Isolation of Indigenous Phytophthora palmivora from Indonesia, Their Morphological and Pathogenicity Characterization

Isolasi Phytophthora palmivora Asal Asli Indonesia, Karakterisasi Morfologis dan Patogenisitasnya

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Summary

Pathogenicity of Phytophthora palmivora isolates from various cacao production centers has not been evaluated. Moreover, isolates of this pathogen may change in time. Therefore, collection and identification of the existing P. palmivora need to be done from time to time. The specific objectives of this research were: (1) to collect indigenous isolates of P. palmivora from a number of cacao production centers in Indonesia, (2) to characterize the Indonesian indigenous isolates using various morphological characters, and (3) to evaluate pathogenicity of the indigenous isolates. The indigenous isolates of P. palmivora were isolated from diseased cacao pod from cacao plantations at 21 districts and 13 provinces in Indonesia. Morphological and pathogenicity characterization were conducted on the identified isolates. Results of the activities showed 24 indigenous isolates of P. palmivora were identified from various cacao production centers in 13 districts and 8 provinces in Indonesia. These isolates produced ellipsoid, globoid, or ovoid spores. On the other hand, they exhibited less apparent differences in their papillae and pedicels. Although these indigenous isolates of P. palmivora were morphologically similar, they exhibited a diverse pathogenicity against cacao clones GC 7, ICS 60, and TSH 858. The LbSBR isolate of P. palmivora from Lubuk Basung, West Sumatra were identified as very pathogenic against the three cacao clones tested, while JkBwi(12) and KgBwi(8) isolates from Banyuwangi, East Java; PtBdg(7) isolate from Badung, Bali; SsSpg(36) and AgSpg1(35) from Sopeng, South Sulawesi, and PwMnw from Manokwari, West Papua were characterized as either pathogenic or very pathogenic against evaluated pods of the three cacao clones, respectively. Unless properly managed, these pathogenic or very pathogenic indigenous isolates of P. palmivora might become future major constraints in cacao production in Indonesia.

Ringkasan

Patogenisitas isolat Phytophthora palmivora dari berbagai sentra produksi kakao di Indonesia belum banyak dievaluasi, apalagi isolat P. palmivora dapat...
berubah dari waktu ke waktu, sehingga koleksi dan identifikasi keberadaan P. palmivora di lapangan perlu secara periodik dilakukan. Tujuan spesifik penelitian yang dilakukan adalah: (1) mengkoleksi isolat Phytophthora palmivora dari sejumlah sentra produksi kakao di Indonesia, (2) mengkarakterisasi isolat P. palmivora dari Indonesia menggunakan berbagai karakter morfologi, dan (3) mengevaluasi patogenisitas isolat P. palmivora asal asli terhadap buah kakao. Isolat P. palmivora diisolasi dari buah kakao terinfeksi yang berasal dari kebun kakao di 21 kabupaten dan 13 provinsi di Indonesia. Isolat yang didapat selanjutnya dikarakterisasi morfologi dan patogenisitasnya. Hasil penelitian menunjukkan 24 isolat P. palmivora telah berhasil diisolasi dari 13 kabupaten dan 8 provinsi di Indonesia. Isolat yang didapat mempunyai bentuk spora jorong (ellipsoid), membulat (globoid), atau bulat telur (ovoid). Sebaliknya, antarisolat asal asli tidak terdapat perbedaan yang jelas untuk papila dan tangkainya. Meskipun isolat P. palmivora asal asli Indonesia yang didapat secara morfologi hampir sama, terdapat perbedaan yang besar dalam tingkat patogenisitasnya terhadap kakao klon GC 7, ICS 60 atau TSH 858. Isolat P. palmivora LbSBR dari Lubuk Basung, Sumatra Barat diketahui sangat patogen terhadap ketiga kultivar kakao yang diuji. Sedangkan isolat JkBwi(12) dan KgBwi(8) dari Banyuwangi, Jawa Timur; PtBdg(7) dari Badung, Bali; SsSpg(36) dan AgSpg1(35) dari Sopeng, Sulawesi Selatan, serta PwMnw dari Manokwari, Papua Barat bersifat patogen atau sangat patogen terhadap buah dari tiga klon kakao yang diuji. Kecuali dilakukan pengendalian yang sesuai, isolat asal Indonesia P. palmivora yang bersifat sangat patogen atau patogen dapat berkembang menjadi kendala utama dalam budi daya kakao di Indonesia di masa mendatang.

