Asian Journal of Plant Pathology

ISSN 1819-1541

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Plant Pathology 9 (2): 59-71, 2015
ISSN 1819-1541 / DOI: 10.3923/ajppaj.2015.59.71

Identification of *Pythium* and *Phytophthora* Associated with Durian (*Durio* sp.) in Indonesia: Their Molecular and Morphological Characteristics and Distribution

[1,2]Panca J. Santoso, [1]I. Nyoman P. Aryantha, [1]Adi Pancoro and [1]Sony Suhandono

[1]School of Life Science and Technology, Institute Teknologi Bandung, Jl. Ganeca 10 Bandung, 40132, Indonesia

[2]Indonesian Tropical Fruit Research Institute, IAARD, Jl. Raya Solok-Aripan Km. 8, P.O. Box 5 Solok, Sumatra Barat, 27301, Indonesia

Corresponding Authors: Panca J. Santoso and I. Nyoman P. Aryantha, School of Life Science and Technology, Institute Teknologi Bandung, Jl. Ganeca 10 Bandung, 40132, Indonesia

ABSTRACT

Phytophthora and *Pythium* are reported as pathogen causing tree-decline to durian. Survey relating to their diversity and distribution in Indonesia is very limited. A research to identify their molecular, morphological characteristic and distribution was undertaken from 2011 to 2014. Molecular identification was based on ITS-nrDNA sequences. Morphological characteristics observed were colony motif, shape and sporangium size. A total of 36 isolates were successfully baited from 32 durian fields represent of 17 provinces in Indonesia. Based on ITS-nrDNA sequences, the isolates correspond to six *Pythiaceae* species, namely *Pythium cucurbitacearum*, *Pythium vexans*, *Pythium* sp. D37, *Pythium deliense*, *Phytophthora cinnamomi* var., parvispora and *Phytophthora palmivora*. These species demonstrated the diversity of *Pythiaceae* associated with durian in Indonesia. The diversity was also confirmed by the morphological characteristics such as colony motif, shape and sporangium size. *Pythium cucurbitacearum* were found in 13 (76.5%) provinces and *Pythium vexans* were in 10 (58.8%). The findings concerning the distribution of both pathogens are indicating that these two species could be more dangerous than *Phytophthora palmivora*. This is the first time; *Pythium cucurbitacearum*, *Pythium* sp., D37, *Pythium deliense*, *Phytophthora cinnamomi* var., parvispora reported their association with durian.

Key words: Durian, tree-decline, *Phytophthora*, *Pythium*, diversity, distribution

INTRODUCTION

Durian (*Durio* sp.) is a tropical fruit crop which is highly prized culturally and economically in South East Asia. The fruit is very famous not only due to the taste richness but also the strong odour. Durian is believed native to Kalimantan or Borneo Island (Kostermans, 1958; Brown, 1997) and standard Indonesia as the third largest durian producer in the world after Thailand and Malaysia (Somsri, 2014). Total area planted durian in this country reached about 69.045 ha which produce fruit a total of 883.969 t in year 2011 (MoA., 2012).

Durian culture practice in Indonesia is now moving from traditionally toward more intensively. This practice is delivering hope to increase the fruit quantity and quality. On the other hand,

intensive practices such as monoculture planting and high fertilizer applications could lead the increment of disease incidence caused by fungi such as *Phytophthora palmivora* and *Pythium vexans* (Drenth and Guest, 2004; Vawdrey *et al*., 2005).

Phytophthora palmivora is more frequently reported as pathogens in durian. This fungus causes a variety of symptoms such as leaf spot, root rot, stem cancer and fruit rot pre- and post-harvest (Drenth and Guest, 2004; Lee and Lum, 2004; Sivapalan *et al*., 1997). It has characters that demonstrate it as an effective pathogen, such as: Produces a sporangium which able to survive and spread through the air, rapid sporulation in host plants, its zoospore able to enter the roots chemostatically and electrostatically and able to live a long life inside and outside the host plant (Drenth and Guest, 2004; Kong *et al*., 2010; Kueh and Khew, 1982). *Phytophthora palmivora* was reported to have damaged the durian orchard in Penang (Hasan and Siew, 2000) and durian collection in Australia (Zappala *et al*., 2002). Yield losses due to this defect in Southeast Asian countries are estimated to be around 20-25% (Drenth and Sendall, 2004). Unlikely, only a few informations is relating to the existence and attack of *Pythium* on durian. Vawdrey *et al*. (2005) reported the discovery of *Pythium vexans* along with *Phytophthora palmivora* on durian orchard in Queensland, Australia. Whilst, Lim (1990) described that the *Pythium vexans* is potential pathogens in the nursery and mature durian.

In Indonesia, *Phytophthora palmivora* is only the pathogen reported to be the causes of durian tree-decline (Muryati *et al*., 2009; Purwantara *et al*., 2004; Emilda, 2007; Sunarwati *et al*., 2007). However, information from the field officer and other informal sources indicate a different pathogen has invaded durian. It has characteristics similar to *Phytophthora palmivora* in general and is called as dry-phytophthora, since it absent of gummosis and remains dry instead of wet stem cancer symptom. This uncertainty may occur due to limited research and observation has been conducted and little is known about the pathogens.

The family Pythiaceae, especially both genus *Pythium* and *Phytophthora* have more than 300 described species which being pathogenic on plants and other organism (Van der Plaats-Niterink, 1981; Park *et al*., 2013). Identification to the species level morphologically requires a high level of expertise, since some similar species share same characteristics lead to misinterpretation (Godfrey *et al*., 2003; Spies *et al*., 2011). Certain technique which provides faster and more accurate identification such as molecular tools (Baldauf, 2008; Crous *et al*., 2003) therefore, should be implemented. For this purpose, a barcoding conserved region, Internal Transcribed Spacer (ITS) of nucleous ribosomal DNA (nrDNA) is frequently used (Cooke and Duncan, 1997; McLeod *et al*., 2009; Spies *et al*., 2011). Baldwin *et al*. (1995) reported that the ITS region has the advantages that support widely used to study phylogeny. This gene has been used for identification of eukaryotes including fungi turn to the intra-species levels (Appiah *et al*., 2004; Belbahri *et al*., 2008).

