.47	1.40± 0.20	LC176473
ASR24	Asymptomatic Butter head	Nakhon Ratchasima	No pattern	5.07± 0.56	7.56± 2.14	23.38± 2.54	1	16.51± 1.38	1.36± 0.39	LC176474
ASR25	Asymptomatic Butter head	Nakhon Ratchasima	No pattern	5.46± 0.78	7.93± 2.14	25.14± 2.65	1	18.33± 1.73	1.29± 0.32	LC176475
ASR26	Asymptomatic Green oak	Nakhon Ratchasima	No pattern	5.52± 0.89	8.14± 2.18	21.62± 1.21	1	15.38± 1.36	1.52± 0.29	LC176476
ASR27	Asymptomatic Red coral	Bangkok	No pattern	5.39± 0.82	8.04± 1.65	24.47± 2.01	1	17.58± 1.85	1.35± 0.33	LC176477
SR28	Symptomatic Red oak	Bangkok	No pattern	6.36± 1.47	6.48± 1.92	33.78± 3.13	4-10	21.21± 2.42	1.26± 0.30	LC176478
SR29	Symptomatic Red oak	Bangkok	No pattern	5.24± 1.41	7.15± 1.19	39.19± 4.89	4-10	22.99± 3.79	1.68± 0.37	LC176479
SR30	Symptomatic Red oak	Bangkok	No pattern	6.21± 0.99	8.83± 1.61	33.54± 2.15	4-10	23.21± 2.39	1.54± 0.25	LC176480
SR31	Symptomatic Red oak	Bangkok	No pattern	6.62± 1.73	9.28± 2.52	24.86± 3.65	4-10	21.78± 2.60	1.49± 0.20	LC176481
SR32	Symptomatic Red oak	Bangkok	No pattern	5.74± 0.84	7.68± 1.66	29.94± 3.97	4-10	22.77± 2.93	1.33± 0.20	LC176482
SR33	Symptomatic Red oak	Bangkok	Rosette pattern	No Data	No Data	7.50± 0.91	No Data	No Data	No Data	LC176483
SR34	Symptomatic Red oak	Bangkok	No pattern	4.69± 0.74	7.00± 2.37	36.31± 3.93	4-10	24.58± 4.59	1.37± 0.27	LC176484

Isolates	Occurrence		Morphological descriptions							Accession number
	Host	Geographical origin	Colony	Hyphal width (µm)	Sporangia (µm)	Oogonia diameter (µm)	Antheridia	Oospores		
								Diameter (µm)	Wall (µm)	
SR35	Symptomatic Red oak	Bangkok	No pattern	5.74± 1.22	6.32± 2.08	34.54± 4.77	4-10	19.52± 3.31	1.56± 0.36	LC176485
SR36	Symptomatic Red oak	Bangkok	Rosette pattern	No Data	No Data	No Data	No Data	No Data	No Data	LC176486
IS37	Infested soil Green oak	Bangkok	No pattern	5.37± 0.79	10.85± 1.8	26.77± 2.19	1	19.03± 1.35	1.37± 0.22	LC176487
IS38	Infested soil Green oak	Bangkok	No pattern	5.94± 0.79	9.56± 2.07	19.01± 1.81	1	12.64± 1.87	0.93± 0.24	LC176488

3. Results and Discussion

Among the 38 isolates of *Pythium* species, 36 were recovered from a hydroponic system and 2 from soil. The identification was performed by examining both morphological and molecular features. The *Pythium* species isolated from hydroponics were morphologically identified as *P. aphanidermatum* (25 isolates) and *P. myriotylum* (10 isolates), with 3 isolates of unidentified species which is concordant with the findings of morphological identification [8, 26]. *P. aphanidermatum* was found mostly on asymptomatic lettuce roots and *P. myriotylum* was isolated mostly from symptomatic lettuce roots. Based on morphological characteristics, the representative isolates of *P. aphanidermatum* (ASR21) (Fig. 1) and *P. myriotylum* (SR31) (Fig. 2) showed similar colony patterns on PDA (Fig. 1A, 2A). The colonies had aerial cottony mycelium with no special colony pattern, and the sporangia had filamentous inflated, simple or branched, terminals or intercalary sporangia (Fig. 1B-C, 2B-C). Additionally, the different characteristics of both *Pythium* isolates were observed on oogonia, antheridia, and oospores. The oogonia of *P. aphanidermatum* were intercalary or terminal, spherical, 23.14 ± 2.85 µm in diameter, and smooth walled. Antheridia were mostly intercalary, sometimes terminal, antheridial branches monoclinous with one antheridium per oogonium (Fig. 1D-E). Oospores were aplerotic, spherical, 17.59 ± 2.12 µm in diameter, with smooth walls 1.81 ± 0.34 µm thick (Fig. 1F). The oogonia of *P. myriotylum* were intercalary or terminal, spherical, 34.27 ± 5.97 µm in diameter, and smooth walled. Antheridia were present, antheridial stalk branched, mostly diclinous and 4 to 10 antheridia per oogonium (Fig. 2D-E). Oospores were aplerotic, spherical, 23.85 ± 2.56 µm in diameter, with smooth walls 1.52 ± 0.28 µm thick (Fig. 2F). The other representative isolates from *Pythium* sp. isolate SR36 developed as a cottony mycelial colony on PDA with a chrysanthemum pattern but the reproductive structures were not exposed.