**Key Words:** Cacao, back pod, detached pod assay, Phytophthora palmivora, characterization, isolates, pathogenicity, clones, GC 7, ICS 60, TSH 858.

**INTRODUCTION**

Black pod disease of cacao is one of the major diseases associated with cacao cultivation in the field (Prawirosoemardjo & Purwantara, 1992). The cacao disease caused by Phytophthora palmivora significantly reduced pod and bean yields of cacao. Phytophthora palmivora is also capable of infecting stems, young flushes, and leaves of cacao in the field (Purwantara, 1990; Sri-Sukamto, 1985). Yield reduction due to P. palmivora infection in Indonesia was epidemiology of this disease is very complex (Tey, 1991). A number of factor supporting development of black pod disease in cacao plantation in Indonesia are (1) the cultivated cacao genotypes are mostly susceptible against black pod disease, (2) the cacao pods require 5–7 months of development before harvesting and they could get infected by black pod disease at any stage of their development, (3) The relative humidity in Indonesia usually very high all year round, therefore it is favorable for infection and disease development, (4) The sources of infection are generally always available in the field because of the favorable environment factors and the presence of many alternative hosts in the field, and...
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(5) the ability of P. palmivora to infect all plant parts of cacao (vander Vosen, 1997).

Although P. palmivora could preventively be controlled with fungicides (Holderness, 1990), the required cost for controlling this pathogen could reach up to 40% out of total cost of cacao cultivation (Sunaryo & Situmorang, 1980). Therefore, availability of alternative methods for controlling P. palmivora is necessary. A number of antagonistic microbes were able to inhibit development of P. palmivora (Sri-Sukamto et al., 1997; Tondje et al., 2006). However, their effectiveness for controlling black pod disease in cacao needs further evaluation.

Information regarding P. palmivora isolates in Indonesia and other places has been reported (Umayah & Purwantara, 2006). However, pathogenicity of P. palmivora isolates from various cacao production centers has not been evaluated. Moreover, isolates of this pathogen may change in time (Goodwin, 1997). Therefore, collection and identification of the existing P. palmivora need to be done from time to time to determine the possible occurrences of new and more pathogenic isolates in the field.

Understanding of the existing P. palmivora isolates infecting cacao and their characters is needed in order to develop control strategies for the pathogen and to support cacao resistance breeding program for black pod disease (Appiah, 2001; Iwaro et al., 1998; Surujdeo-Maharaj et al., 2001). Therefore, isolation and characterization of pathogenicity of indigenous P. palmivora isolates from various places in Indonesia need to be conducted. Field isolates of P. palmivora from various cacao production centers in Indonesia may also be used as reference isolates. Subsequently, they can be used to evaluate resistance of cacao genotypes against infection of P. palmivora.

Research activities supported by the Partnership Cooperation with University in Agricultural Research Project (KKP 3T) have been conducted to develop effective control strategies for black pod disease in cacao through various approaches (Sudarsono et al., 2007) such as pathogenicity of the indigenous isolates.