The objectives of this research were (1) To identify molecular characteristic of Pythiaceae associated with durian decline in Indonesia, (2) To identify morphological characteristic of Pythiaceae associated with durian decline in Indonesia and (3) To determine the geographical distribution of Pythiaceae associated with durian decline in Indonesia.

Improving understanding of these areas will provide basic information for the development of integrated disease management for durian decline in the country.

MATERIALS AND METHODS

Sampling and isolates collection: Samples collection was conducted from 2011 to 2014 covered 32 durian fields in 17 provinces in Indonesia. A number of 43 soil, two leaf and one fruit samples

were picked up for fungi isolation. The sites are generally a backyard durian field, except for Subang Experimental Farm at Wera which is a germplasm collection field and Sagalaherang orchard which is a private company.

Isolation of the fungi was conducted using fruit baiting method. The soil sample was firstly mixed with sterile water, then put small part of the sample into green sour apples. The inoculated apple was incubated for several days in room temperature until brown-mold occurred surrounding inoculation spot. Pieces of inoculated apple flesh were put onto the Potato-Carrot-Agar (PCA) medium. Monoculture was obtained by excising a peace of mycelium and cultured on PDA medium containing 5 μg pimaricin, 250 μg ampicillin, 10 μg rifampicin and 50 μg hyemexazol (P5ARH), a modification of P5ARPH medium by Jeffer and Martin (1986). Isolation of fungi from leaf and fruit were conducted by put the piece of fungus-attacked organ directly onto PDA medium containing antibiotic P5ARH.

Molecular identification: Molecular identification of Pythiaceae from durian was mainly based on the sequence of the internal transcribed spacers of the ribosomal DNA (ITS-nrDNA). The DNA template isolation and amplification of ITS region was carried out according to the protocol modified by Grunwald *et al.* (2011) with few modifications. Isolation of DNA was conducted using boiling method as follow: (1) An amount of mycelium as much of 1-2 cm scraped pipette tip along the surface of growing culture was put into PCR tube containing 100 μL dH_2O, (2) The tube containing sample was then put floated onto boiled water (at 96-97°C) for 10 min and (3) Lets the water down to room temperature then ready to be use as DNA template. The ITS region was amplified using the universal primers ITS6 (5'-GAA GGT GAA GTC GTA ACA AGG-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The PCR reaction mixture was consisted of 10 μL DNA (boiled mycelium), 25 μL PCR mix (KAPA Biosystems), each of 0.4 μM ITS6 and ITS4 primers and 8% DMSO. Amplification was proceed using Applied Biosystem 2720 Thermal Cycler with condition of pre-denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec. Successful amplification was checked by running 5 μL of amplicon and 1 μL loading dye on a 1% agarose gel in 1×TAE buffer at 100 VA for 30 min.

Sequencing and phylogenetic analysis: The PCR products were submitted to 1st BASE Singapore for direct sequencing using Applied Biosystems genetic analyzer with the BigDye® Terminator v3.1 cycle sequencing kit chemistry. Species identification was then conducted by aligning the sequences with deposited sequences data at National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tools (BLAST). The first top entries of sequences of the corresponding species in GenBank were downloaded. Phylogeny tree was constructed for all samples sequences and corresponding species, with *Rizoctonia solani* as rooted outgroup. Sequences were first aligned with ClustalW. Genetic distance was using Tamura-Nei model, whilst the tree was built using Neighbor-Joining method. The bootstrap was generated using 1000 replications. All of the constructing tree processes were conducted using pc-software Geneious R6 and the softwares mention before are available as plug-in.

Colony motif: In order to identify variation of colony motif amongst isolates, observations were conducted at room temperature (22-25°C). A piece of isolate cultures sized 5 mm were grown on PDA medium amended with P5ARH antibiotic formula. The motif was observed on days 3 to 5 upon the isolate growth reached fully the dish.

Sporangium shape and size: To facilitate for comparison the difference of isolate morphologically to support molecular identification, microscopic observations were conducted to identify the shape and size of the sporangium using a light microscope. Initiation of sporangium development was conducted by grown the isolate in sterile soil extract under luminous room for 48 h. A piece of colony was then put on object glass and impressed with lactophenol blue. The observation was then carried out at 400x magnification.

RESULTS

Pythiaceae isolates: Isolation of fungi conducted in the years 2011 to 2014 was successfully obtaining a total of 36 isolates from 32 durian fields. Of those, 34 were baited from soil and each of 1 isolate from leaf and fruit. They represented 17 provinces in five major islands in Indonesia, namely Kalimantan, Sumatra, Jawa, Sulawesi and Papua with the number of 7, 7, 14, 4 and 2 isolates, respectively, as well as two islands of Bangka-Belitung and the Maluku each of 1 isolate (Table 1). A total of 24 isolates obtained from durian fields which were occurring tree-decline incidence and the remaining 12 isolates were not occurring tree-decline incidence.

Molecular characterization: Generated sequences of ITS region using primers ITS6 and ITS4 for 36 isolates separated them into 7 species of Pythiaceae (Table 1). Fifteen isolates showed 98-99% identity to *Pythium cucurbitacearum* strain 1341pc (HQ237483.1), except for Pp-21, Pp-17 and Pp-33 isolates which showed 88, 95 and 97% identity, respectively. Nine isolates showed 95-98% identity to 5 different strains of *Pythium vexans*, namely *Pythium vexans* (GU931701.1), *Pythium vexans* (JQ898479.1), *Pythium vexans* Lev3100 (HQ643954.1), *Pythium vexans* STE-U6728 (GU133594.1) and *Pythium vexans* WPC3980 (FJ801894.1). Seven isolates showed 94-99% identity to *Phytopythium* sp., strain AL-2010 (HQ643400.2). Two isolates showed 100% identity to *Phytophthora cinnamomi* strain parvispora (GU191211.1). Three isolate showed 99, 98 and 99% identity to *Phytophthora palmivora* (AM422704.1), *Pythium* sp. D37 (JN863978.1) and *Pythium deliense* CBS413.33 (AY598674.2), respectively.

