Jurnal Perlindungan Tanaman Indonesia, Vol. 22, No. 1, 2018: 13–19
DOI: 10.22146/jpti.24728

**Research Article**

# Identification of the Causal Agent of Cocoa Pod Rot Disease from Various Locations

## *Identifikasi Penyebab Penyakit Busuk Buah Kakao dari Beberapa Lokasi*

**Indri Komalasari[1)]*, Suryanti[2)], & Bambang Hadisutrisno[2)]**

*[1)]Tarakan Agricultural Quarantine Service, Indonesian Agricultural Quarantine Agency, Ministry of Agriculture*
*Jln. Yos Sudarso No. 11, Lingkas Ujung, Kota Tarakan, North Kalimantan, 77126*

*[2)]Department of Crop Protection, Faculty of Agriculture, Universitas Gadjah Mada*
*Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281*

**Corresponding author. E-mail: indri.hira01@gmail.com*

Submitted May 2, 2017; accepted September 20, 2017

## ABSTRACT

Cacao (*Theboroma cacao* L.) is an important estate commodity in Indonesia with high economic value. The interference of cocoa pod rot disease which was affected by *Phytophthora palmivora* Butl. resulted in the reduction of the quantity and quality of cocoa beans, with losses up to 44%. This research was aimed to figure out the variation in morphology of *P. palmivora* isolates from cacao. The research was carried out by collecting samples of cocoa pod with rot symptoms in several cacao growing areas in Java, then the pathogen was isolated and cultured on Potato Dextrose Agar (PDA) medium. The observation was performed on morphological characteristics of isolates macroscopically (colony shape) and microscopically (size of sporangium and chlamydospores). All tested isolates showed various colony shape such as stellate, cottony and irregular as well as sporangium varying from obpyriform, globose, ellipsoid, ovoid and distorted with various size between 30.8×21.9–65.5×46.5 µm in range.

Keywords: cacao, morphological variation, *Phytophthora palmivora*

## *INTISARI*

*Kakao (*Theboroma cacao *L.) merupakan komoditas perkebunan unggulan di Indonesia dengan nilai ekonomi tinggi. Gangguan penyakit busuk buah kakao yang disebabkan oleh* Phytophthora palmivora *Butl. mengakibatkan penurunan kuantitas dan kualitas biji kakao, dengan kerugian mencapai 44%. Penelitian ini bertujuan untuk mengetahui variasi morfologi isolat* P. palmivora *asal kakao. Penelitian dilakukan dengan mengambil sampel buah kakao bergejala busuk buah di beberapa area perkebunan kakao di Jawa, kemudian patogen diisolasi dan dikulturkan pada media Potato Dextrose Agar (PDA). Pengamatan dilakukan terhadap karakteristik morfologi isolat secara makroskopis (bentuk koloni) dan mikroskopis (ukuran sporangium dan klamidospora). Semua isolat yang diuji menunjukkan bentuk koloni seperti* stellate*,* cottony*, dan* irregular *serta sporangium yang bervariasi dari* obpyriform*,* globose*,* ellipsoid*,* ovoid*, dan* distorted *dengan ukuran bervariasi antara 30,8×21,9−65,5×46,5 µm.*

*Kata kunci: kakao,* Phytophthora palmivora*, variasi morfologi*

## INTRODUCTION

Cocoa pod rot which was caused by *Phytophthora palmivora* Butl. was the most important disease in cultivating cacao in Indonesia (Semangun, 2008). Yield losses resulted in the interference of cocoa pod rot disease in Indonesia reached 44% due to the reduction in quality and quantity of cocoa bean production (Rubiyo & Amaria, 2013). *P. palmivora* could attack almost whole parts of cacao plant, such as stem, flower cushion and leaves. The most destructive invasion occurred on pod since it directly related to yield loss (Opeke & Gorenz, 1974; Sri-Sukamto, 1985; Purwantara, 1990; Priyatmojo & Subandiyah, 1996; Rubiyo & Amaria, 2013). The initial symptom was spot on pod, then developed quickly and extended until covered whole surface of pod (Guest, 2007). On further infection, pathogen could invade the beans with symptom of blackening and wrinkling cocoa beans (Bowers *et al*., 2001).