MATERIALS AND METHODS

Collection of diseased cacao pods

Samples of cacao pods infected with black pod disease were collected from cacao plantations at 21 districts and 13 provinces in Indonesia. The provinces and districts were selected because they were known as the center of cacao production in Indonesia or in the process of developing cacao as one of the major crops in the locations. Within certain province and district, locations where concentrations of cacao plantations were selected for collecting diseased pods. List of locations of diseased cacao pod collection was presented in Table 1. From each location, 2—3 cacao pods showing symptoms of black pod infection (Fig. 1.a) were collected. The sampled cacao pods were either brought back directly or sent through express mail service to Jember. Subsequently, the diseased pods were used to isolate indigenous P. palmivora in subsequent experiments.
Isolation of Indigenous \textit{P. palmivora}

Isolation of \textit{P. palmivora} from diseased cacao pod was conducted through three steps, such as: (1) baiting step, (2) isolation step, and (3) identification and propagation steps, respectively. For the baiting step, the diseased cacao pods were disinfected using 70\% alcohol. A piece of tissue was cut from diseased pod and used to inoculate a healthy pod of cacao clone GC 7 (4 months after pollination) (Fig. 1.b.). To maintain humidity, the inoculated site of the healthy pods was padded with wet paper towel. Subsequently, the inoculated cacao pods were wrapped with newspaper and incubated for 5–7 days in plastic boxes. The relative humidity in the plastic boxes was maintained at > 90\%.

Once the inoculated pods showed spegal \textit{P. palmivora} isolate identified from previous experiment was grown on a 9-cm petridish containing solid PDA medium. The fungal cultures were incubated for seven days under dark condition in an incubation room. Temperature in the incubation room was set at 26\textdegree C day and night. To induce sporulation, mycelia of \textit{P. palmivora} grown on solid PDA medium were cooled at 4\textdegree C for 15 minutes in a refrigerator. Observation for the spore morphology was conducted under binocular microscope with 100x magnification. In addition, the shape of the fungal spore, the presence of pedicels and papillae were also recorded as part of morphological characters of the \textit{P. palmivora} isolates.

\textbf{Table 1. List of locations of sampled cacao pods infected with black pod disease, the number of fungal isolates and the number of indigenous \textit{Phytophthora palmivora} isolates identified from each location}

\begin{tabular}{|c|c|c|c|}
\hline
No. & Province & Regency & No. of fungal isolate (\textit{P. palmivora}) \\
\hline
1 & North Sumatera (Sumatera Utara) & Deli Serdang & 3 \\
2 & West Sumatera (Sumatera Barat) & Lubuk Basung, Agam & 2 \\
3 & Lampung (Lampung) & Lampung Tengah & 1 \\
4 & West Java (Jawa Barat) & Sukabumi & 4 \\
5 & Central Java (Jawa Tengah) & Wonosobo, Temanggung & 2 \\
6 & East Java (Jawa Timur) & Jember, Banyuwangi & 9 \\
7 & Bali (Bali) & Jembrana, Tabanan, Badung & 6 \\
8 & South Sulawesi (Sulawesi Selatan) & Sopeng & 3 \\
9 & Southeast Sulawesi (Sul. Tenggara) & Konawe, Kolaka, Kendari & 10 \\
10 & Central Sulawesi (Sulawesi Tengah) & Toli-toli, Donggala & 2 \\
11 & West Sulawesi (Sulawesi Barat) & Mamuju & 1 \\
12 & West Papua (Papua Barat) & M anokwari & 1 \\
13 & Nangroe Aceh Darussalam (NAD) & Saree & - \\
\hline
Total & & & 44 \\
\hline
\end{tabular}