Variation of species obtained was not only from difference site but also from the same durian field. Isolates Pp-15 and Pp-37 both were obtained from Wera but they identical to two different species, *Pythium* sp. D37 and *Pythium cucurbitacearum*. These two isolates are also different with two other samples from Wera, Pp-43 and Pp-45 which identical to *Phytophthora palmivora* (AM422704.1) and *Pythium delinse* (AY598674.2), respectively which both isolated from leaf and fruit host. Similarly, the different species was also obtained from three other locations namely Kaumrejo, Waturejo and Luwu Utara, where each were found two isolates identical to two species *Phytopythium* sp. (HQ643400.2) and *Pythium cucurbitacearum* (HQ237483.1).

The parsimoniously phylogenetic tree constructed from the consensus sequence of 36 isolates and their seven corresponding species was forming six clades (Fig. 1), of which two big clades consisted of *Pythium cucurbitacearum* and *Pythium vexans* isolates and five small clades consisted of *Phytophthora palmivora*, *Phytophthora cinnamomi*, *Pythium deliense*, *Pythium* sp., D37 and Pp-19 Krajan isolates. The two large clades are then subdivided into each five smaller clades. The isolates identical to *Phytopythium* sp., all joined to the clade of *Pythium vexans*. The corresponding species *Phytopythium* sp. (HQ643400.2) is very close to *Pythium vexans* (JQ898479.1), where both stand in the same subclade with bootstrap value of 100. Therefore, it is considered six corresponded species instead of seven as first stated above.

Table 1: Molecular characteristic of *Pythium* and *Phytophthora* associated with durian in Indonesia

Isolate	Sample	Origin	Province	Tree-decline incidence	Year of isolation	GenBank Acc. No.	BLAST result		
							Homolog	Acc. No.	Identity (%)
Pp-01	Soil	Nunukan Slt	Kalimantan Utara	+	2011	KP183929	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-02	Soil	Karang Intan	Kalimantan Selatan	+	2011	KP183930	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	99
Pp-03	Soil	Deli Serdang	Sumatra Utara	-	2011	KP183931	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-07	Soil	Muntok	Bangka	+	2011	KP183935	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-09	Soil	Luwu Utara	Sulawesi Selatan	+	2011	KP183937	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	99
Pp-17	Soil	Bagan Petai	Jambi	+	2011	KP183945	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	95
Pp-18	Soil	Mekarsari	Jawa Barat	+	2012	KP183946	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	99
Pp-21	Soil	Aripan	Sumatra Barat	+	2011	KP183948	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	88
Pp-23	Soil	Jebu	Bangka	+	2011	KP183950	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-26	Soil	Batuah	Kalimantan Timur	-	2011	KP183953	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-27	Soil	Amban	Papua Barat	+	2011	KP183954	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-33	Soil	Anjungan BBI	Kalimantan Barat	-	2011	KP183955	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	97
Pp-34	Soil	Waturejo	Jawa Timur	+	2011	KP183956	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	98
Pp-35	Soil	Kaumrejo	Jawa Timur	+	2011	KP183957	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	99
Pp-37	Soil	Wera	Jawa Barat	+	2011	KP183959	*Pythium cucurbitacearum* 1241Pc	HQ237483.1	99
Pp-10	Soil	Palopo	Sulawesi Selatan	+	2011	KP183938	*Pythium vexans* Lev3100	HQ643954.1	99
Pp-12	Soil	Tanjungan	Sumatra Selatan	-	2011	KP183940	*Pythium vexans*	GU931701.1	97
Pp-14	Soil	Pendahara	Kalimantan Tengah	+	2011	KP183942	*Pythium vexans*	GU931701.1	95
Pp-16	Soil	Sungai Elang	Kalimantan Tengah	-	2011	KP183944	*Pythium vexans*	GU931701.1	97
Pp-19	Soil	Krajan	Jawa Barat	-	2011	KP183947	*Pythium vexans* STE-U6728	GU133594.1	96
Pp-22	Soil	Ujung Ladang	Sumatra Barat	-	2011	KP183949	*Pythium vexans*	GU931701.1	96
Pp-24	Soil	Tanete	Sulawesi Selatan	+	2011	KP183951	*Pythium vexans* clone WPC3980	FJ801894.1	97
Pp-25	Soil	Selat	Jambi	-	2011	KP183952	*Pythium vexans*	GU931701.1	97
Pp-40	Soil	Kemranjen	Jawa Tengah	-	2013	KP183960	*Pythium vexans*	JQ898479.1	98
Pp-04	Soil	Kaumrejo	Jawa Timur	+	2011	KP183932	*Phytopythium* sp. AL-2010	HQ643400.2	94
Pp-05	Soil	Waturejo	Jawa Timur	+	2011	KP183933	*Phytopythium* sp. AL-2010	HQ643400.2	99
Pp-06	Soil	Luwu Utara	Sulawesi Selatan	+	2011	KP183934	*Phytopythium* sp. AL-2010	HQ643400.2	99
Pp-13	Soil	Keroncong	Banten	-	2011	KP183941	*Phytopythium* sp. AL-2010	HQ643400.2	99
Pp-36	Soil	Seram	Maluku	-	2012	KP183958	*Phytopythium* sp. AL-2010	HQ643400.2	98
Pp-41	Soil	Cagak	Jawa Barat	+	2013	KP183961	*Phytopythium* sp. AL-2010	HQ643400.2	99
Pp-42	Soil	Cagak	Jawa Barat	+	2013	KP183962	*Phytopythium* sp. AL-2010	HQ643400.2	99
Pp-08	Soil	Sumani	Sumatra Barat	+	2011	KP183936	*Phytophthora cinnamomi* var. parvispora	GU191211.1	100
Pp-11	Soil	Prafi SP 4	Papua Barat	-	2011	KP183939	*Phytophthora cinnamomi* var. parvispora	GU191211.1	100
Pp-43	Leaf	Wera	Jawa Barat	+	2013	KP183963	*Phytophthora palmivora*	AM422704.1	99
Pp-15	Soil	Wera	Jawa Barat	+	2011	KP183943	*Pythium* sp. D37	JN863978.1	98
Pp-45	Fruit	Wera	Jawa Barat	+	2014	KP183964	*Pythium deliense* CBS 314.33	AY598674.2	99