Wahyuno *et al*. (2007) reported that isolate of *Phytophthora* possessed various shape of sporangium, i.e. spherical to pear or lemon-shape with distinct papillae on the tip of sporangium. The investigation revealed that *P. capsici* from white pepper had the length of sporangium between 20 – 88.8 µm, breadth of

17.5–55 µm, and length (l) to breadth (b) ration of sporangium between 0.9–2.8. This research was aimed to figure out the variation on morphological characteristics of *P. palmivora* isolates, causal agent of cocoa pod rot disease, from several locations of cacao plantations.

## MATERIALS AND METHODS

This experiment was carried out in Laboratory of Plant Disease, Department of Crop Protection, Faculty of Agriculture, Universitas Gadjah Mada. Samples of *P. palmivora*-infected cocoa pod were collected from several cacao plantations, i.e. West Java (Sumedang and Cianjur), Central Java (Batang and Wonosobo), Daerah Istimewa Yogyakarta (Kulon Progo and Gunung Kidul), as well as East Java (Jember).

### *Preparaton of Isolates*

Pathogen was isolated from part of cocoa pod showing symptom of pod rot. Symptomatic pods were washed with tapping water, and then surface-disinfected using ethanol 96%. The skin was peeled and pulp was cut in small size on adjacent part of healthy and diseased tissue, and then cultured on *Potato Dextrose Agar* (PDA) medium and incubated at temperature of 24°C. The emerged mycelium was then observed and directly identified under compound microscope. The growing mycelium was sub-cultured on the same medium to get the pure culture.

### *Molecular Identification*

Molecular identification was aimed to ensure that the obtained isolate was *P. palmivora*. This analysis was conducted using PCR method with specific primers for *P. palmivora* (pal1s and pal2a) with target size of 650 bp (Chirapongsatonkul *et al*., 2015). DNA extraction of *P. palmivora* isolates was performed using CTAB method (Subandiyah, 2003).

### *Morphological Identification of Isolates*

Morphological identification involved the observation of isolate characteristic macroscopically and microscopically. The observation of macroscopic morphology was performed on the growth of hyphae by daily measurement on the diameter of colony until 5 days after subculture and shape of colony on PDA medium. Meanwhile, the observation of microscopic morphology was performing by taking and putting the small cut of isolates from various locations on object glass which had been previously dropped with methylene blue solution and then warmed by passing them on Bunsen fire until melting and then covered with cover glass. Morphological characteristics were observed under compound microscope which was connected to Optilab software on computer. Shape and size of sporangium and chlamydospores were recorded.

### *Analysis of UPGMA*

Morphological variation of tested isolates was analyzed with Unweighed Pair Group Method with Arithmatic mean (UPGMA) using NTSys program.

## RESULTS AND DISCUSSION

*Phytophthora palmivora* was recognized having wide range of host plants such as coconut, cacao, papaya, durian, citrus, quinine and areca nut (Zentmyer, 1974; Agrios, 2005; Semangun, 2008). On cacao, the infection could occur in almost whole parts of plant, i.e. stem, flower, pod and leaf surface. Cocoa pod was the most susceptible part against the invasion of *P. palmivora*. Pathogen could infect at all stages of pod development, and immature pod was the most susceptible phage toward pathogen attack. The initial symptom of infection was spot on cocoa pod which would quickly develop within 14 days and then could extend covering whole pod's surface. Such symptoms were various on pod, i.e. from the tip of pod, base of pod close to stalk and irregular pattern. Further infection was indicated with the emergence of white powder which was the sporangium of *P. palmivora* on surface of diseased pod (Figure 1). At the same time, neighboring pod either on the same or different trees would express varying symptoms (Bowers *et al*., 2001; Guest, 2007; Rubiyo *et al*., 2008; Vanegtern *et al*., 2015).