Notes (Catatan): The fungal isolates were identified as isolate of \textit{P. palmivora} based on their ability to produce typical spore on solid PDA medium and to infect cacao pods (Isolat jamur diidentifikasi sebagai isolat \textit{P. palmivora} berdasarkan pada kemampuannya membentuk spora khusus setelah ditumbuhkan dalam media PDA padat).
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Figure 1. Steps of indigenous Phytophthora palmivora isolation from a sample of cacao pod infected with black pod disease in the field and morphological characters of the isolates. (a) A sample of cacao pod infected with black pod disease; (b) Baiting step - inoculation of healthy pod with sap from disease cacao pod; (c) Occurrences of necrotic symptoms on healthy cacao pod inoculated with sap of diseased pod and the tissue in the perimeter of diseased and healthy pod used as inoculums for isolation step; (d) Fungal colonies growing from the inoculums tissues on solid PDA medium; (e) Example of typical spore formation from fungal isolate suspected as *P. palmivora*. Microscopic observation for the abilities to form those typical spores was conducted to verify the identity of the isolates as *P. palmivora*; and (f) Example of the ovoid (O) and ellipsoid (E) types of *P. palmivora* spore morphologies.

Gambar 1. Langkah-langkah isolasi *P. palmivora* dari contoh buah kakao terinfeksi di lapangan dan karakterisasi morfologi isolat. (a) Contoh buah terserang *P. palmivora*; (b) Inokulasi buah sehat dengan cairan penyakit buah terserang; (c) Gejala nekrosis hasil inokulasi (b); (d) Koloni jamur tumbuh dalam medium PDA padat; (e) Spora *P. palmivora*; (f) Contoh bentuk spora bulat telur (O) dan henjang (E).
Pathogenicity Test of Indigenous
P. palmivora

Identified isolates of P. palmivora were grown on solid PDA medium in petri dishes, and subsequently were used to inoculate healthy cacao pods in the pathogenicity test. Prior to inoculation, the pods of cacao clones GC 7 (susceptible), ICS 60 (moderately resistant) and TSH 858 (resistant - against infection of P. palmivora) (Suhendi et al., 2005) were rinsed in running tap water and damped using tissue towels. The pods were inoculated with actively growing mycelia on solid PDA medium (0.5x0.5 cm²) and the inoculated site was padded with wetted paper towels. After inoculation, cacao pods were incubated for 7 days in wooden boxes with > 90% relative humidity. To maintain relative humidity, 10 cm thick of foam was laid at the base of wooden boxes and wetted with sterile water. The boxes were covered with plastic covers and maintained at 28°C.

Starting at 3 days after inoculation (DAP), observations for the occurrences of necrotic symptoms were conducted daily up to 8 DAP. The observations were conducted on incubation periods, number of pods showing necrotic symptoms, and average width of necrotic symptoms on the surfaces of cacao pods. Pathogenicity of the isolates was determined based on the diameter of the necrotic symptoms measured at 8 DAP. The pathogenicity of the isolates was grouped based on criteria developed by Waterhouse (1975), such as: (1) non-pathogenic if there is no necrotic symptom on the inoculated cacao pods; (2) less pathogenic if the symptom was < 25%; (3) pathogenic if the symptom was between 25-50%; and (4) very pathogenic if the symptom was > 50% of the infected cacao pods.

RESULTS AND DISCUSSION

Isolation of Indigenous P. palmivora

Isolation of the pathogens from cacao pods infected with black pod disease was conducted up to November 2007, and a total of 44 fungal isolates exhibiting mycelia similar to P. palmivora were obtained. These fungal isolates were obtained from cacao production centers at 21 districts and 13 provinces in Indonesia (Table 1).

The ability to produce typical spores on PDA medium was evaluated to verify the identity of the isolated fungi as P. palmivora. Results of identification indicated that 24 out of 44 fungal isolates were positively identified as P. palmivora. These 24 isolates of P. palmivora were obtained from diseased pods originated from 13 districts and 8 provinces, such as Jembrana, Tabanan, and Badung districts (Bali Province), Banyuwangi and Jember (East Java), Sukabumi (West Java), Lubuk Basung and Agam (West Sumatera), Deli Serdang (North Sumatera), Soppeng (South Sulawesi), Kolaka and Konawe (Southeast Sulawesi), and Manokwari (West Papua) (Table 1).