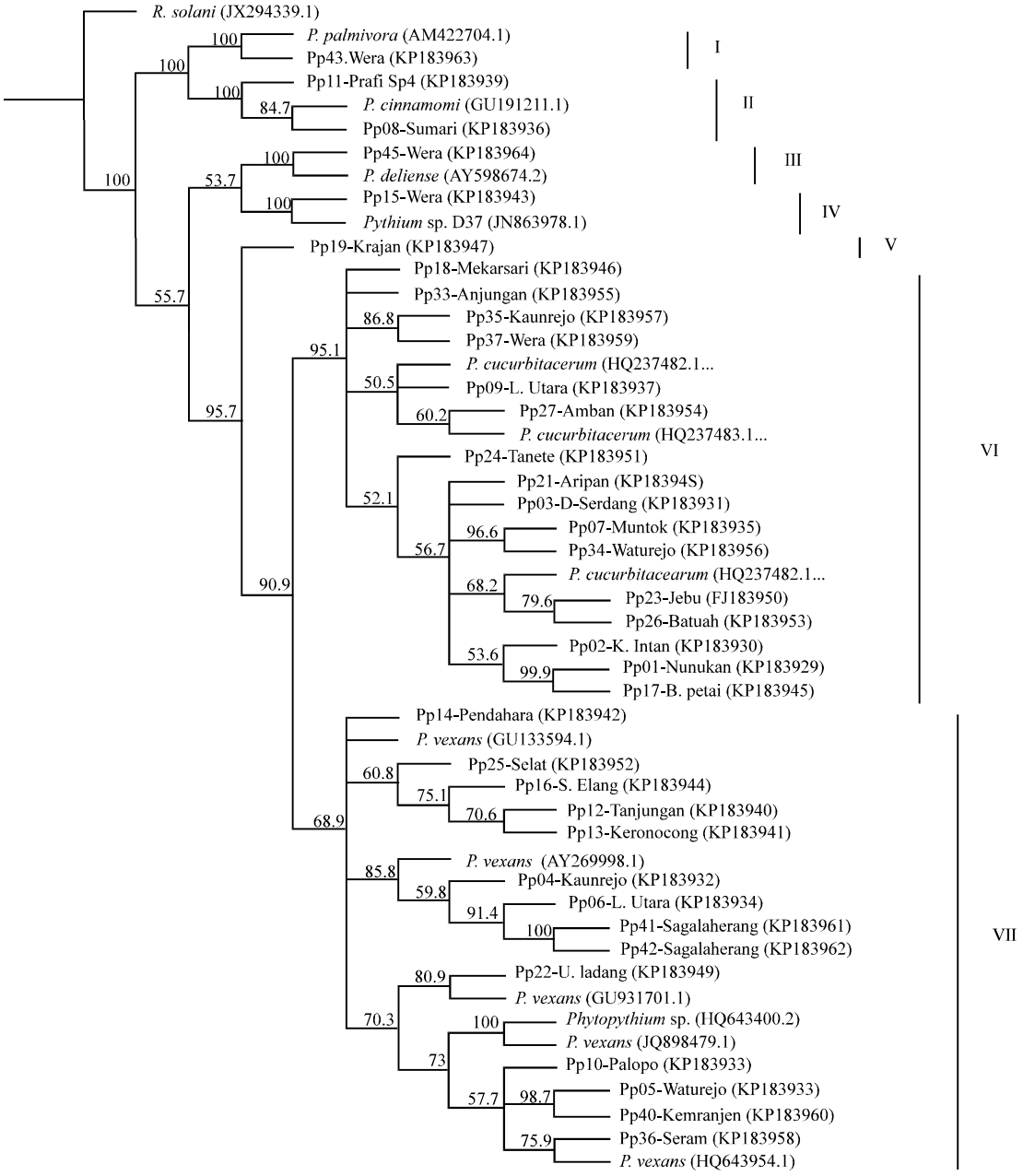

Fig. 1: Phylogenetic tree representing the relationship of *Pythium* and *Phytophthora* associated with durian in Indonesia. I: *Phytophthora palmivora*, II: *Phytophthora cinnamomi* var. parvispora, III: *Pythium deliense*, IV: *Pythium* sp., D37, V: Pp-19 Krajan, VI: *Pythium cucurbitacearum*, VII: *Pythium vexans*

Amongst the isolates identical to *Pythium vexans*, Pp-19 Krajan stands a distinct clade outside the groups. This isolate is considered to be different to the six species obtained, or might be a new phylotype since the alignment has only 96% identity (Pryor and Gilbertson, 2000; Liu *et al*., 2010) with the corresponding species *Pythium vexans* STE-U6728 (GU133594) and lower identity value with another *Pythium vexans* strains.