### *Molecular Identification*

The PCR test showed that DNA from all isolates could be amplified at 650 bp (Figure 2). It proved that 13 collected isolates from rotting pods of 8 surveyed locations were *P. Palmivora*, the causal agent of black pod diseases on cocoa. The previous research of Chirapongsatonkul *et al*. (2015) also found that molecular identification of *P. palmivora* isolates with PCR method using specific primers of Pal1s and Pal2a could amplify the target DNA at 650 bp.

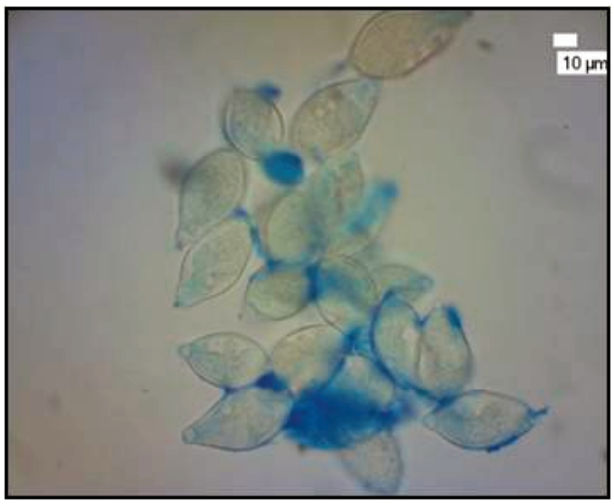


Figure 1. Sporangium which were directly isolated from the surface of infected cacao pod

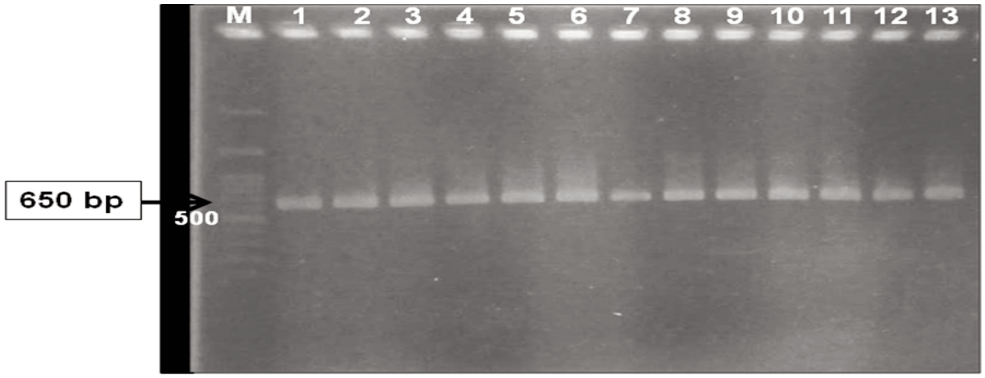


Figure 2. PCR results of *Phytophthora palmivora* isolates with spesific primer Pal1s and Pal2a; (M) DNA marker; isolat (1) JB.611; (2) SGL.164.1a; (3) SGL.289.1b; (4) GK.254.1a; (5) GK.274.1b; (6) PTK.2811; (7) SGY.213.a; (8) SGY.213.b; (9) WB.163; (10) SMD.218; (11) CJR.113.a; (12) CJR.113.b; (13) CJR.113.c

### *Macroscopic Characteristics*

Macroscopic observation was conducted on the shape and diameter of colony which was cultured on PDA media at 5 day after subculture. There were variations in mycelia growth and shape of colony from each isolate which were revealed on Table 1.

Table 1 showed that PTK.2811 isolate had the slowest growth of colony on PDA medium compared to other isolates, i.e. 37 mm at 5 day after subculture. This research grouped the variation in shape of colony into stellate, cottony and irregular (Figure 3).

