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BIOLOGY OF *Botryodiplodia theobromae* OF CASHEW (*Anacardium occidentale*)

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ABSTRACT

Gumming disease, caused by *Botryodiplodia theobromae*, has recently been found in many cashew plantations in Indonesia. Economic importance of the disease has not been studied, however, anticipation to understand some aspects on biology of the pathogen to support the study on controlling the disease has been done. Aspects studied were cultural characteristics of the pathogen, virulence in young cashew seedlings, host range and effect of controlled temperature and humidity to infection. Experiments were conducted in the laboratory and glass house of the Research Institute for Spices and Medicinal Crops in 1995. The cultural characteristics of the fungus were studied on PDA under continuous fluorescent light (800 lux). Response of inoculation and test of virulence of *B. theobromae* isolates on different plant tissues were studied using young cashew seedlings, whereas host range of the fungus was tested in detached leaves of various plants. Effect of temperature and relative humidity to infection was studied in controlled humidity chambers containing saturated salt solutions. The research results suggested that *B. theobromae* (taken from diseased cashew) was considered as wound-pathogen. Cultural characteristics of the pathogen varied slightly. The pathogen was able to infect cashew leaves incubated at different temperatures (24 and 30°C) and relative humidity (55 - 100%). Fruit, leaf and young stem of cashew were all susceptible to the inoculation. Infection on fruit caused fruit rot, on leaf and stem caused a necrotic lesion with occasional dieback in the stem in particular. The fungus was able to incite disease not only in cashew, but also in other plants, such as carambola, cocoa, nutmeg, papaya, coconut, mango, avocado, rubber and jackfruit. This indicated that *B. theobromae* is a potentially important pathogen to various crops.

Key words: *Botryodiplodia theobromae*, *Anacardium occidentale*, relative humidity, host

RINGKASAN

Biologi *Botryodiplodia theobromae* pada jambu mente (*Anacardium occidentale*)

Penyakit gumpis yang disebabkan oleh *Botryodiplodia theobromae* baru-baru ini telah ditemukan menyerang per-tanaman jambu mente di Indonesia. Nilai kerugian karena penyakit ini belum diketahui, tetapi untuk mengantisipasi telah dipelajari beberapa aspek biologi patogen dalam rangka menyokong penelitian komponen penanggulangan penyakit. Aspek biologi yang dipelajari adalah karakteristik pertumbuhan dan morfologi konidia patogen, virulensi pada bibit jambu mente, kisaran tanaman inang, dan pengaruh suhu dan kelembaban terhadap infeksi. Percobaan dilakukan di laboratorium dan rumah kaca Balai Penelitian Tanaman Rempah dan Obat tahun 1995. Karakteristik pertumbuhan cendawan dipelajari

dengan menumbuhkan cendawan pada medium PDA dan diinkubasikan di bawah sinar lampu neon (800 lux). Pengaruh jaringan tanaman melalui uji inokulasi dan virulensi isolat-isolat cendawan dipelajari dengan menggunakan bibit jambu mente, dan kisaran inangnya diuji dengan menginokulasikannya pada daun-daun dari beberapa jenis tanaman. Pengaruh suhu dan kelembaban udara relatif terhadap infeksi cendawan pada daun jambu mente dipelajari dalam kotak kelembaban (*humidity chamber*) yang telah diatur kelembabannya dengan menambahkan larutan jenuh senyawa-senyawa garam. Hasil percobaan menunjukkan bahwa *B. theobromae* yang diisolasi dari tanaman jambu mente sakit adalah patogen jaringan luka, artinya infeksi mudah terjadi melalui jaringan tanaman yang terluka. Perbedaan ciri pertumbuhan antara isolat-isolat *B. theobromae*, hanya sedikit. Patogen ini mampu menginfeksi daun mente pada suhu 24 dan 30 °C dan kelembaban udara relatif 55-100%. Jaringan-jaringan buah, daun dan batang muda sama kerentanannya terhadap patogen ini. Infeksi pada buah menyebabkan buah busuk, sedang infeksi pada daun dan batang mengakibatkan jaringan tanaman mati dan kadang-kadang menimbulkan gejala *dieback*. Cendawan patogen ini dapat menimbulkan gejala sakit tidak hanya pada jambu mente, tetapi juga pada beberapa jenis tanaman lainnya, antara lain belimbing, coklat, pala, pepaya, kelapa, mangga, alpukat, dan nangka. Hal ini menunjukkan bahwa *B. theobromae* yang berasal dari jambu mente berpotensi menimbulkan kerusakan pada tanaman-tanaman tersebut.