The identified indigenous isolates of P. palmivora could be used as reference isolates for evaluating response of cacao germplasm collections and breeding lines against
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More indigenous isolates of P. palmivora would be re-isolated from diseased cacao pods originated from districts and provinces that have not been represented in this activity. However, results of characterization of only 24 identified indigenous isolates of P. palmivora were presented in

black pod disease in Indonesia. The reference isolates could be used to inoculate cacao germplasm collections and identify the resistance clones. The resistance clones could then be planted and cultivated in the target areas where the reference isolates existed.

Table 2. Morphological characteristics of indigenous isolates of Phytophthora palmivora based on the spores shape and the presence of pedicels and papillae

| Isolate of P. palmivora | Origin of isolate | Morphological characters (Sifat morfologi) |
|-------------------------|------------------|------------------------------------------|
|                         | Asal isolat      | Spore (Spora) Pedicel Papillae (Papila) |
| AdinSU                  | Deli Serdang, Noth Sumatra (Sumut) | O + + |
| LbSBR                   | Lubuk Basung, West Sumatra (Sulbar) | O/E + + |
| AgSBR                   | Agam, West Sumatra (Sulbar) | O/E + + |
| BISkim3B(15)            | Sukabumi, West Java (Jawa Barat) | O + + |
| BLSkim4A (16)           | Sukabumi, West Java (Jawa Barat) | O + + |
| KgBwi(8)                | Banyuwangi, East Java (Jawa Timur) | O + + |
| JkBwi(12)               | Banyuwangi, East Java (Jawa Timur) | O + + |
| Koajbr(10)              | Jember, East Java (Jawa Timur) | O + + |
| Kwjbr1(29)              | Jember, East Java (Jawa Timur) | O/G + + |
| Kwjbr2(30)              | Jember, East Java (Jawa Timur) | O/E + + |
| Kwjbr3(31)              | Jember, East Java (Jawa Timur) | O/E + + |
| Kwjbr4(32)              | Jember, East Java (Jawa Timur) | O/E + + |
| PsTbn                   | Tabanan, Bali | O/G + + |
| Mjbrn                   | Jembrana, Bali | E + + |
| Pjbrn                   | Jembrana, Bali | O + + |
| AsBdg(6)                | Badung, Bali | O/G + + |
| PtBdg(7)                | Badung, Bali | O + + |
| EgSpg(37)               | Sopeng, South Sulawesi (Sulsel) | O/G + + |
| SsSpg(36)               | Sopeng, South Sulawesi (Sulsel) | O/G + + |
| AgSpg1(35)              | Sopeng, South Sulawesi (Sulsel) | O/G + + |
| OkKnw(26)               | Konawe, Southeast Sulawesi (Sultra) | O/G + + |
| TuKnw(27)               | Konawe, Southeast Sulawesi (Sultra) | O/G + + |
| TlKlk(20)               | Kolaka, Southeast Sulawesi (Sulsel) | O/G + + |
| PwMnw                   | Manokwari, West Papua (Papua Barat) | O/G + + |
this report. Twenty fungal isolates evaluated did not produce typical spores of P. palmivora. Therefore, they may not be the isolates of P. palmivora.

**Morphological Characterization of Indigenous P. palmivora**

All fungal isolates positively identified as P. palmivora were evaluated for the shape of their spores and for the presence of the pedicels and the papillae. Results of evaluations indicated that indigenous isolates of P. palmivora from various cacao production centers in Indonesia have spore shape either as ellipsoid (E), globoid (G), or ovoid (O). Out of 24 isolates of P. palmivora identified, nine isolates have O spores and only two has E spores (Table 2). Results of the observation also indicated nine identified isolates of P. palmivora have a mixture of O and G spores and six isolates have a mixture of O and E spores (Table 2).