Hyphae of *P. palmivora* on PDA medium was white and would grow downward or into medium, so that the colony looked thin on the surface of medium. In line with the research of Manti (2009), macroscopically colony of *P. palmivora* on PDA medium would grow slowly, round in shape with wavy margin, like cotton, white and elastic when it was cut using scalpel.

### *Microscopic Characteristics*

The result of microscopic observation on morphological features showed that there was difference of species characteristics between sampled isolates from several locations, i.e. variation in size as shown in Table 2.

Shape of sporangium on PDA medium varied, i.e. obpyriform, globose, ellipsoidal, disorted and ovoid in common (Figure 4). Size of sporangium was in range of 30.8×21.9–65.5×46.5 µm, and l/b ratio was between 1.4–1.8. *P. palmivora* had papillae on the tip of sporangium with range of 3.3–12.6 µm in size and pedicel measuring between 2.7–5 µm. Chlamydospores were spherical with size of 26.7–47.6 µm in range and were commonly established on the tip of hyphae. Hyphae was nonseptate (coenocytic), hyaline, ±13 µm in width and had swelling region (also known as hyphal swelling).

Table 1. Difference in type of symptoms, colony growth and the emergence of sporangium on PDA medium

| Code | Originating area | Altitude (m asl) | Type of symptom | Colony | |
|---|---|---|---|---|---|
| | | | | Diameter (mm) | Shape |
| JB.611 | Jember | 75 | Base | 75 | *Stellate* |
| SGL.164.1a | Kulonprogo | 470 | Tip | 65 | *Cottony* |
| SGL.289.1b | Kulonprogo | 575 | Base | 70 | *Cottony* |
| GK.254.1a | Gunung Kidul | 311 | Tip | 65 | *Cottony* |
| GK.274.1b | Gunung Kidul | 373 | Tip | 74 | *Cottony* |
| PTK.2811 | Gunung Kidul | 210 | Tip | 37 | *Cottony* |
| SGY.213.a | Batang | 90 | Tip | 60 | *Cottony* |
| SGY.213.b | Batang | 90 | Tip | 65 | *Cottony* |
| WB.163 | Wonosobo | 482 | Base | 44 | *Cottony* |
| SMD.218 | Sumedang | 910 | Base | 75 | *Cottony* |
| CJR.113.a | Cianjur | 525 | Tip | 65 | *Irregular* |
| CJR.113.b | Cianjur | 525 | Base | 60 | *Irregular* |
| CJR.113.c | Cianjur | 525 | Irregular | 55 | *Irregular* |

Remark: asl is abbreviation for above sea level

Table 2. Comparison on morphological size of isolates at 5 days after isolation

| Code of Isolates | Originating area | Sporangium | | | | | Diameter of chlamydospore (µm) |
|---|---|---|---|---|---|---|---|
| | | Pedicel (µm) | Length × breadth (µm) | l/b ratio *) | Length of papillae | Shape **) | |
| JB.611 | Jember | 3.6 | 59.2 × 39.8 | 1.5 | 12.6 | *Ob, G* | 31.2 |
| SGL.164.1a | Kulon Progo | 4.1 | 65.5 × 46.5 | 1.4 | 5.9 | *Ob, G* | 47.6 |
| SGL.289.1b | Kulon Progo | 3.6 | 61.5 × 36.7 | 1.7 | 6.8 | *O, G* | 43.1 |
| GK.254.1a | Gn. Kidul | 3.5 | 61.6 × 34.2 | 1.8 | 10.2 | *Ob, El, Dis* | 34.4 |
| GK.274.1b | Gn. Kidul | 2.4 | 56.6 × 36.7 | 1.5 | 8.7 | *O, G* | 40.9 |
| PTK.2811 | Gn. Kidul | 4.7 | 57.2 × 38.3 | 1.5 | 9.5 | *O, Dis* | 36.1 |
| SGY.213.a | Batang | 3.4 | 40.9 × 29.5 | 1.4 | 4.5 | *O, G* | 39.7 |
| SGY.213.b | Batang | 4.5 | 51.7 × 40.0 | 1.3 | 7.0 | *G* | 35.5 |
| WB.163 | Wonosobo | 3.2 | 45.9 × 30.0 | 1.5 | 5.3 | *O, El* | 32.8 |
| SMD.218 | Sumedang | 2.7 | 30.8 × 21.9 | 1.4 | 3.3 | *O* | 34.8 |
| CJR.113.a | Cianjur | 5.0 | 46.6 × 29.1 | 1.6 | 4.4 | *O, El* | 26.7 |
| CJR.113.b | Cianjur | 3.0 | 55.0 × 30.8 | 1.8 | 5.8 | *O, El* | 33.7 |
| CJR.113.c | Cianjur | 3.3 | 48.0 × 30.0 | 1.6 | 4.5 | *O, El* | 38.5 |