Kata kunci : *Botryodiplodia theobromae*, *Anacardium occidentale*, kelembaban relative, inang

INTRODUCTION

Botryodiplodia theobromae Pat. *Lasiodiplodia theobromae* is the causal agent of gumming disease of cashew plant (*Anacardium occidentale* L.), and associated with twig die-back and flower blight (SUPRIADI *et al.*, 1994). The disease is widespread in cashew plantations in Java, Bali, Kupang and Sumbawa. The pathogen also attacks cashew plant in Nigeria (OLUNLOYU and ESURUOSO, 1975 and OHLER, 1979). The pathogen attacks various plants under different names, such as *Diplodia gummosis* of citrus, pod-rot of cocoa, and stem end rot of mango (HILL and WALLER, 1988), leaf blight of clove (ONIKI *et al.*, 1988), leaf blight of coconut (WARWICK *et al.*,

1991), fruit rot of banana and stem end rot of papaya, *Botryodiplodia* rot of soursop and carambola (AICAF, 1995). The pathogen is also reported to cause diseases in many other crops in the South Pacific Areas (DINGLEY *et al.*, 1981). *Botryodiplodia theobromae* is common its performance to tropical area and is responded to be a weak pathogen which related to attacks stressed host plants and due to involvement of other diseases or pests or environmental conditions (SINCLAIR *et al.*, 1987; HILL and WALLER, 1988). Preliminary study on *B. theobromae* isolates from cashew plant in West Java showed that the fungus infects plant through wound, and sporulation of the fungus was induced by fluorescent light (SUPRIADI *et al.*, 1995). This paper reports further study on the biology of *B. theobromae* isolates of diseased cashew plants from various origins.

MATERIAL AND METHODS

Cultures

Cultures of *B. theobromae* were isolated from diseased cashew plant showing gummosis at Cikampek, West Java, Bali, Sumbawa and Kupang, Nusa Tenggara Timur (NTT) as described earlier (SUPRIADI *et al.*, 1994). The isolates were maintained on slopes of potato dextrose agar (PDA - Difco) and cultured on plates of PDA and incubated under continuous fluorescent light (800 lux) in woody box. All isolates were maintained on slopes of PDA medium under sterile paraffin and kept at room temperature (20 °C).

Cultural characteristics

The fungus was grown on PDA medium at 28-30 °C. Mycelia growth and pycnidia production were observed. Conidia were released from pycnidia by breaking them on slide glass then stained with trypan blue (0.05%) in lactophenol (JOHNSTONE and BOOTH, 1983). Size of conidia (20 conidia) was measured under an ordinary light microscope.

Susceptibility of different plant tissues to infection

Four different isolates of *B. theobromae*, code numbers C031 isolated from diseased cashew in Cikampek, C019 and C069 from Bali, and C075 from NTB, were inoculated in young stems (light green color) and leaves of cashew seedlings (50 cm height) grown in plastic bags containing garden soil under a screen house. The virulence of *B. theobromae* isolates from different origins i.e. Bali (3 isolates), Cikampek (4 isolates), Kupang (1 isolate), NTB (2 isolates) and Sumbawa (3 isolates) were also compared by inoculating leaves of cashew seedlings. A piece of agar block (1 cm x 1 cm) containing two day-old mycelia was placed on wounded tissues by scraping a scalpel on the epidermis of very young (light green tissue) stem tips and midrib of leaves. Inoculated stem and leaves were covered with wet cotton and the plant was then covered with transparent plastic bag to keep humid condition. The plastic cover was removed 3-4 days following inoculations and the infected area was measured after removing the wetted cotton from inoculation sites. Inoculated cashew plants were watered daily.