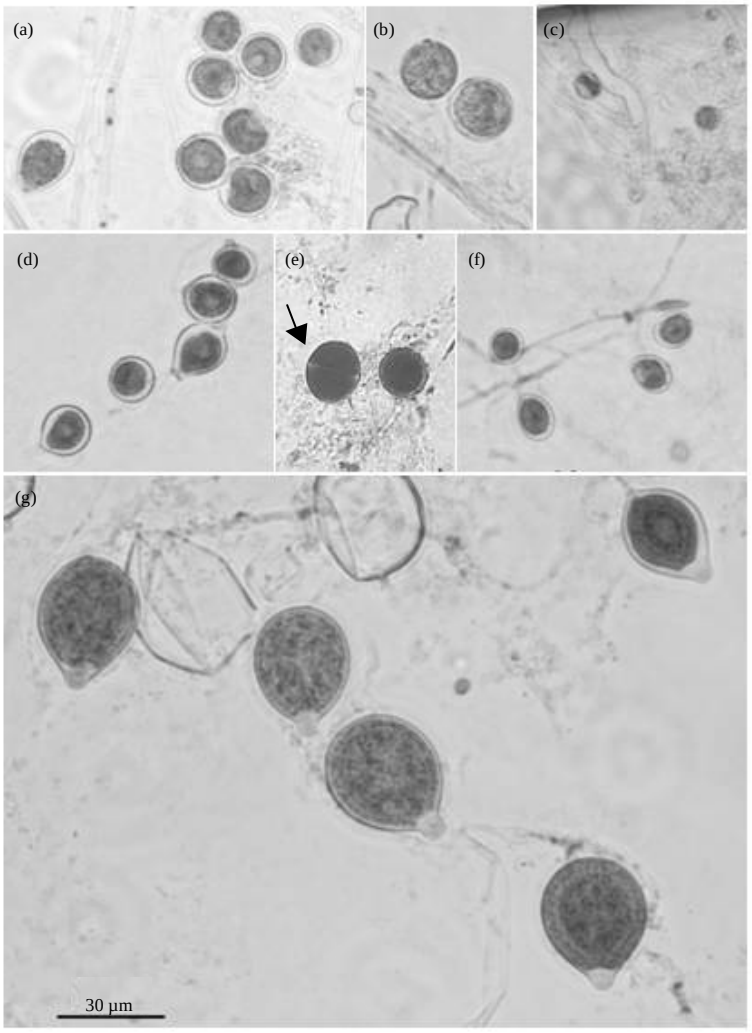

Fig. 2(a-g): Sporangium shapes of *Pythium* and *Phytophthora* associated with durian in Indonesia, (a) *Pythium cucurbitacearum*, (b) *Pythium* sp., D37, (c) *Phytophthora cinnamomi*, (d) *Pythium vexans*, (e) *Pythium deliense*, (f) *Phytopythium* sp. and (g) *Phytophthora palmivora*

Morphological characterization: Morphological characterizations were conducted through observation of colony motif and sporangia apparent (Table 2). Three basic types of colony motif were found on the isolate culture. Those are petallate, stellate and stoloniferous, each of 20, 15 and 1 isolate, respectively. Petallate motif consists of 3 sub-motifs; they were narrow petallate, medium and wide petallate, each of 4, 14 and 2 isolates, respectively. The stellate consists of 2 sub-motifs namely normal stellate and fluffy stellate, each of 14 and 1 isolate, respectively. Two main motifs, patellate and stellate, were found on the 35 isolates identical to the six species of *Pythium* and *Phytophthora*. Whilst, stoloniferous was only found as the motif of isolate Pp-15 Wera.

Sporangiums of the isolates identical to *Pythium cucurbitacearum* (Fig. 2a) were dominated by globose shape. Of which could also be found another minor shape such as ovoid, ellipsoid,

Table 2: Morphological characteristics of *Pythium* and *Phytophthora* associated durian in Indonesia

				Sporangium			
Isolate	Origin	GenBank Acc. No.	Colony motif	Shape	Length (µm)	Wide (µm)	Papilla
Pp-01	Nunukan	KP183929	Petalate	Globose-ovoid	11-17	9-15	+
Pp-02	Karang Intan	KP183930	Petalate	Globose-ovoid-ellipsoid-lemonform	14-18	13-18	+/-
Pp-03	Deli Serdang	KP183931	Petalate	Globose-ellipsoid-obpyriform-ovoid	12-19	11-18	+/-
Pp-07	Muntok	KP183935	Petalate	Globose-ellipsoid-ovoid	9-15	8-14	-
Pp-09	Luwu Utara	KP183937	Narrow petallate	Globose-ovoid-oblong	7-15	5-12	+/-
Pp-17	Bagan Petai	KP183945	Petalate	Globose-lemonform-ovoid	11-16	10-15	-
Pp-18	Mekarsari	KP183946	Stellate	Globose-ovoid-elipsoid-oblong	12-23	11-22	+/-
Pp-21	Aripan	KP183948	Stelatte	-na-	-na-	-na-	-na-
Pp-23	Jebu	KP183950	Stellate	-na-	-na-	-na-	-na-
Pp-26	Batuah	KP183953	Stellate	-na-	-na-	-na-	-na-
Pp-27	Amban	KP183954	Stellate	Globose-ellipsoid	11-19	9-17	-
Pp-33	Anjungan BBI	KP183955	Stellate	Globose-ovoid-lemonform	14-21	13-17	+/-
Pp-34	Waturejo	KP183956	Petalate	Globose-ovoid-ellipsoid-obpyriform-irreguler	8-25	8-21	+/-
Pp-35	Kaumrejo	KP183957	Narrow petallate	Globose-ovoid	12-23	11-21	+/-
Pp-37	Wera	KP183959	Stellate	Globose-lemonform-ovoid	11-19	10-17	+/-
Pp-10	Palopo	KP183938	Stellate	Globose	7-22	7-21	+/-
Pp-12	Tanjungan	KP183940	Petalate	Globose-ovoid-irreguler	12-19	11-18	-
Pp-14	Pendahara	KP183942	Petalate	Globose-ovoid	12-21	11-18	+/-
Pp-16	Sungai Elang	KP183944	Stellate	Globose-ovoid-irreguler	14-18	13-16	-
Pp-19	Krajan	KP183947	Narrow petallate	Globose-ellipsoid	12-27	12-24	-
Pp-22	Ujung Ladang	KP183949	Petallate	Globose-ellipsoid-ovoid-obpyriform-irreguler	10-28	10-16	+/-
Pp-24	Tanete	KP183951	Petalate	Globose-ellipsoid-ovoid-obpyriform-irreguler	12-20	11-13	+/-
Pp-25	Selat	KP183952	Narrow petallate	Globose-ovoid-lemorform-oblong-ellipsoid	10-20	9-18	+/-
Pp-40	Kemranjen	KP183960	Stellate	Globose-lemonform-ovoid	9-18	8-17	+/-
Pp-04	Kaumrejo	KP183932	Petalate	Globose-ovoid-oblong-irreguler	9-16	8-13	+/-
Pp-05	Waturejo	KP183933	Petalate	Globose-oblong-ellipsoid	9-15	8-14	+/-
Pp-06	Luwu Utara	KP183934	Petalate	Globose	8-17	8-16	+/-
Pp-13	Keroncong	KP183941	Petalate	Globose-ovoid-obpyriform-oblong-irreguler	7-15	6-14	+/-
Pp-36	Seram	KP183958	Stellate	Globose	17-23	16-22	-
Pp-41	Cagak	KP183961	Stellate	Globose-ovoid	11-17	10-16	+/-
Pp-42	Cagak	KP183962	Stellate	Globose-ovoid-oblong	7-16	6-14	+/-
Pp-08	Sumani	KP183936	Wide petallate	Globose-irreguler-ovoid-ellipsoid-oblong	8-15	7-12	-
Pp-11	Prafi SP 4	KP183939	Fluffy stellate	Globose	8-26	8-25	-
Pp-43	Wera	KP183963	Stellate	Ovoid-lemonform-obpyriform-globose-irreguler	28-51	19-39	+
Pp-15	Wera	KP183943	Stoloniferous	Globose-ovoid-obpyriform-lemonform	15-25	15-23	-
Pp-45	Wera	KP183964	Wide petallate	Globose-ellipsoid	18-26	17-22	-

na: Data not available, +: Papillated sporangium, -: Unpapillated sporangium

obpyriform, lemon form and irregular. The sporangia were generally did not have papillae except for some sporangia which have papillae. In general, the sporangium size of the isolates in this group ranged from the smallest of 7 μm length×5 μm width, to the largest of 25 μm length×22 μm width.