Remark:
*) length – breadth ratio
**) *Ob* = Obpyriform; *G* = Globose; *El* = Ellipsoidal; *Dis* = Disorted; *O* = Ovoid

Ellipsoidal sporangium had lengthening and slender form (Figure 3A), found on isolates of GK.254.1a, WB.163, CJR.113.a, CJR.113.b, and CJR.113.c. Obpyriform sporangium had broad and spherical form and its neck part prior to papillae was slight swelling and a little bit long (Figure 3B). This shape was observed on isolates of JB.611, SGL.164.1a and GK.254.1a. Disorted sporangium possessed irregular form (imperfect), found on isolates of GK.254.1a and PTK.2811 (Figure 3C). Globose sporangium (Figure 3D) had round form with the prominent papillae on the tip of sporangium. Such sporangium was observed on isolate of JB. 611, SGL.164.1a, SGL.289.1b, GK.274.1b, SGY.213.a and b. In general, the tested isolates on this experiment had ovoid sporangium (Figure 3E), indicated by its spherical form like egg and distinct papillae on the tip of sporangium.

Microscopically, this pathogen had non septate (coenocytic) hyphae with excessive and stiff branches (Manti, 2009). *P. palmivora* was characterized with

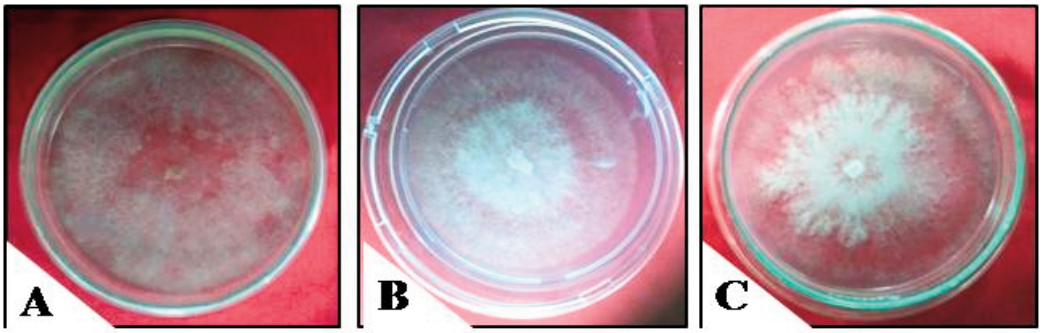


Figure 3. Variation of colony shape of isolates on PDA medium; (A) Stelate, (B) Cottony, (C) Irregular