Regarding the molecular characteristics, genomic DNA of the *Pythium* isolates was extracted and the ITS rDNA region was amplified by PCR. After visualization of the PCR product, a specific DNA band was shown at the size of 650 – 750 bp. All *Pythium* isolate identifications were confirmed by sequence comparison of the ITS region retrieved from the GenBank database. The data revealed that all sequences of *Pythium* isolate had a 50-100% identity match to the reference species. Among the sequencing data of 38 isolates, 24 isolates were closely related to those of *P. aphanidermatum* (KT336808, MK311253, MK326548, and MH393376) with 54-97% similarity (Fig. 3; clade 1).

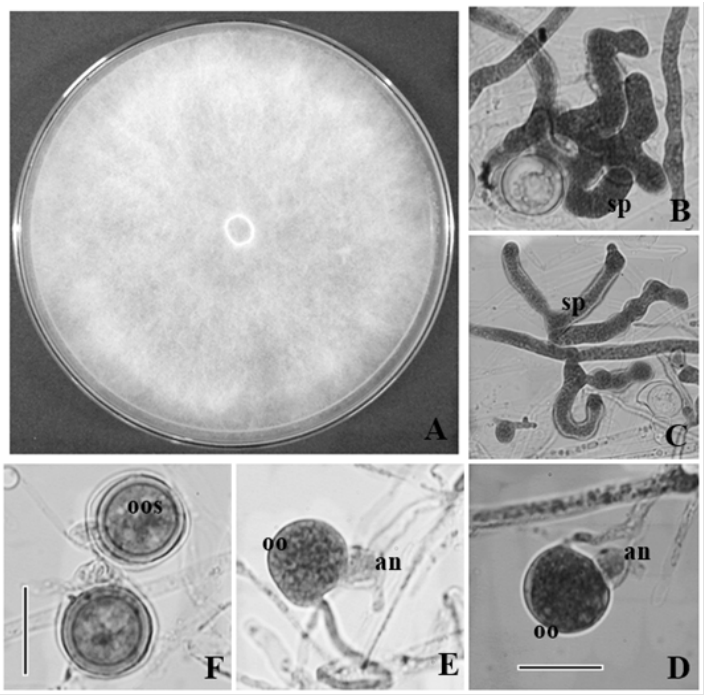

Fig. 1. *Pythium aphanidermatum*: A. colony on PDA for 4 days at 26 °C, B-C. filamentous and lobulate sporangia, D. oogonium with terminal monoclinous antheridium, E. oogonium with intercalary monoclinous antheridium F. Aplerotic oospores. Scale Bars = 20 µm. *oo = oogonium; an = antheridium; oos = oospore; sp = sporangium.