Based on the observed data, except for spore shape, fungal morphology variation was not apparent. All fungal isolates positively identified as P. palmivora have pedicel and papillae and produced ellipsoid, globoid, or ovoid shape of spores. However, there was unclear association, if any, among shape of the spore, the origin of the isolates, and the level of their pathogenicity. Previous report has attempted to evaluate the spore shape of P. palmivora as an isolate identity. However, result of the evaluation also indicated the absence of strong association between spore shape and isolate identity (Appiah et al., 2003; Drenth & Sendall, 2001; Umayah & Purwantara, 2006).

Characterization of Phytophthora sp. has also been done using DNA sequence of intergenic transcribed spacer (ITS) and the 5S rRNA gene. Although it is possible to differentiate among Phytophthora species, this technique was not able to differentiate different isolates of P. palmivora (Appiah et al., 2004; Ristaino et al., 1998).

| Cacao clone | Number of isolates with certain pathogenicity level: (Jumlah isolat dg patogenisitas): |
|-------------|----------------------------------------------------------------------------------|
|             | NP | LP | PT | VP |
| GC 7        | 8  | 3  | 3  | 10 |
| ICS 60      | 3  | 9  | 8  | 4  |
| TSH 858     | 4  | 7  | 7  | 6  |

**Remark (Catatan):** NP - non-pathogenic (tidak patogenis), LP - less pathogenic (kurang patogenis), PT - pathogenic (patogenik), and VP - very pathogenic (sangat patogenik). Pathogenicity groupings were based on the size of necrotic areas of the tested cacao pods, 8 days after inoculation (Patogenisitas berdasarkan ukuran area nekrosis buah yg diinokulasi, 8 hari setelah inokulasi).
Pathogenicity of Indigenous *P. palmivora*

Pathogenicity of indigenous isolates of *P. palmivora* was characterized based on their ability to induce necrotic symptoms and the width of the symptoms on inoculated cacao pods.

Results of pathogenicity test indicated the 20 fungal isolates that did not produce typical *P. palmivora* spores did not result in necrotic symptoms on cacao pods. Such results supported previous suspicions that these fungal isolates were not *P. palmivora*.

These 20 isolates might be other fungi co-inhabiting cacao pods with *P. palmivora* in the field. Previous report has indicated the presence of various types of fungi inhabiting cacao pods, including endophytic fungi that show antagonistic activities against *P. palmivora* (Crozier et al., 2006; Sri-Sukamto et al., 1997). However, no further attempt of identifying these fungal isolates was conducted in this experiment.

On the other hand, all indigenous isolates identified as *P. palmivora* based on their ability to form typical spores resulted in various degrees of necrotic symptoms on evaluated cacao pods. Summary of the pathogenicity test results on the indigenous isolate of *P. palmivora* from various cacao production centers in Indonesia was presented in Table 3.

Based on results of pathogenicity test using pods of cacao clone GC 7, 10 indigenous isolates of *P. palmivora* were grouped as very pathogenic, 3 were pathogenic, 3 were less pathogenic, and 8 were non-pathogenic (Table 3). Using pods of cacao clone ICS 60, the same indigenous isolates were grouped as very pathogenic (4 isolates), pathogenic (8 isolates), less pathogenic (14 isolates), and non-pathogenic (3 isolates) (Table 3). On the other hand, the grouping of indigenous isolates based on results of pathogenicity test using pods of cacao clone TSH 858 were very pathogenic (6 isolates), pathogenic (7 isolates), less pathogenic (7 isolates), and non-pathogenic (4 isolates) (Table 3).

Results of the pathogenicity test also indicated that LbSBR isolate of *P. palmivora* was very pathogenic against pods of cacao clones GC 7, ICS 60, and TSH 858. This isolate originated from Lubuk Basung district, West Sumatera Province (Table 4). Moreover, six indigenous isolates, JkBwi(12) and KgBwi(8) isolates from Banyuwangi, East Java; PtBdg(7) isolate from Badung, Bali; and SsSpg(36) and AgSpg1(35) from Sopeng, South Sulawesi, and PwMnw from Manokwari, West Papua (Table 4) were characterized as either pathogenic or very pathogenic against evaluated pods of the three cacao clones, respectively.