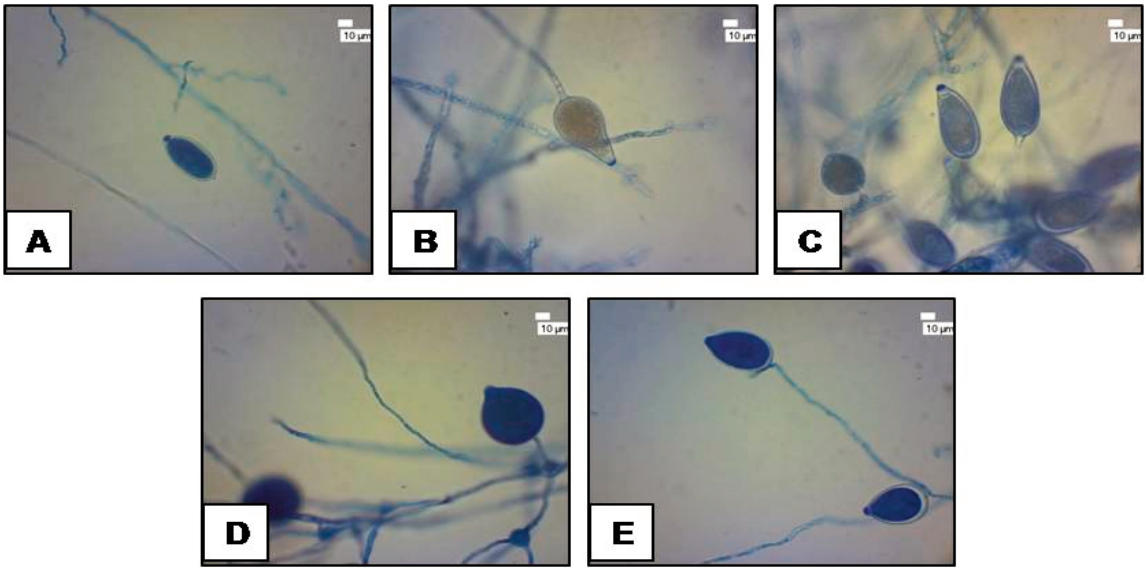


Figure 4. Variation on sporangium shape of *Phytophthora palmivora*; (A) ellipsoidal, (B) obpyriform, (C) disorted, (D) globose, (E) ovoid

commonly pear-shape sporangium (ovoid) about 30−60 × 20−53 µm in size, clear papillae, l/b ratio of 1.4 – 2, spherical and thick-walled chlamydospores, as well as transparent hyphae either on V8 (Mchau & Coffey, 1994) or PDA media (Umayah & Purwantara, 2006; Liswarni, 2011; Khairum *et.al*., 2016). Its sporangium was caducous (easy to be liberated from stalk of sporangium or sporangiofor) (Umayah & Purwantara, 2006).

All isolates were incubated on bright room under TL lightening to enhance the establishment of sporangium. Brasier (1969) explained that the formation of sporangium either in nature or artificial medium could be triggered by the presence of light.

The variation in morphological characteristics of *P. palmivora* isolates either macroscopic or microscopic was not influenced by environmental factors and altitude of cacao plantations. Variation in shape of colony and size of sporangium was also characteristics of *P. palmivora*. These were stated as well by Erwin and Ribeiro (1996) that the difference in morphological characteristics of *P. palmivora* species depended on those isolates of species.

Based on UPGMA analysis using 7 morphological combinations, 13 isolates were grouped into two clusters with similarity of 70% (Figure 5). The first group consisted of all isolates from Java Island excluded isolates from Cianjur (West Java) which was clustered into the second one together with one isolate from Gunung Kidul (Daerah Istimewa Yogyakarta). However, these clusters did not show the variablity on morphology of isolates against altitude and originating areas.