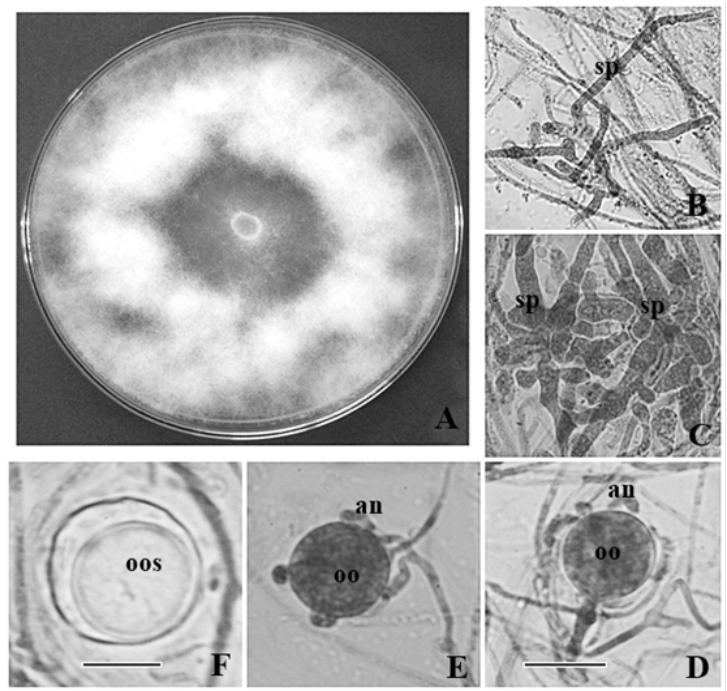

Fig. 2. *Pythium myriotylum*: A. colony on PDA for 4 days at 26 °C, B-C. filamentous and lobulate sporangia, D-E. oogonia surrounded by club or crook shaped diclinous antheridia, F. aplerotic oospore. Scale Bars = 20 µm. *oo = oogonium; an = antheridium; oos = oospore; sp = sporangium.

Ten isolates were closely related to *P. myriotylum* (KT364295 and KM434129) with 24-57% similarity (Fig. 3; clade 4). The isolates SR33, SR36, and ASR17 were unidentified species but matched CLE-2015d (KT247392) with 48-96% similarity (Fig. 3; clade 3). The ITS sequences of *Pythium* isolates were submitted to the DDBJ under the accession numbers of LC176451 to LC176488. Regarding the molecular characteristics, the identity of isolate IS38 was a 100% match to *P. deliense* (AJ233442) (Fig. 3; clade 2) while the morphological characteristics were related to *P. aphanidermatum*. These results correspond to previous reports confirming the morphological characteristics of Pythia by ITS region confirmation [27-29]. This is generally supported by Mutsumoto et al. [14], who examined the relationship of *Pythium* species by their ITS sequences and sporangia characteristics. The two species, *P. aphanidermatum* and *P. deliense*, were seen to be similar to each other and to share some morphological features like lobulate sporangia, zoospore formation, terminal oogonia, and aplerotic oospores [30]. Therefore, morphological characteristics alone are insufficient for the identification of *P. aphanidermatum* and *P. deliense*. Previously, Levesque et al. [31] suggested that the taxonomic status of *P. aphanidermatum* and *P. deliense* should be further classified. In 2002, Moorman et al. [32] revealed that the ITS sequences of these two species were identical, and as such could not be used for their differentiation. Regarding the selected three unknown *Pythium* isolates (ASR17, SR33, and SR36), they were re-identified by COI region (Fig. 4). The amplified COI gene was approximately 690 bp. The tree revealed that *Pythium* isolates ASR17, SR33, and SR36 were 100% identical to *Pythium* sp. (KT247393) (Fig. 4; clade 3). The representative isolates ASR23 and SR31 were included in clade 1 and 2, as well as *P. aphanidermatum* and *P. myriotylum*, respectively. According to morphology and