Similar to isolates identical to *Pythium cucurbitacearum*, the sporangiums of the isolates identical to *Pythium vexans* (Fig. 2d) were also dominated by globose shape, meanwhile another shape such as ovoid, ellipsoid, oblong, lemon form, obpyriform and irregular were also there in very small number. The sporangia were generally absent of papillae except for a small number of sporangia which were present. In general, the sizes of sporangium of the isolates in this group were ranging from the smallest of 7 μm length×5 μm width, to the largest of 28 μm length×24 μm width. Isolates identical to *Phytopythium* sp. (Fig. 2f) also have characteristic similar to previous isolate groups *Pythium cucurbitacearum* and *Pythium vexans* in shape and the presence or absence of papillae. The sporangium size was ranging from the smallest of 7 μm length×6 μm width to the largest of 23 μm length×22 μm width. Characteristic similarity of the isolates with those identical to *Pythium vexans* are in conformity with the phylogenetic tree where generally the two groups merge into one clade.

Sporangium shape of isolate identical to *Pythium* sp. D37 (Fig. 2b) were all globose. Unlike the previous three isolate groups, the only isolates (Pp-15) obtained identical to this species does not have the papillae. It has sporangium width of 15-23 μm which is greater than the three preceding groups.

The sporangia of isolates identical to *Pythium deliense* (Fig. 2e) were globose and ellipsoidal. They did not have any papillae. The sizes were larger than the average of all *Pythium* species observed in this research. They were 18-26 μm length and 17-22 μm width. Unique characteristic of this isolate compared to all other isolates observed is that in the ellipsoid sporangium apparently composed of two compartments.

Isolates identical to *Phytophthora cinnamomi* var., parvispora (Fig. 2c) have sporangium which dominated by globose shape. The other shapes such as ovoid, ellipsoid, oblong and irregular were also found. They did not have any papillae at all. In general, the uniqueness of these isolate is the small size of sporangiums which were about 7-8 μm width or length, except for irregular shapes which were generally longer.

Unlike the six previous groups, the sporangiums of Pp-43 isolate which identical to *Phytophthora palmivora* (Fig. 2g), were dominated by ovoid and obpyriform shapes. Although other shapes such as globose and irregular also could be found. In general the sporangium seems more prominent because of the relatively larger size with length and width ranging 28-51 and 19-39 μm, respectively. It is 3-5 times fold the size of the sporangium of the other isolates. Another specific characteristic of this isolate is all sporangium has papillae, even though the globose shape.

Geographical distribution: Amongst six corresponding species, two species *Pythium cucurbitacearum* and *Pythium vexans* were dominant isolates found association with durian in Indonesia. Of the 17 provinces observed, *Pythium cucurbitacearum* were found in 13 provinces (76.5%) and *Pythium vexans* were in 10 provinces (58.8%). Both species were also found to be existed together in six provinces. *Phytophthora cinnamomi* were found in two provinces, whilst another three species, *Phytophthora palmivora*, *Pythium deliense* and *Pythium* sp., D37 were only found in one location (Fig. 3).

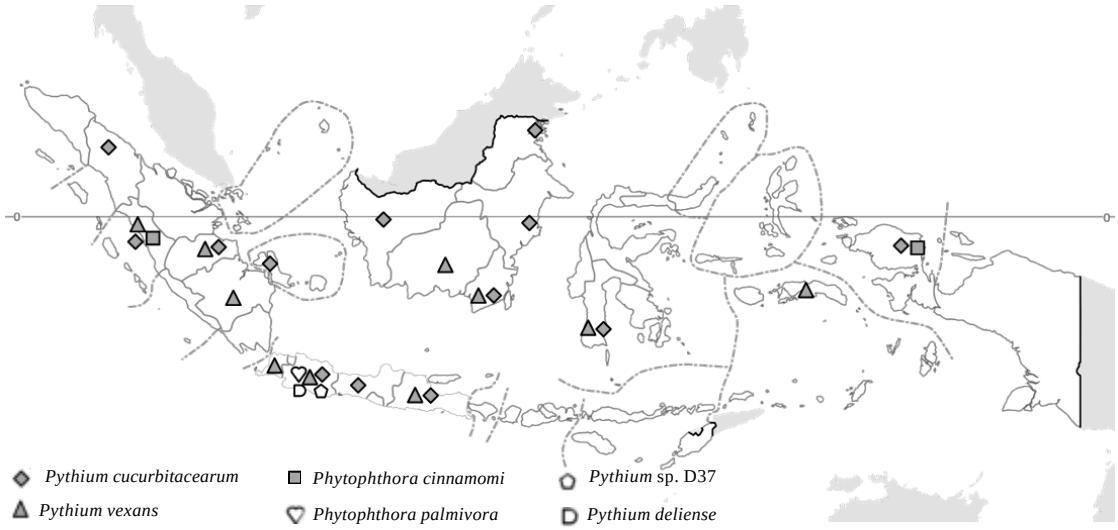

Fig. 3: Distribution maps of *Pythium* and *Phytophthora* associated with durian in Indonesia

DISCUSSION

Tree decline is a serious disease of durian tree in Indonesia. However, due to the culture practices is still dominated by non-intensive cultivation; the disease has not been widely perceived by the growers. This disease is almost related to *Phytophthora palmivora* as the responsible pathogen (Muryati *et al*., 2009; Purwantara *et al*., 2004; Emilda, 2007; Sunarwati *et al*., 2007). Meanwhile, Vawdrey *et al*. (2005) reported the *Pythium vexans* also found associated with durian tree-decline.