Effects of temperature and relative humidity on infection of *B. theobromae*

Four different degrees of air relative humidity (r.h.) i.e. 100%, 84%, 78% and 55% were tested. Humidity chambers were set according to the methods described by LIDDELL and BURGESS (1985) using transparent bread plastic boxes. Each chamber contained 100 ml of saturated aqueous solutions to control the relative humidity (r.h.) i.e. distilled water (100% r.h.), KCl (84% r.h.), NaCl (78% r.h.), MgCl₂ (55% r.h.) in distilled water (Table 1) and a platform for holding leaf samples. Actual value was measured by using a hair thermohygrometer (IUCHI, CITIZEN). Detached leaves of cashew taken from young seedlings were wounded as previously described, than a block of PDA containing two-day culture of *B. theobromae* isolate C031 was placed on the wounded tissue. Each end

Table 1. Relative humidity of salt solutions at 24 and 30°C

Tabel 1. Kelembaban relatif dari berbagai larutan garam pada 24 dan 30°C

| Temperature Suhu | Saturated solution Larutan jenuh | | | |
|------------------------|----------------------------------|-------|-------|------------------------------|
| | H2O | KCl | NaCl | MgCl2 |
| 24 °C | | | | |
| Nominal r.h. | 100.0% | 84.0% | 75.0% | not known Tidak diketahui |
| Observed, average r.h. | 100.0% | 84.2% | 78.0% | 54.9% |
| Teramati, rata-rata | | | | |
| 30 °C | | | | |
| Nominal r.h. | 100.0% | 84.0% | 75.0% | not known Tidak diketahui |
| Observed, average r.h. | 96.0% | 82.5% | 75.0% | 54.9% |
| Teramati, rata-rata | | | | |

of the petiole was wrapped with wet cotton then covered with cling film to minimize dryness of the leaf. All inoculated leaves were then placed on the platform in the humidity chamber at 30 °C. Leaf inoculated with agar block was only used as control. Four leaves were used in each treatment. Infected area of the leaves was measured two days after inoculation. All infected leaves were then transferred to the platforms in plastic boxes containing distilled water (r.h. 95- 100 %) and incubated under continuous fluorescent light to induce production of pycnidia and conidia.

Host range

Pathogenicity of *B. theobromae* isolate C031 was tested in several hosts using wound detached leaves or fruits as described above. Samples were kept at humid plastic boxes at 28-30 °C. Host plants tested were carambola (*Averrhoa carambola*), cocoa (*Theobroma cacao*), nutmeg (*Myristica fragrans*) and papaya (*Carica papaya*), fruits and leaves of coconut (*Cocos nucifera*), mango (*Mangifera indica*), avocado (*Persea americana*), rubber (*Hevea brasiliensis*) and jack-fruit (*Artocarpus heterophyllus*). Four samples were inoculated for each host plant. Samples inoculated with PDA on wounded tissue and block of mycelia on un-wounded tissue were used as control.

RESULTS AND DISCUSSION

Cultural characteristic

All isolates of *B. theobromae* tested produced thick white mycelial mats when young and turned dark grey or black when old, produced numerous pycnidia on PDA medium at 28-30 °C. Pycnidia production induced by fluorescent light. Conidia were ellipsoid to obovoid, hyaline, 1-celled when young, some turned brown, 2-celled when old. Size of conidia showed that their length were almost twice as their width (Table 2). Variability of the size of conidia amongst different isolates of *B. theobromae* was little, and they may not be related with their origins.