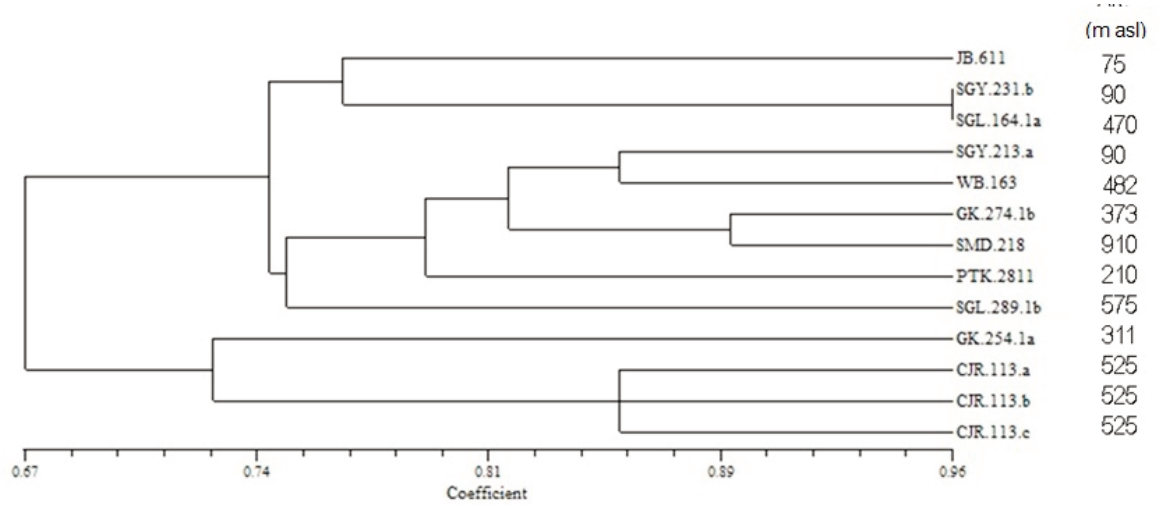


Figure 5. Dendrogram of tested *Phytophthora palmivora* isolates from cacao in Java Island

## CONCLUSION

There were variations in type of rotting symptoms on pod, i.e. from the tip of pod, base of pod close to stalk and irregular pattern. On PDA media, colony growth of *P. palmivora* varied, i.e. *stellate*, *cottony* and *irregular*. *P. palmivora* had several shapes of sporangium varying from obpyriform, globose, ellipsoidal, ovoid and distorted with various size between 30.8×21.9–65.5×46.5 µm in range.

## ACKNOWLEDGEMENTS

This study was conducted as part of research for program of master degree. Author would like to thank to Agricultural Institute for Extension and Human Resource Development (*Badan Penyuluhan dan Pengembangan Sumber Daya Manusia Pertanian* – BPPSDMP) Ministry of Agriculture for funding support in this research. The grateful expression goes to Director of PT Pagilaran, Indonesian Coffee and Cocoa Research Institute, as well as the owners of private cacao plantations for facilitating the sampling of cocoa pod in the farms.

## LITERATURE CITED

Agrios, G.N. 2005. *Plant Pathology*, 5thed. Acad. Press, USA. 922 p.

Bowers, J. H., B.A. Bailey, P.K. Hebbar, S. Sanogo & R. Lumsden. 2001. The Impact of Plant Diseases on World Chocolate Production. Online. *Plant Health Progress*. Doi: 10.1094/PHP-2001-07090-01-RV.http://www.apsnet.org/publications/apsnetfeatures/Pages/WorldChocolateProduction.aspx., modified 3/5/17.

Brasier, C.M. 1969. The Effect of Light and Temperature on Reproduction in vitro in Two Tropical Species of *Phytophthora*. *Transactions of the British Mycological Society* 52: 105−113.

Chirapongsatonkul, N., K. U-taynapun, T. Chanwun, & N. Churngchow. 2015. Development of a Multiplex PCR Assay for Rapid and Simultaneous Detection of Rubber Tree Pathogens *Phytophthora* spp. and *P. palmivora*. *Science* Asia 41: 170−179.

Erwin, D.C. & O.K. Ribeiro. 1996. *Phytophthora Diseases Worldwide*. APS Press, USA. 562 p.

Guest, D. 2007. Black Pod: Diverse Pathogens with a Global Impact on Cocoa Yield. *Phytopathology* 97: 1650−1653.

Khairum, A., A. Tharapreuksapong, S. Wongkaew & P.A. Tantasawat. 2016. Cultural Characteristics and Pathogenicity Analysis of Phytophthora palmivora, Causal Pathogen of Black Rot in Orchids, p. 105−110. *In* M.A. Rahman & P. Dasic (eds.), *International Conference on Agricultural, Food, Biological and Health Sciences* (AFBHS-16). Kuala Lumpur, August 22−24, 2016 .