Recent development of durian cultivation in Indonesia which is starting to shift from subsistence towards intensive culture practices need to prepare disease management control. Because of intensive agriculture practices usually lead to increase disease problems (Drenth and Guest, 2004). At the beginning is necessary to identify the pathogen accurately and its distribution to create more specific and effective strategic control.

In this study, a total of 36 Pythiaceae isolates were found associated with durian in Indonesia. These isolates were found both in the location that there is disease incidence and no incidence at the time of collection. The presence of pathogens in both types of locations is indicating that the fungi are able to survive in the soil for long time. Vawdrey *et al*. (2005) reported that two species of Pythiaceae, *Phytophthora palmivora* and *Pythium vexans* were found in two climatic conditions, wet and dry, however no information how long these fungi can survive in the soil. As comparison, *Fusarium oxysporum* (foc) that attack banana can survive for 30 years in the soil without a host (Ploetz, 2006). Further research to identify how long they could survive in the soil is important to diseases management.

Molecular identification using ITS sequences found all 36 isolates correspond to six Phythiaceae species: *Pythium cucurbitacearum*, *Pythium vexans*, *Phytophthora cinnamomi*, *Phytophthora palmivora*, *Pythium* sp., D37 and *Pythium deliense*. Two species *Pythium cucurbitacearum* and *Pythium vexans* consisted of ten distinct strains or phylotypes. This is more numerous than those found by Vawdrey *et al*. (2005) in Queensland, Australia which

found *Phytophthora palmivora* and *Pythium vexans*. Variations which arise at the species level and at strain or phylotype are not surprisingly, because this country is known as one of the mega-biodiversity in the world. Beside as the center of origin and diversity of the genus *Durio* (Kostermans, 1958; Brown, 1997), Indonesia also grows many other potential hosts for Pythiaceae. For a long time this condition, therefore, could emerge new pathogen species as a result of reciprocated interaction between them (Frank, 1992).

The diversity is also indicated by the variation in colony motifs. There are six motifs were found which is slightly different with that found by Pongpisutta and Sangchote (2004). However, comparison amongst colony motifs and molecular identification results seem no correlation were found. Unlike Pp-15 isolate (correspond to *Pythium* sp., D37) from Wera which has the only stoloniferous motif, another isolates might have a stellate or patellate motif even though they are *Pythium* sp., or *Phytophthora* sp. Based on this finding, it was found that the motive colony could not be used as an identifier to distinguish between *Phytophthora* and *Pythium* species.

Among the isolates observed, it was generally found six types of sporangium, those are globose, ovoid, ellipsoid, oblong, lemon form and obpyriform, as found by Pongpisutta and Sangchote (2004) on the isolate of *Phytophthora palmivora* from durian. However, there is prominent variation in the size of sporangium found among isolates in this study (Fig. 2). Isolates in a group of identical species generally have diverse sporangium and almost all the basic shapes are there in the same identical group. It leads difficulty to differentiate morphologically. Amongst inter-species isolates are generally also difficult to distinguish. Such isolates identical to the two species, *Pythium cucurbitacearum* and *Pythium vexans*, they share identical characteristics of sporangium shape and size. However, by using the molecular tools such as ITS region was able to distinguish amongst the species (Appiah *et al*., 2004; Belbahri *et al*., 2008), even though at lower taxa as shown by the phylogenetic tree (Fig. 1).

Isolate Pp-43 which identical to *Phytophthora palmivora* is only obtained from leaf samples, while Pp-45 which identical to *Pythium deliense* is from the fruit. Both are different from another 34 isolates were obtained from the soil, even though from two isolates obtained from the same location (Pp-15 and Pp-37). It is, therefore, assumed that each fungus has different preferences on plant organs, or they have different modes of transmission. *Phytophthora palmivora* is able to survive and spread through the air (Drent and Guest 2004), is a reasonable answer for the finding isolate Pp-43 from the leave.

The present study demonstrated the first publication for the association of *Pythium cucurbitacearum* with tree-decline of durian. *Pythium cucurbitacearum* together with *Pythium vexans* provide evidence their present throughout the region observed (Fig. 3). Based on these findings we could infer that pathogen that attack durian which called as the dry-*Phytophthora* is referring to the *Phythium* species, it could be *Pythium vexans* or *Pythium cucurbitacearum*, as both species are more commonly found in most of the soil samples compared to *Phytophthora*. This is supported by the symptom found on the durian stem which showed absent of gummosis or wet cancer (Thompson, 1934, 1938).

ACKNOWLEDGMENT

This study was funded by Indonesian Agency for Agricultural Research and Development through program KKP3T Fiscal Year 2011-2013.

REFERENCES

Appiah, A.A., J. Flood, S.A. Archer and P.D. Bridge, 2004. Molecular analysis of the major *Phytophthora* species on cocoa. Plant Pathol., 53: 209-219.

Baldauf, S.L., 2008. An overview of the phylogeny and diversity of eukaryotes. J. Syst. Evol., 46: 263-273.

Baldwin, B.G., M.J. Sanderson, J.M. Porter, M.F. Wojciechowski, C.S. Campbell and M.J. Donoghue, 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Ann. Missouri Bot. Garden, 82: 247-277.

Belbahri, L., A. Le McLeod, B. Paul, G. Calmin and E. Moralejo *et al.*, 2008. Intraspecific and within-isolate sequence variation in the ITS rRNA gene region of *Pythium mercurial* sp. nov. (Pythiaceae). FEMS Microbiol. Lett., 284: 17-27.

Brown, M.J., 1997. *Durio*-A Bibliographic Review. International Plant Genetics Research Institute, New Delhi, ISBN-13: 978-9290433187, pp: 188.

Cooke, D.E.L. and J.M. Duncan, 1997. Phylogenetic analysis of *Phytophthora* species based on ITS1 and ITS2 sequences of the ribosomal RNA gene repeat. Mycol. Res., 6: 667-677.

Crous, P.W., J.Z. Groenewald and W. Gams, 2003. Eyespot of cereals revisited: ITS phylogeny reveals new species relationships. Eur. J. Plant Pathol., 109: 841-850.

Drenth, A. and B. Sendall, 2004. Economic Impact of *Phytophthora* Diseases in Southeast Asia. In: Diversity and Management of *Phytophthora* in Southeast Asia, Drenth, A. and D.I. Guest (Eds.). Australian Centre for International Agricultural Research, Australia, ISBN-13: 9781863204057.