Susceptibility of leaf and stem tissues to infection

Leaves or stems inoculated with isolates of *B. theobromae* showed similar diseasesymptoms (Table 3). First symptoms appeared two days after inoculation as necrotic and brown lesion around the inoculation site, then progressively the infected leaf yellowing or wilting. In both infected leaves and stems, numerous submerged dark pycnidia were produced. Development of the lesion in infected young stem caused part of the stem become wilt, dropped most of the leaves and die-back. In many occasions, however, the lesion

Table 2. Measurement of conidia of *B. theobromae* from different origins

Tabel 2. Ukuran konidia *B. theobromae* dari berbagai daerah asal

| Isolate | Origin | Size of conidia (in cm) * |
|---------|-------------|---|
| Isolat | Daerah asal | long x wide Ukuran konidia (dalam cm) panjang x lebar |
| C001 | Bali | 18.25 x 10.62 |
| C019 | Sumbawa | 21.00 x 11.75 |
| C020 | Sumbawa | 21.87 x 12.92 |
| C027 | Cikampek | 22.62 x 13.00 |
| C028 | Cikampek | 24.37 x 12.69 |
| C030 | Cikampek | 21.65 x 11.67 |
| C031 | Cikampek | 24.25 x 12.50 |
| C065 | Cikampek | 26.00 x 12.75 |
| C067 | Kupang | 24.80 x 13.05 |
| C069 | Bali | 20.75 x 11.00 |
| C072 | Bali | 20.00 x 9.62 |
| C075 | NTB | 23.12 x 11.50 |

* Average of at least 20 conidia.
Data dari 20 konidia atau lebih

developed only in limited areas, not reaching the base of the stem (older tissue), and then new shoots were produced from uninfected stem. This phenomena may explain why the fungus is considered to be a weak pathogen because it can not infect older tissues.

The tissues of the leaf and young stem were both susceptible to the infection and therefore the technique could be used for further tests such as resistant screening, especially when number of plants to be tested is limited. The technique is being tested to screen the resistance different numbers of cashew to the inoculation of the pathogen using detached leaves. It is important to note that *B. theobromae* infected plants only through wounded tissue. Such entry for infection of the fungus in cashew tissues is common in the nature i.e. damage caused by sucking insects such as tea mosquito, *Helopelthis* spp. which is common on cashew plantations, such as due to wind. This results suggest that it is necessary to protect wound (e.g. using fungicide) from infection of the pathogen. The fungus inoculated in plant tissue without wounding did not incite infection although mycelia of the fungus grew over the site of inocula-

Table 3. Infected areas of cashew leaves and young stems inoculated with different isolates of *B. theobromae*

Tabel 3. Luas infeksi pada daun dan batang muda jambu mente yang diinfeksi dengan berbagai isolat *B. theobromae*

| Isolate/ Origin | Length of infected area (in cm) | |
|-----------------|---------------------------------|-------------|
| | leaf Daun | stem Batang |
| C031 (Cikampek) | 5.8 a | 5.0 a |
| C069 (Bali) | 3.7 b | 3.4 a |
| C019 (Bali) | 2.7 b | 3.4 a |
| C075 (NTB) | 2.1 b | 3.4 a |
| Control (PDA) | 0.5 c | 0.5 b |

Note : Numbers followed by the same letter in the same column are not significantly different at P = 0.01 according to Duncan's multiple range test.

Keterangan : Angka yang diikuti oleh huruf yang sama pada tiap kolom, tidak berbeda nyata pada P = 0.01 berdasarkan uji berganda Duncan

tions. This finding was in agreement with other workers and therefore they believed that *B. theobromae* is a wound pathogen which attacked stressed plants (HILL and WALLER, 1987). The mechanism of infection of *B. theobromae* on cashew has not been studied, however, it can be suggested that the fungus is unable to break the cuticular membrane of plant hosts due to lack of enzymes for degrading the cuticular layers as stated by JONES (1987).