On the other hand, PsTbn isolate from Tabanan, MJbrn from Jembrana, Bali Province and BISkbm3B (15) from Sukabumi, West Java were identified as non-pathogenic against pods of cacao clone GC 7, but either less pathogenic or pathogenic against that of ICS 60 and TSH 858 (Table 4). The AdlnSU isolate originated from Deli Serdang, North Sumatera; AgSBR from Agam, West Sumatra; BISkbm4A (16) from...
Sukabumi, West Java, KoaJbr (10), KwJbr1 (29), KwJbr2 (30), KwJbr3 (31), and KwJbr4 (32) isolates from Jember, East Java were identified either as less pathogenic or non-pathogenic against three cacao clones tested (Table 4). Overall results of the observation indicated the indigenous isolates of P. palmivora from various cacao production centers in Indonesia showed various levels of pathogenicity against pods of cacao.

Table 4. Pathogenicity grouping of indigenous isolates of Phytophthora palmivora isolated from various cacao production centers in Indonesia based on the response of pods of cacao clones GC 7 (susceptible), ICS 60 (moderately resistance), and TSH 858 (resistance - against P. palmivora infection)

| Isolate no. | Isolate origin | Pathogenicity on cacao clone: (Patogenisitas thd. klon): |
|-------------|----------------|--------------------------------------------------------|
|             |                | GC 7         | ICS 60 | TSH 858 |
| AdinSU      | Deli Serdang, North Sumatra (Sumut) | N | L | P |
| LlbSR       | Lubuk Basung, West Sumatra (Sumbar) | V | V | P |
| AgSBR       | Agam, West Sumatra (Sumatra Barat) | L | P | L |
| BlSkbr3B(15) | Sukabumi, West Java (Jawa Barat) | N | L | P |
| BLSkbr4A(16) | Sukabumi, West Java (Jawa Barat) | L | L | P |
| KgBwi(8)    | Banyuwangi, East Java (Jawa Timur) | P | T | V |
| JkBwi(12)   | Banyuwangi, East Java (Jawa Timur) | V | P | T |
| KoaJbr(10)  | Jember, East Java (Jawa Timur) | V | T | L |
| KwJbr1(29)  | Jember, East Java (Jawa Timur) | N | L | N |
| KwJbr2(30)  | Jember, East Java (Jawa Timur) | N | N | L |
| KwJbr3(31)  | Jember, East Java (Jawa Timur) | N | N | N |
| KwJbr4(32)  | Jember, East Java (Jawa Timur) | V | V | P |
| PsTbn       | Tabanan, Bali | N | P | T |
| M Jbrn      | Jembrana, Bali | N | L | P |
| P Jbrn      | Jembrana, Bali | V | L | L |
| AsBdg(6)    | Badung, Bali | L | P | T |
| PtbBdg(7)   | Badung, Bali | V | V | P |
| EgSpg(37)   | Sopeng, South Sulawesi (Sulsel) | P | L | V |
| SoSpg(36)   | Sopeng, South Sulawesi (Sulsel) | V | P | V |
| AgSpg(31)   | Sopeng, South Sulawesi (Sulsel) | N | N | N |
| OIKnw(26)   | Konawe, Southeast Sulawesi (Sultra) | P | L | T |
| TuKnw(27)   | Konawe, Southeast Sulawesi (Sultra) | V | P | T |
| TKlk(20)    | Kolaka, Southeast Sulawesi (Sultra) | V | P | L |
| PwMnw       | Manokwari, West Papua (Papua Barat) | V | V | V |

Notes (Catatan): NP - non-pathogenic (tidak patogenik), LP - less pathogenic (kurang patogenik), PT - pathogenic (patogenik), and VP - very pathogenic (sangat patogenik). Pathogenicity groupings were based on the size of necrotic areas of the tested cacao pods, 7 days after inoculation (Patogenisitas berdasarkan ukuran area nekrosis buah yg diinokulasi, 7 hari setelah inokulasi).
Based on RAPD data using six random primers and 14 polymorphic amplified DNA fragments, Umayah et al. (2007) reported that P. palmivora isolates from six provinces in Indonesia were genetically similar. Subsequently they concluded that there might be less possibility of occurrence of new physiological isolates of P. palmivora. However, results of this experiment might not support the hypotheses proposed by Umayah et al. (2007).