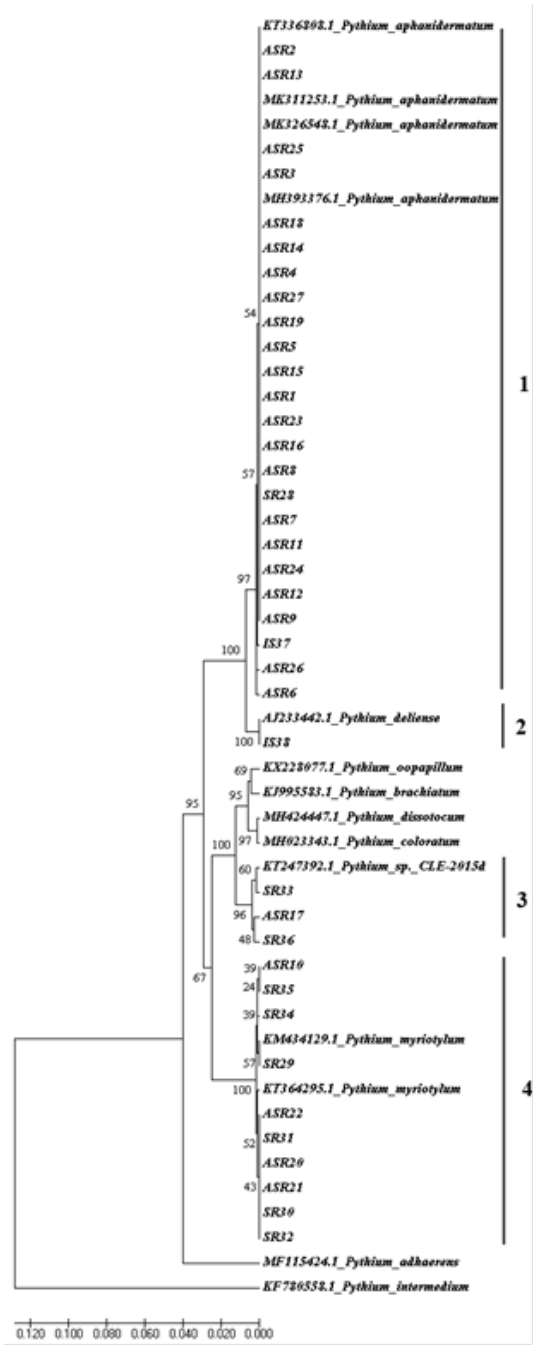

Fig. 3. The UPGMA tree performed on the ITS sequences of Pythium species. *Pythium intermedium* was used as outgroup. Bootstrap values (1,000 reps) were shown at branches. Data obtained from GenBank were represented by GenBank accession numbers.

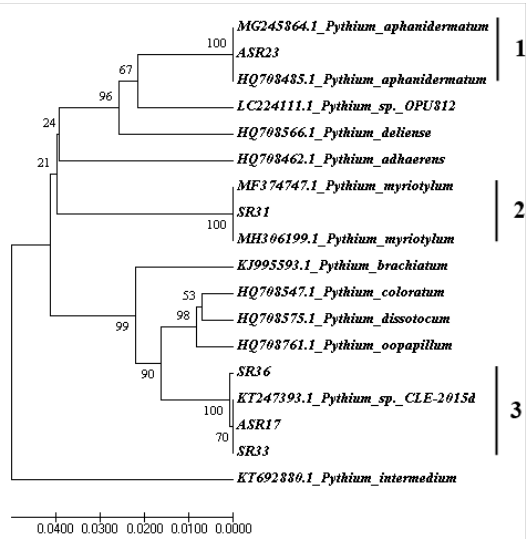

Fig. 4. The UPGMA tree performed on the COI sequences of Pythium species. *Pythium intermedium* was used as outgroup. Bootstrap values (1,000 reps) were shown at branches. Data obtained from GenBank were represented by GenBank accession numbers.

ITS data, three unknown isolates of *Pythium* were unidentified species. Robideau et al. [18] reported that the sequence analyses for the oomycetes were completed using ITS and COI sequences. The data from the ITS and COI region analyses of the *Pythium* isolates were correlated to the morphological features of each isolate applicable for identification confirmation.

The disease severity seen in the seedling rot test significantly differed among the three *Pythium* isolates ASR23, SR36, and SR31 (Table 2). After 4 days, *P. myriotylum* SR31 showed strong pathogenicity to all tested seedlings, with 90 to 100% disease severity. The symptoms manifested as necrotic roots, browning tissue, and seed rot. Control seedlings were asymptomatic and healthy (Fig. 5). *Pythium* sp. SR36 exhibited low pathogenicity to seedling roots of tomato and kale (disease severity less than 20%), butterhead and red oak (disease severity less than 10%), cucumber and green oak (asymptomatic). Regarding the effect of *P. aphanidermatum* ASR23 to the non- and weakly pathogenic *Pythium* groups, it was non- and rarely pathogenic to all tested plants (Table 2). Nonetheless, no symptoms were observed in control plants. Gharieb et al. [33] reported on the variations in the virulence of four *Pythium* spp. that cause cucumber seedling rot and pre- and post-emergent damping-off. Similarly, Rossman et al. [34] showed the variations of pathogenicity of *Pythium* isolates in dry bean plants that cause seed rot and pre-emergence damping-off. Two species of *Pythium*, *P. myriotylum* and *P. aphanidermatum*, were abundant in summer from diseased tomato root in hydroponics [35]. The data are correlated to those from of Koohakan et al. [36], reporting that *P. myriotylum* was the causal agent of root rot in lettuce and cucumber grown on commercial hydroponic farms in Thailand. In addition, the results of this study also correspond to the findings of Kageyama [12], that *P. myriotylum* could be found along with other *Pythium* spp. and distributed in warm regions worldwide. Additionally, it is capable of causing economic damage from crop losses (such as with tomatoes) by having a very wide host range. This indicates that *P. myriotylum* SR31 caused seedling rot in various crops. However, this seedling rot was not severe in *P. aphanidermatum* ASR23 and *Pythium* sp. SR36. There were no disease symptoms in uninoculated controls.