Virulence of isolates of *B. theobromae* on cashew seedlings

All isolates of *B. theobromae* inoculated on the leaves of cashew seedlings were able to induce disease symptoms and the isolates were proved to be virulent (Table 4). The symptoms were necrotic brown lesions on the leaves or young stem. In the infected leaves, the lesion could develop into larger lesion over the whole leaves, whereas in the infected young stems it could develop into die-back over few centimeter from the stem tips. Disease symptoms developed more rapidly in the younger tissues than in the older ones. Numerous black pycnidia were

Table 4. Virulence of different isolates of *B. theobromae* on leaves of cashew

Tabel 4. Virulensi berbagai isolat *B. theobromae* pada daun jambu mente

| Isolate | Origin Daerah asal | Infected area Luas infeksi (cm ²) |
|---------------|--------------------|---|
| C031 | Cikampek | 5.8 a |
| C028 | Cikampek | 3.9 ab |
| C076 | NTB | 3.9 ab |
| C066 | Sumbawa | 3.8 ab |
| C069 | Bali | 3.7 ab |
| C072 | Bali | 3.0 b |
| C067 | Kupang | 2.9 b |
| C019 | Sumbawa | 2.7 b |
| C027 | Cikampek | 2.5 b |
| C020 | Sumbawa | 2.2 bc |
| C001 | Bali | 2.2 bc |
| C075 | NTB | 2.1 bc |
| C030 | Cikampek | 1.6 bc |
| Control (PDA) | | 0.5 c |

Note : Numbers followed by the same letters are not significantly different at P = 0.05 according to Duncan's multiple range test

Keterangan : Angka yang diikuti oleh huruf yang sama tidak berbeda nyata pada luas P = 0,05 berdasarkan uji berganda Duncan

found on the infected tissues few days following inoculation. All the pycnidia contained one-celled or two-celled conidia. This result showed that isolates of *B. theobromae* tested were all virulent to cashew.

Effect of temperature and relative humidity on infection of *B. theobromae*

Isolate of *B. theobromae* was able to induce disease symptoms in detached cashew leaves incubated at all levels (treatments) of relative humidity and temperature tested (Table 5). This showed that the pathogen can develop at wider range (r.h. 55- 100 %). The optimum temperature for fungus development was reported around 20-31 °C (AICAF). It is surprising that disease lesion in the leaves incubated at r.h. 100% was smaller than that incubated at r.h. 75%. At the lowest r.h. (54%) the leaves were easily dried after two days. All infected leaves taken from different boxes were able to produce pycnidia when incubated at suitable conditions i.e. humid one. This indi-

Table 5. Diseased lesion in the cashew leaves induced by *B. theobromae*

Tabel 5. Pelukaan pada daun jambu mente yang disebabkan oleh *B. theobromae*

| Saturated solution Larutan jenuh (nominal r. h.) | Length of infected area (cm) of the leaf incubated at Panjang daerah infeksi (cm) pada daun yang diinokulasikan pada | |
|--|--|--------|
| | 24 °C | 30 °C |
| H2O (r.h. 100%) | 1.95 * | 2.67 * |
| KCl (r.h. 85%) | 2.67 | 2.44 |
| NaCl (r.h. 75%) | 5.60 | 4.83 |
| MgCl2 (Actual r.h. 54.9%) | 4.13 | 2.67 |

* Average of 4 samples Rata-rata dari 4 contoh

cated that the fungus could survive at dry conditions. Study on the survival of the pathogen at different is under investigation.