Drenth, A. and D.I. Guest, 2004. Diversity and Management of *Phytophthora* in Southeast Asia. Australian Centre for International Agricultural Research, Australia, ISBN-13: 9781863204057, Pages: 238.

Emilda, D., 2007. Protocol for *In-vitro* rapid identification of durian variety resistancy to *Phytophthora palmivora*. Buletin Teknik Pertanian, 12: 59-62, (In Indonesian).

Frank, S.A., 1992. Models of plant-pathogen coevolution. Trends Genet., 8: 213-219.

Godfrey, S.A.C., R.D. Monds, D.T. Lash and J.W. Marshall, 2003. Identification of *Pythium oligandrum* using species-specific ITS rDNA PCR oligonucleotides. Mycol. Res., 107: 790-796.

Grunwald, N.J., F.N. Martin, M.M. Larsen, C.M. Sullivan and M.D. Press *et al.*, 2011. Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Dis., 95: 337-342.

Hasan, N.M. and L.B. Siew, 2000. Integrated management of durian cancer. Proceedings of the Durian Seminar 2000: Toward Stability of Quality Output and Marketing, August 1-3, 2000, Ipoh, Perak, Malaysia.

Jeffer, S.N. and S.B. Martin, 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis., 70: 1038-1043.

Kong, P., B.M. Tyler, P.A. Richardson, B.W.K. Lee, Z.S. Zhou and C. Hong, 2010. Zoospore interspecific signaling promotes plant infection by *Phytophthora*. BMC Microbiol., Vol. 10. 10.1186/1471-2180-10-313

Kostermans, A.J.G.H., 1958. The genus *Durio* adans. (Bombac.). Reinwardtia, 4: 47-153.

Kueh, T.K. and K.L. Khew, 1982. Survival of *Phytophthora palmivora* in soil and after passing through alimentary canals of snails. Plant Dis., 66: 897-899.

Lee, B.S. and K.Y. Lum, 2004. *Phytophthora* Diseases in Malaysia. In: Diversity and Management of *Phytophthora* in Southeast Asia, Drenth, A. and D.I. Guest (Eds.). Australian Centre for International Agricultural Research, Australia, ISBN-13: 9781863204057.

Lim, T.K., 1990. Durian: Diseases and Disorders. Tropical Press, Malaysia, Pages: 95.

Liu, A., F. Bao, C. Zhang, M. Li, M. Shi, J. Saising and P. Shen, 2010. Biodiversity of cultivable fungi in hair samples from tree shrews. Afr. J. Microbiol. Res., 4: 2704-2707.

McLeod, A., W.J. Botha, J.C. Meitz, C.F. Spies, Y.T. Tewoldemedhin and L. Mostert, 2009. Morphological and phylogenetic analyses of Pythium species in South Africa. Mycol. Res., 113: 933-951.

MoA., 2012. Agricultural statistic 2012. Ministry of Agriculture Republic of Indonesia, Center for Agricultural Data and Information System.

Muryati, L. Octriana, D. Emilda, P.J. Santoso and D. Sunarwati, 2009. Effect of organic fertilizers on susceptibility of potted durian seedlings to *Phytophthora* diseases. J. Fruit Ornamental Plant Res., 17: 67-77.

Park, B., F. Martin, D.M. Geiser, H.S. Kim and M.A. Mansfield *et al.*, 2013. Phytophthora database 2.0: Update and future direction. Phytopathology, 103: 1204-1208.

Ploetz, R.C., 2006. Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum* f. sp. cubense. Phytopathology, 96: 653-656.

Pongpisutta, R. and S. Sangchote, 2004. Morphological and Host Range Variability in *Phytophthora palmivora* from Durian in Thailand. In: Diversity and Management of *Phytophthora* in Southeast Asia, Drenth, A. and D.I. Guest (Eds.). Australian Centre for International Agricultural Research, Australia, ISBN-13: 9781863204057.

Pryor, B.M. and R.L. Gilbertson, 2000. Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. Mycol. Res., 104: 1312-1321.

Purwantara, A., D. Manohara and J.S. Waroka, 2004. Phytophthora Diseases in Indonesia. In: Diversity and Management of *Phytophthora* in Southeast Asia, Drenth, A. and D.I. Guest (Eds.). Australian Centre for International Agricultural Research, Australia, ISBN-13: 9781863204057, pp: 70-76.

Sivapalan, A., F.H. Hamdan and M.A.H.M. Junaidy, 1997. Patch canker of *Durio zibethinus* caused by *Phytophthora palmivora* in brunei darussalam. Plant Dis., 81: 113-113.

Somsri, S., 2014. Current status of durian breeding program in Thailand. Acta Hortic., 1024: 51-60.

Spies, C.F.J., M. Mazzola and A. McLeod, 2011. Characterisation and detection of Pythium and Phytophthora species associated with grapevines in South Africa. Eur. J. Plant Pathol., 131: 103-119.

Sunarwati, D., P. J. Santoso and D. Emilda, 2007. Identification of pathogen causing root rot and stem cancer of durian (*Durio zibethinus* Murr.) in several production center. Proceeding of National Seminar: Innovation and Technology Transfer of Specific Location Supporting Agricultural Revitalization, June 5, 2007, Medan, pp: 330-337.

Thompson, A., 1934. A disease of durian trees. Malaysian Agric. J., 22: 369-371.

Thompson, A., 1938. A root disease caused by *Pythium complectens* braun. Malaysian Agric. J., 26: 460-464.

Van der Plaats-Niterink, A.J., 1981. Monograph of the genus *Pythium*. Stud. Mycol., 21: 1-244.

Vawdrey, L.L., P. Langdon and T. Martin, 2005. Incidence and pathogenicity of *Phytophthora palmivora* and *Pythium vexans* associated with durian decline in far northern Queensland. Aust. Plant Pathol., 34: 127-128.

Zappala, G., A. Zappala and Y. Diczbalis, 2002. Durian germplasm evaluation for tropical Australia *Phase* 1. RIRDC Project No. ZTR-1A, A report for the Rural Industries Research and Development Corporation, July 2002, Australia, pp: 1-100.