Liswarni, Y. 2011. Insidensi Penyakit Busuk Buah (*Phytophthora palmivora* Butl.) pada Tanaman Kakao (*Theobroma cacao* L.) di Sentra Produksi Kakao Kabupaten Pasaman Barat. *Manggaro* 12: 43–48.

Manti, I. 2009. *Jenis dan Tingkat Serangan Penyakit Busuk Buah Kakao di Kabupaten Padang Pariaman.* http://sumbar.litbang.pertanian.go.id/ index.php/publikasi-1/40-karya-ilmiah-peneliti-dan-penyuluh/139-jenis-dan-tingkat-serangan-penyakit-busuk-buah-kakao-di-kabupaten-padang-pariaman, modified 21/6/16.

McHau, G.R.A & M.D. Coffey. 1994. Isozyme Diversity in *Phytophthora palmivora*: Evidence for a Southeast Asia Centre of Origin. *Mycological Research* 98: 1035–1043.

Opeke, L. K & A. M. Gorenz. 1974. *Phytophthora* Pod Rot: Symtoms and Economic Importance, p. 117−124. *In* P.H. Gregory (ed.), *Phytophthora Disease of Cocoa*. Longman Group Limited, London.

Priyatmojo, A & S. Subandiyah. 1996. Ekstraksi DNA *Phytophthora palmivora* Isolat Kelapa [Extraction of Coconut Isolate of *Phytophthora palmivora* DNA]. *Jurnal Perlindungan Tanaman Indonesia* 2: 1–4.

Purwantara, A. 1990. Pengaruh Beberapa Unsur Cuaca terhadap Infeksi *Phytophthora palmivora* pada Buah Kakao [Effect of Some Meteorological Elements on the Infection of *Phytophthora palmivora* on Cocoa Pod]. *Menara Perkebunan* 58: 78–83.

Rubiyo; A. Purwantara; S. Sukamto &S. Sudarsono. 2008. Isolation of Indigenous *Phytophthora palmivora* from Indonesia, their Morfological and Pathogenicity Characterization. *Pelita Perkebunan* 24: 35–48.

Rubiyo & W. Amaria. 2013. Ketahanan Tanaman Kakao terhadap Penyakit Busuk Buah (*Phytophthora palmivora* Butl.) [Resistance of Cocoa to Black Pod Disease (*Phytophthora palmivora* Butl.)]. *Perspektif* 12: 23–36.

Semangun, H. 2008. *Penyakit-Penyakit Tanaman Perkebunan di Indonesia*. Gadjah Mada University Press, Yogyakarta. 808 p.

Sri-Sukamto. 1985. *Phytophthora palmivora* Butl. Salah Satu Jamur Penyebab Penyakit pada Tanaman Kakao. *Menara Perkebunan* 53: 7–11.

Subandiyah, S. 2003. *Cara Kerja Ekstraksi DNA Menggunakan CTAB*. Workshop and Training Course on Molecular Detection for Plant and Environmental Protection. Fakultas Pertanian Universitas Gadjah Mada, Yogyakarta, December 15−20, 2003.

Umayah, A. & A. Purwantara. 2006. Identifikasi Isolat *Phytophthora* Asal Kakao [Identification of Isolates of *Phytophthora* from Cocoa]. *Menara Perkebunan* 74: 76 – 85.

Vanegtern, B.; M. Rogers & S. Nelson. 2015. Black Pod Rot of Cacao Caused by *Phytophthora palmivora*. *Plant Disease* 108: 1−5.

Wahyuno, D., D. Manohara & D.N. Susilowati. 2007. Variasi Morfologi dan Virulensi *Phytophthora capsici* Asal Lada. *Buletin Plasma Nutfah* 13: 70–81.

Zentmyer, G .A. 1974. Variation, Genetics, and Geographical Distribution of Mating Types, p. 89−101. *In* P.H. Gregory (eds.), *Phytophthora Disease of Cocoa*. Longman Group Limited, London.