Host range

Botryodiplodia theobromae isolated from diseased cashew showed that the pathogen was a potentially important for crops other than cashew. The pathogen was able to cause damage in the inoculated leaves, stems and fruits through wounding, whereas inoculation in unwounded tissues did not produce any symptoms. The inoculated fruits of carambola, cashew, cocoa, nutmeg and papaya produced water soaked which then developed over the whole fruits caused wet-rot. Thick dark mycelia of the fungus were grown over the diseased tissues, and pycnidia were produced few days later.

Results of this experiment showed that *B. theobromae* isolated from diseased cashew could become an important pathogen not only in cashew but also in other crops, such as carambola, cocoa, nutmeg, papaya, coconut, mango, avocado, rubber and jackfruit (Table 6). Fruit, leaf and stem tissues were all susceptible to infection. For successful infection, the pathogen required wounding. Such wounding could be caused by environmental problems such as strong wind, or due to damage by insects.

Table 6. Pathogenicity of *B. theobromae* isolate C031 in different host plants
 Tabel 6. Patogenitas *B.theobromae*, isolat C031 pada berbagai tanaman inang

| Host plant Tanaman inang | Part of the plant used for inoculation Bagian tanaman untuk inokulasi | Disease symptoms Gejala penyakit |
|-----------------------------|---|---|
| Avocado Alpokah | leaf daun | necrotic, spreading nekrosis, menyebar |
| Carambola Belimbing | fruit buah | water soaked, rot basah, busuk |
| Cashew Jambu mente | leaf daun | necrotic, spreading nekrosis, menyebar |
| Clove Cengkeh | apple buah semu | water soaked, rot basah, busuk |
| Cocoa Kakao | stem, leaf batang, daun | limited spot bercak terbatas |
| Coconut Kelapa | fruit buah | water soaked, rot basah, busuk |
| Jack fruit Nangka | leaf daun | necrotic, spreading nekrosis, menyebar |
| Mango Mangga | leaf daun | necrotic, spreading nekrosis, menyebar |
| Nutmeg Pala | leaf daun | necrotic, spreading nekrosis, menyebar |
| Papaya Pepaya | fruit buah | water soaked, rot basah, busuk |
| Rubber Karet | leaf daun | water soaked, rot basah, busuk |
| | | necrotic, spreading nekrosis, menyebar |

The fact that *B. theobromae* has not been reported to be an important pathogen in these plants in Indonesia needs to be studied further. However, the report from Brazil showed that *B. theobromae* was one of the important pathogens in coconut and significant yield losses from 25-40% equal to production of around 10.000 - 15.000 nuts/ha (WARWICK et al., 1991).

Studied of some cultural and physiological characteristics of *B. theobromae* of cashew showed that the pathogen was found in cashew plantations in Bali, Cikampek, Kupang, NTB and Sumbawa. Growth and conidia size of *B. theobromae* isolated from these areas were similar, but their virulence was varied based on the inoculation in leaves and young stems of cashew seedlings. The most virulent was isolate C031 from Cikampek.

Temperature and relative humidity suitable for development of infection ranged between 24-30 °C and r.h. 75-100%. At these conditions, the pathogen may caused severe damage in cashew. Furthermore, since the pathogen survived at lower r.h. (54%) by forming pycnidia in inoculated cashew leaves or infected in other hosts, field sanitation is necessary to reduce sources of inoculum for further infection. Application of fungicide to protect wound due to insect damages or wind may

be necessary to prevent infection from the pathogen.

CONCLUSION

B. theobromae isolated from diseased cashewnut was pathogenic to cause fruit rot, necrotic lesion and die back in inoculated leaves or young stems. The pathogen can be considered as specific in term of infection only occurred through wounded tissue. Cultural characteristics of the fungus taken from different origins were little varied. The fungus was successfully infected detached leaves at incubation conditions of 24 and 30°C with relative humidity ranged from 55-100%. Isolate of *B. theobromae* was able to cause disease not only in cashewnut but also in other crops such as avocado, carambola, coconut, cocoa, jackfruit, mango, nutmeg, papaya and rubber leaves or fruits.

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