Umayah et al. (2007) proposed conclusions might not necessarily correct because of the following arguments: (a) The number of RAPD markers utilized in the genetic similarity analysis were only 16 markers. Considering size of the genome of P. palmivora, 16 markers were relatively too small. These markers would only represent 16 loci in the P. palmivora genome and most probably might not be in the same loci as those of gen(s) controlling pathogenicity characters. (b) If the above argument is true, the statement associating high genetic similarity to less probable occurrences of new P. palmivora isolates might not be valid. If gene(s) associated with pathogenicity and the RAPD markers were in different loci, P. palmivora isolates with different levels of pathogenicity might exhibit high genetic similarity based on the markers. Moreover, occurrences of new physiological isolates of P. palmivora might also be possible without changing the level of genetic similarity based on the RAPD markers. (c) The pathogenicity of the tested P. palmivora isolates were also not reported in the Umayah et al. (2007). Therefore, correlation among genetic similarity and isolate pathogenicity should not have been discussed. Such reasoning further support our arguments that genetic similarity data based on 16 RAPD markers should not be associated with isolate pathogenicity and possibility of new isolate occurrences.

In conclusion, high level of pathogenicity among isolates of P. palmivora as shown in this experiment could not be nullified by Umayah et al. (2007) data. Differences in isolate pathogenicity might not be attributed to the environmental factors as it was suggested by Umayah et al. (2007). In this experiment, pathogenicity tests were conducted under controlled environment and optimized for infection of P. palmivora. Hence, response differences were observed among the tested isolates on pod of either cacao clone GC 7, ICS 60, or TSH 858.

The diverse pathogenicity exhibited by indigenous isolates of P. palmivora and the absence of diversity on fungal morphology indicated that pathogenicity of the isolates of P. palmivora might not be associated with fungal morphology. Therefore, fungal morphology might not be used to predict pathogenicity of indigenous isolate of P. palmivora originated from various cacao production centers in Indonesia.

Cacao clones ICS 60 and TSH 858 have been identified as resistant against P. palmivora infection. On the other hand, cacao clone GC 7 was regarded as susceptible (Suhendi et al., 2005). Results of the pathogenicity test against pods of cacao clones GC 7, ICS 60, and TSH 858 indicated the
existence of isolates that were very pathogenic or pathogenic against ICS 60 and TSH 858. These isolates were identified from Badung-Bali, Banyuwangi-East Java, and Lubuk Basung-West Sumatra. The existence of such P. palmivora isolates would become the major constrain for cacao cultivation in the areas. Even if the available P. palmivora resistance cacao clones such as ICS 60 and TSH 858 were planted, such identified isolates would be able to significantly reduce cacao production in the regions.

CONCLUSIONS

Indigenous isolates of P. palmivora originated from North and West Sumatra, East Java, Bali, South, and Southeast Sulawesi, and West Papua exhibited diverse pathogenicity levels against cacao pods. The LbSBR isolate of P. palmivora from Lubuk Basung, West Sumatra was identified as the most pathogenic isolates identified in this research. The existende of highly pathogenic isolates in Lubuk Basung - West Sumatra; Banyuwangi - East Java; Badung - Bali; Sopeng - South Sulawesi, and Manokwari, West Papua need to be closely monitored to prevent their further widespread across cacao production centers in Indonesia. Unless properly managed, these pathogenic or very pathogenic indigenous isolates of P. palmivora might become future major constrains in cacao production.

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