Table 2. Comparison of disease severity indexes (0 to 5) recorded on seedlings inoculated with non-pathogenic and pathogenic *Pythium* at 4 DAI.

Isolates	Tomato	Cucumber	Kale	Butterhead	Red oak	Green oak
P. aphanidermatum ASR23	0.10a[1/]	0.00a	0.00a	0.61b	0.10ab	0.11b
Pythium sp. SR36	0.95b	0.01a	0.88b	0.17a	0.17b	0.01a
P. myriotylum SR31	5.00c	5.00b	4.34c	5.00c	5.00c	5.00c
Uninoculated control	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

[1/]Values followed by different letters within a column show a statistically significant difference at probability level 0.01 by Duncan's multiple range test.

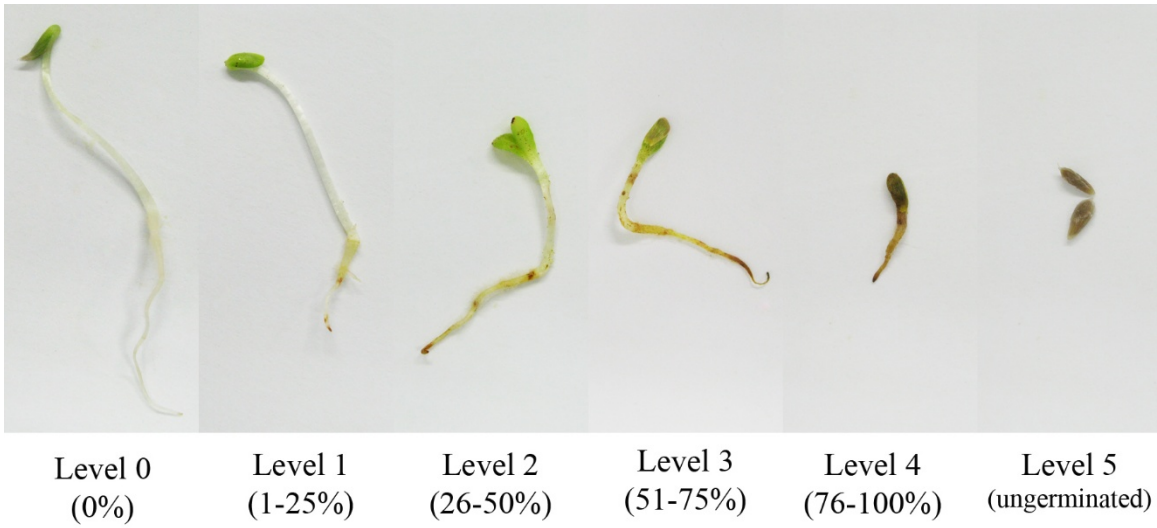

Fig. 5. The disease index of pathogenicity test of *Pythium* isolates on seedling using plate assay. The rating scale was used as follows: 0 = asymptomatic root, 1 = 1 to 20% of root rot, 2 = 21 to 40% of root rot, 3 = 41 to 60% of root rot, 4 = 61 to 80% of root rot, and 5 = 81 to 100% of root rot or died seedling.

4. Conclusion

This paper presents detailed information on the distribution of *Pythium* in hydroponic systems. On the basis of morphological characteristics and molecular techniques using ITS and COI primers, our results revealed that *P. aphanidermatum* and *P. myriotylum* were the two most commonly found *Pythium* species in hydroponically cultivated lettuce in Thailand. It is worth mentioning that morphological identification supplemented with molecular profiles was of great help in confirming the identifications of *Pythium* species. According to the pathogenicity tests done on six kinds of vegetables seeds *in vitro*, *P. myriotylum* (SR31) was found to be highly pathogenic to all tested seedlings (kale, cucumber, tomato, butterhead, green oak, and red oak) while *Pythium* sp. (SR36) and *P. aphanidermatum* (ASR23) were moderately to non-pathogenic to all tested plants. From the results, the *Pythium* distribution data can be used for advanced study or further field assessment. Therefore, it is of interest to focus on the isolation of the non-pathogenic *P. aphanidermatum* isolate ASR23 and its role as a beneficial microorganism or resistance inducer to lettuce root rot diseases in plants grown on hydroponics.

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